EuroMAbNet
European Monoclonal Antibodies Network

7th Antibody Validation Workshop
13th EuroMAbNet Meeting

17th - 19th of June, 2024
Montpellier, France

BOOK OF ABSTRACTS

EuroMAbNet

European Monoclonal Antibodies Network

Book of Abstracts of the 13th EuroMAbNet Meeting

TIMES AND VENUES

7th Antibody Validation Workshop: Monday, 17th June 2024
IRCM, 208 Rue des Apothicaires, F-34298 Montpellier Cedex 5

13th EuroMAbNet Meeting: Tuesday, 18th June 2024 - Wednesday, 19th June 2024
University of Montpellier, Place Eugène Bataillon, Montpellier,
Building 36, Amphitheater A36.01

ORGANIZER

Pierre Martineau

CO-ORGANIZERS

Giovanna Roncador
Berislav Lisnić
Vanda Juranić Lisnić
Sophia Karagiannis

HOSTED BY

Institut de Recherche en Cancérologie de Montpellier (IRCM)
Université de Montpellier
Institut National de la Santé et de la Recherche Médicale (INSERM)
Institut Régional du Cancer

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Welcome to the 13th EuroMabNet Meeting in Montpellier, France!

Dear participants,

On behalf of the organizing committee, I would like to extend a warm welcome to all of you to the 13th EuroMabNet Meeting, which is being held in the beautiful city of Montpellier, France. We are delighted to have you join us at the University of Montpellier for this exciting event.

The field of antibodies has undergone remarkable growth over the past three decades, with their use in both research and clinical settings expanding at an unprecedented rate. EuroMabNet has always placed a strong emphasis on proper antibody validation and knowledge dissemination to young scientists and newcomers to the field. This is the primary goal of the 7th Antibody Validation Workshop, which will be held the day before at the IRCM.

I would like to express my sincere gratitude to Giovanna Roncador for her unwavering dedication to the development of EuroMabNet and for her preparation of the scientific program. I am confident that you will find the program informative and engaging. I also hope that you will take advantage of the opportunity to network with colleagues, make new connections, and initiate new collaborations.

Our deepest appreciation goes to our sponsors, whose unwavering support is essential for the success of this meeting. I would like to extend a special thank you to the IRCM and the University of Montpellier for providing us with free access to their facilities, as well as to the French MabImprove network for their generous support.

I hope you will have a memorable time at the conference and enjoy the vibrant atmosphere of Montpellier's city, with its renowned student life, stunning beaches, flamingo-filled lagoons, and pleasant climate.

Sincerely,
Pierre Martineau

Head of "Functional screening and targeting in cancer" team
Deputy-Director of Labex MabImprove
IRCM, Montpellier France

On behalf of the EuroMabNet Organizing Committee

WORKSHOP AND MEETING SCHEDULE

7th ANTIBODY VALIDATION WORKSHOP - MONDAY, JUNE 17th

MORNING SESSION

9:30 **Giovanna Roncador**
Monoclonal Antibodies Unit, CNIO, Madrid, Spain
Opening introduction

9:40 **Friedrich Koch-Nolte**
Immunology and Molecular Biology, Institute of Immunology, Hamburg, Germany
Basic introduction to antibodies

10:00 **Pierre Martineau**
Functional screening and targeting in cancer, Institut de Recherche en Cancérologie de Montpellier, France
Generation and engineering of recombinant antibodies

10:20 **Alicia Chenoweth**
Translational Cancer Immunology and Immunotherapy, King's College, UK
How to approach an antibody project

10:40 **Giovanna Roncador**
Monoclonal Antibodies Unit, CNIO, Madrid, Spain
Principles of antibody validation

COFFEE BREAK (11:00-11:30)

11:30 **Paola Kučan Brlić**
University of Rijeka, Faculty of Medicine, Rijeka, Croatia
Reproducibility and dissemination of data

11:50 **Yael Glasson**
ICM, Plateforme de Cytométrie Et d'Imagerie de Masse, Inserm Montpellier, France
About antibody validation on FFPE tissues in immunohistochemistry and imaging mass cytometry

12:10 **Myriam Croze & Véronique Giudicelli**
IMGT Montpellier, France
IMGT tools to analyze antibody sequences, structures and repertoires

12:30 **SPONSOR TALK**
Simone Kidger
Abcam
Recombinant antibodies: our approach to characterization, quality and reproducibility

12:40 **SPONSOR TALK**
Maxime Jacquet
Cell Signalling Technology
More Stringent Antibody Validation Yields More Reliable Results

LUNCH (12:50-14:00)

7th ANTIBODY VALIDATION WORKSHOP - MONDAY, JUNE 17th

AFTERNOON SESSION

14:00 **Interactive Session I (75 min)**
Meet the expert - Giovanna Roncador
Expert in immunohistochemistry

COFFEE BREAK (15:15-15:45)

15:45 **Interactive Session II (75 min)**
Meet the expert - Vanda Juranić Lisnić
Expert in flow cytometry

END OF THE WORKHSOP (17:00)

13th EUROMABNET MEETING
SCIENTIFIC SECTION - TUESDAY, JUNE 18th

MORNING SESSION
Chair: Pierre Martineau

9:00 **Pierre Martineau**
Functional screening and targeting in cancer, Institut de Recherche en Cancérologie de Montpellier, France
Opening and welcome remarks

9:10 **INVITED SPEAKER**
Professor Sofia Kossida
Director IMGT at CNRS - Centre National de la Recherche Scientifique, University of Montpellier, France
IMGT®, the international ImMunoGeneTics information system® current endeavors and future perspective

9:40 **Luis Álvarez-Vallina**
Cancer Immunotherapy Unit (UNICA), 12 de Octubre, University Hospital, Madrid, Spain
Antibody-based T cell redirecting strategies

10:10 **Bruno Robert & Tristan Mangeat**
INSERM U896/Montpellier University/Val d'Aurelle Oncology Center, Montpellier, France
Optimizing antibodies to improve tumor targeting and decrease on-target off-tumor toxicity

10:40 **Selected student short talk**
Julia Hambach
Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
The highway to cancer treatment: Biparatopic CD38-specific nanobody-based CARNK and CAR-T cells for treatment of multiple myeloma

COFFEE BREAK (10:55-11:30)

MORNING SESSION - Cont'd
Chair: Vanda Juranić Lisnić

11:30 **Valerija Kovač**
Center for Immunology and Development and research group Biomedicine, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
Antibody-based insight into prion protein-related neurodegenerative diseases

11:50 **Sophia Karagiannis**
Translational Cancer Immunology and Immunotherapy, King's College, UK
IgE immunotherapy for cancer translated to the clinic

12:10 **Poster Slam Session**

LUNCH BREAK AND POSTER VIEWING (13:00-14:00)

MEETING SCHEDULE

13th EUROMABNET MEETING
SCIENTIFIC SECTION - TUESDAY, JUNE 18th

AFTERNOON SESSION
Chair: Sophia Karagiannis

14:00 **Selected student short talk**
Benjamina Esapa
St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London, UK
Antigenic Epitope and Antibody Isotype Selection for the Design of Anti-CSPG4 Antibody and ADC Therapies for TNBC

14:15 **Indrė Kučinskaitė-Kodzė**
Department of Immunology, Institute of Biotechnology, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
The use of bacteriophage-derived nanotubes as a carrier of target epitopes for tailored antibody production

14:35 **Roberto Perris**
Centre for Molecular and Translational Oncology & Department of Chemical and Life Sciences and Environmental Sustainability, University of Parma, Italy
Deconvoluted discovery of cancer-elective antigens for immunotherapeutic targeting

14:55 **Jose-Ignacio Rodriguez-Barbosa**
Section of Transplantation Immunobiology, Leon, Spain
LIGHT (TNFSF14), a member of the TNF superfamily that interacts with HVEM and LTbR

15:20 **SPONSOR TALK**
Tatjana Tufedžić
Kuhner
Excellent Scalling up: Essential equipment in mAb production

COFFEE BREAK (15:30-16:00)

AFTERNOON SESSION - Cont'd
Chair: Vladka Čurin Šerbec

16:00 **SPONSOR TALK**
Julian Kirschstein
PEPperPRINT
Antibody Validation by High-Density Peptide and Protein Microarrays

16:10 **Anjana Kushwaha**
IMGT at CNRS - Centre National de la Recherche scientifique, Montpellier, France
IMGT Robust Training Approach for class-I MHC Peptide Binding Prediction

16:30 **Giovanna Roncador**
Monoclonal Antibodies Unit CNIO, Madrid, Spain
Enhancing Transparency in Antibody Research: The Ab ID CARD Initiative

EUROMABNET ROUNDTABLE (17:00-18:00)
EUROMABNET DINNER (20:00)

13th EUROMABNET MEETING
SCIENTIFIC SECTION - WEDNESDAY, JUNE 19th

WELCOME COFFEE (08:00-09:00)

MORNING SESSION
Chair: Pablo Engel

9:00 **INVITED SPEAKER**
Anne Poupon
Research director of French National Institute for Agriculture, Food, and Environment (INRAE) and CTO and co-founder at MAbSilico Parice, France
Applying artificial intelligence to accelerate and de-risk antibody discovery

9:30 **INVITED SPEAKER**
Pierre Cosson
Centre Médical Universitaire, Dpt of Cell Physiology and Metabolism, Geneva, Switzerland
The ABCD toolbox: a database, a journal and much more

10:00 **Friedrich Koch-Nolte**
Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Nanobody discovery from camelids and mice

10:20 **Selected student short talk**
Alessa Schaffrath
Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
LaMice - An appealing alternative for nanobody discoveries

10:35 **Selected student short talk**
Nicola Koch
Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia
Establishing an antibody toolbox for murine cytomegalovirus

10:50 **SPONSOR TALK**
Radha Parmar
Twist Bioscience
Leveraging cutting-edge DNA technologies to build precision libraries for antibody engineering

COFFEE BREAK (11:00-11:30)

13th EUROMABNET MEETING
SCIENTIFIC SECTION - WEDNESDAY, JUNE 19th

MORNING SESSION - Cont'd
Chair: Luis Álvarez-Vallina

11:30 **Selected student short talk**
Rodrigo Lázaro-Gorines
Cancer Immunotherapy Clinical Research Unit, H12O-CNIO, Madrid, Spain
Bispecific trimerbodies combining viral neutralization and dendritic cell-mediated crosspriming boost cytotoxic T cell responses and protect mice against lethal virus challenge

11:45 **SPONSOR TALK**
Marcelo Viegas
ProteoGenix
Advances in human antibody discovery and production

11:55 **Paola Kučan Brlić**
Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia
Therapeutic anti-PVR mAb restores DNAM-1 expression and enhances antitumor immunity

AWARD CEREMONY (12:15-13:00)

END OF THE MEETING (13:00)

ABSTRACTS
INVITED SPEAKERS

Tuesday
18. 06. 2024.
09:10-09:40

INVITED SPEAKER

Sophia Kossida

IMGT®, the international ImMunoGeneTics information system® - current endeavors and future perspective

Sofia Kossida
IMGT®, The International ImMunoGeneTics Information System®, Institute of Human Genetics (IGH), National Center for Scientific Research (CNRS), University of Montpellier (UM), 34000 Montpellier, France

IMGT®, known as the international ImMunoGeneTics information system®, has been a pioneering force in the fields of immunogenetics and immunoinformatics for over three decades. With a wealth of experience, IMGT® offers a comprehensive array of databases and tools to the scientific community, all centered around the adaptive immune response and built upon the IMGT-ONTOLOGY.

Our primary focus is on the latest advancements within the IMGT® databases, tools, reference directories, and web resources, which revolve around three key aspects of IMGT® research and development.

- Axis I centers on unraveling the mysteries of the adaptive immune response by identifying and characterizing the genes responsible for immunoglobulins (IG) and T cell receptors (TR) in jawed vertebrates. This foundational axis serves as the cornerstone for the other two axes.
- Axis II delves into the analysis and exploration of expressed IG and TR repertoires, drawing upon comparisons with IMGT reference directories in both normal and pathological contexts.
- Axis III concentrates on scrutinizing amino acid modifications and the functionalities of 2D and 3D structures in antibody and TR engineering.

