EuroMAbNet

European Monoclonal Antibodies Network

6th Antibody Validation Workshop
12th EuroMAbNet Meeting

22nd - 24th of September, 2022

Hamburg

BOOK OF ABSTRACTS

EuroMAbNet

European Monoclonal Antibodies Network

Book of Abstracts of the 12th EuroMabNet meeting

VENUE

September 22nd-24th, 2022

Thursday 22nd - Friday 23rd of September: Universitätsklinikum Hamburg-Eppendorf
N61 Institut für Anatomie, Martinistr. 52, 20246 Hamburg

Saturday 24th of September: Universitätsklinikum Hamburg-Eppendor
W29 Erika-Haus, Martinistr. 52, 20246 Hamburg

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Friedrich Koch-Nolte

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Pablo Engel
Sophia Karagiannis
Pierre Martineau
Berislav Lisnić
Vanda Juranić Lisnić

SCIENTIFIC COMMITTEE

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Friedrich Koch-Nolte
Pablo Engel
Sophia Karagiannis
Pierre Martineau
Berislav Lisnić
Vanda Juranić Lisnić

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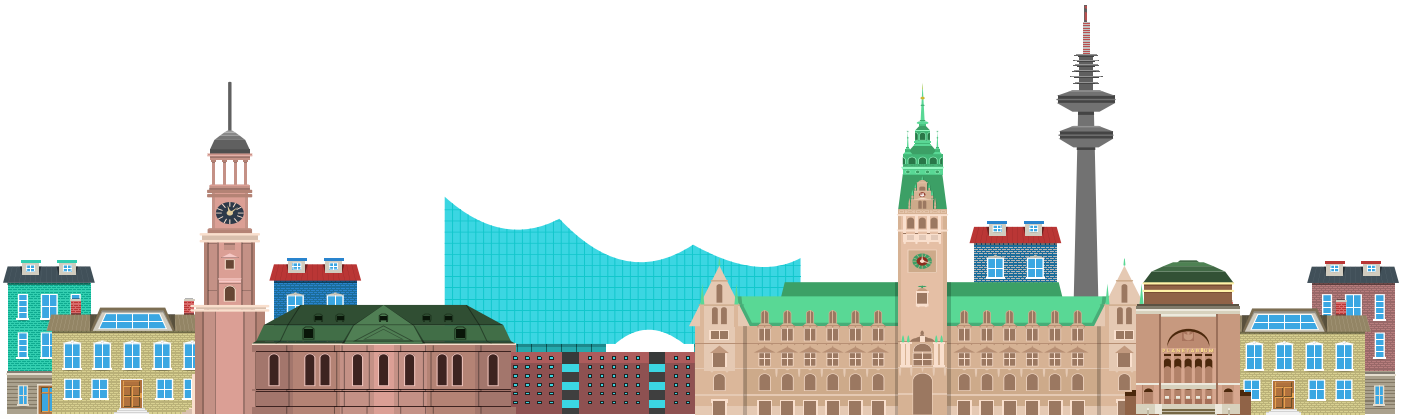
WELCOME PAGES



UKE HAMBURG

6th EuroMabNet Antibody Validation Workshop
12th EuroMAbNet Meeting

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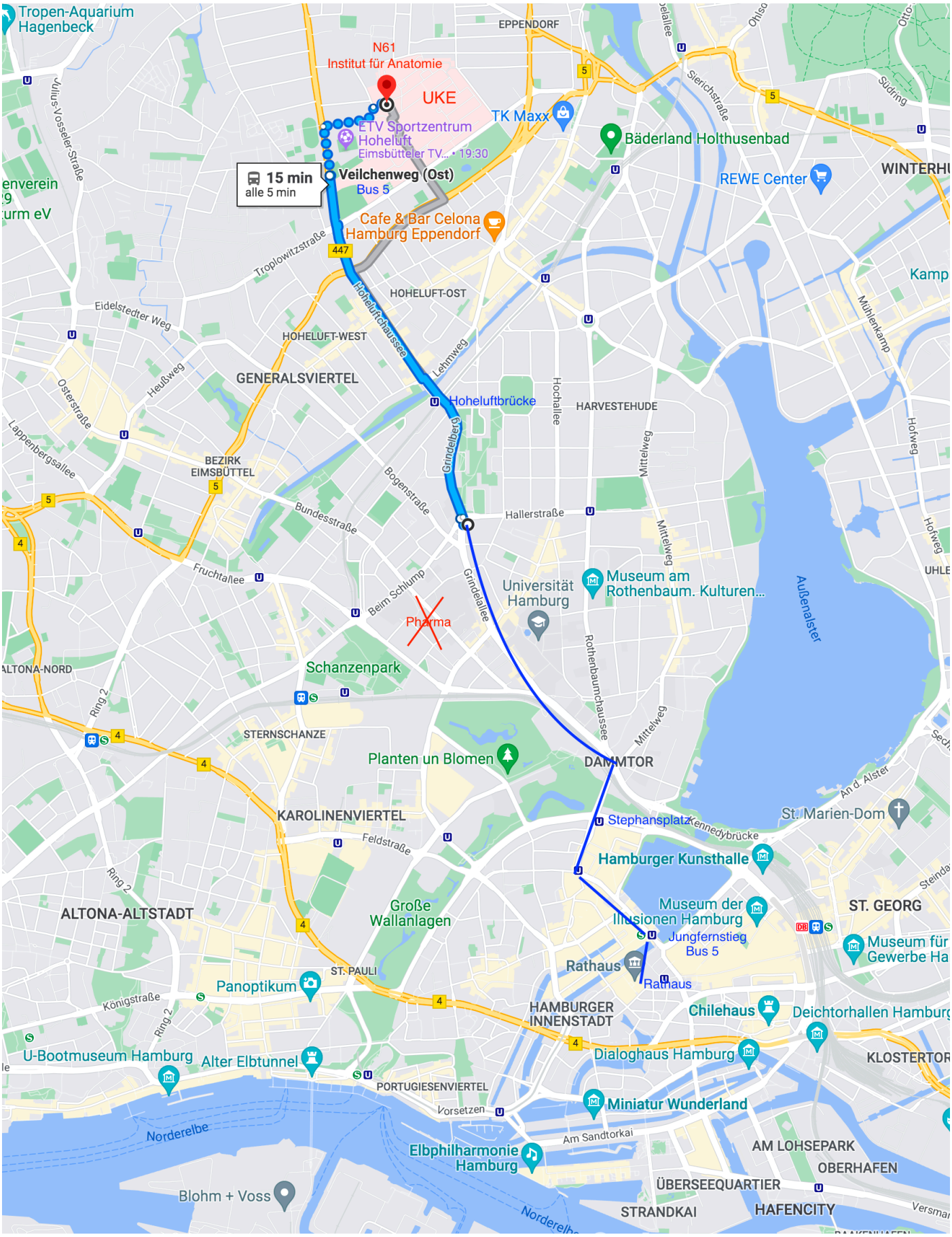
Location of the Universitätsklinikum Hamburg-Eppendorf (UKE) in the city of Hamburg



Moin Moin, dear antibody-fans,

welcome to Hamburg, its University Medical Center Hamburg-Eppendorf with its Institute of Anatomy and its historical Erika-Haus!

Thanks to Giovanna Roncador, the program of the 2022 EuroMabNet Meeting again has gems for both, veterans of and new-comers to the field of antibodies. We hope that the talks, posters and exhibits will arouse your curiosity. May you find inspiration for new ways of using antibodies in your research and/or to translate antibodies into biotech and the clinic.

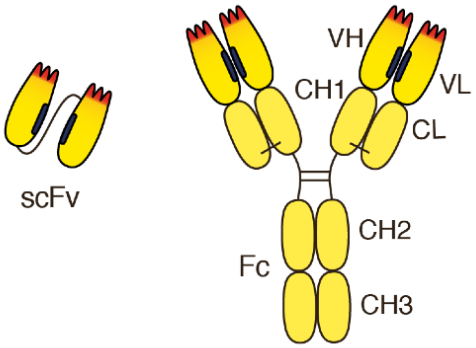
Hamburg is a green, lively, city with a maritime flair.

Stay healthy and enjoy your visit!

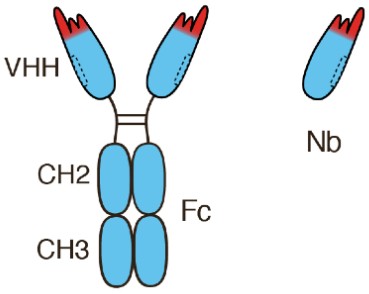
Hummel Hummel!

Friedrich Koch-Nolte and the nanobody team

Hummel Hummel



human mAb
conventional antibody



camelid hcAb
heavy chain antibody

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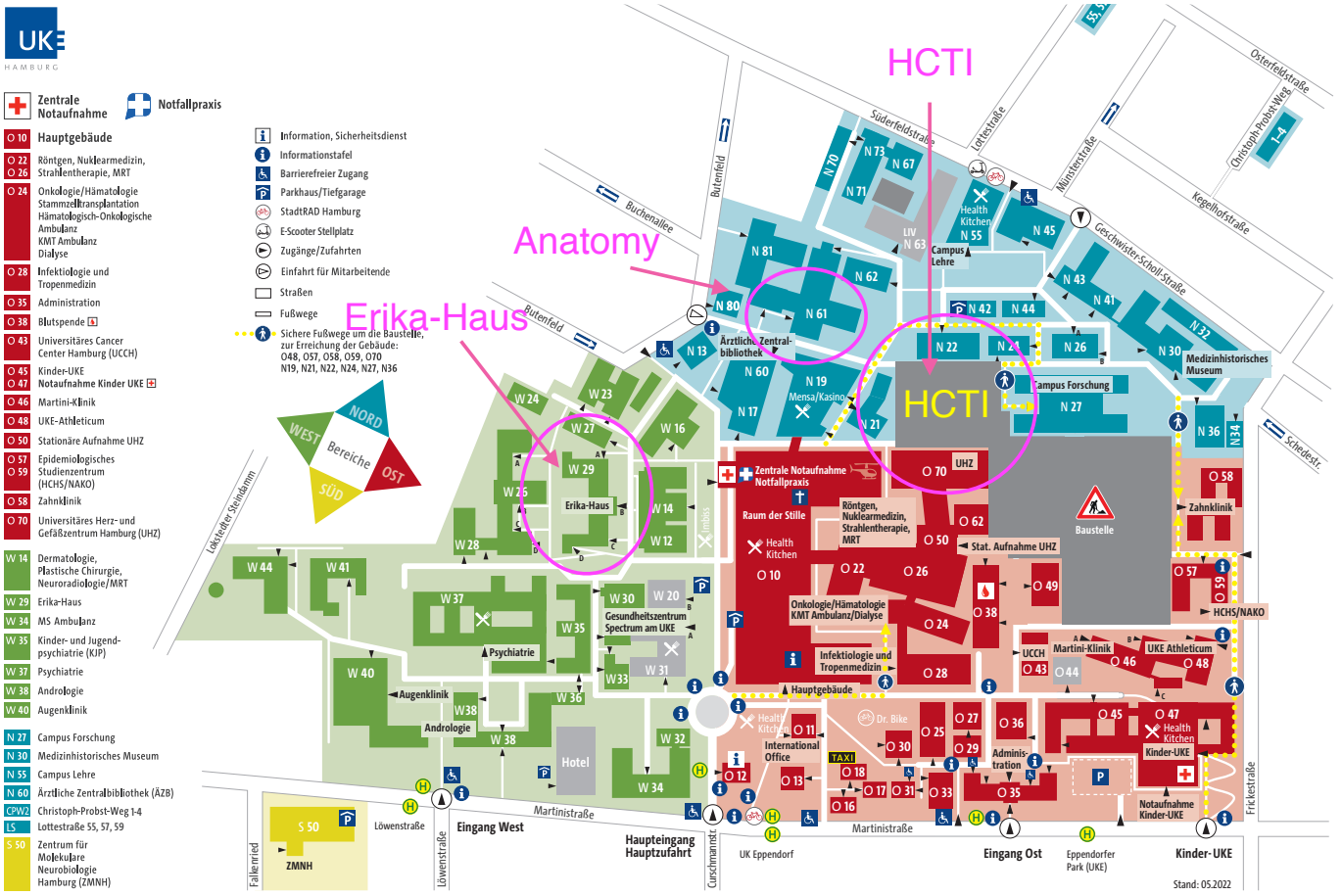


University Medical Center Hamburg-Eppendorf (UKE)

The UKE is one of the largest and the most modern hospitals in Europe and a leader in research and medical treatment. Founded in 1889 in a park, the hospital became a university medical center in 1934. Today, the UKE comprises 78 interdisciplinary collaborative clinics and institutes in 13 centers and has about 14,400 employees. Each year, approximately 90,000 inpatients and 407,000 outpatients are treated, in addition to more than 110,000 emergencies.

From the Laboratory to the Hospital Bed. Medical treatment, science and research at the University Medical Center Hamburg-Eppendorf enjoy an excellent international reputation. Finding new solutions to treat disease and improve medical care – that is the focus of intensive research at the UKE. The UKE subsidiary, MediGate, acts as a technology transfer center to support researchers in bringing their research results to patients, quickly and safely.

