LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN

CAMPUS INNENSTADT

KINDERKLINIK UND KINDERPOLIKLINIK IM DR. V. HAUNERSCHEN KINDERSPITAL

Thursday, July 4th 2019, 16:00

Dr. von Hauner Children`s Hospital

Klinikum der Universität München

KUBUS and inner yard

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- 16:00 to 16:15 Welcome address
- 16:15 to 18:15 Poster session
- 18:30 to 20:00 Dinner
- 20:00 to 20:30 Poster prize
- 20:30 to 22:00 Party

Organizing committee

Dr. Carolin Ruther
Persönliche Referentin Direktion
Kinderklinik und Kinderpoliklinik im
Dr. von Haunerschen Kinderspital

Dr. Maria Izquierdo-Arcusa
Project Manager International Affairs
Kinderklinik und Kinderpoliklinik im
Dr. von Haunerschen Kinderspital

Dr. Katharina Dennemarck
Projektmanagement, Koordination i-Target
Abteilung für Klinische Pharmakologie

Welcoming words

Dear colleagues,

We are very pleased to welcome you to the 2nd i-Target-Kubus Conference.

This conference brings together the hosting Divisions of Pediatrics and Clinical Pharmacology to share enthusiasm for translational science and to build bridges across disciplines. Members of the international doctoral program i-Target (Immunotargeting of Cancer), the Else Kröner Fresenius Clinical Research School Munich and more than 20 research groups will convene to learn about each other's research fields, build collaborative bridges, and understand disease-driven research from various perspectives.

To generate a stimulating environment and scientific discussions, there will be three poster sessions, followed by social events. The best poster presentations will be awarded a prize.

We are looking forward to connect people and their research to give rise to prospective support, enhancement and interdisciplinary exchange among all different projects and their inventive groups.

Prof. Dr. Stefan Endres

Prof. Dr. Christoph Klein

Posters

No.	Presenter	Group	Title
1.	Mohamed-Reda Benmebarek	Kobold/Endres	**Bispecific antibodies that drive synthetic agonistic receptor - transduced T cells to mediate specific and conditional therapy in human pancreatic cancer models**
2.	Daniel Böhmer	König/Schnurr	**Immunostimulatory double-stranded RNA triggers anti-tumoral cytotoxicity through RLR-independent signaling**
3.	Yanxin Fan	Klein	**Location matters: the role of HAX1 protein in mitochondria – implications for differentiation of neutrophil granulocytes and severe congenital neutropenia (SCN)**
4.	Dr. Julia von Frowein	Schmid	**MiR-492 regulates metastatic properties of hepatoblastoma via CD44**
5.	Maryam Ghalandary	Kotlarz/Klein	**Analysis of NOD2 sequence variants in patients with primary immunodeficiency**
6.	Kathrin Gürlich	Koletzko	**Associations of sleep duration with internalizing and externalizing behaviour in eight-year-old children in the European Childhood Obesity Project**
7.	Dr. Sebastian Hesse	EKFS Alumni	**Multi-omics analysis of human neutrophil granulocytes in monogenic disease**
8.	Duc Huynh	Kobold/Endres	**Dominant negative transforming growth factor β receptor 2 enhances T cell efficacy in a murine pancreatic tumor model**
9.	Ermioni Kalfopoulou	Hübner	**Production of mouse monoclonal antibodies against enterococcal polysaccharides**
10.	Johana Krusche	Schaub	**DUSP1 plays a pivotal role in MAPK signaling in childhood asthma development and environment-mediated**

			protection
11.	Kristina Laubhahn	Schaub	**Pathway analysis - Genetic and immunological influences of gene polymorphisms in 17q21 locus on childhood wheeze**
12.	Diana Laverde	Hübner	**Identification of novel polysaccharide targets from *Enterococcus faecium***
13.	Stefani Lesch	Kobold/Endres	**Engineering tumor specific T cells with CXCR6 enables access to CXCL16-producing solid tumors**
14.	Yue Li	Klein	**Inherited loss-of-function of RIPK1 causes immunodeficiency and intestinal inflammation**
15.	Yang Li	Griese	**Metabolic labelling of choline phospholipids probes ABCA3 transport in lamellar bodies**
16.	Dr. Thomas Magg	Hauck	**Heterozygous OAS1 Gain-of-Function Variants Cause a Polymorphic Autoinflammatory and Immunodeficiency Syndrome**
17.	Jair Marques	Koletzko	**GC QTOF Profile of stool metabolome and machine learning algorithms - a pilot study to diagnose Crohn's disease**
18.	Dorothea Mock	Braun	**Defining Brain Cancer Vulnerabilities with CRISPRi Screens**
19.	Natascha Röhrle	Anz	**GM-CSF is a potent inducer of the Treg-attracking chemokine CCL22**
20.	Anne Maria Senz	König/Schnurr	**Indoleamine-2,3-Dioxygenase in T cells**
21.	Kolja Siebert	Schwerd	**Impact of exclusive enteral nutrition on microbiome signatures and function in pediatric Crohn's disease**
22.	Tanja Stief	Feuchtinger	**TCR reprogramming for the treatment of refractory adenovirus infections**
23.	Dr. Megumi Tatematsu	Klein	**Genome wide knockout screening to identify the novel genes involved in the ER function and secretory pathway**

24.	Martina Totzauer	Koletzko	**Cesarean section and its impact on childhood BMI modified by infant feeding: 8 years follow-up of the CHOP trial**
25.	Alexandra Wagner	Kappler	**SP8 promotes an aggressive phenotype in hepatoblastoma**
26.	Dr. Semjon Willier	Feuchtinger	**Analysis of bone marrow CD8 T cells from pediatric leukemia patients reveals targets for improved adoptive T cell therapy**
27.	Magdalena Zaucha	Rothenfußer	**Yellow fever vaccination as a model to study the immune response to the acute viral infection**

Bispecific antibodies that drive synthetic agonistic receptor - transduced T cells to mediate specific and conditional therapy in human pancreatic cancer models

Mohamed-Reda Benmebarek[1] C. Karches[1], M.L. Schmidbauer[1], M. Kurzay[1], R. Klaus[1], M. Geiger[2], F. Rataj[1], B. Cadilha[1], S. Lesch[1], C. Heise[1], R. Murr[2], J. vom Berg[3], M. Jastroch[4], D. Lamp[4], G. Niederfellner[5], C. Sustmann[5], S. Endres[1], C. Klein[2] and S. Kobold[1]

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Background: Despite marked success in haematological malignancies, the inadequate delivery of T cells into the tumour milieu, as well as a lack of specificity and persistence of their action has hindered the potential of adoptive T cell therapy (ACT) in solid tumors. To overcome these issues via a more controlled approach, we propose to arm T cells with synthetic agonistic receptors (SARs) that are conditionally activated only in the presence of a target tumour associated antigen, and a cross-linking bispecific antibody (BiAb) specific for both (SAR) T cell and tumour cell.