In essence, IMGT® is dedicated to advancing our understanding of the adaptive immune response and providing invaluable resources and tools to further research and development in these critical fields. .

Wednesday
19. 06. 2024.
09:00-09:30

INVITED SPEAKER

Anne Poupon

Applying artificial intelligence to accelerate and de-risk antibody discovery

Anne Poupon[1,2]
[1] MAbSilico, 37000 Tours, France
PRC, CNRS, INRAE, 37380 Nouzilly, France

As in all sectors of science and industry, artificial intelligence (AI) is meant to have a high impact in the discovery of antibodies in the coming years. Antibody discovery was traditionally conducted through a succession of experimental steps: animal immunization, screening of relevant clones, in vitro testing, affinity maturation, in vivo testing in animal models, then different steps of humanization and maturation generating the candidate that will be tested in clinical trials. This scheme suffers from different flaws, rendering the whole process very risky, with an attrition rate over 95%. The rise of in silico methods, among which AI, has been gradually proven to reliably guide different experimental steps with more robust processes. They are now capable of covering the whole discovery process.

Wednesday
19. 06. 2024.
09:30-10:00

INVITED SPEAKER

Pierre Cosson

The ABCD toolbox: a database, a journal and much more

Pierre Cosson[1,2]
Centre Médical Universitaire, Dpt of Cell Physiology and Metabolism, Geneva, Switzerland

Our aim is to make recombinant antibodies as widely accessible as possible to the research community. The Geneva Antibody Facility has created a database of recombinant antibodies with known targets: the ABCD database currently includes 27'000 antibodies against 4'300 different targets. The scientific journal Antibody Reports publishes characterization of recombinant antibodies. We also developed specific tools for production of antibodies. I will discuss how these tools and materials could be used by the community of antibody developers and by the whole research community.

ABSTRACTS
ORAL PRESENTATIONS

Tuesday
18. 06. 2024.
09:40-10:10

ORAL PRESENTATION

PRESENTER
Luis Álvarez-Vallina

Antibody-based T cell redirecting strategies

Luis Álvarez-Vallina
Cancer Immunotherapy Unit Hospital Universitario 12 de Octubre/Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain

Immunotherapies based on redirecting T cell activity against tumor cells are under active investigation. Bispecific T cell engagers (TCE) are engineered antibodies with at least one arm targeting a tumor-associated antigen (TAA) and another arm directed against the CD3e chain of the TCR/CD3 complex. The first TCE, the anti-CD19 blinatumomab, was approved in 2014, but no other reach the market until 2022. Now the area is heating up, with several approvals in the last three years and many more under review. TCEs are likely to transform the treatment of hematological malignancies in the short term, as they are significantly more effective than conventional monoclonal antibodies which recognize the same TAA. The field is expanding with a plethora of different formats and targets, and more than 100 TCEs are already in clinical trials. The impressive clinical success of engineered T cells expressing chimeric antigen receptors (CAR T cells) has led to renewed interest in a novel cancer immunotherapy strategy that combines features of antibody and cell-based therapies. This emerging approach is based on the endogenous secretion of TCE by engineered T cells (STAb-T cells). Adoptive transfer of STAb-T cells has demonstrated potent anti-tumor activity in both solid and hematological preclinical models..

Tuesday
18. 06. 2024.
10:10-10:40

ORAL PRESENTATION

PRESENTERS
Bruno Robert & Tristan Mangeat

Optimizing antibodies to improve tumor targeting and decrease on-target off-tumor toxicity

Bruno Robert & Tristan Mangeat[1]
INSERM U896/Montpellier University/Val d'Aurelle Oncology Center, Montpellier, France

Given the difficulty of identifying tumor-specific antigens, therapeutic antibody-based immunotherapy mostly targets tumor-associated antigens overexpressed by cancer cells. However, the off-targeting of the antigen weakly expressed by healthy tissues frequently results in unwanted side effects for patients. This "on-target, off-tumor" targeting could even lead to fatal toxicities, especially with antibody-drug conjugates and T-cell engager. Therefore, our team is focused on the development of innovative strategies to optimize the tumor targeting specificity of antibodies.

Firstly, we developed an approach based on the specific acidification of the tumor microenvironment to avoid the binding of antibodies to healthy tissue. This well-known phenomenon, known as the Warburg effect, results from the preferential tumor metabolism of glucose into lactic acid by tumor cells. The export of the metabolite results in an acidification of the tumor microenvironment. Our strategy consists of developing pH-dependent antibodies, i.e. antibodies that are able to bind to their target at the acidic tumor microenvironment (pH 5.5-6.8), but have very weak or, ideally, no binding at all to the same target present on healthy tissue at physiological pH (pH 7.2-7.4). We have developed a method for the selection of antibody fragments by phage display from a naïve synthetic scFv library, and the screening of pH-dependent antibodies against a tyrosine kinase receptor. Several scFvs were selected for their acid pH binding and their acid /physiological pH binding ratio. The two best antibodies also displayed a much stronger binding at acidic pH than at physiological pH on different tumor cell lines, and no binding to the receptor expressed by immortalized healthy cells at physiological pH. Mutagenesis studies were performed to have a better understanding of the pH-dependent binding of antibodies.

Secondly, we investigated whether Fc silencing influenced antibody biodistribution. We compared recombinant 89Zr-labeled antibodies with wild-type Fc and with mutated Fc (LALAPG triple mutation to prevent binding to Fc gamma receptors). After antibody injection in mice harboring xenografts of different tumor cell lines or of immortalized human myoblasts, we analyzed antibody biodistribution by PET-CT and conventional biodistribution analysis. Accumulation in liver was strongly reduced and tumor-specific targeting was increased for the antibodies with mutated Fc compared with wild-type Fc. Finally, the combination of antigen-binding site and Fc modification could be a promising strategy to improve the safety profile of antibody-drug conjugates.

Tuesday
18. 06. 2024.
10:40-10:55

SELECTED STUDENT SHORT TALK

PRESENTER
Julia Hambach

The highway to cancer treatment: Biparatopic CD38-specific nanobody-based CAR-NK and CAR-T cells for treatment of multiple myeloma

J Hambach[1,2], **AJ Gebhardt**[1,2], **LJ Pape1,2**, **AJ Duttmann**[1,2], **T Stähler**[1], **G Adam**[2], **K Riecken**[3], **B Fehse**[3], **K Weisel**[3], **F Koch-Nolte**[1], **P Bannas**[2]
[1] Institute of Immunology
[2] Department of Diagnostic and Interventional Radiology and Nuclear Medicine
[3] Department of Stem Cell Transplantation
[4] Department of Oncology and Hematology, University Medical Center, Hamburg, Germany

Nanobodies represent the antigen-binding variable domains of camelid heavy-chain antibodies. Nanobodies have distinct advantages over conventional antibody variable domains, such as enhanced solubility and smaller size. Compared with the binding domains of conventional antibodies, nanobodies facilitate convenient reformatting into mono-, bi-, and multispecific proteins. This renders nanobodies particularly suitable as binding domains for chimeric antigen receptors (CARs) (1). CD38 is a type II membrane protein overexpressed on multiple myeloma cells. CD38-specific nanobodies binding three distinct epitopes on CD38 were used to create CD38-specific nanobody-based CARs in NK92 cells. These nanobody-based CAR-NK cells showed potent cytotoxicity against CD38-expressing cell lines in vitro and primary multiple myeloma cells ex vivo (2). Cilta-Cel (Ciltacabtagene Autoleucel), a breakthrough nanobody-based CAR therapy, contains two genetically linked nanobodies targeting different BCMA epitopes. Significantly fewer allogenic T cells expressing Cilta-Cel are required to achieve complete remission compared with monoparatopic nanobody-based BCMA-specific CARs (3).

We now developed biparatopic CD38-specific nanobody-based CAR-NK and CAR-T cells to assess the potential enhancement of cytotoxicity against myeloma cells. These cells exhibit biparatopic binding to CD38. Both CAR-NK and CAR-T cells mediated effective lysis of CD38-expressing cells in vitro. The orientation of the two nanobodies within the CAR appeared to influence the killing capacity of CAR-NK and CAR-T cells. We plan further investigation using patient-derived primary myeloma cells ex vivo. Our findings underscore the feasibility of generating functional biparatopic CAR-NK and CAR-T cells using CD38-specific nanobodies. CD38-specific nanobody-based CAR cells might be promising for future multiple myeloma therapy.

1. Hambach J, Mann AM, Bannas P, Koch-Nolte F. Targeting multiple myeloma with nanobody-based heavy chain antibodies, bispecific killer cell engagers, chimeric antigen receptors, and nanobody-displaying AAV vectors. Front Immunol. 2022;13:1005800.
2. Hambach J, Riecken K, Cichutek S, Schütze K, Albrecht B, Petry K, et al. Targeting CD38-Expressing Multiple Myeloma and Burkitt Lymphoma Cells In Vitro with Nanobody-Based Chimeric Antigen Receptors (Nb-CARs). Cells. 2020;9(2).
3. Bao C, Gao Q, Li LL, Han L, Zhang B, Ding Y, et al. The Application of Nanobody in CAR-T Therapy. Biomolecules. 2021;11(2).

Tuesday
18. 06. 2024.
11:30-11:50

ORAL PRESENTATION

PRESENTER
Valerija Kovač

Antibody-based insight into prion protein-related neurodegenerative diseases

Valerija Kovač[1,*], Feizhi Song[2,*], Behnam Mohammadi[2,*], Maja Černilec[1], Hermann C. Altmeppen[2,§], Vladka Čurin Šerbec[1,§]
[1] Center for Immunology and Development and research group Biomedicine, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
[2] Institute of Neuropathology, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany
[*] Shared first authorship
[§] Co-correspondance: h.altmeppen@uke.de, vladka.curin@ztm.si

Antibodies are essential for studying proteins and their involvement in protein-related mechanisms. They help distinguish between proteins, protein fragments, and protein isoforms and enable diagnostics for target-based disease. Prion protein (PrP) remains the culprit behind prion diseases. Furthermore, PrP shed forms have recently received attention as aggregation-related agents in other neurodegenerative diseases. In our previous studies, we prepared monoclonal antibody V5B2 that selectively recognizes a released form of PrP that ends with amino acid residue Tyr226, PrP226*/shed PrP. Using the antibody, we undoubtedly confirmed that in humans, PrP is cleaved at Tyr226, producing a physiologically present and strictly ADAM10-dependent shed form of PrP. With the antibodies directed against human shed PrP, we also detected the shed form in all of the most relevant species naturally affected by fatal and transmissible prion diseases (cattle, sheep, deer). Apart from human and animal prion diseases, we could also show that shed PrP co-localizes with extracellular aggregates of misfolded proteins in patients with Alzheimer`s disease. These findings provide new insight into the role of PrP shedding and the involvement of shed PrP in neurodegeneration and neuroprotection.

This presentation will describe PrP shed by protease ADAM10 and its potential involvement in diseases. We will also discuss the possible application of the new knowledge to understanding and diagnosis of neurodegenerative diseases.

Tuesday
18. 06. 2024.
11:50-12:10

ORAL PRESENTATION

PRESENTER
Sophia Karagiannis

IgE immunotherapy for cancer translated to the clinic

Sophia Karagiannis
Translational Cancer Immunology and Immunotherapy, King's College, UK

Monoclonal antibodies for cancer treatment are designed as IgGs. Antibodies of the IgE class are known for pathogenic roles in allergies but physiologically IgE antibodies are also known for their contributions to anti-parasitic immune responses. IgEs can exert potent immunological effects in tissues through very high affinity for cognate Fcε receptors on immune effector cells such as macrophages, which are also known to infiltrate tumours. We hypothesised that directing these powerful effector mechanisms against cancer-associated antigens may activate tissue resident immune cells against tumours. We engineered monoclonal IgE class antibodies directed against cancer-associated antigens. IgE-based immunotherapy significantly reduced tumour burden in different rodent models of solid tumours such as ovarian cancer and melanoma. Tumour growth restriction associated with induction of pro-inflammatory immunological mechanisms and macrophage recruitment in the tumour microenvironment. The first-in-class IgE antibody has been translated to clinical testing and encouraging data have been generated in the first-in-man clinical trial. Together, pre-clinical and clinical studies point to IgE as a promising antibody class for cancer immunotherapy.