Research at the UKE involves a close interaction between basic research and clinical research. The "translational idea" for the rapid translation of research findings for the benefit of the patients at the UKE has a high priority. Research at the UKE benefits from a close sharing of research infrastructure, including core facilities in the campus research building. UKE researchers closely collaborate with other research institutions in northern Germany, e.g. faculties of the University of Hamburg, other universities, Helmholtz (DESY) and Leibniz institutes (HPI, BNI) in the region.

The Hamburg Center for Translational Immunology (HCTI) at the UKE.

With the scientific and clinical focus on the research and treatment of inflammatory and infectious diseases the UKE hosts a competence network of institutes and clinics. Their common goal is to develop an improved understanding of the etiology and pathogenesis of inflammatory and infectious processes in order to design new options for diagnosis and treatment of our patients in a translational approach. To this end, the HCTI, a new research building with excellent facilities is being constructed and is expected to open in 2024. In a nationally and internationally unique combination of organ immunology, patient cohorts, new comparative analytical approaches and comprehensive systems immunology, the HCTI aims to achieve a paradigm shift for the understanding and therapy of autoimmune diseases.

The Erika-Haus (1913), and its architecht, Fritz Schumacher (1869 - 1947)

The institute of anatomy (1958)

The HCTI (2024)

UKE HAMBURG

IP Management

TECHNOLOGY AND KNOWLEDGE TRANSFER

Production of new llama-heavy chain only antibodies in transgenic mice

The advantages of llama VHH
with the genetic versatility of mice

FOCUS SECTORS

- Transgenic mice
- Nanobody production
- Nanobody therapy/diagnostics

PROJECT KEY WORDS

- Llama VHH/Nanobodies
- Transgenic mouse model
- Production of VHH antibodies

DEVELOPMENT STATUS

- Immunized mice produce affinity matured, soluble heavy chain antibodies containing the best natural and synthetic VHH frameworks

PATENT PROCEDURE STATUS

- Patent application filed in AU, CA, EP, JP, KR, NZ, US

POTENTIAL FOR COOPERATION

- R&D Cooperation
- Transfer of rights
- Licensing

UKE270

Background & Innovation

The use of **antibodies** for therapeutic and diagnostic purposes is well established.

Nanobodies / llama VHHs offer an attractive solution due to their unique characteristics that **outperform monoclonal antibodies**:

- higher stability and solubility
- easier formatting of bispecifics
- higher expression yields
- better in vivo tissue penetration
- better binding to crevices

Nanobodies are currently produced in camelids, requiring higher **costs** compared to transgenic mice without **genetic engineering possibilities**.

Our technology offers **novel transgenic mouse models to produce llama VHHs**, paired with the vast genetic engineering possibilities of the mouse.

Competitive advantages

Nanobody discovery from our transgenic mice features:

- only VHH, no conventional antibodies
- full VHH repertoire from lymphatic organs
- direct sequence information
- no VH-VL pairing -> better developability
- use of established mouse immunization and hybridoma technologies
- use of target-ko mice to address conserved epitopes

Technical Description

We present novel genetically engineered mice for the production of nanobodies.

This is the **first available** genetic construct and transgenic animal that comprises: camelid VHH, D, J and constant heavy chain regions, which are **not rearranged**.

This mouse model was generated by insertion of an engineered llama IgH locus into the mouse genome by **BAC-recombineering**.

The usage of transgenic mice allows **flexible and efficient generation** of VHHs by:

- **hybridoma technology**
- **phage display technology**
- direct cloning and sequencing
- high yield production in CHO & HEK cells

Nanobodies can be produced in **mice deficient for the target antigen (knock-out)**.

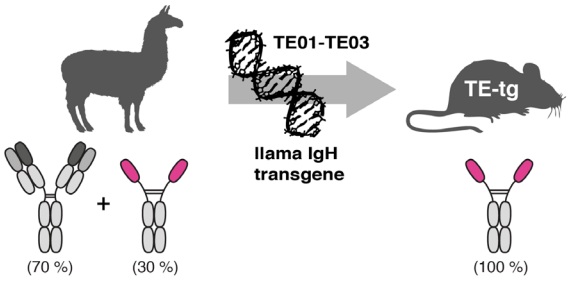
Summary

Thanks to their **unique features**, nanobodies are becoming more and more important for biomedical applications.

We are presenting a **unique platform** for the production of camelid VHHs in genetically engineered mice

with **greater technical flexibility** and **lower costs** than nanobody discovery in camelids.

Discovery of specific VHH by
- **hybridoma technology**
- **expression in HEK-cells**
- **phage display**



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Gefördert durch:
Bundesministerium für Wirtschaft und Energie
aufgrund eines Beschlusses des Deutschen Bundestages
WIPANO

Hamburg

With its 1.8 million inhabitants, Hamburg is the second largest city in Germany, and many consider it one of the most beautiful cities in the world. Our Hanseatic city is multifaceted, exciting and safe. Extensive bodies of water and green spaces as well as attractive recreational facilities offer room to live and air to breathe. These features, combined with maritime atmosphere, open-minded residents, and proximity to the North Sea and the Baltic Sea, make Hamburg a gem among international cities.

Woods and meadows in the city center

Lush green is the dominant color even in the city center, whether along the streets or in the city park, the Jenischpark or the verdant areas around the Outer Alster. In addition to the numerous parks, there are vast forests in the metropolitan area.

Water as far as the eye can see

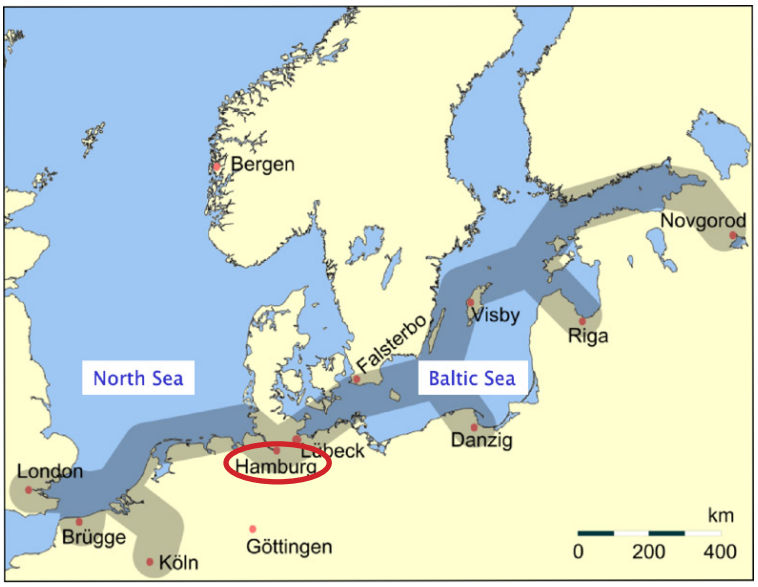
Nearly 2,500 bridges cross the extensive labyrinth of canals between the Elbe, Alster and Bille Rivers. The waterways themselves are perfectly suited for exploring the hustle and bustle of the harbor or the languorous nooks of the Hanseatic city.

Culture – around the clock

Musicians, artists and other creative people make Hamburg Germany's foremost cultural capital. Modern theatre productions, operas, popular musicals, and a variety of museums and galleries ensure ever new heights. In the new HafenCity a monument to culture nicknamed "Elphi" was opened in 2017: a concert hall of international stature.

Easy to reach

Hamburg is located in the heart of Europe. In only 90 minutes on a train you can reach Berlin; a one hour flight will take you to London, Prague, Brussels, or Paris.



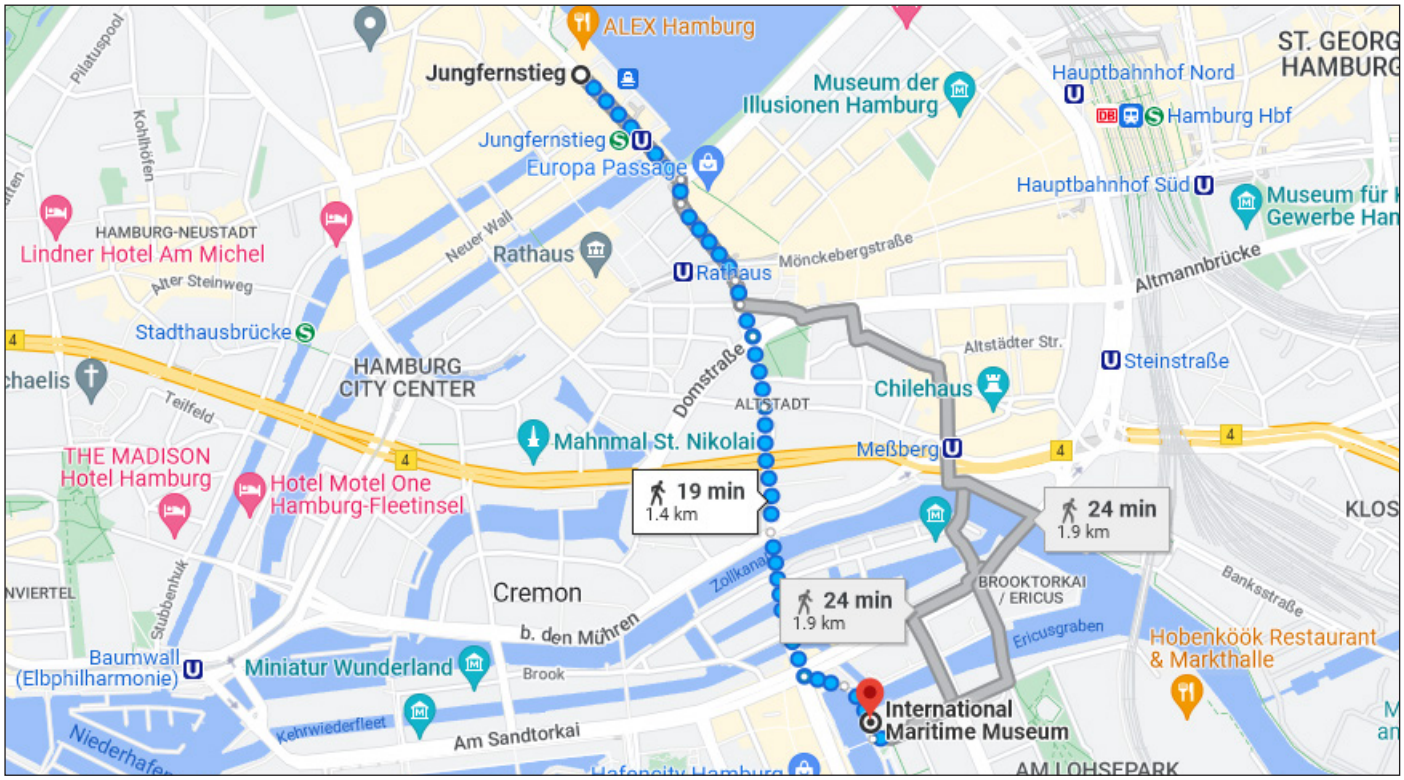
Hanseatic League

Elphi

River Elbe and its tributaries

EuroMabNet Dinner
Maritim-Museum
Friday 19:00

Take Bus 5 to **Jungfernstieg**, and continue walking in the same direction past the **Petri-Kirche**, **Domplatz**, **Columbus** & **Vasco Da Gama**, the **customs border**, the Unesco heritage "**Speicherstadt**", and the statue of **Klaus Störtebeker**, Hamburg's 'Robin-Hood' pirate.



