10th International Symposium
on the Clinical Use of Cellular Products

Final Program and Abstract Book

Cellular Therapy 2019

March 22 and 23, 2019, Erlangen, Germany

CELLULAR THERAPY
International Symposium
Erlangen

Universitätsklinikum Erlangen

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International Symposium Erlangen

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10th International Symposium on the Clinical Use of Cellular Products

Cellular Therapy 2019

March 22 and 23, 2019

Department of Internal Medicine 5 – Hematology/Oncology
University of Erlangen-Nuremberg

Department of Dermatology
University of Erlangen-Nuremberg

Department of Internal Medicine 3 – Hematology/Oncology
University of Regensburg

Department of Internal Medicine 2 – Hematology/Oncology
University of Würzburg

Unter der Schirmherrschaft der

DGHO
DEUTSCHE GESELLSCHAFT FÜR HÄMATOLOGIE UND MEDIZINISCHE ONKOLOGIE

Cooperating Institutions

Comprehensive Cancer Center Erlangen
European Metropolitan Region Nürnberg

SFB Transregio 221

SFB1181
Checkpoints for Resolution of Inflammation

MICE Medical Immunology Campus Erlangen

GK1660 Adaptive Immunity

i-Target, Elitenetzwerk Bayern

Deutsches Zentrum Immuntherapie

Supported by the FAU Visiting Professor Programme

Welcome Address

Dear Colleagues.

On behalf of the organizing committee we would like to welcome you to the 10th International Cellular Therapy Symposium in Erlangen, Germany. The Cellular Therapy Symposium is co-organized by the Universities of Erlangen-Nuremberg (A. Mackensen; G. Schuler), Regensburg (W. Herr) and Würzburg (H. Einsele).

Recent advances in protein engineering and molecular biology have increased the feasibility of cellular therapies such as genetically engineered T cells (CARs & TCRs) to treat malignant diseases.

Over the past years the meeting has evolved to a widely respected event, attracting experts from all over the world to discuss recent topics of the field in a pleasant environment. The upcoming meeting 2019 will focus on preclinical and clinical aspects of CAR T-cell therapy, TCR-engineered T-cell therapy, immunological checkpoints, and the tumor immune microenvironment.

We look forward to sharing the newest data with the aim to improve the translation of ideas into therapy.

Hopefully you will also have the chance to explore the beautiful area of Franconia.

Welcome to Erlangen!
Yours sincerely

Prof. Andreas Mackensen

General Information

Organizer:

Prof. Dr. A. Mackensen
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Conference Site

Hörsaalgebäude
Universitätsklinikum Erlangen
Ulmenweg 18
91054 Erlangen
Germany

Coffee Break and Lunch

During session breaks, coffee and cake will be served to participants wearing their badges. The coffee break will take place in the lobby.
Lunch on March 22 and brunch on March 23 is included in the registration fee and will also take place in the lobby.

Social Event

A social evening with dinner and live music will be organized at the restaurant Schloss Atzelsberg, Marloffstein-Atzelsberg, on Friday, March 22 at 18.30 h (6.30 pm). The social evening event is included in the registration fee.

General Information

Opening Hours of the Conference Office

Thursday, March 21, 2019	15.00 – 17.00 h
Friday, March 22, 2019	08.00 – 18.00 h
Saturday, March 23, 2019	08.30 – 15.00 h

During the conference you can contact us by phone or fax

Phone: +49-91 31-85 43100
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Registration:

Participants can register onsite (**cash only**)

Full Registration:	350 €
Students:	225 €
Employees of the Universities Erlangen & Regensburg & Würzburg:	100 €

The registration fee covers conference volume, coffee breaks, lunch, brunch and social event.

Tourist Information Erlangen

Erlanger Tourismus und Marketing Verein e.V.
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CELLULAR THERAPY
International Symposium Erlangen

Universitätsklinikum Erlangen

Organizer:

Andreas Mackensen, Erlangen
Gerold Schuler, Erlangen
Wolfgang Herr, Regensburg
Herrmann Einsele, Würzburg

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Location:

Hörsaalgebäude
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Ulmenweg 18
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Friday March 22, 2019

09.00 h

Opening

Andreas Mackensen

Director of the Department of Internal Medicine 5 – Hematology and Oncology, University of Erlangen-Nuremberg

Friday March 22, 2019

Session I

CAR T-Cells - Preclinical

9.15 h - 11.15 h

Chairpersons: Evelyn Ullrich (Frankfurt)
Claudia Rössig (Münster)

Friday March 22, 2019

Session I

01 **09.15–09.45** Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for AML
Saar Gill, Philadelphia

02 **09.45–10.15** Beyond hematologic malignancies: challenges and opportunities
Barbara Savoldo, Chapel Hill

03 **10.15–10.45** Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo
Renier Brentjens, New York

04 **10.45–10.55** A Novel Pharmacologic “Remote Control” to prevent CAR T cell related CRS
Katrin Mestermann, Würzburg

05 **10.55–11.05** Engineering tumor-specific T cells with CXCR6 enables access to CXCL16-producing solid tumors
Stefanie Lesch, Munich

11.15-13.30 **Lunch and Poster Discussion**
Authors are requested to be present at their poster

Friday March 22, 2019

Session II

CAR T-Cells - Clinical

13.30 h - 15.30 h

Chairpersons: Michael Hudecek (Würzburg)
Simone Thomas (Regensburg)

Friday March 22, 2019

Session II

06 **13.30-14.00** Next generation CAR T cells for lymphoma/myeloma
Carl June, Philadelphia

07 **14.00-14.30** GD2-targeting CAR T-cell therapy for childhood solid tumours
Karin Straathof, London

08 **14.30-15.00** HER2neu CAR T cells in sarcoma patients
Nabil Ahmed, Houston

09 **15.00-15.10** Liquidation of solid tumors by in vivo expanded CLDN6-CAR T cells
Benjamin Rengstl, Mainz

10 **15.10-15.20** T4 Immunotherapy of Head and Neck Squamous Cell Carcinoma using Pan-ErbB Targeted CAR T-cells
Marc Davies, London

15.30-16.00 **Coffee Break**

Friday March 22, 2019

Session III

TCR-engineered T-Cell Therapy

16.00 h - 18.00 h

Chairpersons: **Matthias Theobald (Mainz)**
Wolfgang Uckert (Berlin)

Friday March 22, 2019

Session III

11 **16.00-16.30** Overcoming barriers to therapy of solid tumors with CAR/TCR engineered T cells
Stanley Riddell, Seattle

12 **16.30-17.00** TCR-engineerd T-cell immunotherapy of cancer
Thomas Blankenstein, Berlin

13 **17.00-17.30** NY-ESO-1 TCR single edited stem and central memory T cells to treat multiple myeloma without graft-versus-host disease
Chiara Bonini, Milano

14 **17.30-17.40** Dual orthotopic T cell receptor alpha and beta chain replacement enables engineering close to physiological T cells
Thomas Müller, Munich

15 **17.40-17.50** Mutated nucleophosmin 1 as immunotherapy target in acute myeloid leukemia
Dyantha van der Lee, Leiden

18.30 **Social Event:**
Schloss Atzelsberg, Marloffstein-Atzelsberg

Saturday March 23, 2019

Session IV

Immunological Checkpoints

09.30 h – 11.30 h

Chairpersons: Peter Brossart (Bonn)
Robert Zeiser (Freiburg)

Saturday March 23, 2019

Session IV

16 **09.30-10.00** Exploiting the gut phagome for T cell based-therapy
Laurence Zitvogel, Villejuif

17 **10.00-10.30** Determinants of immunological response to immune checkpoint blockade in human cancer
Daniela Thommen, Amsterdam

18 **10.30-11.00** Tumor suppressor signaling by PD-1
Jürgen Ruland, Munich

19 **11.00-11.10** Development of a novel, highly effective trifold immuno-therapeutic approach using autologous humanized PDX models and genetically-engineered mouse models of cancer
Sven Borchmann, Cologne

20 **11.10-11.20** Identification of AR1 as a Novel Immune modulator in Lung Cancer Using an RNA-i based High-Throughput Discovery Platform
Anachana Rathinasamy, Regensburg

11.30-13.00 **Brunch and Poster Discussion**
Authors are requested to be present at their poster

Saturday March 23, 2019

Session V

Modulation of the Tumor-Immune-Microenvironment

13.00 h - 15.00 h

Chairpersons: Alexander Steinkasserer (Erlangen)
Andreas Beilhack (Würzburg)

Saturday March 23, 2019

Session V

21 **13.00-13.30** T-cell based immunotherapy and the tumor microenvironment
Hans Schreiber, Chicago

22 **13.30-14.00** Understanding the tumor immune microenvironment for effective therapy
Dmitry Gabrilovich, Philadelphia

23 **14.00-14.30** Analysis of immune - tumor cell interactions using patient-specific explant models
Nils Halama, Heidelberg

24 **14.30-14.40** ß2-microglobulin - a trigger for NLRP3 inflammasome activation within macrophages promoting multiple myeloma cell progression
Daniel Hofbauer, Erlangen

25 **14.40-14.50** Targeting regulatory T cells fosters immune control and disrupts multiple myeloma disease progression
Julia Hartweg, Würzburg

14.50 **Summary**
A. Mackensen

Friday March 22, 2019

Poster Session

11.15 h - 13.30 h

Saturday March 23, 2019

Poster Session

11.15 h - 13.00 h

Poster Session A

CAR T-Cells

A1 A clinical trial with SLAMF7 CAR T cells generated by sleeping beauty gene transfer to treat multiple myeloma – The CARAMBA project
S. Prommersberger, Würzburg

A2 A lipocalin-based ON-switch for controlling CAR-T cells with an orally available drug
M. Lehner, Wien

A3 CAR-T Cells directed against tumor associated antigens in B cell neoplasms and beyond
A. Rehm, Berlin

A4 Clinical-scale and GMP-compliant production of CAR-T cells for the treatment of melanoma patients by mRNA transfection of a CSPG4-specific chimeric antigen receptor
N. Schaft, Erlangen

A5 FLT3 inhibitor treatment increases FLT3 expression that exposes FLT3-ITD+ AML blasts to elimination by FLT3 CAR-T cells
H. Jeatni, Würzburg

A6 Combinatorial targeting of multiple shared antigens by adapter-CAR-T cells (AdCAR-T) allows target cell discrimination and specific lysis based on differential expression profiles
C. Seitz, Tübingen

A7 CSPG4-specific CAR T cells react against leukemia cells
D. Harrer, Erlangen

A8 Engineering chimeric antigen receptor T cells with enhanced specificity and reactivity towards Aspergillus fumigatus
M. Seif, Würzburg

A9 High-level MYCN expression in neuroblastoma impairs CD171-directed CAR-T cell therapy in vitro
L. Grunewald, Berlin

A10 Tolerance in xenotransplantation by use of regulatory T cells with a SLA-specific chimeric antigen receptor
F. Noyan, Hannover

A11 Highly multiplexed, single-Cell functional profiling of CAR-T cells enables more predictive product characterization, cell manufacturing analysis, and cellular biomarkers across product types
P. Djali, Branford

A12 Inducible universal chimeric antigen receptor T cells combined with a CD123-specific costimulatory targeting module acting in trans are highly efficient against acute myeloid leukemia
J. Meyer, Dresden

A13 Minicircle DNA – New Tool for Cell Therapy
M. Schleef, Bielefeld

A14 Mode of action of a novel modular platform for adoptive T cell therapy combining bispecific antibodies with synthetic agonistic receptors
C. Karches, Munich

A15 The simultaneous siRNA-mediated downregulation of the inhibitory receptors PD-1 and CTLA-4 to boost anti-tumor CAR T-cell activity
U. Uslu, Erlangen

A16 Transfection of natural killer T cells with a CSPG4-specific CAR for cancer immunotherapy
B. Simon, Erlangen

A17 Using insight into TCR functioning for an improvement of CARs
W. Schamel, Freiburg

A18 Antigen loss as potential escape mechanism following CD171-directed CAR-T cell therapy
L. Andersch, Berlin

A19 Arming T cells with C-X-C-motive receptor 6 enhances infiltration of pancreatic cancer patient-derived organoids
J. Ogonek, Munich

A20 CrispR/Cas9-mediated deletion of the endogenous TCR reduces alloreactivity of a CAR based on T-cell signaling via endogenous CD3 and enhances its expression and tumor recognition
R. Voss, Mainz

A21 Decentralised manufacturing of CD19 CAR T cells using the automated and robust CliniMACS Prodigy® platform for a multicenter clinical trial in Germany
M. Aktas, Bergisch Gladbach

A22 Pre-clinical validation of ROR2-specific CAR T cells for the treatment of breast cancer
J. Weber, Würzburg

A23 VEGFR2 as a target for CAR T cell therapy of Ewing sarcoma
A. Englisch, Münster

Poster Session B

TCR-engineered T-Cell Therapy

B1 A high throughput approach for the parallel identification of TCRs recognizing multiple antigens with clinical relevance for the treatment of B-cell malignancies
M. Meeuwsen, Leiden

B2 Adoptive cell transfer in metastatic melanoma patients at the NKI-AVL in Amsterdam; from autologous TIL towards neoantigen directed T cell therapies
J. van der Berg, Amsterdam

B3 Adoptive therapy with TCR gene-engineered T cells to treat patients with MAGE-C2-positive melanoma and head and neck cancer
C. Lamers, Rotterdam

B4 Effective NY-ESO-1-specific MHC II-restricted T cell receptors from antigen-negative hosts enhance tumor regression
L. Poncette, Berlin

B5 Effective re-routing of NK cell cytotoxicity against B-cell malignancies upon TCR gene transfer
L. Morton, Leiden

B6 Exploiting clonal tracking of WT1-specific T cells to generate a library of tumor-specific T cell receptors (TCR) for TCR gene editing of acute leukemia
E. Ruggiero, Milan

B7 Identification of a neoantigen targeted by tumor-infiltrating lymphocytes in a patient with HER2+ breast cancer
H. Reimann, Erlangen

B8 HA-1-specific T-cell receptors for the treatment of hematological malignancies
D. Sommermeyer, Planegg-Martinsried

B9 Redirecting CD4 and gamma-delta T cells by RNA electroporation of mRNAs encoding CD8 and an MHC class I-restricted Wilms' Tumor 1-specific TCR
G. Roex, Antwerp

B10 Revealing efficacy and toxicity of high affinity WT1-specific TCRs
R. A. van Amerongen, Leiden

B11 Targeting mutant p53 in spontaneous cancer by T cell receptor gene therapy
V. Anastasopoulou, Berlin

B12 Analysis of potential epitopes from drug-selected mutations in chronic myeloid leukemia for T cell receptor gene therapy
M. Hsu, Heidelberg

B13 Selective inhibition of the transcription factor NFAT in mitigating graft-versus-host disease
R. Seal, Würzburg

B14 Targeting FLT3 with T cell receptor-modified T cells for treatment of hematological malignancies: potential on target toxicity in clinical applications
N. Çakman-Görür, Berlin

B15 TCR gene therapy of CD22-positive B cell malignancies
S. Rhein, Berlin

B16 With great power comes great vulnerability:
Functional comparison of TCR and CAR transduced T cells targeting CD20
T. Wachsmann, Leiden

Poster Session C

Immunological Checkpoints

C1 1,25-dihydroxyvitamin-D3 levels measured at the time of allogeneic hematopoietic stem cell transplantation predict one-year survival
K. Peter, Regensburg

C2 A screening for novel immune-checkpoints identifies a serine/threonine kinase to confer immune resistance in multiple myeloma
V. Volpin, Regensburg

C3 Alternative splicing of immune receptor SLAMF6 – a new regulatory mechanism with the potential to enhance T cell transfer
M. Lotem, Jerusalem

C4 Patterns of immune checkpoint expression by primary tumor cells and tumor infiltrating lymphocytes across different tumor entities
M. Thelen, Cologne

C5 In vivo studies of immunomodulatory antibody CP-870,893 in a humanized mouse model
C. Reitinger, Erlangen

C6 PD-L1 expression in oral leukoplakia: New player in prediction of malignant transformation and new starting point for treatment?
J. Ries, Erlangen

C7 Tumor microenvironment confers enhanced immune privilege of CLL cells by upregulation of PD-L1
M. Böttcher, Erlangen

C8 Regulation of the immune checkpoint molecule PD-L1 in cancer cells
V. Strauch, Erlangen

C9 Tumor immune microenvironment drives prognostic relevance correlating with bladder cancer subtypes
M. Eckstein, Erlangen

C10 Allergen and Rhinovirus (RV) regulation of PDL1 and IFNß in allergic asthma
J. Koelle, Erlangen

C11 Emerging immuno-oncology targets for glioma and multiple sclerosis
A. Menevse, Regensburg

C12 Immunomodulatory effect of vitamin D during allogeneic hematopoietic stem cell transplantation
C. Flamann, Erlangen

C13 Testing human Fc-Fcgamma R contributions to agonistic antibodies in a humanized mouse model system
A. Ipsen Escobedo, Erlangen

Poster Session D

Modulation of the Tumor-Immune Microenvironment

D1 Accelerated glucose metabolism is associated with less T cell infiltration in human OSCC
K. Singer, Regensburg

D2 Development of a murine, myc-driven lymphoma model expressing human CD22 enables testing of targeted therapies and their effects on tumor immune microenvironment
F. Wagner, Erlangen

D3 Ex vivo hyperthermia of B16 melanoma and MCF-7 / MDA-MB-231 breast cancer cells by microwave irradiation compared to warm-water in a closed-loop system
M. Hader, Erlangen

D4 Extracellular depletion of hydrogen peroxide leads to induction of regulatory dendritic cells capable of suppressing T cell proliferation
A. Menzner, Erlangen

D5 Multiple myeloma (MM) is considered a chronic and incurable disease due to its highly complex and heterogeneous molecular abnormalities. In recent years, integrating proteasome inhibitors and immunomodulatory drugs into MM frontline therapy has signi
H. Bruns, Erlangen

