74th Mosbacher Kolloquium

Immune Engineering – from Molecules to Therapeutic Approaches

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Abstracts

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Content

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O 07
Engineering of the immunomodulatory adenovirus protein E3/49K

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The early transcription unit 3 (E3) of human adenoviruses (HAdVs) encodes immunomodulatory proteins. One of these proteins is E3/49K, a type I transmembrane protein that is uniquely expressed by species D HAdVs. E3/49K is proteolytically processed on the cell surface by matrix metalloproteases and its N-terminal ectodomain sec49K is released. Sec49K interferes with leukocyte activation through binding to receptor-like protein tyrosine phosphatase CD45. Therefore, the E3/49K-mediated immunomodulation may provide a therapeutic strategy to treat autoimmune diseases or to prevent graft rejection after transplantation. It remains elusive whether the interaction of CD45 and E3/49K can also occur on the cell surface of infected cells with membrane-anchored full-length E3/49K. This question may be addressed by engineering an E3/49K variant that is no longer shed at the cell surface. In order to create such a variant we analyzed the proteolytic cleavage sites by mass spectrometry. Subsequently, we generated deletion mutants lacking these sites to inhibit cleavage of E3/49K. The effect of these mutations on E3/49K cleavage were investigated by digestion with recombinant ADAM10 and in transfected alveolar epithelial A549 cells and human embryonic kidney 293T cells. Although the shedding was strongly reduced in these mutants, it was not abrogated in the mutants investigated so far. Thus, E3/49K may contain several metalloprotease cleavage sites ensuring efficient secretion of sec49K.

C 04
Rational engineering of a folding pathway changes mechanism and chaperone dependency of interleukin secretion

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In our work we focus on the human IL-12 family which contains four heterodimeric members, IL-12, IL-23, IL-27, and IL-35. These four cytokines are made up of only five subunits, with the four-helix bundle α-subunits being secretion-incompetent in isolation and the β-subunits being secreted alone. In the context of heterodimer formation, the β-subunit assists in assembly-induced folding of the α-subunit and subsequent secretion. This biogenesis is

accompanied by chaperones and cytokine release is strictly regulated by the ER quality control system.

Based on structural studies that revealed why IL-23α fails to fold correctly alone, we applied in silico methods together with rational engineering to design an autonomously folding- and secretion-competent IL-23α protein. We succeeded in obtaining a highly stable IL-23α, introducing a disulfide bond that stabilizes its first α-helix. Stability of the variant is increased not only intracellularly but also of the purified subunit. Importantly, this mutant can still assemble with IL-12β with low-µM affinity. Besides this, the optimized IL-23α mutant shows a different intracellular folding behaviour, which goes hand in hand with an altered chaperone repertoire acting on it. This study allowed us to address the question, whether a molecular competition of IL-12 versus IL-23 formation exists in cells, as both contain IL-12β, and why IL-12 family subunits have evolved for assembly-dependent instead of independent folding and secretion.

O 03
Computational design of specific binding pockets for phosphorylated amino acids within Armadillo repeat proteins

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Current biomedical research and diagnostics critically depend on detection agents for specific recognition and quantification of protein molecules. Due to the shortcomings of state-of-the-art commercial reagent antibodies such as low specificity or cost-inefficiency, we aim to develop an alternative recognition system based on a regularized armadillo repeat protein scaffold. Its modular architecture can be exploited for the specific binding of linear epitopes, potentially targeting various peptides and proteins based on their amino acid sequence [1,2]. To make such a powerful system universally applicable, individual modules must be engineered to recognize specific amino acids. As part of this project, we developed a framework to computationally predict, graft, and test binding pocket modules that are complemented by library synthesis, directed evolution and experimental screening. The project is a close collaborative effort with the groups of Anna Hine (Aston, UK) and Andreas Plückthun (Zürich, CH).

Here, we present our computational workflow for detection and analysis of interaction sites to design new binder proteins for peptides that include phosphorylated amino acids.

[1] Reichen et al. (2014) J. Struct. Biol. 185: 147–162,

[2] Gisdon et al. (2022) Biol Chem, doi: 10.1515/hsz-2021-0384

C 02

Forced-dimerization of cytokine receptors using the transmembrane domain of constitutively active IL-7R

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Mutations with insertion of cysteines have been found in the transmembrane domain (TMD) of the IL-7R leading to constitutive activation and thus an augmented proliferation.

To find out if this mutated TMD could activate different receptors, we exchanged the natural TMD of several cytokine receptors (IL-23R, gp130, IFNAR2) with the IL-7R TMD containing the L234PPCL insertion known for leading to constitutive activation in the transmembrane domain (TMD) of the IL-7 receptor.

Additionally, these mutated receptors (IL-23R, gp130, IFNAR2) were transformed into synthetic cytokine receptors (SyCyRs) with a GFP nanobody as extracellular domain, which can only be activated by the synthetic ligand GFP.

In both cases, natural and synthetic cytokine receptors showed a constitutive activation bearing the IL-7R-PPCL-TMD. Mutated natural and synthetic gp130, IL-23R and IL-7R showed ligand-independent activation of the JAK/STAT pathway and proliferation of Ba/F3 cells.

Although in some cases constitutive activation of cytokine receptors can lead to disease through the overexpression and activation of certain pathways, the presented strategy could be a useful tool to improve e.g. CAR T cell therapy.

I 17

Preclinical Validation of alphaSEPT, an engineered human Cytokine, as a Next-Generation Immunotherapy

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Sepsis is an indication with an enormous unmet medical need. In Western societies more people die of sepsis than of most common cancers combined. No treatment is available yet. To address this, we develop alphaSEPT. alphaSEPT is a rationally engineered human immune signalling molecule. It has immunomodulatory functions that are key in indications caused by an immune system out of balance. Sepsis is such an indication: the immune dysbalance in sepsis involves both, overreaction to an infection and extensive functional defects leading to immune paralysis. Patients die either of multiorgan dysfunction due to hyperinflammation or of secondary infections due to immune paralysis. The immunobalancing functions of alphaSEPT provide a new mode of action (MoA) that addresses both mortality drivers. Studies with the mouse protein in mice demonstrated a reduction of sepsis mortality of up to 70%. Our studies with abdominal sepsis patients at the Klinikum rechts der Isar, the university hospital of the TUM, demonstrated that alphaSEPT can precisely restore the immune competence of immune cells isolated from the patients: alphaSEPT restores the antigen presenting capacities of patients´ monocytes as well as the antigen-dependent proliferation of T-cells. With this novel MoA alphaSEPT achieves the potential of precision medicine via active immunomodulation. We now aim to perform indication extension studies and to bring alphaSEPT as a next-generation biopharmaceutical into the clinics.

C 06

Leveraging chemically-controlled protein switches for increased safety of cytokine-based cancer immunotherapeutics

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Cytokines are key signal mediators of the immune system playing an essential role in the orchestration of immune responses. Despite their unique ability to modulate the immune system, the translation of cytokine-based therapies to the clinic has been greatly hindered by severe toxicities due to the pleiotropy and off-targeting effects of many cytokines.

Here, we present a general strategy that enables precise control over cytokine activity. We control their activity by selectively masking the receptor binding site with a fused chemically-responsive domain, which could be unmasked with a competing molecule (Venetoclax). To achieve this, Bcl-2 was fused to the cytokine and the BIM-BH3 interaction motif was transplanted to sites in close proximity to the cytokine's receptor binding site. In absence of Venetoclax, Bcl-2 bound the cytokine with high affinity blocking the interaction site between the cytokine and its receptor. Upon addition of Venetoclax, the interaction between Bcl-2 and the BIM-BH3 motif was disrupted, so restoring the cytokine's activity. We have developed switchable mutants for a range of different cytokines (IL-2, IL-10, and IL-15) used in cancer immunotherapy. Moreover, we showed that in presence of Venetoclax, their activities can be selectively and fully restored.

Overall, this drug-responsive switch strategy may achieve spatiotemporal control of cytokine activities in vivo and thus improve the safety and clinical applicability of cytokine therapeutics.

O 18
The S-palmitoylation switch in activated T cells

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While T cell activation has been intensively studied on protein, DNA and RNA level, much less is known about the contribution of lipids and protein-lipid interactions to this process. Nevertheless, several findings (e.g., changes in the cellular lipidome after activation of T cells) suggest that lipids play important roles in T cell activation. To shed light on the contribution of lipid modifications on T cell signaling, we are investigating palmitoylation switches during T cell activation. One of the proteins described to undergo a palmitoylation switch after T cell stimulation, is the type II membrane protein CKAP4 (Morrison et al., 2020). To investigate how dynamic depalmitoylation of CKAP4 affects T cell signaling, we first validated depalmitoylation of CKAP4 in activated T-cells using a click chemistry approach and plan to investigate its functional role by using palmitoylation-deficient mutants. The effects of palmitoylation of CKAP4 on T cell signaling will be examined by measuring phosphorylation signals, calcium mobilization, and cytokine production in combination with cell migration assays. Furthermore, we will investigate the stimulation-dependent dynamics of CKAP4 subcellular localization. We plan to investigate this in T cells by using super resolution microscopy, proximity ligation assays and detergent resistant membrane fractionation. Together, these experiments will help elucidate functional consequences of palmitoylation switches during by T cell activation.

I 20
Investigating The T Cell Repertoire In Melanoma Immune Checkpoint Inhibitor Therapy Patients and Their Response To Therapy

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In the study presented, changes to $CD4^+$ T Cells, $CD8^+$ T Cells and their reactivity to stimuli by Immune Checkpoint Inhibitor (ICI) Therapy are investigated. Findings are compared to healthy

donors and patients' course of therapy, to better predict response to therapy, development of immune-related adverse effects (irAEs), patient benefit and survival.

The study is part of an ongoing Master Thesis.

Tumors can exhibit immune-evasion mechanisms by expressing checkpoint ligands, e.g. PD-L1 and CTLA-4 ligands. This can be abrogated by ICIs, which bind immune checkpoint molecules and prevent their ligands from interacting, restoring anti-tumor responses.

Since 2011, multiple ICIs have been approved for treatment of melanoma in Germany. However, irAEs may arise in up to 45% of ICI patients, depending on the dose, time and type of agent used.

To characterize the T cell department, samples from healthy donors and melanoma patients were intra- and extracellularly analyzed using Flow Cytometry (FACS) by staining surface markers of $CD4^+$ subsets, $CD8^+$ cells and transcription factors Tbet, GATA3, RORγt and FoxP3. So far, characterizing assays have been developed and verified for healthy donors.

Currently, healthy donor samples are being incubated with anti-CD3/CD28 or melanoma cell culture supernatant to investigate their reactivity to stimuli. Results will subsequently be compared to melanoma ICI therapy patients.

T 06
Fast identification of clincally relevant T cell receptors for adoptive T cell therapy

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T cell engineering with antigen-specific T cell receptors (TCRs) allows the generation of increasingly specific and reliable T cell products for cancer and viral infections. However, the number of TCRs available for clinical use is still limited and often of suboptimal potency. Here, we developed platforms to support a rapid selection of candidate TCRs for therapy from donor material.

The highly diverse antigen-unexperienced TCR repertoire of healthy donors represents a suitable source for identifying tumor-specific TCRs. Rare antigen-specific naïve T cells are enriched from large-size T cell apheresis and single-cell sorted according to pMHC class I multimer staining. During cell sorting, our developed flow cytometry-based functional screening estimates the structural avidity of each individual pMHC multimer-reactive TCR, which correlates with functionality. Altogether, epitope-specific TCRs can be isolated and

concurrently ranked according to predicted avidity/functionality. Virus-specific TCRs are, instead, more proficiently isolated from seropositive donors. Considering the higher frequencies of these memory repertoires, we developed a high-throughput, single-cell sequencing-based approach capable of discriminating highly functional and bystander TCRs according to transcriptional shifts in T cell activation genes induced by recent peptide stimulation prior to cell sorting (Wagner et al., Cell Reports 2022; Mateyka et al., Vaccines 2022).

I 02
A modular approach for intracellular protein delivery via a cell- penetrating antibody

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Many disease-based protein-protein interactions occur in the cytoplasm, which is not commonly targetable by antibodies. In this study, the cell penetrating properties of a heparan sulfate-binding antibody (CPAb) were investigated and compared with the well-known internalizing but not cytosol-penetrating antibody Trastuzumab. Furthermore, the function of CPAb as an intracellular transporter was verified by LplA-mediated GFP coupling. Finally, a coupling mechanism based on the high affinity of streptavidin and biotin was tested for cell-penetration to allow up to three cargo proteins to be transported into cells.

C 01
Respiratory syncytial virus (RSV)-approved monoclonal antibody Palivizumab as ligand for anti-idiotype nanobody-based synthetic cytokine receptors

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Synthetic cytokine receptors can modulate cellular functions based on an artificial ligand to avoid off-target and/or unspecific effects. We developed a synthetic cytokine/cytokine receptor pair based on the antigen-binding domain of Palivizumab as synthetic cytokine and a set of anti-idiotype nanobodies as synthetic receptors. Palivizumab targets the fusion (F) glycoprotein responsible for the fusion of viral and host cell membranes. Importantly, Palivizumab is neither cross-reactive with human proteins nor immunogenic. In the synthetic receptors, AIPVHH were fused to the activating Interleukin (IL-)6 cytokine receptor gp130 and

the apoptosis-inducing receptor Fas. The synthetic cytokine receptor AIPVHHgp130 was efficiently activated by cross-linked Palivizumab but not by Palivizumab alone. After dimerization of reformatted Palivizumab single-chain variable fragments (scFv (PscFv)), the miniaturized synthetic PscFv efficiently activated AIPVHHgp130. Since the distance between two antigen-binding arms in Palivizumab and PscFv is critical for efficient receptor activation, Palivizumab was used as an inhibitor of PscFv-induced receptor activation. Moreover, PscFv specifically induced apoptosis in cells expressing synthetic AIPVHHFas death receptors. Here, we developed a non-immunogenic full-synthetic cytokine/cytokine receptor pair for in vivo therapeutic strategies involving non-physiological targets during immunotherapy.

O 02
Nanoscale signaling platform for spatiotemporal control of receptor tyrosine kinase activity in live cells

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Dynamic assembly of signaling complexes in the plasma membrane plays prominent roles in immune cell signaling. We here developed a novel surface nanopatterning approach to spatiotemporally control the assembly of signalling complexes at nanoscale in live cells. Based on efficient surface biofunctionalization via adhesive proteins and biopolymers, capillary nanostamping of biofunctional nanodot arrays (bNDAs) with representative 400 nm-diameter and a spacing of optic microscopy resolution limit was obtained. High-density enrichment of signalling protein complexes in the bNDAs was achieved by engineering specific adaptor proteins for orthogonal covalent and non-covalent capturing of target proteins. We exploited these tools for nanoscale assembly of active signalling complexes in the plasma membrane of live cells. To the end, spatiotemporally controlled triggering of receptor tyrosine kinase activity in live cell bNDAs was confirmed via recruitment of the critical effector proteins Grb2, SOS and Ras. The established nanoscale signaling platform with desired time-zero of signaling paves the way for unveiling the crucial time-dependent protein-protein interaction cascades in signaling pathways, offering new insights into the development of immunotherapeutic approaches.

I 13
Human OX40 targeting Nanobodies for diagnostic imaging

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In the last decades, the fight against cancer led to the emergence of various innovative approaches including immunotherapies which deploy the patient's T lymphocytes to waging war on cancer cells. Since the outcome of such cost intensive therapies is highly dependent on the individual immune status, there is an increasing need to stratify patients and monitor therapeutic progress. Recent data showed that the immune checkpoint molecule OX40, which plays a strong role in proliferation, survival and effector function of T cells, can be a prognostic marker. Taking advantage of the favorable characteristics of single-domain antibodies (Nanobodies, Nbs) for application as potential in vivo imaging probes, we identified four unique Nbs targeting human OX40 (hOX40). In-depth biochemical and biophysical characterization showed a strong affinity and stability of all Nbs at physiological conditions. Epitope binning revealed that these binders address two different domains of hOX40, while two of them block binding of its natural ligand OX40L. Using flow cytometry, we confirmed binding of the Nbs to natively expressed OX40 present on activated T lymphocytes. Addressing a potential modulating effect on the T cell functionality, we performed proliferation tracking and analyzed the cytokine profile after Nb treatment. These studies led to the selection of a leading candidate for the development of Nb-based probes for in vivo application.

T 05
Targeted adenovirus-mediated transduction of human T cells in vitro and in vivo

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Clinical success in T cell therapy has stimulated widespread efforts to increase safety, potency and extend this technology to solid tumors. Yet progress in cell therapy remains restricted by the limited payload-capacity, specificity of target cell transduction and transgenic gene expression efficiency of applied viral vectors. This renders complex reprogramming or direct in vivo applications difficult. Here, we developed a synergistic combination of trimeric adapter constructs enabling T cell-directed transduction by the human adenoviral vector serotype C5

in vitro and in vivo. Rationally chosen binding partners showed receptor-specific transduction of otherwise non-susceptible human T cells by exploiting activation stimuli. This platform remains compatible with high-capacity vectors for up to 37 kb DNA delivery, increasing payload capacity and safety due to the removal of all viral genes. Together, these findings provide a tool for targeted delivery of large payloads in T cells as potential avenue to overcome current limitations of T cell therapy.

O 20
Tracing early stage cancers by detection of specific antigens in blood monocytes using novel antibodies

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Detection of malignant lesions in an as early as possible stage is of paramount importance for the patients' chances of survival and cure. In addition to high sensitivity and specificity, approaches to achieve this task ideally have to be fast, non-invasive and economic so that they can potentially be applied in a patient screening format.

Intense research has been devoted to the the employment of blood samples ("liquid biopsies") to detect circulating tumour cells or protein markers, DNA or RNA indicative of cancerous tissue within the body. Striving for enhanced sensitivity, the recently introduced Epitope Detection in Monocytes (EDIM) technique exploits the naïve immune system's property of accumulating potentially harmful antigens in blood monocytes. Cytometric determination of general cancer-associated antigens TKTL1 (Transketolase-like 1) and Apo10 (epitope of DNAse X) using fluorescent antibodies can reveal occult malignomas with noteworthy efficiency. However, this technique does not disclose information on type and tissue localisation of detected tumors.

Addressing this shortcoming, we generated and characterized panels of novel antibodies to specific tissues and tumor entities and employed them to the cytometric analysis of blood monocytes from cancer patients. Using the examples of colorectal and pancreas carcinoma, we show that this approach can identify tumor patients with high sensitivity and reliably discrimitates between malignoma entities.

T 04
Image guided cell therapy: How PET-imaging can improve CAR-T treatments

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Chimeric Antigen Receptor (CAR)-T cell therapies were first approved in 2017 and are increasingly entering clinical development and routine care. However, prospectively these therapies necessitate a reliable method to analyze preclinical animal experiments and to monitor therapy outcomes in patients. The use of a universal reporter gene for these applications would facilitate and accelerate the implementation of CAR-T therapies. We have developed a novel reporter gene system encoding a radiochelate-binding protein DTPA-R that binds the radioligand [^{18}F]F-DTPA [see Abstract by V. Morath]. Based on an existing αCD19-CAR-T product, the reporter gene was genetically integrated replacing the second membrane protein on these CAR-T cells (EGFRt). The reporter protein can also be used for magnetic activated cell sorting (MACS) in the production process. Functionality was confirmed by comparing modified CAR-TDPTA-R and original CAR-TEGFRt cells. The reporter protein did not alter proliferation, viability or cytotoxic effector function as confirmed by flow cytometry assays and killing assays. PET studies of CD19 lymphoma bearing mice allowed quantitative tracking of these CAR-T cell over a 30-day period providing information on the precise location and concentration of therapeutic cells. Different ex vivo validations using the V5-tag confirmed the results. In summary, this novel reporter gene provides a promising tool to accelerate the development of CAR-T therapies.

I 15
Multiplexed autoantibody profiling to identify markers for clinical outcome of Immune Checkpoint Inhibitors in metastatic Urothelial Carcinoma patients

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Background: Immune checkpoint inhibitors (ICI) have improved the treatment of a broad spectrum of cancers as metastatic Urothelial Carcinoma (mUC). However, the durable responses to ICIs are only observed in a subset of patients. Therefore, there is an unmet need to identify biomarkers predictive of outcome to therapy. In this study, we addressed the role

of autoantibodies (AAb) as potential predictive biomarkers for clinical outcome stratification in mUC patients following ICI therapy.

Methods: We analysed AAb profiles of pre- (n=66) and post-treatment (n=65) serum samples from mUC patients receiving ICIs or platinum-based chemotherapy (PBC) utilizing Oncimmune's ImmunoINSIGHTS bead-based AAb profiling platform and protein array containing 1150 tumour- and self-antigens.

Results: Multiplexed profiling identified AAbs with significantly higher levels in patients with mUC in comparison to healthy controls. Baseline level of AAbs targeting antigens such as known bladder cancer antigens KDM6B, BRCA1 and BCL2 were associated with earlier progression on ICI. Patients on ICI showed a greater fold change from pre- to post-therapy across all AAbs compared to patients receiving chemotherapy and the higher overall AAb reactivity was associated with improved response to ICI.

Conclusion: AAbs may serve as candidate biomarkers to predict clinical outcome for ICI therapy in patients suffering from mUC. These findings contribute to the understanding of the immune response in mUC and could potentially assist in selection of patients who would benefit from ICI treatment.