Jungfernstieg

Petri Kirche

Domplatz

Columbus

Vasco da Gama

Speicherstadt Customs-border

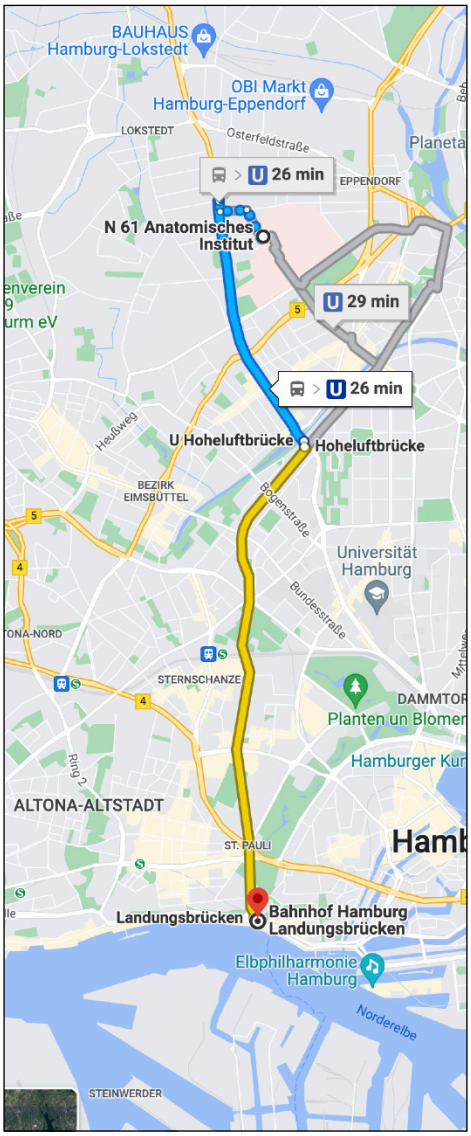
Störtebeker

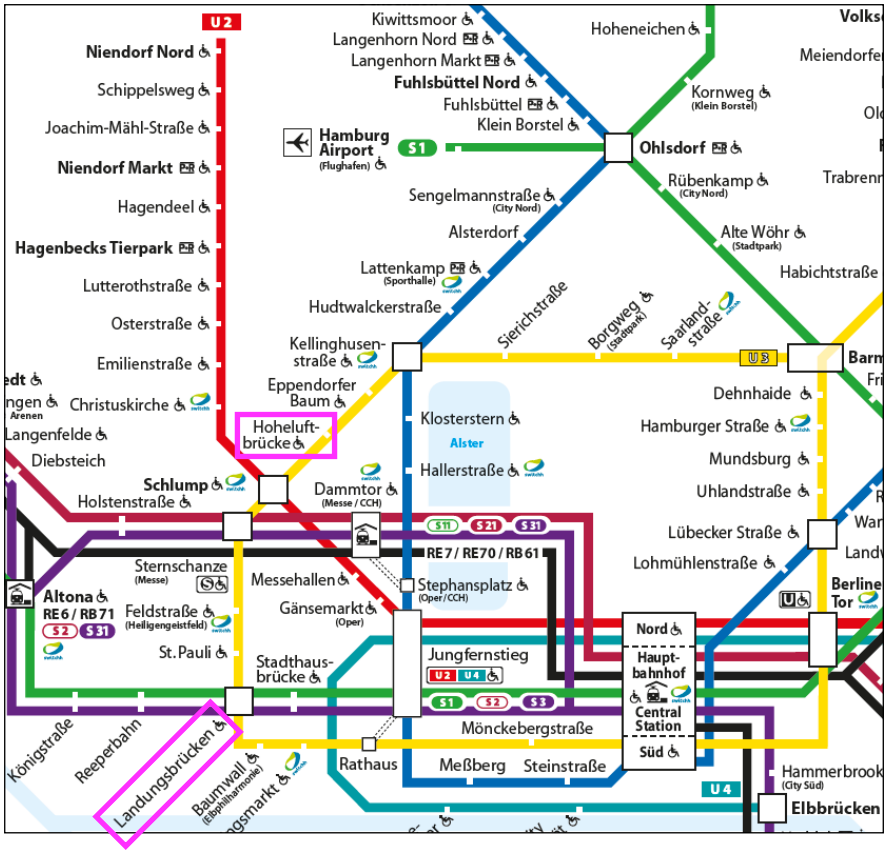
Maritim Museum

Christa Glitscher - our boat

Harbor tour by boat
Brücke 6
Saturday 14:00
Landungsbrücken

Take **Bus #5** to **Hoheluftbrücke**, switch to the subway (**U-Bahn #3**) take the **yellow line** to **Landungsbrücken**, walk over to the piers on the Elbe river grab a **Bier** and a **Fischbrötchen**, catch the boat at **bridge #6**.





WORKSHOP
AND
MEETING SCHEDULE

12th EUROMABNET MEETING
6th ANTIBODY VALIDATION WORKSHOP - THURSDAY, SEPTEMBER 22nd

MORNING SESSION

9:00 Giovanna Roncador
Monoclonal Antibodies Unit, CNIO, Madrid, Spain
Opening introduction

9:10 Pablo Engel
Department of Biomedical Sciences, School of Medicine, University of Barcelona, Spain
The problems arising from poor antibody validation

9:30 Friedrich Koch-Nolte
Immunology and Molecular Biology, Institute of Immunology, Hamburg, Germany
Generation and engineering of recombinant antibodies

10:00 Alicia Chenoweth
Translational Cancer Immunology and Immunotherapy, St. John's Institute of Dermatology
School of Basic & Medical Biosciences, King's College, UK
How to approach an antibody project

COFFEE BREAK (10:30-11:00)

11:00 Giovanna Roncador
Monoclonal Antibodis Unit, CNIO, Madrid, Spain
Principles of antibody validation

11:40 Paola Kučan Brlić
Center for proteomics, University of Rijeka, School of Medicine, Rijeka, Croatia
Principles of antibody validation

12:00 Sponsor talk
Will Howat
Abcam
Why choose a recombinant antibody

12:00 Sponsor talk
Yannick Nossin
Cell Signalling Technology
The Hallmarks of Validation: 6 Complementary Strategies to Verify Antibody Performance

LUNCH (12:30-14:00)

AFTERNOON SESSION

14:00 Interactive Session "Meet the Experts I" (45 minutes each)
1. Meet the expert in immunohistochemistry
2. Meet the expert in flow cytometry

COFFEE BREAK (15:30-16:00)

3. Meet the expert in western blotting

WORKSHOP END (16:45)

12th EUROMABNET MEETING
SCIENTIFIC SECTION - FRIDAY, SEPTEMBER 23rd

MORNING SESSION
Chair: Friedrich Koch-Nolte

9:00 Friedrich Koch-Nolte
Immunology and Molecular Biology, Institute of Immunology, Hamburg, Germany
Opening and welcome remarks

9:10 INVITED SPEAKER
Luis Alvarez-Vallina
Head of the Cancer Immunotherapy Unit (UNICA),
12 de Octubre University Hospital, Madrid
Next-Generation 4-1BB Agonistic Antibodies for Cancer Immunotherapy

9:40 INVITED SPEAKER
Sahil Adriouch
Université de Rouen Faculté de Médecine et Pharmacie, Rouen, France
Using AAV gene therapy vectors for persistent, long term expression of nanobodies and heavy chain antibodies in vivo

10:10 Valerija Kovač and Maja Černilec
Center for Immunology and Development and research group Biomedicine, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia
Finding the right antibody for the selected target– a lesson from prion protein

10:40 Selected student short talk
Raphaelle Drean
Antibody engineering Platform, Institute Pasteur, France
Advances in cancer immunotherapy development: new VHH-based immune checkpoint inhibitors targeting the receptor ILT4

COFFEE BREAK (10:55-11:30)

11:30 Paola Kučan-Brlić
Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia
Translational Opportunities for Antibodies targeting PVR (CD155)

12:00 Selected student short talk
Pablo Hernández-Luis
Department of Biomedical Sciences, School of Medicine, University of Barcelona, Spain
Development of neutralizing human chimeric monoclonal antibodies against the ACE2 receptor to broadly neutralize SARS-CoV-2 variants and future emerging coronaviruses for therapeutic use

12:15 Poster Slam Session
Friedrich Koch-Nolte

LUNCH BREAK AND POSTER VIEWING (12:30-14:00)

AFTERNOON SESSION
Chair: Roberto Perris

NEW MEMBERS SECTION

14:00 Regina Feederle
Monoclonal Antibody Core Facility, Helmholtz Center Munich, Germany
Activities of the Helmholtz Munich Monoclonal Antibody Core Facility. A new member of EuroMabNet

14:20 Aurelija Žvirblienė
Department of Immunology and Cell Biology Institute of Biotechnology, Vilnius, Lithuania
Virus-like particles as a platform for antigen design and antibody engineering

14:40 Tara Hiltke
Office of Cancer Clinical Proteomics Research, National Cancer Institute, National Institutes of Health, USA
The National Cancer Institute's Antibody Program: Fit-for-Purpose Community Affinity Reagents

15:00 Ilse Hoffman
GPCF Unit Antibodies, German Cancer Research Center, Heidelberg, Germany
Advantages of the recombinant antibody technology: Insights into 14 BMMF Rep specific antibodies

15:20 Sponsor talk
Andreas Weise
Genovac
Genetic Immunization and Single Cell Screening: Combining two powerful technologies for the discovery of monoclonal antibodies against challenging targets

COFFEE BREAK (15:30-16:00)

AFTERNOON SESSION - Cont'd
Chair: Gholamreza Hassanzadeh-Ghassabeh

16:00 Sponsor talk
Kathrin Günther
Absolute Antibody
Why go recombinant? Take your antibody experiments further with high reproducibility and engineering

16:10 Armand Bensussan
INSERM, Saint Louis Hospital and OREGA Biotech, Paris, France
New targets for cutaneous T cell lymphomas treatments : Lacutamab opened the field of economical valorisation of therapeutic monoclonal antibodies issued from academic laboratories

16:30 Giovanna Roncador
Monoclonal Antibodies Unit, CNIO, Madrid, Spain
Licensing antibodies: dream or nightmare?

EUROMABNET ROUNDTABLE (17:00-18:30)

EUROMABNET DINNER (19:30)

12th EUROMABNET MEETING
SCIENTIFIC SECTION - SATURDAY, SEPTEMBER 24th

MORNING SESSION
Chair: Pablo Engel

9:00 INVITED SPEAKER
Jeannette Leusen
Center for Translational Immunology, UMC Utrecht, The Netherlands
Engineered IgA as new antibody isotype to treat cancer

9:30 Sophia Karagiannis
Translational Cancer Immunology and Immunotherapy, St. John's Institute of Dermatology
School of Basic & Medical Biosciences, King's College, UK
Humoral immunity in patients with solid tumours: opportunities for antibody design

10:00 Pierre Martineau
INSERM U896/Montpellier University/Val d'Aurelle Oncology Center, Montpellier, France
Phage and Mammalian Display for improved therapeutic antibodies

10:30 Selected student short talk
Alicia Chenoweth
Translational Cancer Immunology and Immunotherapy, St. John's Institute of Dermatology
School of Basic & Medical Biosciences, King's College, UK
Fc Engineered Monoclonal Antibodies for Triple Negative Breast Cancer Therapy

10:45 Selected student short talk
Julia Hambach
Immunology and Molecular Biology, Institute of Immunology, Hamburg, Germany
CD38-specific nanobody-based half-life extended bispecific killer cell engagers (HLEnano-BiKEs) for the treatment of multiple myeloma

COFFEE BREAK (11:00-11:30)

MORNING SESSION - Cont'd
Chair: Pierre Martineau

11:30 Sponsor talk
Erik Stahl
Preclinics
From antigen to preclinics - development and preclinical analysis of therapeutic monoclonal antibodies

11:40 Sponsor talk
Thorsten Zacher
PEPperPRINT GmbH, HeidelBerg, Germany
Custom peptide microarrays for in-depth mapping and validation of antibody epitopes

11:50 Friedrich Koch-Nolte
Immunology and Molecular Biology, Institute of Immunology, Hamburg, Germany
Nanobody discovery from llama IgH-transgenic mice

12:20 Award ceremony

EUROMABNET HARBOR BOAT TOUR (14:00-15:00)

END OF THE MEETING (15:00)

ABSTRACTS
INVITED SPEAKERS

Morning session
Friday, September 23rd
09:10-09:40

INVITED SPEAKER

Luis Alvarez-Vallina

Next-Generation 4-1BB Agonistic Antibodies for Cancer Immunotherapy

Luis Alvarez-Vallina[1,2]
[1] Immuno-Oncology and Immunotherapy Group, Fundación de Investigación "12 de Octubre", Madrid, Spain
[2] Cancer Immunotherapy Unit, Hospital Universitario "12 de Octubre", Madrid, Spain

Costimulation of tumor-infiltrating T lymphocytes by anti-4-1BB (CD137) monoclonal antibodies (mAbs) has shown anti-tumor activity in human trials, but can be associated with significant off-tumor toxicities involving FcγR interactions. New strategies aiming to confine 4-1BB costimulation to the tumor microenvironment and avoid FcγR interactions are under clinical development. These approaches are based on bispecific molecules, targeting 4-1BB as well as tumor cells or tumor stromal cells, designed without Fc regions or with either engineered effector-silent Fc regions. We have designed bispecific antibodies aiming to mimic the 4-1BB ligand structure to promote tumor-specific hyper-clustering, as well as highly compact small bispecific molecules fully adapted to the immune synapse context. Our results demonstrate that NextGen 4-1BB agonistic antibodies induce in vitro cognate target engagement, tumor-specific costimulatory activity, and in vivo tumor inhibition, with no indication of 4-1BB mAb-associated toxicity. Furthermore, we show a greater therapeutic effect when used in combination with immune checkpoint blocking antibodies. These findings demonstrate the feasibility of tumor-specific 4-1BB agonistic antibodies for safe and effective costimulatory strategies in cancer immunotherapy.