Material and methods: A SAR composed of an extracellular EGFRvIII, trans-membrane CD28, and intracellular CD28 and CD3z domains was fused via overlap-extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. We validated our approach in three human cancer models expressing our target antigen mesothelin (MSLN). We confirmed conditional and specific stimulation and proliferation of our T cells, as well as their tumour-antigen- directed cytotoxicity, in vitro and in vivo. We further investigated the safety profile of our approach in vitro and in vivo.

Results: Crosslinking MSLN-EGFRvIII BiAb, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation of SAR-T cells and directed tumour cell lysis with specificity towards three different MSLN-expressing human pancreatic cancer cells. In vivo, anti-tumoural activity was mediated by the co-administration of SAR-T cells and BiAb, in three pancreatic cancer cell xenograft models. In this treated group, SAR T cells were shown to infiltrate and persist within solid tumors. We could further demonstrate reversibility of T cell activation upon antibody depletion in vivo and in vitro.

Conclusion: Here we describe a novel approach in ACT that delivers specific and conditional activation of agonistic receptor transduced T cells, and targeted tumour cell lysis. It is mediated via a modular platform, which is fundamental in our drive towards personalised immunotherapies. Further, with safety concerns aplenty, this

approach offers an intrinsic safety switch through its BiAb facet. Moreover, by using an approach that specifically targets the transduced T cell population, we demonstrate potential to circumvent pan-T cell activation.

Double-stranded RNA triggers cell death through an RLR-dependent priming and an RLR-independent effector phase

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Introduction: Cytoplasmic double-stranded RNA released by many viruses during infection is sensed by RIG-I-like receptors (RLR). Activation of RLR signaling ultimately leads to the induction of Type I interferons (IFN), proinflammatory cytokines and apoptosis. While IFN and cytokine induction through RLR signaling is well described, there is contradictory data on the mechanisms leading to cell death. Particularly the mechanisms of IFN induction have not been convincingly discriminated from cell death pathways. In the study presented, we elucidate the differential signaling mechanisms downstream of RLR activation leading to either cytokine secretion and/or cell death induction.

Results: IFN production and cell death were strongly dependent on intact RLR signaling. Surprisingly, co-culturing KO and wildtype cells or priming cells with IFN rescued the ability of RIG-I-, MAVS- and IRF3-deficient cell lines to undergo apoptosis in response to 3p-RNA. Affinity purification followed by mass spectrometry revealed 3p-RNA specific binding of oligoadenylate synthetase 1 (OAS1). Overexpression of OAS1 alone was sufficient to trigger apoptosis in RIG-I KO cells in response to 3p-RNA and cells deficient for RNAseL showed profoundly impaired ability to undergo cell death. Finally, we show, that the concerted action of translational arrest, triggered by RNAseL, and upregulation of NOXA by RIG-I is needed to rapidly deplete the anti-apoptotic BCL-2 family member MCL-1 and thereby induce intrinsic apoptosis in melanoma cells.

Conclusion: Our thorough analysis of RLR-induced signaling pathways using KO cell lines provides clear evidence, that cytokine release and cell death induction are two separable events downstream of cytoplasmic 3p-RNA recognition. While direct RLR signaling leads to transcriptional priming of the OAS/RNaseL pathway and mitochondrial priming via NOXA, the execution of cell death is dependent on translational arrest triggered by activity of the OAS/RNAseL system. This two-step

mechanism consisting of priming and effector phase reminiscent of NLRP3 inflammasome activation appears to be a common mechanism in innate immunity allowing the cell to either cope or perish depending on the insult taken.

Location matters: the role of HAX1 protein in mitochondria – implications for differentiation of neutrophil granulocytes and severe congenital neutropenia (SCN)

Yanxin Fan

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Loss-of-function mutations in HAX1 (HS1-associated protein X1) in humans result in autosomal recessive severe congenital neutropenia (SCN), yet the exact molecular function of HAX1 remains unclear. Here, we aimed to determine the subcellular localization of HAX1 and its interacting proteome network. Using mitochondrial swelling and carbonate extraction methods, we demonstrate that HAX1 is localised predominantly in the mitochondrial inter membrane space. We performed mass-spectrometry studies and identified Caseinolytic peptidase B protein homolog (CLPB) as a novel interacting protein. CLPB is a member of the ATP-ase superfamily associated with diverse cellular activities (AAA+). Biallelic mutations in CLPB cause a rare neurological disorder associating impaired cognitive development, 3-methylglutaconic aciduria, and congenital neutropenia. Human mutations leading to SCN in either HAX1 or CLPB disrupt the mutual interactions of the corresponding proteins. Further investigations revealed that the activity of mitochondrial complex I is reduced in the absence of HAX1. Our data provide new evidence on the critical function of HAX1 in mitochondria. In ongoing studies, we investigate imbalances of the proteome and biochemical pathways in a systems biology perspective in order to define new ways to develop targeted therapies.

MiR-492 regulates metastatic properties of hepatoblastoma via CD44

Julia von Frowein[1], S.M. Hauck[2], R. Kappler[3], P. Pagel[4], K.K. Fleischmann[1], T. Magg[1], S. Cairo[5], A. Roscher[6], D. von Schweinitz[3], I. Schmid[1]

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MicroRNAs are important genetic regulators of physiological and pathophysiological processes including cancer initiation and progression of hepatoblastoma, the most common liver tumour in childhood. We aimed to identify malignant and metastasis promoting effects of miR-492, a miRNA, previously reported to be overexpressed in metastatic hepatoblastoma. Furthermore, we intended to evaluate its diagnostic and prognostic potential.