I 09
Dodecin is a carrier protein for immunizations

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In bioengineering, scaffold proteins have been increasingly used to recruit molecules to parts of a cell, or to enhance the efficacy of biosynthetic or signaling pathways. For example, scaffolds can be used to make weak or non-immunogenic small molecules immunogenic by attaching them to the scaffold, in this role called carrier. We have recently presented the 8 kDa small protein dodecin from Mycobacterium tuberculosis (mtDod) as a new scaffold protein (1). MtDod forms a homododecameric complex of spherical shape, high stability and robust assembly, enabling the attachment of cargo at its surface. MtDod, either directly loaded with cargo or equipped with domains for non-covalent and covalent loading of cargo, can be produced recombinantly in high quantity and quality in Escherichia coli. Proteins as large as the monomeric superfolder green fluorescent protein can be recruited to the dodecin surface. In a case study, we harnessed mtDod-peptide fusions for producing antibodies against human heat shock proteins and the C-terminus of heat shock cognate 70 interacting protein (CHIP). Future studies will show to what extent dodecin can be used for immunotherapeutic applications.

(1) Bourdeaux, F. et al. Dodecin as carrier protein for immunizations and bioengineering applications. Sci Rep 10, 13297 (2020).

I 01
A generic strategy to generate trifunctional two-in-one antibodies by chicken immunization

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In this work, we generated chicken-derived trispecific antibodies based on a two-in-one antibody targeting three antigens simultaneously with two Fab arms. These NK cell engagers inhibit EGFR signaling, block the PD-1/PD-L1 immune checkpoint and show a potent ADCC effect. Based on this method, further therapeutic antibodies derived from avian immunization with novel and tailored binding properties and target combinations can be developed.

I 16
PACMAN - Proteolytic Antigen Cleavage Mediated AmplificatioN: Introducing a novel class of proteolytic immunotherapeutics to tackle amyloid diseases

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In amyloid diseases, a pathological build up of aggregated proteins or peptides leads to toxicity and triggers a plethora of mislead processes ultimately leading to the diseases' symptomatic expressions. In Alzheimer's disease, the amlyoid-β (Aβ) peptide is one of the disease's amyloid culprits. Conventional immunotherapeutics, with monoclonal antibodies, aim to bind the amyloid antigen and remove it via endogenous degradation pathways. Unfortunately, these approaches have consistently proven insufficient in clinical trials.

We propose proteolytically active antibodies as a novel therapeutic approach to tackle amyloid diseases which might supersede and fundamentally outcompete conventional immunotherapeutics. Proteolytic antibodies offer a great advantage – they cleave their antigen and do not merely bind to their antigen. Furthermore, proteolytic antibodies do not form stable

immune complexes with their amyloid antigen, which have been linked to severe negative side effects experienced in recent clinical trials using conventional antibodies. Here we present the PACMAN method as means to develop this novel class of proteolytic immunotherapeutics to utilize their potential against amyloid diseases.

I 06
Cryo-EM structure of the NLRP3 decamer bound to the cytokine release inhibitor CRID3

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Innate immune cells recognise invading pathogens via germline-encoded pattern recognition receptors (PRRs). NOD-like receptor protein 3 (NLRP3) is a cytosolic PRR that senses a diverse set of stimuli, including pathogen-derived molecular patterns or endogenous danger signals. Upon activation, NLRP3 forms a multiprotein signalling platform called the inflammasome, which induces the production of proinflammatory cytokines and pyroptotic cell death. NLRP3 is the clinically most implicated inflammasome protein with its overdue activation being involved in a broad range of chronic inflammatory diseases, including atherosclerosis, diabetes and Alzheimer's disease. Although NLRP3 is a highly relevant drug target, the mechanisms leading to NLRP3 activation and the functioning of antagonistic small molecules remain poorly understood. We investigated the structures of different NLRP3 functional states to delineate NLRP3 inflammasome formation. We found that inactive, ADP-bound NLRP3 forms a spherical decamer with a size of 1.2 megadaltons. Binding of the NLRP3-specific antagonist CRID3 at the backside of the NLRP3 nucleotide binding domain locks NLRP3 in its inactive conformation and prevents downstream activation. With the determination of the binding site of this lead therapeutic, specific targeting of NLRP3 for the treatment of autoinflammatory and autoimmune diseases is within reach.

I 10
The impact of Blinatumomab treatment on immune cell dynamics in children and adolescents with BCP-ALL

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Blinatumomab is a bispecific T cell engager for the therapy of B cell precursor acute lymphoblastic leukemia (BCP-ALL) that targets the B cell specific antigen CD19. Therefore, the B cells are depleted whereas the T cells expand. Since T cells with naïve phenotypes also expand, we assessed thymic output under Blinatumomab treatment in 117 patients of the clinical study AIEOP-BFM ALL 2017, that tests Blinatumomab as a first-line therapy for children and adolescents with high (HR) and medium risk (MR) BCP-ALL. Both the TCR repertoire analyses and the ddPCR-based quantification of T cell receptor excision circles (TRECs), that are markers for recent thymic emigrants, do not indicate a significant thymic output under Blinatumomab but a polyclonal proliferation of both αβ and γδ T cells. Interestingly, the proliferation tends to be stronger in MR patients than in HR patients as measured by the decrease in the TREC content of the T cells. That might be due to the higher intensity of the prior chemotherapy and the significantly lower T and B cell counts in the HR patients at the time of Blinatumomab application compared to their levels in MR patients. This result indicates that the intensity of the prior immunosuppressive chemotherapy might influence the response to Blinatumomab by affecting both target and effector immune cell compartments. The pending clinical data will reveal the clinical relevance of these immune cell dynamics regarding optimization of treatment protocols.

O 04
Cysteine-less split inteins for the engineering of functionalized nanobodies

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Author(s):
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Split inteins are capable to mediate the virtually traceless ligation of peptides or recombinant proteins by protein-trans splicing with their own concomitant removal. Inteins became a powerful tool in chemical biology and protein chemistry as a chemo-enzymatic approach or for protein semi-synthesis, even in complex mixtures. However, their entry into the fields of cell biology and biomedicine stumbles due to several pitfalls arising when inteins are used for protein trans-splicing on or in the living cell or the whole organism. Here, we focus on the rare cysteine-less split inteins, which have the advantage of being insensitive to extracellular, oxidizing environments, in contrast to the more common cysteine-dependent counterparts. (Ref1. Bhagawati PNAS 2019) We report on engineering cysteine-less split inteins for the functionalization of single-domain antibody fragments, also termed nanobodies. We aim to site-specifically install nanobodies on cells and to prepare bispecific nanobodies with light-activatable properties (Ref2. Jedlitzke Angew Chem Int Ed. 2019) through genetic code expansion with non-canonical amino acids (Figure 1).

Ref1: Bhagawati et al., A mesophilic cysteine-less split intein for protein trans-splicing applications under oxidizing conditions. PNAS 116, 22164-22172 (2019).

Ref2: Jedlitzke et al., Photobodies: Light-Activatable Single-Domain Antibody Fragments. Angew Chem Int Ed 59, 1506-1510 (2019).

O 16

The interferon-inducible antiviral MxB GTPase promotes herpesvirus capsid disassembly and premature genome release in cells

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We have reconstituted capsid-host protein complexes, and identified the IFN-inducible GTPase MxB to interact with herpes simplex virus (HSV) capsids and induce their disassembly (Serrero et al. 2022, eLife). Here, we report on the potential targets of MxB on capsid surfaces, the timeline of MxB-induced capsid disassembly, and MxB's contribution to expose viral genomes to pattern recognition receptor sensing.

Using immunoelectron microscopy, we detected MxB on the vertices of HSV-1 capsids. The portal cap, located at one vertex, is a highly conserved structure among herpesviruses and formed by two pentamers of pUL25 in HSV-1 (pORF19 in KSHV). Pentameric pORF19 impaired MxB's ability to disassemble HSV-1 capsids, suggesting that MxB interacts directly with the portal cap. Transgenic epithelial cell lines (RPE, A549) stably expressing MxB, mutated MxB, or MxA were used to measure the impact of Mx proteins on the subcellular fate of incoming capsids, genomes, viral gene expression, capsid formation, and the induction of IFN-β and ISGs. Using click chemistry in MxB expressing cells, infected with EdC/EdA labelled HSV-1 virions, we detected disintegrated capsids and cytoplasmic genomes.

Our data suggest that MxB senses herpesviral capsids on their portal caps to mediate capsid disassembly. This premature release of viral genomes from capsids may enhance the activation of DNA sensors, that play a key role in the amplification and regulation of host innate immune defences to infection.

O 21

Uptake of peptides investigated on a model system of the nasal mucosa

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Pharmacodynamic properties often limit therapeutic application of peptides. Nevertheless, this group of compunds allows an improved pharmacological profile by high target-specificity compared to small molecules, and lead to non-toxic degradation products. Further, allergic reactions are rare. However, pharmacodynamics improvement of peptides is contrasted by rapid degradation and low bioavailability. Cell permeation through epithelial (e.g. intestinal and nasal mucosa) and endothelial (e.g. blood brain barrier) barriers is a key factor in drug uptake and distribution. Compounds can cross these tissues by different routes, including para- and transcellular as well as passive and active transport mechanism. In vitro models allow to screen a high number of peptides for permeability and transport mechanism. Differentiated Calu-3 cells provide properties of respiratory epithelium. We have applied this model system to different peptides with fluorescent labels and stabilizing motives such as lipidation and non-natural amino acids. Permeation of these compounds through Calu-3 cell layer, differentiated by culturing on air-liquid interface, was determined by fluorescence, mass spectrometry and reversed phase high performance liquid chromatography. Further, subcellular localization was analyzed with fluorescence microscopy. Interestingly, minor changes, such as different lipidation forms, display major effects on cellular uptake and permeation.

I 19
Droplet microfluidic antibody screening platform for functional antibodies at the single-cell level

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Antibody screening has become an essential role in modern medicine. Screenings are usually limited to a small subset of target proteins and are based on antibody binding, which does not necessarily correlate with their functionality. Being able to assess the effect of thousands of antibodies on the transcriptome of target cells would allow target-agnostic screens for a variety of functions in a highly multiplexed way. While droplet microfluidic systems have already become a powerful tool for single-cell RNA sequencing and antibody discovery, many key problems remain. First, the sparsity of the single-cell RNA sequencing data hardly allows the detection of individual outliers showing differential gene expression after exposure to an antibody. Furthermore, there are currently no systems for the detection of altered single-cell transcriptomic signatures after stimulation in droplets. Here we address all these challenges and conceptualize a microfluidic workflow for in-droplet stimulation of target cells with antibodies expressed by antibody-secreting cells. In addition, we developed a computational

workflow that can detect outliers that remain hidden in commonly used clustering approaches. Lastly, we investigated the effect of labeling newly synthesized mRNA to distinguish between transcriptomic signatures before and after antibody stimulation of a target cell. Taken together, this should pave the way for RNA sequencing-driven functional antibody discovery.

T 02
siRNA-mediated downregulation of WAPAL inhibits T cell proliferation upon allogeneic stimulus

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Graft-versus-Host-Disease (GvHD) is a common complication after allogeneic hematopoietic stem cell transplantation where alloreactive T cells recognize and attack host antigens. Current GvHD drugs have widespread toxicities arising from unspecific inhibition of immune cell function and proliferation. Wings apart-like homolog (WAPAL) emerged as a potential regulator of GvHD. Another therapeutic moiety, extracellular vesicles (EVs) derived from mesenchymal stem cells (MSC) have been shown to 1) facilitate siRNA delivery to difficult-to-transfect cell types and 2) to improve GvHD. Here I used fully chemically modified siRNA to silence WAPAL and assessed its effect on T cell proliferation in cell culture and in an allogeneic in a GvH in vitro model. I also tested siRNA formulated into MSC-derived EVs. We screened 12 siRNAs and identified a lead showing an IC50 of 400nM and a maximal silencing of 65% in primary T cells. Cellular fractionation in primary T cells and in Hela showed 30% mRNA localization in the nucleus, potentially explaining the silencing plateau at 65%. WAPAL downregulation inhibited T cell proliferation up to 70% upon allogeneic stimulus (co-incubation with irradiated allogeneic dendritic cells). Upon formulation into EVs, the inhibitory effect could be enhanced to nearly 100%. Interestingly, when T cells were stimulated with activation beads, cell proliferation was not affected by WAPAL silencing, suggesting an allogeneic-stimulus-specific role of WAPAL.

O 12
Novel cytosolic LPS sensors: GBPs break down bacterial membranes and activate the non-canonical inflammasome

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Gram-negative bacterial pathogens are protected from antimicrobials by surface exposed lipopolysaccharides (LPS), the main building block of the outer membrane. Besides transforming the bacterial envelope into an effective permeability barrier, LPS is also a potent inducer of innate immunity. Interferon-inducible guanylate-binding proteins (GBPs) are key players of innate immunity and promote host defense against cytosol invading Gram-negatives through bacterial lysis, blocking of bacterial actin-based dissemination, and activation of the non-canonical inflammasome caspase-4. The molecular mechanisms underlying these various antibacterial functions were elusive. Our cell-free and cell-based studies identified GBP1 as novel LPS sensor that targets cytosolic bacteria directly to form an antimicrobial protein coat. This GBP1 microcapsule breaks down the protective outer membrane barrier for antimicrobial recognition and killing. Interference with the integrity of the bacterial envelope further affects localization and function of outer membrane virulence factors thereby inhibiting bacterial actin-based motility. Finally, we show that binding to the bacterial surface is dispensable for GBPs to promote caspase-4-induced pyroptosis and demonstrate that detergent-like GBP1 and GBP2 form GBP-LPS complexes that serve as hubs for non-canonical inflammasome activation. Together, our studies provide a novel mechanistic framework of how GBPs fulfill their antibacterial functions.

I 17
Preclinical Validation of alphaSEPT, an engineered human Cytokine, as a Next-Generation Immunotherapy

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Sepsis is an indication with an enormous unmet medical need. In Western societies more people die of sepsis than of most common cancers combined. No treatment is available yet. To address this, we develop alphaSEPT. alphaSEPT is a rationally engineered human immune signalling molecule. It has immunomodulatory functions that are key in indications caused by an immune system out of balance. Sepsis is such an indication: the immune dysbalance in sepsis involves both, overreaction to an infection and extensive functional defects leading to immune paralysis. Patients die either of multiorgan dysfunction due to hyperinflammation or of secondary infections due to immune paralysis. The immunobalancing functions of alphaSEPT provide a new mode of action (MoA) that addresses both mortality drivers. Studies with the mouse protein in mice demonstrated a reduction of sepsis mortality of up to 70%. Our studies with abdominal sepsis patients at the Klinikum rechts der Isar, the university hospital of the TUM, demonstrated that alphaSEPT can precisely restore the immune competence of immune

cells isolated from the patients: alphaSEPT restores the antigen presenting capacities of patients´ monocytes as well as the antigen-dependent proliferation of T-cells. With this novel MoA alphaSEPT achieves the potential of precision medicine via active immunomodulation. We now aim to perform indication extension studies and to bring alphaSEPT as a next-generation biopharmaceutical into the clinics.

T 03
Gene expression signatures can predict SARS-CoV-2 T cell receptor functionality

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T cell immunity is crucial in conveying protective immunity against infection with SARS-CoV-2. Especially $CD8^+$ T cells play a major role in combating SARS-CoV-2 infections. To better understand and predict the level of protection, it is of utmost importance to characterize in more depth the quality of recruited $CD8^+$ T cell receptors (TCRs) and the functional profile of often highly polyclonal antigen-specific T cell populations.

To achieve this, we identified SARS-CoV-2-specific $CD8^+$ TCRs from mild COVID-19 patients to immunogenic epitopes of different HLA restrictions. Re-expression of those TCRs via CRISPR/Cas9-mediated orthotopic TCR replacement (OTR) in healthy donor PBMCs allowed us to perform extensive structural and functional characterizations. Combining these data with single cell RNAseq, we identified 'gene signatures of recent activation' to differentiate high and low functional TCRs. We demonstrate that these 'gene signatures of activation' can predict TCR functionality.

We then challenged this model by performing experimental validation on a set of TCRs isolated from a second single cell RNAseq experiment; and we could show that affinity, functionality and cellular avidity, which is assumed to predict in vivo functionality, correlate with our predictive scores. We hope that by combing different TCR characterization methods, we can speed up the process of bringing the most effective TCRs into clinic for SARS-CoV-2 as well as for other viral and non-viral diseases.

O 10
Multiscale Origami Structures as Interfaces for Cells

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Over the last two decades, DNA origami has evolved into a well-established, versatile method for the specific arrangement of molecules in the nanometre range.

Within the framework of MOSAIC (Multiscale Origami Structures as Interfaces for cells), we structure DNA-Origami on a surface, to form micro arrays for the analysis of biological processes Here, we focus on the phenomenon of receptor clustering, where the specific grouping of receptors can amplify the sensitivity of a signaling response. With the complete control over the number and spatial arrangement of ligands bound on DNA-Origami at a lower nanoscale, MOSAIC is a compelling way to study this process. The easy adaptability of the origami system allows a wide range of spatial arrangements and ligand-receptor relationships to be investigated.

For MOSAIC, we prepare a surface with an oligonucleotide pattern via polymer pen lithography (PPL). DNA origami structures are then applied to the surface using DNA-directed immobilisation (DDI) and decorated with epidermal growth factor (EGF) in various arrangements. In the further course, the specific activation of EGFR in adherent MCF7 cells is investigated. The focus lies on different intensities of activity and how these can be influenced by the spatial arrangement of EGF on DNA origami structures.

MOSAIC combines a variety of innovative technologies such as DNA origami, PPL and DDI to investigate fundamental aspects of early-stage cell signalling in living cells.

C 03
Synthetic receptor platform to identify loss-of-function single nucleotide variants and designed mutants in the death receptor Fas/CD95

Presenting author: **Anna Rita Minafra**

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Synthetic biology has emerged as a useful technology for studying cytokine signal transduction. Recently, we described fully synthetic cytokine receptors to phenocopy trimeric receptors such as the death receptor Fas/CD95. Using a nanobody as an extracellular binding domain for mCherry fused to the natural receptor's transmembrane and intracellular domain, trimeric mCherry-ligands were able to induce cell death. Among the 17,889 single nucleotide

variants (SNVs) in the SNP database (dbSNP) for Fas, 337 represent missense mutations that functionally remained largely uncharacterized. Here, we developed a workflow for the Fas synthetic cytokines receptor (Fas-SyCyR) system to functionally characterize missense SNPs within the transmembrane and intracellular domain of Fas. To validate our system, we selected 5 functionally assigned loss-of-function polymorphisms and included 15 additional unassigned SNPs. Moreover, based on structural data, 15 gain- or loss-of-function candidate mutations were additionally selected. All 35 nucleotide variants were functionally investigated through cellular proliferation, apoptosis, and caspases 3 and 7 cleavage assays. Collectively, our results showed that 30 variants resulted in partial or complete loss-of-function (LOF) while 5 lead to a gain-of-function (GOF). In conclusion, we demonstrated that synthetic cytokine receptors are a suitable tool for functional SNPs/mutations characterization in a structured workflow.

O 08
Formulation of DNA Nanocomposites: Towards Functional Materials for Protein Expression

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DNA based hydrogels are a new class of biomaterials with high potential for a broad range of biotechnological applications. Recently DNA nanocomposites originating from DNA-functionalized silica nanoparticles and carbon nanotubes have been developed. These materials are characterized by the unique properties of nucleic acids, such as programmability, and excellent biocompatibility. Among other interesting characteristics, their mechanical properties can be tuned by adjusting the ratio of the composites' starting materials. These properties enable applications in cell culture as well as usage as a substrate for cell-free protein synthesis (CFPS).

The presented work investigates the scope of DNA materials, which are synthesized using nanoparticles as the starting point of the polymerization of long DNA single strands, which are produced by rolling circle amplification. The integration of plasmids coding for fluorescent marker proteins provides materials with genetic transcribable information. Flow cytometry and confocal microscopy showed a very efficient uptake of the nanocomposites by different eukaryotic cell lines, which continue to divide while the ingested material is distributed among the daughter cells. Although the encoded protein was not expressed in living cells, DNA based hydrogels prove to be efficient templates for CFPS. This work contributes to the understanding of the molecular interactions between complex DNA materials and the functional cellular machinery.

T 07
Seeing is believing: Imaging of cell and gene therapies using a novel PET reporter gene system

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Advanced Therapy Medicinal Products (ATMPs), such as cell and gene therapies necessitate a reliable diagnostic method to image transgenes quantitatively. We developed a novel positron emission tomography (PET) reporter gene system consisting of a membrane-anchored Anticalin binding protein (DTPA-R) and a corresponding radiohybrid ligand [^{18}F]F-DTPA•terbium. The simple design of the reporter protein yields high receptor densities of up to ~1×10^{6} receptors per cell, small gene size and high affinity binding of [^{18}F]F-DTPA. The physical detection limit for JurkatDTPA^{-}R is 500 cells. [^{18}F]F-DTPA showed a high tumor uptake of 22.1 %ID/g in PC3DTPA^{-}R xenografts compared to 0.2 %ID/g for a DTPA-R negative tumor 90 min p.i. (ratio = 125) and cleared nearly exclusively via the renal route . The reporter system allowed the quantitative, depth-independent imaging of gene therapy with AAV9 viral vectors with a linear dose-to-signal relation. Expansion and migration of CD19-CAR-T cells in a systemic Raji animal model was monitored over a four-week therapy, demonstrating a linear relationship between PET signal and CAR-T cell number. Furthermore, immunohistochemistry of these animals confirmed that PET imaging allowed identification of CAR-T infiltration into individual vertebrae. Our novel & proprietary reporter gene system is a promising tool to elevate the understanding of cell and gene therapies and support the development of precision medicine.