Morning session
Saturday, September 24th
09:00-09:30

INVITED SPEAKER
Jeannette Leusen

Engineered IgA as new antibody isotype to treat cancer

Jeannette Leusen
Center for Translational Immunology, UMC Utrecht, The Netherlands

Therapeutic antibodies can be applied in a variety of diseases, ranging from cancer to autoimmunity and allergy. All current therapeutic monoclonal antibodies used in the clinic are of the IgG isotype. IgG antibodies can induce the killing of cancer cells by growth inhibition, apoptosis induction, complement activation (CDC) or antibody-dependent cellular cytotoxicity (ADCC) by NK cells, antibody-dependent cellular phagocytosis (ADCP) by monocytes/macrophages, or trogoptosis by granulocytes. To enhance these effector mechanisms of IgG, protein and glyco-engineering has been successfully applied. As an alternative to IgG, antibodies of the IgA isotype have been shown to be very effective in tumor eradication. Using the IgA-specific receptor FcαRI expressed on myeloid cells, IgA antibodies show superior tumor-killing compared to IgG when granulocytes are employed. However, reasons why IgA has not been introduced in the clinic yet can be found in the intrinsic properties of IgA posing several technical limitations: (1) IgA is challenging to produce and purify, (2) IgA shows a very heterogeneous glycosylation profile, and (3) IgA has a relatively short serum half-life. Next to the technical challenges, pre-clinical evaluation of IgA efficacy in vivo is not straightforward as mice do not naturally express the FcαR. In the presentation, I will provide a concise overview of the latest insights in these engineering strategies overcoming technical limitations of IgA as a therapeutic antibody: developability, heterogeneity, and short half-life. In addition, the challenge to activate myeloid derived suppressor cells to kill cancer cells will be addressed. Furthermore, the cytokines involved in IgA-mediated neutrophils will be elucidated *in vitro* and *in vivo*. Finally, GD2 as a target for IgA-based therapy for neuroblastoma alternative approaches will be demonstrated.

Morning session
Friday, September 23rd
09:40-10:10

INVITED SPEAKER
Sahil Adriouch

Using AAV gene therapy vectors for persistent, long term expression of nanobodies and heavy chain antibodies in vivo

Mélanie Demeules[1], Henri Gondé[1], Romain Hardet[1], Catalina Abad[1], Charlotte Guillou[1], Yossan-var Tan[1], Carolina Pinto-Espinoza[2], Anna Marei Eichhoff[2], Waldemar Schäfer[2], Friedrich Koch-Nolte[2] and Sahil Adriouch[1]

[1] University of Rouen, INSERM U1234, Physiopathologie et biothérapies des maladies inflammatoires et autoimmunes (PANTHER), Normandie Univ, UNIROUEN, 76000 Rouen, France
[2] Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Numerous nanobodies have been developed we the aim to antagonise or to stimulate the functions of their target proteins and to induce therapeutic benefit in vivo. Their potential advantages over small-molecule drugs include high specificity, lower off-target effects, and tuneable in vivo half-life. Besides modulating their target function, nanobody based heavy-chain antibodies can also be used to deplete cells that express their specific target. Adeno-associated viral vectors (AAV) represent an efficient mean to express antibodies or nanobody-based biologics in vivo. We developed a methodological approach (termed AAVnano) based on AAV vector coding for selected nanobody-based biologics with the aim to rapidly validate their functionality, their safety profile, and their biological effects directly in vivo in mouse models during the preclinical evaluation phases. As an example, illustrating this methodological approach, we present data obtained using nanobody-based biologics targeting the P2X7/ARTC2 pathway. Single intramuscular injection of AAVnano allow long-term expression of the selected nanobody-based biologics for at least 120 days resulting in the long-term potentiation or blockade of the function of their target protein, or depletion of the specific cells expressing their targets. The AAVnano approach has been applied to appreciate the importance of P2X7 as a druggable target in acute colitis and in different tumour models. We propose that the AAVnano methodological approach can similarly be used to rapidly validate the relevance of any given potential target during the preclinical evaluation phase of nanobody-based biologics.

ABSTRACTS
ORAL PRESENTATIONS

Morning session
Friday, September 23rd
10:10-10:40

ORAL PRESENTATION

PRESENTERS
Valerija Kovač and Maja Černilec

Toward a therapy of neurodegenerative diseases - a lesson learned from prion diseases

Valerija Kovač, Maja Černilec, Vladka Čurin Šerbec
Center for the Production of Diagnostic Reagents and for Research and research group Biomedicine, Blood Transfusion Centre, Slovenia

Prion protein (PrP) is a highly conserved ubiquitous glycoprotein. It exists in two forms; the normal or cellular isoform, PrPC, and the disease-associated infectious isoform or scrapie PrP, PrPSc. PrPC is expressed in a variety of different organs and tissues with high expression levels in the central and peripheral nervous systems. It is abundantly present on the cell surface of neurons and has been shown to be involved in many physiological mechanisms. PrPC is cleaved at the cell membrane by proteases, forming released and attached forms. The protein can undergo four posttranslational cleavages – α-cleavage, β-cleavage, γ-cleavage and PrP shedding. α-cleavage, β-cleavage and the related fragments have already been intensively studied, whereas the mechanisms behind γ-cleavage and PrP shedding remain to be elucidated. . In recent years, prion protein and prion protein released forms have received attention in correlation with neuroprotection in neurodegenerative diseases.

Antibodies are important tools in studying prion protein and its fragments, as specific antibodies enable identification of protease cleavage sites and fragment involvement in vitro and in vivo. In our previous studies, we prepared monoclonal antibody V5B2, which selectively recognizes a released form of PrP that ends with amino acid residue Tyr226, PrP226*. We speculated that PrP226* is produced by shedding and may be one of, if not the only form of shed PrP.

In this presentation, we will describe PrP226* and V5B2 and present their connection to shedding of PrP by protease ADAM10. We will also discuss the possible application of the new knowledge to understanding and diagnosis of neurodegenerative diseases.

Morning session
Friday, September 23rd
10:40-10:55

SHORT ORAL PRESENTATION

PRESENTER
Raphaelle Dréan

Advances in cancer immunotherapy development: new VHH-based immune checkpoint inhibitors targeting the receptor ILT4

Raphaelle Dréan[1, 2, 3], Gabriel Aymé[2], Alix Jacquier[1], Odessa Fayet[1], Martin Lecomte[1], Julien Caumartin[1], Maria Loustau[1]* and Pierre Lafaye[2]*
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MILT4 (Immunoglobulin-Like Transcript 4) is an immune checkpoint receptor mainly expressed by myeloid immune cells. In cancer context, ILT4 participates in tumor development by maintaining a pro-tumoral immuno-microenvironment and directly promoting tumor cell proliferation.

ILT4 interaction with the non-classical MCH class I molecule HLA-G induces an immunosuppressive microenvironment by promoting tolerogenic myeloid cells. Moreover, the ectopic expression of ILT4 has been reported in several solid tumors. The activation of ILT4 by Angiopoietin-like-2 (ANGPTL2) promotes non-small cell lung tumor cell proliferation and inhibits cell apoptosis.

Targeting this new immune checkpoint with blocking antibodies is therefore a promising cancer immunotherapy approach. In light of several drawbacks of classical IgG blocking antibodies in solid cancer, we investigated the potential of VHH-based inhibitors. This small monoclonal antibody format, derived from camelid homodimeric antibodies, is devoid of Fc region and presents increased tumor penetrability.

After immunization of an alpaca and phage-display screening, we selected a VHH with high affinity and specificity to ILT4 that inhibits the interaction of the receptor with both ligands. We validated the VHH's biological antagonist activity on tumor cells and monocytederived pro-tumoral M2 like macrophages in vitro. These results support the potential of this new VHH-based antibody targeting ILT4 in cancer immunotherapy.

Morning session
Friday, September 23rd
11:30-12:00

ORAL PRESENTATION

PRESENTER
Paola Kučan Brlić

Translational Opportunities for Antibodies targeting PVR (CD155)

Paola Kučan Brlić[1], Anas Atieh[2], Akram Obeidat[2], Keren Paz[2], Guy Cinamon[2], Lea Hiršl[1], Marija Mazor[1], Tihana Lenac Roviš[1], Stipan Jonjić[1], Ofer Mandelboim[3], Pini Tsukerman[2]
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[2] Nectin Therapeutics LTD, Jerusalem, Israel
[3] Hebrew University, Jerusalem, Israel

Poliovirus receptor (PVR, CD155), has recently been gaining considerable scientific interest because of its intrinsic and extrinsic roles in tumor progression. The intrinsic functions of PVR in tumor cells promote tumor progression and metastasis, whereas its extrinsic functions involve interaction with the activating and inhibitory (checkpoint) immune cell receptors. Therefore, targeting PVR by blocking monoclonal antibodies (mAbs) offers an attractive therapeutic approach for patients with advanced cancer. Here we describe a first-in-class, potent therapeutic blocking antibody to human PVR called NTX-1088, developed by Nectin Therapeutics LTD that is being investigated for the treatment of solid tumors. The antibody blocks PVR interaction with inhibitory receptors TIGIT and CD96, interrupting their immunosuppressive signaling. Additional advantage of the NTX-1088 is manifested through its ability to block the interaction between PVR and the costimulatory receptor DNAM-1 (CD226), preventing PVR-induced internalization of DNAM-1, thus restoring its expression on the surface of immune cells leading to robust antitumor activation. Efficacy of the NTX-1088 was validated on several human tumor cell lines both in in vitro co-culture systems and in humanized murine models *in vivo*. Our in vitro data shows that NTX-1088 increases activation of both CD8 T-cells and NK-cells, even to superior levels than anti-TIGIT mAb (tiragolumab) and has potentially synergistic effect with anti-PD-1 (pembrolizumab). In vivo, NTX-1088 induces a robust tumor growth inhibition that is accompanied by higher prevalence of DNAM1+, CD8+ tumor infiltrating cells. Altogether, NTX-1088 shows, for the first time, exclusive triple mechanism of action, whereby simultaneous and effective blockade of TIGIT and CD96 is complemented by the efficient restoration of DNAM1. This is a step change in antitumor immune activation, which is currently being validated in the clinic, in a Phase 1, First-in-Human Study of NTX-1088 as monotherapy and combined with Pembrolizumab, in patients with advanced solid malignancies (NCT05378425).

In addition to well characterized membrane PVR, in humans, PVR also exists as less characterized, soluble form (sPVR) that was shown to be increased in sera of cancer patients and proposed as a potential tumor biomarker. However, it is less clear if serum soluble PVR derives from shedding of the membrane PVR ectodomain or by the alternate splicing isoforms that have been identified. Furthermore, potential correlation between membrane and soluble PVR expression has not been studied in detail. To investigate this, we generated antibodies against different PVR isoforms and developed sandwich ELISAs that are able to discriminate total PVR in biological fluids from "true" soluble PVR (generated by alternate splicing). Indicated ELISAs are currently being validated on serum and urine samples from cancer patients to select the one that is better prognostic tool and eventual candidate for liquid biopsy, which would facilitate patient selection and allow monitoring of therapeutic efficacy in studies targeting membrane PVR.

Morning session
Friday, September 23rd
12:00-12:15

SHORT ORAL PRESENTATION

PRESENTER
Pablo Hernández-Luis

Development of neutralizing human chimeric monoclonal antibodies against the ACE2 receptor to broadly neutralize SARS-CoV-2 variants and future emerging coronaviruses for therapeutic use

Pablo Hernández-Luis[1], Mar Tolós[1], Pablo Engel1, and Ana Angulo[1]
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SARS-CoV-2 has produced unprecedented global health problems with devastating economic consequences and the situation is far from being yet under control. Moreover, the emergence of new viral variants has put the world on an even higher alert. Thus, there is an urgent need to find effective therapeutics to fight infections caused by present and future SARS-CoV-2 variants. SARS-CoV-2 enters host cells through binding of its spike protein (S protein) to the cell surface receptor human angiotensin-converting enzyme 2 (ACE2). In this study, we have developed neutralizing chimeric monoclonal antibodies (mAbs) that recognize and block the human ACE2 receptor. BALB/c mice were immunized with a stably transfected murine cell line (300.19) that expresses at high levels human ACE2 on its cell surface. 649 murine hybridoma supernatants were initially screened by flow cytometry for their capacity to interact with the receptor, using the ACE2-transfected cells, and 26 showed specific ACE2 binding. Employing an in vitro pseudo-neutralization flow cytometry-based assay with RBD (receptor binding domain)-Fc fusion proteins, we obtained two mAbs, clone 1.48 and clone 2.96, able to block the RBD-ACE2 interaction. Both mAbs recognized different ACE2 genetic variants. Moreover, they presented neutralizing activity against multiple RBD variants. Cross-competition assays showed that clones 1.48 and 2.96 targeted overlapping receptor epitopes. The variable regions of the two blocking antibodies and one non-blocking mAb were sequenced and subcloned in an expression vector containing the constant regions of human IgG4 and light chain k, to generate human chimeric antibodies. We are producing these chimeric mAbs to analyze their neutralizing potential in a transgenic mouse expressing human ACE2 with S-protein pseudotyped lentiviruses. We predict that a therapy based on this type of mAbs could be especially useful for infected elderly persons or immunocompromised patients, which present poor immune responses to vaccination.