D6 PD-L1-specific immunocytokines augment functionality and antitumor activity of CAR-engineered NK cells
A. Lindner, Frankfurt

D7 PKC-ß dependent changes in the metabolism of the bone marrow niche after CLL contact
G. Lutzny-Geier, Erlangen

D8 Reduction of CLL-induced immunosuppression by DUSP1/6 inhibition via BCI
M. Braun, Munich

D9 Stroma cells promote a S100A8/A9high-subset of AML blasts with distinct metabolic features in a Jak/STAT3-dependent manner
K. Panagiotidis, Erlangen

D10 Whole tumor cell-based vaccines generated with high hydrostatic pressure act synergistically with radiotherapy by generating a favorable immune microenvironment
M. Rückert, Erlangen

Poster Session E

Immune Effector Cells (T-Cells, NK-Cells)

E1 Adoptive transfer of CMV - and EBV - specific peptide-stimulated T cells after allogeneic stem cell transplantation: update to the phase I/IIa clinical trial [MULTIVIR-01]
R. Gary, Erlangen

E2 A novel mouse model for superagonistic anti-CD28 monoclonal antibody-induced cytokine release syndrome in humans
N. Beyersdorf, Würzburg

E3 AML immune evasion is mediated by soluble factors interfering with CD8+ T cell metabolism
F. Uhl, Freiburg

E4 Evolution of a GMP-compliant allogeneic EBV peptide-specific T cell bank and the use of multi-parameter flow cytometry for product analysi
A. Fraser, Edinburgh

E5 New whole genome association scanning approach for the discovery of HLA class I-restricted minor histocompatibility antigens
K. Fuchs, Leiden

E6 NF-kappaB activation triggers NK-cell stimulation by monocyte-derived dendritic cells
J. Doerrie, Erlangen

E7 Oncogene inactivating drug combined with irradiation and vaccination breaks tolerance against autochthonous tumors
K. Anders, Berlin

E8 Metabolic stress related immune alterations with impact on the GvL effect and GvHD in allo-HSCT
F. Karl, Erlangen

E9 Two distinct NK cells subsets differ in cytokine expression without effect on cytotoxic potential
M. Holubova, Pilsen

E10 Successful ex vivo expansion of BK-virus-specific T-cells from immuno-suppressed patients after kidney transplantation indicates potential for adoptive T-cell therapy
R. Geyeregger, Vienna

E11 The importance of NFATc1 for anti-tumoral immune responses during the development of lung cancer
L. Heim, Erlangen

E12 The NK cell compartment in multiple myeloma patients – a phenotypic and functional analysis
B. Jacobs, Erlangen

E13 TNF-α based adoptive T cell therapy
S. Stamova, Regensburg

E14 Tumor-specific immune responses following microwave ablation in patients with hepatocellular carcinoma
K. Leuchte, Cologne

E15 Immune cell infiltration predicts malignant transformation of precursor lesions of oral cancer
M. Weber, Erlangen

E16 A database of computationally predicted T cell epitopes from overexpressed proteins in cutaneous melanoma
J. Vera-González, Erlangen

E17 Cellular and molecular changes in the anti-tumor immune response after therapeutic vaccination with CpG and αGC
L. Norkus, Bonn

E18 Characterization of bispecific antibodies that drive synthetic agonistic receptor - transduced T cells to mediate specific and conditional therapy in human pancreatic cancer models
M. Benmebarek, Munich

E19 Development of an off-the-shelf cell product for antigen-dependent cytokine secretion to increase anti-cancer immune responses
C. Faust Akl, Berlin

E20 Diet-induced Vitamin D insufficiency alters immune cell composition of Balb/c mice
C. Matos, Regensburg

E21 Specific PRMT5 inhibitors suppress human CD8+ T cells by upregulation of p53 and impairment of the AKT pathway similar to the tumor metabolite MTA
C. Strobl, Erlangen

E22 LDHB overexpression alters mitochondrial metabolism in human CD4+ T cells
S. Decking, Regensburg

E23 Strategies for enhancing Vγ9Vδ2 T-cell functions against cancer
T. Hoeres, Nürnberg

Poster Session F

Antigen Presenting Cells

F1 A phase I/II vaccine clinical trial in metastatic malignant melanoma patients with step by step optimized mRNA-electroporated dendritic cells: an increase in survival correlates with eosinophilia and upregulation of PEBP1
J. Doerrie, Erlangen

F2 Antigen targeting of Fc receptors induces strong and functional relevant T cell responses in vivo independent of ITAM signaling but dependent on dendritic cell subsets
C. Lehmann, Erlangen

F3 Characterizing the immunogenicity of DM-sensitive and DM-resistant antigens
S. Kretschmann, Erlangen

F4 Dendritic cell vaccination in metastatic uveal melanoma as compassionate treatment - immunological and clinical responses
A. Moreira, Erlangen

F5 Induction of anti-tumor responses via antigen targeting of dendritic cells in vivo
L. Amon, Erlangen

F6 Randomized, open-label Phase III multi-center study to evaluate the adjuvant vaccination with tumor RNA-loaded autologous dendritic cells versus observation of patients with resected monosomy 3 uveal melanoma
B. Schuler-Thurner, Erlangen

F7 The generation of GMP-compliant human monocyte-derived dendritic cells by using the Quantum® hollow fiber bioreactor system
U. Uslu, Erlangen

F8 Automated closed-system generation of monocyte-derived dendritic cells by CliniMACS Prodigy® for the use in cancer immunotherapy
M. Erdmann, Erlangen

F9 Blood eosinophilia is an on-treatment biomarker in patients undergoing vaccination with dendritic cells (DC) that correlates with long-term patient outcome
A. Moreira, Erlangen

F10 CD86+ antigen-presenting B cells are increased in solid cancers and induce tumor antigen-specific T cell responses
K. Wennhold, Cologne

F11 Dendritic cell vaccination of a type III gastric neuroendocrine tumor and a microsatellite-stable colorectal carcinoma patient: immunological and clinical responses
S. Gross, Erlangen

F12 Potent target cell effects make Dendritic Cell (DC)-derived extracellular vesicles (EV) an attractive tool in cellular therapy
A. Baur, Erlangen

F13 Tissue derived non-classical monocyte derived host macrophages protect against murine intestinal acute graft-versus-host disease
D. Le, Würzburg

F14 Transendothelial migration of monocytes as potential therapeutical target for acute Graft-Versus-Host-Disease of the liver
T. Rottmar, Erlangen

Poster Session G

Suppressor Cells (Treg-Cells, MDSCs, MSCs)

G1 AML exosomes promote TLR2-mediated induction of glycolytic MDSCs
S. Tohumeken, Erlangen

G2 Exposing TNF receptor super family members as therapeutic targets for pancreatic cancer
T. Steinfatt, Würzburg

G3 Human double-negative regulatory T cells modulate functionality of conventional T cells via inhibition of mTOR signaling and metabolic reprogramming
T. Haug, Erlangen

G4 T cell receptor repertoires and gene expression profiles of regulatory T cells after polyclonal or allo-specific in vitro expansion
D. Dittmar, Regensburg

G5 Definition of inflamed, immune-excluded and immune desert head and neck squamous cell carcinomas based on intratumoral cytotoxic and regulatory T-cells
M. Hecht, Erlangen

G6 Functional conversion of cytotoxic T cells into iTreg in breast cancer patients
M. Xydia, Regensburg

Invited Speakers

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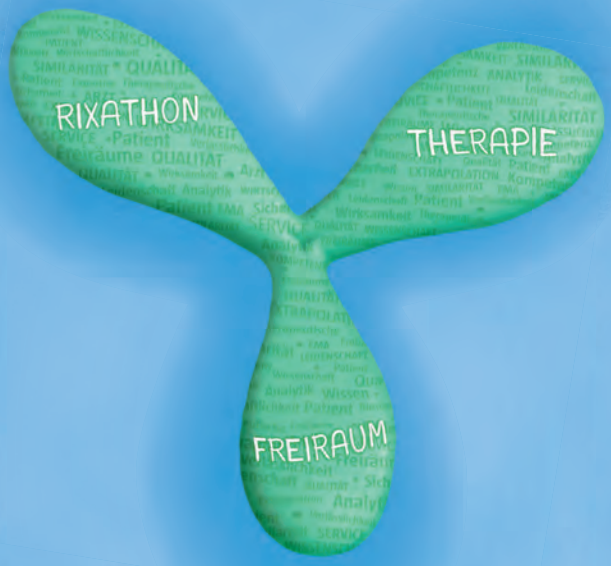
Novartis Pharma GmbH	10.000 €
PeproTech GmbH	2.500 €
Pfizer Pharma GmbH	2.500 €
Pharmacosmos GmbH	2.500 €
Roche Pharma AG	10.000 €
Sanofi-Aventis Deutschland GmbH	2.500 €
Therakos (UK) Limited	5.000 €

RIXATHON®
rituximab

Jede Behandlung ein Beitrag
zu mehr **Therapiefreiraum**



- Wirtschaftliche Therapiealternative für Ihre Rituximab-Patienten[1]
- Mehr Flexibilität in der Behandlung durch verlängerte Haltbarkeit[2]
- Entwicklung und Herstellung Sandoz/Hexal in Europa

Referenzen (mod. nach): 1. Gegenüber dem Referenzprodukt Mabthera® (Roche), Stand Lauer-Taxe: 01.01.2019 Parallelimporte und Rabatte aus eventuell bestehenden Rabattverträgen sind nicht berücksichtigt. **2.** Fachinformtation Rixathon®, Stand November 2017; 30 Tage Haltbarkeit bei 2°-8° Grad bei aseptischer Verdünnung in 0,9% NaCl-Lösung.

Rixathon® 100 mg/- 500 mg Konzentrat zur Herstellung einer Infusionslösung. Zusammensetz.: Wirkstoff: Rituximab. Jede Durchstechfl. m. 10 ml/50 ml enth. 100 mg/ 500 mg Rituximab. Sonstige Bestandteile: Na-citrat, Polysorbat 80, Na-chlorid, Na-hydroxid, Salzsäure, Wasser f. Inj.-zw. **Anwendungsgeb.:** Erw. Pat. in Komb. m. einer Chemother. als Erstbehandl. des follikulären Lymphoms im Stad. III-IV. Erhalt.-ther. b. Pat. m. follikulärem Lymphom nach Ansprechen auf eine Indukt.-ther. Als Monother. b. Pat. m. follikulärem Lymphom im Stad. III-IV, die geg. eine Chemother. resistent sind od. nach einer solchen einen zweiten od. neuerlichen Rückfall haben; Ther. v. Pat. m. CD20-pos. diffusem großzelligen B-Zell-Non-Hodgkin-Lymphom (DLBCL) in Komb. m. einer CHOP-Chemother. In Komb. m. einer Chemother. b. nicht vorbehand. Pat. u. b. Pat. m. rezidivier./refraktärer chron. lymphat. Leukämie (CLL). Begrenzte Daten zur Wirksamk. u. Sicherh. für Pat. m. CLL, die bereits m. monoklon. Antikörpern einschl. Rituximab behand. wurden od. für Pat. m. CLL, die refraktär auf eine Vorbehandl. m. Rituximab in Komb. m. einer Chemother. sind. In Komb. m. Methotrexat b. erw. Pat. m. schwerer aktiver rheum. Arthritis, die ungenüg. auf DMARDs einschl. einer od. mehrerer anti-TNF-Ther. angesprochen od. diese nicht vertragen haben. Rituximab verhindert in Komb. m. MTX das Fortschreiten der radiolog. nachweisbaren Gelenkschäd. u. verbessert die körperl. Funkt.-fähigk. In Komb. m. Glucocorticoiden zur Indukt. einer Remiss. b. erw. Pat. m. schwerer aktiver Granulomatose m. Polyangiitis (Wegenersche Granulomatose, GPA) u. mikroskop. Polyangiitis (MPA). **Gegenanz.:** Überempf. geg. Inhaltsst. od. Maus-Proteine. Aktive, schwere Infekt. Stark geschwächte Immunabwehr. Indikat. RA/GPA/MPA zusätzl.: schwere Herzinsuff. (NYHA Kl. IV) od. schwere unkontroll. Herzerkrank. **Nebenwirk.:** Bakt. u. virale Infekt., Bronchitis, Sepsis, Pneumonie, febrile Infekt., Herpes zoster, Infekt. d. Respirationstrakts, Pilzinfekt., Infekt. unbekannter Genese, akute Bronchitis, Sinusitis, Hepatitis B (einschl. Reaktivier., primäre Infekt. u. fulminante Hepatitis m. tödl. Ausgang), schwerwieg. Virusinfekt., Pneumocystis jirovecii, PML, Harnwegsinfekt., Gastroenteritis, Tinea pedis, Nasopharyngitis; Neutrop., Leukop., febrile Neutrop., Thrombozytop., Anämie, Panzytop., Granulozytop., Gerinnungsstör., aplast. Anämie, hämolyt. Anämie, Lymphadenopathie, vorübergeh. Anstieg d. IgM-Serumsp., späte Neutrop., Serumkrankh.-ähnl. Reakt.; infusionsbed. Reakt. (auch bis zu 24h nach d. Infus. u. m. tödl. Ausgang), Angioödem, Überempfindlichk., Anaphylaxie, Tumorlysesyndr., Zytokin-Freisetzungs-Syndr., Serumkrankh., infusionsbed. akute Thrombozytop.; Hyperglykämie, Gewichtsverlust, peripheres Ödem, Gesichtsödem, erhöhte LDL-Werte, Hypokalzämie, Hypercholesterinämie, Hyperkaliämie, Depression, Nervosität, Angst, Insomnie; Parästhesie, Hypästhesie, Erreg., Schlaflosigk., Vasodilatat., Schwindel, Angstgefühle, Stör. d. Geschmacksempf., periph. Neuropathie, Gesichtsnervenlähm., kraniale Neuropathie, Verlust and. Sinne, Kopfschm., Migräne, Ischialgie, Zittern; Stör. d. Tränenbild., Konjunktivitis, schwerer Sehverlust; Tinnitus, Ohrenschm., Gehörverlust; Myokardinfarkt, Arryth., Vorhofflimmern, Tachykard., Herzerkrank., linksventrikul. Versagen, supraventrikul. Tachyk., Angina pectoris, Myokardischämie, Bradyk., schwere Herzerkrank., Herzinsuff., Vorhofflattern; Hypertonie, orthostatische Hypotonie, Hypotonie, Vaskulitis (vorwieg. kutan), leukozytoklast. Vaskulitis, Hautröt.; Bronchospasmus, Atemwegserkrank., Brustschm., Dyspnoe, vermehrtes Husten, Rhinitis, Asthma, Bronchiolitis obliterans, Lungenerkrank., Hypoxie, interstit. Lungenerkrank. (einschl. Todesfälle), respiratorische Insuff., Lungeninfiltrate, Epistaxis, verstopfte Nase; Übelk., Erbr., Diarrhö, Abdominalschm., Dysphagie, Obstipat., Dyspepsie, Anorexie, Rachenreiz., Abdomenvergrößer., Magen-Darm-Perforat. (einschl. Todesfälle), gastroösophag. Reflux, Mundulzerat., Oberbauchschm.; Pruritus, Exanthem, Alopezie, Urtikaria, Schwitzen, Nachtschweiß, Hauterkrank., schwere bullöse Hautreakt., SJS, TEN (Lyell-Syndrom), Akne; Hypertonie, Myalgie, Arthralgie, Rückenschm., Nackenschm., Schmerzen, Skelettmuskulaturschm., Osteoarthritis, Bursitis, Muskelspasmen, Muskelschwäche, Schm. in d. Gliedmaßen; Nierenversagen; Fieber, Schüttelfrost, Asthenie, Kopfschm., Tumorschm., Röt., Unwohls., Erkältungserschein., Fatigue, Frösteln, Multiorganversagen, Schm. an d. Inf.-stelle, periphere Ödeme; vermind. IgG-Serumsp., verringerte IgM-Spiegel, verringertes Hämoglobin. **Warnhinw.:** Enthält Natrium. Weit. Einzelh. u. Hinw. s. Fach- u. Gebrauchsinfo. Verschreibungspflichtig. **Mat.-Nr.:** 3/51010899 **Stand:** November 2017. Hexal AG, 83607 Holzkirchen, www.hexal.de

HEXAL
A Sandoz Brand

www.hexal.de

KEYTRUDA® als Monotherapie bei Erwachsenen:
a mit fortgeschrittenem (nicht resezierbaren oder metastasierenden) Melanom; **b** zur adjuvanten Behandlung des Melanoms im Tumorstadium III mit Lymphknotenbeteiligung nach vollständiger Resektion; **c** zur Erstlinienbehandlung des metastasierenden NSCLC mit hoher Tumor-PD-L1-Expression (TPS ≥ 50 %) ohne EGFR oder ALK-positive Tumormutationen; **d** zur Behandlung des lokal fortgeschrittenen oder metastasierenden PD-L1-positiven NSCLC (TPS ≥ 1 %) nach vorheriger Chemotherapie. Patienten mit EGFR- oder ALK-positiven Tumormutationen sollten vor der Therapie mit KEYTRUDA® ebenfalls eine auf diese Mutationen zielgerichtete Therapie erhalten haben; **e** beim rezidivierenden oder refraktären klassischen Hodgkin-Lymphom nach Versagen einer autologen Stammzelltransplantation (auto-SZT) und Behandlung mit Brentuximab Vedotin (BV); oder nach Versagen einer Behandlung mit BV, wenn auto-SZT nicht in Frage kommt; **f** mit lokal fortgeschrittenem oder metastasierendem Urothelkarzinom mit PD-L1 kombiniertem positiven Score (CPS) ≥ 10 bei nicht für eine Cisplatin-basierte Therapie geeigneten Patienten in der Erstlinie; **g** mit lokal fortgeschrittenem oder metastasierendem Urothelkarzinom nach vorheriger Platinbasierter Therapie; **h** beim rezidivierenden oder metastasierenden Plattenepithelkarzinom der Kopf-Hals-Region mit hoher Tumor-PD-L1-Expression (TPS ≥ 50 %) und fortschreitender Krebserkrankung während/nach vorheriger Platin-basierter Therapie