We show that miR-492 significantly enhances cell proliferation, anchorage-independent growth, migration and invasion of hepatoblastoma cells. We also identified and validated CD44, a transmembrane adhesion receptor for hyaluronan, as direct and functional target of miR-492. This miRNA has a strong direct impact on two CD44 isoforms (standard and v10). High miR-492 expression correlates with high-risk or aggressive tumours and further bears potential for predicting reduced event-free survival. We identified miR-492 and its target CD44 as regulators of a number of biological features important for malignancy and metastasis. Furthermore, we demonstrated the diagnostic and prognostic potential of miR-492, a promising novel therapeutic target and biomarker for hepatoblastoma.

Analysis of NOD2 sequence variants in patients with primary immunodeficiency

Maryam Ghalandary

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Nucleotide-binding oligomerization domain 2 (NOD2) is a member of the Nod-like receptor (NLR) protein family that recognizes intracellular peptidoglycan fragments from bacteria. Mutations in NOD2 have been associated with Crohn's disease, Blau syndrome, and autoinflammatory diseases (Philpott DJ et al., Nat Rev Immunol 2014). Here, we report a novel rare homozygous missense mutation in the first CARD domain of NOD2 in a patient presenting with primary immunodeficiency. Our studies have unraveled VCP as a new interaction partner of NOD2 by employing immunoprecipitation-coupled mass spectrometry. Biochemical assays showed that knockdown of VCP in coloncarcinoma cell lines leads to reduced NF-kB activation and decreased expression of the proinflammatory cytokine IL-8 upon stimulation of the NOD2 pathway.

Associations of sleep duration with internalizing and externalizing behaviour in eight-year-old children in the European Childhood Obesity Project.

Kathrin Gürlich[1], D. Gruszfeld[2], P. Socha[3], V. Luque[4], R. Closa-Monasterolo[4], F. Martin[5], P. Poncelet[6], B. Mariani[7], E. Verduci[7], B. Koletzko[1] and V. Grote[1]

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Objective: The aim of the present study was to examine whether sleep duration is associated with emotional or behavioural symptoms in eight-year-old children.

Methods: The study is a cross-sectional analysis of data from 406 children from the European Childhood Obesity Project. Sleep duration was objectively measured using the SenseWear Armband 2 at the age of eight years. At the same time point mothers rated their child's emotional (internalizing) and behavioural (externalizing) problems on the Child Behaviour Checklist. Logistic regression models were applied to test the association between sleep duration and internalizing or externalizing problems.

Results: Average sleep duration was 9.24 hours per night (SD: ±0.66 h). 66.7% of children fulfilled the sleep duration recommendation of the National Sleep Foundation (NSF) (9-11 hours for school-aged children per night). Children who did not adhere to the NSF recommendation had an increased risk for internalizing problems (adjusted OR=2.12; 95% CI: 1.01, 4.45). Sleep duration and externalizing problems showed no significant association.

Conclusion: Results highlight the importance of adequate sleep duration throughout primary-school years for an optimal emotional development of children.

Novel antibody derivative locally blocking the PD-1/PD-L1 Multi-omics analysis of human neutrophil granulocytes in monogenic disease

Sebastian Hesse

Genetic disease of neutrophil granulocytes, the most abundant white blood cell in humans, are only partially solvable with current short-read based sequencing methods. We hypothesize that employment of proteome and transcriptome analysis of neutrophils in addition to whole exome sequencing may allow us to understand the RNA and protein phenotypes of different disease causing genotypes. Our recent publication demonstrated specificity of neutrophil proteotypes in distinct neutrophil disease (chronic granulomatous disease, leukocyte adhesion deficiency and severe congenital neutropenia). We were able to use proteome findings in two patients without diagnostic exome result to direct targeted sequencing methods that resulted in successful variant detection in both cases.

Currently we are analyzing proteome states of two putative SCN causing genes and find their proteotypes to overlap with a known, recently published SCN genotype that affects function of a protein working in complex with the two novel genotypes (indicating proteotypic mimicry for closely interacting genes). Other SCN genotypes show distinct proteotypes and may allow further diagnosis and classification of genetically unknown SCN patients.

Dominant negative transforming growth factor β receptor 2 enhances T cell efficacy in a murine pancreatic tumor model

Duc Huynh, B. Cadilha, K. Dorman, S. Endres and S. Kobold

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Introduction: Adoptive T cell transfer (ACT) with tumor-specific cytotoxic T lymphocytes is a promising treatment modality for a number of malignancies. However, so far, the success of ACT on solid tumors is poor. The lack of efficacy of ACT in solid entities is in part due to tumor-microenvironment induced T cell exhaustion and anergy. Transforming Growth Factor - β (TGF-β) is produced in large amounts by pancreatic cancer cells and their surroundings and is thought to suppress T cell activity through binding to the ubiquitously expressed TGF-β receptor-1 and -2 tetramers. We hypothesize that a truncated TGF-β-receptor may shield T cells from TGF-β effects.

Material and methods: As the segment responsible for TGF-β signaling resides in the intracellular domain of TGF-β receptor 2 (TGF-β-R2), a stop codon has been inserted after the tenth intracellular amino acid to prevent further transcription, this receptor is also known as Dominant Negative TGF- β-R2 (DNR). The product was then cloned in the pMP71 retroviral vector. The generated receptor can be constitutively expressed in T cells upon transduction and competes with the endogenous TGF-β R2 for the formation of tetramer receptor complexes. We have expressed through retroviral transduction the DNR in primary murine T cells specific for the model antigen ovalbumin (OT-1 cells) and tested the transduced cells in-vitro with cultures in the presence of TGF- β and in-vivo as treatment for PancO2OVA sub-cutaneous tumors.

Results and discussion: When cultivated with TGF-β, DNR transduced cells are unaffected by TGF-β in terms of proliferation, as opposed to untransduced cells which stop proliferating in the presence of TGF-β. This is mirrored by an inexistent SMAD2/3 complexes phosphorylation in DNR T cells as opposed to untransduced T cells upon TGF-β addition. In vivo, ACT of DNR transduced cells in mice bearing Panc02-OVA tumors, ameliorates tumor control and overall survival compared to untransduced OT1 T cells.

Conclusion: Adoptive T cell therapy efficacy can be improved in a pancreatic cancer model by introducing a Dominant Negative TGF-β Receptor 2 into antigen-specific T cells. This receptor may be combined with other promising strategies to improve the efficacy of cellular approaches.