Afternoon session | New members section
Friday, September 23rd
14:00-14:20

ORAL PRESENTATION

PRESENTER
Regina Feederle

Activities of the Helmholtz Munich Monoclonal Antibody Core Facility: A new member of EuroMabNet

Regina Feederle
Monoclonal Antibody Core Facility, Helmholtz Center Munich, Germany
Monoclonal Antibody Core Facility, German Center for Neurodegenerative Diseases, Munich, Germany

The Monoclonal Antibody Core Facility at Helmholtz Munich generates custom monoclonal antibodies in rats and mice for internal and external partners from academia and industry. We provide support from antigen design to antibody validation, purification and labelling with more than 25 years of experience.

We employ hybridoma technology to generate ten-thousands of clones per immunized animal which are then analyzed in an automated high-throughput flow cytometry screening platform according to the specific project requirements. We also design ELISA assays to identify high-affinity binders or neutralizing antibodies in the primary screening. A thorough antibody validation is performed in collaboration with our partners. We frequently license antibodies with high value for the research community to industry partners. To archive the DNA sequences or for further antibody engineering, we also provide antibody sequencing and recombinant cloning.

We have generated antibodies against many difficult targets such as transmembrane proteins, post-translationally modified proteins, small molecules and surface proteins on cells, virus-like particles or extracellular vesicles. A selection of antibodies with therapeutic or diagnostic value are presented.

Afternoon session | New members section
Friday, September 23rd
14:20-14:40

ORAL PRESENTATION

PRESENTER
Aurelija Žvirblienė

Virus-like particles as a platform for antigen design and antibody engineering

Aurelija Žvirblienė
Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

Viral structural proteins produced in yeast expression system are capable to self–assemble to highly-organized virus-like particles (VLPs) that mimic native viruses in terms of their antigenicity and imunogenicity. Due to their repetitive structure, VLPs are extensively used for protein engineering.

Research team of Vilnius University develops and investigates recombinant VLPs with desired features. These studies involve construction of chimeric VLPs harbouring foreign epitopes and the use of VLPs as a carrier for antibody molecules.

Chimeric VLPs harboring foreign protein sequences at certain surface-exposed positions are employed as highly efficient immunogens for tailored antibody production. It was demonstrated that the insertion of non-immunogenic protein sequences into polyomavirus-derived VLPs significantly increases their ability to induce a strong B cell response and provides an alternative to chemical coupling of synthetic peptides to carrier proteins. This approach has been proven successful to develop antibodies against short non-immunogenic peptides, viral glycoproteins and other target antigens. The generated antibodies were reactive with native full-length target proteins thus demonstrating the surface localization and proper folding of the inserted sequences. Another approach used by Vilnius University team is generation of multimeric antibody molecules exposed on the surface of VLPs. For this purpose, so-called pseudotype VLPs are designed where polyomavirus major capsid protein VP1 that mediates VLP formation is co-expressed with polyomavirus minor capsid protein VP2 fused with antibody molecule. The capacity of VP1 protein to self-assemble to VLPs and interact with the modified VP2 protein has been exploited to generate pseudotype VLPs displaying either single-chain fragments variable (scFvs) or Fc-engineered scFvs. The successful examples include neutralizing multimeric antibodies against hepatitis B surface antigen and bacterial virulence factors. The VLPs harbouring multiple functionally active antibody molecules may represent a promising platform for developing novel diagnostic or therapeutic tools.

Afternoon session | New members section
Friday, September 23rd
14:40-15:00

ORAL PRESENTATION

PRESENTER
Tara Hiltke

The National Cancer Institute's Antibody Program: Fit-for-Purpose Community Affinity Reagent

Tara Hiltke
Office of Cancer Clinical Proteomics Research, National Cancer Institute, National Institutes of Health, USA

Central to reproducibility in biomedical research is being able to use well-characterized and defined reagents. The National Cancer Institute (NCI)'s Antibody Program serves as a community resource of unbiased antibody characterization in a centralized location for a large variety of renewable antibodies. To date, 907 antibodies to 543 protein targets have been generated and characterized. Antibodies are characterized for specificity and performance and align with the National Institutes of Health's Rigor and Reproducibility Principles and Guidelines.

Approximately every 12 to 18 months, the NCI solicits the cancer research community to submit requests for specific cancer-related protein or peptides to be used for antibody generation. Reagents are generated using a fit-for-purpose approach and the requestor is encouraged to participate in both reagent screening and selection. Regardless of the intended end use application, each affinity reagent is evaluated using a variety of characterization methods and all data and standard operating procedures are made available on the NCI's Antibody Portal (antibodies.cancer.gov). The Antibody Characterization Laboratory (ACL) at the Frederick National Laboratory for Cancer Research (FNLCR) oversees antibody screening and characterization. All antibodies are characterized using methods that include Enzyme Linked ImmunoSorbent Assay (ELISA), Western blot (recombinant/cell lysate/native), Affinity Measurements by Surface Plasmon Resonance (SPR) and Biolayer Interferometry, Immuno Precipitation Western Blot and Mass Spectrometry, Immuno Mass Spectroscopy (iMRM), Reverse Phase Protein Array (RPPA), Immunofluorescence and Immunohistochemistry. The broad range of specialized assays, use of standardized methods, and making public the complete characterization data (positive and negative results) to the researcher community are what make this a unique public resource. The availability of data and methods allows researchers to select appropriate antibodies for their particular use and provides researchers with tools to quality control materials for experiments. Antibodies are made available for research use only through the Developmental Studies Hybridoma Bank (DSHB), with a subset distributed through commercial venders.

Afternoon session | New members section
Friday, September 23rd
15:00-15:20

ORAL PRESENTATION

PRESENTER
Ilse Hoffman

Advantages of the recombinant antibody technology: Insights into 14 BMMF Rep specific antibodies

Ilse Hofmann[1], Tanja Schlechter[1], Claudia Tessmer[1], Timo Bund[2]
[1] Antibody Core Facility, German Cancer Research Center, 69120 Heidelberg, Germany
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Bovine meat and milk factors (BMMF) are small episomal DNA molecules frequently isolated from bovine sera and milk products, and recently, also from colon cancer. To be able to detect proteins coding for open reading frames on these episomal DNA molecules we generated mouse monoclonal antibodies against the replication (Rep) protein of H1MSB.1 (BMMF1). For immunization we used two different peptides or a protein fragment and obtained in total 14 BMMF Rep monoclonal antibodies that were used to analyze BMMF presence in different cohorts of CRC peritumor and tumor tissues and cancer-free individuals by immunohistochemistry and Western blot.

To get insights into the question how different the 14 antibodies are, we isolated RNA from the hybridomas and performed RNA sequencing and identified the transcripts coding for the heavy and light chain. The variable region especially the CD3 domain was carefully compared. Interestingly, the sequence of four antibodies derived from the same peptide immunization were identical regarding heavy and light chain. In contrast, the 9 antibodies obtained by immunization with a protein fragment showed a higher diversity. The variable regions of the heavy and light chains were expressed as fusion proteins in HEK cells with human, rabbit or mouse Fc-backbone. Upon testing all turned out to be functional. This approach allows a broader application spectrum as the recombinant antibodies with rabbit Fc-backbone may now be used on mouse tissues.

Afternoon session | New members section
Friday, September 23rd
15:20-15:30

SPONSOR SHORT TALK

PRESENTER
Andreas Weise

Genetic Immunization and Single Cell Screening: Combining two powerful technologies for the discovery of monoclonal antibodies against challenging targets

Andres Weise
Genovac

G-Protein-Coupled Receptors (GPCRs) and other multi-membrane spanning proteins (MMPs) are attractive targets for the development of therapeutic antibody drugs due to their cell-surface expression and their critical roles in multiple (patho) physiological processes. However, due to their multi-transmembrane topology and low area of exposed extracellular epitopes, it is challenging to develop therapeutically relevant antibodies against MMPs.

Genovac's core technology, genetic immunization, combined with single B cell screening provides the most rapid and robust generation of high-quality monoclonal antibodies (mAbs) directed against native (conformational) epitopes.

Identification of antibodies directed against native epitopes is achieved using transfected mammalian cells as reporter cells that present the target antigen on the cell surface. Genovac now applies its optimized genetic immunization and screening protocols to two highly advanced B cell platforms for more efficient sampling of the antibody repertoire and significantly shorter timelines. Plasma cells (PCs) from wild type or transgenic mice and rats are isolated and screened on our Beacon® platform and/or in-house developed microfluidic platform Genovac Nano for target specific candidates. Both allow automated identification and retrieval of target-specific PCs for cloning and sequencing of their variable heavy and light chain sequences using traditional (Sanger) and next generation sequencing (NGS) methods.

We have recently expanded our single B cell screening capabilities through the acquisition of a 10x Chromium instrument. This platform is used to generate barcoded, naturally paired sequencing libraries of antibody variable regions, enabling full repertoire screening.

Taken together Genovac offers state-of-the-art solutions for the discovery of rare and functional antibodies against challenging targets.

Afternoon session
Friday, September 23rd
16:00-16:10

SPONSOR SHORT TALK

PRESENTER
Kathrin Günther

Why go recombinant?
Take your antibody experiments further with high reproducibility and engineering

Kathrin Günther
Absolute Antibody

Recombinant antibodies offer significant benefits compared to traditional hybridoma-produced monoclonal antibodies, including ensured batch-to-batch reproducibility, manufacturability and reliable long-term antibody performance. Recombinant antibody production also enables antibody engineering, allowing researchers to improve experimental design and develop antibodies in diverse isotypes, and with tailored effector function. Absolute Antibody was founded in 2012 with the vision to make recombinant antibody technology accessible to all. Rather than raise a whole new generation of recombinant antibodies, which we believe may further exacerbate the reproducibility crisis by severing methodological links to past studies, we sequence well-established monoclonal antibodies and produce them recombinantly in mammalian cells. This ensures critical clones are not lost while allowing the research community to harness all the benefits of recombinantly produced antibodies.

Afternoon session
Friday, September 23rd
16:10-16:30

ORAL PRESENTATION

PRESENTER
Armand Bensussan

New targets for cutaneous T cell lymphomas treatments: Lacutamab opened the field of economical valorisation of therapeutic monoclonal antibodies issued from academic laboratories

Armand Bensussan
INSERM U976, Hôpital St Louis, 75475 Paris Cedex 10, France

KIR3DL2 is expressed in all subtypes of cutaneous T-cell lymphomas (CTCL), irrespective of clinical stage, with the highest prevalence of expression in Sézary syndrome (SS) and transformed mycosis fungoides (MF), two subgroups of patients with high unmet need of clinically meaningful therapies. KIR3DL2 belongs to the killer immunoglobulin-like receptor (KIRs) family and is expressed on minor subpopulations of normal NK, CD8 and CD4 T cells.

Lacatumab is a first-in-class anti-KIR3DL2 monoclonal antibody (mAb). It selectively depletes KIR3DL2-expressing cells by recruiting immune effectors. Its main modes of action include antibody-dependent cell-cytotoxicity (ADCC) and -phagocytosis (ADCP). Lacatumab has shown potent efficacy in preclinical models, in particular ex vivo autologous assays using primary CTCL cells.

The partnership between an academic laboratory and a biotech company for the development of a therapeutic antibody as well as the phase I study of the targeted immune therapy will be presented and discussed at the meeting.

Afternoon session
Friday, September 23rd
16:30-17:00

ORAL PRESENTATION

PRESENTER
Giovanna Roncador

Licensing antibodies: dream or nightmare?

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

Antibodies licensing has essential advantages for researchers involved in this field, as it allows to give value to the products they generate, and to make them widely available to the scientific community, contributing to the advance of science. In addition, it can provide the researcher and the institution with financial resources to support research activities. However, in some cases, licensing your antibodies can become quite challenging, due to the complexity of the process and the lack of expertise and resources. In this talk, I will present an overview of my 18 years of personal experience in antibody licensing. This will include the approaches that you can use to make antibodies more attractive to the market, the different marketing strategies, the choice of the right company for licensing, as well as some examples of legal issues that you can face during the antibody licensing process.

Morning session
Saturday, September 24th
09:30-10:00

ORAL PRESENTATION

PRESENTER
Sophia Karagiannis

Humoral immunity in patients with solid tumours: opportunities for antibody design

Sophia Karagiannis
Translational Cancer Immunology and Immunotherapy, St. John's Institute of Dermatology School of Basic & Medical Biosciences, King's College, UK

B cells are emerging as key players of anti-tumour adaptive immune responses, especially in immunogenic tumours such as melanoma and aggressive triple-negative subtypes of breast cancer (TNBC). We investigated circulating and tumour-associated B cell phenotypes, including class-switched subsets and their expressed antibodies in cancer patients. Gene expression, immunohistochemical and cell-based evaluations revealed active B cell recruitment and isotype-switched B cell populations with distinct immunoglobulin isotype profiles in cutaneous melanoma and in breast cancer. In melanoma, we identified IgG4 as an antibody subclass elicited by melanoma-associated interleukin-10-driven inflammation. IgG4 antibodies exhibit inefficient immunostimulatory capacity and could block the cytotoxic activities of other antibodies. Consistent with emerging evidence for immunosuppressive roles of IgG4, elevated IgG4 serum levels were associated with disease progression and less favourable clinical outcomes in melanoma. In breast cancers, tumour-infiltrating B lymphocytes were observed assembled in clusters, undergoing B cell receptor-driven activation, proliferation, and isotype switching. Clonally expanded, IgG1 isotype-biased humoral immunity were associated with favourable prognosis, primarily in triple-negative breast cancers. These findings point to dynamic but often dysregulated B cell responses, implicating considerable biological and associated prognostic heterogeneity in solid tumours. Dissecting the humoral response and immunoglobulin profiles in cancers may uncover new mechanisms of tumour-associated immune suppression, reveal novel prognostic biomarkers, and inform monoclonal antibody therapy design and translation.