Tuesday
18. 06. 2024.
14:00-14:15

SELECTED STUDENT SHORT TALK

PRESENTER
Benjamina Esapa

Antigenic Epitope and Antibody Isotype Selection for the Design of Anti-CSPG4 Antibody and ADC Therapies for TNBCa

Benjamina Esapa[1], Alicia M. Chenoweth[1,2], Anthony Cheung[1,2], Yi Liu1, Kristina M. Ilieva[1], Katie Stoker[1,3], Roman Laddach[1,3,4], Patrycja Gazinska[2], Pablo Romero Clavijo[1], Silvia Crescioli[1], Melanie Grandits[1], Annelie Johansson[2,4], Anita Grigoriadis[2,4], Sophia Tsoka[3], Paul J. M. Jackson[5], David E. Thurston[5,*], and Sophia N. Karagiannis[1,2,3,*]
[1] St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London, Tower Wing, 9th Floor, Guy's Hospital, London SE1 9RT, UK
[2] Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King's College London, Guy's Cancer Centre, London SE1 9RT, UK
[3] Department of Informatics, Faculty of Natural, Mathematical and Engineering Sciences, King's College London, Bush House, London, WC2B 4BG, UK
[4] Cancer Bioinformatics, School of Cancer & Pharmaceutical Sciences, King's College London, Guy's Cancer Centre, London SE1 9RT, United Kingdom
[5] Institute of Pharmaceutical Science, School of Cancer and Pharmaceutical Sciences, King's College London, London SE1 9NH,

Monoclonal antibodies and antibody-drug conjugates (ADCs) targeting cancer cell surface antigens are emerging therapeutic modalities for Triple-negative breast cancer (TNBC) for which effective treatments are urgently required. Chondroitin Sulphate Proteoglycan 4 (CSPG4) is overexpressed in several malignancies and may provide a target for antibody and ADC therapies. Most clinically approved antibodies and ADCs utilise the IgG1 and IgG4 antibody isotype. However, there is no accepted dogma for selection of the optimal subclass or how the antigenic epitope affects pertinent functions such as affinity, internalisation, and cytotoxicity. Here, we evaluated CSPG4 expression in TNBC and compared antibody clones and isotypes for therapeutic development. Immunohistochemical, transcriptomic and spatial transcriptomic evaluations revealed elevated CSPG4 expression in TNBCs compared with other subtypes. CSPG4 expression was retained after chemotherapy, rationalising targeting CSPG4 in treatment-resistant disease. To identify a suitable antibody and ADC construct, we produced three anti-CSPG4 clones, each binding to a distinct epitope, in a human IgG1 format. Live cell imaging, and cytotoxicity studies after conjugation to payloads revealed Clone A to exhibit superior internalisation and payload delivery to cancer cells. Comparative studies of Clone A generated in human IgG1 and IgG4 formats showed IgG1 to exhibit superior internalisation and higher cytotoxicity when conjugated to a cytotoxic payload. In a human TNBC xenograft grown orthotopically in the mammary fat pad of mice reconstituted with human immune cells, anti-CSPG4 IgG1 Clone A significantly restricted tumour growth compared to isotype control. Together, the choice of antigenic epitope and antibody isotype affects essential antibody and ADC features, informing the development of anti-CSPG4 therapies to meet the clinical need presented by TNBC.

Tuesday
18. 06. 2024.
14:15-14:35

ORAL PRESENTATION

PRESENTER
Indrė Kučinskaitė-Kodzė

The Use of Bacteriophage-Derived Nanotubes as a Carrier of Target Epitopes for Tailored Antibody Production

Indrė Kučinskaitė-Kodzė[1,*], Karolina Bielskė[1], Silvija Juciutė[2], Aliona Avižinienė[1], Rasa Petraitytė Burneikienė[1], Aurelija Žvirblienė[1]
[1] Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania
[2] Faculty of Chemistry and Geosciences, Institute of Chemistry, Vilnius University, Naugarduko g. 24, Vilnius, Lithuania
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Multimeric nanostructures such as virus-like particles (VLPs) can present foreign epitopes of interest on the surface, offering a technique to produce immunogens for developing monoclonal antibodies (mAbs) with the desired specificity and functionality. Besides of VLPs, other multimeric proteins have a potential for epitope presentation. In this study, yeast-derived recombinant tail tube protein gp39 from vB_EcoS_NBD2 bacteriophage, known for its self-assembly into extremely long (up to 3.95 μm), flexible and stable polytubes was selected as the scaffold to develop immunogens displaying multivalent foreign target epitopes. To prove this approach, a highly conserved sequence of bacterial β-lactamases was selected as a target epitope. With antibiotic resistance reaching alarming levels globally, rapid detection of resistance determinants is crucial for administering appropriate antimicrobial medications. In this context, a universal 17 amino acid (aa) sequence (positions 77-93 aa of DHA-1 β-lactamase (GenBank no. JN638038.1)) conserved among various bacterial β-lactamases responsible for degradation of β-lactam antibiotics was displayed on bacteriophage-derived nanotubes composed of gp39 protein, aiming to develop mAbs capable of recognising a broad spectrum of β-lactamases in biological samples.

Chimeric nanotubes, consisting of gp39 protein with a C-terminal insertion of the 17 aa-long universal peptide of β-lactamases, were produced in yeast and used to immunise Balb/c mice. Thirteen hybridoma clones producing target peptide-specific mAbs of IgG isotype were generated. These mAbs were further validated using various immunoassays. To assess mAb reactivity with target proteins, purified enzymatically active DHA-1, PDC-195, ACT-14, CMY-34, and ADC-144 β-lactamases expressed in E. coli were utilised. Eleven out of thirteen mAbs were reactive with all used lactamases in indirect ELISA and Western blot assays. Two mAbs showed lower cross-reactivity, particularly not reacting with PDC-195 and ADC-144. The ability of the mAbs to recognise natural β-lactamases was tested using the lysate of Citrobacter portucalensis isolate producing CMY-34. All mAbs efficiently detected natural CMY-34 in Western blot assay. This reactivity was further confirmed by immunoprecipitation of CMY-34 from bacterial lysate. Two epitopes of the mAbs within 17 aa-long conserved sequence were determined. Epitope mapping results were consistent with the cross-reactivity of the mAbs. The group of broadly reactive mAbs recognised an 11 aa-long epitope at positions 79-89 aa of DHA-1, while the two mAbs showing lower cross-reactivity recognised a 9 aa-long epitope at positions 85–93 aa of DHA-1. Highly cross-reactive mAbs targeting natural β-lactamases were utilised in optical (spectral ellipsometry) and acoustic (quartz crystal microgravimetry with dissipation) immunosensors to detect β-lactamases in bacterial lysates.

This study highlights the potential of chimeric nanotubes formed by the tail tube protein gp39 of vB_EcoS_NBD2 bacteriophage as a novel immunogen for producing epitope-specific mAbs.

Tuesday
18. 06. 2024.
14:35-14:55

ORAL PRESENTATION

PRESENTER
Roberto Perris

Deconvoluted discovery of cancer-elective antigens for immunotherapeutic targeting

Roberto Perris
Centre for Molecular and Translational Oncology & Department of Chemical and Life Sciences and Environmental Sustainability, University of Parma, Italy

Identification of ideal targets for passive immunotherapy directed against the cancer cells of solid tumours, or their microenvironment (i.e., the tumour ecosystem), is notoriously challenging because of the difficulty in disclosing discrete molecules that are truly exclusive components of the tumour lesion. In hematological neoplasia, this difficulty is further exarcebated by the need to target surface-associated antigens electively expressed by the neoplastic lymphocytes/leukocytes.

Although the majority of the immunotherapeutics clinically approved for treatment of lymphomas and leukemia are directed against fairly common hematopoietic surface molecules, and their anti-tumour efficacy relies upon their exhuberance when compared to healthy cells, a recurrent clinical caveat is the high levels of collateral toxicity detected in patients over the years (post-treatment). This limitation incites the search for truly tumour-specific antigens for antibody drug targeting.

There are presently more than 200 types of empirically determined post-translational modifications (PTMs) in the human proteome (and even more are predicted in silico). Accordingly, a crucial role in tumorigenesis has been assigned to aberrant transformation-dependent variations of the glycome and glycoproteome of the cancer cell. We have leveraged on the fact that glycosylation is the most abundant and diverse form of PTM of proteins in eukaryotes and the cancer cells to approach the production of antibodies against antigenic glycoforms of cell surface proteins of neoplastic lymphocytes.

To this end, we have devised a method to derive immunogen complexes enriched for PTM components and, as a proof-of-concept, we have adopted a follicular lymphoma cell line to explore the potential of the method for generating cancer cell surface glycoprotein-specific mAbs. Screening of the produced mAbs on an ample panel of hematologic cell lines, patient-derived cells and healthy PBL revealed up to 80% cancer-specificity of the mAbs and the concurrent detection of glyco-antigens common to various lymphoma and leukemia variants. Antibody engagement of some of these antigens produces distinct biological effects on the cancer cells, suggesting that the approach is capable of creating functional mAbs to be further developed preclinically because of their potential anti-tumour activity.

Tuesday
18. 06. 2024.
14:55-15:15

ORAL PRESENTATION

PRESENTER
Jose-Ignacio Rodriguez-Barbosa

LIGHT (TNFSF14), a member of the TNF superfamily that interacts with HVEM and LTbR

Maria-Luisa del Rio[1], Pascal Schneider[2] and Jose-Ignacio Rodriguez-Barbosa[1]
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LIGHT binds to two receptors HVEM (TNFSFR14, CD270) and LTβR (TNFSFR3). The bidirectional interaction of HVEM/LIGHT delivers costimulatory and survival signals to T cells, whereas the engagement of LTβR by LTα1β2 and LIGHT has been involved in lymph node development during embryo development and in the formation of ectopic tertiary lymphoid structures at sites of chronic inflammation.

The classical approach to produce soluble recombinant proteins bound to Fc fragment of immunoglobulin G (IgG) turned out to be functionally inactive when applied to the production of mouse LIGHT (dimeric Ig-LIGHT). To overcome this hurdle and gain insight into the preclinical potential of LIGHT as a proinflammatory cytokine expressed in tumor cells and taking into account that trimerization of the members of the TNFR/TNF superfamily and clustering is required for efficient signal transduction, various genetic constructs encoding multimeric recombinant mouse LIGHT protein were expressed in eukaryotic cells (trimeric FF (Flag-Foldon)-LIGHT and hexameric Ig.FF-LIGHT) and were then tested for their ability to bind and engage HVEM and LTbR, the two known ligand partners of LIGHT. Whereas the dimeric format of recombinant LIGHT (Ig-LIGHT) neither bind nor kill target cells that expressed a chimeric construct composed of mouse HVEM bound to human FAS transduced into Fas deficient Jurkat cells, the trimeric and hexameric recombinant versions of LIGHT exhibited effective binding and functional activity in this cell-based reporter bioassay. The cytotoxic effect observed upon LIGHT interaction with its receptors was abrogated in a dose-dependent manner by antibody-mediated blockade of LIGHT.

Membrane bound LIGHT and two different configurations of soluble recombinant LIGHT (trimeric and hexameric LIGHT) were then transduced into the poorly immunogenic B16.F10 melanoma cells and tumor growth was monitored in syngeneic recipients. Whereas cell-bound membrane LIGHT did not delay or prevent tumor growth, the bioactive hexameric configuration of recombinant soluble LIGHT exhibited a significant potent anti-tumor effect delaying tumor growth.

In conclusion, in-situ secretion of hexameric LIGHT by tumor cells promotes immune cell infiltration and control of tumor growth likely by stimulating HVEM expressed on T cells and LTbR expressed on stromal and myeloid cells of the tumor microenvironment.