Production of mouse monoclonal antibodies against enterococcal polysaccharides

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Multidrug-resistant Enterococci are major causes of hospital-acquired infections. Immunotherapies utilizing monoclonal antibodies (mAbs) that target bacterial polysaccharides have emerged as a valuable treatment and/or prevention of infectious diseases. This study describes the production of mouse mAbs that target well-characterized polysaccharides of E. faecalis. For this purpose, BALB/c mice were immunized using different immunization protocols combining plain and conjugated forms of the native capsular polysaccharide diheteroglycan, DHG, as well as protein conjugated forms of the synthetic teichoic acid, WH7, a previously described molecule that mimics lipoteichoic acid from E. faecalis. The splenocyte cultures were screened by ELISA against the bacterial polysaccharides. In total, two anti-capsular mAbs and one mAb against the synthetic teichoic acid were obtained. All mAbs belonged to the IgG1 subclass and exhibited variability in the type of the light chain. The mAbs against the capsular polysaccharide were IgG1 kappa and IgG1 lambda whereas the mAb against the synthetic teichoic acid had a kappa light chain. In conclusion, three mAbs against enterococcal polysaccharides were developed through hybridoma technology after immunizing mice with polysaccharide-protein conjugates. The discovery of these mAbs against E. faecalis polysaccharides will enable us to explore the interactions between mAbs and their epitopes.

DUSP1 plays a pivotal role in MAPK signaling in childhood asthma development and environment-mediated protection

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While childhood asthma prevalence is rising in westernized countries, children from farming areas are protected. We aimed to investigate the role of DUSP1 (MKP-1), the central negative regulator of the pro-inflammatory mitogen-activated protein kinase (MAPK) in childhood asthma development and its environment-mediated protection. Gene expression of unstimulated and farm dust or LPS stimulated PBMCs (qPCR) and isolated DCs and Tregs (Nanostring) from asthmatic (N=40) and healthy (N=50) 4-14 year old children and protein levels of phosphorylated MAPKs were analyzed (mass cytometry, CyTOF). Site-specific histone acetylation was assessed by chromatin immunoprecipitation. Asthmatics expressed significantly reduced levels of anti-inflammatory DUSP1 ($p=0.005$) and site-specific H4-acetylation ($p=0.002$). Stimulation with LPS and farm dust extracts upregulated DUSP1 expression to healthy levels, while pro-inflammatory MAPKs were downregulated on gene and protein levels in various cell populations, indicating a regulatory capacity of DUSP1 for future anti-inflammatory therapy

Pathway analysis - Genetic and immunological influences of gene polymorphisms in 17q21 locus on childhood wheeze

Kristina Laubhahn [a,b], A. Böck[a], J.K. Eckert[a], V. Hildebrand[a], S. Ackstaller[a], E.S. Klucker[a], S. Kunze[c], B. Schaub[a,b]

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Genome-wide association studies identified genetic variants in chromosome 17q21 locus, being strongly associated with childhood onset asthma. We assessed the impact of SNPs at 17q21 locus and related genes and cytokines on the immune regulation in cord blood (CBMCs) of German newborns (n=221) with subsequent wheeze (multitrigger/MTW and viral/VW). Mediation analysis was performed to derive direct and indirect effects of genetic variations mediated through gene expression/cytokine secretion on wheeze risk. Risk-allele carriers in tagging SNPs of GSDMA, GSDMB and ORMDL3 had a higher risk for subsequent MTW (ORs: 2.75-5.01, $p \leq 0.01$), but not for VW vs. HC. Upregulation of IL-17 secretion (OR: 2.22) and downregulation of TLR2 expression (OR: 1.85) were observed in risk-allele carriers in subsequent VW vs. HC. The increased risk of VW vs. HC in ORMDL3 and GSDMA SNP-carriers was mediated by both IL-17 and TLR2. In MTW vs. VW, STAT6 expression was increased (OR: 1.79) and IL-2 secretion was decreased (OR: 1.43). For all reported risk-genotypes, mediation analysis revealed a significant role for IL-2 secretion in later MTW vs. VW. This study showed a strong association between genetic variants in the asthma-associated 17q21 locus with different wheeze phenotypes and altered immune regulations with characteristic patterns in CBMCs. These findings may be a promising tool for a more specific characterization and early prediction of childhood wheeze phenotypes.

Identification of novel polysaccharide targets from *Enterococcus faecium*

Diana Laverde[1], F. Romero-Saavedra[1], Z. Kaczynski[2], O. Holst[3] and J. Hübner[1]

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Enterococci are among the most common isolated pathogens associated with healthcare infections. Since the beginning of 1990s E. faecium has dramatically increased in frequency and has become a serious concern because of their reduced susceptibility to first and second line antibiotics. Therefore, identification of surface antigens for vaccine development is a promising strategy to fight this pathogen. In this work we identified a novel polysaccharide from E. faecium U0317 that enable them to resist opsonophagocytosis. The polysaccharides were purified by size exclusion and anion exchange chromatography. Pooling of the fractions was performed according to the different organic groups detected by colorimetric assays, and the immunoreactivity of fractions with the anti-U0317 serum. The resulting polysaccharide I22 completely inhibited the opsonic killing activity of anti-U0317 serum. Structural elucidation of I22 by one and two-dimensional NMR spectroscopy showed that this polysaccharide has a novel structure, making it a promising vaccine target. Antibodies raised in mice with I22-glycoconjugates mediated opsonic killing of the target strain, indicating its feasibility for active immunotherapy.

Engineering tumor specific T cells with CXCR6 enables access to CXCL16-producing solid tumors

Stefanie Lesch, V. Blumenberg, S. Stoiber, J. Ogonek, B. Cadilha, Z. Dantes, F. Rataj, K. Dorman, J. Lutz, C.H. Karches, C. Heise, M. Kurzay, S. Grassmann, M. Rapp, R.T.A. Megens, K.P. Janssen, M. Jastroch, D. Lamp, S. Ruehland, M. Di Pilato, J.N. Pruessmann, S. Ormanns, A. Reischer, M. Hristov, S. Rothenfusser, P. Duewell, M. Schnurr, M. Subklewe, M. Reichert, T.R. Mempel, S. Endres and S. Kobold

Introduction: Chimeric antigen receptor (CAR) T cell therapies are approved for the treatment of different hematological malignancies. In solid tumors, however, this approach has failed so far. A major limitation of CAR T cells is their ineffective infiltration into solid tumors. A prerequisite for an efficient migration and tumor homing of transferred T cells is the expression of matching chemokine receptors to the chemokines secreted by the tumor tissue. In this study, we genetically engineered tumor-specific T cells with the C-X-C chemokine receptor 6 (CXCR6) to enhance T cell trafficking and to improve adoptive tumor immunotherapy.