KEYTRUDA® als Kombinationstherapie bei Erwachsenen:
i mit Pemetrexed und Platin-Chemotherapie zur Erstlinienbehandlung des metastasierenden nicht-plattenepithelialen NSCLC ohne EGFR- oder ALK-positive Tumormutationen

KEYTRUDA® 50 mg Pulver für ein Konzentrat zur Herstellung einer Infusionslösung | KEYTRUDA® 25 mg/ml Konzentrat zur Herstellung einer Infusionslösung
Wirkstoff: Pembrolizumab **Zus.:** *Arzneil. wirks. Bestandt.: -50 mg Pulver:* 1 Durchstechfl. enth. 50 mg Pembrolizumab. Nach Rekonstitution enth. 1 ml Konzentrat 25 mg Pembrolizumab. *-25 mg/ml Konzentrat:* 1 Durchstechfl. (4 ml) enth. 100 mg Pembrolizumab. 1 ml Konzentrat enth. 25 mg Pembrolizumab. *Sonst. Bestandt.:* L-Histidin, L-Histidinhydrochlorid-Monohydrat, Sucrose, Polysorbat 80. *-25 mg/ml Konzentrat zusätzl.:* Wasser für Injekt.-zwecke. **Anw.:** Als Monother. zur Behandl. d. fortgeschrittenen (nicht resezierbaren od. metastasierenden) Melanoms b. Erw. Als Monother. zur adjuvanten Behandl. des Melanoms im Tumorstadium III mit Lymphknotenbeteilig. nach vollständ. Resektion bei Erw. Als Monother. zur Erstlinienbehandl. d. metastasierenden nicht-kleinzelligen Lungenkarzinoms (NSCLC) m. PD-L1 exprimierenden Tumoren (Tumor Proportion Score [TPS] ≥ 50 %) ohne EGFR- od. ALK-pos. Tumormutationen b. Erw. In Komb. m. Pemetrexed u. Platin-Chemotherapie zur Erstlinienbehandl. d. metastasierenden nicht-plattenepithelialen NSCLC ohne EGFR- od. ALK-pos. Tumormutationen bei Erw. Als Monother. zur Behandl. d. lokal fortgeschrittenen od. metastasierenden NSCLC m. PD-L1 exprimierenden Tumoren (TPS ≥ 1 %) nach vorheriger Chemother. b. Erw. Pat. m. EGFR- od. ALK-pos. Tumormutationen sollten vor Ther. ebenfalls eine auf diese Mutationen zielgericht. Ther. erhalten haben. Als Monother. zur Behandl. d. rezidivierenden od. refraktären klassischen Hodgkin-Lymphoms (HL) b. Erw. nach Versagen einer autologen Stammzelltransplantation (auto-SZT) u. einer Behandl. m. Brentuximab Vedotin (BV), od. nach Versagen einer Behandl. m. BV, wenn eine auto-SZT nicht in Frage kommt. Als Monother. zur Behandl. d. lokal fortgeschrittenen od. metastasierenden Urothelkarzinoms nach vorheriger Platin-basierter Ther. b. Erw. Als Monother. zur Behandl. d. lokal fortgeschrittenen od. metastasierenden Urothelkarzinoms bei Erw., die nicht für e. Cisplatin-basierte Ther. geeignet sind u. deren Tumoren PD-L1 m. einem kombinierten positiven Score (CPS) ≥ 10 exprimieren. Als Monother. zur Behandl. d. rezidivierenden od. metastasierenden Plattenepithelkarzinoms der Kopf-Hals-Region (HNSCC) m. PD-L1 exprimierenden Tumoren (TPS ≥ 50 %) und einem Fortschreiten der Krebserkrank. während od. nach vorheriger Platin-basierter Ther. b. Erw. **Gegenanz.:** Überempf.-keit gg. d. Wirkstoff od. e. d. sonst. Bestandt. **Vorsicht bei:** Pat. ≥ 75 J. Schwerer Einschränk. d. Nierenfunkt.; moderater od. schwerer Einschränk. d. Leberfunkt. Melanom d. Auges. Anamnest. bek. immunvermittelte Myokarditis. Behandl. nach Risikoabwägung b. Pat. m.: aktiven ZNS-Metastasen; ECOG-Performance-Status ≥ 2 (außer bei Urothelkarzinom); HIV-, HBV- od. HCV-Infekt.; aktiven, system. Autoimmunerkrank.; interstit. Lungenkrankh.; einer früheren Pneumonitis, d. system. Kortikoidbehandl. erforderte; schwerer Überempf.-keit gg. e. and. monoklonalen Antikörper in d. Anamnese; laufender Ther. m. Immunsuppressiva; schweren immunvermittelten Nebenw. unter Ipilimumab in d. Anamnese (jegliche Grad 4 od. Grad 3 Toxizität), d. eine Kortikosteroid-Behandl. über mehr als 12 Wo. erforderte (mehr als 10 mg/Tag Prednison od. Äquivalent in entspr. Dosierung); aktiv. Infekt.-erkrank.; Pat., d. unter vorhergeh. Krebsbehandl. m. immunstimulierenden Arzneim. schwere od. lebensbedrohl. Nebenw. d. Haut hatten; Pat., d. ein solides Organtransplantat empfangen haben; Pat. m. allo-HSZT in Krankengesch. Komb. m. Pemetrexed u. e. Platin-Chemother. Hinweise zu Schwangerschaft/Stillzeit beachten. Unter den immunvermittelten Nebenw. waren schwere Fälle u. Todesfälle. *Zusätzl. bei HNSCC:* Pat. m. vorheriger Strahlenther. *Zusätzl. bei klass. HL:* Pat. ≥ 65 J. Pat. m. rezidivierendem od. refraktärem klassischen HL, für die aus and. Gründen als dem Versagen einer Salvage-Chemother. eine auto-SZT nicht in Frage kommt. Bei allo-HSZT bei klassischem HL nach Ther. m. Pembrolizumab sorgfältige Nutzen-Risiko-Abwägung (GVHD u. schwere Lebervenenverschlusskrankheit als Komplikat. beobachtet). *Zusätzl. bei Urothelkarzinom n. vorh. Platin-basierter Ther.:* Pat. m. schlechterer Prognose u./od. aggressiv. Krankheitsverl. *Zusätzl. bei Pat. m. Urothelkarzinom, d. nicht f. Cisplatin-basierte Ther. geeignet sind u. deren Tumoren PD-L1 m. einem CPS ≥ 10 exprimieren:* gebrechliche Pat. (z. B. ECOG-Performance-Status 3). **Nebenw.:** *Monother.: Sehr häufig:* Anämie. Hypothyreose. Vermind. Appetit. Kopfschm. Dyspnoe; Husten. Diarrhö; Abdominalschm.; Übelk.; Erbr.; Obstipat. Hautausschl.; Pruritus. Muskuloskelett. Schm.; Arthralgie. Müdigk./Erschöpf.; Asthenie; Ödeme; Fieber.*Häufig:* Pneumonie. Thrombozytopenie; Lymphopenie. Infusionsbedingte Reakt. Hyperthyreose. Hyponatriämie; Hypokaliämie; Hypokalzämie. Schlaflosigk. Schwindelgef.; periph. Neuropathie; Lethargie; Dysgeusie. Trock. Augen. Hypertonie. Pneumonitis. Kolitis; Mundtrockenh. Schwere Hautreakt.; Erythem; Vitiligo; trock. Haut; Alopezie; Ekzem; akneiforme Dermatitis. Schm. in d. Extremitäten; Myositis; Arthritis. Grippeähnl. Erkrank.; Schüttelfrost. Erhöh. Werte von: AST, ALT; Hyperkalzämie; erhöh. Werte von: alkal. Phosphatase im Blut, Bilirubin im Blut, Kreatinin im Blut. *Gelegentl.:* Neutropenie; Leukopenie; Eosinophilie. Sarkoidose. Hypophysitis; Thyreoiditis; Nebenniereninsuff. Typ-1-Diabetes mellitus. Epilepsie. Uveitis. Perikarderguss; Perikarditis. Pankreatitis. Hepatitis. Lichenoide Keratose; Psoriasis; Dermatitis; Papeln; Änd. d. Haarfarbe. Tendosynovitis. Nephritis. Erhöh. Werte von Amylase. *Selten:* Immunthrombozytopen. Purpura; hämolyt. Anämie; isolierte aplast. Anämie. Guillain-Barré-Syndrom; Myasthenie-Syndrom; Meningitis (aseptisch); Enzephalitis. Vogt-Koyanagi-Harada-Syndrom. Myokarditis. Dünndarmperforation. TEN; SJS; Erythema nodosum. *Nicht bekannt:* Abstoßung eines soliden Organtransplantats. *Zusätzl.:* Hinweise zu Abw. bei Laborwerten beachten. *B. Komb. m. Chemother.: Sehr häufig:* Anämie; Neutropenie; Thrombozytopenie. Vermind. Appetit. Dysgeusie. Diarrhö; Übelk.; Erbr.; Obstipation. Hautausschl.; Pruritus. Müdigk./Erschöpf.; Asthenie; Ödeme. Erhöh. Werte von ALT. *Häufig:* Pneumonie. Febrile Neutropenie; Leukopenie; Lymphopenie. Infusionsbed. Reakt. Hypothyreose; Hyperthyreose. Hypokaliämie; Hyponatriämie; Hypokalzämie. Schwindelgef.; Kopfschm.; Lethargie; periphere Neuropathie. Trock. Augen. Pneumonitis; Dyspnoe; Husten. Kolitis; Abdominalschm.; Mundtrockenh. Hepatitis. Schwere Hautreakt.; Alopezie; akneiforme Dermatitis; trock. Haut; Erythem. Arthralgie; Myositis; Arthritis; muskuloskelett. Schm.; Schm. in d. Extremitäten. Nephritis; akutes Nierenvers. Fieber. Erhöh. Werte von: AST, Kreatinin im Blut, alkal. Phosphatase im Blut. *Gelegentl.:* Hypophysitis; Thyreoiditis; Nebenniereninsuff. Typ-1-Diabetes mellitus. Schlaflosigk. Perikarderguss. Hypertonie. Pankreatitis. Dermatitis; Ekzem; Änd. d. Haarfarbe; lichenoide Keratose; Vitiligo. Schüttelfrost; grippeähnl. Erkrank. Erhöh. Werte von Amylase; Hyperkalzämie. **Warnhinw.:** KEYTRUDA® 25 mg/ml Konzentrat zur Herstellung einer Infusionslösung: Nicht schütteln. **Hinw.:** Untersuch. der PD-L1-Tumor-Expression mittels eines validierten Tests bei Pat. mit NSCLC, zuvor unbehandeltem Urothelkarzinom od. HNSCC. Zuverlässige Verhütungsmethode b. Frauen im gebärf. Alter währ. u. bis 4 Mon. nach Behandl.-ende.
Verschreibungspflichtig.

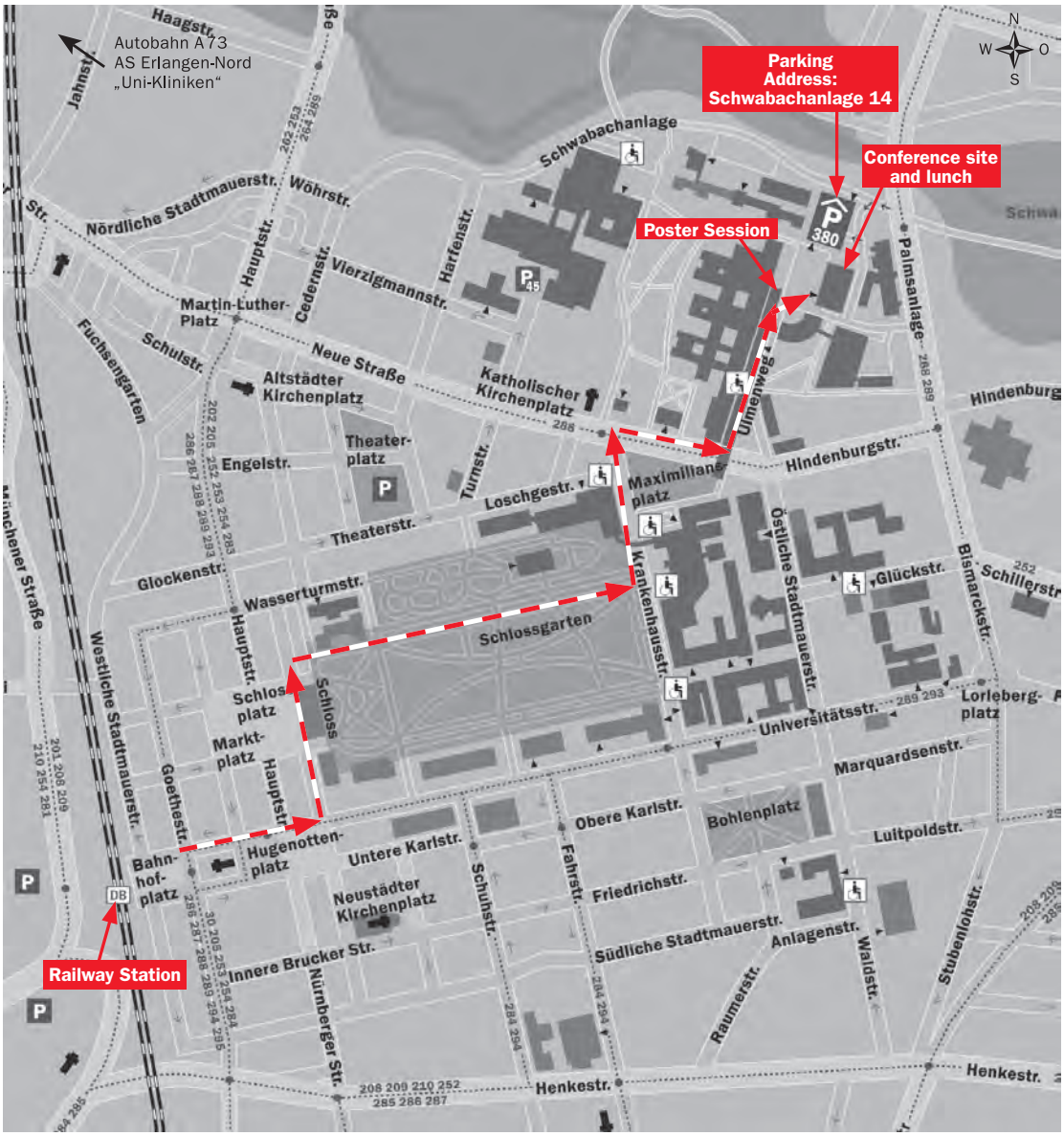
Stand: 01/2019

Bitte lesen Sie vor Verordnung von KEYTRUDA® die Fachinformation!

Pharmazeutischer Unternehmer: Merck Sharp & Dohme B.V., Waarderweg 39, 2031 BN Haarlem, Niederlande; Lokaler Ansprechpartner: MSD SHARP & DOHME GMBH, Lindenplatz 1, 85540 Haar

ONCO-1190862-0010 01/19

Map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and lunch
Poster Session
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Jahnstr.
Schwabachanlage
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Wöhrstr.
Hauptstr.
Cedernstr.
Vierzigmannstr.
Harfenstr.
Martin-Luther-Platz
Fuchsengarten
Schulstr.
Neue Straße
Altstädter Kirchenplatz
Katholischer Kirchenplatz
Ulmenweg
Palmsanlage
Hindenburg
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Theater-platz
Engelstr.
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Loschgestr.
Maximilians-platz
Theaterstr.
Glockenstr.
Wasserturmstr.
Krankenhausstr.
Östliche Stadtmauerstr.
Glückstr.
Bismarckstr.
Schillerstr.
Schlossgarten
Hauptstr.
Schloss-platz
Schloss
Markt-platz
Universitätsstr.
Lorleberg-platz
Marquardsenstr.
Goethestr.
Westliche Stadtmauerstr.
Münchener Straße
Obere Karlstr.
Bohlenplatz
Luitpoldstr.
Bahn-hof-platz
Hugenotten-platz
Untere Karlstr.
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Schuhstr.
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Nürnberger Str.
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Notes

10th International Symposium
on the Clinical Use of Cellular Products

Abstract Book

Cellular Therapy 2019

March 22 and 23, 2019, Erlangen, Germany

CELLULAR THERAPY
International Symposium
Erlangen

Universitätsklinikum Erlangen

Session I

CAR T-Cells - Preclinical

1

Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for AML

S. Gill
[1] University of Pennsylvania, Hematology-Oncology, Philadelphia, USA

The absence of cancer-restricted surface markers is a major impediment to antigen-specific immunotherapy using chimeric antigen receptor (CAR) T cells. Targeting the canonical myeloid marker CD33 in acute myeloid leukemia (AML) results in toxicity from destruction of normal myeloid cells. We created a leukemia-specific antigen by deleting CD33 from normal hematopoietic stem and progenitor cells (HSPCs), thereby generating a hematopoietic system resistant to CD33-targeted therapy and enabling specific targeting of AML with CAR T cells. We generated CD33-deficient human HSPCs and demonstrated normal engraftment and differentiation in immunodeficient mice. Autologous CD33 KO HSPC transplantation in rhesus macaques demonstrated long-term multilineage engraftment of gene-edited cells with normal myeloid function. CD33-deficient cells were impervious to CD33-targeting CAR T cells, allowing for efficient elimination of leukemia without myelotoxicity. Thus we developed a novel approach to antigen-specific immunotherapy by genetically engineering the host to avoid on-target, off-tumor toxicity.