I 05
Computational engineering of stabilized VαVβ single chain TCRs

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Recent improvements in computational algorithms for protein design and bioengineering have paved the way for new strategies for protein-based drug development. Computational sequence design has already demonstrated success in improving the stability and expression

of proteins of therapeutic interest such as antibodies, single-chain variable fragments, and T-cell receptors. However, stabilization of single-chain VαVβ TCRs (scTv) still remains a major challenge. We have developed a pipeline that combines deep learning-based structure prediction (AlphaFold) with biophysical scoring functions (Rosetta) to first characterize TCR antigen binding and specificity. In a second step, neural networks for sequence design (ProteinMPNN, ESM) are used to stabilize the variable regions of TCRs of interest as scTvs. scTv designs will be expressed on yeast surfaces and sorted for expression and antigen binding. In this way, we aim to develop a simple and rapid approach to stabilize scTvs for screening and therapeutic applications.

O 05
Designed Ankyrin Repeat Proteins as a tool to reactivate p53 in cancer

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Inactivation of the tumor suppressor p53 is one of the most common hallmarks of human cancer. In more than 50 % of all tumors this inactivation is caused by mutations which are mainly located at several hotspots within the DNA binding domain. Some of the hotspot mutations do not only lead to inactivation of p53 but also to its destabilization causing conformational changes of the DBD or even unfolding and the exposure of aggregation prone regions. Those mutants are described as temperature sensitive mutants as they adopt a wild type conformation at lower temperatures but get destabilized at elevated temperatures. Unfolding can lead to co-aggregation with other proteins including p53 family members, thus repressing their transcriptional function and hence promote cancer cell survival and metastasis. In order to target and stabilize those cancer hotspot mutants we selected Designed Ankyrin Repeat Proteins (DARPins) against the wild type and mutant p53 DBD. Characterization of the selected DARPins revealed that DARPin C10 stabilizes a variety of common cancer mutants of p53. Furthermore, we were able to show that DARPin C10 restores the transcriptional activity of p53 in transactivation assays. Additionally, we found that DARPin C10 can stabilize and restore the transcriptional activity of p53 in HeLa cells by blocking the HPV-E6 mediated degradation of p53. The described DARPins could present a new approach to stabilize p53 mutants and to target HPV induced tumorigenesis.

I 04
Complementary antibody fragments (hemibodies) for effective targeting of breast cancer

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Antibody-based immunotherapy has become an integral part of modern cancer treatment. We have developed a new generation of T-cell-engaging antibodies, called hemibodies, for tumour targeting. The hemibody comes as a logic-gated antibody pair that primarily targets cells co-expressing two tumour-associated antigens (TAA). This AND exclusive dual-targeting allows for highly efficient tumour elimination. Each hemibody consists of an antigen-binding domain fused to either the variable heavy (VH) or variable light (VL) chain domain of a T-cell activating anti-CD3 antibody. Upon binding to the respective target antigens, the complementary antibody halves come into close proximity and reconstitute a T-cell activating unit, followed by the formation of an immunological synapse and lysis of the target cell. In the treatment of haematopoietic neoplasms, the hemibodies have shown exceptional efficacy. This success encouraged us to use the hemibodies for treatment of breast cancer. Breast cancer is characterised by a high degree of heterogeneity and plasticity, making effective treatment difficult. Here we report on the development of a hemibody-based strategy for the treatment of breast cancer using the HER2, EpCAM, EGFR and ROR1 receptors as target antigens.

O 19
Toward Reproducible Enzyme Modeling with Isothermal Titration Calorimetry

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To apply enzymes in technical processes, a detailed understanding of the molecular mechanisms is required. Isothermal titration calorimetry (ITC) allows the label-free and highly sensitive analysis of kinetic and thermodynamic parameters of individual steps in the catalytic cycle of an enzyme reaction. The presented ITC-dependent workflow was used to determine these parameters for the cofactor-dependent ketoreductase Gre2p under different reaction conditions. Using a standardized approach with the implementation of sample quality control by DLS, we obtained high-quality data suitable for the advanced modeling of the enzyme reaction mechanism. The results revealed that this enzyme operates with an ordered sequential mechanism in which the cofactor NADPH binds first, which then allows binding of the substrate NDK. In addition, the enzyme was found to be affected by substrate or product inhibition depending on the reaction buffer. Data reproducibility, a mandatory prerequisite to achieve robust modeling, is ensured by specifying standard operating procedures, using programmed

workflows for data analysis, and storing all data in a F.A.I.R. repository. Because they can be automated and scaled for high-throughput, the combination of different approaches such as the one presented will provide the high-quality data needed for the development of enzymes and biocatalytic processes through machine learning to accelerate the future development of industrial biocatalysis.

I 08
Do immunoglobulin M (IgM) antibodies represent a natural immune mechanism against amyloid diseases?

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Amyloid diseases represent a range of severe, yet incurable diseases. In patients suffering from those diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Diabetes mellitus Type 2 (DMT2), amyloid aggregates are found as small soluble, but highly toxic oligomers as well as insoluble, structurally highly ordered fibrils. For yet unknown reasons, these aggregates accumulate in amyloid diseases, possibly due to an altered balance of the protein homeostasis or due to increased production of the respective amyloid proteins.

We explore a novel approach to eliminate these amyloid structures by utilizing catalytic antibodies. Catalytic antibodies combine the function of proteases and the superior specificity of antibodies. They are a natural phenomenon and have been found in humans. In immunoglobulin M (IgM) class antibodies a particularly high proteolytic activity has been observed.

In first studies of our research group, the amyloid peptides amyloid-β (AD associated), α-synuclein (PD associated) and islet amyloid polypeptide (DMT2 associated) were incubated with polyclonal IgM antibodies from serum and analysed by RP-HPLC. It was found that the purified IgM antibodies were catalytic against all those peptides, but not against globular proteins. This observation raises the question whether proteolytically active antibodies represent a natural immune mechanism against amyloidogenic peptides.

O 13
Spatiotemporal dynamics of IL-17 family receptor complex formation

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IL-17 family cytokines have emerged as central mediators of inflammatory and autoimmune conditions. While most family members remain understudied, therapeutic antibodies targeting IL-17A or IL-17 Receptor A have demonstrated high clinical efficacy in various diseases, indicating the high medical potential of IL-17 cytokines. A common feature of IL-17 family cytokines is that they recruit the shared receptor subunit IL-17RA and a second chain that is assumed to mediate ligand specificity to initiate signaling. Despite being a critical step for the activation of signaling, the assembly of IL-17 receptor complexes in the plasma membrane is poorly understood so far. Here, we have explored the spatiotemporal organization and dynamics of IL-17RA, IL-17RB and IL-17RC in living cells by single molecule TIRF microscopy in combination with nanobody-based labeling. These studies suggest ligand-induced formation of homo- and heteromeric complexes with higher stoichiometries as compared to homo- and heterodimers that have previously been assumed. In line with recent structural studies, a novel receptor-receptor interface that mediates complex formation spatially distant from the ligand-binding site was confirmed by mutagenesis. Overall, our results support a new paradigm for signaling complexes of the IL-17 family with formation of higher-order complex stoichiometries, which is orchestrated by distinct extracellular receptor interfaces.

C 05
Engineering of synthetic cytokine chimeras (cytokimeras)

Presenting author: **Puyan Rafii, Patricia Rodrigues Cruz**

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Synthetic cytokines like IC7 have proven beneficial in mouse model diseases like type 2 diabetes devoid of significant side effects. All except one cytokine of the Interleukin (IL-)6 family share gp130 as the common b-receptor chain. Whereas IL-6, IL-11 or ciliary neurotrophic factor (CNTF) require previous attachment to its a-receptor via a binding site I following b-receptor homo- or hetero dimerization, other members of IL-6 family bind b-receptors chains directly via site II and site III. Using IL-6 or IL-11 as a scaffold, we exchanged the gp130 binding site III of IL-6 or IL-11 with the binding site III of leukemia inhibitory factor (LIF) or oncostatin-M (OSM) in a structure-based approach. The engineered cytokine chimeras (cytokimera) GIL-6, GIO-6, and GIL-11 are able to efficiently recruit non-natural receptor complexes resulting in signal transduction and proliferation of factor-depending Ba/F3 cells. Human GIL-11 exhibits cross-reactivity to mouse and rescued IL-6R deficient mice following partial hepatectomy,

demonstrating gp130:IL11-R:LIFR signaling efficiently induced liver regeneration. The development of these cytokimera has enabled new potential avenues for therapeutic application by assembling novel artificial cytokine receptor complexes.

I 03
Cell-free production of targeted toxins for future medical treatments

Presenting author: **Franziska Ramm**

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Toxins are generally considered as harmful substances as they appear in our everyday lives as well as in our work environment including food associated toxins. Nonetheless, many toxins may also provide valuable characteristics for therapeutic approaches when combining a toxic moiety with a targeting moiety thus resulting in targeted toxins, and more specifically immunotoxins when using antibodies. Toxic proteins comprise a variety of different substances with diverse mechanisms of action. Thus, toxin synthesis and characterization are rather difficult. Cell-free protein synthesis (CFPS) offers a fast and cost-efficient alternative to synthesize a variety of toxins while using a cell lysate rather than viable cells. Here, we describe CFPS as a platform technology for the production of targeted toxins using different eukaryotic cell-free systems based on mammalian CHO, insect Sf21 and fungal Pichia pastoris lysates. One example is the ribosome-inactivating toxin Dianthin from the ornamental plant clove pink combined with an EGF or a single-chain antibody fragment. Another example is the combination of the active subunit of the Shiga toxin combined with an interleukin. They were synthesized in a cell-free manner and further characterized in diverse assays. The data presented here show the versatility of CFPS to synthesize, modify and investigate proteinaceous toxins. These data further facilitate the screening of novel targeted toxins for therapeutic applications.

O 15
Synthetic Glycans as Vaccine Candidates and Diagnostic Tool for Candida Infections

Presenting author: **Emelie Ellen Reuber**

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Fungal diseases affect more than one billion people and claim around 1.5 million lives worldwide every year - more than prostate or breast cancer. The fungal species Candida is responsible for the majority of cases. The World Health Organization (WHO) classifies Candida albicans and Candida auris as pathogens of the "critical priority group" because they show increasing resistance to antifungal drugs. Therefore, the development of an effective vaccine against and an early diagnostic tool for Candida infections is highly desirable. We synthesized a series of beta-glucans and mannans, which are essential components of the cell wall of Candida, with diagnostic and preventive potential for Candida infections. We screened sera from infected patients and mice for antibodies to these structures on glycan arrays and found structures that could serve as candidates for semisynthetic conjugate vaccines and also for early diagnosis of infection.

O 17
The influence of glycation on the expression of the bi-functional enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE)

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GNE-myopathy (GNEM) is a very rare autosomal recessive genetic disorder – prevalence 9:1 000 000 (Carillo et al., 2018) – that results from a variety of more than 200 different mutations in the GNE gene. This gene encodes the bi-functional enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) – the key enzyme of sialic acid biosynthesis. The disease manifests in early adulthood and shows a relatively slow progression characterized by an atrophy of distal and later also proximal skeletal muscles. It leads to most patients becoming wheelchair dependent. In particular, this progression and the late onset of the disease indicates an influence of age. Methylglyoxal (MGO) and glyoxal (GO) are two metabolites, which accumulate in cells over time and with aging. Both are glycation agents by interacting with free amino groups of amino acids in proteins and lead to the formation of advanced glycation endproducts (AGEs). Thereby AGEs account in part of age-related tissue dysfunction. To investigate the effect of ageing on GNE expression, murine C2C12 cells were treated with different concentrations of MGO and GO and corresponding qPCR analyzes were performed. It could be shown that a treatment of the cells with the two glycation agents in the non-lethal range did not significantly change the GNE expression, suggesting other age-derived effects on the GNE.

C 05
Engineering of synthetic cytokine chimeras (cytokimeras)

Presenting author: **Puyan Rafii, Patricia Rodrigues Cruz**

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Synthetic cytokines like IC7 have proven beneficial in mouse model diseases like type 2 diabetes devoid of significant side effects. All except one cytokine of the Interleukin (IL-)6 family share gp130 as the common b-receptor chain. Whereas IL-6, IL-11 or ciliary neurotrophic factor (CNTF) require previous attachment to its a-receptor via a binding site I following b-receptor homo- or hetero dimerization, other members of IL-6 family bind b-receptors chains directly via site II and site III. Using IL-6 or IL-11 as a scaffold, we exchanged the gp130 binding site III of IL-6 or IL-11 with the binding site III of leukemia inhibitory factor (LIF) or oncostatin-M (OSM) in a structure-based approach. The engineered cytokine chimeras (cytokimera) GIL-6, GIO-6, and GIL-11 are able to efficiently recruit non-natural receptor complexes resulting in signal transduction and proliferation of factor-depending Ba/F3 cells. Human GIL-11 exhibits cross-reactivity to mouse and rescued IL-6R deficient mice following partial hepatectomy, demonstrating gp130:IL11-R:LIFR signaling efficiently induced liver regeneration. The development of these cytokimera has enabled new potential avenues for therapeutic application by assembling novel artificial cytokine receptor complexes.

O 09
Molecular and biochemical characterization of MHC class I non-covalent clusters at the plasma membrane of mammalian cells

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At the plasma membrane of mammalian cells, major histocompatibility complex class I molecules (MHC-I) present peptides to T cells. Following the loss of the peptide and the light chain beta-2 microglobulin (β2m), the resulting free heavy chains (FHCs) can associate into

homotypic complexes at the plasma membrane. Here, we investigated the stoichiometry and dynamics of MHC-I FHCs assemblies by combining a micropattern assay with fluorescence recovery after photobleaching (FRAP) and with single molecule co-tracking. We identified transient, non-covalent MHC-I FHC dimers, with dimerization mediated by the α3 domain, as the prevalent species at the plasma membrane. MHC-I FHC dimer formation correlates with a moderate decrease in the diffusion coefficient and an increased immobile fraction at the cell surface. In vitro studies with isolated proteins in conjunction with molecular docking and dynamics simulations suggest that in the complexes, the α3 domain of one FHC binds to another FHC in a manner similar to that seen for β2m. We propose distinct functions of these MHC I free heavy chain dimers in signaling and in the endocytic sorting at the end of their lifespan.

I 15

Multiplexed autoantibody profiling to identify markers for clinical outcome of Immune Checkpoint Inhibitors in metastatic Urothelial Carcinoma patients

Presenting author: **Jana Gajewski, Elena Rupieper**

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Background: Immune checkpoint inhibitors (ICI) have improved the treatment of a broad spectrum of cancers as metastatic Urothelial Carcinoma (mUC). However, the durable responses to ICIs are only observed in a subset of patients. Therefore, there is an unmet need to identify biomarkers predictive of outcome to therapy. In this study, we addressed the role of autoantibodies (AAb) as potential predictive biomarkers for clinical outcome stratification in mUC patients following ICI therapy.

Methods: We analysed AAb profiles of pre- (n=66) and post-treatment (n=65) serum samples from mUC patients receiving ICIs or platinum-based chemotherapy (PBC) utilizing Oncimmune's ImmunoINSIGHTS bead-based AAb profiling platform and protein array containing 1150 tumour- and self-antigens.

Results: Multiplexed profiling identified AAbs with significantly higher levels in patients with mUC in comparison to healthy controls. Baseline level of AAbs targeting antigens such as known bladder cancer antigens KDM6B, BRCA1 and BCL2 were associated with earlier progression on ICI. Patients on ICI showed a greater fold change from pre- to post-therapy across all AAbs compared to patients receiving chemotherapy and the higher overall AAb reactivity was associated with improved response to ICI.

Conclusion: AAbs may serve as candidate biomarkers to predict clinical outcome for ICI therapy in patients suffering from mUC. These findings contribute to the understanding of the immune response in mUC and could potentially assist in selection of patients who would benefit from ICI treatment.

I 14
Therapeutic Targeting of TREM2 - a Novel Approach for Treatment of Alzheimer's disease?

Presenting author: **Kai Schlepckow**

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Several independent studies indicate that boosting TREM2 signaling by agonistic anti-TREM2 antibodies may be a promising novel therapeutic approach for the treatment of Alzheimer's disease. We have developed such an antibody, which is antibody 4D9. 4D9 binds 12 amino acids N-terminal of the ectodomain cleavage site, inhibits shedding of TREM2, and potently activates downstream pSYK signaling. Boosting TREM2 signaling by 4D9 leads to improved survival of macrophages, enhanced phagocytic activity of primary micoglia, lower amyloid burden in an amyloidosis mouse model as well as accelerated recovery in mouse models of demyelination. In more recent work we have focussed on the development of a version of 4D9 with an engineered Fc part, which allows very efficient transcytosis of the antibody across the blood brain barrier (ATV:4D9; ATV: antibody transport vehicle). We demonstrate in an amyloidosis mouse model by TSPO and FDG PET imaging that a human specific version of the antibody enhances microglial activation and brain glucose metabolism, respectively.

I 12
Hapten-decorated DNA nanostructures decipher the distance-dependent antigen-binding of antibodies on the nanoscale

Presenting author: **Leonie Schneider**

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The immunological response of mast cells is regulated by multivalent binding of antigens to IgE antibodies bound to the high-affinity receptor FcεRI on the cell membrane. However, the spatial organization of antigen-antibody-receptor complexes at the nanometer scale and the structural constraints involved in the initial events at the cell surface are not yet fully understood. We report the use of DNA origami nanostructures (DON) functionalized with the haptenic 2,4-dinitrophenyl (DNP) ligand to generate multivalent artificial antigens with full control over valency and nanoscale ligand architecture. We arranged DNP ligands in distinctive patterns on the DON to induce receptor clustering and investigated the relation between antigen-binding affinity and mast cell activation, an important feature for tailoring next generation monoclonal antibodies (mAb) for immunotherapy. To study the spatial requirements for mast cell activation, we performed real-time binding kinetics of free and FcεRI-bound antibodies with the DNP-DON and could show how the interplay between antigen, antibody and receptor promotes an oligovalent type of interaction. Finally, the use of DNP-DON complexes for mast cell activation revealed that antigen-directed tight assembly of antibody-receptor complexes is the critical factor for triggering degranulation, even more critical than ligand valence. Our study emphasizes the significance of DNA nanostructures for the study of fundamental biological processes.

I 11
Generation of IgM-directed antibodies for the treatment of B cell cancers

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Facing the challenge of eliminating cancerous B cells while retaining the non-malignant B cell population for immune system maintenance we generated IgM-directed antibodies for the treatment of B cell cancers. By targeting the IgM-positive B cell subpopulation and introducing an anti-IgM masking unit which is only released in the tumor microenvironment we achieved precise tumor targeting. Due to antibody drug conjugation we further ensured high cytotoxicity towards lymphoma cells.

T 01
Chemical optimization of miR-146a mimic for the treatment of Graft versus Host disease

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Mice transplanted with miR-146a-/- T cells show more aggressive GvHD. Pretreatment of T cells a commercial miR-146a mimic (nucleofection), lower GvHD mortality and severity can be achieved. We generated cholesterol-conjugatedmiR-146a mimics spanning chemical scaffolds from miRNA-like to siRNA-like in order to identify a clinically relevant scaffold for GvHD therapy. We tested the impact of the length of the sense strand, and the complementarity of the sense strand on miRNA efficacy in reporter and in functional assays. We cloned a fully complementary target site 4x in tandem into the 3'UTR of a dual luciferase reporter system to test an siRNA-like effect. In this fully complementary target setting a shorter sense strand or a fully complementary sense strand led to less efficient silencing. Unexpectedly, a more siRNA-like structure induced poorer siRNA-like effect. Then, we used an in vitro model of GvHD, mixed lymphocyte reactions, to compare T cell inhibition functionality of miRNA mimics. We saw that a further enhancement could be achieved by increasing the number phosphorotioate (PS) modifications in the antisense strand. We achieved the most potent T cell inhibition (up to 80 %, IC 50 216 nM) using a full length fully complementary sense strand and increased PS in the antisense strand. We demonstrate that some elements of siRNA chemical design augment miRNA mimic efficacy in a functional setting, and antisense strand metabolism is rate-limiting for miRNA functionality.

O 14
Stable empty MHC class I proteins for Immunobiotechnology

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Peptide-bound complexes of recombinant MHC (major histocompatibility complex) class I proteins are useful for the detection of antigen-specific T cells (as MHC tetramers or multimers) and also for their isolation and stimulation, as well as for affinity measurements and for the safety screening of T cell receptors (TCRs). Production of such recombinant peptide-MHC complexes is slow since each complex has to be manufactured individually in an in vitro folding reaction. Alternatives, such as peptide exchange on folded complexes, are expensive and can be unreliable. We have designed disulfide-stabilized MHC class I proteins that can be produced without peptides and that are stable in the peptide-empty state. Specific peptides are rapidly bound, and the interaction with the TCR is not altered by the stabilization. They have been successfully used in several studies.

I 18
Design and generation of antibody-drug conjugates using non-canonical amino acids

Presenting author: **Marlitt Stech**

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The concept of antibody-drug conjugates (ADCs) is theoretically simple, but it is difficult to combine their components into an optimized and functional therapeutic agent. With most conjugation technologies, the choice of the conjugation site is limited, or pre-functionalization of the conjugation site is required. This leads to limited possibilities of optimization and extensive downstream processes, respectively. Against this background, we develop biochemical tools for the site-specific modification of antibodies using the amber suppression technology. We use two different approaches: Mammalian cell-based as well as novel mammalian cell-free systems. Here we demonstrate the synthesis of "ready-to-conjugate" antibodies containing site-specifically introduced 2-azidoethoxycarbonyl-L-lysine (AECK) by using an orthogonal tRNA/synthetase pair from the archaea Methanosarcina mazei in a mammalian cell-free system for the first time, as well as in a cell-based system. Using the cell-free system, we show the efficient one-step synthesis of radiolabeled and ready-to-click antibody. Using cell-based expression, we investigated the developability of an antibody-drug conjugate and we proof the position-specific coupling of DBCO-PEG3-MMAE to cell-based produced AECK labeled antibodies by hydrophobic interaction chromatography.