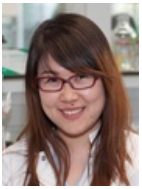
Materials and methods: Murine and human T cells were transduced with the chemokine receptor CXCR6. Transwell migration assays validated the migratory capacity of CXCR6+ T cells. Furthermore, in vitro penetration of spheroids or pancreatic cancer patient-derived organoids by CXCR6+ T cells was investigated using selective plane illumination microscopy (SPIM) or laser confocal scanning microscopy (LCSM). Cytotoxicity assays and real-time impedance measurements were performed to determine anti-tumor effectiveness in vitro. Various murine and human tumor models expressing the chemokine ligand CXCL16 were used to characterize the therapeutic potential of CXCR6- modified tumor specific T cells. Flow cytometry, 2-photon microscopy and intravital live cell tracking analysis were done to monitor tumor trafficking of CXCR6+ T cells.

Results: CXCL16 was found to be expressed by murine and human pancreatic cancer cell lines and effectively attracts T cells transduced to express CXCR6. This migratory effect was not only observed in trans-well migration assays, but also in co-cultures with spheroids and pancreatic cancer patient-derived organoids, resulting in an increased penetration of 3D structures by CXCR6+ T cells in vitro. Introducing CXCR6 in antigen-specific T cells (conferred by TCR as well as by murine and human CAR) led to improved anti-tumor activity and overall survival improvements. The therapeutic response was attributed to increased T cell infiltration into the tumor tissue, as validated by flow cytometry, 2-photon microscopy and intravital live cell tracking.

Conclusion: Forced expression of CXCR6 enhanced homing of adoptively transferred tumor-specific T cells towards CXCL16-secreting tumors, which resulted in improved

T cell infiltration and decreased tumor growth. These results demonstrate that CXCR6 is a promising target to selectively redirect tumor-specific T cells to CXCL16-expressing tumors and might overcome the hurdle of limited tumor infiltration in adoptive cell therapies of solid tumors.

Inherited loss-of-function of RIPK1 causes immunodeficiency and intestinal inflammation



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Children with very early onset inflammatory bowel diseases (VEO-IBD; onset <6 years) often show life-threatening conditions refractory to conventional treatment. VEO-IBD may result from inborn errors of immunity, yet most patients still lack genetic diagnosis. To unravel novel genetic signatures of VEO-IBD, we have conducted whole exome sequencing of 600 patients. Our screen revealed RIPK1 loss-of-function mutations as a novel molecular cause for VEO-IBD in 8 patients from 6 unrelated families. While 2 patients expressing truncated RIPK1 presented with immunodeficiency and diarrhea, 6 patients with missense mutations in the death domain primarily showed signs of VEO-IBD. All patients showed increased susceptibility to infections.

Immunophenotypical analysis of patients' peripheral blood mononuclear cells exhibited impaired lymphocyte differentiation, as demonstrated by reduced frequency of CD45RO+CCR7- effector memory T cells, CD45RO+HLA-DR+ memory activated regulatory T cells, CXCR3+CCR6- and CXCR3-CCR6+ T helper cells as well as IgD-CD27+ class-switched B cells. Patient-derived monocytes and monocyte-like BLaER1 cells with overexpression of RIPK1 mutant variants showed increased IL-1beta secretion upon LPS priming, without requirement of second stimuli (e.g., ATP, nigericin). Blockade of NLRP3 and MLKL by small molecule inhibitors reduced the secretion of IL-1beta, suggesting that both pathways are implicated in the dysregulated proinflammatory responses. In addition to immune dysfunctions, we could also detect intrinsic defects in RIPK1-deficient epithelial cells. Coloncarcinoma cells with transgenic expression of patient-specific mutations exhibited impaired TNF-alpha-mediated NF-kappa-B signaling and TNF-alpha-induced cell death responses.

Our study demonstrates that RIPK1 deficiency is a life-threatening Mendelian disorder with defects in the adaptive and innate immune system as well as the intestinal epithelium. The characterization of rare patients with RIPK1 deficiency highlights the critical role of RIPK1 in human immune and intestinal homeostasis.

Metabolic labelling of choline phospholipids probes ABCA3 transport in lamellar bodies

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Background: In the metabolism of pulmonary surfactant, the ATP-binding cassette sub-family A member 3 (ABCA3) is a crucial protein in the formation of the storage compartment for surfactant, the lamellar body (LB), and the transport of phospholipids in it. Mutations in ABCA3 not only disturb surfactant metabolism but also cause chronic interstitial lung diseases. Assays for ABCA3 transport function are needed to investigate pathophysiology of the mutations and treatment options for the patients.

Methods and results. We metabolically labeled choline (Cho) head phospholipids with the Cho analogue, propargyl-Cho. The universal incorporation of propargyl-Cho was confirmed by mass spectrometry and labeled lipids was visualized by click reaction with an azide fluorophore in confocal microscopy. After pulse-labeling propargyl-Cho labeled lipids accumulated in ABCA3+ vesicles in a time and dose dependent manner. Lipids intensity inside ABCA3+ vesicles decreased when treated with the choline kinase inhibitor MN58b in the first 12 hours, whereas intensity was unchanged when treated after 12 hours. Miltefosine, a substrate of ABCA3, decreased the uptake of labeled lipids in ABCA3+ vesicles at all time points. The lipids intensity inside the mutated (p.N568D or p.L1580P) ABCA3+ vesicles was decreased compared to WT, while the intensity outside of vesicles showed no difference.

Conclusion: Propargyl-Cho can metabolically pulse-label cho phospholipids. Visualization and quantification of fluorescence intensity of the labeled lipids inside ABCA3+ vesicles at equilibrium can specifically assess the transport function of ABCA3.