Tuesday
18. 06. 2024.
16:10-16:30

ORAL PRESENTATION

PRESENTER
Anjana Kushwaha

IMGT Robust Training Approach for class-I MHC Peptide Binding Prediction

Anjana Kushwaha[1], Patrice Duroux[1], V´eronique Giudicelli[1], Konstantin Todorov[2], Sofia Kossida[1]
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National Center for Scientific Research (CNRS),France

The interaction between the Major Histocompatibility Complex (MHC) and antigen peptide sequences is crucial in the immune response, as it involves presenting peptides on the cell surface to T cells. The MHC gene family is divided into two major subgroups: MHC Class I and MHC Class II. MHC class I molecules consist of one membrane-spanning α chain produced by MHC genes, and one β chain produced by the β2-microglobulin gene. MHC class II molecules consist of two membrane-spanning chains, α and β both produced by MHC genes. Prediction of MHC binding peptides is important to vaccine design and targeted therapy development in immunology and cancer immunotherapy, however MHC-II binding peptides is much more challenging than MHC-I because HLA-II are highly polymorphic and the size of the presented peptides differ. Computational scanning of peptide sequences that bind to a specific major histocompatibility complex (MHC) can speed up the peptide-based vaccine development process, as a result various methods are being actively developed for prediction. Recently, more sophisticated neural network based models have been proposed for peptide-HLA class I binding prediction, they have two shortcomings: (i) they rely on hand-crafted pseudo-sequence extraction, (ii) they do not generalise well to different datasets, which limits the practicality of these approaches. While existing methods rely on a 34 amino acid pseudosequence, our findings uncover the involvement of 147 positions in direct interactions between MHC and peptide. I further demonstrate that neural architectures are capable of learning the intricacies of peptide-MHC binding using full sequences. In my research, I have demonstrated the efficacy of my transformer-based perceiver model in predicting peptide-HLA class I binding probabilities, particularly when considering full MHC sequences. My model employs cross-attention mechanisms within deep neural networks, therefore exhibits capability to learn comprehensive representations of MHC sequences, underscoring the potential of efficient transformer architectures like perceiver architecture in computational immunology. Furthermore, I also incorporate self-supervised learning by training the network with mutations that empowered my model to capture subtle inter-dependencies between peptide and HLA sequences, enabling my model to generalize effectively and resulting in significant improvements in binding affinity predictions. I extensively evaluate RobustpMHC on 8 different datasets and showcase an overall improvements of over 6% in binding prediction accuracy compared to state-of-the-art approaches. Notably, my model maintains robust performance in the presence of mutations, which is vital for real-world applications. My work provides a new perspective towards more accurate peptide-MHC binding prediction.

Keywords— pMHC, self-supervised, robust, deep learning

Tuesday
18. 06. 2024.
16:30-17:00

ORAL PRESENTATION

PRESENTER
Giovanna Roncador

Enhancing Transparency in Antibody Research: The Ab ID CARD Initiative

Giovanna Roncador
Monoclonal Antibodies Unit, Biotechnology Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

The Ab ID CARD initiative (https://www.euromabnet.com/antibody-identity-card/) stands as an example in the area of antibody research, addressing the ongoing challenges of antibody selection and validation. Promoted by the EuroMAbNet organization, this initiative aims to improve transparency and reproducibility in scientific experiments involving antibodies.

The Ab ID CARD provides a unique identity for each antibody, offering detailed information on its properties, performance, and validation data. The platform is a user-friendly interface enables researchers to find detailed information such as immunogen details, epitope information, concentration used and application specific protocols.

In line with EuroMAbNet's validation guidelines (https://www.euromabnet.com/guidelines/), our approach includes using multiple positive and negative controls for each application. For example, transfected cell lines are used to discard antibody cross-reactivity, while CRISPR/Cas9-modified cell lines confirm antibody specificity. Additionally, controls with different levels of protein expression and the use of cell lines and tissues with known presence or absence of the target protein help confirm the endogenous expression of the target.

The initiative also employs a multi-faceted approach to antibody validation. It includes parallel testing of different antibodies against the same target, utilizing various antigens and species. This not only enhances the confidence in validation data but also allows researchers to observe common patterns across different antibody clones.

Furthermore, the Ab ID CARD initiative aligns with other initiatives that help increasing research traceability and reproducibility. By encouraging researchers and industry stakeholders to share comprehensive information about each antibody, it fosters a collaborative environment that benefits the entire scientific community.

Wednesday
19. 06. 2024.
10:00-10:20

ORAL PRESENTATION

PRESENTER
Friedrich Koch-Nolte

Nanobody discovery from camelids and mice

Friedrich Koch-Nolte
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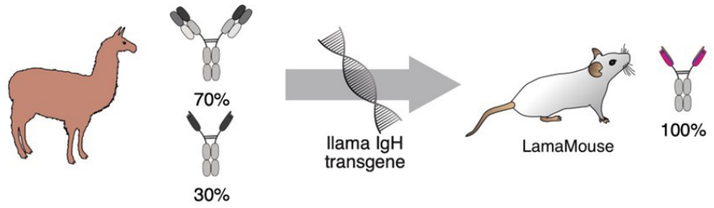
Nanobody discovery from immunized animals benefits from clonal expansion and affinity maturation in natural immune responses. For nanobody discovery from immunized camelids (llamas, alpacas, dromedaries and camels), the nanobody-repertoire typically is PCR-amplified from peripheral blood lymphocytes in a blood sample obtained a few days after the final boost (1). The PCR-product is cloned into a phage display vector and target specific nanobodies are selected by panning of the phage library on the target. To this end, the target can be immobilized on beads, cells, or wells of an ELISA-plate. The husbandry of camelids is labor-intensive, time-consuming and expensive.

Mice offer facile breeding as well as easy access to lymphoid organs and to genetic tools. To combine the advantages of nanobodies and mice, we inserted an engineered llama immunoglobulin heavy chain (IgH) gene locus into IgH deficient mice (2). We refer to this transgenic mouse line as 'LamaMouse'. LamaMice express llama IgM and IgG molecules without association to Igκ or Igλ light chains. We obtained affinity-matured antigen-specific nanobodies from LamaMice immunized with IgE or CLELC9a using phage display technologies. Moreover, we readily recovered specific nanobodies against a variety of targets from immunized LamaMice also by classical hybridoma technology, as well as by Beacon® single B cell screening and by directly cloning the nanobody-repertoire into a eukaryotic expression vector that drives transiently transfected HEK cells to secrete high levels of heavy chain antibodies.

1. Eden T, et al. 2018. A cDNA Immunization Strategy to Generate Nanobodies against Membrane Proteins in Native Conformation. Front Immunol. 8:1989.
2. Eden T., et al. 2024. Generation of Nanobodies from Transgenic 'LamaMice' Lacking an Endogenous Immunoglobulin Repertoire. Nat Commun. in press



Wednesday
19. 06. 2024.
10:20-10:35

SELECTED STUDENT SHORT TALK

PRESENTER
Alessa Schaffrath

LaMice - An appealing alternative for nanobody discoveries

Alessa Schaffrath[1], Natalie Tode[1], Thomas Eden[1], Janusz Wesolowski[1], Tobias Stähler[1], Julia Hambach[1], Josephine Gebhardt[1], Fabienne Seyfried[1], Jannis Woens[4], Irm-Hermanns Borgmeyer[3], Kristoffer Riecken[4], Björn Rissiek[1,2], Boris Fehse[4], Friedrich Haag[1], Friedrich Koch-Nolte[1]
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[2] Dept. of Neurology,
[3] ZMNH
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A nanobody or VHH is a recombinantly expressed variable immunoglobulin domain of an antibody consisting of two heavy chains only (hcAb). In contrast to conventional antibodies and their derivatives, nanobodies are highly stable, soluble and small. These favorable biophysical properties allow them to be easily assembled into various formats, e.g. by fusing them to other nanobodies and/or proteins. Traditionally, nanobodies are derived from synthetic libraries or immunized camelids. Here, we demonstrate an appealing alternative that benefits from the affinity maturation during a natural antibody response and from the established technologies for genetic engineering and antibody discoveries in mice – the llama hcAb-producing "LaMice". LaMice were established by introducing an engineered llama immunoglobulin heavy chain (IgH) locus into B cell-deficient IgH-knock-out mice. We show that the transgene restores B cell development and that these B cells express and secrete immunoglobulins, which are solely composed of llama IgHs, without any detectable association with kappa or lambda light chains. We also demonstrate the suitability of our LaMice for the discovery of antigen-specific and functional nanobodies by immunizing them with the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein as model antigen. For the discovery, we directly cloned the PCRamplified VHH repertoire from spleen into a eukaryotic expression vector, upstream of the Fc region of rabbit IgG. Nanobody-rabbit IgG hcAbs were produced in HEK cells and their supernatants were tested for binding to different variants of the spike protein by flow cytometry, and for neutralization using a pseudovirus neutralization assay. Among several RBD-reactive hcAb families, we identified one that neutralizes both the Wuhan strain and its omicron variant. We conclude that LaMice present a promising, flexible and cost-effective new platform for nanobody discoveries.

Wednesday
19. 06. 2024.
10:35-10:50

SELECTED STUDENT SHORT TALK

PRESENTER
Nicola Koch

Establishing an antibody toolbox for murine cytomegalovirus

Nicola Koch, Tina Ružić, Jelena Železnjak, Magdalena Medved, Vanda Juranić Lisnić
Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia

Human cytomegalovirus (HCMV) is a human specific herpesvirus which causes severe disease in immunocompromised individuals and neonates. Murine cytomegalovirus (MCMV) can infect mice and is used to model cytomegalovirus infections to study pathogenesis and immune reaction as it shares biological similarities with HCMV.

Flow cytometry is a powerful and high-throughput method to analyze the expression of proteins on the surface and inside the cell. This is often done by staining cells with antibodies and secondary antibodies labeled with fluorescent molecules. Thus, differences in the phenotype of cells during virus infection can be analyzed. However, as rarely 100% of cells in a sample are infected, it is important to distinguish between infected and uninfected cells to be able to identify effects of infection without confounding the data with bystander cells. In this study, MCMV specific antibodies were tested in flow cytometry to find suitable antibodies for the use as infection control. Four antibodies were identified which could be used as infection controls. These antibodies were m04-17, m04-16, targeting the viral protein m04, IE1.01, targeting IE1, and M45.01, targeting the protein M45.

As MCMV expresses a viral FCγ receptor (m138), including proper negative controls is important. Often, isotype control is used. However, it was previously reported that this control is not reliable for all applications. Testing of the isotype controls showed great differences in the behavior of different antibodies. While the specific antibodies were mostly very little affected by m138, some of the tested isotype controls for IgG2a and IgG2b antibodies were affected by m138. Furthermore, differences in the behavior between staining conditions (surface, intracellular or intranuclear staining) were noticed. However, not all differences in the behavior can be explained with a sensitivity for m138, which highlights that controls have to be tested in order to be able to trust them.