I 07
Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC

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The T cell receptor (TCR) expressed by T lymphocytes initiates protective immune responses to pathogens and tumors. To explore the structural basis of how TCR signaling is initiated when the receptor binds to peptide-loaded major histocompatibility complex (pMHC) molecules, we used cryogenic electron microscopy to determine the structure of a tumor-reactive TCRαβ/CD3δγε2ζ2 complex bound to a melanoma-specific human class I pMHC at 3.08 Å resolution. The antigen-bound complex comprises 11 subunits stabilized by multivalent

interactions across three structural layers, with clustered membrane-proximal cystines stabilizing the CD3-εδ and CD3-εγ heterodimers. Extra density sandwiched between transmembrane helices reveals the involvement of sterol lipids in TCR assembly. The geometry of the pMHC/TCR complex suggests that efficient TCR scanning of pMHC requires accurate pre-positioning of T cell and antigen-presenting cell membranes. Comparisons of the ligand-bound and unliganded receptors, along with molecular dynamics simulations, indicate that TCRs can be triggered in the absence of spontaneous structural rearrangements.

Sušac L, Vuong MT, Thomas C, von Bülow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampé R, Davis SJ. Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC. Cell. 2022

O 06
Development of a Cell-Based Allergy Screening System

Presenting author: **Steffen Tank**

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Allergies are on the rise, demanding potent diagnostic tools for screening of allergen-specific IgE antibodies in blood samples. The aim of this project is to develop a non-invasive, fast, and easily adaptable allergen screening system.

To study IgE-mediated degranulation in vitro without the need to isolate mast cells from blood and tissues, we chose the rat basophil leukemia RBL 2H3 cell line as it carries the FcεRI receptor and releases mediators comparable to mast cells. As the system needs to respond to human IgE antibodies, RBL cells were transfected with a chimeric FcεRI receptor and its functionality was verified in degranulation assays after stimulation with human IgE. For an easy and sensitive read-out of IgE-triggered degranulation events, a stable cell line expressing a functional fusion of β-hexosaminidase subunit a (Hexa), mCherry, and β-lactamase (bla), which couples a direct read-out (mCherry) with a sensitive enzymatic assay (bla), was created using a modified CRISPR/Cas9 system.

Additionally, a split-version of bla is evaluated, where one fragment is packaged in the vesicles as fusion to WinZipB1 and Hexa, while the other fragment is fused to WinZipA2 and FcεRIα. Consequently, bla is only reconstituted via cognate interaction of WinZipA2 and B1, which is directly coupled to the degranulation event.

Our RBL-based allergy screening system combines easy and fast handling by omitting separate incubation steps with higher sensitivity due to reduced background.

O 01
Biochemical analysis of transcription factor protein-protein interactions for inhibitor design

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Transcription factors are vital regulatory proteins for healthy hematopoiesis. Mutated or dysregulated activity of these proteins can lead to autoimmune disease and cancer. Acute myeloid leukemia (AML) remains one of the deadliest forms of cancer worldwide. The transcription factors IRF8 and PU.1 form a regulatory protein complex recently identified as a necessity for AML maintenance. Although transcription factors are responsible for many diseases, including cancer, and are found to be potential therapeutic targets, not many of them are being drugged in the clinic.

In contrast to enzymes or receptors that often have well-defined interaction sites that can be targeted, transcription factors usually have more extensive and more complicated interaction interfaces. How these transcription factors physically interact and regulate each other is poorly understood, and direct inhibition of protein-protein interactions for cancer therapy relies on the structural identification of these interaction interfaces. Using X-ray crystallography, in vitro interaction assays, and Hydrogen Deuterium Exchange coupled to Mass Spectrometry (HDX-MS) we are currently elucidating the IRF8/PU.1 complex formation and want to use structure-based peptide-inhibitor design to aid the treatment of AML.

C 01

Respiratory syncytial virus (RSV)-approved monoclonal antibody Palivizumab as ligand for anti-idiotype nanobody-based synthetic cytokine receptors

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Synthetic cytokine receptors can modulate cellular functions based on an artificial ligand to avoid off-target and/or unspecific effects. We developed a synthetic cytokine/cytokine receptor pair based on the antigen-binding domain of Palivizumab as synthetic cytokine and a set of anti-idiotype nanobodies as synthetic receptors. Palivizumab targets the fusion (F) glycoprotein responsible for the fusion of viral and host cell membranes. Importantly, Palivizumab is neither cross-reactive with human proteins nor immunogenic. In the synthetic receptors, AIPVHH were fused to the activating Interleukin (IL-)6 cytokine receptor gp130 and the apoptosis-inducing receptor Fas. The synthetic cytokine receptor AIPVHHgp130 was efficiently activated by cross-linked Palivizumab but not by Palivizumab alone. After dimerization of reformatted Palivizumab single-chain variable fragments (scFv (PscFv)), the miniaturized synthetic PscFv efficiently activated AIPVHHgp130. Since the distance between two antigen-binding arms in Palivizumab and PscFv is critical for efficient receptor activation, Palivizumab was used as an inhibitor of PscFv-induced receptor activation. Moreover, PscFv specifically induced apoptosis in cells expressing synthetic AIPVHHFas death receptors. Here, we developed a non-immunogenic full-synthetic cytokine/cytokine receptor pair for in vivo therapeutic strategies involving non-physiological targets during immunotherapy.

C 02

Forced-dimerization of cytokine receptors using the transmembrane domain of constitutively active IL-7R

Presenting author: **Lynn Baumgärtner**

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Mutations with insertion of cysteines have been found in the transmembrane domain (TMD) of the IL-7R leading to constitutive activation and thus an augmented proliferation.

To find out if this mutated TMD could activate different receptors, we exchanged the natural TMD of several cytokine receptors (IL-23R, gp130, IFNAR2) with the IL-7R TMD containing the L234PPCL insertion known for leading to constitutive activation in the transmembrane domain (TMD) of the IL-7 receptor.

Additionally, these mutated receptors (IL-23R, gp130, IFNAR2) were transformed into synthetic cytokine receptors (SyCyRs) with a GFP nanobody as extracellular domain, which can only be activated by the synthetic ligand GFP.

In both cases, natural and synthetic cytokine receptors showed a constitutive activation bearing the IL-7R-PPCL-TMD. Mutated natural and synthetic gp130, IL-23R and IL-7R showed ligand-independent activation of the JAK/STAT pathway and proliferation of Ba/F3 cells.

Although in some cases constitutive activation of cytokine receptors can lead to disease through the overexpression and activation of certain pathways, the presented strategy could be a useful tool to improve e.g. CAR T cell therapy.

C 03
Synthetic receptor platform to identify loss-of-function single nucleotide variants and designed mutants in the death receptor Fas/CD95

Presenting author: **Anna Rita Minafra**

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Synthetic biology has emerged as a useful technology for studying cytokine signal transduction. Recently, we described fully synthetic cytokine receptors to phenocopy trimeric receptors such as the death receptor Fas/CD95. Using a nanobody as an extracellular binding domain for mCherry fused to the natural receptor's transmembrane and intracellular domain, trimeric mCherry-ligands were able to induce cell death. Among the 17,889 single nucleotide variants (SNVs) in the SNP database (dbSNP) for Fas, 337 represent missense mutations that functionally remained largely uncharacterized. Here, we developed a workflow for the Fas synthetic cytokines receptor (Fas-SyCyR) system to functionally characterize missense SNPs within the transmembrane and intracellular domain of Fas. To validate our system, we selected 5 functionally assigned loss-of-function polymorphisms and included 15 additional unassigned SNPs. Moreover, based on structural data, 15 gain- or loss-of-function candidate mutations were additionally selected. All 35 nucleotide variants were functionally investigated through cellular proliferation, apoptosis, and caspases 3 and 7 cleavage assays. Collectively, our results showed that 30 variants resulted in partial or complete loss-of-function (LOF) while 5 lead to a gain-of-function (GOF). In conclusion, we demonstrated that synthetic cytokine receptors are a suitable tool for functional SNPs/mutations characterization in a structured workflow.

C 04
Rational engineering of a folding pathway changes mechanism and chaperone dependency of interleukin secretion

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In our work we focus on the human IL-12 family which contains four heterodimeric members, IL-12, IL-23, IL-27, and IL-35. These four cytokines are made up of only five subunits, with the four-helix bundle α-subunits being secretion-incompetent in isolation and the β-subunits being secreted alone. In the context of heterodimer formation, the β-subunit assists in assembly-induced folding of the α-subunit and subsequent secretion. This biogenesis is accompanied by chaperones and cytokine release is strictly regulated by the ER quality control system.

Based on structural studies that revealed why IL-23α fails to fold correctly alone, we applied in silico methods together with rational engineering to design an autonomously folding- and secretion-competent IL-23α protein. We succeeded in obtaining a highly stable IL-23α, introducing a disulfide bond that stabilizes its first α-helix. Stability of the variant is increased not only intracellularly but also of the purified subunit. Importantly, this mutant can still assemble with IL-12β with low-μM affinity. Besides this, the optimized IL-23α mutant shows a different intracellular folding behaviour, which goes hand in hand with an altered chaperone repertoire acting on it. This study allowed us to address the question, whether a molecular competition of IL-12 versus IL-23 formation exists in cells, as both contain IL-12β, and why IL-12 family subunits have evolved for assembly-dependent instead of independent folding and secretion.

C 05
Engineering of synthetic cytokine chimeras (cytokimeras)

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Synthetic cytokines like IC7 have proven beneficial in mouse model diseases like type 2 diabetes devoid of significant side effects. All except one cytokine of the Interleukin (IL-)6 family share gp130 as the common b-receptor chain. Whereas IL-6, IL-11 or ciliary neurotrophic factor (CNTF) require previous attachment to its a-receptor via a binding site I following b-receptor homo- or hetero dimerization, other members of IL-6 family bind b-receptors chains directly via site II and site III. Using IL-6 or IL-11 as a scaffold, we exchanged the gp130 binding site III of IL-6 or IL-11 with the binding site III of leukemia inhibitory factor (LIF) or oncostatin-M (OSM) in a structure-based approach. The engineered cytokine chimeras (cytokimera) GIL-6, GIO-6, and GIL-11 are able to efficiently recruit non-natural receptor complexes resulting in signal transduction and proliferation of factor-depending Ba/F3 cells. Human GIL-11 exhibits cross-reactivity to mouse and rescued IL-6R deficient mice following partial hepatectomy, demonstrating gp130:IL11-R:LIFR signaling efficiently induced liver regeneration. The development of these cytokimera has enabled new potential avenues for therapeutic application by assembling novel artificial cytokine receptor complexes.

C 06
Leveraging chemically-controlled protein switches for increased safety of cytokine-based cancer immunotherapeutics

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Cytokines are key signal mediators of the immune system playing an essential role in the orchestration of immune responses. Despite their unique ability to modulate the immune system, the translation of cytokine-based therapies to the clinic has been greatly hindered by severe toxicities due to the pleiotropy and off-targeting effects of many cytokines.

Here, we present a general strategy that enables precise control over cytokine activity. We control their activity by selectively masking the receptor binding site with a fused chemically-responsive domain, which could be unmasked with a competing molecule (Venetoclax). To achieve this, Bcl-2 was fused to the cytokine and the BIM-BH3 interaction motif was transplanted to sites in close proximity to the cytokine's receptor binding site. In absence of Venetoclax, Bcl-2 bound the cytokine with high affinity blocking the interaction site between the cytokine and its receptor. Upon addition of Venetoclax, the interaction between Bcl-2 and the BIM-BH3 motif was disrupted, so restoring the cytokine's activity. We have developed switchable mutants for a range of different cytokines (IL-2, IL-10, and IL-15) used in cancer immunotherapy. Moreover, we showed that in presence of Venetoclax, their activities can be selectively and fully restored.

Overall, this drug-responsive switch strategy may achieve spatiotemporal control of cytokine activities in vivo and thus improve the safety and clinical applicability of cytokine therapeutics.

I 01
A generic strategy to generate trifunctional two-in-one antibodies by chicken immunization

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In this work, we generated chicken-derived trispecific antibodies based on a two-in-one antibody targeting three antigens simultaneously with two Fab arms. These NK cell engagers inhibit EGFR signaling, block the PD-1/PD-L1 immune checkpoint and show a potent ADCC effect. Based on this method, further therapeutic antibodies derived from avian immunization with novel and tailored binding properties and target combinations can be developed.

I 02
A modular approach for intracellular protein delivery via a cell- penetrating antibody

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Many disease-based protein-protein interactions occur in the cytoplasm, which is not commonly targetable by antibodies. In this study, the cell penetrating properties of a heparan sulfate-binding antibody (CPAb) were investigated and compared with the well-known internalizing but not cytosol-penetrating antibody Trastuzumab. Furthermore, the function of CPAb as an intracellular transporter was verified by LplA-mediated GFP coupling. Finally, a coupling mechanism based on the high affinity of streptavidin and biotin was tested for cell-penetration to allow up to three cargo proteins to be transported into cells.

I 03
Cell-free production of targeted toxins for future medical treatments

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Toxins are generally considered as harmful substances as they appear in our everyday lives as well as in our work environment including food associated toxins. Nonetheless, many toxins may also provide valuable characteristics for therapeutic approaches when combining a toxic moiety with a targeting moiety thus resulting in targeted toxins, and more specifically immunotoxins when using antibodies. Toxic proteins comprise a variety of different substances with diverse mechanisms of action. Thus, toxin synthesis and characterization are rather difficult. Cell-free protein synthesis (CFPS) offers a fast and cost-efficient alternative to synthesize a variety of toxins while using a cell lysate rather than viable cells. Here, we describe CFPS as a platform technology for the production of targeted toxins using different eukaryotic cell-free systems based on mammalian CHO, insect Sf21 and fungal Pichia pastoris lysates. One example is the ribosome-inactivating toxin Dianthin from the ornamental plant clove pink combined with an EGF or a single-chain antibody fragment. Another example is the combination of the active subunit of the Shiga toxin combined with an interleukin. They were synthesized in a cell-free manner and further characterized in diverse assays. The data presented here show the versatility of CFPS to synthesize, modify and investigate proteinaceous toxins. These data further facilitate the screening of novel targeted toxins for therapeutic applications.

I 04
Complementary antibody fragments (hemibodies) for effective targeting of breast cancer

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Antibody-based immunotherapy has become an integral part of modern cancer treatment. We have developed a new generation of T-cell-engaging antibodies, called hemibodies, for tumour targeting. The hemibody comes as a logic-gated antibody pair that primarily targets cells co-expressing two tumour-associated antigens (TAA). This AND exclusive dual-targeting allows for highly efficient tumour elimination. Each hemibody consists of an antigen-binding domain fused to either the variable heavy (VH) or variable light (VL) chain domain of a T-cell activating anti-CD3 antibody. Upon binding to the respective target antigens, the complementary antibody halves come into close proximity and reconstitute a T-cell activating unit, followed by the formation of an immunological synapse and lysis of the target cell. In the treatment of haematopoietic neoplasms, the hemibodies have shown exceptional efficacy. This success encouraged us to use the hemibodies for treatment of breast cancer. Breast cancer is characterised by a high degree of heterogeneity and plasticity, making effective treatment

difficult. Here we report on the development of a hemibody-based strategy for the treatment of breast cancer using the HER2, EpCAM, EGFR and ROR1 receptors as target antigens.

I 05
Computational engineering of stabilized VαVβ single chain TCRs

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Recent improvements in computational algorithms for protein design and bioengineering have paved the way for new strategies for protein-based drug development. Computational sequence design has already demonstrated success in improving the stability and expression of proteins of therapeutic interest such as antibodies, single-chain variable fragments, and T-cell receptors. However, stabilization of single-chain VαVβ TCRs (scTv) still remains a major challenge. We have developed a pipeline that combines deep learning-based structure prediction (AlphaFold) with biophysical scoring functions (Rosetta) to first characterize TCR antigen binding and specificity. In a second step, neural networks for sequence design (ProteinMPNN, ESM) are used to stabilize the variable regions of TCRs of interest as scTvs. scTv designs will be expressed on yeast surfaces and sorted for expression and antigen binding. In this way, we aim to develop a simple and rapid approach to stabilize scTvs for screening and therapeutic applications.

I 06
Cryo-EM structure of the NLRP3 decamer bound to the cytokine release inhibitor CRID3

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Innate immune cells recognise invading pathogens via germline-encoded pattern recognition receptors (PRRs). NOD-like receptor protein 3 (NLRP3) is a cytosolic PRR that senses a diverse set of stimuli, including pathogen-derived molecular patterns or endogenous danger signals. Upon activation, NLRP3 forms a multiprotein signalling platform called the inflammasome, which induces the production of proinflammatory cytokines and pyroptotic cell death. NLRP3

is the clinically most implicated inflammasome protein with its overdue activation being involved in a broad range of chronic inflammatory diseases, including atherosclerosis, diabetes and Alzheimer's disease. Although NLRP3 is a highly relevant drug target, the mechanisms leading to NLRP3 activation and the functioning of antagonistic small molecules remain poorly understood. We investigated the structures of different NLRP3 functional states to delineate NLRP3 inflammasome formation. We found that inactive, ADP-bound NLRP3 forms a spherical decamer with a size of 1.2 megadaltons. Binding of the NLRP3-specific antagonist CRID3 at the backside of the NLRP3 nucleotide binding domain locks NLRP3 in its inactive conformation and prevents downstream activation. With the determination of the binding site of this lead therapeutic, specific targeting of NLRP3 for the treatment of autoinflammatory and autoimmune diseases is within reach.

I 07
Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC

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The T cell receptor (TCR) expressed by T lymphocytes initiates protective immune responses to pathogens and tumors. To explore the structural basis of how TCR signaling is initiated when the receptor binds to peptide-loaded major histocompatibility complex (pMHC) molecules, we used cryogenic electron microscopy to determine the structure of a tumor-reactive TCRαβ/CD3δγε2ζ2 complex bound to a melanoma-specific human class I pMHC at 3.08 Å resolution. The antigen-bound complex comprises 11 subunits stabilized by multivalent interactions across three structural layers, with clustered membrane-proximal cystines stabilizing the CD3-εδ and CD3-εγ heterodimers. Extra density sandwiched between transmembrane helices reveals the involvement of sterol lipids in TCR assembly. The geometry of the pMHC/TCR complex suggests that efficient TCR scanning of pMHC requires accurate pre-positioning of T cell and antigen-presenting cell membranes. Comparisons of the ligand-bound and unliganded receptors, along with molecular dynamics simulations, indicate that TCRs can be triggered in the absence of spontaneous structural rearrangements.

Sušac L, Vuong MT, Thomas C, von Bülow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampé R, Davis SJ. Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC. Cell. 2022

I 08
Do immunoglobulin M (IgM) antibodies represent a natural immune mechanism against amyloid diseases?

Presenting author: **Anne Pfitzer**

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Amyloid diseases represent a range of severe, yet incurable diseases. In patients suffering from those diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Diabetes mellitus Type 2 (DMT2), amyloid aggregates are found as small soluble, but highly toxic oligomers as well as insoluble, structurally highly ordered fibrils. For yet unknown reasons, these aggregates accumulate in amyloid diseases, possibly due to an altered balance of the protein homeostasis or due to increased production of the respective amyloid proteins.

We explore a novel approach to eliminate these amyloid structures by utilizing catalytic antibodies. Catalytic antibodies combine the function of proteases and the superior specificity of antibodies. They are a natural phenomenon and have been found in humans. In immunoglobulin M (IgM) class antibodies a particularly high proteolytic activity has been observed.

In first studies of our research group, the amyloid peptides amyloid-β (AD associated), α-synuclein (PD associated) and islet amyloid polypeptide (DMT2 associated) were incubated with polyclonal IgM antibodies from serum and analysed by RP-HPLC. It was found that the purified IgM antibodies were catalytic against all those peptides, but not against globular proteins. This observation raises the question whether proteolytically active antibodies represent a natural immune mechanism against amyloidogenic peptides.

I 09
Dodecin is a carrier protein for immunizations

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In bioengineering, scaffold proteins have been increasingly used to recruit molecules to parts of a cell, or to enhance the efficacy of biosynthetic or signaling pathways. For example, scaffolds can be used to make weak or non-immunogenic small molecules immunogenic by attaching them to the scaffold, in this role called carrier. We have recently presented the 8 kDa small protein dodecin from Mycobacterium tuberculosis (mtDod) as a new scaffold protein (1). MtDod forms a homododecameric complex of spherical shape, high stability and robust

assembly, enabling the attachment of cargo at its surface. MtDod, either directly loaded with cargo or equipped with domains for non-covalent and covalent loading of cargo, can be produced recombinantly in high quantity and quality in Escherichia coli. Proteins as large as the monomeric superfolder green fluorescent protein can be recruited to the dodecin surface. In a case study, we harnessed mtDod-peptide fusions for producing antibodies against human heat shock proteins and the C-terminus of heat shock cognate 70 interacting protein (CHIP). Future studies will show to what extent dodecin can be used for immunotherapeutic applications.

(1) Bourdeaux, F. et al. Dodecin as carrier protein for immunizations and bioengineering applications. Sci Rep 10, 13297 (2020).