Heterozygous OAS1 gain-of-function variants cause a polymorphic autoinflammatory and immunodeficiency syndrome

Thomas Magg

The genetic, molecular, and cellular analysis of autoinflammatory and immunodeficiency disorders contributes to our understanding of human immunity and leads to the development of targeted therapies. Oligoadenylate synthase 1 (OAS1) is an intracellular double-stranded RNA sensor that generates the second messenger 2'-5'-oligoadenylate to activate RNaseL as a means of antiviral defense. We found three de novo heterozygous OAS1 variants in four unrelated patients with a polymorphic syndrome of fever, dermatitis, pulmonary alveolar proteinosis, inflammatory bowel disease, and hypogammaglobulinemia. Variant OAS1 proteins showed double-stranded RNA-independent in vitro gain-of-function enzyme activity. They constitutively activated RNaseL resulting in increased RNA cleavage in a heterologous cell system as well as in primary monocytes and B cells. This lead to an increased interferon response, spontaneous monocyte and B cell apoptosis, impaired monocyte and B cell differentiation and function, and impaired cellular co-stimulation towards T cells. Allogeneic hematopoietic stem cell transplantation corrected the otherwise lethal phenotype.

GC QTOF Profile of stool metabolome and machine learning algorithms - a pilot study to diagnose Crohn's disease

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Objectives and Study: The stool metabolome might provide information on the pathophysiology and activity of Crohn's disease (CD) and related microbial metabolism. We aimed to explore the potential of this non-invasive tool for the diagnosis of paediatric CD.

Methods: Stool samples from 31 healthy subjects and 28 paediatric CD patients in remission were used. Samples were extracted in methanol. Metabolomic profiling was carried out using GC QTOF after derivatisation in a 37 min chromatographic run. Chromatograms were extracted and compound annotation was done by matching with the library of the US National Institute of Standards and Technology (NIST). Statistical analysis and quality control were performed using Mass Profiler Professional® software. Principal component analysis was performed to detect outliers, and a filter by frequency was used to retain compounds present in 100% of the samples in at least one group, followed by moderated t-test prior to building the prediction models. Three approaches were evaluated: Partial Least Square Discriminant Analysis (PLS-DA), Support Vector Machine (SVM) and Decision Tree (DT). A training set of 28 samples from healthy children and 25 samples from CD patients was used to build and validate the models, and 3 samples of each group were randomly chosen and left out to test the model.

Results: The GC QTOF data provided a total of 7951 features. After quality control and t-test, this number was reduced to 35 compounds (34 annotated and one unidentified). A corrected p value < 0.05 (Benjamini-Hochberg) was used to build the prediction models. The three models were able to correctly classify all CD patients and healthy subjects based on stool sample metabolome with overall confidence measure = 1.

Conclusions: Untargeted stool metabolomics using GC QTOF is a promising tool for the diagnosis of paediatric CD. The method detected 35 significant compounds that may serve as diagnostic biomarkers. Further investigation of fecal metabolomic biomarkers could shade more light on the pathophysiology of CD and microbiota-host interaction, which might facilitate the development of new therapeutic approaches.

Defining brain cancer vulnerabilities with CRISPRi screens

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Many children suffering from brain cancer eventually die. “Targeted” therapeutics, which in a perfect world inhibit tumor processes while leaving healthy cells unaffected, spark the hope that a personalized therapy for brain cancer is possible. For a long time, RNAs were mostly seen as mere link between the DNA and proteins. It has now become clear that RNA molecules are subject to a vast regulatory machinery. Some tumors “hijack” such regulations in order to fuel their pro-oncogenic needs, which can be exploited therapeutically (Braun et al., Cancer Cell, 2017). We aim to conduct inhibitory pooled CRISPRi screens in tumor lines directly derived from patients. By targeting our custom libraries specifically towards regulators of RNA stability and localization, we will contribute to a comprehensive functional model elucidating the role of RNA-regulators in pediatric brain cancer

GM-CSF is a potent inducer of the Treg-attracking chemokine CCL22

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The chemokine CCL22 is an important mediator of many immunoregulatory processes involved in pathological conditions including autoimmunity and cancer. Under homeostatic conditions it is constitutively expressed in many lymphoid organs. CCL22 is believed to mediate immune suppression by attracting and orchestrating the distribution of regulatory T cells (Tregs) via their chemokine receptor CCR4. However, the exact mechanisms of CCL22 regulation in homeostasis and disease are still not fully understood.

We were able to reveal that cytokines of the common beta chain receptor family including GM-CSF, IL-3 and IL-5 were capable of inducing CCL22 secretion in vitro. Since GM-CSF is involved in different pathological processes, we wanted to further investigate the GM-CSF-CCL22 axis. Here, we show that in GM-CSF knockout splenocytes, CCL22 secretion under homeostatic conditions is significantly reduced compared to wildtype splenocytes. This finding supports the ambivalent nature of GM-CSF which exhibits both pro-inflammatory but also immune suppressive functions that may in part be mediated via CCL22 induction. Of note, we could show that the application of recombinant GM-CSF significantly increased CCL22 secretion from murine dendritic cells (DCs) in vitro and in vivo. Importantly, we were also able to validate these findings in human blood-derived DCs.

This work elucidates a novel mechanism of immunosuppressive CCL22 induction in DCs under homeostatic conditions and in GM-CSF-based therapeutic approaches. Our results provide the basis for evaluating CCL22 as a target for future immunotherapeutic interventions in autoimmunity and cancer.

Indoleamine-2,3-Dioxygenase in T cells

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Indoleamine-2,3-dioxygenase 1 (IDO1) is a cytosolic enzyme involved in the tryptophan catabolism catalyzing the rate limiting reaction in the kynurenine pathway. Many cell types have been described to express IDO1 including tumor cells, dendritic cells, macrophages, epithelial cells and fibroblasts. In general, the expression of IDO1 by these cell types in the context of the tumor microenvironment promotes tryptophan starvation and accumulation of kynurenines which result in the inhibition of T cell proliferation and an induction of regulatory T cells. Additionally, IDO1 possesses two ITIMs (immunoreceptor tyrosine-based inhibitory motifs), that upon phosphorylation can act as docking sites for the recruitment and activation of the tyrosine phosphatases SHP-1 and SHP-2 and ultimately to an activation of the non-canonical NF-κB pathway. This pathway is described to determine the tolerogenic phenotype of plasmacytoid dendritic cells upon TGF-β signaling.