Wednesday
19. 06. 2024.
11:30-11:45

SELECTED STUDENT SHORT TALK

PRESENTER
Rodrigo Lázaro-Gorines

Bispecific trimerbodies combining viral neutralization and dendritic cell-mediated crosspriming boost cytotoxic T cell responses and protect mice against lethal virus challenge

Rodrigo Lázaro-Gorines[1,2,3], Patricia Pérez, Ignacio Heras-Murillo, Irene Adán-Barrientos, Guillermo Albericio, David Astorgano, Sara Flores, Joanna Luczkowiak, Nuria Labiod, Seandean L. Harwood, Alejandro Segura-Tudela, Laura Rubio-Pérez, Carlos Alfonso, Rocío Navarro, Marta Compte, Laura Sanz, Francisco J. Blanco, Mariano Esteban, Raquel Godoy-Ruiz, Inés G. Muñoz, Rafael Delgado, David Sancho, Juan García-Arriaza, Luis Álvarez-Vallina
[1] Cancer Immunotherapy Unit (UNICA), Department of Immunology, Hospital Universitario12 de Octubre, 28041 Madrid, Spain
[2] Immuno-Oncology and Immunotherapy Group, Instituto de Investigación Sanitaria 12 de Octubre (imas12), 28041 Madrid, Spain
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The administration of neutralizing antibodies (nAbs) has proved to be effective against different viral driven diseases by providing immediate transferred host immunity. Beyond of this, dual antibody-based strategies combining virus neutralization with immune stimulation to enhance specific cytotoxic T cell responses, such as dendritic cell (DC) cross-priming, represent a promising approach for improved immunotherapies against viruses. As a starting point for this aim, focusing on SARS-CoV-2 and HIV virus, we have developed a new class of superneutralizing antibodies, formed by grafting single or tandem virus-blocking nanobodies onto a trimerbody scaffold. In both cases, the Spike (S) and Envelope (Env) targeted trimerbodies, TNT and J3T, respectively; showed efficient and broadly neutralizing activities, consequence of their multivalent high avidity interactions with viral antigens. This capacity was confirmed for anti-SARS-CoV-2 TNT, as it binds simultaneously to all six RBD S protein epitopes according to cryo-EM structure. Subsequently, by C-terminal fusion of an anti-DNGR-1 (CLEC9A) scFv, we generated bispecific trimerbodies for targeting the neutralized virions to type 1 conventional DCs (cDC1s) and promote T cell cross-priming. Both bispecific trimerbodies demonstrated DNGR-1-mediated targeting of viral antigens to cDC1s in vitro, resulting in rapid internalization of the antigen-receptor complexes, and increased cytotoxic T cell responses in antibody/antigen conjugate immunization experiments, compared to just-neutralizing trimerbodies. Finally, regarding COVID-19 prototypes, the therapeutic administration of TNTDNGR-1, but not TNT, completely protected K18-hACE2 mice from a lethal SARS-CoV-2 challenge, promoting long-term adaptative immune responses through the generation of S protein-specific CD8+ T cells while allowing the efficient development of humoral anti-viral responses. Collectively, our results reinforce the mechanistic necessity of immune interactions for the efficacy of antiviral neutralizing antibodies, while providing an Fc-free effective and alternative strategy that could be advantageously used to provide both immediate and long-term therapeutic protection against SARS-CoV-2 and other viral infections.

Wednesday
19. 06. 2024.
11:55-12:15

ORAL PRESENTATION

PRESENTER
Paola Kučan Brlić

Therapeutic anti-PVR mAb restores DNAM-1 expression and enhances antitumor immunity

Paola Kucan Brlic[1], Anas Atieh[2], Akram Obeidat[2], Keren Paz[2], Guy Cinamon[2], Vitenshtein Alon[2], Chechik Simona[2], Lea Hirsl[1], Marija Mazor[1], Tihana Lenac Rovis[1], Stipan Jonjic[1], Ofer Mandelboim[3], Pini Tsukerman[2]
[1] Center for Proteomics, University of Rijeka Faculty of Medicine, Rijeka, Croatia
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[3] Hebrew University, Jerusalem, Israel

PVR (CD155), a membrane protein significantly upregulated in numerous tumor types, has been linked to poorer patient outcomes due to its central role in immune suppression. Recently, PVR has emerged as a primary mechanism of resistance to approved immune checkpoint inhibitors (ICIs). Its impact is mediated through interactions with the key stimulatory receptor, DNAM-1 (CD226), on T and NK cells, resulting in DNAM-1 internalization and degradation. PVR also interacts with inhibitory immune checkpoint receptors such as TIGIT, CD96, and KIR2DL5A.

NTX1088 is an innovative, first-in-class anti-PVR monoclonal antibody, developed by Nectin Therapeutics.. By effectively binding PVR with high affinity, NTX1088 exhibits a multifaceted role in immune stimulation. It disrupts the interaction between PVR and its receptors, thereby restoring DNAM-1 expression and its immune activation function, while concurrently counteracting inhibitory signals from TIGIT, CD96 and KIR2DL5A in immune cells. In vitro studies have shown that NTX1088, as a standalone treatment, significantly enhances immune cell activation, resulting in increased immune-mediated tumor cell killing, IFNγ secretion, and CD137 induction which surpass the effects of TIGIT, CD112R, and PD1 antibody blockade. Numerous humanized murine xenograft models also demonstrated robust inhibition of tumor growth with NTX1088 as a monotherapy and in combination with PD1 blockade. Identification of DNAM-1 downmodulation by PVR as a critical immune surveillance mechanism underscores the unique capacity of NTX1088 to restore DNAM-1 expression, a feature not observed with any other therapies. NTX1088 is presently undergoing assessment in a Phase 1, open-label, multi-center clinical trial (NCT05378425). The primary objectives of the trial are to evaluate safety and determine optimal dosage for following clinical trials. The study also assesses pharmacokinetics and efficacy, with particular emphasis on, pharmacodynamics and biomarker discovery. To this date, the ongoing dose escalation phase has shown a favorable safety profile across the tested dose levels.

ABSTRACTS
POSTER PRESENTATIONS

POSTER PRESENTATION

PRESENTER
Heather J. Bax

Melanoma-targeting CSPG4 IgE anti-tumoral effects associated with pro-inflammatory immune phenotypes and signalling pathways

Jitesh Chauhan[1,2], Melanie Grandits[1], Lais C. G. F. Palhares[1], Silvia Mele[1], Mano Nakamura[1], Jacobo López-Abente[1], Silvia Crescioli[1], Roman Laddach1,3, Pablo Romero-Clavijo[1,4], Anthony Cheung[1,5], Jenny L. C. Geh[6], Alastair D. MacKenzie Ross[6], Sophia Tsoka[3], Panagiotis Karagiannis[1,7], Katie E. Lacy[1], James Spicer2, Sophia N. Karagiannis[1,5*], Heather J. Bax[1,2]
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[7]Department of Oncology, Haematology and Bone

Despite existing immune and targeted therapies, outcomes for patients with metastatic melanoma remain poor. The tumour-associated antigen, chondroitin sulfate proteoglycan 4 (CSPG4), is overexpressed in up to 63% of malignant melanoma, consequently antibodies targeting CSPG4- expressing tumours may benefit patients. Alongside known roles in allergy, the field of AllergoOncology has demonstrated that IgE class antibodies can exert potent anti-tumour responses associated with enhanced pro-inflammatory immunomodulation. We therefore engineered a monoclonal IgE antibody recognising CSPG4 and confirmed binding to human melanoma tissues by immunohistochemistry (IHC). Fc-mediated immune effector functions and anti-tumour activity were assessed in vitro and in vivo.

Cross-linking of CSPG4 IgE on the surface of FcεRI-expressing human monocytes promoted secretion of pro-inflammatory cytokines and enhanced expression of co-stimulatory cell-surface markers. CSPG4 IgE mediated antibody-dependent cellular cytotoxicity (ADCC) of melanoma tumour cells by patient-derived monocytes and PBMCs, with ADCC by PBMCs being ablated by monocyte depletion. Likewise, CSPG4 IgE significantly inhibited the growth of subcutaneous A375 tumours in immunodeficient mice engrafted with human peripheral blood immune cells, but this efficacy was significantly diminished by depletion of monocytes within the PBMC engraftment. Compared to control-treated mice, immunohistochemical studies showed elevated levels of human CD45+ leukocytes and CD68+ macrophages in tumours excised from animals treated with CSPG4 IgE. Transcriptomic analyses also revealed enhanced monocyte and macrophage signatures, and activation of Fcε-receptor and pro-inflammatory immune pathways, in tumours from mice treated with CSPG4 IgE.

POSTER PRESENTATION

PRESENTER
Taismara Kustro Garnica

Genetic Landscape of Immunoglobulin Loci in Dog Breeds: Implications for B-Cell Lymphoma Susceptibility

T.K. GARNICA[1,2], A. Papadaki[1], M. Georga[1], T. Samadova[1], G. Zeitoun[1], G. Folch[1], J. J. Michaloud[1], V. Giudicelli[1], S. Kossida1, H. Fukumasu[2]
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B-cell lymphoma (BCL) is a prevalent and often fatal hematopoietic cancer in dogs, marked by unpredictable therapeutic responses and high mortality rates. Certain breeds, including Labrador Retriever, Golden Retriever, Bernese Mountain Dog, and German Shepherd, exhibit a predisposition to BCL, prompting investigations into the underlying genetic factors. Considering the pivotal role of the B-cell receptor in BCL development, our hypothesis suggested that allelic polymorphisms and structural variations within immunoglobulin (IG) loci might contribute to an elevated risk of B-cell lymphoma in specific dog breeds. To address this hypothesis, our study aimed to comprehensively annotate IG loci, encompassing the heavy-chain (IGH) and light-chain (IGK and IGL) regions, across a diverse range of dog breed assemblies. Meticulous biocuration was conducted using internally developed tools (IMGT/LIGMotif, NtiToVald, and IMGT/Automat) grounded in IMGT-ONTOLOGY axioms and concepts. The research focused on nine dog assemblies from NCBI, including Basenji, Bernese Mountain Dog, Boxer, Cairn Terrier, German Shepherd, Great Dane, and Labrador Retriever. Initial gene annotation concentrated on the IGK locus located on chromosome 17 in the forward orientation, revealing variable spans (average: 298 kb, range: 235-369 kb). The analysis identified 40 new alleles and 9 novel genes, with significant contributions of Bernese Mountain Dog assemblages (BD_1.0 and OD_1.0). The majority of newly identified genes and alleles were associated with the V2 subgroup of the V-region. Subsequent steps involve annotating the IGH and IGL loci and making all sequences accessible in the IMGT database and tools. This study marks the inaugural exploration of variations in immunoglobulin loci in various dog breeds, providing valuable insights into the genetic diversity of the IGK locus and laying the foundation for further investigations into the study of expressed IG repertoires by dogs and their genetic susceptibility to BCL.

POSTER PRESENTATION

PRESENTER
Isabel Corraliza-Gorjón

Evaluation of the specific inhibitory effects of an antibody in a tumor progression model

Corraliza-Gorjón, I.[1], Martín, M.T.1, Domínguez, V.[1,2], Llorente, M.[1], Osteso-Ibanez, T.[1], Pintado, B.[1,2], García-Sanz, J.A.[3] and Kremer, L.[1]

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A significant fraction of leukemia patients exhibit resistance to traditional chemotherapy treatments or experience relapses, leaving hematopoietic stem cell transplantation as the last viable treatment option. Therefore, it is imperative to devise novel therapeutic strategies that are both less harmful and more likely to succeed. Monoclonal antibodies (mAbs) represent promising candidates as antitumor agents due to their high specificity, safety profile and efficacy. The CCR9 receptor is overexpressed in the tumor cells of near 80% of individuals diagnosed with T-cell acute lymphoblastic leukemia. Our group has generated mAbs using this receptor as immunogen, with certain mAbs showing considerable potential as anti-tumor therapeutics in preclinical studies. In order to assess whether the in vivo protective effects of a selected mAb are solely caused by the specific antibody-receptor interaction, we genetically modified the CCR9 molecule from the MOLT-4 leukemia cells using the CRISPR/Cas9 system to substitute an epitope in CCR9. Both cell and in vivo-based studies support that substituting the second and fourth amino acids within the six-residue CCR9 minimal epitope is sufficient to prevent antibody binding. Flow cytometry and immunofluorescence analyses have revealed that the mAb failed to recognize the CCR9 mutant MOLT-4 cells. However, these cells retained their responsiveness to the ligand CCL25. In an orthotopic mouse xenograft model using the modified cells, the protective efficacy of the antibody was completely lost. The findings confirm that modification in the antigen of the critical amino acids necessary for antibody-antigen interaction allows the assessment of the side effects of therapeutic antibodies in preclinical studies.

POSTER PRESENTATION

PRESENTER
Shamsa Batool

Genomic study and Biocuration of antigen receptor loci in Orangutans

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IMGT, The International ImMunoGeneTics Information System, Institute of Human Genetics (IGH), National Center for Scientific Research (CNRS), University of Montpellier (UM), Montpellier, France

Antibodies drive the adaptive immune system of jawed vertebrates to efficiently detect and fightagainst a diverse array of pathogens. Antibody diversity results from VDJ gene recombination inimmunoglobulin (IG) loci and is further enhanced by somatic hypermutations. Annotating non-human primate IG loci and predicting V, D, and J genes is essential for cross-species immunogenetic insights.