Morning session
Saturday, September 24th
10:00-10:30

ORAL PRESENTATION

PRESENTER
Pierre Martineau

Humoral immunity in patients with solid tumours: opportunities for antibody design

Pierre Martineau
INSERM U896/Montpellier University/Val d'Aurelle Oncology Center, Montpellier, France

Most display methods are based on antibody fragments binding activity. However, the preferred format for therapeutic applications is the IgG whose binding properties are affected by reformatting. We thus developed a display vector and an engineered mammalian cell line that allow both phage display and direct generation of cells stably secreting a monoclonal human IgG for functional screening. This system allows the screening of phage-derived antibody fragments as full-length IgG. Using this system, we show that Fab display gets rise to better binders than more classical scFv display, and that mammalian display allows to isolate rare binders impossible to obtain in a traditional phage display experiment.

Morning session
Saturday, September 24th
10:30-10:45

SHORT ORAL PRESENTATION

PRESENTER
Alicia Chenoweth

Fc Engineered Monoclonal Antibodies for Triple Negative Breast Cancer Therapy

Alicia Chenoweth[1,2], Anthony Cheung[1,2], Gabriel Osborn[2], Silvia Crescioli[2], Kristina Ilieva[1,2], Rebecca Marlow[1], Jennifer Trendell[1], Anita Grigoriadis[1], Andrew Tutt[1,3], and Sophia Karagiannis[1,2]

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[2] St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London, London, United Kingdom
[3] Breast Cancer Now Toby Robins Research Centre, Institute of Cancer Research, London, United Kingdom

Triple negative breast cancers (TNBCs), which represent 10-20% of all breast carcinomas, are highly aggressive and have a poor prognosis compared to the other breast cancer types. Monoclonal antibody (mAb) therapy has revolutionised the treatment of certain types of breast cancer, by targeting tumour cell surface molecules such as the human epidermal growth factor receptor 2 (HER2). However, there are very few mAb-based therapeutic treatments available for TNBC. Thus, the development of novel and more effective treatments are urgently needed.

In this study we report the design and engineering of novel mAbs targeting TNBC tumour surface markers. We identified the cell surface molecule folate receptor alpha (FRα) and engineered anti-FRα mAbs as either an IgG1 or an Fc engineered IgG1 designed to improve the antibody's Fc-mediated effector functions. We also engineered anti-HER2 mAbs with the same Fc mutations to compare to the clinically available trastuzumab IgG1. Our Fc engineered antibodies elicited stronger immune mediated effector functions as compared to wildtype IgG1, inducing greater breast cancer cell killing by NK cell mediated antibody-dependent cell-mediated cytotoxicity (ADCC). In addition, our Fc engineered antibodies also increased production of pro-inflammatory cytokines by NK cells and macrophages, altering the immune microenvironment towards a more pro-inflammatory state.

In summary, we have developed novel Fc engineered mAbs targeting TNBC with greater potential treatment efficacy compared to the original wildtype versions.

Morning session
Saturday, September 24th
10:45-11:00

SHORT ORAL PRESENTATION

PRESENTER
Julia Hambach

CD38-specific nanobody-based half-life extended bispecific killer cell engagers (HLEnano-BiKEs) for the treatment of multiple myeloma

Julia Hambach[1,2], Anna Josephine Gebhardt[1,2], Luca Pape[1,2], Anya Duttmann[1,2], William Fumey[1,2], Tobias Stähler[1], Gerhard Adam[2], Katja Weisel[3], Friedrich Koch-Nolte[1], and Peter Bannas[2]

[1] Institute of Immunology, University Medical Center, Hamburg, Germany
[2] Department of Diagnostic and Interventional Radiology and Nuclear Medicine, University Medical Center, Hamburg, Germany
[3] Department of Oncology and Hematology, University Medical Center, Hamburg, Germany

CD38 is an established target for the immunotherapy of multiple myeloma. Nanobodies allow easy reformatting into mono-, bi-, and multispecific proteins. We generated half-life extended nanobody-based bispecific killer cell engagers (HLE-nano-BiKEs) by fusing a CD38-specific nanobody to a CD16-specific nanobody to mediate binding to the Fc-receptor on NK cells. To extend the half-life of this construct in vivo, we fused this nanobody dimer to an albumin-specific nanobody. HLE-nano-BiKEs targeting one of the three different epitopes (E1, E2, E3) of CD38 were expressed in transiently transfected HEK cells. Specific binding to CD38 on myeloma cells, CD16 on NK cells, and serum albumin was verified by flow cytometry. We confirmed the capacity of these HLE-nano-BiKEs to mediate cytotoxicity against CD38-expressing multiple myeloma and Burkitt lymphoma cell lines as well as primary myeloma cells from human bone marrow biopsies using NK92 cells as effector cells. The results reveal specific time- and dose-dependent killing of CD38-expressing cell lines and effective depletion of CD38-expressing myeloma cells from human bone marrow samples. Binding of albumin did not impact killing efficacy. Our results show the utility of CD38-targeted HLE-nano-BiKES ex vivo and raise hope for further clinical development of CD38-specific HLE nano-BiKEs as a therapy for multiple myeloma.

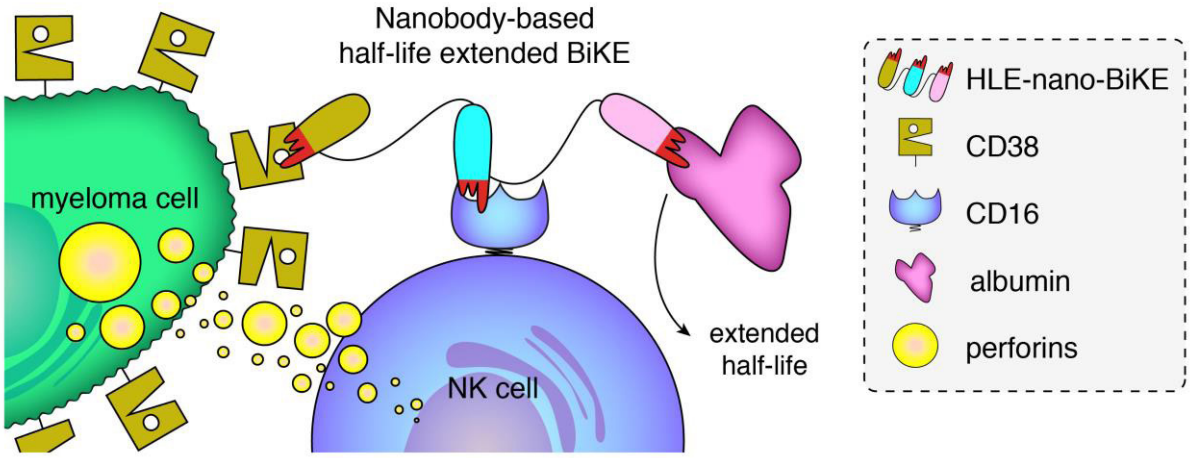

Figure 1: Proposed mode of action of the CD38-specific HLE-nano-BiKEs. The first nanobody specifically binds CD38 on a myeloma cell. Binding of the second nanobody to CD16 on an NK cell leads to the activation of the NK cell and release of perforins. The C-terminal nanobody extends the half-life of the construct by binding to albumin.

Supported by DFG grants No310/16 and Ba5893/7

Morning session
Saturday, September 24th
11:30-11:40

SPONSOR SHORT TALK

PRESENTER
Erik Stahl

From antigen to preclinics - development and preclinical analysis of therapeutical monoclonal antibodies

Erik Stahl
Preclinics

Like other therapeutic compounds, monoclonal antibodies have to pass through an intensive development process. It all begins with the selection of the antigen, the immunization strategy, and leads to an extensive process of screening and characterization. preclinics is an experienced CRO, providing the complete portfolio – from concept to proof of principle. From antigen to preclinics.

Morning session
Saturday, September 24th
11:40-11:50

SPONSOR SHORT TALK

PRESENTER
Thorsten Zacher

Custom peptide microarrays for in-depth mapping and validation of antibody epitopes

Thorsten Zacher
[1]PEPperPRINT GmbH, Heidelberg, Germany

To be disclosed.

Morning session
Saturday, September 24th
11:50-12:20

ORAL PRESENTATION

PRESENTER
Friedrich Koch-Nolte

Nanobody discovery from llama IgH-transgenic mice

Thomas Eden[1], Janusz Wesolowski[1], Alessa Schaffrath[1], Björn Rissiek[2], Nathalie Richter[1], Anna Mann[1], Waldemar Schäfer[1], Julia Hambach[1], Sabine Wendler[3], Christian Linke-Winnebeck[3], Tobias Stähler[1], Stephan Menzel[1], Irm Hermans-Borgmeyer[4], Natalie Tode[1], Fabienne Seyfried[1], Lynn Dieckow[1], Tim Magnus[2], Friedrich Haag[1], Felix Hartlepp[3], Friedrich Koch-Nolte[1]*
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[2] Department of Neurology, University Medical Center, Hamburg, Germany
[3] Chromotek GmbH, Martinsried, Germany
[4] Center for Molecular Neurobiology Hamburg, University Medical Center, Hamburg, Germany

Nanobodies exhibit exceptional solubility and stability, making them ideal building blocks for bispecific antibodies, chimeric antigen receptors (CARs), retargeted Adeno-associated viral vectors (AAV) and other diagnostic or therapeutic antibody formats. Nanobody discovery from immunized animals benefits from clonal expansion of B cells and affinity maturation of antibodies in natural immune responses. Immunoglobulin-transgenic (Ig-tg) mice offer access to lymphoid organs and use of monoclonal antibody discovery technologies. The goal of our study was to combine the advantages of nanobodies and Ig-tg mice. For this, we introduced an engineered bacterial artificial chromosome (BAC) covering the essential components of the llama immunoglobulin heavy chain (IgH) locus into mice harbouring an inactivated endogenous IgH locus (JHT mice). This rescued B cell development in the resulting llama Ig-tg mice (LaMice). B cells of these mice express cell surface B cell receptors composed solely of llama IgHs without any detectable association with endogenous lambda or kappa light chains. Using AAV, the receptor binding domain (RBD) of the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) Spike protein, IgE, and mouse IgG2c heavy chain antibodies as model antigens, we generated affinity-matured, antigen-specific nanobodies from immunized LaMice (see Posters by Schaffrath et al and by Richter et al). Nanobodies could readily be recovered using a variety of established technologies, including hybridoma fusion and phage display. Moreover, we demonstrate that antigen-specific nanobodies can easily be recovered from immunized mice by directly cloning the PCR-amplified nanobody repertoire from total RNA of spleen, lymph node or bone marrow cells into a eukaryotic expression vector that drives transiently transfected HEK cells to secrete heavy chain antibodies into the serum-free culture supernatant. LaMice represent a promising cost-effective and flexible new platform for the production of innovative nanobody-based biologics.

POSTER ABSTRACTS

PRESENTER
Alessa Schaffrath

B cell development and nanobody discovery in LaMice

Alessa Schaffrath[1], Thomas Eden[1], Janusz Wesolowski[1], Björn Rissiekl[1,2], Natalie Tode[1], Tobias Stähler[1], Josephine Gebhardt[1], Fabienne Seyfried[1], Irm-Hermanns Borgmeyer[3], Kristoffer Riecken[4], Jannis Woens[4], Boris Fehse[4], Friedrich Haag[1], Friedrich Koch-Nolte[1]
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Nanobodies or VHHs are single variable immunoglobulin domains derived from camelid heavy-chain antibodies. Their high stability, solubility and small size allows nanobodies to be readily fused genetically to other nanobodies and/or proteins. Traditionally, nanobodies are derived from immunized camelids or synthetic libraries. To benefit from the affinity maturation during a natural antibody response, and from the established technologies for antibody discovery in mice, we introduced engineered versions of the llama immunoglobulin heavy chain (IgH) locus into B cell-deficient IgH-knock-out mice. We analysed B cell development in the resulting LaMice by flow cytometry. The results show that B cell development is restored in LaMice and that B cells express cell surface B cell receptors and secretory immunoglobulins that are solely composed of llama IgHs, without any detectable association with kappa or lambda light chains. Using the receptor binding domain (RBD) of the SARS-CoV-2 Spike protein as model antigen, we demonstrate the facile recovery of affinity-matured, antigen-specific, neutralizing nanobodies from immunized LaMice. For nanobody discovery, we directly cloned the PCR-amplified VHH repertoire from spleen into a eukaryotic expression vector, downstream of a signal peptide and upstream of the hinge, CH2 and CH3 domains of rabbit IgG. From single E. coli colonies, we prepared plasmid DNA for sequencing and transient transfection of HEK cells on 96-well plates.. Five days after transfection, cell supernatants were screened by flow cytometry for the presence of Spike protein-specific nanobody-rabbit IgG heavy chain antibodies (hcAbs). Positive clones were then tested for binding to different variants of the Spike protein by flow cytometry, and for neutralization using a pseudoneutralization assay with ACE2-expressing HEK cells and lentiviral vectors displaying the Spike protein. Among several RBD-reactive hcAb families, we identified one that neutralizes both the Wuhan strain and its omicron variant. We conclude that LaMice represent a promising, cost-effective and flexible new platform for the nanobody discovery.