2

Beyond hematologic malignancies: challenges and opportunities

B. Savoldo
[1] Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, USA

3

Targeted delivery of a PD-1-blocking scFvby CAR-T cells enhances anti-tumor efficacyin vivo

R. Brentjens
[1] Memorial Sloan Kettering Cancer Center, New York, USA

4

A Novel Pharmacologic 'Remote Control to prevent CAR T cell related CRS

K. Mestermann[1], T. Giavridis[2], J. Rydzek[1], S. Frenz[1], J. Weber[1], T. Nerreter[1], H. Einsele[1], M. Sadelain[2], M. Hudecek[1]

[1] Universitätsklinikum Würzburg, Medizinische Klinik und Poliklinik II, Würzburg, Germany

[2] Memorial Sloan Kettering Cancer Center, Center for Cell Engineering, New York, USA

Background: Immunotherapy with chimeric antigen receptor T cells (CAR-T) is emerging as a transformative novel treatment for hematologic malignancies. In contrast to conventional agents, CAR-T are a living drug that is essentially out of the physician's (and patient`s) control after infusion. At present, there is a lack of technologies to maintain control over CAR-T, both for steering efficacy and for preventing toxicity. Here, we present a novel strategy of pharmacologic 'remote control' to precisely steer CAR-T activity and function in real-time. Methods: We considered that an effective way for controlling CAR-T function was to interfere with signal transduction through the CAR. We assembled a library of tyrosine kinase inhibitors and screened for their ability to block CAR-T function without affecting CAR-T viability. We performed functional testing with CD8+ and CD4+ CAR-T (n=3 donors) in the presence of titrated doses of the lead compound, and employed CD19- and ROR1-specific CARs comprising 4-1BB or CD28 costimulatory moieties. Results: We identified a lead compound (TCI-1), that stood out through its ability to confer a dose-dependent (partial at lower, complete at higher doses) blockade of all CAR-T effector functions, i.e. cytolytic activity, cytokine secretion and proliferation. We confirmed that this lead compound is effective in both CD8+ and CD4+ CAR-T, and capable of completely blocking CAR-T function independent from CAR specificity and costimulatory moiety included in the signaling module. The onset of CAR-T blockade is immediate after exposure to the drug and is caused by interference with early phosphorylation events in the CAR-CD3ζ module as demonstrated by western blot, and by interference with the induction of transcription factors, as demonstrated with an NFAT-inducible reporter gene. Blockade of CAR-T function is effective for several days if the compound is continuously supplied and instantaneously and fully reversible after removal of the compound. Short- and long-term exposure to TCI-1 does not lead to a reduction in CAR-T viability, and does not hinder the subsequent ability of CAR-T to exert their effector functions. For further proof-of-concept, we employed a xenograft model in immunodeficient mice (NSG/Raji) and demonstrate that our lead compound is capable of controlling the function of CD19 CAR-T cells in vivo. We administered TCI-1 between d3 and d5 after CAR-T infusion and show that TCI-1 is able to arrest activated CAR-T during this time interval and pause the antitumor effect. Importantly, CAR-T immediately resumed their antitumor function once administration of TCI-1 was discontinued (function ON-OFF-ON sequence). Subsequently, we evaluated the potential of TCI-1 to mitigate life threatening CRS by using a CRS mouse model recently published by Giavridis et al. The data show that TCI-1 prevents the induction of CRS by blocking the secretion of T-cell-released cytokines, and additionally hinders the stimulation adjacent macrophages by activated CAR-T. Thus, CRS was less severe in mice during and after TCI-1 injection, leading to improved overall survival. Conclusions: Our data introduce TCI-1 as a universally applicable pharmacologic 'remote control' for CAR-T. We show that TCI-1 is capable to exert real-time control over CAR-T function in vitro and efficiently prevents CAR-T related CRS in vivo. TCI-1 has a favorable pharmacokinetic and safety profile in humans, suggesting the potential for rapid clinical implementation. The qualities of TCI-1 with rapid-onset mode of action in addition to complete but fully reversible CAR-T inhibition surpass the qualities of steroids that are toxic to T cells and provide only incomplete control.

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

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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. One major limitation of CAR T cells is their insufficient infiltration into solid tumors resulting in a limited therapeutic effectiveness. A requirement for an efficient migration and tumor homing of transferred T cells is the expression of chemokine receptors matching the chemokine profile produced 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. Methods: Murine and human T cells were transduced with the chemokine receptor CXCR6. Trans-well migration assays validated the migratory capacity of CXCR6-transduced T cells. Furthermore, in vitro penetration of spheroids or pancreatic cancer patient-derived organoids by CXCR6-transduced T cells was investigated using selective plane illumination microscopy (SPIM) or laser confocal scanning microscopy (LCSM). Cytotoxicity assays were performed to determine the 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-transduced T cells. Results: CXCL16 was found to be expressed by murine and human pancreatic cancer cell lines and effectively attracted 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. Therefore, CXCR6 mediates an improved penetration of T cells into 3D structures in vitro. Introducing CXCR6 in antigen-specific T cells (conferred by TCR as well as by murine and human CAR) led to enhanced 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 solid tumors resulting in an intratumoral accumulation of transferred T cells and a 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 a limited tumor infiltration in adoptive cell therapies of solid tumors.

Session II

CAR T-Cells - Clinical

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Next generation CAR T cells for lymphoma/myeloma

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Engineered T cells that encode transgenic T cell receptors (TCR) or chimeric antigen receptors (CAR) are increasingly being tested is a new modality for cancer immunotherapy. In this presentation we will review emerging patterns of efficacy and toxicity after adoptive cell transfer (ACT), with a focus on blood cancers. Striking efficacy has been observed in acute and chronic B cell leukemia and lymphoma. More unexpectedly, strong antitumor effects are being observed in multiple trials in patients with advanced refractory and relapsed myeloma. In some cases, toxicity has been predicted in preclinical models. In other cases, toxicity was only uncovered after results from Phase 1 clinical trial results were in hand. Onset of toxicity can be immediately observed following infusion of cells or maybe delayed. Toxicity usually occurs coincident with peak (Cmax) levels of adoptively transferred cells. Today with more than 1,000 patients having been infused with genetically engineered human T cells, genotoxicity has not been reported. To date, the induction of irAE appears to be less than with checkpoint therapies, although more experience with ACT is required to determine the ultimate incidence of autoimmune toxicities. Funding: Parker Institute for Cancer Immunotherapy, NIH/NCI and Tmunity Therapeutics

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GD2-targeting CAR T-cell therapy for childhood solid tumours

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Neuroblastoma is the second most common solid tumour after brain tumours in childhood. Treatment of high-risk disease consists of a combination of chemotherapy, surgery, radiotherapy and more recently immunotherapy. This highly intensive treatment is associated with significant side effects and still less than half of patients will be long-term survivors. Neuroblastoma uniformly expresses disialoganglioside GD2, while its expression on normal tissue is highly restricted. Redirecting specificity of patient T-cells to GD2 using a chimeric antigen receptor (CAR) provides an attractive approach to induce durable anti-tumour immunity. I will discuss the results to date of our clinical study of GD2-CAR T-cell therapy in patients with relapsed/refractory neuroblastoma (NCT02761915). Anti-tumour activity can indeed be induced in this patient group. Importantly, tumour responses in (bone) marrow and soft tissue sites of disease were achieved without neurotoxicity. However, responses were short-lived. This is likely due to immune inhibitory mechanisms employed by the tumour. Multiple approaches are being developed to overcome these obstacles. As these cannot all be evaluated in the clinical setting, selection of the optimal strategies to apply in neuroblastoma is required. I will discuss our work in progress of using a transgenic model of neuroblastoma to study immune evasion strategies employed in the neuroblastoma tumour microenvironment (TME) upon treatment with GD2-directed CAR T-cells to inform the design of next generation GD2-CAR T-cell therapy.

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HER2neu CAR T cells in sarcoma patients

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Liquidation of solid tumors by in vivo expanded CLDN6-CAR T cells

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Adoptive cell therapy based on genetically engineered T cells armed with chimeric antigen receptors (CARs) shows tremendous clinical success in patients with B-cell malignancies. However, only limited anti-tumoral activity was observed in multiple clinical trials upon CAR T-cell therapy in patients suffering from solid tumors. Two key hurdles of CAR T-cell therapy targeting non-hematological neoplasia are i) the lack of highly cancer selective targets providing challenges for safe treatment protocols and, ii) the insufficient expansion and long-term persistence of CAR T cells probably due to inefficient co-stimulation upon antigen-exposure in vivo. Especially the latter point displays a fundamental difference comparing CAR-based treatment of B cell derived hematological malignancies and solid tumors. We introduce here a CAR T-cell therapy against Claudin 6 (CLDN6), an oncofetal cell surface antigen expressed in embryonic stem cells during fetal development. The gene encoding CLDN6 is strictly silenced and not expressed in healthy adult tissues but it is re-activated in different cancers with a high medical need including ovarian, endometrial, testicular and lung cancers. Based on in vitrostudies, we selected a 4-1BB containing second generation CAR that exhibited a highly specific and sensitive recognition and killing of CLDN6 positive cells. T cells equipped with CLDN6-CAR were able to repetitively clear tumor spheroids in a stress test scenario recently proven to be predictive for in vivo efficiency. Accordingly, therapeutic studies in immunocomprimised mice showed that CLDN6-CAR T cells mediate a complete eradication of advanced human tumors. To further improve the activity we developed a novel approach for stimulation of CAR T cells in vivo by antigen-exposure on professional antigen presenting cells. To this end we used on liposomally formulated mRNA ($RNA_{(LIP)}$) to systemically deliver the corresponding antigen to dendritic cells (DCs) in secondary lymphoid tissues. Repetitive $RNA_{(LIP)}$ treatment can expand CAR T cells in vivo in a dose-dependent manner resulting in an improved persistence. Similarly, we demonstrated an expansion of CAR T cells recognizing other antigens proving the broad applicability of the approach. Further studies demonstrated that stimulation of CAR T cells by $RNA_{(LIP)}$ treatment accelerates anti-tumor activity and can restore anti-tumoral efficacy even at insufficient CAR T-cell doses. Quantitative analyses of CAR-T cells demonstrated that repetitive antigen-exposure on lymphoid DCs in vivo allows a controlled expansion of CAR T cells thereby supporting a safer clinical translation of novel CAR T-cell therapies. A clinical trial investigating CLDN6-CAR therapy in combination with $RNA_{(LIP)}$ treatment is currently planned in patients with advanced CLDN6 positive human cancers.

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T4 Immunotherapy of Head and Neck Squamous Cell Carcinoma using Pan-ErbB Targeted CAR T-cells

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Striking progress has been achieved in CD19+ malignancies using chimeric antigen receptor (CAR) T-cells. However, substantial challenges remain in using CAR T-cells for the treatment of solid malignancies, including the paucity of suitable targets, tumour heterogeneity and the homing, penetration and persistence of the T-cells within a profoundly immunosuppressive tumour microenvironment. Cognisant of these obstacles, we designed T4 immunotherapy. T4+ T-cells co-express (i) T1E28ζ, a CAR coupling a ligand that engages 8/9 possible ErbB dimers to a fused CD28+CD3ζ endodomain; and (ii) 4αβ, a chimeric cytokine receptor enabling IL-4-driven enrichment and expansion during closed manufacture. Pre-clinical data have demonstrated potent anti-tumor activity in a broad range of solid tumour models. To de-risk T4 immunotherapy in man, a dose-escalation, intra-tumoural Phase I trial was commenced, without lymphodepletion. Head and neck squamous cell carcinoma (HNSCC) was selected due to the unmet need and the propensity for localised recurrence. Despite prevalent lymphopenia, T4 immunotherapy was consistently generated from a 130mL blood draw, with no batch failures to date. Intra-tumoural injections of T4 immunotherapy were administered at doses between 0.1-10x10e8. Treatment-related AEs were < grade 2, with no DLTs, even at the highest dose. The disease control rate from 15 patients is 60% (9/15), with all three patients in cohort 3 and cohort 4 achieving stable disease.

Session III

TCR-engineered T-Cell Therapy

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Overcoming barriers to therapy of solid tumors with CAR/TCR engineered T cells

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TCR-engineered T-cell Immunotherapy of cancer

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NY-ESO-1 TCR single edited stem and central memory T cells to treat multiple myeloma without graft-versus-host disease

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Dual orthotopic T cell receptor alpha and beta chain replacement enables engineering close to physiological T cells

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T cells can be re-directed through the introduction of antigen-specific receptors to fight infections or cancer. Engineering of T cell receptors (TCRs) is particularly attractive due to the vast diversity of potential targets. However, therapeutic TCR engineering has remained challenging. TCRs are complex heterodimeric receptors and competition and mispairing between endogenous and transgenic receptors can occur. Moreover, conventional TCR introduction does not support TCR fine regulation as known for natural T cells. Advanced genomic engineering tools now allow elimination of endogenous TCR chains and even introduction of TCRs into endogenous TCR genes. We engineered human T cells using CRISPR-Cas9 to delineate the consequences of endogenous TCR elimination and orthotopic TCR placement (into the natural gene locus). We validated non-viral CRISPR-Cas9-mediated knock-in with five different TCRs and compared this technology to conventional TCR engineering strategies. We found that, while transgenic TCRs placed in the endogenous TCR α chain are functional, single-chain editing still results in significant mispairing. However, simultaneous editing of α and β chains overcomes this problem and, combined with orthotopic TCR placement, enables T cell engineering with accurate TCR pairing and regulation close to natural T cells.

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Mutated nucleophosmin 1 as immunotherapy target in acute myeloid leukemia

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The most frequent subtype of acute myeloid leukemia (AML) is defined by mutations in the nucleophosmin (NPM1) gene. Mutated NPM1 (ΔNPM1) is a driver gene that is essential for malignant transformation early in leukemogenesis. ΔNPM1 arises from 4 base pair frameshift insertions in exon 12, resulting in a novel C-terminal alternative reading frame of 11 aa (CLAVEEVSLRK). The insertions occur at restricted positions in the coding sequence and although their exact sequence can differ, the majority of mutations encode the same alternative reading frame. Since ΔNPM1 is a clonal driver mutation in 30-35% of AML, its alternative reading frame is an attractive target for immunotherapy. To investigate whether ΔNPM1 peptides are processed and presented on AML, we searched the HLA class I ligandome of 12 primary AML for ligands matching a region spanning 10 N-terminal aa in the normal reading frame followed by the 11 C-terminal aa in the alternative reading frame (MTDQEAIQDLCLAVEEVSLRK). This revealed the presence of 5 peptides (VEEVSLRK, AVEEVSLR, CLAVEEVSL, AVEEVSLRK and CLAVEEVSLRK) in AML with ΔNPM1, but not in AML with wild type NPM1 (wtNPM1). All 5 ligands as eluted from 7 of 8 AML with ΔNPM1 were validated by comparing tandem mass spectra with synthetic peptides. The eluted CLAVEEVSL and CLAVEEVSLRK peptides were validated upon cysteinylation of the first residue of the synthetic peptide. Online algorithms predicted binding of CLAVEEVSL to HLA-A*02:01 and binding of both AVEEVSLRK and CLAVEEVSLRK to HLA-A*03:01 as well as A*11:01, which was confirmed by appropriate folding of the respective HLA class I monomers. Since HLA-A*02:01 is expressed in 50% of the Caucasian population, we selected CLAVEEVSL to explore its relevance as therapeutic neoantigen and searched for specific T cells in patients with AML. Peptide-HLA tetramers were produced for CLAVEEVSL and its cysteinylated variant and a mix of tetramers was used to isolate specific T cells from PBMC from 6 HLA-A*02:01 positive patients with ΔNPM1 AML who were in remission after chemotherapy. No specific T cells could be isolated. In addition to direct T cell isolation, we also screened for tetramer-positive T cells after in vitro peptide stimulation, but failed to detect these T cells above background in the 4 patients analyzed. These data indicate that T cells for ΔNPM1 did not exist at frequencies that could be detected in limited numbers of PBMC from AML patients. We subsequently screened large numbers of PBMC from 6 HLA-A*02:01 positive healthy individuals and directly isolated various tetramer positive T cell clones. One of these clones, clone 1A2, specifically reacted against HLA-A*02:01 positive AML with ΔNPM1, but lacked reactivity against AML with wtNPM1 in IFN-γ ELISA. To investigate CLAVEEVSL as target for T cell receptor (TCR) gene transfer, the variable regions of the TCR α and β chains from clone 1A2 were sequenced, synthesized and cloned into the MP71-TCR-flex retroviral vector. The TCR for ΔNPM1 and, as a control, the TCR for HLA-A*02:01-restricted CMV peptide NLVPMVATV, were introduced in CD8 and CD4 cells that were isolated from healthy HLA-A*02:01 positive individuals. TCR-transduced T cells were tested for anti-tumor reactivity against HLA-A*02:01 positive AML in IFN-γ ELISA. Upon transduction with the ΔNPM1 TCR, CD8 and CD4 cells both showed recognition of AML with ΔNPM1, while there was no reactivity against AML with wtNPM1. T cells transduced with the ΔNPM1 TCR also failed to recognize HLA-A*02:01 negative AML and HLA-A*02:01 positive autologous monocyte-derived mature dendritic cells. Next, we tested the cytolytic capacity of TCR-transduced T cells in a 9 hrs 51chromium release assay. Both CD8 and CD4 cells transduced with the ΔNPM1 TCR showed specific lysis of AML with ΔNPM1, but did not kill AML with wtNPM1. Finally, the efficacy of TCR-transduced T cells was tested in immunodeficient mice engrafted with a human AML cell line expressing ΔNPM1. A clear antitumor effect was observed in mice treated with the ΔNPM1 TCR, resulting in significantly better overall survival than untreated mice or mice treated with the CMV TCR. In conclusion, our data show that various ΔNPM1 peptides are presented on AML and that CLAVEEVSL is a neoantigen that can be efficiently targeted by ΔNPM1 TCR gene transfer. Immunotherapy targeting ΔNPM1 may therefore contribute to treatment of AML.