In this study, we conducted a comprehensive comparative analysis of IG loci in two orangutan species, Pongo abelii and Pongo pygmaeus, using the extensive resources provided by global reference in immunogenetics and immunoinformatics the International ImMunoGeneTics Information System (IMGT). IMGT® genomic annotated data are classically displayed in IMGT Databases, tools and IMGT Repertoire Web Resources (Locus description, Locus representation, Gene tables, Alignments of alleles).

Orangutans, one of the great apes, provide invaluable insights into the evolution and diversity of immune system genes. P.abelii and P.pygmaeus, once considered subspecies, are now regarded as distinct species. Their genetic differences and shared sequences highlight both their evolutionary divergence and relatedness within the genus Pongo.

Through a combination of computational methods and manual curation, the IG loci were extracted from whole-genome assemblies available on NCBI, namely Susie_PABv2 and NHGRI_mPonPyg2-v2.0_pri, and assigned an IMGT accession number. Our analysis focused on identifying the chromosomal positions of IG loci, characterization of V, D (for IGH), J and C genes using standardized gene nomenclature, identifying gene content, defining allele functionality, and displaying the conventional recombination signal (RS) sequences.

A total of 395 immunoglobulin (IG) genes were annotated in P.abelii and 426 IG genes in P.pygmaeus, revealing slight differences despite overall genome similarity. Notably, P.pygmaeus exhibits a higher count of V genes, impacting the number of functional genes in each species. Further research on genomic organization in available assemblies as well as in other nonhuman primate genomes is vital to determine gene proportions and trace their evolutionary trajectories.

Key words: orangutans, immunogenetics, comparative analysis, Immunoglobulin loci, IMGT

POSTER PRESENTATION

PRESENTER
Lucy Booth

B cell phenotype and immunoglobulin isotype predict immune related adverse events and response to checkpoint inhibitor therapy in advanced melanoma

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Immune checkpoint inhibitor (CPI) immunotherapy has transformed the treatment of metastatic melanoma in recent years. Yet, more than half of patients do not respond, and many develop severe toxicities that result in discontinued treatment. We have previously shown that the humoral immune response shows alternatively activated features in melanoma. Here we compared circulating B cell and antibody features in a cohort of patients and healthy volunteers, and we investigated whether circulating humoral responses in patients may predict the onset of immune related adverse events (irAEs), treatment outcomes and overall survival.

We comprehensively characterised the circulating humoral compartment in healthy volunteers (N=22) and in a cohort of patients with advanced melanoma (III/IV, N=52) treated with anti-PD1 antibodies (CPI). Patients were stratified based on subsequent development of irAEs throughout checkpoint blockade, categorised as either 'no toxicity' or 'high grade toxicity'. Phenotyping of circulating B cells was performed using mass cytometry (CyTOF); and immunoglobulin isotyping was conducted using multiplex immunoassay.

At baseline, naïve CD21lo B cells, plasmablasts and double negative B cells were enriched in melanoma patient circulation compared to healthy controls, showing similarities to the phenotypes enriched in classical autoimmune diseases. These B cell phenotypes, in particular a subset of IL-10+ CD95hi plasmablast-like cells were enriched in the 'no toxicity' group. Conversely, PD-1+ CD5hi Bregs, previously reported to promote T cell dysfunction and tumour progression, were shown to be enriched in the circulation of 'high grade toxicity' patients. Consistent with IL-10 enriched, Th2 biased conditions, serum antibody isotyping revealed an enrichment of IgG4 at baseline in patients compared with healthy controls, and this enrichment was found to be negatively associated with overall survival in patients treated with anti PD-1 therapy. Furthermore, baseline enrichment of IgA and IgE were found to be protective against the development of irAE.

Taken together, our data identify distinct B cell signatures in patients with advanced melanoma that may be predictive of irAE and treatment responses in the context of checkpoint blockade. Furthermore, skewed circulating antibody isotype levels in patients may be relevant to both toxicity and treatment outcomes.

PRESENTER
Petra Kern

Development of monoclonal antibodies against membrane proteins: challenges and solutions

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Monoclonal antibodies against membrane proteins are essential diagnostic tools and are part of emerging strategies in targeted cell therapies like CAR-T cell therapy. Membrane proteins are proteins that are associated with the cell membrane or interact with it. They are often specific for certain cell types and physiological states, making them ideal diagnostic markers or therapeutic targets.

Production of antibodies against membrane proteins is challenging. Membrane proteins are difficult to work with, because their structure, function, and mobility intertwine with intermolecular interactions (protein-lipid, protein-protein) and membrane composition. Little is known about the structure and function of membrane proteins, compared to soluble proteins. Further, the isolation and handling of membrane proteins are complex and usually result in alterations in the proteins' three-dimensional structure, compared to the native form expressed on live cells. Inappropriate hybridoma screening strategies for membrane protein-directed monoclonal antibody development can result in the selection of antibodies that do not bind to living cells and can be unsuited for most applications.

To ensure the selection of suitable antibody candidates with the ability to bind to proteins expressed on cells in native form, high-throughput cell-based selection assays are needed. Although flow cytometry is usually the method of choice when targeting native proteins on cells, it is labor-intensive, time-consuming, rather expensive and unsuitable for rapid screening of hundreds of hybridoma candidates.

To test large numbers of antibodies on live cells, we developed a high-throughput screening method for testing hybridoma that produce antibodies targeting membrane proteins, called cell ELISA. Cell ELISA is conducted similarly to ELISA, and with it, we can easily recognize antibodies that bind to membrane proteins in vivo in the primary screening process. It is a suitable, high-throughput, inexpensive, and easy-to-perform method for screening hybridomas that produce antibodies targeting membrane proteins.

POSTER PRESENTATION

PRESENTER
Turkan Samadova

Full annotation of IG loci in *Lemur catta* and evolution

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Innate and adaptive immunity collectively protects the organism, crucial for maintaining health and vitality. The innate immune system protects against infection during the initial exposure to a pathogen, while the adaptive immune system remembers previous encounters with specific pathogens and can respond quickly in the case of repeated exposure. Immunoglobulins (IG) and T cell receptors (TR) are crucial in identifying antigens and eliminating threats. An IG is a heterodimer composed of two heavy chains (IGH) and two light chains produced by B cells and can be found on the membrane or in the soluble form known as the antibody. Studying immune responses in different species is essential for developing vaccines and therapeutic and diagnostic tools.

Lemurians are early-branching organisms in primate evolution, and they have been isolated in Madagascar. Different species of Lemurians exhibit different types of social behavior. Lemur catta is a species living in groups. Studying Lemur catta's adaptive immune response enables a better understanding of the evolutionary history of immune response in primates.

The IG loci of the Lemur catta are currently being analyzed. Sequences coding for key proteins in the acquired immune response have been retrieved from databases and annotated. Semi-automated annotation of the IG genes has identified 221 functional genes, 72 pseudogenes, and 23 ORFs.

In this work, the annotation of the three loci of IG (IGH, IGL, and IGK) is presented after following the IMGT-ONTOLOGY. The V-GENES at the IGH locus are mainly of subgroup 3, which can be part of antibodies in humans. IGK locus has subgroup 1 and 2 V-GENES. Most paralogues have a single lemurian origin.

Keywords: immunogenetics, immunoglobulin (IG), biocuration, adaptive immune system, T cell receptors (TR), *Lemur catta*

POSTER PRESENTATION

PRESENTER
Scherezade Jiménez

Unravelling TOX2 expression in B and T Cell lymphomas

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Background: TOX2, a member of the Thymocyte selection-related HMG box protein family, shares structural similarities with TOX, TOX3, and TOX4. While TOX is linked to T cell exhaustion, TOX2 exhibits diverse functions, playing a critical role in human NK cell maturation by positively regulating TBX21 expression. Additionally, TOX2 modulates T follicular helper cells (Tfh) cell differentiation by suppressing inflammation-related gene pathways.

Despite the importance of TOX2 in immune regulation, its distribution in reactive and neoplastic lymphoid tissues remains largely unexplored.

Purpose of the study: To address this gap, we have investigated TOX2 expression in normal and neoplastic lymphoid tissue using a novel rat monoclonal antibody (TOM924D) that recognizes its target molecule in paraffin-embedded tissue sections. A comprehensive study was conducted on a large number of normal tissues and B and T-cell lymphomas (comprising more than 300 cases), utilizing both whole tissue sections and tissue microarrays.

Results: We identified a restricted expression pattern of TOX2 within lymphocytes. In tonsil, we observed its presence in PD1-positive follicular helper cells. In the thymus cortex TOX2 was expressed in double-positive CD8/CD4 T cells, while in the medulla, TOX2-positive cells were predominantly CD4. Furthermore, in T lymphomas, TOX2 was expressed in NK/T Cell Lymphomas, whereas in B cell lymphomas, it was found in Diffuse Large B Cell Lymphoma. In the other types of lymphomas, its expression was confined to the tumour microenvironment, especially in follicular and Hodgkin's lymphoma, indicating its potential role in tumour response.

Conclusions: By unravelling its expression patterns, this study not only expands our knowledge of TOX2 roles in health and disease, but also lays the foundations for potential diagnostic and therapeutic applications, especially in the context of lymphomas originating from NK cells.

POSTER PRESENTATION

PRESENTER
Lais C. G. F. Palhares

The therapeutic potential of a CSPG4 IgE: in vitro anti-tumour activity, impact on CSPG4 antigen shedding and early safety indicators

Lais C. G. F. Palhares[1], Jitesh Chauhan[1,2], Melanie Grandits[1], Pablo Romero-Clavijo[1], Reneè Gitsaki-Taylor[1], Silvia Mele[1], Sophia N. Karagiannis[1,3], Heather J. Bax[1,2]
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Immunoglobulin G (IgG) are often the antibody class of choice for cancer immunotherapy. However, IgEs are emerging as potential anti-cancer candidates due to biological advantages, including high affinity for cognate Fcε receptors expressed on tumour-resident immune cells, and a lack of inhibitory Fc receptors. These properties may lead to activation of anti-tumour responses within tissues. An IgE antibody, MOv18 (recognizing the tumour antigen, folate receptor α, FRα), has shown efficacy in the first-in-human, first-in-class Phase I clinical trial.

Transcriptomic analyses of publicly available datasets confirmed elevated gene expression of the melanoma-associated antigen hondroitin sulfate proteoglycan 4 (CSPG4) in melanoma as compared to other tumour types and normal skin. Additionally, mmunohistochemistry revealed CSPG4 staining in up to 63% of melanoma tissues. We engineered a human monoclonal IgE antibody targeting CSPG4 and validated its binding to melanoma cancer cell lines. CSPG4 IgE mediated in vitro Fab-mediated anti-tumour effects, and antibody-dependent cellular cytotoxicity (ADCC) against melanoma cells by human immune effector cells.

CSPG4 antigen may be cleaved and released from the surface of tumour cells, and bind to anti- CSPG4-targeting antibodies, thereby hindering their ability to interact with tumour cells and reducing their anti-tumour efficacy. However, minimal shedding of the CSPG4 antigen was detected in melanoma cell supernatants. Additionally, complexes of shed CSPG4 may crosslink anti-CSPG4 IgE on the surface of basophils, which could potentiate degranulation, leading to type I hypersensitivity. Comparable low levels of CSPG4 were detected in the circulation of melanoma patients and healthy subjects. We investigated whether CSPG4 IgE could stimulate degranulation of RBL-SX38 cells when exposed to sera, however no RBL-SX38 degranulation was triggered in the presence of sera from healthy volunteers or melanoma patients.

Our findings indicate that despite tumour antigen shedding, CSPG4 IgE could be an effective and safe immunotherapeutic candidate for melanoma.