I 10
The impact of Blinatumomab treatment on immune cell dynamics in children and adolescents with BCP-ALL

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Blinatumomab is a bispecific T cell engager for the therapy of B cell precursor acute lymphoblastic leukemia (BCP-ALL) that targets the B cell specific antigen CD19. Therefore, the B cells are depleted whereas the T cells expand. Since T cells with naïve phenotypes also expand, we assessed thymic output under Blinatumomab treatment in 117 patients of the clinical study AIEOP-BFM ALL 2017, that tests Blinatumomab as a first-line therapy for children and adolescents with high (HR) and medium risk (MR) BCP-ALL. Both the TCR repertoire analyses and the ddPCR-based quantification of T cell receptor excision circles (TRECs), that are markers for recent thymic emigrants, do not indicate a significant thymic output under Blinatumomab but a polyclonal proliferation of both αβ and γδ T cells. Interestingly, the proliferation tends to be stronger in MR patients than in HR patients as measured by the decrease in the TREC content of the T cells. That might be due to the higher intensity of the prior chemotherapy and the significantly lower T and B cell counts in the HR patients at the time of Blinatumomab application compared to their levels in MR patients. This result indicates that the intensity of the prior immunosuppressive chemotherapy might influence the response to Blinatumomab by affecting both target and effector immune cell compartments. The pending clinical data will reveal the clinical relevance of these immune cell dynamics regarding optimization of treatment protocols.

I 11
Generation of IgM-directed antibodies for the treatment of B cell cancers

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Facing the challenge of eliminating cancerous B cells while retaining the non-malignant B cell population for immune system maintenance we generated IgM-directed antibodies for the treatment of B cell cancers. By targeting the IgM-positive B cell subpopulation and introducing an anti-IgM masking unit which is only released in the tumor microenvironment we achieved precise tumor targeting. Due to antibody drug conjugation we further ensured high cytotoxicity towards lymphoma cells.

I 12
Hapten-decorated DNA nanostructures decipher the distance-dependent antigen-binding of antibodies on the nanoscale

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The immunological response of mast cells is regulated by multivalent binding of antigens to IgE antibodies bound to the high-affinity receptor FcεRI on the cell membrane. However, the spatial organization of antigen-antibody-receptor complexes at the nanometer scale and the structural constraints involved in the initial events at the cell surface are not yet fully understood. We report the use of DNA origami nanostructures (DON) functionalized with the haptenic 2,4-dinitrophenyl (DNP) ligand to generate multivalent artificial antigens with full control over valency and nanoscale ligand architecture. We arranged DNP ligands in distinctive patterns on the DON to induce receptor clustering and investigated the relation between antigen-binding affinity and mast cell activation, an important feature for tailoring next generation monoclonal antibodies (mAb) for immunotherapy. To study the spatial requirements for mast cell activation, we performed real-time binding kinetics of free and FcεRI-bound antibodies with the DNP-DON and could show how the interplay between antigen, antibody and receptor promotes an oligovalent type of interaction. Finally, the use of DNP-DON complexes for mast cell activation revealed that antigen-directed tight assembly of antibody-receptor complexes is the critical factor for triggering degranulation, even more critical than ligand valence. Our study emphasizes the significance of DNA nanostructures for the study of fundamental biological processes.

I 13
Human OX40 targeting Nanobodies for diagnostic imaging

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In the last decades, the fight against cancer led to the emergence of various innovative approaches including immunotherapies which deploy the patient's T lymphocytes to waging war on cancer cells. Since the outcome of such cost intensive therapies is highly dependent on the individual immune status, there is an increasing need to stratify patients and monitor therapeutic progress. Recent data showed that the immune checkpoint molecule OX40, which plays a strong role in proliferation, survival and effector function of T cells, can be a prognostic marker. Taking advantage of the favorable characteristics of single-domain antibodies (Nanobodies, Nbs) for application as potential in vivo imaging probes, we identified four unique Nbs targeting human OX40 (hOX40). In-depth biochemical and biophysical characterization showed a strong affinity and stability of all Nbs at physiological conditions. Epitope binning revealed that these binders address two different domains of hOX40, while two of them block binding of its natural ligand OX40L. Using flow cytometry, we confirmed binding of the Nbs to natively expressed OX40 present on activated T lymphocytes. Addressing a potential modulating effect on the T cell functionality, we performed proliferation tracking and analyzed the cytokine profile after Nb treatment. These studies led to the selection of a leading candidate for the development of Nb-based probes for in vivo application.

I 14
Therapeutic Targeting of TREM2 - a Novel Approach for Treatment of Alzheimer's disease?

Presenting author: **Kai Schlepckow**

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Several independent studies indicate that boosting TREM2 signaling by agonistic anti-TREM2 antibodies may be a promising novel therapeutic approach for the treatment of Alzheimer's disease. We have developed such an antibody, which is antibody 4D9. 4D9 binds 12 amino acids N-terminal of the ectodomain cleavage site, inhibits shedding of TREM2, and potently activates downstream pSYK signaling. Boosting TREM2 signaling by 4D9 leads to improved survival of macrophages, enhanced phagocytic activity of primary micoglia, lower amyloid burden in an amyloidosis mouse model as well as accelerated recovery in mouse models of demyelination. In more recent work we have focussed on the development of a version of 4D9 with an engineered Fc part, which allows very efficient transcytosis of the antibody across the blood brain barrier (ATV:4D9; ATV: antibody transport vehicle). We demonstrate in an amyloidosis mouse model by TSPO and FDG PET imaging that a human specific version of the antibody enhances microglial activation and brain glucose metabolism, respectively.

I 15

Multiplexed autoantibody profiling to identify markers for clinical outcome of Immune Checkpoint Inhibitors in metastatic Urothelial Carcinoma patients

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Background: Immune checkpoint inhibitors (ICI) have improved the treatment of a broad spectrum of cancers as metastatic Urothelial Carcinoma (mUC). However, the durable responses to ICIs are only observed in a subset of patients. Therefore, there is an unmet need to identify biomarkers predictive of outcome to therapy. In this study, we addressed the role of autoantibodies (AAb) as potential predictive biomarkers for clinical outcome stratification in mUC patients following ICI therapy.

Methods: We analysed AAb profiles of pre- (n=66) and post-treatment (n=65) serum samples from mUC patients receiving ICIs or platinum-based chemotherapy (PBC) utilizing Oncimmune's ImmunoINSIGHTS bead-based AAb profiling platform and protein array containing 1150 tumour- and self-antigens.

Results: Multiplexed profiling identified AAbs with significantly higher levels in patients with mUC in comparison to healthy controls. Baseline level of AAbs targeting antigens such as known bladder cancer antigens KDM6B, BRCA1 and BCL2 were associated with earlier progression on ICI. Patients on ICI showed a greater fold change from pre- to post-therapy across all AAbs compared to patients receiving chemotherapy and the higher overall AAb reactivity was associated with improved response to ICI.

Conclusion: AAbs may serve as candidate biomarkers to predict clinical outcome for ICI therapy in patients suffering from mUC. These findings contribute to the understanding of the immune response in mUC and could potentially assist in selection of patients who would benefit from ICI treatment.

I 16

PACMAN - Proteolytic Antigen Cleavage Mediated AmplificatioN: Introducing a novel class of proteolytic immunotherapeutics to tackle amyloid diseases

Presenting author: **Filip Hasecke**

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In amyloid diseases, a pathological build up of aggregated proteins or peptides leads to toxicity and triggers a plethora of mislead processes ultimately leading to the diseases' symptomatic expressions. In Alzheimer's disease, the amlyoid-β (Aβ) peptide is one of the disease's amyloid culprits. Conventional immunotherapeutics, with monoclonal antibodies, aim to bind the amyloid antigen and remove it via endogenous degradation pathways. Unfortunately, these approaches have consistently proven insufficient in clinical trials.

We propose proteolytically active antibodies as a novel therapeutic approach to tackle amyloid diseases which might supersede and fundamentally outcompete conventional immunotherapeutics. Proteolytic antibodies offer a great advantage – they cleave their antigen and do not merely bind to their antigen. Furthermore, proteolytic antibodies do not form stable immune complexes with their amyloid antigen, which have been linked to severe negative side effects experienced in recent clinical trials using conventional antibodies. Here we present the PACMAN method as means to develop this novel class of proteolytic immunotherapeutics to utilize their potential against amyloid diseases.

I 17

Preclinical Validation of alphaSEPT, an engineered human Cytokine, as a Next-Generation Immunotherapy

Presenting author: **Julia Behnke, Melanie Laschinger**

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Sepsis is an indication with an enormous unmet medical need. In Western societies more people die of sepsis than of most common cancers combined. No treatment is available yet. To address this, we develop alphaSEPT. alphaSEPT is a rationally engineered human immune signalling molecule. It has immunomodulatory functions that are key in indications caused by an immune system out of balance. Sepsis is such an indication: the immune dysbalance in sepsis involves both, overreaction to an infection and extensive functional defects leading to immune paralysis. Patients die either of multiorgan dysfunction due to hyperinflammation or of secondary infections due to immune paralysis. The immunobalancing functions of alphaSEPT provide a new mode of action (MoA) that addresses both mortality drivers. Studies with the mouse protein in mice demonstrated a reduction of sepsis mortality of up to 70%. Our studies with abdominal sepsis patients at the Klinikum rechts der Isar, the university hospital of the TUM, demonstrated that alphaSEPT can precisely restore the immune competence of immune cells isolated from the patients: alphaSEPT restores the antigen presenting capacities of patients´ monocytes as well as the antigen-dependent proliferation of T-cells. With this novel MoA alphaSEPT achieves the potential of precision medicine via active immunomodulation. We now aim to perform indication extension studies and to bring alphaSEPT as a next-generation biopharmaceutical into the clinics.

I 18
Design and generation of antibody-drug conjugates using non-canonical amino acids

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The concept of antibody-drug conjugates (ADCs) is theoretically simple, but it is difficult to combine their components into an optimized and functional therapeutic agent. With most conjugation technologies, the choice of the conjugation site is limited, or pre-functionalization of the conjugation site is required. This leads to limited possibilities of optimization and extensive downstream processes, respectively. Against this background, we develop biochemical tools for the site-specific modification of antibodies using the amber suppression technology. We use two different approaches: Mammalian cell-based as well as novel mammalian cell-free systems. Here we demonstrate the synthesis of "ready-to-conjugate" antibodies containing site-specifically introduced 2-azidoethoxycarbonyl-L-lysine (AECK) by using an orthogonal tRNA/synthetase pair from the archaea Methanosarcina mazei in a mammalian cell-free system for the first time, as well as in a cell-based system. Using the cell-free system, we show the efficient one-step synthesis of radiolabeled and ready-to-click

antibody. Using cell-based expression, we investigated the developability of an antibody-drug conjugate and we proof the position-specific coupling of DBCO-PEG3-MMAE to cell-based produced AECK labeled antibodies by hydrophobic interaction chromatography.

I 19
Droplet microfluidic antibody screening platform for functional antibodies at the single-cell level

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Antibody screening has become an essential role in modern medicine. Screenings are usually limited to a small subset of target proteins and are based on antibody binding, which does not necessarily correlate with their functionality. Being able to assess the effect of thousands of antibodies on the transcriptome of target cells would allow target-agnostic screens for a variety of functions in a highly multiplexed way. While droplet microfluidic systems have already become a powerful tool for single-cell RNA sequencing and antibody discovery, many key problems remain. First, the sparsity of the single-cell RNA sequencing data hardly allows the detection of individual outliers showing differential gene expression after exposure to an antibody. Furthermore, there are currently no systems for the detection of altered single-cell transcriptomic signatures after stimulation in droplets. Here we address all these challenges and conceptualize a microfluidic workflow for in-droplet stimulation of target cells with antibodies expressed by antibody-secreting cells. In addition, we developed a computational workflow that can detect outliers that remain hidden in commonly used clustering approaches. Lastly, we investigated the effect of labeling newly synthesized mRNA to distinguish between transcriptomic signatures before and after antibody stimulation of a target cell. Taken together, this should pave the way for RNA sequencing-driven functional antibody discovery.

I 20
Investigating The T Cell Repertoire In Melanoma Immune Checkpoint Inhibitor Therapy Patients and Their Response To Therapy

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In the study presented, changes to $CD4^+$ T Cells, $CD8^+$ T Cells and their reactivity to stimuli by Immune Checkpoint Inhibitor (ICI) Therapy are investigated. Findings are compared to healthy donors and patients' course of therapy, to better predict response to therapy, development of immune-related adverse effects (irAEs), patient benefit and survival.

The study is part of an ongoing Master Thesis.

Tumors can exhibit immune-evasion mechanisms by expressing checkpoint ligands, e.g. PD-L1 and CTLA-4 ligands. This can be abrogated by ICIs, which bind immune checkpoint molecules and prevent their ligands from interacting, restoring anti-tumor responses.

Since 2011, multiple ICIs have been approved for treatment of melanoma in Germany. However, irAEs may arise in up to 45% of ICI patients, depending on the dose, time and type of agent used.

To characterize the T cell department, samples from healthy donors and melanoma patients were intra- and extracellularly analyzed using Flow Cytometry (FACS) by staining surface markers of $CD4^+$ subsets, $CD8^+$ cells and transcription factors Tbet, GATA3, RORγt and FoxP3. So far, characterizing assays have been developed and verified for healthy donors.

Currently, healthy donor samples are being incubated with anti-CD3/CD28 or melanoma cell culture supernatant to investigate their reactivity to stimuli. Results will subsequently be compared to melanoma ICI therapy patients.

O 01
Biochemical analysis of transcription factor protein-protein interactions for inhibitor design

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Transcription factors are vital regulatory proteins for healthy hematopoiesis. Mutated or dysregulated activity of these proteins can lead to autoimmune disease and cancer. Acute myeloid leukemia (AML) remains one of the deadliest forms of cancer worldwide. The transcription factors IRF8 and PU.1 form a regulatory protein complex recently identified as a necessity for AML maintenance. Although transcription factors are responsible for many diseases, including cancer, and are found to be potential therapeutic targets, not many of them are being drugged in the clinic.

In contrast to enzymes or receptors that often have well-defined interaction sites that can be targeted, transcription factors usually have more extensive and more complicated interaction

interfaces. How these transcription factors physically interact and regulate each other is poorly understood, and direct inhibition of protein-protein interactions for cancer therapy relies on the structural identification of these interaction interfaces. Using X-ray crystallography, in vitro interaction assays, and Hydrogen Deuterium Exchange coupled to Mass Spectrometry (HDX-MS) we are currently elucidating the IRF8/PU.1 complex formation and want to use structure-based peptide-inhibitor design to aid the treatment of AML.

O 02
Nanoscale signaling platform for spatiotemporal control of receptor tyrosine kinase activity in live cells

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Dynamic assembly of signaling complexes in the plasma membrane plays prominent roles in immune cell signaling. We here developed a novel surface nanopatterning approach to spatiotemporally control the assembly of signalling complexes at nanoscale in live cells. Based on efficient surface biofunctionalization via adhesive proteins and biopolymers, capillary nanostamping of biofunctional nanodot arrays (bNDAs) with representative 400 nm-diameter and a spacing of optic microscopy resolution limit was obtained. High-density enrichment of signalling protein complexes in the bNDAs was achieved by engineering specific adaptor proteins for orthogonal covalent and non-covalent capturing of target proteins. We exploited these tools for nanoscale assembly of active signalling complexes in the plasma membrane of live cells. To the end, spatiotemporally controlled triggering of receptor tyrosine kinase activity in live cell bNDAs was confirmed via recruitment of the critical effector proteins Grb2, SOS and Ras. The established nanoscale signaling platform with desired time-zero of signaling paves the way for unveiling the crucial time-dependent protein-protein interaction cascades in signaling pathways, offering new insights into the development of immunotherapeutic approaches.

O 03
Computational design of specific binding pockets for phosphorylated amino acids within Armadillo repeat proteins

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Current biomedical research and diagnostics critically depend on detection agents for specific recognition and quantification of protein molecules. Due to the shortcomings of state-of-the-art commercial reagent antibodies such as low specificity or cost-inefficiency, we aim to develop an alternative recognition system based on a regularized armadillo repeat protein scaffold. Its modular architecture can be exploited for the specific binding of linear epitopes, potentially targeting various peptides and proteins based on their amino acid sequence [1,2]. To make such a powerful system universally applicable, individual modules must be engineered to recognize specific amino acids. As part of this project, we developed a framework to computationally predict, graft, and test binding pocket modules that are complemented by library synthesis, directed evolution and experimental screening. The project is a close collaborative effort with the groups of Anna Hine (Aston, UK) and Andreas Plückthun (Zürich, CH).

Here, we present our computational workflow for detection and analysis of interaction sites to design new binder proteins for peptides that include phosphorylated amino acids.

[1] Reichen et al. (2014) J. Struct. Biol. 185: 147–162,

[2] Gisdon et al. (2022) Biol Chem, doi: 10.1515/hsz-2021-0384

O 04
Cysteine-less split inteins for the engineering of functionalized nanobodies

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Split inteins are capable to mediate the virtually traceless ligation of peptides or recombinant proteins by protein-trans splicing with their own concomitant removal. Inteins became a powerful tool in chemical biology and protein chemistry as a chemo-enzymatic approach or for protein semi-synthesis, even in complex mixtures. However, their entry into the fields of cell biology and biomedicine stumbles due to several pitfalls arising when inteins are used for protein trans-splicing on or in the living cell or the whole organism. Here, we focus on the rare cysteine-less split inteins, which have the advantage of being insensitive to extracellular, oxidizing environments, in contrast to the more common cysteine-dependent counterparts. (Ref1. Bhagawati PNAS 2019) We report on engineering cysteine-less split inteins for the functionalization of single-domain antibody fragments, also termed nanobodies. We aim to site-specifically install nanobodies on cells and to prepare bispecific nanobodies with light-

activatable properties (Ref2. Jedlitzke Angew Chem Int Ed. 2019) through genetic code expansion with non-canonical amino acids (Figure 1).

Ref1: Bhagawati et al., A mesophilic cysteine-less split intein for protein trans-splicing applications under oxidizing conditions. PNAS 116, 22164-22172 (2019).

Ref2: Jedlitzke et al., Photobodies: Light-Activatable Single-Domain Antibody Fragments. Angew Chem Int Ed 59, 1506-1510 (2019).

O 05
Designed Ankyrin Repeat Proteins as a tool to reactivate p53 in cancer

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Inactivation of the tumor suppressor p53 is one of the most common hallmarks of human cancer. In more than 50 % of all tumors this inactivation is caused by mutations which are mainly located at several hotspots within the DNA binding domain. Some of the hotspot mutations do not only lead to inactivation of p53 but also to its destabilization causing conformational changes of the DBD or even unfolding and the exposure of aggregation prone regions. Those mutants are described as temperature sensitive mutants as they adopt a wild type conformation at lower temperatures but get destabilized at elevated temperatures. Unfolding can lead to co-aggregation with other proteins including p53 family members, thus repressing their transcriptional function and hence promote cancer cell survival and metastasis. In order to target and stabilize those cancer hotspot mutants we selected Designed Ankyrin Repeat Proteins (DARPins) against the wild type and mutant p53 DBD. Characterization of the selected DARPins revealed that DARPin C10 stabilizes a variety of common cancer mutants of p53. Furthermore, we were able to show that DARPin C10 restores the transcriptional activity of p53 in transactivation assays. Additionally, we found that DARPin C10 can stabilize and restore the transcriptional activity of p53 in HeLa cells by blocking the HPV-E6 mediated degradation of p53. The described DARPins could present a new approach to stabilize p53 mutants and to target HPV induced tumorigenesis.

O 06
Development of a Cell-Based Allergy Screening System

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Allergies are on the rise, demanding potent diagnostic tools for screening of allergen-specific IgE antibodies in blood samples. The aim of this project is to develop a non-invasive, fast, and easily adaptable allergen screening system.

To study IgE-mediated degranulation in vitro without the need to isolate mast cells from blood and tissues, we chose the rat basophil leukemia RBL 2H3 cell line as it carries the FcεRI receptor and releases mediators comparable to mast cells. As the system needs to respond to human IgE antibodies, RBL cells were transfected with a chimeric FcεRI receptor and its functionality was verified in degranulation assays after stimulation with human IgE. For an easy and sensitive read-out of IgE-triggered degranulation events, a stable cell line expressing a functional fusion of β-hexosaminidase subunit a (Hexa), mCherry, and β-lactamase (bla), which couples a direct read-out (mCherry) with a sensitive enzymatic assay (bla), was created using a modified CRISPR/Cas9 system.

Additionally, a split-version of bla is evaluated, where one fragment is packaged in the vesicles as fusion to WinZipB1 and Hexa, while the other fragment is fused to WinZipA2 and FcεRIα. Consequently, bla is only reconstituted via cognate interaction of WinZipA2 and B1, which is directly coupled to the degranulation event.

Our RBL-based allergy screening system combines easy and fast handling by omitting separate incubation steps with higher sensitivity due to reduced background.

O 07
Engineering of the immunomodulatory adenovirus protein E3/49K

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The early transcription unit 3 (E3) of human adenoviruses (HAdVs) encodes immunomodulatory proteins. One of these proteins is E3/49K, a type I transmembrane protein that is uniquely expressed by species D HAdVs. E3/49K is proteolytically processed on the cell surface by matrix metalloproteases and its N-terminal ectodomain sec49K is released. Sec49K interferes with leukocyte activation through binding to receptor-like protein tyrosine phosphatase CD45. Therefore, the E3/49K-mediated immunomodulation may provide a therapeutic strategy to treat autoimmune diseases or to prevent graft rejection after transplantation. It remains elusive whether the interaction of CD45 and E3/49K can also occur

on the cell surface of infected cells with membrane-anchored full-length E3/49K. This question may be addressed by engineering an E3/49K variant that is no longer shed at the cell surface. In order to create such a variant we analyzed the proteolytic cleavage sites by mass spectrometry. Subsequently, we generated deletion mutants lacking these sites to inhibit cleavage of E3/49K. The effect of these mutations on E3/49K cleavage were investigated by digestion with recombinant ADAM10 and in transfected alveolar epithelial A549 cells and human embryonic kidney 293T cells. Although the shedding was strongly reduced in these mutants, it was not abrogated in the mutants investigated so far. Thus, E3/49K may contain several metalloprotease cleavage sites ensuring efficient secretion of sec49K.