Session IV

Immunological Checkpoints

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Exploiting the gut phagome for T cell based-therapy

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Determinants of immunological response to immune checkpoint blockade in human cancer

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Reinvigoration of tumor-specific T cells by cancer immunotherapies, in particular PD-(L)1 blocking agents, has greatly improved clinical outcome in multiple cancer types. Nevertheless, durable clinical benefit is currently limited to a small number of patients. Recent evidence from chronic viral infection and tumor models indicates that not all dysfunctional T cell populations can respond equally well to immune checkpoint blockade (ICB). To achieve a better understanding of how heterogeneity in intratumoral T cells may impact on the response to ICB, we developed a platform using human tumor explants that preserves the human tumor microenvironment but allows us to visualize immunological responses to ICB on a patient-specific level. The observed ICB-induced changes can then be linked to the inherent qualities of a tumor and its infiltrating T cell populations, thereby contributing to the identification of determinants for effective response to ICB across human cancers.

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Tumor suppressor signaling by PD-1

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Development of a novel, highly effective trifold immunotherapeutic approach using autologous humanized PDX models and genetically-engineered mouse models of cancer

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Less than 10% of all cancer patients respond to immune-checkpoint-inhibitors and less than 30% to any form of immunotherapy. Therefore, novel approaches are needed and ought to be developed rationally using appropriate model systems. Genetically-engineered murine cancer models can be used to develop immunotherapeutic interventions for cancer with the tumor being in its natural environment. However, profound differences between human and murine immunity limit translational success. In contrast, humanized mouse models of cancer enable the study of interventions affecting the human cancer-immune-interaction, but only in a xenogeneic host and with potential alloreactivity unless the immune-graft and the tumor originate from the same donor. Here, we develop a combination-immunotherapy of intratumorally delivered TLR agonists, aPD1-checkpoint-blockade and a multi-cellular therapy of innate and adaptive effectors utilizing genetically-engineered mouse models of cancer, cell-line based humanized mouse models and, importantly, an autologous humanized PDX transplant mouse model of lung cancer. Using TCGA-data, we found that combined infiltration of activated NK-cells, γδT-cells and non-regulatory αβT-cells confers a uniform survival benefit across cancers. Thus, we adapted protocols to selectively expand NK-like-, γδT- and tumor-specific αβT-cells in vitro from human PBMCs or mouse splenocytes. Tumor-specific T-cells were induced in-vitro in a coculture system with the respective tumor target. We performed in vitro experiments confirming cytotoxicity, specificity, functionality and adequate expansion of subsets. We found that NK-like-, γδT- and tumor-specific αβT-cells act synergistically in combination to kill lung-, breast-, and lymphoid cancer cells and exhibit increased cytotoxic activity when exposed to aPD1-antibodies in vitro. Next, we performed in vivo experiments in humanized NSG-mice carrying lung- (H441, H1975), breast- (JimT1), and lymphoid (KMH2) cancers, in a syngeneic melanoma model (B16F10) and in a difficult to treat K-ras / p53 driven (KP) genetically-engineered mouse model of lung cancer as well as an autologous humanized PDX lung cancer model. We sequentially combined intratumoral TLR-agonist therapy targeting TLR-3, TLR-7 and TLR-9, a combined adoptive cellular therapy of NK-like-, γδT- and tumor-specific αβT-cells and aPD1 immune checkpoint blockade. Strikingly, we were able to show that all 3 elements were needed in order to effectively eradicate or at least reduce tumor growth in all models, while mono- or dual-immunotherapies were only effective in some models. Aiming to elucidate the mechanism of this synergy, we performed immunohistochemistry analyses of tumors and used flow cytometry of tumors and spleens to enumerate and characterize immune cell subsets. We found that combination treatment leads to a notable increase in tumor-infiltrating γδT-cells and marked increases in CD4+ and CD8+ T-cells as well as NK-cells across models. Furthermore, we used multiplex cytokine arrays to evaluate both changes in cytokines associated with tumor regression and those associated with certain treatment combinations. Our most prominent finding was a pattern of increased cytokine levels of MIP-1a, IL-5, IL-4, IL-9 and IL-15 which were associated with both reduced tumor growth and combination immunotherapy. We did not observe any overt toxicity with more detailed studies ongoing. In conclusion, we show that a combination-immunotherapy of intratumorally delivered TLR agonists, aPD1-checkpoint-blockade and a multi-cellular therapy of innate and adaptive effectors is highly effective across cancer entities and model systems, including PDX models as well as in genetically-engineered mouse models of cancer. Importantly, we were able to develop a novel, highly effective, trifold immunotherapy combination treatment regimen with broad efficacy that is completely agnostic of the precise target antigen and thus translationally highly relevant especially where a precise target antigen for cancer immunotherapy is not known. Merely requiring a tumor biopsy and peripheral blood, our study has high translational relevance.

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Identification of AR1 as a Novel Immune modulator in Lung Cancer Using an RNA-i based High-Throughput Discovery Platform

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Immunotherapy has emerged as an effective treatment modality in oncology, particularly in non-small cell lung cancer. Though immunotherapy holds considerable promise resulting in tumor regression and improved overall survival in a fraction of patients, many patients still remain refractive or develop resistance towards such therapies. In order to better understand, predict and achieve tumor regression, identifying and intervening novel immune checkpoints is crucial. Towards this aim, we performed a high throughput RNA-i based screening assay to identify novel immune check points in the lung tumor entity. We transfected the luciferase expressing H23 Lung adenocarcinoma cell line with siRNA library targeting 4160 genes and co-cultured them with Tumor infiltrating lymphocytes derived from HLA matched patients. Among the top hits, we identified AR1 as a novel immune modulator, whose knock down sensitized lung tumors to Tumor infiltrating lymphocyte (TIL) mediated cytotoxicity. This was also reconfirmed in a subsequent secondary screen and further followed up for mechanistic analysis. AR1 is an essential component in the protein degradation machinery and our preliminary findings show that AR1 promoted intrinsic tumor resistance to Trail mediated cell death. Taken together, we propose that AR1 is a novel immune modulator that could potentially be targeted for immunotherapy.

Session V

Modulation of the Tumor-Immune-Microenvironment

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T-cell based immunotherapy and the tumor microenvironment

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The objective of this project is to examine whether neoantigens, which arise from single point mutations in cancer, can sensitize stroma for destruction by CD4+ T cells and how this affects tumor growth. To test this notion, we used the cancer 6132A as model of one of the many cancers that escape immunological destruction by attracting and stimulating non-malignant cells in the tumor stroma. These activated stromal cells are not only immunosuppressive but also release growth factors that fuel cancer cell growth. This vicious circle allows cancer cells to escape immune destruction while retaining immunogenic neoantigens. The mechanism is particularly important for cancers that harbor therapeutically attractive mutations that cannot be lost because they are essential for malignant growth. 6132A harbors such an immunogenic driver mutation, a point mutation in a tumor suppressor gene L9 (mL9), which is unmanipulated in its expression level. Here we show that this antigen is released from viable cancer cells into the surrounding stromal cells and regularly presented by CD11b+ stromal cells, mostly F4/80+ tumor-associated macrophages. Thus, we made use of tetramers to detect T cells specific for mL9. Indeed, neoantigen-specific T cells infiltrated the tumor stroma, yet the cancer grows progressively in normal immunocompetent mice. T cells exposed to neoantigen in the tumor microenvironment often become epigenetically locked irreversibly in a dysfunctional state that prevents damage to the cancer cells. We therefore isolated the TCR from such neoantigen-specific T cells and transduced the TCR into competent peripheral T cells that express unrelated TCRs and are therefore not disabled. These TCR-transduced T cells were then adoptively transferred into mice bearing large long-established 6132A tumors. Remarkably, even though we targeted just the stroma and not the cancer cells directly, the cancers shrunk in size considerably and remained small as long-term 'stable disease'. Thus, destroying the tumor microenvironment with TCR-transduced neoantigen-specific T cells offers an alternative and novel approach for immunotherapy of patients who fail adoptive transfer of tumor-infiltrating T cells or checkpoint blockade therapy. Supported by NIH grants R01-CA22677 and R01-CA37156, the Cancer Research Foundation, Harriet and Allan Wulfstat and the Gerald O. Mann Foundation, the Berlin Institute of Health, the Einstein-Stiftung Berlin, Chicago Biomedical Consortium Catalyst Award to J.H.

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Understanding the tumor immune microenvironment for effective therapy

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Myeloid cells are a critical component of the tumor microenvironment. In cancer, the myeloid compartment is dramatically affected, which is now considered as one of the major immunological hallmarks of cancer. Accumulation of immunosuppressive macrophages (MΦ), defective dendritic cells (DC) function and expansion of pathologically activated immune suppressive immature myeloid cells - myeloid-derived suppressor cells (MDSC) are major changes in the myeloid compartment in cancer. The total population of MDSC consists of three groups of cells: the most abundant (>75%) immature, pathologically activated neutrophils (PMN-MDSC); the less abundant population of pathologically activated monocytes - (M-MDSC). Tumor associated macrophages (TAM) and DCs can persist in tissues for a long time, whereas PMN-MDSC have short lifespan (< 48 hours) and are constantly replaced from bone marrow (BM). The role of MDSC in mouse tumor models is well established. In most of the studies PMN-MDSC were cells that expanded the most. In recent years, the clinical role of MDSC has emerged. Results showed positive correlation of MDSC in peripheral blood with cancer stage and tumor burden. In a meta-analysis, elevated MDSC in the circulation was found to be an independent indicator of poor outcomes in patients with solid tumors. It is widely accepted that the population of myeloid cells in cancer is heterogenic. It consists of cells able to exert antitumor and pro-tumorigenic activity. Based on available data on the very short lifespan of MDSC in animal models and cancer patients as well as on functional data, we have postulated that at any given moment, myeloid cells in tissues are comprised of classically activated neutrophils (PMN) and monocytes, with pro-inflammatory and antitumor activity, and pathologically activated MDSC with potent immune suppressive and pro-tumorigenic activity. The balance between these cells defines the tumor microenvironment as immunosuppressive. This balance depends on the stage of cancer. It is plausible that in tissues, MDSC are present not in a binary state but in a range of functional states that are characterized by different markers and different functional activities. We found that bone marrow neutrophils from mice with early-stage cancers exhibited high spontaneous migration to tissues. These cells lacked immunosuppressive activity but had elevated rates of oxidative phosphorylation and glycolysis, and much more production of ATP. Their enhanced spontaneous migration was mediated by the binding of ATP to purinergic receptors. In ectopic tumor models and the late stages of cancers, bone marrow neutrophils demonstrated immunosuppressive activity. However, these cells had metabolic and migratory activity indistinguishable from that of control neutrophils. A similar pattern of migration was observed in neutrophils and PMN-MDSC from patients with cancer. These results elucidate the dynamic changes that neutrophils undergo in cancer and demonstrate the mechanism of neutrophils' contribution to early tumor dissemination.

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Analysis of immune-tumor cell interactionsusing patient-specific explant models

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Immunotherapy has changed the standard of care for many solid Tumors, especially malignant melanoma and lung cancer. Other cancer entities like pancreatic cancer or colorectal cancer are largely unresponsive to immunotherapy so far. One problem in developing new immunotherapies is the inter-species difference between humans and animal model systems, especially rodent models. In order to cope with this problem, a new approach utilizing fully human explant models systems was developed. Cellular composition and cytokine production as well as generall stability were optimized and a prospective validation Trial could show repdroducible and robust results, showing the possibilities of explant models in the development of new immunotherapies as a proof-of-concept. The CCL5-CCR5 axis was identified as a possible target and data from human patients with advanced metastatic colorectal cancer confirmed the proposed mode-of-action of a small molecule inhibitor. Modulation of myeloid cells with anti-tumoral activation is proposed as a new treatment paradigm for gastrointestinal cancers.

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β-microglobulin - a trigger for NLRP3 inflammasome activation within macrophages promoting multiple myeloma cell progression

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Serum β2-microglobulin (B2M), a component of major histocompatibility complex (MHC) class I molecules, serves as reliable marker in multiple myeloma (MM) prognostication. To promote MM progression, we previously demonstrated that bone marrow (BM) stroma of MM patients consists of a considerable proportion of tumor-associated macrophages (TAMs). Since increasing levels of various growth factors and proinflammatory cytokines, such as interleukin-1β (IL-1β) and IL-18, were detected in tumor BM stroma as well, we focused on the correlation between B2M, inflammasome activation in human monocyte-derived macrophages (HMDMs) triggering IL-1β and IL-18 release, and its impact on MM progression. Therefore, we identified B2M as NLRP3 inflammasome inducer within HMDMs leading to assembly of adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), caspase-1 activation and release of IL-1β and IL-18. By stimulation of murine BM deficient in NLRP3 inflammasome, we confirmed B2M dependency on NLRP3 inflammasome and caspase-1 activation. Subsequently, we revealed that the B2M signaling pathway comprises phagocytosis of B2M and aggregation to B2M amyloid fibrils within lysosomes due to acidic conditions. A consequent lysosomal rupture leads to release of cathepsin B which activates the NLRP3 inflammasome. Furthermore, stimulation of HMDMs with the B2MW60G mutant, which displays a distinctly lower tendency to form B2M amyloid fibrils, thus indicated no induction of the NLRP3 inflammasome. To confirm our hypothesis, 5TMM mice were treated with the NLRP3-specific inhibitor MCC950 during course of disease. As a consequence, a significant reduction of the serum cytokines IL-1β and IL-18, as well as serum B2M was determined. In addition, IL-1β and IL-18 depletion in murine co-culture experiments led to a decline of MM cell proliferation. Our findings expose a novel role of B2M in triggering NLPR3 inflammasome activation within TAMs which induce inflammation and promote MM cell progression.

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Targeting regulatory T cells fosters immune control and disrupts multiple myeloma disease progression

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Multiple Myeloma (MM) remains an incurable disease of clonally expanding malignant plasma cells. The bone marrow (BM) microenvironment harbors treatment resistant myeloma cells, which eventually results in disease relapse in patients. CD4+FoxP3+ regulatory T cells (Tregs) are highly abundant amongst CD4+ T cells in the BM providing an immune protective niche for different long-living cell populations, e.g. hematopoietic stem cells. Although previous studies in MM patients have mostly focused on peripheral blood analyses without factoring in the BM niche environment, employed therapy regimens and functional Treg-MM analyses, it becomes clear that in the BM Tregs in MM are more abundant. Here, we addressed whether Treg-mediated immune-regulation fosters MM resilience and progression and, consequently, whether targeting Tregs in established MM would foster intrinsic immune mechanisms to control or even eradicate this malignant disease. To investigate the immune regulation of MM, we utilized a syngeneic immunocompetent murine MM model (eGFP+luc+ MOPC-315.BMP2.FUGLW in BALB/c mice), which allowed non-invasive in vivo bioluminescence imaging of disease progression in combination with fluorescence microscopy techniques and multi-parameter flow cytometry. DEREG mice provided a system to selectively deplete FoxP3+ Tregs upon diphtheria toxin administration at different time points of MM progression. First, we found that Tregs accumulate in the BM of MM and display an highly activated phenotype. Tregs upregulated activation marker such as CD44, CD69 and ICAM as well as several checkpoint receptors such as PD-1 and LAG-3. Notably, this switch in Treg phenotype solely occurred in the BM of MM bearing mice, but neither in spleen nor peripheral blood, emphasizing the relevance of the microenvironment. Second, we asked whether Tregs create a suppressive environment that enables tumor engraftment/dissemination and disease progression. To this end, we depleted Tregs before MM injection and found that preemptive Treg depletion completely protected mice from MM development. This finding is supported by recent studies in another mouse model. Third, and most importantly, we asked whether targeting Tregs in mice with established MM can trigger an intrinsic immune response to control MM disease. Remarkably, even a short-term depletion of Tregs in mice with established MM resulted in complete regression of MM below the detection limit (10/10). Longitudinal follow-up monitoring with sensitive bioluminescence imaging of a sub-cohort of five mice revealed that 40% (2/5) of mice remained entirely tumor free for at least 150 days. 60% of the animals (3/5) remained for an average of 57 days progression-free before relapse. Importantly, subsequent rechallenge resulted again in MM control in two mice of this cohort. Cellular analyses of the BM after Treg depletion in MM bearing mice, pinpointed to natural killer cells and neutrophils as immune effector cells either eradicating MM or suppressing MM progression. Conclusively, our preclinical in vivo study emphasizes that therapeutic Treg targeting poses an attractive treatment strategy for patients suffering from multiple myeloma.