POSTER PRESENTATION

PRESENTER
Miriam Velasco-Sidro

Dual-Targeted STAb-T Cells for the Treatment of Hematological Malignancies

Miriam Velasco-Sidro[1,2,3], Javier Arroyo-Ródenas[1,2,3], Laura Díez-Alonso[1,2,3], Ángel Ramírez- Fernández[1,2,3], Luis Álvarez-Vallina[1,2,3]
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Despite recent advances in the treatment of B-cell malignancies, such as chimeric antigen receptor-engineered T-cells (CAR-T), most patients relapse, and new approaches are urgently needed. Among these strategies, dual-targeting approaches are being investigated and tested in clinical trials. Here, we present for the first time a dual-targeting STAb-T strategy based on the secretion of two different T cell engagers (TCEs). Our aim is to verify that the two TCEs do not compete for binding to CD3 on transduced and non-transduced T lymphocytes and to test the efficacy of this dual strategy in vitro. We performed single lentiviral transduction (to obtain singletargeted TAA1- or TAA2-STAb-T cells) or lentiviral co-transduction (CoT-STAb-T) of Jurkat T cells or primary T cells and then co-cultured effector T cells with tumor cells in contacting and noncontacting co-culture systems to conduct activation, cytotoxicity and tumor escape assays. We demonstrated that TAA1- and TAA2-specific TCEs secreted by pooled STAb-T and CoT STAb-T do not interfere with each other in the recruitment of both transduced STAb-T cells and nontransduced bystander T cells, and that dual-targeted STAb-T cells (pooled and CoT) reduce tumor escape in vitro compared to single-targeted TAA1- or TAA2-STAb-T cells. In conclusion, both dual-targeted STAb-T strategies demonstrate efficacy in vitro and may have potential as an alternative for patients suffering from relapsed or refractory hematological malignancies.

POSTER PRESENTATION

PRESENTER
Laura Rubio-Pérez

Characterization of a trispecific PD-L1 blocking antibody that exhibits EGFR-conditional 4-1BB agonist activity

Laura Rubio-Pérez[1,2,3,4], Susana Frago[5], Marta Compte5, Rocío Navarro[5], Seandean L. Harwood[6], Rodrigo Lázaro-Gorines[1,2,3], Marina Gómez-Rosel[1,2,3], Oana Hangiu[1,2,5], Noelia Silva-Pilipich[7,8], Lucía Vanrell[9,10], Cristian Smerdou[7,8], Luis Álvarez-Vallina[1,2,3,4]
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Immune checkpoint blockade has changed the treatment paradigm for advanced solid tumors, but overall response rates are still limited. The combination of checkpoint blockade with anti-4-1BB antibodies to stimulate tumor-infiltrating T cells has shown anti-tumor activity in human trials. However, the further clinical development of these antibodies has been hampered by significant off-tumor toxicities. Here, we have generated an anti-4- 1BB/EGFR/PD-L1 trispecific antibody consisting of a triple-targeting tandem trimerbody (TT) fused to an engineered silent Fc region. This antibody (IgTT-4E1-S) is designed to combine blockade of the PD-L1/PD-1 axis with conditional 4-1BB co-stimulation specifically confined to the tumor microenvironment (TME). The antibody demonstrated simultaneous binding to purified EGFR, PD-L1 and 4-1BB in solution, effective blockade of PD-L1/PD1 interaction and potent 4-1BB-mediated co-stimulation, but only in the presence of EGFR-expressing cells. These results demonstrate the feasibility of IgTT-4E1-S to specifically block the PDL1/ PD-1 axis and to induce EGFR-conditional 4-1BB agonist activity.

POSTER PRESENTATION

PRESENTER
Chilanay Alakbarova

Genomic study and Biocuration of antigen receptor loci in Norway rat (*Rattus norvegicus*)

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Currently, a substantial number of genetically well-defined inbred rat strains have been established, serving as models for significant human disease traits, including susceptibility to cancer, hypertension, ischemia, obesity, diabetes, and autoimmune diseases. Thus, the rat has undoubtedly become one of the most frequently used experimental animals in transplantation as well as autoimmunity research and biomedical research. This study encompasses a comprehensive genomic investigation and biocuration of antigen receptor loci in different assemblies of Rattus norvegicus, such as mRatBN7.2; UTH_Rnor_SHRSP_BbbUtx_1.0, employing advanced sequencing techniques, bioinformatics tools and using IMGT® (The international ImMunoGeneTics information system) standarts and resources. By investigating these antigen receptor loci, which play a pivotal role in immune response induction, we identify and annotate genes, including immunoglobulin (Ig) loci, and other immune-related genes using IMGT nomenclature for named genes and alleles. This nomenclature follows established rules, which include specific prefixes and suffixes denoting gene types, species, and allele variation. Additionally, IMGT numbering is a system used to assign identification to different regions and positions within immunoglobulin sequences. Furthermore, we provide insights into biocuration of these antigen receptor loci. Our findings not only enhance our understanding of immune gene organization and regulation in Rattus norvegicus but also provide valuable resources for comparative immunogenomics and translational research in human health and disease.

Keywords: Norway rat (Rattus norvegicus), IMGT, biocuration, immunoglobulin, loci, annotation.

POSTER PRESENTATION

PRESENTER
Gabriel Osborn

IgE induces hyperinflammatory repolarisation of ovarian cancer patient macrophages to restrict an immunosuppressive macrophage:Treg axis

Gabriel Osborn[1], Jacobo López-Abente[1], Rebecca Adams[1], Roman Laddach[1,2], Heather J Bax[1], Jitesh Chauhan[1], James Spicer[3], Debra H Josephs[1,3], Sophia N Karagiannis[1,4]
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Ovarian cancer is the most lethal gynaecological cancer and treatment options remain limited. In a recent first-in-class Phase I trial, IgE monoclonal antibody (mAb) MOv18, specific for the tumour-associated antigen Folate Receptor-α, was well tolerated in patients and preliminary anti-tumoural activity was seen. In pre-clinical rodent models of cancer, a macrophage-mediated mechanism has been identified for IgE class mAbs, via tumour cell killing and pro-inflammatory activation. However, IgE engagement and activation of macrophages in human ovarian cancer and the downstream effects on tumour immunity, remain unclear.

Here we study the tumour immune microenvironment in ovarian cancer patients naïve to IgE therapy, via high-dimensional flow cytometry and RNA-seq analyses. We demonstrate immunosuppressive phenotypes for patient macrophages. These phenotypes are consistent with IL-10 macrophage polarisation and associate with poor patient prognosis, as well as importantly, expression of the high affinity IgE receptor gene, FCER1A. RNA-seq interaction analyses and ex vivo co-cultures reveal an immunosuppressive association between patient macrophages and regulatory T cells (Tregs). MOv18 IgE-engaged patient macrophages undergo pro-inflammatory repolarisation ex vivo, and display induction of a de novo hyperinflammatory subset enriched for T cell stimulation markers. IgE reverses macrophage-mediated Treg induction and suppressive function, to increase CD8+ T cell expansion. This ex vivo IgE-immune activation signature is recapitulated in transcriptomic analyses of MOv18 IgE-treated tumours from an immunocompetent rat model and associates with improved patient prognosis.

We demonstrate that IgE induces hyperinflammatory and de novo repolarised states of patient macrophages, to reverse Treg induction and promote CD8+ T cell activity. Collectively, these novel effector functions of IgE may promote a tumour immunity activation of potential clinical utility in cancer patients receiving IgE therapy.

POSTER PRESENTATION

PRESENTER
Ivana Zagorac

mRNA-engineered T lymphocytes secreting bispecific T cell engagers with therapeutic potential in solid tumors

Ivana Zagorac[1,2,3], Ángel Ramírez-Fernández[1,2#], Antonio Tapia-Galisteo[1,2,3], Laura Rubio-Pérez[1,2,3], Marina Gómez-Rosel[1,2,3], Montserrat Grau[4], José Luis Rodríguez Peralto[5,6,7,8], Luis Álvarez-Vallina[1,2,3], Belén Blanco[1,2,3,9]
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Background: in the last decade, chimeric antigen receptor (CAR)-modified T cells have revolutionized the treatment of hematologic malignancies. However, antitumor responses in solid tumors remain poor, and the difficulty in finding truly tumor-specific target antigens leads to a high risk of on-target/off-tumor toxicity. Transient modification with mRNA is gaining momentum as an alternative approach to viral transduction in order to achieve a better safety profile. On the other hand, generation of T cells secreting bispecific T cell engagers (TCEs) has been reported to improve the antitumor efficacy of T lymphocytes expressing membrane-anchored CARs, due to the ability of the soluble TCEs to recruit unmodified bystander T cells.

Methods: we have electroporated human primary T cells with in vitro transcribed mRNA encoding an anti-TAA x anti-CD3 bispecific T cell engager. Such mRNAmodified T cells (STAR-T cells) have been analyzed for anti-TAA bispecific TCE secretion and for their ability to drive anti-tumor responses against TAA-expressing cells, both in vitro and in vivo.

Results: STAR-T cells transiently secrete bispecific TCEs capable of recruiting both modified and bystander T lymphocytes to exert tumor cell-specific killing in in vitro assays. Moreover, STAR-T cells efficiently control tumor growth in an in vivo xenograft model of solid malignancy.

Conclusions: our results provide proof of concept that mRNA engineered TCEsecreting T cells represent a therapeutic approach for solid tumors.

POSTER PRESENTATION

PRESENTER
Jitesh Chauhan

AllergoOncology: Application of the basophil activation test (BAT) in pre-clinical testing and the first-in-class clinical trial of anti-cancer IgE immunotherapy

Jitesh Chauhan, Melanie Grandits, Lais C. G. F. Palhares, Chara Stavraka, Debra H. Josephs, James Spicer, Sophia N. Karagiannis* & Heather J. Bax*
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Introduction: Novel tumour-targeting IgE antibodies are undergoing pre-clinical and clinical testing. IgE anJbodies are known to be involved in type I hypersensitivity reactions, and therefore there is a perceived risk with this therapeutic modality. The basophil activation test (BAT) utilises unfractionated whole human blood, meaning that any mediators of basophil activation are present alongside the test therapeutic ex vivo. Here, we evaluated potenJal hypersensitivity to MOv18 IgE, the first IgE evaluated in a Phase I clinical trial (specific for folate receptor alpha (FRα) overexpressed in ovarian cancer); and CSPG4 IgE (recognising chondroitin sulfate proteoglycan 4 (CSPG4) overexpressed by human melanomas).

Methods: Using the BAT, we interrogated the capacity of basophils from cancer patients (melanoma: n=15; ovarian: n=71) to be acJvated ex vivo by IgE and non-IgE immune stimuli, and assessed the potenJal for hypersensitivity to anti-cancer IgE, including in patients receiving MOv18 IgE treatment (n=24). Possible mechanisms of hypersensitivity were evaluated by assessing concurrent serum samples for potential cross-linking mediators (by ELISA and ImmunoCAP).

Results: We demonstrated activation of basophils with IgE- and non-IgE-mediated immune stimulation in melanoma and ovarian cancer paJent blood. CSPG4 IgE did not trigger basophil activation in the melanoma paJent blood samples assessed, however basophils in 3 of 71 ovarian cancer patient samples were reactive to MOv18 IgE (<5% risk of hypersensitivity). The BAT was predictive of the anaphylaxis experienced by one trial patient during their first infusion of MOv18 IgE. Subsequently, a positive baseline BAT was used as a exclusion criteria for the trial. In BAT positive patients, the mechanism of hypersensiJvity to MOv18 IgE was independent of circulating FRα, antigen-specific autoantibodies, anti-alpha-GAL IgE, or anti-drug antibodies.

Conclusion: We provide evidence of a lack of basophil acJvaJon by anJ-cancer IgEs in the majority of paJents. The BAT can evaluate the propensity for and predict hypersensitivity to MOv18 IgE.