O 08
Formulation of DNA Nanocomposites: Towards Functional Materials for Protein Expression

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DNA based hydrogels are a new class of biomaterials with high potential for a broad range of biotechnological applications. Recently DNA nanocomposites originating from DNA-functionalized silica nanoparticles and carbon nanotubes have been developed. These materials are characterized by the unique properties of nucleic acids, such as programmability, and excellent biocompatibility. Among other interesting characteristics, their mechanical properties can be tuned by adjusting the ratio of the composites' starting materials. These properties enable applications in cell culture as well as usage as a substrate for cell-free protein synthesis (CFPS).

The presented work investigates the scope of DNA materials, which are synthesized using nanoparticles as the starting point of the polymerization of long DNA single strands, which are produced by rolling circle amplification. The integration of plasmids coding for fluorescent marker proteins provides materials with genetic transcribable information. Flow cytometry and confocal microscopy showed a very efficient uptake of the nanocomposites by different eukaryotic cell lines, which continue to divide while the ingested material is distributed among the daughter cells. Although the encoded protein was not expressed in living cells, DNA based hydrogels prove to be efficient templates for CFPS. This work contributes to the understanding of the molecular interactions between complex DNA materials and the functional cellular machinery.

O 09

Molecular and biochemical characterization of MHC class I non-covalent clusters at the plasma membrane of mammalian cells

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At the plasma membrane of mammalian cells, major histocompatibility complex class I molecules (MHC-I) present peptides to T cells. Following the loss of the peptide and the light chain beta-2 microglobulin (β2m), the resulting free heavy chains (FHCs) can associate into homotypic complexes at the plasma membrane. Here, we investigated the stoichiometry and dynamics of MHC-I FHCs assemblies by combining a micropattern assay with fluorescence recovery after photobleaching (FRAP) and with single molecule co-tracking. We identified transient, non-covalent MHC-I FHC dimers, with dimerization mediated by the α3 domain, as the prevalent species at the plasma membrane. MHC-I FHC dimer formation correlates with a moderate decrease in the diffusion coefficient and an increased immobile fraction at the cell surface. In vitro studies with isolated proteins in conjunction with molecular docking and dynamics simulations suggest that in the complexes, the α3 domain of one FHC binds to another FHC in a manner similar to that seen for β2m. We propose distinct functions of these MHC I free heavy chain dimers in signaling and in the endocytic sorting at the end of their lifespan.

O 10

Multiscale Origami Structures as Interfaces for Cells

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Over the last two decades, DNA origami has evolved into a well-established, versatile method for the specific arrangement of molecules in the nanometre range.

Within the framework of MOSAIC (Multiscale Origami Structures as Interfaces for cells), we structure DNA-Origami on a surface, to form micro arrays for the analysis of biological processes Here, we focus on the phenomenon of receptor clustering, where the specific grouping of receptors can amplify the sensitivity of a signaling response. With the complete control over the number and spatial arrangement of ligands bound on DNA-Origami at a lower nanoscale, MOSAIC is a compelling way to study this process. The easy adaptability of the origami system allows a wide range of spatial arrangements and ligand-receptor relationships to be investigated.

For MOSAIC, we prepare a surface with an oligonucleotide pattern via polymer pen lithography (PPL). DNA origami structures are then applied to the surface using DNA-directed immobilisation (DDI) and decorated with epidermal growth factor (EGF) in various arrangements. In the further course, the specific activation of EGFR in adherent MCF7 cells is investigated. The focus lies on different intensities of activity and how these can be influenced by the spatial arrangement of EGF on DNA origami structures.

MOSAIC combines a variety of innovative technologies such as DNA origami, PPL and DDI to investigate fundamental aspects of early-stage cell signalling in living cells.

O 11
Contribution withdrawn

O 12
Novel cytosolic LPS sensors: GBPs break down bacterial membranes and activate the non-canonical inflammasome

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Gram-negative bacterial pathogens are protected from antimicrobials by surface exposed lipopolysaccharides (LPS), the main building block of the outer membrane. Besides transforming the bacterial envelope into an effective permeability barrier, LPS is also a potent inducer of innate immunity. Interferon-inducible guanylate-binding proteins (GBPs) are key players of innate immunity and promote host defense against cytosol invading Gram-negatives through bacterial lysis, blocking of bacterial actin-based dissemination, and activation of the non-canonical inflammasome caspase-4. The molecular mechanisms underlying these various antibacterial functions were elusive. Our cell-free and cell-based studies identified GBP1 as novel LPS sensor that targets cytosolic bacteria directly to form an antimicrobial protein coat. This GBP1 microcapsule breaks down the protective outer membrane barrier for antimicrobial

recognition and killing. Interference with the integrity of the bacterial envelope further affects localization and function of outer membrane virulence factors thereby inhibiting bacterial actin-based motility. Finally, we show that binding to the bacterial surface is dispensable for GBPs to promote caspase-4-induced pyroptosis and demonstrate that detergent-like GBP1 and GBP2 form GBP-LPS complexes that serve as hubs for non-canonical inflammasome activation. Together, our studies provide a novel mechanistic framework of how GBPs fulfill their antibacterial functions.

O 13
Spatiotemporal dynamics of IL-17 family receptor complex formation

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IL-17 family cytokines have emerged as central mediators of inflammatory and autoimmune conditions. While most family members remain understudied, therapeutic antibodies targeting IL-17A or IL-17 Receptor A have demonstrated high clinical efficacy in various diseases, indicating the high medical potential of IL-17 cytokines. A common feature of IL-17 family cytokines is that they recruit the shared receptor subunit IL-17RA and a second chain that is assumed to mediate ligand specificity to initiate signaling. Despite being a critical step for the activation of signaling, the assembly of IL-17 receptor complexes in the plasma membrane is poorly understood so far. Here, we have explored the spatiotemporal organization and dynamics of IL-17RA, IL-17RB and IL-17RC in living cells by single molecule TIRF microscopy in combination with nanobody-based labeling. These studies suggest ligand-induced formation of homo- and heteromeric complexes with higher stoichiometries as compared to homo- and heterodimers that have previously been assumed. In line with recent structural studies, a novel receptor-receptor interface that mediates complex formation spatially distant from the ligand-binding site was confirmed by mutagenesis. Overall, our results support a new paradigm for signaling complexes of the IL-17 family with formation of higher-order complex stoichiometries, which is orchestrated by distinct extracellular receptor interfaces.

O 14
Stable empty MHC class I proteins for Immunobiotechnology

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Peptide-bound complexes of recombinant MHC (major histocompatibility complex) class I proteins are useful for the detection of antigen-specific T cells (as MHC tetramers or multimers) and also for their isolation and stimulation, as well as for affinity measurements and for the safety screening of T cell receptors (TCRs). Production of such recombinant peptide-MHC complexes is slow since each complex has to be manufactured individually in an in vitro folding reaction. Alternatives, such as peptide exchange on folded complexes, are expensive and can be unreliable. We have designed disulfide-stabilized MHC class I proteins that can be produced without peptides and that are stable in the peptide-empty state. Specific peptides are rapidly bound, and the interaction with the TCR is not altered by the stabilization. They have been successfully used in several studies.

O 15
Synthetic Glycans as Vaccine Candidates and Diagnostic Tool for Candida Infections

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Fungal diseases affect more than one billion people and claim around 1.5 million lives worldwide every year - more than prostate or breast cancer. The fungal species Candida is responsible for the majority of cases. The World Health Organization (WHO) classifies Candida albicans and Candida auris as pathogens of the "critical priority group" because they show increasing resistance to antifungal drugs. Therefore, the development of an effective vaccine against and an early diagnostic tool for Candida infections is highly desirable. We synthesized a series of beta-glucans and mannans, which are essential components of the cell wall of Candida, with diagnostic and preventive potential for Candida infections. We screened sera from infected patients and mice for antibodies to these structures on glycan arrays and found structures that could serve as candidates for semisynthetic conjugate vaccines and also for early diagnosis of infection.

O 16
The interferon-inducible antiviral MxB GTPase promotes herpesvirus capsid disassembly and premature genome release in cells

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We have reconstituted capsid-host protein complexes, and identified the IFN-inducible GTPase MxB to interact with herpes simplex virus (HSV) capsids and induce their disassembly (Serrero et al. 2022, eLife). Here, we report on the potential targets of MxB on capsid surfaces, the timeline of MxB-induced capsid disassembly, and MxB's contribution to expose viral genomes to pattern recognition receptor sensing.

Using immunoelectron microscopy, we detected MxB on the vertices of HSV-1 capsids. The portal cap, located at one vertex, is a highly conserved structure among herpesviruses and formed by two pentamers of pUL25 in HSV-1 (pORF19 in KSHV). Pentameric pORF19 impaired MxB's ability to disassemble HSV-1 capsids, suggesting that MxB interacts directly with the portal cap. Transgenic epithelial cell lines (RPE, A549) stably expressing MxB, mutated MxB, or MxA were used to measure the impact of Mx proteins on the subcellular fate of incoming capsids, genomes, viral gene expression, capsid formation, and the induction of IFN-β and ISGs. Using click chemistry in MxB expressing cells, infected with EdC/EdA labelled HSV-1 virions, we detected disintegrated capsids and cytoplasmic genomes.

Our data suggest that MxB senses herpesviral capsids on their portal caps to mediate capsid disassembly. This premature release of viral genomes from capsids may enhance the activation of DNA sensors, that play a key role in the amplification and regulation of host innate immune defences to infection.

O 17
The influence of glycation on the expression of the bi-functional enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE)

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GNE-myopathy (GNEM) is a very rare autosomal recessive genetic disorder – prevalence 9:1 000 000 (Carillo et al., 2018) – that results from a variety of more than 200 different mutations in the GNE gene. This gene encodes the bi-functional enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) – the key enzyme of sialic acid biosynthesis. The disease manifests in early adulthood and shows a relatively slow progression characterized by an atrophy of distal and later also proximal skeletal muscles. It leads to most patients

becoming wheelchair dependent. In particular, this progression and the late onset of the disease indicates an influence of age. Methylglyoxal (MGO) and glyoxal (GO) are two metabolites, which accumulate in cells over time and with aging. Both are glycation agents by interacting with free amino groups of amino acids in proteins and lead to the formation of advanced glycation endproducts (AGEs). Thereby AGEs account in part of age-related tissue dysfunction. To investigate the effect of ageing on GNE expression, murine C2C12 cells were treated with different concentrations of MGO and GO and corresponding qPCR analyzes were performed. It could be shown that a treatment of the cells with the two glycation agents in the non-lethal range did not significantly change the GNE expression, suggesting other age-derived effects on the GNE.

O 18
The S-palmitoylation switch in activated T cells

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While T cell activation has been intensively studied on protein, DNA and RNA level, much less is known about the contribution of lipids and protein-lipid interactions to this process. Nevertheless, several findings (e.g., changes in the cellular lipidome after activation of T cells) suggest that lipids play important roles in T cell activation. To shed light on the contribution of lipid modifications on T cell signaling, we are investigating palmitoylation switches during T cell activation. One of the proteins described to undergo a palmitoylation switch after T cell stimulation, is the type II membrane protein CKAP4 (Morrison et al., 2020). To investigate how dynamic depalmitoylation of CKAP4 affects T cell signaling, we first validated depalmitoylation of CKAP4 in activated T-cells using a click chemistry approach and plan to investigate its functional role by using palmitoylation-deficient mutants. The effects of palmitoylation of CKAP4 on T cell signaling will be examined by measuring phosphorylation signals, calcium mobilization, and cytokine production in combination with cell migration assays. Furthermore, we will investigate the stimulation-dependent dynamics of CKAP4 subcellular localization. We plan to investigate this in T cells by using super resolution microscopy, proximity ligation assays and detergent resistant membrane fractionation. Together, these experiments will help elucidate functional consequences of palmitoylation switches during by T cell activation.

O 19
Toward Reproducible Enzyme Modeling with Isothermal Titration Calorimetry

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To apply enzymes in technical processes, a detailed understanding of the molecular mechanisms is required. Isothermal titration calorimetry (ITC) allows the label-free and highly sensitive analysis of kinetic and thermodynamic parameters of individual steps in the catalytic cycle of an enzyme reaction. The presented ITC-dependent workflow was used to determine these parameters for the cofactor-dependent ketoreductase Gre2p under different reaction conditions. Using a standardized approach with the implementation of sample quality control by DLS, we obtained high-quality data suitable for the advanced modeling of the enzyme reaction mechanism. The results revealed that this enzyme operates with an ordered sequential mechanism in which the cofactor NADPH binds first, which then allows binding of the substrate NDK. In addition, the enzyme was found to be affected by substrate or product inhibition depending on the reaction buffer. Data reproducibility, a mandatory prerequisite to achieve robust modeling, is ensured by specifying standard operating procedures, using programmed workflows for data analysis, and storing all data in a F.A.I.R. repository. Because they can be automated and scaled for high-throughput, the combination of different approaches such as the one presented will provide the high-quality data needed for the development of enzymes and biocatalytic processes through machine learning to accelerate the future development of industrial biocatalysis.

O 20
Tracing early stage cancers by detection of specific antigens in blood monocytes using novel antibodies

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Detection of malignant lesions in an as early as possible stage is of paramount importance for the patients' chances of survival and cure. In addition to high sensitivity and specificity, approaches to achieve this task ideally have to be fast, non-invasive and economic so that they can potentially be applied in a patient screening format.

Intense research has been devoted to the the employment of blood samples ("liquid biopsies") to detect circulating tumour cells or protein markers, DNA or RNA indicative of cancerous tissue within the body. Striving for enhanced sensitivity, the recently introduced Epitope Detection in Monocytes (EDIM) technique exploits the naïve immune system's property of accumulating

potentially harmful antigens in blood monocytes. Cytometric determination of general cancer-associated antigens TKTL1 (Transketolase-like 1) and Apo10 (epitope of DNAse X) using fluorescent antibodies can reveal occult malignomas with noteworthy efficiency. However, this technique does not disclose information on type and tissue localisation of detected tumors.

Addressing this shortcoming, we generated and characterized panels of novel antibodies to specific tissues and tumor entities and employed them to the cytometric analysis of blood monocytes from cancer patients. Using the examples of colorectal and pancreas carcinoma, we show that this approach can identify tumor patients with high sensitivity and reliably discrimitates between malignoma entities.

O 21
Uptake of peptides investigated on a model system of the nasal mucosa

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Pharmacodynamic properties often limit therapeutic application of peptides. Nevertheless, this group of compunds allows an improved pharmacological profile by high target-specificity compared to small molecules, and lead to non-toxic degradation products. Further, allergic reactions are rare. However, pharmacodynamics improvement of peptides is contrasted by rapid degradation and low bioavailability. Cell permeation through epithelial (e.g. intestinal and nasal mucosa) and endothelial (e.g. blood brain barrier) barriers is a key factor in drug uptake and distribution. Compounds can cross these tissues by different routes, including para- and transcellular as well as passive and active transport mechanism. In vitro models allow to screen a high number of peptides for permeability and transport mechanism. Differentiated Calu-3 cells provide properties of respiratory epithelium. We have applied this model system to different peptides with fluorescent labels and stabilizing motives such as lipidation and non-natural amino acids. Permeation of these compounds through Calu-3 cell layer, differentiated by culturing on air-liquid interface, was determined by fluorescence, mass spectrometry and reversed phase high performance liquid chromatography. Further, subcellular localization was analyzed with fluorescence microscopy. Interestingly, minor changes, such as different lipidation forms, display major effects on cellular uptake and permeation.

T 01
Chemical optimization of miR-146a mimic for the treatment of Graft versus Host disease

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Mice transplanted with miR-146a-/- T cells show more aggressive GvHD. Pretreatment of T cells a commercial miR-146a mimic (nucleofection), lower GvHD mortality and severity can be achieved. We generated cholesterol-conjugatedmiR-146a mimics spanning chemical scaffolds from miRNA-like to siRNA-like in order to identify a clinically relevant scaffold for GvHD therapy. We tested the impact of the length of the sense strand, and the complementarity of the sense strand on miRNA efficacy in reporter and in functional assays. We cloned a fully complementary target site 4x in tandem into the 3'UTR of a dual luciferase reporter system to test an siRNA-like effect. In this fully complementary target setting a shorter sense strand or a fully complementary sense strand led to less efficient silencing. Unexpectedly, a more siRNA-like structure induced poorer siRNA-like effect. Then, we used an in vitro model of GvHD, mixed lymphocyte reactions, to compare T cell inhibition functionality of miRNA mimics. We saw that a further enhancement could be achieved by increasing the number phosphorotioate (PS) modifications in the antisense strand. We achieved the most potent T cell inhibition (up to 80 %, IC 50 216 nM) using a full length fully complementary sense strand and increased PS in the antisense strand. We demonstrate that some elements of siRNA chemical design augment miRNA mimic efficacy in a functional setting, and antisense strand metabolism is rate-limiting for miRNA functionality.

T 02
siRNA-mediated downregulation of WAPAL inhibits T cell proliferation upon allogeneic stimulus

Presenting author: **Anastasia Kremer**

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Graft-versus-Host-Disease (GvHD) is a common complication after allogeneic hematopoietic stem cell transplantation where alloreactive T cells recognize and attack host antigens. Current GvHD drugs have widespread toxicities arising from unspecific inhibition of immune cell function and proliferation. Wings apart-like homolog (WAPAL) emerged as a potential regulator of GvHD. Another therapeutic moiety, extracellular vesicles (EVs) derived from mesenchymal stem cells (MSC) have been shown to 1) facilitate siRNA delivery to difficult-to-transfect cell types and 2) to improve GvHD. Here I used fully chemically modified siRNA to silence WAPAL and assessed its effect on T cell proliferation in cell culture and in an allogeneic

in a GvH in vitro model. I also tested siRNA formulated into MSC-derived EVs. We screened 12 siRNAs and identified a lead showing an IC50 of 400nM and a maximal silencing of 65% in primary T cells. Cellular fractionation in primary T cells and in Hela showed 30% mRNA localization in the nucleus, potentially explaining the silencing plateau at 65%. WAPAL downregulation inhibited T cell proliferation up to 70% upon allogeneic stimulus (co-incubation with irradiated allogeneic dendritic cells). Upon formulation into EVs, the inhibitory effect could be enhanced to nearly 100%. Interestingly, when T cells were stimulated with activation beads, cell proliferation was not affected by WAPAL silencing, suggesting an allogeneic-stimulus-specific role of WAPAL.

T 03
Gene expression signatures can predict SARS-CoV-2 T cell receptor functionality

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T cell immunity is crucial in conveying protective immunity against infection with SARS-CoV-2. Especially $CD8^+$ T cells play a major role in combating SARS-CoV-2 infections. To better understand and predict the level of protection, it is of utmost importance to characterize in more depth the quality of recruited $CD8^+$ T cell receptors (TCRs) and the functional profile of often highly polyclonal antigen-specific T cell populations.

To achieve this, we identified SARS-CoV-2-specific $CD8^+$ TCRs from mild COVID-19 patients to immunogenic epitopes of different HLA restrictions. Re-expression of those TCRs via CRISPR/Cas9-mediated orthotopic TCR replacement (OTR) in healthy donor PBMCs allowed us to perform extensive structural and functional characterizations. Combining these data with single cell RNAseq, we identified 'gene signatures of recent activation' to differentiate high and low functional TCRs. We demonstrate that these 'gene signatures of activation' can predict TCR functionality.

We then challenged this model by performing experimental validation on a set of TCRs isolated from a second single cell RNAseq experiment; and we could show that affinity, functionality and cellular avidity, which is assumed to predict in vivo functionality, correlate with our predictive scores. We hope that by combing different TCR characterization methods, we can speed up the process of bringing the most effective TCRs into clinic for SARS-CoV-2 as well as for other viral and non-viral diseases.

T 04
Image guided cell therapy: How PET-imaging can improve CAR-T treatments

Presenting author: **Katja Fritschle**

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Chimeric Antigen Receptor (CAR)-T cell therapies were first approved in 2017 and are increasingly entering clinical development and routine care. However, prospectively these therapies necessitate a reliable method to analyze preclinical animal experiments and to monitor therapy outcomes in patients. The use of a universal reporter gene for these applications would facilitate and accelerate the implementation of CAR-T therapies. We have developed a novel reporter gene system encoding a radiochelate-binding protein DTPA-R that binds the radioligand [^{18}F]F-DTPA [see Abstract by V. Morath]. Based on an existing αCD19-CAR-T product, the reporter gene was genetically integrated replacing the second membrane protein on these CAR-T cells (EGFRt). The reporter protein can also be used for magnetic activated cell sorting (MACS) in the production process. Functionality was confirmed by comparing modified CAR-TDPTA-R and original CAR-TEGFRt cells. The reporter protein did not alter proliferation, viability or cytotoxic effector function as confirmed by flow cytometry assays and killing assays. PET studies of CD19 lymphoma bearing mice allowed quantitative tracking of these CAR-T cell over a 30-day period providing information on the precise location and concentration of therapeutic cells. Different ex vivo validations using the V5-tag confirmed the results. In summary, this novel reporter gene provides a promising tool to accelerate the development of CAR-T therapies.

T 05
Targeted adenovirus-mediated transduction of human T cells in vitro and in vivo

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Clinical success in T cell therapy has stimulated widespread efforts to increase safety, potency and extend this technology to solid tumors. Yet progress in cell therapy remains restricted by

the limited payload-capacity, specificity of target cell transduction and transgenic gene expression efficiency of applied viral vectors. This renders complex reprogramming or direct in vivo applications difficult. Here, we developed a synergistic combination of trimeric adapter constructs enabling T cell-directed transduction by the human adenoviral vector serotype C5 in vitro and in vivo. Rationally chosen binding partners showed receptor-specific transduction of otherwise non-susceptible human T cells by exploiting activation stimuli. This platform remains compatible with high-capacity vectors for up to 37 kb DNA delivery, increasing payload capacity and safety due to the removal of all viral genes. Together, these findings provide a tool for targeted delivery of large payloads in T cells as potential avenue to overcome current limitations of T cell therapy.