Poster Session A

CAR T-Cells

A1

A clinical trial with SLAMF7 CAR T cells generated by sleeping beauty gene transfer to treat multiple myeloma –The CARAMBA project

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Multiple myeloma is a rare hematologic disease, arising from the clonal proliferation of degenerated plasma cells. In advanced disease stages, the overproduced plasma cells and monoclonal immunoglobulins cause immunosuppression and versatile end-organ damages. There are conventional strategies to treat patients suffering from myeloma, such as proteasome inhibitors, immunostimulatory drugs or stem cell transfer. Despite these clinical options most patients cannot be completely cured. In recent clinical trials, CAR T cells have been employed to treat multiple myeloma patients. For this promising approach B-cell maturation antigen (BCMA) has been chosen as prevalent target antigen for CAR therapy. The treatment leads to partial and complete responses in the major part of myeloma patients. However, clinical results were not always durable and relapses with antigen-negative myeloma cells occurred. These data highlight the therapeutic potential of CAR T cell therapy against multiple myeloma, but also emphasise the need for alternative strong target antigens. We have shown before that SLAMF7 might be an ideal target antigen for CAR therapy of multiple myeloma: The SLAMF7 molecule is highly expressed on myeloma cells and plays a critical role in their homing to the bone marrow niche. We are therefore planning to conduct a phase I/IIa clinical trial utilizing a 1:1 mixture of CD4+ and CD8+ SLAMF7 CAR T cells to treat multiple myeloma patients. In preparation of this clinical trial, we generated and tested different constructs of SLAMF7-targeting CARs. The CAR we will ultimately employ comprises the antigen-binding domain of the monoclonal antibody huLuc63 (elotuzumab), which is clinically utilised in myeloma treatment and shows a favourable safety profile. Apart from a long, modified spacer and a CD28 transmembrane domain, the CAR contains a CD28 and a CD3 ζ signalling domain. The gene cassette also includes a truncated EGFR sequence. After successful gene transfer, the EGFR protein is expressed as surrogate marker on the cell surface and allows us to stain and track CAR-modified T cells. We generated SLAMF7 CAR T cells from blood of healthy donors and myeloma patients, and proved their functionality and antigen-specificity in vitro and in a MM.1S/NSG mouse model. CAR T cells efficiently eradicated SLAMF7+ target cells and were able to proliferate and to secrete cytokines after binding to their antigen. Gene transfer by viral vectors, is cost-intensive and time-consuming in production. The potential risk of inducing genotoxicity raises doubts of regulatory authorities and increases translational hurdles. For that reason, we are producing CAR T cells by non-viral sleeping beauty-mediated gene transfer. For this approach we transfect activated T cells with two vehicles: an RNA, which is expressing the sleeping beauty 100x transposase and a plasmid, which encodes the SLAMF7 CAR and the truncated EGFR tag. After refining this technique and highly reducing the size of the plasmids, this method proves to be very efficient in CAR T cell production and shows a safer gene integration profile in comparison to gene modification by lentiviral vectors. In the dose escalation phase of the SLAMF7 CAR clinical trial, we are planning to use a starting dose of 2x104 CAR+ T cells per kg and end with the highest dose of 1x106 CAR+ T cells per kg. To produce the SLAMF7 CAR T cells in this clinical context, we refined the parameters of our manufacturing protocol. Therefore, we tested different strategies and time intervals for T cell activation, distinct transfection programs and subsequent culturing conditions. With our improved protocol, we are currently validating the cell manufacturing process under GMP conditions. Primary results confirm, that we can successfully generate the highest therapeutic cell doses and that the formulated cell product of a 1:1 mixture of CAR+ CD4+ and CD8+ T cells is highly reactive and antigen-specific in functional assays. The Horizon 2020 research and innovation program of the European Union recently funded the CARAMBA project to transfer SLAMF7 CAR T cells to the clinic. A consortium of ten multinational partners with clinical, regulatory, biotechnological and administrative expertise, as well as the umbrella patient organisation MPE (myeloma patients Europe) conducts the CARAMBA project. The preparation of this clinical trial is in progress. The manufacturing process developed in this project constitutes the basis for this CAR T cell trial.

A2

A lipocalin-based ON-switch for controlling CAR-T cells with an orally available drug

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The regulation of a protein-protein interaction with a small molecule represents a molecular ON-switch and enables the control of cellular functions. However, current ON-switches for conditional protein-heterodimerization lack essential prerequisites for therapeutic application, such as the usage of human protein components and small molecules suited for clinical application. Here we report the development of an allosteric ON-switch system that for the first time is based on a human protein and can be regulated by an orally applicable drug. This allosteric ON-switch system relies on the selection of binders which recognize conformational changes induced in a small molecule specific manner in a lipocalin protein. Human lipocalins have a deep binding pocket surrounded by flexible loops and have evolved for binding of a multitude of diverse small molecules, and we hypothesized that a lipocalin would be ideally suited for generating an allosteric ON-switch. Here we prove this concept with the human lipocalin retinol binding protein-4 (hRBP4), the orally applicable drug A1120 and two different binder scaffolds which we engineered for highly specific interaction with the A1120-induced conformation of hRBP4. We integrated this prototypic hRBP4-based ON-switch into a chimeric antigen receptor (CAR) and thereby could regulate the activity of primary human CAR-T cells for the first time by an orally applicable drug. Our molecular ON-switch system is the first one combining the benefits of a non-toxic, orally applicable drug with human protein components (hRBP4) and has the potential to be broadly applied for the control of cellular therapeutics such as CAR-T cells.

A3

CAR-T Cells directed against tumor associated antigens in B cell neoplasms and beyond

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Chimeric antigen receptor (CAR)-T cell therapy is is based on the adoptive transfer of autologous T cells genetically modified with an engineered immunoglobulin-derived receptor that recognizes tumor-associated antigens. CAR-T cell therapies targeted at the broadly expressed CD19 antigen on leukemia and lymphoma B cells have brought about substantial clinical efficacy and currently, two CD19 CAR-T cell products have received approval by the FDA for the treatment of B-NHL and B-ALL. The most challenging problem remains the identification and development of novel CAR-T cells targeted at suitable target antigens which should be uniformly expressed on the malignant cells, but absent from healthy tissues, also referred to as on-target/off-tumor specificity. We and others recently identified the B cell maturation antigen (BCMA) as a tumor-associated antigen that binds B cell growth factors and maintains survival of long-lived plasma cells and their malignant counterparts, the multiple myeloma (MM) cells. Based on our previously generated humanized and sequence-optimized anti-BCMA antibody of high affinity (Oden et al., 2015), we designed a humanized scFv that was incorporated into a second-generation CAR backbone. Our construct showed that cytotoxic activity of CAR-transduced T cells was not only efficiently directed against MM cells in-vitro and in-vivo, but also against mature B-NHL entities expressing BCMA at much lower densities in the range of 100 BCMA molecules on target cells. This suggests that BCMA is an alternative target structure for B-NHL (Bluhm et al., 2018). In addition, we identified a B cell homing receptor as an alternative target for immunotherapy of mature B cell lymphomas. Moreover, this homing receptor is also expressed on T follicular helper (Tfh) cells in germinal centers and its abrogation may also limit the microenvironmental support for follicle-located B cell lymphomas. We successfully generated an anti-B cell homing receptor CAR-T cell product and the first pre-clinical characterization revealed that this novel CAR construct confers human T cells with a high cytotoxic activity against defined mature B-NHLs. The on-target/off-tumor safety profile was characterized extensively, indicating that this novel CAR offers a feasible strategy to complement current CD19-dependent approaches to cure B-NHLs and other antigen-carrying hematologic malignancies.

A4

Clinical-scale and GMP-compliant production of CAR-T cells for the treatment of melanoma patients by mRNA transfection of a CSPG4-specific chimeric antigen receptor

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Autologous T cells, reprogrammed to target malignant cells via the expression of a chimeric antigen receptor (CAR-T cells) represent a promising tool in the adoptive cellular therapy of cancer. Tremendous clinical regressions, mostly of leukemia or lymphoma, have been achieved using CAR-T cells in several clinical trials. While the FDA and EMA approved two CD19-directed CAR-T-cell therapies (Kymriah and Yescarta) recently, the development of CAR-T cells against solid tumors lags behind. Here we present the clinical-scale and GMP-compliant production of CAR-T cells for the treatment of melanoma. In this approach a CAR, specific for chondroitin sulfate proteoglycan 4 (CSPG4), also known as melanoma-associated chondroitin sulfate proteoglycan (MCSP), is intentionally transiently expressed by mRNA electroporation for safety reasons. We have enhanced this clinical-scale protocol for an optimal combination of several parameters: i) activation and expansion of T cells (yield), ii) electroporation efficiency, iii) viability, iv) cryopreservation, and v) potency. Together, this resulted in a procedure that generates CAR-modified T cells in clinically sufficient numbers under full GMP-compliance. This protocol allows the generation of 2.4x10e9 CAR-expressing T cells starting from 1.77x10e8 PBMCs. However, we only used approximately 1/20 of PBMCs isolated from one leukapheresis. Therefore, using a semi-automated electroporation system, one can considerately increase the yield of CAR-positive T cells. The optimized CAR-T cell product was tested in four consistency runs and showed an average expansion of 13.6x, an electroporation efficiency of 85.6% CAR-positive cells, a survival of 74.1% after electroporation, and a viability of 84% after cryopreservation. Purity was 98.7% CD3+ cells, with 78.1% CD3+/CD8+ T cells and minor contaminations of 1.2% NK cells and 0.6% B cells. The resulting CAR-T cells were tested for cytolytic activity after cryopreservation and showed antigen-specific and very efficient lysis of tumor cells. Taken together, we provide a clinically applicable protocol to generate sufficient numbers of mRNA-transfected CAR-T cells for use in adoptive cell therapy of cancer. BST and NS contributed equally

A5

FLT3 inhibitor treatment increases FLT3 expression that exposes FLT3-ITD+ AML blasts to elimination by FLT3 CAR-T cells

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Background: FMS-like tyrosine kinase 3 (FLT3) is a transmembrane protein uniformly expressed on leukemic blasts in acute myeloid leukemia (AML), and driver of leukemia-genesis in FLT3-ITD+ (Internal tandem duplication) AML. There is an increasing body of pre-clinical and clinical data suggesting that FLT3-ITD+ AML blasts respond to FLT3-inhibitor treatment by augmenting FLT3 expression in order to sustain the survival signal provided by this mutation. Here, we analyzed FLT3 expression on FLT3 wild type and FLT3-ITD+ AML cells after treatment with the FLT3-inhibitors midostaurin, quizartinib and crenolanib, and determined the antileukemia efficacy of combination treatment with FLT3-inhibitors and FLT3 CAR-T cells in vitro and in vivo. Methods: AML cell lines MOLM-13 and MV4;11 (both FLT3-ITD+) were cultured in the presence of IC50 doses of midostaurin, quizartinib and crenolanib, respectively to induce resistance. A FLT3-CAR comprising a BV10 scFv binding domain, CD28-CD3ζ signal module and EGFRt marker was encoded in a lentiviral vector and expressed in CD8+ and CD4+ T cells of healthy donors (n=5) and patients (n=3). T-cell mediated cytolytic activity was evaluated in luminescence-based assay, cytokine production analyzed by ELISA and proliferation assessed by CFSE dye dilution. NSG mice (n= 4-6 per group) were engrafted with MOLM-13/ffLuc AML cells and treated with 5x106 CAR-T cells alone or in combination with FLT3 inhibitors. Results: We detected a significant increase in FLT3-expression on both MV4;11 and MOLM-13 AML cells after treatment with each of the inhibitors as assessed by mean fluorescence intensity (quizartinib > crenolanib > midostaurin). The increase in FLT3 expression occurred specifically on these FLT3-ITD+ AML cell lines and was not observed on FLT3 wt AML (THP-1), acute lymphoblastic leukemia (NALM-16), mixed lineage leukemia (KOPN-8, SEM) cell lines and normal hematopoietic stem cells. We applied single-molecule sensitive super-resolution microscopy to demonstrate that the average number of FLT3 molecules (per μm2) on MV4;11 AML cells had increased from 0.80 (untreated) to 10.7 (quizartinib), 4.7 (crenolanib), and 3.3 (midostaurin). Of interest, midostaurin induced clustering of FLT3, while FLT3 was still present as monomers after quizartinib and crenolanib treatment. Intriguingly, the higher FLT3 density after FLT3-inhibitor treatment translated into superior antileukemia reactivity of FLT3 CAR-T cells against AML cell lines and primary AML cells in vitro and in vivo. We observed the strongest increase in cytolytic activity, cytokine production and proliferation by CD8+ and CD4+ FLT3 CAR-T cells after treatment with crenolanib or quizartinib, followed by midostaurin. We confirmed that upregulation of FLT3 occurred on MOLM-13 cells during FLT3 inhibitor therapy in NSG mice in vivo, and observed synergistic antileukemia efficacy of FLT3 CAR-T cells in combination with each of the compounds. The mean frequency of FLT3 CAR-T cells in mice that received FLT3 CAR-T cells and an FLT3 inhibitor was 2-4 fold higher compared to mice had received FLT3 CAR-T cells alone and was the highest in the cohort of mice that had received FLT3 CAR-T cells in combination with crenolanib. FLT3 CAR-T cells alone and each of the combination treatments of FLT3 CAR-T & FLT3-inhibitor achieved 100% response rate which compares favorably to untreated (0%) or FLT3-inhibitor alone (0%). However, the mean fold reduction in leukemia burden was greater in all three combination treatment compare to only CAR treatment. The most potent combination was FLT3 CAR-T cell & crenolanib that accomplished the strongest reduction in leukemia burden as assessed by bioluminescence imaging and flow cytometry of bone marrow. Conclusion: Collectively, the data show that FLT3 inhibitors augment cell surface expression of FLT3 in FLT3-ITD+ AML cells which leads to enhanced recognition and elimination by FLT3 CAR T-cells. This is, to our knowledge, the first demonstration that small molecule inhibitors and CAR-T cell immunotherapy can be used synergistically to treat a hematologic malignancy. We confirmed this principle with each of the FLT3 inhibitors in our panel, and observed the strongest antileukemia activity of FLT3 CAR-T cells in combination with crenolanib. Our data encourage the clinical evaluation of this combination treatment in high risk patients with FLT3-ITD+ AML.

A6

Combinatorial targeting of multiple shared antigens by adapter-CAR-T cells (AdCAR-T) allows target cell discrimination and specific lysis based on differential expression profiles

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Despite tremendous clinical success in B-cell malignancies, broader application of chimeric antigen receptor (CAR) expressing T cell (CAR-T) is limited by the lack of exclusive tumor surface antigens and the inability of CAR-T to differentiate between cancerous and healthy tissue expressing the same antigens. For instance in AML, targeting of most promising surface antigens would eventually lead to detrimental myelosuppression due to co-expression on hematopoietic stem- and progenitor cells. To address these issues, we have developed the adapter CAR-T cell (AdCAR-T) system. By splitting antigen recognition and CAR-T activation, introducing adapter molecules (AMs), the system allows precise quantitative (on-/off-switch) as well as qualitative (change and combine target antigens) regulation of CAR-T function. AdCAR-T target an epitope structure consisting of the endogenous vitamin biotin in the context of a specific linker, referred to as Linker-Label-Epitope (LLE), conjugated to AMs. We have previously demonstrated that AdCAR-T allow simultaneous targeting of various antigens ("OR"-gate), preventing antigen evasion by selection of epitope-loss variants. In the present study we intended to investigate whether AdCAR-Ts are capable to identify and differentiate target cells due to multivariate antigen expression profiles ("AND"-gate). In theory, AdCAR-T activation is the result of the binding of AdCARs to LLE-tags presented by AMs bound to surface antigens on a target cell. AM titration experiments have revealed that a certain threshold concentration or LLE presentation density (ρT) on the target cell is required to activate AdCAR-T. We hypothesize that the threshold ρT can not only be reached by increasing concentrations of one specific AM, but also as a result of the assembly of AMs against different antigens (ρT = ρA+ ρB+ ρC+ ... + ρX) on the target cell's surface.

To test this hypothesis, we have generated LLE-AMs against ALL/NHL (CD10, CD19, CD20, CD22, CD138, ROR1) and AML (CD32, CD33, CD38, CD56, CD123, CD135, CD305, CLL1) associated antigens. Individual threshold concentrations for AdCAR-T activation by different AMs, targeting the model cell lines Nalm-6 (ALL), JeKo1 (NHL), HL-60 (AML), U973 (AML), Molm13 (AML), were analyzed. Cut offs were found to be between 10 and 100 pg/ml, dependent on target antigen expression and target cell line. Importantly, combinations of 2, 3 or 5 AMs, targeting different antigens expressed on the same target cell, caused complete target cell lysis at concentrations below the activation threshold for single AMs. Moreover, we found that combinations of CD10, CD19 and CD138 sufficiently eliminate Nalm-6 BCP-ALL cells, while sparing healthy B cells in coculture experiments. Similar results were obtained in coculture experiments of U937 AML cells vs monocytes as well as CD34-enriched hematopoietic progenitor cells, applying combinations of CD32, CD33, CD56 CD123, CD305 and CLL1. In conclusion, we demonstrate that combinatorial AM targeting translates complex and multiple antigen-dependent target cell identification into AdCAR-T activation. Our results indicate that AdCAR-T have the ability to identify and specifically eliminate cancer cells while sparing healthy tissue based on aberrant antigen expression profile

A7

CSPG4-specific CAR T cells react against leukemia cells

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J.D. and N.S. share senior authorship The advent of CD19-specific chimeric antigen receptor (CAR) T cells has proven to be a powerful asset in the arsenal of cancer immunotherapy, culminating in FDA approval for acute lymphoblastic leukemia and some B cell lymphomas in mid-2017 based on a myriad of complete remissions in patients with no other therapeutic options left. A very efficient way, however, by which cancer cells can escape CAR-T cell mediated destruction, is antigen-shutdown, which is evident in a sizable portion of those patients that do not respond to, or relapse after CD19-CAR T cell therapy, necessitating the quest for backup antigens. Chondroitin sulfate proteoglycan 4 (CSPG4) expression has been reported on various leukemic blasts, most notably on those bearing the ill-fated MLL 11q23 rearrangement. Capitalizing on our experience with targeting melanoma cells with CSPG4-specific CAR T cells, we aimed at exploring the use of those T cells against MLL-rearranged leukemic blasts, using the precursor B cell leukemia cell line KOPN-8 (KMT2A-MLLT1 translocation) as a model. T cells were expanded for 10-11 days using OKT3 and IL-2 from cryoconserved non-adherent fractions of healthy volunteer donor blood. Transfer of a CSPG4-specific CAR was intentionally carried out via mRNA electroporation for transient CAR expression to allow safe testing in a phase I clinical trial. T cells transfected with a CEA-specific 28ζ control CAR, as well as mock-electroporated T cells were incorporated as controls. Additional target cell lines included CSPG4+ melanoma cells A375M and CSPG4-negative T2.A1 cells. Upon co-culture with KOPN-8 cells, CSPG4-specific CAR T cells upregulated activation markers and secreted Th1 cytokines TNF and IFNy in an antigen-specific manner. More importantly, CSPG4-specific CAR T cells evinced specific cytotoxicity towards KOPN-8 cells reflected by a significantly higher degranulation and granzyme B production in comparison to T2.A1 cells. CSPG4 is a well-established CAR target in cutaneous melanoma, now we wanted to stir up initial attention for the use of CSPG4-specific CAR T cells in hematological malignancies as a backup antigen for CD19, with special emphasis on MLL-translocated leukemias.