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POSTER PRESENTATION

PRESENTER
Luca Pape

Nanobody-based bispecific antibody recruiters: Raising the BAR for CD38-specific myeloma therapeutics

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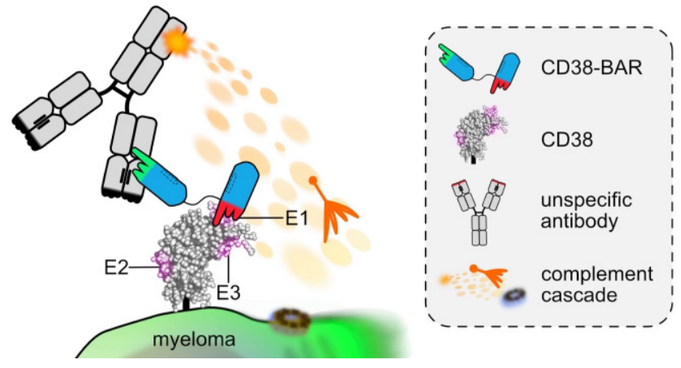
Bispecific Antibody Recruiters (BARs) represent an innovative class of immunotherapeutics that redirect endogenous antibodies toward tumor cells. Comprising an antibody-binding terminus and a tumor-binding terminus, BARs effectively harness non-specific endogenous
antibodies to opsonize target cells. Antibodies recruited to the tumor cell surface can then trigger FC-dependent effector functions, including complement-dependent cytotoxicity (CDC) and antibody-dependent cellular phagocytosis (ADCP).

CD38 is highly expressed on multiple myeloma cells, thereby making it a suitable target antigen for immunotherapy. This is illustrated by the clinical success of CD38-specific monoclonal antibodies Daratumumab and Isatuximab. Nanobodies are derived from camelid heavy chain antibodies. Their low molecular weight and ease of formatting make them particularly suited for constructing bispecific antibody constructs such as BARs.

We have developed two sets of CD38-specific BARs. Each BAR consists of a nanobody specific for one of CD38's three epitopes, fused by a glycine-serine linker to a nanobody specific for human immunoglobulin kappa (CD38-kappa-BAR) or lambda light chain (CD38- lambda-BAR). Both CD38-kappa-BARs and CD38-lambda-BARs exhibit specific and simultaneous binding to CD38-expressing myeloma cells and polyclonal human IgG in vitro. While a single CD38-BAR induces only limited CDC by polyconal IgG, the combination of two BARs targeting distinct eptiopes induces potent CDC. Similarly, a BAR that targets a distinct epitope potently enhances CDC mediated by the mAbs daratumumab and isatuximab.

Our results demonstrate the capacity of nanobody-based CD38-specific BARs to engage polyclonal endogenous antibodies for the killing of multiple myeloma cells.



POSTER PRESENTATION

PRESENTER
Melanie Grandits

Anti-tumoral activity of anti-HER2 IgE against high and medium/low HER2-expressing breast cancer

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Background
Monoclonal antibody therapies have revolutionised cancer treatment, including for human epidermal growth factor 2 (HER2)-positive breast cancer. These antibodies are generally of the IgG isotype, however, in recent years potential benefits of other isotypes have also been explored. Monoclonal IgE-based therapies have been shown to be efficacious in reducing tumour burden in in vivo models of melanoma and ovarian cancer. Furthermore, the first-in-class clinical trial of MOv18 IgE, an IgE targeting ovarian cancer, has shown good tolerability, as well as evidence of anti-tumour efficacy in one patient.
Here we investigated whether anti-HER2 IgE can be efficacious against HER2-positive breast cancer, as treatment failure still occurs in several patients despite the availability of a variety of treatments.

Methods
We produced IgE versions of trastuzumab and pertuzumab, both commonly used in an IgG1 format for the treatment of HER2+ breast cancer. In vitro, direct effects were assessed in phosphorylation and cell proliferation assays, and the immune cell-mediated functions, degranulation and antibody-dependent cellular cytotoxicity, were measured. The Basophil Activation Test (BAT) was employed to make early assessments of the potential for type 1 hypersensitivity to these IgEs.

Results
We confirmed that both IgEs retained their binding affinity to HER2 and reduced levels of HER2 phosphorylation by blocking HER2 dimerisation, resulting in reduced cell proliferation in vitro. We assessed and classified the HER2 cell surface expression of nine different human cancer cell lines (HER2 1+, 2+ or 3+) and showed that cross-linking of the two anti-HER2 IgEs by these cancer cells, triggered degranulation of a basophilic cell line. We further demonstrated immune cell-mediated anti-tumour activity in an antigen-density dependent manner in vitro. In the BAT, no basophil activation has been triggered by trastuzumab and pertuzumab to-date.

Conclusion
HER2-targeting IgE antibodies may offer a promising avenue for treatment of HER2-positive tumours, including those with low expression levels.

POSTER PRESENTATION

PRESENTER
Nathalie Richter

AAV-specific nanobodies from Llama-IgH transgenic mice (LaMice) for the retargeting of AAV gene therapy vectors

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Adeno-associated viruses (AAV) are widely used, non-pathogenic gene therapy vectors. The low cell specificity of natural AAV serotypes can result in off-target effects and limits the application of AAV-based therapeutics. We have shown that the insertion of a membrane protein-specific nanobody into the capsid of AAV vectors strongly increases the transduction of target cells expressing the cognate membrane protein (1). Nanobodies or VHHs are the single variable domains from camelid heavy chain antibodies (hcAbs). Due to their small size, high solubility and easy reformatting options nanobodies are valuable tools in research and therapy. Our lab generated transgenic mice (LaMice) that carry the llama IgH gene locus instead of their endogenous immunoglobulin locus and therefore solely produce llama hcAbs (2). We immunized LaMice with recombinant AAV and selected AAV-specific nanobodies via classical hybridoma technology. After screening of hybridoma supernatants for AAV-specific hcAbs by ELISA, cDNA was prepared from positive clones and the respective VHH-coding region was amplified by PCR and sequenced. The VHH-coding regions were cloned into a eukaryotic expression vector to fuse each VHH to the hinge, CH2 and CH3 domains of rabbit IgG. Specificity of the resulting nanobody-rabbit IgG hcAbs were verified by ELISA, immunofluorescence microscopy and flow cytometry.

Selected AAV-specific nanobodies were genetically fused to membrane-protein specific nanobodies via a flexible glycine-serin linker and produced as secretory proteins in HEK cells. These bispecific adaptors strongly enhanced AAV transduction of cells expressing the respective target protein on the surface. The modular composition of such nanobody-based adaptors allows the flexible adjustment to new target proteins and AAV serotypes making them interesting tools in research and, if validated in vivo, possibly also in the clinic.

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2) Eden, T. et al. (2024) Nature Communications. in press

PRESENTER
Rebecca Adams

Monocytes and macrophages in the melanoma tumour microenvironment express Fc receptors which can be engaged with monoclonal antibodies of different isotypes

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Introduction and Aim

Melanoma is the deadliest form of skin cancer with an increasing worldwide incidence. It is well established that the melanoma tumour microenvironment (TME) has a large immune cell infiltrate, including a prominent monocytic compartment. Monocytic cells within the TME support melanoma growth, survival and metastasis. Contrastingly, such cells can be stimulated to promote tumour killing when engaged with monoclonal antibodies (mAbs) against cancer antigens, due to their expression of multiple Fc receptors (FcRs). However, little is known about the monocytic subsets present within melanoma, the FcRs they express and how engaging their FcRs may influence function.

Methods

Flow cytometry on peripheral monocytes isolated from melanoma patients and healthy volunteers enabled the characterisation of cell surface markers. Next, publicly available single cell RNA-seq datasets were analysed to explore the transcriptome of melanoma associated macrophages and these phenotypes were recapitulated in vitro. Finally, monocytes and macrophages were co-cultured with melanoma cells, with or without the addition of mAbs against a melanoma antigen, in order to assess the effect of FcR crosslinking on monocyte and macrophage phenotype and function.

Results

Circulating monocytes in patients with melanoma feature an inhibitory phenotype, and express multiple FcRs, including Fcε and Fcγ receptors, which could be targeted with mAbs of multiple isotypes. Secondly, monocytic cells within the melanoma TME express pro-tumour genes, including metalloproteinases and angiogenic factors. However, FcRs expression suggests that tumour-promoting subsets can be targeted with mAbs. Finally, in ex vivo co-cultures with melanoma cells, the FcRs of both human monocytes and macrophages are able to engage anti-melanoma mAbs of multiple isotypes, inducing differential phenotype change. This is demonstrated by the upregulation of activation markers and co-stimulatory cell surface markers.

Conclusion

Together, these data suggest that tumour-promoting monocytic cells within the melanoma TME can be engaged and harnessed using different isotypes of mAbs targeting melanoma.

PRESENTER
Saruul Jargalsaikhan

Insertion of membrane protein-specific nanobodies into the capsid of AAV gene therapy vectors enhances transduction specificity and efficacy

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Nanobodies are single variable immunoglobulin domains derived from camelid heavy-chain antibodies. Due to their small size and high solubility, nanobodies can be readily fused to other proteins. We have shown that nanobodies can be inserted into exposed surface loops of the capsid of Adeno-associated virus (AAV) gene therapy vectors (1).

AAVs are small, single-stranded DNA viruses that can drive long-term expression of therapeutic proteins and/or RNA in dividing and non-dividing cells with minimal immunogenicity and toxicity. A limiting factor for clinical application of AAVs is the broad tropism of the natural serotypes, i.e., the low tissue and cell specificity.

Here, we show that the insertion of membrane protein specific nanobodies into exposed surface loops on the capsids of various AAV serotypes dramatically improves their cell specificity. AAVs displaying a nanobody against structurally different membrane proteins strongly enhance the transduction of cells expressing the corresponding membrane protein, e.g., PD-L1, CD38, P2X7, and CD73. These results highlight the utility of membrane protein-specific nanobodies as tools to improve targeting of AAV gene therapy vectors.

1. Eichhoff AM et al. 2019. Nanobody-Enhanced Targeting of AAV Gene Therapy Vectors. Mol Ther Methods Clin Dev. 15:211-220.

Supported by the BMBF (COMMUTE)

POSTER PRESENTATION

PRESENTER
Marie Eggers

Targeting autoantibody-producing B cells with chimeric nanobody-mouse IgG heavy chain antibodies as therapeutic approach for glomerular autoimmune diseases

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Many autoimmune diseases are driven by autoantibodies produced by B and plasma cells. Thus, the depletion of autoantibody-producing B cells provides a promising causal therapeutic strategy. Our study introduces nanobody-based heavy chain antibodies as novel tools for depleting (auto)antibody-producing plasma cells. Nanobodies correspond to the single variable immunoglobulin domains of heavy chain antibodies (hcAbs) that naturally occur in llamas and other camelids. Fusion of a nanobody to the hinge and Fc domains of human or mouse IgG results in chimeric human or murine hcAbs, respectively.

The aim of this project was to engineer plasma cell depleting hcAbs and to evaluate their potential as therapeutics in a mouse model of autoimmune glomerular disease. Flow cytometry analyses revealed a novel target protein on the cell surface of primary murine plasma cells and mAb-producing hybridoma cells. Following intravenous injection, Nb-hcAbs against this target protein induced potent depletion of plasma cells in bone marrow and spleen ex vivo and in vivo.

In a murine model of autoimmune membranous glomerulonephropathy i.v. injection of hcAbs lead to reduction of autoantibody titers and clinical symptoms in vivo. Moreover, first analyses of human samples confirmed strong expression of this target protein on human plasma cells.

Our results promote hcAbs against a novel target protein as promising new tools for the depletion of autoantibody-producing plasma cells in the treatment of autoimmune diseases. Considering the global burden of severe autoimmune diseases, further investigation of this potential therapeutic strategy is warranted.

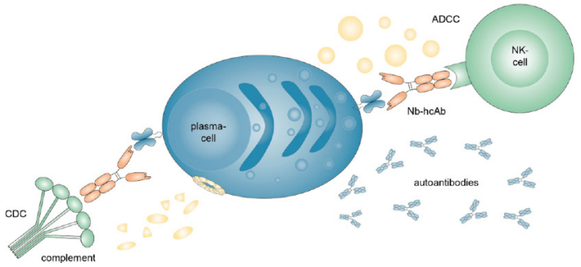

Figure 1: Nanobody-based hcAbs lead to the depletion of plasma cells via the induction of immune effector functions such as antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

Supported by DFG SFB1192.

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