To date, the expression of IDO1 in T cells has been poorly documented in the literature and there is little to no information on its effect on T cell biology. We found that IDO1 is inducible in primary human and mouse T cells upon T cell receptor activation and type I and type II interferon signaling. In a tumor immunotherapeutic setting, we could show that IDO1 knockout T cells have an increased cytotoxic activity in vitro and in vivo. As we could not find any evidence that IDO1 acts via its enzymatic activity in T cells, we are focusing on a putative signaling function that might have an inhibitory effect or might foster an immunosuppressive phenotype on T cell biology.

Impact of exclusive enteral nutrition on microbiome signatures and function in pediatric Crohn's disease

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Crohn's disease (CD) is one of the two major entities of inflammatory bowel disease (IBD) with a rising incidence in industrialized countries. The etiology of CD is multifactorial and based on dysregulated interactions between the immune system, the microbiome and additional environmental factors (e.g. westernized diet) in genetically susceptible individuals. Symptoms of CD include abdominal pain, fever or diarrhea. For IBD no curative treatment is currently available.

In pediatric CD, exclusive enteral nutrition (EEN) is the first line therapy for active luminal CD. EEN is a liquid formula-based dietary intervention, which induces remission and mucosal healing. Compared to standard immunosuppressive treatments, EEN has almost no related side effects. Yet, the protective and remission inducing mechanisms behind this dietary therapy remain unclear. Of note, previous studies have shown a substantial change in the microbiome composition during EEN.

The aim of our project is to understand the functional role of the intestinal microbiome for the clinical efficacy of EEN. To reach this goal, we conduct an open label prospective study at Dr. von Hauner Children's Hospital. We longitudinally follow newly diagnosed CD patients and regularly collect various biomaterials (blood, saliva, stool and biopsies) over one year to understand changes in the microbiome, the metabolome and the immune system related to EEN-mediated resolution of inflammation. Based on this knowledge, we aim to develop a maintenance treatment using the EEN-conditioned microbiome or its metabolites to induce long-term remission in pediatric CD patients.

TCR reprogramming for the treatment of refractory adenovirus infections

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Absent T cell-immunity against persistent viruses like Adenovirus (AdV) is a major cause for virus-related mortality in patients after haematopoietic stem cell transplantation. We aimed to redirect primary human T cells by replacing endogenous T cell receptors (TCRs) with an AdV-specific TCR using CRISPR/Cas9. The simultaneous knock-out (KO) of the endogenous TCR will prevent harmful TCR mispairing.

Highly efficient and stable KO of the endogenous TCR was confirmed on genetic as well as on protein level. TCR KO T cells were unable to produce IFNγ upon stimulation whereas CRISPR/Cas9-mediated knock-in of protective AdV-specific TCR rescued IFNγ production upon AdV-specific stimulation and showed AdV-specific cytotoxic capacity.

In summary, redirecting primary human T cells from seronegative donors by replacing the endogenous TCR with a virus-specific TCR and characterization of these engineered virus-specific T cells reveals that combined CRISPR/Cas9 mediated TCR KO and knock-in presents a powerful tool for future treatment of refractory viral infections in the immunocompromised host.

Genome wide knockout screening to identify the novel genes involved in the ER function and secretory pathway

Megumi Tatematsu

Although more than 10 genes have been identified as responsible genes for severe congenital neutropenia (SCN), the pathophysiology of SCN remains unclear. So far, it has been reported that endoplasmic reticulum (ER) homeostasis is crucial in neutrophil development and related to the neutropenia.

In this study, we designed a genome-wide knockout screening to identify genes involved in the regulations of ER function and secretory pathway in human myeloid cells. We established CD2-expressing KBM-7 cell line, a near haploid human myeloid cell. Since the expression level of CD2 on the cell surface is controlled by secretion from the ER, reduced CD2 expression reflects ER damage.

In the screening, CD2-expressing KBM7 cells were transduced with a guide RNA pooled library targeting 19,114 genes. About 3% of the transduced cells showed a low expression of CD2 were collected as a ER damaged population. We are investigating the function of genes enriched in CD2-low expressing cells.

Cesarean section and its impact on childhood BMI modified by infant feeding: 8 years follow-up of the CHOP trial.

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Background: Early life factors affecting early growth and metabolism are of growing interest to face the obesity epidemic. Beside infant nutrition, delivery mode causing different gut colonization is shown to be associated to early weight gain and obesity risk. Therefore we examine whether infant feeding and delivery mode have interactive long-term programming effects on weight gain and body-mass-index.

Design: The CHOP study is a multicenter randomized European intervention trial. Healthy infants who could not be breastfed were randomized within the first two months of life to receive infant and follow-on formula with different protein content (higher protein (HP: 1.6 and 3.2 g/dl): N=550 (Caesarean section 116); lower protein (LP: 1.25 and 2.05 g/dl): N=540 (129)), predominantly breastfed (BF) infants (N=588 (118)) were included as a reference. Regular weight and height measurements were performed from 3 months to 8 years of age. Information on delivery was assessed by questionnaire at inclusion.

Results: Twenty-two percent of the cohort were delivered by Caesarean section and 707 (Caesarean section: 162) children could be followed to 8 years of age. Weight gain in the first year (as z-score difference according to WHO growth standards) was significantly different between feeding groups (P<0.001) and significantly increased by Caesarean section. In longitudinal analysis stratified by feeding group, BMI tracks of children delivered by Caesarean section are significantly increased in the HP group at 6 and 8 years, in the LP group at 8 years and not at all in the breastfed group.

Conclusions: Caesarean section is an early risk factor for later obesity. Early nutrition might offer strategies to interrupt this risk relation since breastfeeding acts protective and LP formula retarding.

SP8 promotes an aggressive phenotype in hepatoblastoma

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Hepatoblastoma (HB) is the most common pediatric liver tumor with a general good prognosis, but if associated with multifocal growth, vessel invasion and metastasis, outcome is still poor. The molecular mechanisms causing this high-risk profile are still unknown. RNA-sequencing of 10 non-metastasized and 7 metastasized HB samples identified the transcription factor SP8 as a significantly upregulated gene in metastatic HB, which was further verified in a cohort of 33 patients by quantitative PCR. Following SP8 overexpression in hepatoma cell lines, we found a significant increase in motility, stemness and invasive capacity, presumably by inducing the expressing of FGF8. Consequently, chromatin-immunoprecipitation showed binding of SP8 to the FGF8 promoter and CRISPRi-mediated knockdown of FGF8 rescued the phenotype. Collectively, our data define SP8 as an important factor provoking aggressive behaviour of HB cells.