T 06
Fast identification of clincally relevant T cell receptors for adoptive T cell therapy

Presenting author: **Elvira D´Ippolito**

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T cell engineering with antigen-specific T cell receptors (TCRs) allows the generation of increasingly specific and reliable T cell products for cancer and viral infections. However, the number of TCRs available for clinical use is still limited and often of suboptimal potency. Here, we developed platforms to support a rapid selection of candidate TCRs for therapy from donor material.

The highly diverse antigen-unexperienced TCR repertoire of healthy donors represents a suitable source for identifying tumor-specific TCRs. Rare antigen-specific naïve T cells are enriched from large-size T cell apheresis and single-cell sorted according to pMHC class I multimer staining. During cell sorting, our developed flow cytometry-based functional screening estimates the structural avidity of each individual pMHC multimer-reactive TCR, which correlates with functionality. Altogether, epitope-specific TCRs can be isolated and concurrently ranked according to predicted avidity/functionality. Virus-specific TCRs are, instead, more proficiently isolated from seropositive donors. Considering the higher frequencies of these memory repertoires, we developed a high-throughput, single-cell sequencing-based approach capable of discriminating highly functional and bystander TCRs according to transcriptional shifts in T cell activation genes induced by recent peptide stimulation prior to cell sorting (Wagner et al., Cell Reports 2022; Mateyka et al., Vaccines 2022).

T 07

Seeing is believing: Imaging of cell and gene therapies using a novel PET reporter gene system

Presenting author: **Volker Morath**

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Advanced Therapy Medicinal Products (ATMPs), such as cell and gene therapies necessitate a reliable diagnostic method to image transgenes quantitatively. We developed a novel positron emission tomography (PET) reporter gene system consisting of a membrane-anchored Anticalin binding protein (DTPA-R) and a corresponding radiohybrid ligand [^{18}F]F-DTPA•terbium. The simple design of the reporter protein yields high receptor densities of up to ~1×10^{6} receptors per cell, small gene size and high affinity binding of [^{18}F]F-DTPA. The physical detection limit for JurkatDTPA^{-}R is 500 cells. [^{18}F]F-DTPA showed a high tumor uptake of 22.1 %ID/g in PC3DTPA^{-}R xenografts compared to 0.2 %ID/g for a DTPA-R negative tumor 90 min p.i. (ratio = 125) and cleared nearly exclusively via the renal route . The reporter system allowed the quantitative, depth-independent imaging of gene therapy with AAV9 viral vectors with a linear dose-to-signal relation. Expansion and migration of CD19-CAR-T cells in a systemic Raji animal model was monitored over a four-week therapy, demonstrating a linear relationship between PET signal and CAR-T cell number. Furthermore, immunohistochemistry of these animals confirmed that PET imaging allowed identification of CAR-T infiltration into individual vertebrae. Our novel & proprietary reporter gene system is a promising tool to elevate the understanding of cell and gene therapies and support the development of precision medicine.

Thu., March 23, 09:00

Antibody Evasion Properties of SARS-CoV-2 Variants

Presenting author: **David Ho**

The COVID-19 pandemic has been devastating to the human population over the past three years. The scientific community has responded brilliantly in rapidly developing effective treatments and preventive measures, including highly protective vaccines against the etiologic agent, SARS-CoV-2. However, this virus has continued to evolve, producing variants that not only escape from our antibody responses but also capable of more efficient transmission. Earlier variants like Beta and Delta already led to greater antibody resistance, resulting in lower degree of protection against symptomatic infection by SARS-CoV-2. But this challenge became greatly exacerbated by the advent of the Omicron variant that was dramatically more resistant to antibody neutralization. Its subsequent subvariants (BA2, BA4/5) again developed even greater antibody resistance while gaining a transmissible advantage. The most recent dominant Omicron subvariants (BQ.1.1 and XBB.1.5) are the most extreme in evading existing antibodies. Essentially, they are refractory to neutralization by all authorized/approved monoclonal antibodies; they are also about 100-fold more resistant to neutralization by vaccinee sera when compared with the ancestral strain of SARS-CoV-2. Yet by detailed examinations of how the virus is evolving to antibodies, there are indeed strategies we could employ to attack conserved sites on the viral spike and to improve our chance of countering future variants.

Thu., March 23, 09:30 & Poster I 18

Design and generation of antibody-drug conjugates using non-canonical amino acids

Presenting author: **Marlitt Stech**

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The concept of antibody-drug conjugates (ADCs) is theoretically simple, but it is difficult to combine their components into an optimized and functional therapeutic agent. With most conjugation technologies, the choice of the conjugation site is limited, or pre-functionalization of the conjugation site is required. This leads to limited possibilities of optimization and extensive downstream processes, respectively. Against this background, we develop biochemical tools for the site-specific modification of antibodies using the amber suppression technology. We use two different approaches: Mammalian cell-based as well as novel mammalian cell-free systems. Here we demonstrate the synthesis of "ready-to-conjugate" antibodies containing site-specifically introduced 2-azidoethoxycarbonyl-L-lysine (AECK) by using an orthogonal tRNA/synthetase pair from the archaea Methanosarcina mazei in a

mammalian cell-free system for the first time, as well as in a cell-based system. Using the cell-free system, we show the efficient one-step synthesis of radiolabeled and ready-to-click antibody. Using cell-based expression, we investigated the developability of an antibody-drug conjugate and we proof the position-specific coupling of DBCO-PEG3-MMAE to cell-based produced AECK labeled antibodies by hydrophobic interaction chromatography.

Thu., March 23, 09:45

Immune-Cell Engagement in Tumour Therapy: From Bispecific to Multispecific Antibodies

Presenting author: **Harald Kolmar**

Therapeutic strategies for cancer treatment have led to the rise of bispecific and multispecific antibodies in recent years that are now gaining importance due to their power of binding multiple tumor-associated antigens (TAA) simultaneously and/or redirecting immune cells to the tumor microenvironment to enhance anti-tumor activities and combining different mechanisms of action. The design and generation of multifunctional antibodies is often hampered by the structural complexity of these molecules that require correct synthesis and assembly of different heavy and light chains thereby limiting their developability. We recently established a strategy for the generation of antibodies, where the light chain and the heavy chain binds different target proteins simultaneously. Furthermore, we established a procedure for expeditious generation of so-called common light chain antibodies where target binding is mainly mediated by the variable domain of the antibody heavy chain. By combining these formats and strategies we were able to generate tetrafunctional antibodies with prescribed binding characteristics aimed at optimizing TAA binding, immune cell engagement and simultaneous mitigating unwanted side effects such as overshooting cytokine release.

Thu., March 23, 10:45

Engineering antibodies for immune stimulation

Presenting author: **Mark Cragg**

Agonistic antibodies directed to immunostimulatory receptors are a currently untapped source for immunotherapy. Whereas checkpoint blockers have translated into the clinic, the rules for agonistic antibodies have been more difficult to discern and these reagents await further optimisation. Here we highlight the salient properties of monoclonal antibodies (mAb) required to strongly agonise these receptors and discuss potential strategies for leveraging them for immune activation and anti-tumour efficacy. Using TNFR superfamily receptors as a paradigm the following key aspects will be discussed: The role of isotype; properties of the epitope; the importance of antibody hinge flexibility and the impact of affinity on delivering receptor agonism.

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Thu., March 23, 11:15 & Poster I 19

Droplet microfluidic antibody screening platform for functional antibodies at the single-cell level

Presenting author: **Leonie Kolmar**

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Antibody screening has become an essential role in modern medicine. Screenings are usually limited to a small subset of target proteins and are based on antibody binding, which does not necessarily correlate with their functionality. Being able to assess the effect of thousands of antibodies on the transcriptome of target cells would allow target-agnostic screens for a variety of functions in a highly multiplexed way. While droplet microfluidic systems have already become a powerful tool for single-cell RNA sequencing and antibody discovery, many key problems remain. First, the sparsity of the single-cell RNA sequencing data hardly allows the detection of individual outliers showing differential gene expression after exposure to an antibody. Furthermore, there are currently no systems for the detection of altered single-cell transcriptomic signatures after stimulation in droplets. Here we address all these challenges and conceptualize a microfluidic workflow for in-droplet stimulation of target cells with antibodies expressed by antibody-secreting cells. In addition, we developed a computational workflow that can detect outliers that remain hidden in commonly used clustering approaches. Lastly, we investigated the effect of labeling newly synthesized mRNA to distinguish between transcriptomic signatures before and after antibody stimulation of a target cell. Taken together, this should pave the way for RNA sequencing-driven functional antibody discovery.

Thu., March 23, 11:30

In vivo engineered B cells secrete high titers of broadly neutralizing anti-HIV antibodies in mice

Presenting author: **Adi Barzel**

Transplantation of B cells engineered ex vivo to secrete broadly neutralizing antibodies (bNAbs) has shown efficacy in disease models. However, clinical translation of this approach would require specialized medical centers, technically demanding protocols and major histocompatibility complex compatibility of donor cells and recipients. Here we report in vivo B cell engineering using two adeno-associated viral vectors, with one coding for Staphylococcus aureus Cas9 (saCas9) and the other for 3BNC117, an anti-HIV bNAb. After intravenously injecting the vectors into mice, we observe successful editing of B cells leading to memory retention and bNAb secretion at neutralizing titers of up to 6.8 μg ml-1. We observed minimal clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 off-target cleavage as detected by unbiased CHANGE-sequencing analysis, whereas on-target cleavage in undesired tissues is reduced by expressing saCas9 from a B cell-specific promoter. In vivo B cell engineering to express therapeutic antibodies is a safe, potent and scalable method, which may be applicable not only to infectious diseases but also in the treatment of noncommunicable conditions, such as cancer and autoimmune disease.

Thu., March 23, 14:30

Enhancing endogenous and synthetic immunity with engineered antibody-fusion proteins for cancer immunotherapy

Presenting author: **Pablo Umaña**

A new generation of engineered fusion proteins is currently being developed to enhance endogenous and synthetic immunity approaches for cancer immunotherapy The talk with cover both a PD-1-cis-targeted IL-2 variant used to differentiate antigen-specific, stem-like T cells into better effectors, and also the combination of tumor-targeted costimulatory receptor agonists with T-cell engaging bispecific antibodies as an off-the-shelf approach for enhanced T cell redirection. Both of these approaches are currently being tested in first-in-class clinical trials.

Thu., March 23, 15:00

Novel developments on immunocytokines

Presenting author: **Dario Neri**

Antibody-cytokine fusions (also called “immunocytokines”) are a new class of biopharmaceuticals, which are being considered for the therapy of cancer and of other serious conditions [Neri (2019) Cancer Immunol. Res., 7, 348]. When designing immunocytokine products, a number of parameters have to be considered, including the choice of antigen and the format for the antibody-cytokine fusion.

In this lecture, I will review antibody-cytokine fusions which have been developed at Philogen [www.philogen.com] and which are being investigated in clinical trials in patients with cancer. Specifically, I will focus on immunocytokines based on interleukin-2, interleukin-12 and tumor necrosis factor as payloads. The tumor targeting properties, as well as emerging preclinical and clinical findings, will be discussed.

Thu., March 23, 16:00

T cell Engaging Cancer Immunotherapies

Presenting author: **Paul Adam**

Therapeutic antibodies that antagonize immune cell checkpoint proteins such as PD-1 and PD-L1 have established a new paradigm for cancer treatment. However, the clinical effectiveness of checkpoint inhibitor therapy is generally restricted to patients whose tumors are recognized as foreign by their cytotoxic immune cells, with immunogenicity typically due to high mutational burden in cancer cells. Most tumors across cancer indications are poorly immunogenic and this has led to the discovery and development of therapeutic strategies that can promote the patients' cytotoxic immune cells to recognize and attack tumor cells. T cell Engaging bispecific antibodies (TcEs) that can re-direct cytotoxic T cells selectively to tumor cells have been validated in clinical studies for hematologic cancers, leading to promise for TcE approaches targeting solid cancer indications that have a more complex tumor microenvironment. This presentation will highlight the discovery of novel TcEs for solid cancers.

Thu., March 23, 16:30 & I 20

Investigating The T Cell Repertoire In Melanoma Immune Checkpoint Inhibitor Therapy Patients and Their Response To Therapy

Presenting author: **Meike Bröckelmann**

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Author(s):
Meike Bröckelmann

In the study presented, changes to $CD4^+$ T Cells, $CD8^+$ T Cells and their reactivity to stimuli by Immune Checkpoint Inhibitor (ICI) Therapy are investigated. Findings are compared to healthy donors and patients' course of therapy, to better predict response to therapy, development of immune-related adverse effects (irAEs), patient benefit and survival.

The study is part of an ongoing Master Thesis.

Tumors can exhibit immune-evasion mechanisms by expressing checkpoint ligands, e.g. PD-L1 and CTLA-4 ligands. This can be abrogated by ICIs, which bind immune checkpoint molecules and prevent their ligands from interacting, restoring anti-tumor responses.

Since 2011, multiple ICIs have been approved for treatment of melanoma in Germany. However, irAEs may arise in up to 45% of ICI patients, depending on the dose, time and type of agent used.

To characterize the T cell department, samples from healthy donors and melanoma patients were intra- and extracellularly analyzed using Flow Cytometry (FACS) by staining surface markers of $CD4^+$ subsets, $CD8^+$ cells and transcription factors Tbet, GATA3, RORγt and FoxP3. So far, characterizing assays have been developed and verified for healthy donors.

Currently, healthy donor samples are being incubated with anti-CD3/CD28 or melanoma cell culture supernatant to investigate their reactivity to stimuli. Results will subsequently be compared to melanoma ICI therapy patients.

Thu., March 23, 16:45

Bayer Pharmaceuticals PhD Prize:
Conformational transitions in NLRP3 inflammasome formation

Presenting author: **Inga Hochheiser**

Innate immune cells recognise invading pathogens via germline-encoded pattern recognition receptors (PRRs). NOD-like receptor protein 3 (NLRP3) is a cytosolic PRR that senses a diverse set of stimuli, including pathogen-derived molecular patterns or endogenous danger signals. Upon activation, NLRP3 forms a multiprotein signalling platform called the inflammasome, which induces the production of proinflammatory cytokines and pyroptotic cell death. NLRP3 is the clinically most implicated inflammasome protein with its overdue activation being involved in a broad range of chronic inflammatory diseases, including atherosclerosis, diabetes and Alzheimer's disease. Although NLRP3 is a highly relevant drug target, the mechanisms leading to NLRP3 activation and the functioning of antagonistic small molecules remain poorly understood. We investigated the structures of different NLRP3 functional states to delineate NLRP3 inflammasome formation. We found that inactive, ADP-bound NLRP3 forms a spherical decamer with a size of 1.2 megadaltons. Binding of the NLRP3-specific antagonist CRID3 at the backside of the NLRP3 nucleotide binding domain locks NLRP3 in its inactive conformation and prevents downstream activation. Active NLRP3 via homotypic pyrin domain (PYD) interactions with its adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) oligomerizes into helical, filamentous assemblies. We determined the structure of the human NLRP3-PYD filament, showing that NLRP3- and ASC-PYD filaments exhibit the same symmetry in rotation and axial rise per subunit, allowing a continuous transition between NLRP3 as the nucleation seed and ASC as the elongator. Analysing the NLRP3 to ASC filament transition by cryo-EM, we show that NLRP3-induced inflammatory responses exhibit a particular directionality.

Thu., March 23, 17:00

Karl Lohmann PhD Prize:
Development and Application of Photoswitchable Lipids

Presenting author: **Johannes Morstein**

Photoswitchable lipids are emerging tools for the precise manipulation and study of lipid function. Optical control of lipid structure is attained through the incorporation of a hydrophobic molecular photoswitch (azobenzene) into the fatty acid tails of lipids. I demonstrate that this approach is broadly applicable to glycerolipids, sphingolipids, and other natural and synthetic amphiphilic pharmacophores. These include the sphingolipids ceramide, sphingosine, and sphingosine-1-phospate, the glycerolipids phosphatidic acid, lysophosphatidic acid, and phosphatidylcholine, and multiple nuclear hormone receptor ligands. The applications of these tools include modulation of membrane biophysics, including permeability, fluidity, lipid mobility and domain formation. Photoswitchable lipids are also very useful in lipid physiology and enable optical control of a wide array of lipid receptors, such as ion channels, G protein-coupled receptors, nuclear hormone receptors, and enzymes that translocate to membranes. Enzymes involved in lipid metabolism often process them in a light-dependent fashion. The utility of photoswitchable lipids for the optical control of physiology was demonstrated in a large range of different applications from biochemical and cellular systems to animal models. For example, our photoswitchable analog of sphingosine-1-phosphate has been tested in both cellular assays, and in vivo behavioral assays and was then used by other research groups for the discovery of new signaling function in brain slice models. Photoswitchable lipids are versatile tools that enable optical control of lipid metabolism and function, and meet a critical need to illuminate lipid biology.

Thu., March 23, 19:30

Feodor Lynen Lecture:
Regulated Changes in Lipid Metabolism that Impact T cell Function

Presenting author: **Erika Pearce**

In recent years there has been an intense research focus on ‘immunometabolism’, that is the intracellular metabolic pathways that support or drive immune cell function. We now appreciate that regulated changes in metabolic programs play a major role in immune function. Work from my laboratory investigating lipid metabolism in T cells will be presented, with a focus on recent findings demonstrating phosphoinositides with distinct acyl chain compositions drive important signaling events to first initiate and then sustain effector function during CD8+ T cell differentiation.

Fri., March 24, 09:00

Engineering IL-2 to increase anti-tumor efficacy and mitigate capillary leak

Presenting author: **Martin Oft**

In recent years there has been an intense research focus on 'immunometabolism', that is the intracellular metabolic pathways that support or drive immune cell function. We now appreciate that regulated changes in metabolic programs play a major role in immune function. Work from my laboratory investigating lipid metabolism in T cells will be presented, with a focus on recent findings demonstrating phosphoinositides with distinct acyl chain compositions drive important signaling events to first initiate and then sustain effector function during CD8+ T cell differentiation. High-dose Interleukin-2 (IL-2) monotherapy induces complete responses in cancer patients, but severe acute vascular toxicities limit its application1,2. IL-2 broadly activates all lymphocytes including NK-cells by binding the intermediate-affinity, dimeric IL-2Rβ/γ (CD122/CD132). Upregulation of IL-2Rα on antigen activated T-cells and regulatory T-cells (Treg) leads to the formation of the high-affinity, trimeric IL-2 receptor, IL-2Rα/β/γ (CD25/CD122/CD132) increasing the sensitivity to IL-23. To reduce Treg selectivity, IL-2 variants with reduced CD25-binding (non-α-IL-2) have been studied4, but demonstrate significant toxicities and limited monotherapy efficacy in clinical trials5,6. To avoid this toxicity and deliver IL-2 to tumor specific T cells we develop highly selective IL-2 variants, targeting tumor reactive T cells.

Our novel α/β-biased IL-2s bind selectively to the highly upregulated IL-2Rα/β/γ on antigen activated T-cells resulting in improved efficacy while avoiding vascular toxicity. In contrast, wt-IL-2 and non-α-IL-2 broadly activated all lymphocytes leading to systemic tissue inflammation, pulmonary inflammation and capillary leak syndrome. In contrast, α/β-IL-2s avoided broad systemic lymphocyte activation but resulted in increased intratumoral, tumor specific CD25+PD-1+CD28+CD8+ T cells, eliciting monotherapy complete responses and tumor immune memory. Systemically increased Tregs did not interfere with anti-tumor efficacy, as intratumoral Tregs were inhibited, improving the intratumoral CD8 to Treg ratio.

Our orthogonal IL-2 – IL-2Rb system enables the specific activation of adoptively transferred T cells expressing the human orthogonal IL-2Rb (hoRb). Orthogonal IL-2 (STK-009) does not activate the endogenous IL-2 receptor. As a consequence, adoptively transferred T cells can be expanded in mice for many months, with the persistence of both stem cell memory (TSCM) and effector T cells (TEMRA). The combination of STK-009 with orthogonal CD19-directed CAR T cells (SYNCAR-001) lead to the elimination of bulky B cell lymphoma in mice. Similarly, STK-009 enables orthogonal GPC3-directed CAR T cells (SYNCAR-002) to eliminate large hepatocellular cancers. In addition, orthogonal IL-2 system allows adoptive transfer and expansion of T cells in syngeneic hosts without lymphodepletion of the host.

In summary, IL-2 variants, specifically directed toward the tumor reactive T cell population can unleash the power of IL-2 without the vascular toxicity observed with high dose IL-2 treatment.

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Fri., March 24, 09:30 & C 06

Leveraging chemically-controlled protein switches for increased safety of cytokine-based cancer immunotherapeutics

Presenting author: **Lucia Bonati**

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Cytokines are key signal mediators of the immune system playing an essential role in the orchestration of immune responses. Despite their unique ability to modulate the immune system, the translation of cytokine-based therapies to the clinic has been greatly hindered by severe toxicities due to the pleiotropy and off-targeting effects of many cytokines.