PRESENTER
Alex J. McCraw

Production and Purification of Wild-type and Glycoengineered IgE for Allergy and AllergoOncology

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Optimised technologies to achieve efficient production and purification of IgE antibodies are required to advance a better understanding of their structure and function in Allergy and in the emerging field of AllergoOncology. Here we report the production and purification of wild-type and glycoengineered IgE antibodies with human Fc regions generated in human embryonic kidney (Expi-HEK293F) cells and purified via HiTrap KappaSelect or CaptureSelect IgE affinity matrices. We enhanced purification efficiency and confirmed antibody structural integrity by SDS-PAGE and HPLC. Using neuraminidase-A as a representative glycosidase, we conducted small-scale purification of desialyated IgE as an example of a glycoengineered antibody and assessed desialylation via Lectin Blot and HILIC-HPLC. IgE antibodies specific for the melanoma-associated antigen Chondroitin Sulfate Proteoglycan 4 (CSPG4) were successfully generated at high purities following removal of by-products such as free light chains. Wild-type and glycoengineered IgEs recognised IgE Fc receptors on the surface of the rat basophilic leukaemia cell line RBL-SX38 stably expressing human FcεRI, and of the FcεRII/CD23-expressing human B-lymphoid cell line RPMI-8866. The antibodies bound to the CSPG4-expressing A2058 human melanoma cells, confirming Fab-mediated recognition. Our findings provide an efficient protocol for the production and purification of recombinant IgEs for Allergy and AllergoOncology research.

PRESENTER
Álvaro García-González

Validation and characterisation of LILRB3/ CD85a monoclonal antibody in lymphoid tissues and lymphomas

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Leukocyte Immunoglobulin-Like Receptors (LILRs) proteins are widely expressed in hematopoietic-lineage cells and mediate activation or inhibition of the functions of various immune cells and primarily myeloid cells. It is becoming clear that LILRs, with their capacity to regulate immune responses and mediate protumour functions, represent a new class of receptors that can be targeted for the treatment of a variety of immunologic disorders and cancer.

Targeting one member of the LILRs family with monoclonal antibodies (mAbs) is extremely complex due to the high homology shown among family members and the study of the functional role of LILRs in cancer is hurdled by the lack of suitable mAbs able to recognise specifically each family member.

In the present study, we have investigated LILRB3/CD85a expression in normal and neoplastic lymphoid tissue using a novel rat monoclonal antibodies against LILRB3/ CD85a against the intracellular domain that recognizes its target molecule in paraffin-embedded tissue sections. A large series of normal tissues and B and T-cell lymphomas were studied, using whole sections and tissue microarrays.

In reactive lymphoid tissues, we found that LILRB3/CD85a protein was expressed in the myeloid component, mainly dendritic cells of myeloid origin. In lymphomas, high number of LILRB3/CD85a+ cells were found in diffuse large B cell lymphoma (DLBCL), angioimmunoblastic T-cell lymphoma (AITL) and classical Hodgkin lymphoma (cHL) tumour microenvironment.
In summary, we describe a new mAb that may be used to achieve a better understanding of the pathogenic role of LILRs family members in inflammatory and malignant diseases.

PRESENTER
Bubacarr G Kaira

Unlocking the unique potential of AvidiMAb® in fighting cancer

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Avidity, the combined binding strength of individual interactions, is a key aspect of cancer-targeting by therapeutic antibodies. Strategies to enhance antibody avidity prove valuable in the context of prolonged target occupancy and/or enhanced signalling, two critical pharmacodynamic factors..

Using scancell's proprietary method we have generated a series of anti-glycan monoclonal antibodies (GlyMab®) with cancer therapy potential. These GlyMabs were subsequently avidity-enhanced leading to enhanced anti-tumour activity in vivo, through our Fc-engineering AvidiMab® technology (1) . Briefly, the introduction of select residues from murine IgG3, an isotype known to exhibit cooperative binding, promoted non-covalent Fc:Fc associations by neighbouring target-bound mAbs. We next sought to expand the applicability of our avidity-promoting strategy by focusing on agonistic mAbs with cancer immunotherapy potential such anti-CD40 agonists (2).

CD40, a tumor necrosis factor receptor (TNFR) superfamily member, plays a critical role in B and T cell immunobiology. Agonistic CD40 mAbs act via mimicking CD40L-induced CD40 clustering and signalling thereby activating antigen presenting cells (APC) and expanding cytotoxic T cells(3). The CD40 interaction of parental SEA-CD40 and avidity-enhanced SEA-CD40 (iSEA-CD40), was analysed using SPR and higher order complex formation via DLS. Cell-based proliferation and activation assays were used to validate the observations. iSEA-CD40 displayed more avid CD40 binding and larger CD40 complex formation, in addition to exhibiting B cell superior proliferative capability, compared to SEA-CD40.

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PRESENTER
Danielle N. Maryanski

Nucleosome spike-in controls identify best-in-class antibodies and enable reliable epigenomic mapping studies

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Histone post-translational modifications (PTMs) play a critical role in chromatin regulation. It has long been suspected that the poorly understood capability of 'PTM-specific' antibodies (i.e. their specificity and efficiency) is a prime driver of the reproducibility crisis in biomedical research. Here we confirm the validity of this concern as it applies to epigenomic mapping studies. Extensive spike-in panels of PTM-defined DNA-barcoded nucleosome standards show that >70% of >500 commercial antibodies (and >80% of the most highly cited) to >50 histone lysine methyl and acyl states have failing performance in ChIP (>20% cross reactivity / <5% target recovery; www.ChromatinAntibodies.com). Variable lot behavior (of both polyclonals and monoclonals) shows the danger of focusing on catalog numbers without considering the inherent variability of biological reagents. Ultimately, these studies support the inclusion of in situ standards to control genomic mapping assays as an improved path out of this morass.

The recent development of immunotethering assays, such as Cleavage Under Targets and Release Using Nuclease (CUT&RUN), deliver high signal-to-noise mapping data using a fraction of the required cells and sequencing depth compared to ChIP-seq. These innovations enable epigenomics for new research applications, including precious samples and scaled clinical studies. By adapting our nucleosome spike-in control approach for CUT&RUN assays, we show that while the same antibody problems observed in ChIP-seq also pervade CUT&RUN, continuous use of spike-in controls improves assay rigor and reproducibility to realize the potential of CUT&RUN to advance epigenetic research.

PRESENTER
Jitesh Chauhan

Anti-cancer Effects of an IgE Antibody Specific for the Melanoma-associated Antigen Chondroitin Sulfate Proteoglycan 4

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Outcomes for patients with metastatic melanoma remain poor, with many not benefiting from existing immune and targeted therapies. The prevalence of expression of the melanoma-associated antigen chondroitin sulfate proteoglycan 4 (CSPG4) in malignant melanoma is high at approximately 70%, so an effective antibody immunotherapy directed at this antigen and consequently directly against cancer cells could benefit a large proportion of patients. Based on the ability of IgE class antibodies to exert potent immunological functions in tissues against parasites, we engineered a monoclonal IgE antibody with human constant domains specifically targeting CSPG4 for cancer therapy. We evaluated target binding with immunohistochemistry (IHC), and efficacy by antibodydependent cellular cytotoxicity (ADCC), immune cell stimulation assays and melanoma models in vivo. CSPG4 IgE bound to melanoma tissues, mediated tumoricidal ADCC by immune effector cells from healthy volunteers or patients with melanoma and stimulated human monocytes towards proinflammatory states. CSGP4 IgE demonstrated anti-tumor activity in human melanoma xenograft models engrafted with human immune effector cells, enhanced macrophage infiltration into tumors and activated pro-inflammatory immune signaling pathways in the tumour microenvironment. The antibody also prolonged the survival of mice bearing patient-derived xenografts reconstituted with autologous immune cells from the same patient. Importantly when considering the safety of administration to patients, CSPG4 IgE did not trigger mast cell degranulation in the presence of patient sera, or basophil activation in whole blood from patients with melanoma measured ex vivo. In summary, we describe a novel CSPG4-specific IgE antibody with in vivo anti-tumor activity in melanoma mouse models, which exerted anti-tumor effects in the absence of toxicities including type 1 hypersensitivity. Our findings point to a promising IgE-based immunotherapy for melanoma.

PRESENTER
Karolina Juskaite

Development and Characterization of Monoclonal Antibodies Against β-lactamases Causing Antimicrobial Resistance

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According to World Health Organization (WHO) antimicrobial resistance (AMR) is one of the greatest threats to global health in this century. Currently 700 000 deaths are linked to AMR each year globally. It is estimated that 10 million lives a year may be lost due to AMR by 2050. Misuse of antimicrobials is the main driver in the development of new AMR mechanisms and difficulties in treating common infectious diseases. Therefore, development of reliable and rapid diagnostic tools is a priority in the context of AMR. In the healthcare system, about 60% of prescribed antibiotics consist of β-lactams. Therefore, bacterial β-lactamases that degrade β-lactam antibiotics can be used as potential targets for diagnostics.

The aim of this study was to develop β-lactamase specific monoclonal antibodies (MAbs) to use them as molecular tools for diagnostic purposes in various immunoassays, such as multiplex ELISA, lateral flow or automated fluorescent bead-based immunometric assays. These immunoassays are rapid, simple to perform and highly promising for point-of care diagnostics. Considering the WHO list of antibiotic resistant priority pathogens, we have selected four β-lactamases – ACT-14, NDM-1, PDC-195 and CMY-34 – as targets for MAb development. Recombinant antigens have been produced in Escherichia coli expression system and purified using affinity chromatography. Large collections of mouse MAbs against each target have been generated by hybridoma technology (60 MAbs in total). The specificity and cross-reactivity of the MAbs have been studied by different immunoassays including a competitive ELISA and epitope mapping using truncated target antigens. The most promising pairs of MAbs have been selected to develop sandwich ELISA for a quantitative detection of each target. The assay has been optimized to achieve the highest sensitivity and specificity. Currently the optimized sandwich ELISA systems are being tested with biological samples containing resistant bacteria and natural β-lactamases. The next step of MAb application is development of multiplex quantitative tests for 4 targets in one sample. We believe that well-characterized MAbs against β-lactamases have high diagnostic potential.

PRESENTER
Keli Rodriguez

Novel nucleosome-based methods for rapid screening and identification of best-in-class antibodies: a community resource to improve genomic mapping approaches

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Genomic mapping studies are critically reliant on the ability of antibodies to distinguish related histone post-translational modifications (PTMs). However inadequate validation of these reagents is widely recognized as contributing to the reproducibility crisis in biomedical research. To address this, we created DNA-barcoded semi-synthetic / fully-defined nucleosomes (Nucs; with histone PTMs or variants) as spike-in controls for Sample Normalization and Antibody Profiling in Chromatin ImmunoPrecipitation (SNAP-ChIP®). These Nucs were assembled into rational panel sets for widely studied target classes (e.g. lysine methylation), and spiked into a ChIP experiment prior to immunoprecipitation. This provides a defined in situ metric of antibody capability and target enrichment. In parallel, we developed a high-throughput, multiplexed triage approach that assembles biotinylated Nucs from similar PTM / variant panels onto optically-barcoded Luminex beads to "decipher" antibody binding preference in a physiological context. In this dCypherTM Luminex approach these panels can be washed after antibody incubation under conditions representing a particular genomic approach (e.g. high stringency ChIP buffers). Of note we now have sufficient Nuc diversity to perform detailed 'motif walking' on a histone tail. As an example an antibody targeted to A31 that distinguishes histone H3.1/ 2 from H3.3 (S31) is unable to bind its epitope in the context of K27-me1-me2-me3 or S28phos (together found on >80% of in vivo nucleosomes4). Such characterization will be essential to interpret results with these reagents

PRESENTER
Luca Julius Pape

CD38-specific nanobodies detect myeloma cells under daratumumab therapy *in vivo*

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The CD38-specific monoclonal antibody daratumumab has become a gold standard for the immunotherapy of multiple myeloma (1). However, binding of daratumumab to CD38 blocks binding of antibodies that target the same or an overlapping epitope. This presents a diagnostic challenge as daratumumab therapy prevents the detection of CD38-expressing myeloma cells (2). To circumvent this problem, we have developed a nanobody, designated JK36, that binds to a distinct, non-overlapping epitope of CD38 and binds to CD38 independently of daratumumab (3).Here we show that AlexaFluor680 fluorochrome-conjugated nanobody JK36 allows for the detection of native and daratumumab-treated cells using flow cytometry, fluorescence microscopy, and in vivo near-infrared fluorescence imaging. Binding of JK36AF680 to daratumumab-treated cells was verified on CD38-expressing cell lines in vitro, on bone marrow biopsies from myeloma patients ex vivo, and in a mouse xenograft tumor model *in vivo*.