Analysis of bone marrow CD8 T cells from pediatric leukemia patients reveals targets for improved adoptive T cell therapy

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Introduction: Acute leukemia is the most common malignancy in children. Despite recent therapeutic advances, patients with relapsed or refractory disease have a dismal prognosis and require new treatment strategies. Since its licensing in 2017, commercially available chimeric antigen receptor (CAR) T cells against CD19+ acute leukemia and have revolutionized treatment of those high-risk patients. Recent data, however, suggests that despite initial complete response rates of about 90% in patients treated with CD19 directed CAR T cells, relapse rates of more than 50% occur over 24 months. Those relapses do often result from leukemia CD19 antigen loss or insufficient CAR T cell persistence. We analyzed the changes in bone marrow T cells caused by presence of acute leukemia in order to address the latter cause for relapse by engineering T cells to be specifically functional within the micromilieu of leukemic bone marrow.

Methods: To determine changes driven by the presence of leukemia blasts within the bone marrow, we analyzed T cells in bone marrow samples from pediatric patients with B-precursor ALL (BCP-ALL), T-precursor ALL (TCP-ALL) and acute myelogenous leukemia (AML) at the time of diagnosis and relapse in comparison to healthy bone marrow donors. Cryopreserved bone marrow samples from both pediatric patients with acute leukemia (n= 77; BCP-ALL: 18, TCP-ALL: 23, AML: 36) and age-matched healthy bone marrow donors (BMD, n=23) were identified in our local biobank. We performed flow cytometric protein quantification of CD4 and CD8 T cells and next generation sequencing (RNA-Seq, ATAC-Seq) of sorted CD8 T cell populations. We analyzed T cell maturation by CD62L, CD95 and CD45RO surface expression and T cell exhaustion by TIM3 and PD1 surface expression.

Results: First, we performed unsupervised clustering of RNA-Seq and ATAC-Seq data of sorted CD8 T cells asking, whether CD8 T cells from leukemia patients differ in their transcriptome and chromatin structure from healthy counterparts. Strikingly, principal component analysis (PCA) of RNA-Seq data documented segregation of CD8 T cells from healthy donors, AML patients and BCP-ALL patients. PCA of ATAC-Seq data did document segregation of BCP-ALL CD8 T cells from HD counterparts, while

AML CD8 T cells did not cluster separately from HD. Finally, stacked integration of RNA-Seq and ATAC-Seq yielded an improved segregation of three populations: AML, BCP-ALL and HD. Consequently, CD8 T cells from leukemic bone marrows differ from healthy counterparts by transcriptome and open chromatin structure.

Next, we observed in flow cytometry experiments both CD4 and CD8 T cell phenotype from patients with acute leukemia skewed towards mature T cell phenotypes according to CD95, CD45RO and CD62L surface expression. In particular, frequency of naïve T cells decreased while effector memory T cells increased in leukemic bone marrow. Moreover, T cells from acute leukemia patients expressed more inhibitory surface molecules such as PD1 and TIM3.

Going back to the RNA-Seq data, we asked whether effector T cell transcripts were indeed overexpressed in leukemic bone marrow CD8 T cells. We found CX3CR1 as a marker of effector CD8 T cells with little capacity to proliferate among the top 10 increased transcripts in CD8 T cells from leukemia patients. Moreover, significantly increased expression of many elements of the cytotoxic granule machinery (among others: GZMB, GZMH, PRF1, CTSD), a characteristic of effector CD8 T cells, was observed.

Given that CD8 T cells from leukemic bone marrow differ from healthy counterparts and show an effector phenotype with expression of inhibitory molecules, we asked, whether transcripts of genes important for CD8 T cell proliferation and longevity are expressed differentially in leukemic bone marrow CD8 T cells. Indeed, we found genes crucial for those CD8 T cell functions decreased by RNA-Seq in CD8 T cells from leukemia patients (e.g. RARA, IL21R and BACH2).

Conclusion: Bone marrow CD8 T cells from pediatric leukemia patients segregate from healthy counterparts by RNA-Seq and ATAC-Seq. Moreover, BCP-ALL and AML segregate according to leukemia subtype. T cells from leukemia patients show a mature effector phenotype with expression of inhibitory surface molecules by flow cytometry. This observation is mirrored by increased cytotoxic granule machinery genes and CX3CR1 in CD8 T cells from leukemia patients. We are currently engineering T cells by retroviral transduction and CRISPR/Cas9 to increase T cell proliferation, longevity and sustained cytotoxicity in order to develop new adoptive CAR T cell products for pediatric leukemia patients. Overexpression of genes beneficial for T cell proliferation and longevity and knockout of genes reducing those T cell functions might boost e.g. CD19 CAR T cell efficiency by rendering T cells resistant to exhaustion induced by the leukemic micromilieu. In summary, we aim to develop CAR T cells for pediatric leukemia patients that yield increased relapse free survival rates.

Yellow fever vaccination as a model to study the immune response to an acute viral infection

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Vaccination with the live attenuated yellow fever vaccine (YF-17D) causes an acute self-limiting viral infection, which results in life-long immune protection and thus provides an attractive model to study the innate and adaptive immune responses. This model could be used to identify predictors of the vaccination efficacy and the genetic differences governing the variability in the activation of the innate and adaptive immune responses across individuals.

The viral particles are detected by intracellular pattern recognition receptors (PRR) present on the surface and within the antigen-presenting cell (APCs), which leads to eliciting the pro-inflammatory cytokines and chemokines and stimulation of the adaptive immune response. However, it is still poorly understood how the innate immune system modulates the adaptive immune response and which APC populations are activated. The study would help to identify and characterize cellular and molecular mechanisms activated by YF-17D. Especially interesting are new factors and components of the nucleic acid sensing pathways and epigenetic differences between individual, which would result in different responses to YF-17D vaccination.

In this study, we have collected bio-samples from a cohort of 250 volunteers taken before the vaccination and then successively 1, 3, 7,14 and 28 days after the vaccination, which we are analyzing using high-throughput omics approaches.

We show that immunization with YF17D leads to differences in cytokines and chemokine production before and after the vaccination.

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