Here, we present a general strategy that enables precise control over cytokine activity. We control their activity by selectively masking the receptor binding site with a fused chemically-responsive domain, which could be unmasked with a competing molecule (Venetoclax). To achieve this, Bcl-2 was fused to the cytokine and the BIM-BH3 interaction motif was transplanted to sites in close proximity to the cytokine's receptor binding site. In absence of Venetoclax, Bcl-2 bound the cytokine with high affinity blocking the interaction site between the cytokine and its receptor. Upon addition of Venetoclax, the interaction between Bcl-2 and the BIM-BH3 motif was disrupted, so restoring the cytokine's activity. We have developed switchable mutants for a range of different cytokines (IL-2, IL-10, and IL-15) used in cancer immunotherapy. Moreover, we showed that in presence of Venetoclax, their activities can be selectively and fully restored.

Overall, this drug-responsive switch strategy may achieve spatiotemporal control of cytokine activities in vivo and thus improve the safety and clinical applicability of cytokine therapeutics.

Fri., March 24, 09:45
Synthetic cytokine signaling

Presenting author: **Jürgen Scheller**

Cytokines control immune-related events and are critically involved in a plethora of physiological and pathophysiological processes including autoimmunity and cancer development. Accordingly, modulation of natural cytokine signaling by antibodies and small molecules has improved therapeutic treatment regimens. Synthetic biology set out to optimize immune therapeutics, with CAR T cell immunotherapy being the first example to combine synthetic biology with genetic engineering during therapy. We have developed a modular fully synthetic cytokine signaling system that precisely orchestrated cellular responses by selectively inducing pro- and anti-apoptotic signaling via canonical dimeric receptors of the IL-6 family and non-canonical trimeric receptor complexes of the TNF superfamily. The latest development resulted in a non-immunogenic full-synthetic cytokine/cytokine receptor pair for in vivo therapeutic strategies involving non-physiological targets during immunotherapy.

Fri., March 24, 10:45
Metabolic reprogramming of exhausted T cells by IL-10 enhances anti-cancer immunity

Presenting author: **Li Tang**

Our immune system interacts with many diseases in a multidimensional manner involving substantial biological, chemical, and physical exchanges. Manipulating the disease-immunity interactions may afford novel immunotherapies to better treat diseases. My lab aims to develop novel strategies to engineer the multidimensional immunity-disease interactions (or termed 'immunoengineering') to create safe and effective therapies against cancer. We leverage the power of metabolic bioengineering, chemistry and material engineering, and mechanical engineering to achieve controllable modulation of immune responses. In this talk, I will first share our discovery of IL-10-Fc as a metabolic reprogramming agent that reinvigorates the terminally exhausted CD8+ tumor-infiltrating lymphocytes leading to eradication of established solid tumors and durable cures. This metabolic reprogramming strategy was also exploited to enhance CAR-T cell therapy to better treat solid tumors. I will next talk about our recent efforts in designing a metabolically armored CAR-T cell that secretes IL-10 to counter exhaustion-associated dysfunction in the tumor microenvironment for enhanced anticancer immunity. Our results establish a generalizable approach to counter T cell dysfunction through metabolic programming, which may overcome a major barrier in the current T cell-based immunotherapy in the clinic.

Fri., March 24, 11:15 & C 05
Engineering of synthetic cytokine chimeras (cytokimeras)

Presenting author: **Puyan Rafii**

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Synthetic cytokines like IC7 have proven beneficial in mouse model diseases like type 2 diabetes devoid of significant side effects. All except one cytokine of the Interleukin (IL-)6 family share gp130 as the common b-receptor chain. Whereas IL-6, IL-11 or ciliary neurotrophic factor (CNTF) require previous attachment to its a-receptor via a binding site I following b-receptor homo- or hetero dimerization, other members of IL-6 family bind b-receptors chains directly via site II and site III. Using IL-6 or IL-11 as a scaffold, we exchanged the gp130 binding site III of IL-6 or IL-11 with the binding site III of leukemia inhibitory factor (LIF) or oncostatin-M (OSM) in a structure-based approach. The engineered cytokine chimeras (cytokimera) GIL-6, GIO-6, and GIL-11 are able to efficiently recruit non-natural receptor complexes resulting in signal transduction and proliferation of factor-depending Ba/F3 cells. Human GIL-11 exhibits cross-reactivity to mouse and rescued IL-6R deficient mice following partial hepatectomy, demonstrating gp130:IL11-R:LIFR signaling efficiently induced liver regeneration. The development of these cytokimera has enabled new potential avenues for therapeutic application by assembling novel artificial cytokine receptor complexes.

Fri., March 24, 11:30
Arming T cells with chemokine receptors for cancer therapy

Presenting author: **Sebastian Kobold**

Engineered T cells have changed the treatment landscape in several hematological malignancies. In contrast, beyond anecdotal reports, no efficacy could be demonstrated to this day in solid tumors. A critical difference to the aforementioned leukemia and other lymphoma is the access of therapeutic cells to cancer cells in tumor tissue. Such environment is typically badly vascularized, hypoxic and tends to exclude inflammatory immune cells. In principle, the entry of immune cells to such tissue is a tightly regulated process involving integrins, chemoattractants (chemokines) and their matching receptors. This process does not seem to be deficient per se in most cancer, as they tend to attract different types of immune suppressive population via said mechanism. A major problem rather seem to be the more selective use of attracting pathways not typically expressed on therapeutic or other pro-inflammatory cells. At the same time, this mismatch also presents a window of opportunity for therapeutic exploitation.

In my talk, I will demonstrate how cancer tissue excludes therapeutic T cells by ligand selection and how this capacity can be restored by introducing adequate chemokine receptors into therapeutic T cells. I will show that both pro- and anti-inflammatory chemokine-chemokine receptor axis can be used for the task. Focussing on pancreatic cancer, I will showcase examples how such strategy can boost chimeric antigen receptor

(CAR) T cell activity in a range of syngeneic, xenograft and patient-derived models in vitro and in vivo and point towards an avenue for clinical translation.

Fri., March 24, 12:20
Designing the Energy System of the Future

Presenting author: **Wolfgang Eberhardt**

The secure, sustainable and affordable supply of energy in the face of a growing population and increasing standard of living is one of the grand challenges of our global society. Rising concerns about air pollution and manmade climate change with not exactly predictable consequences demand innovative new technologies in the way we produce electrical power; heat or cool our buildings, and organize mobility and transport. This has to include all aspects of generation (conversion), transport, storage, and efficient utilization of energy. I will outline a general scenario of our future energy system for the transformation. Developing new concepts and materials for electric power storage in conjunction with renewable energy sources, coupled with efficient distribution systems, are one important aspect to meet this challenge. New technology for transport systems and the generation of chemical fuels without increasing the CO2 content in the atmosphere is another key ingredient.

So far the world is not on a path to accomplish this at any foreseeable time. The measures needed to reduce the CO2 in the atmosphere and to start reversing 'climate change' by 2050 will be outlined. These can be accomplished without seriously sacrificing our standard of living, even in view of a growing population worldwide [1].

[1] W. Eberhardt, 'Designing the Energy System of the Future', ISBN 979845872873-7, Amazon Direct Publishing (2021)

Fri., March 24, 12:50
Photosynthetic hydrogen production

Presenting author: **Kirstin Gutekunst**

Hydrogen is an attractive energy carrier, which can react in fuel cells with oxygen to pure water while releasing energy. Sustainable hydrogen production remains challenging. The most elegant approach is to utilize photosynthesis for water splitting and to subsequently save solar energy as hydrogen. Cyanobacteria and green algae are unicellular photosynthetic organisms that contain hydrogenases and thereby possess the enzymatic equipment for this process. These features of cyanobacteria and algae have inspired artificial and semi-artificial in vitro techniques, that connect photoexcited materials or enzymes with hydrogenases or mimics of these for hydrogen production. These in vitro methods have on their part been models for the fusion of cyanobacterial and algal hydrogenases to photosynthetic photosystems I in vivo, which recently succeeded as proofs of principle.

Fri., March 24, 14:30
CAR-T cells for therapy

Presenting author: **Carl June**

Advances in the understanding of basic immunology have ushered in two major approaches for cancer therapy over the past 10 years. The first is checkpoint therapy to augment the function of the natural immune system. The second uses the emerging discipline of synthetic biology and the tools of molecular biology and genome engineering to create new forms of engineered cells with enhanced functionalities. The emergence of synthetic biology approaches for cellular engineering provides a broadly expanded set of tools for programming immune cells for enhanced function. Barriers to therapy of solid tumors will be discussed.

Reference: Good CR, Aznar MA, Kuramitsu S, et al. An NK-like CAR T cell transition in CAR T cell dysfunction. Cell. 2021;184(25):6081-100.e26.

Fri., March 24, 15:00
Multi-specific hybrid T cell receptors for sensitive targeting of hematologic malignancies

Presenting author: **Stanley R. Riddell**

CAR T cells targeting lineage antigens are highly effective therapeutic options for the treatment of B cell malignancies and multiple myeloma. But despite good initial response rates, long term remissions remain infrequent and in the case of multiple myeloma, 50-60% of treated patients will relapse within 1 year after CAR T cell infusion. This is due to heterogeneity of target antigen expression on tumors, which leads to selection and outgrowth of tumor cells with low levels of antigen and/or loss following immune pressure with CAR therapy. Conventional CARs that encode CD3ζ and a costimulatory domain require high levels of antigen for efficient T cell activation and tumor elimination. By contrast, T cell receptors (TCRs) are 10-100 times more sensitive than CARs as they engage a more complex and diversified signaling machinery following antigen recognition. We and others have designed synthetic hybrid T cell receptors by fusing VH and VL recognition domains to TCR alpha and beta constant chains respectively. When combined with efficient genetic knockout of endogenous TCR chains using base editing, these hybrid molecules provided greater antigen sensitivity than conventional CARs but in current iterations only recognize a single antigen target. We have now addressed the problem of antigen heterogeneity by designing bispecific hybrid receptors composed of two scFvs of different specificities, one fused to the TCRα chain and the second scFv fused to TCRβ chain. Bispecific hybrid receptors for pairs of myeloma antigens (BCMA/SLAMF7; BCMA/GPRC5D; BCMA/CD229) were expressed in primary T cells and bind each of their cognate antigens. Bispecific TCR/CARs conferred T cells with specificity for both targets and were superior for killing heterogeneous tumor cell populations in vitro and in vivo compared to mono-specific CAR- or TCR/CAR T cells.

Furthermore, presence of both target antigens on the tumor cells provided increased functional avidity measured by z-movi assay, increased Ca2+ flux and higher amounts of cytokines compared to tumor cell lines expressing a single antigen. Bispecific hybrid T cell receptors embedded with natural TCR signaling machinery represent a promising therapeutic option to address antigen downmodulation/loss and antigen heterogeneity for treatment of MM and other hematologic cancers.

Fri., March 24, 15:30 & T 07

Seeing is believing: Imaging of cell and gene therapies using a novel PET reporter gene system

Presenting author: **Volker Morath**

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Advanced Therapy Medicinal Products (ATMPs), such as cell and gene therapies necessitate a reliable diagnostic method to image transgenes quantitatively. We developed a novel positron emission tomography (PET) reporter gene system consisting of a membrane-anchored Anticalin binding protein (DTPA-R) and a corresponding radiohybrid ligand [^{18}F]F-DTPA•terbium. The simple design of the reporter protein yields high receptor densities of up to ~1×10^{6} receptors per cell, small gene size and high affinity binding of [^{18}F]F-DTPA. The physical detection limit for JurkatDTPA^{-}R is 500 cells. [^{18}F]F-DTPA showed a high tumor uptake of 22.1 %ID/g in PC3DTPA^{-}R xenografts compared to 0.2 %ID/g for a DTPA-R negative tumor 90 min p.i. (ratio = 125) and cleared nearly exclusively via the renal route . The reporter system allowed the quantitative, depth-independent imaging of gene therapy with AAV9 viral vectors with a linear dose-to-signal relation. Expansion and migration of CD19-CAR-T cells in a systemic Raji animal model was monitored over a four-week therapy, demonstrating a linear relationship between PET signal and CAR-T cell number. Furthermore, immunohistochemistry of these animals confirmed that PET imaging allowed identification of CAR-T infiltration into individual vertebrae. Our novel & proprietary reporter gene system is a promising tool to elevate the understanding of cell and gene therapies and support the development of precision medicine.

Fri., March 24, 15:45

RWE CD19 CART: Efficacy, Safety & Next Steps

Presenting author: **Marion Subklewe**

Abstract not submitted

Fri., March 24, 16:45
Eduard Buchner Prize:
Protein design using deep learning

Presenting author: **David Baker**

Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current-day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch to optimally solve the problem at hand. We now use two approaches. First, guided by Anfinsen's principle that proteins fold to their global free energy minimum, we use the physically based Rosetta method to compute sequences for which the desired target structure has the lowest energy. Second, we use deep learning methods to design sequences predicted to fold to the desired structures. In both cases, following the computation of amino acid sequences predicted to fold into proteins with new structures and functions, we produce synthetic genes encoding these sequences, and characterize them experimentally. In this talk, I will describe recent advances in protein design using both approaches.

Fri., March 24, 17:45
Otto Warburg Medal:
Investigating metabolic switches in cancer in situ with single cell tissue proteomics

Presenting author: **Matthias Mann**

Cancer is a complex disease with heterogenous molecular signatures evolving constantly with the disease. Understanding the disease at single cell resolution detailing the molecular features of the cells within the tumors provides a more accurate diagnosis and prognosis of the disease. Mass spectrometry (MS)-based proteomics has made significant technological advancements over the last decade, and machine learning and AI have also transformed the field of bioscience. In this talk, I will describe our efforts in recent years towards understanding the molecular pathways of cancer using Mass spectrometry-based proteomics. I will introduce our open source AlphaPept software suite for ultra-fast data analysis, and focus on recent developments in MS instruments for single cell analysis. We leveraged these technological breakthroughs to develop a new multidisciplinary workflow for the spatial analysis of tissues at the level of single cell types or states. In Deep Visual Proteomics, we use machine learning and deep learning algorithms to recognize and classify cells in high-resolution visual images, as well as extract them using a laser microscope, followed by ultrasensitive proteomics. This allows us to explore functional cellular heterogeneity in great detail directly in the tissue context. Befitting the theme of this lecture, I

will discuss how the metabolic states of single cancer cells can be interrogated by Deep Visual Proteomics.

Sat., March 25, 09:10

Engineering IgA for cancer therapy with neutrophils

Presenting author: **Mitchell Evers**

Therapeutic antibodies can be applied for a variety of diseases. Strikingly, 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 effective in tumor eradication. Using the FcαRI (CD89) expressed on myeloid cells, IgA antibodies show superior tumor-killing compared to IgG when granulocytes are employed as effector cells.

However, reasons why IgA has not been introduced in the clinic yet can be found in the intrinsic properties of IgA which poses several technical limitations: (1) IgA is challenging to produce and purify, (2) IgA shows a heterogeneous glycosylation profile, and (3) IgA has a relatively short plasma half-life. Next to the technical challenges, pre-clinical evaluation of IgA efficacy in vivo is not straightforward as mice do not naturally express FcaRI. In the presentation, I will provide a concise overview of the latest insights in engineering strategies to overcome the technical limitations of IgA as a therapeutic antibody. In addition, I will show how to engage myeloid-derived suppressor cells against cancer using IgA, elucidate on the key cytokines for IgA antibody therapy in vitro and in vivo and will conclude with IgA for the treatment of neuroblastoma using GD2 as a target.

Sat., March 25, 09:35

Novel immunotherapeutic drugs through computational protein design

Presenting author: **Clara Schoeder**

Recent advances in computational protein design have demonstrated its usability for tasks such as designing self-assembling nanoparticles, ß-barrel membrane proteins or the rapid discovery of picomolar binders for the SARS-CoV-2 receptor binding domain as antivirals. We can further leverage these tools to help design new immunotherapeutic drugs, including stabilized viral glycoproteins for rationally designed vaccines, antibodies and antibody fragments for many applications and change the binding properties of adeno-associated virus capsids for targeted gene therapies. While computational protein design has been based on biophysical and knowledge-derived energy terms in the past, new machine learning

methods are emerging with new capabilities. In this study, protein design in Rosetta was combined with the prediction of post-translational modifications using artificial neural networks. We integrated these models in the Rosetta framework, allowing the access to these predictions during design. With this, it is both possible to enrich for intended post-translational modifications while altering the sequence, e.g. for the design of N-linked glycosylation, but also to decrease the occurrence of unintended modifications sites, such as deamidations of asparagine. This new method will be applied during epitope-focused immunogen design for influenza virus vaccines and for the stabilization of antibody therapeutics.

Sat., March 25, 10:30
Recent developments in genetic engineering of T cells

Presenting author: **Alessia Potenza**

The development of genetic engineering technologies dramatically changed the landscape of Adoptive T cell therapy (ACT) for cancer making this treatment accessible to an unprecedent number of patients and tumor types. By inserting a chimeric antigen receptor or an exogeneous tumor reactive T cell receptor into patient's T cells, the specificity can be precisely redirected toward selected tumor antigens. By permitting multiple gene disruption and targeted gene integration, genome editing tools have further increased the range of opportunities for ACT. With selected biotechnological tools and protocols, such as CRISPR/Cas9 and base editing, we could potentially endow T cells with the ability to infiltrate the tumor mass, recognize relevant tumor antigens, persist as memory cells, and resist the immunosuppressive signals present in the tumor microenvironment. This is of major importance in solid tumors, where exhaustion mechanisms, phenotypically resulting in the co-upregulation of multiple inhibitory receptors (IRs), impede the functional capacity of T lymphocytes against tumor cells. In liver metastases from colorectal and pancreatic cancers, we identified and harnessed the drivers of T cell exhaustion to enhance the potency of adoptively transferred T cells. After selection of the proper target antigen and the consequent identification of a panel of tumor-specific TCRs, we proved how TCR-redirected, IRs-disrupted T cells display a functional advantage in eliminating patient-derived tumor organoids in vitro and in vivo. Challenges and opportunities towards the generation of optimal T cell therapy products will be discussed.

Sat., March 25, 11:00
Synthetic Biology in Cell Therapy Engineering

Presenting author: **Yvonne Chen**

The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has demonstrated clinical efficacy in the treatment of advanced cancers, with anti-CD19 CAR-T cells achieving up to 90% complete remission among patients with relapsed B-cell malignancies. However, challenges such as antigen escape and immunosuppression limit the

long-term efficacy of adoptive T-cell therapy. Here, I will discuss the development of and clinical data on next-generation T cells that can target multiple cancer antigens and resist antigen escape. I will also present recent work on tuning CAR signaling activities via rational protein design to achieve greater in vivo anti-tumor efficacy. This presentation will highlight the potential of synthetic biology in generating novel mammalian cell systems with multifunctional outputs for therapeutic applications.

Sat., March 25, 11:30 & T 06

Fast identification of clincally relevant T cell receptors for adoptive T cell therapy

Presenting author: **Elvira D´Ippolito**

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Author(s):
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T cell engineering with antigen-specific T cell receptors (TCRs) allows the generation of increasingly specific and reliable T cell products for cancer and viral infections. However, the number of TCRs available for clinical use is still limited and often of suboptimal potency. Here, we developed platforms to support a rapid selection of candidate TCRs for therapy from donor material.

The highly diverse antigen-unexperienced TCR repertoire of healthy donors represents a suitable source for identifying tumor-specific TCRs. Rare antigen-specific naïve T cells are enriched from large-size T cell apheresis and single-cell sorted according to pMHC class I multimer staining. During cell sorting, our developed flow cytometry-based functional screening estimates the structural avidity of each individual pMHC multimer-reactive TCR, which correlates with functionality. Altogether, epitope-specific TCRs can be isolated and concurrently ranked according to predicted avidity/functionality. Virus-specific TCRs are, instead, more proficiently isolated from seropositive donors. Considering the higher frequencies of these memory repertoires, we developed a high-throughput, single-cell sequencing-based approach capable of discriminating highly functional and bystander TCRs according to transcriptional shifts in T cell activation genes induced by recent peptide stimulation prior to cell sorting (Wagner et al., Cell Reports 2022; Mateyka et al., Vaccines 2022).

Sat., March 25, 11:45

Making a tumor produce highly potent drugs

Presenting author: **Andreas Plückthun**

There are several fundamental limitations in how therapeutic proteins are used today. First, they are usually applied individually and not in combinations. Secondly, when used systemically, they -- at best -- preferentially localize to the site of action, never exclusively. And third, molecules with high systemic toxicity cannot be used, even if they are very efficacious.

To solve all these problems, we have used several protein engineering technologies to devise a new platform, termed SHielded, REtargeted ADenovirus (SHREAD). It is based on virus-like particles that are devoid of any viral genes, but contain 36 kb of DNA that can encode multiple genes and complex regulatory regions. To target particular cells and organs, an adapter strategy has been devised, based on the DARPin platform, to selectively target any surface receptor of interest. To hide the particles from the immune system and to minimize liver targeting, a shield was developed based on a trimerized single-chain Fv fragment, covering the facets of the icosahedron.

Applications will be shown to target tumors in vivo via different cell types and express therapeutic antibodies in situ. Additionally, several therapeutic cytokines were expressed simultaneously, to modulate the tumor microenvironment. Finally, experiments will be discussed to infect and reprogram T-cells in vivo. We believe that this versatile technology holds great promise to change the paradigm of precision delivery of therapeutic proteins.

Sat., March 25, 12:15

Antibiotic resistance - challenge and new therapeutic perspectives

Presenting author: **Gregor Fuhrmann**

Bacterial resistance to clinically used antibiotics has increased significantly in recent decades. Worldwide, the number of annual deaths is estimated at 700,000 and up to 15,000 in Germany, making antibiotic resistance one of the three major threats to humanity, according to the World Health Organisation. A promising way to address this challenge is the discovery of new natural products in combination with their efficient delivery to the site of infection. We are studying vesicles of non-pathogenic bacteria that are naturally loaded with antimicrobial compounds. In my talk, I present their activity against planktonic and intracellular pathogens as well as against biofilms and discuss current challenges in further development.