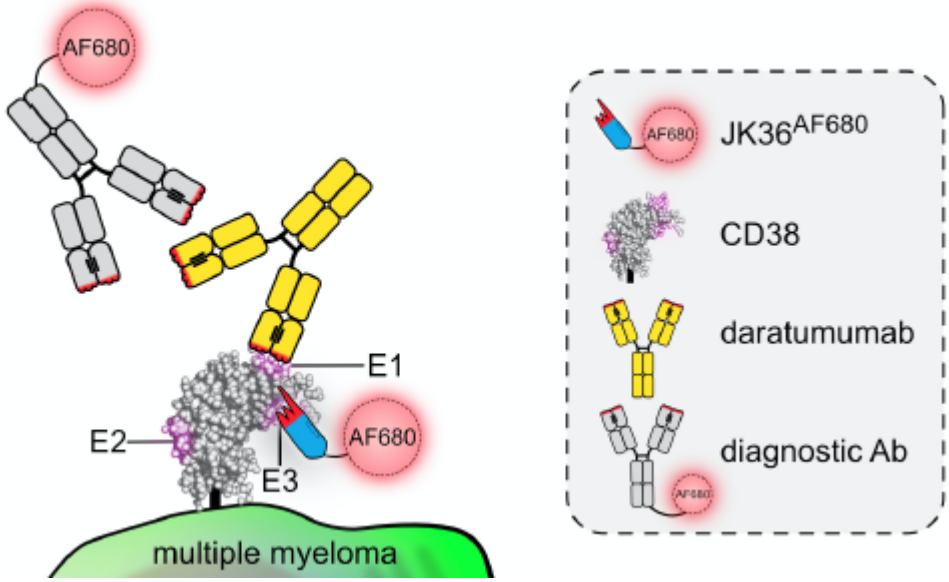

Figure 1: Binding of daratumumab and nanobody JK36AF680 to CD38. Daratumumab binding to CD38 on the surface of multiple myeloma cells prevents the binding of commercially available diagnostic antibodies that target the same epitope (E1). Nanobody JK36 binds to a different, non-overlapping epitope (E3) and can be used to overcome clinical challenges in the detection of residual myeloma cells under daratumumab therapy.

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Supported by DFG grants No310/16 and Ba5893/7

PRESENTER
Nathalie Richter

Selection of AAV-specific nanobodies from Llama-IgH transgenic mice (LaMice)

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Nanobodies or VHHs are single variable immunoglobulin domains derived from camelid heavy-chain antibodies. Since VHHs are highly soluble, small, and readily reformatted, they represent convenient tools for research and therapy. In order to facilitate nanobody discovery, we generated transgenic mice that carry the heavy chain antibody (hcAb) gene locus of the llama, designate LaMice. We immunized four LaMice with non-replicative adeno-associated viral vectors: AAV8 and AAV9. Following classical hybridoma technology, we fused lymph node and spleen cells obtained from these mice three days after the last boost with Sp2/0 mouse myeloma cells and screened the supernatants of growing clones for the presence of AAV-specific llama hcAbs by ELISA. We PCR-amplified and sequenced the VHH-coding region of positive clones. We cloned the VHH-coding region into a eukaryotic expression vector upstream of hinge, CH2 and CH3 domain of rabbit IgG and produced the resulting recombinant nanobody-rabbit IgG heavy chain antibodies (hcAbs) as secretory proteins in transiently transfected HEK cells. Binding of these hcAbs to AAV8, AAV9 and AAV2 serotypes was verified by immunofluorescence microscopy and ELISA. We thereby identified four hcAb families that specifically bind AAV8, two families that recognize AAV9 and one family that recognizes AAV8 and AAV2. In a second approach, we directly cloned the VHH-repertoire obtained by PCR from total spleen and lymph node cDNA into the rabbit IgG expression vector. Sequence analyses of PCR amplified clones revealed dominant hcAb families with extensive somatic hypermutations, including some but not all of the families discovered using the hybridoma technology. Our results illustrate the applicability of the classical hybridoma technology for the discovery of antigen-specific nanobodies from LaMice.

PRESENTER
Ruhul Choudhury

GlyMabs, glycome-targeting monoclonal antibodies for cancer therapy

Ruhul Choudhury
Scancell Ltd

Glycosylation is key in regulating various cellular functions. Aberrant glycosylation plays a significant role in cancer. Current understanding of glycosylation in cancer research have unravelled potential biologics targeting cancer-associated glycans. This unmet need led to Scancell's GlyMab platform of four monoclonal antibodies (mAbs) targeting glycans on lipids and/or proteins.

SC129 targets highly expressed Sialyl-di-lewisa glycan in pancreatic tumour tissue, as well as other forms of cancer, with limited expression on normal human tissue. SC129 targets approximately 85% of pancreatic tumours, with 65.79% showing strong target expression (HS>150), 7.89% moderate binding (HS>50-150) and 13.16% weak binding (HS>5-50). In vitro studies demonstrated antibody-drug conjugate (ADC) for SC129 conjugated with auristatin E or maytansinoids showed (sub)nanomolar potency for colorectal and pancreatic cancer cell lines (1). This coincided with a significant anti-tumour effect in an in vivo COLO205 xenograft model indicating that SC129 has potential to be used for cancer treatment as an ADC.

SC88 targets LecLex, di-Lea, and LeaLex, as well as Lea-containing glycans. This mAb showed strong binding at 64.29%, 28.57% moderate binding and 7.14% weak binding to colorectal tumours. SC88 exhibits strong effector functions (ADCC and CDC) in addition to direct tumour cell killing via a caspase-independent mechanism (2).

SC134 specifically targets Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc-ceramide. This glycolipid is highly expressed in small cells lung carcinoma (SCLC). The absence of healthy tissue binding suggests SC134 may redirect T cells in T cell redirecting bispecific format. SC134 shows strong internalising capabilities in SCLC cell lines and delivers DNA-modifying drugs with subnanomolar efficiency, rendering it a viable candidate for ADC development.

The humanised leads for SC129, SC88 and SC134 candidates recapitulates their parental mAb in their binding and functional characteristics.

SC27, our ultraspecific Lewisy mAb binds to a wide range of cancers, additionally, this mAb has superb internalising ability combined with drug delivery to the lysosomes indicating potential for ADC use.

In summary, Scancell's Glymabs are highly specific for a broad range of cancers making them viable candidates for cancer therapeutics.

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PRESENTER
Tobias Staehler

P2X5-specific antibodies

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P2X purinoreceptors are ATP-gated ion channels mediating calcium influx and potassium efflux. These receptors are activated when ATP is released from stressed cells during sterile inflamation and tissue damage. P2X5 has been identified as a cell surface marker of adipocytes in beige and brown adipose tissue [1]. A mutation in the human P2RX5 gene causes aberrant splicing of exon 10 that encodes the second transmembrane domain, resulting in a functional knock out of P2X5 [2]. This mutation is highly prevalent in East-Asia (EAS), Europe (EUR) and America (AMR), >98% of individual in these populations are homozygous for the defective P2X5 gene. In contrast, most members of subpopulations in Sub-Saharan Africa carry one or two copies of the the functional P2X5 gene. We cloned and expressed mouse and the ancestral variant of human P2X5 as GFP fusion proteins. Using genetic immunization of rats with P2X5 expression vectors, we obtained polyclonal sera that specifically bind to mouse and human P2X5 in native confirmation. This was verified by immunofluorescence microscopy of transfected CHO cells and in vitro differentiated brown adipocytes from mice. From the immunized rats we also isolated a monoclonal antibody, designated R18-A34, that specifically recognizes the functional, ancestral human P2X5 on transient transfected HEK cells. We are currently testing whether mAb R18-A34 also recognizes P2X5 on human cell lines and tissue samples.

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Supported by DFG grants SFB1328/A10 and Z02.

PRESENTER
Vytautas Rudokas

Monoclonal antibodies against house dust mite allergen der p 21 and their application for the analysis of allergen extracts

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The house dust mite (HDM) is one of the most important and widely spread allergen sources causing allergy and its related complications. Recombinant allergens and HDM extracts are being used in allergy diagnostics and immunotherapy. Since allergen extracts from different manufacturers lack proper standardization regarding to their composition, monoclonal antibodies (MAbs) against specific allergen components can be used for their identification and quantification in allergen extracts.

This study aimed to develop recombinant allergen Der p 21 of Dermatophagoides pteronyssinus and generate MAbs against this allergen. Der p 21 allergen fused with maltose-binding protein (MBP) was expressed in E. coli and purified using affinity chromatography of MBP-passenger proteins. The fused protein contained TEV protease cleavage site between the allergen and MBP and the hexahistidine sequence. Hydrolysis reaction was optimized to detach MBP and recombinant Der p 21 was purified using affinity Ni-NTA chromatography.

To investigate the antigenic properties of recombinant Der p 21, its reactivity with blood serum specimens of patients with diagnosed HDM allergy was analyzed. Recombinant Der p 21 was recognized by HDM-specific IgE thus confirming its antigenic similarity with native allergen. Therefore, recombinant purified Der p 21 was a suitable antigen for MAb generation. Five hybridoma cell lines producing high affinity MAbs of IgG isotype were generated using hybridoma technology. Highly specific sandwich ELISA for the quantification of Der p 21 was developed and optimized. Four HDM allergen extracts from different manufacturers were analysed using the newly developed sandwich ELISA and Western blotting. The component Der p 21 was detected in only two of the extracts revealing significant differences of allergen composition. These data demonstrate the importance of allergen-specific MAbs as a tool for the characterization of allergen extracts and the need of an appropriate standardization of extracts before their use for allergy diagnostics or immunotherapy.

PRESENTER
Zaira Vega

Optimized panel of mAbs for the detection of lymphocyte subpopulation in animal species

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Immunohistochemistry (IHC) has proved to be one of the most important ancillary techniques in the characterization of neoplastic diseases in humans, and it has become equally important in veterinary medicine, as oncologists demand more specific diagnoses. The number of immunohistochemical tests offered by veterinary diagnostic laboratories has increased exponentially in the last decade, but the use of this technique has been hampered by the lack of specific monoclonal antibodies (mAbs) able to reliably work across animal species.

In order to increase our knowledge in this area, we have tested the cross reactivity of a panel of commercially available antibodies as immuno-histochemical tools for the detection of different types of immune cells in domestic (n=13) and exotic - wild (n=28) life species.

In this study we have evaluated a panel of more than 100 mAbs, some of them generated in our own laboratory, targeting as many as 16 different antigens (CD3, CD4, CD8, CD19, CD20, CD21, CD79a, CD79b, BCL2, BCL6, FOXP3, BLIMP-1, MUM1, TOX, CD163 and PD-1) suitable as markers for the detection of the most important subpopulations of immune cells, including T and B cells, macrophages and dendritic cells (DCs), in Formalin-Fixed Paraffin-Embedded (FFPE) tissue. The distribution of these antigens was evaluated in samples of lymph node or spleen.

Our study will facilitate further research needed to define the role played by lymphocytes subpopulations in immunological diseases and cancer in animal species.

PRESENTER
Benoit Giquel

How Addgene is Reimagining Antibody Sharing

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Reliable access to antibodies validated for use in research applications is crucial for research reproducibility. Currently available antibodies can be variable in quality and the molecular identity of the material is completely unknown to scientists. Recently Addgene (addgene.org), a nonprofit organization, created an open antibody resource funded by a generous grant from the National Institute of Health's Brain Research Through Advancing Innovative Neurotechnologies® (BRAIN) Initiative. This collection, in collaboration with Dr James Trimmer (University of California Davis School of Medicine), is being developed for scientists studying neuroscience and includes frequently cited antibodies for use in brain imaging. We are planning now to expand this resource with antibodies relevant for scientists working in other fields of research.

Addgene's mission is to accelerate research and biomedical discovery by facilitating access to useful research materials and information. To fulfill this mission, Addgene maintains a repository that distributes >100,000 plasmids contributed by scientists coming from more than 5,000 different labs all over the world. The repository stores, quality controls, and annotates the data associated with the plasmids. Addgene also provides over 700 ready-to-use AAV and lentivirus preparations and now is adding the recombinant antibody collection, which may grow to include recombinant nanobodies and ScFvs. We are a community resource that help material makers and users and we encourage research groups to share their recombinant antibodies via Addgene to maximize the reach and impact of their work.

Addgene advocates for open Science and the sequence information for the antibodies in our catalog is freely shared so that you will know exactly what you are working with. We are sharing our protocols for recombinant antibody production as well as antibody applications. In addition, basic and advanced affinity reagents topics are discussed on Addgene's blog and Antibody Guide. We will also be collecting feedback from the community about how the antibodies work in various applications, enabling you to better plan your experiments. All this information will be compiled in an Antibody Data Hub that Addgene will launch early next year.

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