A8

Engineering chimeric antigen receptor T cells with enhanced specificity and reactivity towards Aspergillus fumigatus

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Immunocompromised patients are susceptible to invasive fungal infections mainly caused by Aspergillus fumigatus (Af). Adoptive transfer of Aspergillus-specific T cells was shown to reduce the burden of invasive aspergillosis. Such specific T cells are hard to isolate and expand. Alternatively, T cells modified to express a chimeric antigen receptor (CAR) can be used. CARs are recombinant receptor constructs composed of an extracellular targeting element linked to an intracellular signaling module. One recent study using Dectin 1 as a targeting element showed the benefits of such an approach in vitro and in vivo. Since Dectin 1 recognizes β glucan a motif that is not specific for Af but rather to a broader range of pathogens and commensal micro-organisms, off-target reactions cannot be excluded. Thus a better specificity is required. Here we aim to enhance the specificity and the reactivity of the CAR T cells towards Aspergillus fumigatus. To redirect specificity towards Af, we designed an scFv derived from an antibody directed against Hyphal cell wall. We constructed CARs containing the scFv fused to extracellular IgG4-Fc spacer domains of different lengths followed by CD28 and CD3-ζ signaling domains. We engineered primary human CD4 and CD8 T cells to express the CARs on their surface using the Sleeping Beauty gene transfer system. We co-cultured CAR T cells with Af germ tubes and evaluated specific T cell activation and direct hyphal damage. We showed that our CAR T cells are specific for Aspergillus fumigatus with no cross-reactivity with other Aspergilli. Upon binding to the target, the cytolytic machinery of CD8 CAR T cells was activated leading to the release of perforin and granzyme B. Furthermore CD8 CAR T cells were able to induce up to 45% hyphal damage in vitro. Activated CD8 CAR T cells secreted mainly Th1 cytokines like IFN-γ, TNFα, IL-8, and IL-2 and chemokines mainly CCL3 and CCL4 and few Th2 cytokines namely high levels of IL-13 and tiny amounts of IL-5. Similarly, CD4 CAR T cells secreted mainly Th1 cytokines (IFN-γ, TNFα, IL-8, and IL-2) and at a lower extend Th2 cytokines like IL-13, IL-4 and low amounts of IL-5. In all our experiments CARs containing long (Hinge-CH2-CH3) extracellular spacer conferred superior T cell activation when compared with CARs having short (Hinge-only) spacer. Noteworthy, mainly activated CAR T cells containing a long spacer underwent proliferation. Taken together, our results show that CD4 and CD8 CAR T cells are specifically activated upon recognition of Af and customizing the spacer design enhanced their effector function. This project is funded by the BMBF (Infect Control 2020—consortium ART4Fun; subproject 2 to HE).

A9

High-level MYCN expression in neuroblastoma impairs CD171-directed CAR-T cell therapy in vitro

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Current treatment protocols have only limited success in patients with neuroblastomas, harboring amplifications of the central oncogenic driver, MYCN. The success achieved by immunotherapies against lymphomas and leukemias have not yet been reached for solid tumors. With neuroblastoma being the most common extra cranial solid tumor in childhood, we aimed to investigate the influence of MYCN amplification on tumor cell response to neuroblastoma-specific CAR-T cell therapy. As a starting point, we used the neuroblastoma cell line, SK-N-AS, with the normal diploid MYCN complement (MYCNnon-amp) and overexpressed MYCN (MYCNamp) in these cells using a tetracycline-inducible system. Neuroblastoma cells with the different MYCN backgrounds were co-cultured with second-generation CD171-targeting CAR-T cells harboring either CD28 or 4-1BB as a costimulatory domain. CD171-CAR-T cell function was assessed in assays for activation/inhibitory receptor expression, cytokine release and cytotoxicity. Co-culture with MYCNamp SK-N-AS cells showed a reduced activation phenotype of CD171-CAR-T cells by reduced CD25 and CD137 expression compared to parental MYCNnon-amp co-culture. Expression of the inhibitory Tim3 receptor was increased by almost two-fold on CD171-CAR-T cells in co-culture with MYCNamp SK-N-AS cells in comparison to the parental MYCNnon-amp cell line. CD171-CAR-T cells released up to two-fold less IFNG and IL2 during co-culture with the MYCNamp SK-N-AS cells and demonstrated less killing potential against the MYCNamp SK-N-AS cells in comparison to the parental cell line. All effects were observed for both CD171-CAR-T cells harboring the 4-1BB and CD28 costimulatory domain whereas the costimulation with CD28 showed a higher efficacy in all functionality assays. Interestingly, a downregulation of the target antigen CD171 on MYCNamp tumor cells was detected. First hints from patient data emphasizes that lower CD171 expression is associated with MYCN amplified high-risk tumors. Therefore, a combinational therapy approach using an AURKA inhibitor, an indirect MYCN inhibitor, and CD171-specific CAR-T cells was tested to restore CD171 expression on MYCNamp neuroblastoma cells. The antigen loss on neuroblastoma cells was not recovered after the combination of targeted drugs while CD171-CAR-T cell-directed neuroblastoma cell killing was increased by up to 40% in MYCNamp SK-N-AS cells. High-level MYCN in SK-N-AS cells results in dampened efficacy of CD171-specific CAR-T cells in vitro. The MYCN driven CD171-downregulation on neuroblastoma cells can be one cause for impaired CD171-specific CAR-T cell therapy. Nevertheless, the combination of an indirect MYCN inhibitor and neuroblastoma-specific CAR-T cells indicated that MYCN exerts additional immunosuppressive mechanism(s), which remains to be discovered.

A10

Tolerance in xenotransplantation by use of regulatory T cells with a SLA-specific chimeric antigen receptor

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* Fatih Noyan and Elmar Jaeckel jointly supervised this work The optimization and improvement of immunosuppressive agents used to protect transplanted organs cannot compensate the still prevailing organ shortage. Alternatives to human-to-human donation are therefore in the focus of transplantation medicine and science. The possibilities of xenotransplantation could counteract this organ shortage. The major hurdle here is constituted by strong immune response of the recipient T and B cells to the xenogeneic solid organ or to cellular xenografts. In line with our hypothesis that the use of antigen-specific regulatory T cells can regulate strong immune responses, we focused on the identification of specific scFv against porcine swine leukocyte antigen (SLA). Based on this scFv we generated xenospecific chimeric antigen receptor (Xeno-CAR). Transduction of Xeno-CARs into nTregs alters the specificity of these cells without a change of the regulatory phenotype or epigenetic stability. On the basis of specificity studies, we were able to prove that the used Xeno-CAR was functional on the one hand and recognized only porcine tissues at the same time. Cross-reactivity with human cells could be excluded. Suppression studies (in vivo and in vitro) demonstrated the increased ability of Xeno-CAR-Tregs over nTregs to significantly regulate T-effector cell proliferation after xenogeneic stimulus. Further, the use of Xeno-CAR-Tregs revealed in our transplantation studies the potential of these therapeutic cells to regulate the recipient immune system and to protect transplanted xenografts. In the human reconstituted NRG mice, adoptive transfer of Xeno-CAR-Tregs resulted in survival of highly immunogenic porcine skin without the need for further immunomodulation (immunosuppressants, etc.), while the transfer of nTregs did not lead to any therapeutically benefit. We conclude that Xeno-CAR-Tregs can induce xenospecific tolerance without perturbation of the general immune competence of the recipient and might thus set the course for the possible use of porcine tissue or grafts in future transplantation medicine.

A11

Highly Multiplexed, Single-Cell Functional Profiling of CAR-T Cells Enables More Predictive Product Characterization, Cell Manufacturing Analysis, and Cellular Biomarkers across Product Types

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Collecting and using a patients' own immune cells is a rapidly emerging immunotherapy approach. Genetically reprograming T cells to express a chimeric antigen receptor (CAR) has already paved the way for successful immunotherapies to fight against leukemia and lymphoma, and research into solid-tumor CAR-T cells are also underway. Nevertheless, a lot is still unknown in terms of exactly how these re-engineered cells will behave once reinfused into a patient, including efficacy and potential side-effects. Moreover, there is a need to effectively characterize the antigen-specific response of these cell products for manufacturing analysis and optimization. In this poster, we review single-cell polyfunctional profiling results obtained from several different sets of pre-infusion CAR-T samples, including anti-CD19 CAR-T samples from Novartis Pharmaceuticals [1] and Kite Pharma (Gilead) [3], GoCAR-T cell products from Bellicum Pharmaceuticals [2], and Bispecific CD19/22 CAR-T cells from the NIH [4]. In each case, CD4+ and CD8+ CAR-T cells were stimulated (details in [1], [2], and [3]) and subsequently analyzed at a single-cell level using IsoPlexis' IsoCode chip. A 16-plex cytokine panel was used with samples in study [2], while samples in studies [1], [3], and [4] used a 32-plex cytokine panel. Single-cell profiling revealed highly polyfunctional and heterogeneous responses across each patient cohorts. In study [1], an association was determined between the polyfunctional strength index (PSI) of the CAR-T samples and the clinical outcome of the patients after receiving the treatment ($p = 0.0119$). Similarly, an association was seen between pre-infusion PSI and post-infusion grade 3+ CRS in the patients ($p = 0.174$). In all studies, the CAR-T cells secreted a wide range of effector, stimulatory, regulatory cytokines; the CD4+ samples of [1] and [3] also secreted inflammatory cytokines. The secretions were highly specific to antigen-stimulation, and a significant portion of the CAR-T cells were polyfunctional (secreted multiple cytokines). In study [4], a consistently higher polyfunctionality was observed in CAR-T samples produced using a modified manufacturing method. The results of these studies demonstrate the potential benefits of single-cell profiling as a way to better understand how these CAR-T products behave in response to antigen-specific stimulation. Analyzing the single-cell polyfunctionality of CAR-T profiles may potentially provide a valuable quality check of the pre-infusion product, may provide key characterization data for optimizing the manufacturing process of the product, and may also help in developing biomarkers to predict eventual patient outcome in response to the therapy. The promise of more predictive product characterization lays the groundwork for greater potential clarity in product release and more proactive administration in the future. References:

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A12

Inducible universal chimeric antigen receptor T cells combined with a CD123-specific costimulatory targeting module acting in trans are highly efficient against acute myeloid leukemia

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Chimeric antigen receptor-modified T cells (CAR-T) are a promising immunotherapeutic approach and have displayed significant success in treatment of patients with refractory or relapsed hematologic malignancies. Complete response rates of up to 90% in clinical trials utilizing CD19 CAR-T cells were conclusive and consequently resulted in FDA approvals. In contrast to B cell derived malignancies, leukemia of myeloid origin like acute myeloid leukemia (AML) lack antigens exclusively expressed on leukemic cells or restricted to non-vital normal cell populations presenting a major limitation. In fact, suitable target antigens are shared between leukemia initiating cells (LICs) and hematopoietic stem and progenitor cells (HSPCs), which complicates CAR-T immunotherapeutic approaches. The α chain of the interleukin-3 receptor (CD123) is known to be highly expressed on AML blasts of 80-90% of patients with a differential expression level on normal HSPCs and LICs making it an attractive antigen for CD123-directed immunotherapy. However, evidence of durable on-target off-tumor toxicities of CD123 CAR-T on HSPCs leading to pan-myeloablation render fine-tuned control mechanisms indispensable for further clinical applications. To safely redirect CAR engineered T cells to non-exclusive targets such as CD123, a switch-controllable universal CAR-T platform (UniCAR) was introduced recently. The UniCAR system consists of two autonomous components: (1) a non-reactive fully humanized inducible second-generation CAR with CD28/CD3ζ stimulation for an inert manipulation of T cells (UniCAR-T) and (2) soluble peptide linker (targeting modules, TMs) enabling UniCAR-T reactivity in an antigen-specific manner. Pre-clinical data for UniCAR-T in combination with a CD123 specific TM (TM123) by Loff et al. demonstrated the efficiency of the molecular safety switch regarding hematotoxic effects in vivo with only limited toxicity towards HSPCs. As co-stimulation via 4-1BB enhances CAR-T expansion, persistence and effector functions, a novel TM123 variant (TM123-4-1BBL) harboring a covalently bound trimeric 4-1BB ligand (4-1BBL) was developed for transient co-stimulation of UniCAR-T at the leukemic site in trans. Thus, TM123-4-1BBL endows UniCAR-T with beneficial properties of third-generation CAR-T cells in an adjustable way. Here we provide pre-clinical data for UniCAR-T in combination with TM123-4-1BBL for treatment of AML. Specific binding of TM123-4-1BBL was demonstrated against native 4-1BB as well as CD123-positive leukemic blasts. Moreover, TM123-4-1BBL-redirected UniCAR-T exhibited a high cytotoxic activity against different AML cell lines. In long-term tumor eradication models, TM123-4-1BBL ameliorated the killing capability of UniCAR-T in vitro. Additionally, the increased hydrodynamic radius of trimeric 4-1BBL-coupled TM123 prolonged plasma half-life and enhanced bioavailability in vivo. Thereby the UniCAR platform technology became more flexible offering short (TM123) to moderate (TM123-4-1BBL) plasma half-life in order to treat various leukemic settings of high and low tumor burden respectively, while minimizing adverse effects. Thus, CD123-directed UniCAR-T combine high anti-leukemic efficacy with therapeutic flexibility introducing a real control mechanism for a customizable therapy of AML patients.

A13

Minicircle DNA –New Tool for Cell Therapy

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We give an overview on our technologies for production and purification of minicircle DNA, a safe and efficient vector system for gene and cell therapy. Our minicircle technology is removing unnecessary sequences like marker genes, the bacterial origin of replication etc., only used for stable maintenance and amplification of plasmids in bacteria. The resulting minicircle DNA consists almost only of the gene of interest, leading to significant size reduction and improved performance. Therefore, it leads to higher transfection efficiencies, no spreading of unwanted bacterial backbone sequences and especially a drastically reduced cell toxicity. Hence, these constructs provide a striking benefit especially for production of CAR-T cells which are among the most promising tools moving forward to clinical applications. Consequently, in close collaboration with the leading experts in the field of CAR-T cell-based therapies, we are developing a process for the production of High Quality Grade (HQ) minicircle DNA, meeting the requirements of most regulating agencies. High Quality Grade minicircle DNA is produced in a dedicated facility. Starting from a characterized Research Cell Bank (RCB), the manufacturing process passes through different well-documented production steps. The HQ fermentation facility is completely separated from purification (chromatography) to ensure that the sensitive DNA downstream processing is not affected by any potential living contamination. The proprietary special purification procedure results in pure, supercoiled (ccc) minicircle monomers, meeting the requirements to have a defined, homogenous and active product, proven by a number of quality controls for the cell bank and to the minicircle DNA product.

A14

Mode of action of a novel modular platform for adoptive T cell therapy combining bispecific antibodies with synthetic agonistic receptors

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Background: In adoptive T cell therapy (ACT), one of the greatest challenges is the recruitment of T cells to tumor tissue. T cells, engineered with chimeric antigen receptors (CAR) haven proven efficacy for hematological malignancies but at the same time induce severe side effects such as cytokine release syndrome. We herein describe a novel MHC-unrestricted modular platform by combining autologous T cells, transduced with a targetable synthetic agonistic receptor (SAR), with crosslinking bispecific antibodies (BiAb) that specifically recruit and activate T cells to the tumor tissue. Methods: The SAR composed of an extracellular inert human EGFRv3 domain fused to the intracellular T cell activation domains CD28 and CD3z was generated by overlap-extension PCR. T cells, retrovirally transduced with SAR, were analyzed for cell proliferation, activation and T cell mediated-lysis in three different murine tumor models in vitro and in vivo. Two different BiAb formats were designed and generated by recombinant expression cloning. Results: Murine T cells, transduced with SAR, could be specifically recruited towards murine EpCAM expressing tumor cells by addition of the respective BiAb. Furthermore, the SAR-transduced T cells showed selective antigen-dependent activation, proliferation and redirected tumor cell lysis mediated by both antibody formats. However, selective activation of SAR-T cells was only achieved with antibodies monovalent for the SAR. Bivalent formats showed unspecific T cell activation due to crosslinking of two SAR-T cells. Interestingly, after investigating the killing mechanism of the platform, FasL appeared to be crucial for SAR-T cell mediated tumor cell lysis. This was in contrast to CAR-T cells utilizing preferentially perforin and granzyme for tumor cell killing. Furthermore, SAR-T cell activity was shown to be reversible upon BiAb resolution. In addition, the EGFRv3 ectodomain of SAR-T cells could be targeted with the monoclonal antibody cetuximab to achieve immediate and complete depletion of T cells in vivo. In vivo, SAR-T cells mediated tumor control and delayed outgrowth only when administered in combination with BiAb. Conclusions: Here we describe a novel, modular platform for enhancing both efficacy and safety in ACT. The combination of SAR-T cells with an exchangeable BiAb can target a broad variety of tumor associated antigens but also supplies a 'safety switch' for T cell activation, thus providing evidence for further translation.

A15

The simultaneous siRNA-mediated downregulation of the inhibitory receptors PD-1 and CTLA-4 to boost anti-tumor CAR T-cell activity

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After the huge success of CAR T cells in hematological malignancies, its efficacy is currently evaluated in different solid tumors. However, first results were not as compelling as for hematological malignancies, due to the fact that CAR T cells need to cope with several challenges when used in solid tumors. One obstacle is defined by the activation of inhibitory immune checkpoint receptors on T cells within the lymphoid tissue and the tumor microenvironment. In this context, programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) represent the two major and highly investigated immune checkpoint molecules. Triggering of these inhibitory receptors may lead to an impaired antitumor activity of CAR T cells resulting in inefficient tumor cell killing. To overcome this problem and to boost anti-tumor CAR T-cell activity, we co-transfected T cells with both, small-interfering RNAs (siRNAs) in order to downregulate the two inhibitory receptors PD-1 and CTLA-4 and additionally with mRNA encoding a CAR recognizing the melanoma-specific antigen chondroitin sulfate proteoglycan 4 (CSPG4) using RNA electroporation. After obtaining suitable ratios for combined siRNA and mRNA electroporation, functionality analysis of these optimized CAR-T cells, e.g. cytokine secretion and cytotoxicity, was performed using PD-L1- and CD80-transfected melanoma cells endogenously expressing CSPG4. After co-transfection of T cells, flow cytometry revealed that activation-induced upregulation of both receptors, PD-1 and CTLA-4, was efficiently suppressed when compared to CAR T cells co-electroporated with negative control siRNA. PD-1 and CTLA-4 expression on CAR T cells was downregulated approximately 50% and 30%, respectively. Importantly, the simultaneous siRNA-transfection did not influence the CAR expression of the engineered T cells. Following antigen-specific stimulation of the CAR-T cells with PD-L1- and CD80-transfected melanoma cell lines endogenously expressing CSPG4, CAR-T cells electroporated with either PD-1 siRNA alone or in combination with CTLA-4 siRNA revealed a significantly higher cytotoxicity when compared to CAR-T cells transfected with negative control siRNA. Additionally, a higher cytokine production was observed in CAR-T cells electroporated with PD-1 siRNA alone, while no significant differences were seen when both receptors were simultaneously downregulated. Co-electroporation of CAR-T cells with CTLA-4 siRNA alone showed no or only little effects. Thus, the effect of PD-1 siRNA seemed to be more crucial in our conducted in-vitro study. Taken together, it is feasible to generate optimized CAR-T-cells by co-transfection of both, CAR-encoding mRNA and additionally siRNAs in order to downregulate inhibitory receptors. The confirmation that the co-transfection of siRNA and mRNA results in an enhanced anti-tumor CAR T-cell activity may open new possibilities and opportunities in cancer immunotherapy. For further evaluation, the use of these optimized CAR-T cells in appropriate animal models will be the next step towards a clinical use in cancer patients.

A16

Transfection of natural killer T cells with a CSPG4-specific CAR for cancer immunotherapy

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Natural killer T (NKT) cells represent an effective cell subpopulation in pathogen and tumor cell defense. Their advantage over conventional T cells is their recognition of cell surface structures independent of MHC presentation and their pronounced intrinsic anti-tumor activity through their endogenous T-cell receptor (TCR). Thus, transfection of NKT cells with a chimeric antigen receptor (CAR), recognizing a tumor-specific surface antigen, could attack tumor cells by multiple means: on the one hand antigen-specifically through the CAR and on the other hand via their endogenous TCR. Moreover, CAR-NKT cells could still be active when tumor cells try to escape immune recognition and their anti-tumor activity may be enhanced due to their intrinsic cytolytic ability. In our study, NKT cells were extracted from PBMCs using magnetic cell separation and expanded over 10 days. Analysis of surface marker expression was performed before and after expansion. At the end of expansion, NKT cells were electroporated with RNA coding for a melanoma-specific second-generation CAR recognizing the surface antigen chondroitin sulfate proteoglycan 4 (CSPG4). CAR-expression on the cell surface of NKT cells was analyzed and the in-vitro functionality after stimulation with melanoma cells, i.e. cytokine secretion and cytotoxicity, was compared to that of conventional CAR-T cells. In addition, CAR-transfected NKT-cells were co-incubated with α-GalCer-loaded target cells in order to analyze their intrinsic cytotoxic activity through their endogenous TCR. Approximately 1-3% NKT cells could be isolated from PBMCs. Through expansion the number of cells could be increased to about tenfold of the starting number. CAR-staining after electroporation of cells showed that NKT cells were efficiently transfected. The highest CAR-expression was seen at 8 hours after transfection. Following antigen-specific stimulation with melanoma cells, CAR-NKT cells produced the cytokines IFNγ and TNF. When compared to conventional CAR-T cells, the cytokine secretion of CAR-NKT cells was in general lower. However, specific lytic capacity was similar in both cell populations with CAR-NKT cells indicating a trend towards an improved cytotoxicity. Moreover, CAR-transfected NKT cells were able to lyse α-GalCer-loaded target cells through their endogenous TCR. Taken together, it is feasible to generate CAR-NKT cells by using mRNA-electroporation. In our study, their CAR-mediated cytotoxicity was at least equal to that of conventional CAR-T cells, while their intrinsic cytotoxic activity was maintained. Thus, CAR-NKT cells may represent a valuable alternative to conventional CAR-T cells in cancer immunotherapy.

A17

Using insight into TCR functioning for an improvement of CARs

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We (Schamel group) study the mechanisms with which the TCR is activated by ligand binding since many years. Our main finding is that the TCR-CD3 complex exists in two different conformations; the resting inactive conformation and the active conformation. In fact, basal signaling by the TCR is suppressed by its quartenary structure. This is missing in chimeric antigen receptors (CARs). Furthermore, the different CD3 subunits contain different signaling domains. Again, in the CD3ζ-based CARs most of them are missing. Having this in mind we (TCR2 Therapeutics and Schamel group) have engineered and studied a new format of CARs called TCR fusion constructs (TRuCs). We show that an intact TCR complex can be effectively reprogrammed for cancer therapy by recombinantly fusing an anti-CD19 scFv to its TCRα, TCRβ, CD3γ, CD3δ or CD3ε subunit. Respective scFv-TCR fusion constructs (termed TRuCs) were integrated into the TCR complex and expressed on the surface of T cells. In the presence of CD19-positive tumor cells, fusion constructs based on CD3ε and CD3γ could specifically and potently activate T cells. Despite the absence of extra signaling domains, TRuC-T cells showed similar in vitro cytotoxicity as CD28- and 4-1BB-based anti-CD19 CAR-T cells. A single CD3ε-TRuC-T cell dose greatly extended the survival of mice with Nalm6 leukemia. In a subcutaneous Raji tumor model, CD3ε-TRuC-T cells outperformed CAR-T cells in terms of anti-tumor activity. Our novel technology for genetically engineering T cells provides an alternative to CARs that can engage the physiological and broad signaling capacity of the entire TCR complex.

A18

Antigen loss as potential escape mechanism following CD171-directed CAR-T cell therapy

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Despite high remission rates following CAR-T cell therapy in hematologic malignancies, relapse due to loss of the targeted antigen is increasingly recognized as a mechanism of immune escape in the clinic. Antigen loss or antigen-low escape is likely to play a role in solid tumor therapy as well. CD171 (also known as L1CAM), an adhesion molecule that plays a fundamental role during brain development, is overexpressed by different tumor entities (e.g. neuroblastoma and retinoblastoma). CD171 is physiologically shed from the cell surface by proteolytic cleavage mediated by ADAM metalloproteases. An epitope of CD171 that is cancer specifically glycosylated is currently targeted by CAR-T cells in a phase I clinical trial for high-risk neuroblastoma at Seattle Children's Hospital, USA. We aimed to evaluate whether CD171 shedding leads to antigen loss and operates as an escape mechanism during CD171-directed CAR-T cell therapy. To this end, we assessed CD171 surface expression on neuroblastoma and retinoblastoma cell lines before and after co-culture with CD171-directed CAR-T cells. We used FACS-based analysis for CD171 tumor surface expression and Westernblot analysis to detect shedded CD171 in the cell culture supernatant. CD171 surface expression was further analyzed by immunohistochemistry in an established intercranial xenograft mouse model after treatment with CD171-CAR-T cells compared to untransduced T-cells. We found in vitro and in vivo a dramatic reduction of CD171-surface expression after co-culture with CD171-specific CAR-T cells. In vitro we detected a reduction of up to 30 fold of CD171 surface expression on human retinoblastoma (n=2) and neuroblastoma (n=2) cell lines after 24-72 hours of co-culture as analyzed by flow cytometry. When co-cultures were maintained for 3 days, cell variants survived that completely lost CD171 surface expression. Under continuous CAR-T cell pressure for 6 days, CD171 negative variants remained in a resting state but resumed growth and CD171 expression within less than two weeks after CAR-T cells were removed. Survival of CD171 negative variants increases the potential of immune escape and may render CAR-T cell therapy ineffective. Next, we plan to analyze whether proteolytic cleavage of CD171 is responsible for the antigen loss phenotype and whether targeted inhibition of ADAM metalloprotease can be combined with CAR-T cell therapy to circumvent the advent of antigen loss variants.

A19

Arming T cells with C-X-C-motive receptor 6 enhances infiltration of pancreatic cancer patient-derived organoids

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Background: Chimeric antigen receptor (CAR)-modified T cells have proven to mediate long-term remissions in hematological malignancies. In contrast, CAR T cells have only limited efficacy in solid tumors, mainly due to poor tumor penetration by T cells. One of the approaches to improve immune cell infiltration into tumors is to take advantage of chemokine receptor signaling. Recently, we were able to demonstrate that overexpression of C-X-C motif chemokine receptor 6 (CXCR6) in anti-mesothelin (MSLN) CAR T cells increases the efficiency of adoptive T cell transfer in a model of human pancreatic cancer with overexpression of C-X-C motif chemokine ligand 16 (CXCL16) – the natural ligand for CXCR6 (manuscript under revision). For further clinical development, we aimed at validation of the proposed therapy in human pancreatic cancer-derived samples. Material and methods: CXCL16 positively stained cells were quantified in pancreatic cancer tissue microarrays. Secretion of CXCL16 by pancreatic cancer patient-derived organoids (PDO) was determined by ELISA. For infiltration analysis, partially digested PDO were resuspended in Matrigel and plated as droplets. Droplets were overlaid with CXCR6-GFP or GFP-transduced T cells, fixed, permeabilized and stained with phalloidin and DAPI after 72 hours. GFP-positive T cells were quantified by confocal microscopy. Single cell suspension of PDO was co-cultured with untransduced, anti-MSLN CAR- or anti-MSLN CAR-CXCR6-transduced T cells, supernatants were analyzed by IFN-γ ELISA. Results and discussion: Analyzing expression of CXCL16 by immunohistochemistry in a cohort of pancreatic cancer patients (n = 438), we detected CXCL16 positive tumor infiltrating immune cells in a majority of patients. Furthermore, CXCL16 was secreted by seven out of nine screened PDO. Both findings suggest that pancreatic cancer might be an apt entity to apply adoptive transfer of CXCR6-transduced T cells. Therefore, we tested the migratory capacity of CXCR6-transduced T cells towards PDO. We found increased penetration of CXCR6 expressing T cells towards these organoids compared to control-transduced T cells. Additionally, co-culture experiments of PDO and anti-MSLN-CAR- or anti-MSLN-CAR-CXCR6-T cells showed specific activation of CAR T cells in all patients tested. This indicates that mesothelin might be a suitable antigen for the combination therapy described. Conclusion: In summary, our results underline the potential of CXCR6 as an enhancer of CAR T cell infiltration into pancreatic cancer. They provide a rationale for the clinical investigation of anti-MSLN CAR T cells overexpressing CXCR6 as a therapy for pancreatic cancer.

A20

CrispR/Cas9-mediated deletion of the endogenous TCR reduces alloreactivity of a CAR based on T-cell signaling via endogenous CD3 and enhances its expression and tumor recognition.

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Chimeric antigen receptor (CAR) based cellular immunotherapy of cancer led to a substantial improvement in curative treatments in particular of hematological neoplasia. Recently, we suggested a novel CAR design that in the style of a natural T-cell receptor (TCR) associates with the endogenous CD3 components to limit T-cell activation to the resources of natural signaling and costimulatory molecules such as CD3ζ, and CD28, 4-1BB, respectively. We hypothesize that this may resemble physiological T-cell signaling thereby minimizing the incidence of adverse reactions such as hyperactivation and 'off-target'-reactivity. Of note, we believe that such a strategy is justified by the generally accepted utilization of tumor-reactive TCRs in a wild type format (ie α/β-heterodimer in a variable/constant V/C-domain order) for adoptive immunotherapy (ADI) in the clinic. Several protein scaffolds were successfully tested relying on the fusion of the antigen-recognizing particle (ARP) to the constant domains of a TCR which accomplishes association to the endogenous CD3 signaling components (CD3z2, CD3ge, CD3de). Since scFv-fragments of the domain order VH-VL do not contribute to stable chain pairing of an ARP-Cα/Cβ heterodimer, we needed to murinize the C-domains. Next, we developed a CAR in which the tandem order of identical V-domains on either Cα/Cβ-chain of a heterodimer provided sufficient interchain affinity to utilize human C-domains (VH-VH-HuCa + VL-VL-HuCb). The latest version of our CD3-dependent CARs exhibited superior characteristics in cytokine secretion, cytotoxicity, proliferation, and eradication of tumors in mice. All these CD3-dependent CARs have in common, that they compete with endogenous TCRs for incorporation into the native CD3 complex and are present as mixed CAR/TCR effector molecules on a T-cell proportionally to their relative protein stabilities, also dictated by their interchain affinities. Since TCRs are supposed to form nanoclusters in a resting state of a T-cell and upon activation convert to microclusters, it is likely that mixed CAR/TCR clusters tend to cross-activate the endogenous TCR in the presence, or even in the absence (depending on the activation status of a T-cell) of the CAR antigen. Eventually, this may lead to alloreactivity against non-syngeneic tumor cell lines in vitro or even autoimmune-reactivity against self antigens in vivo, as hypothesized for TCRs introduced into α/β T-cells (T. Schumacher, Nat. Rev. Immunol. 2002). Here, we used the tight-junction protein Claudin 6 as a model tumor antigen. Bulk CD3+ (ie. TCR+) T-cells clearly demonstrated recognition of the Claudin 6-negative ovarian cancer cell line Skov-3 (RNA-Seq data) in an impedance-based cytotoxicity (Xcelligence) - and IFNg - secretion (ELISA) - based assay, which most likely originated from alloreactivity. Secondly, coexpression of CARs and endogenous TCRs may diminish the number of therapeutic CAR molecules and hence their reactivity. CrispR/Cas9 technology has been exploited for genome editing to delete endogenous TCR expression by targeting the human TRAC locus. For this, in vitro transcribed (IVT)-RNA of Cas9 and synthetic guide RNAs have been electroporated into OKT3/IL-2 preactivated T-cells, and purified to homogeneity for CD3-depletion by means of MACS separation. After 5 days, CD8+CD3- T-cells were electroporated with a CD3-dependent CAR of the Claudin 6 - specificity and assayed for IFNg-secretion against Skov-3 to assess residual alloreactivity, and against Skov-3 electroporated with Claudin 6 to estimate a gain in specific antigen reactivity in the absence of competing TCR. From these experiments we could demonstrate the elimination of potential alloreactivity and a superior expression and effector function of a CD3-dependent CAR in bulk CD8+ T-cells deprived of their endogenous TCRs. In summary, genome editing of human T-cells may not only be exploited for the generation of so-called 'off-the-shelf universal T-cells' to be adoptively transferred between allogeneic donors and recipients but also to minimize the risk of CAR-induced autoimmune-reactivity, and to maximize CAR effector function still being limited by endogenous CD3 expression as a safeguard for prevention of undesignated T-cell hyperactivation.

A21

Decentralised manufacturing of CD19 CAR T cells using the automated and robust CliniMACS Prodigy® platform for a multicenter clinical trial in Germany

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Objectives:
Trials with autologous CAR T cells directed against CD19 have shown remarkable remission rates and increased survival in patients with r/r B cell maligancies. However, limitations still exist for broad access to treatment due to multiple reasons like complex manufacture in central manufacturing models. In order to address these challenges, the fully automated CliniMACS Prodigy for manufacturing and the MACSQuant for flow cytometric quality control has been established at the treatment sites (Berlin, Erlangen, Tübingen) of a Miltenyi Biotec sponsored multi-center and multi-manufacturer clinical trial (EudraCT: 2017-002848-32). Furthermore, Miltenyi Biotec itself acts as additional manufacturing site to allow for a faster recruitment. The decentralised manufacturing model in this trial contributes to easy and affordable access to CAR T products by bringing therapies closer to point-of-care and reducing logistical complexity. Here, we show data from validation runs performed at the different manufacturing sites demonstrating dossier-compliant success in the production of CAR T cells by using a multi-site manufacturing network.

Methods:
The CD19-specific CAR T cells were produced automatically in the closed tubing set TS520 on the CliniMACS® Prodigy using the T cell transduction (TCT) process (all Miltenyi Biotec). For that, leukapheresis products (LP) were sterile welded onto TS520 on day 0 to enrich CD4+ and CD8+ T cells using CliniMACS CD4 and CD8 Reagent (Miltenyi Biotec). Afterwards, 1x108 enriched T cells were activated using MACS GMP T Cell TransAct (Miltenyi Biotec), a CD3/CD28-mediated T cell stimulation reagent. For viral transduction, a second generation anti-CD19 CAR lentiviral vector (Lentigen Technology) was added to the T cells 24 h after activation. During the expansion phase, the transduced T cells were feeded until day 5 with TexMACS medium (Miltenyi Biotec) supplemented with IL-7 and IL-15 (both Miltenyi Biotec) and 3% (v/v) human AB serum (ZKT Tübingen). From day 5, the transduced T cells were expanded in serum-free TexMACS medium incl. IL-7 and IL-15. On day 12, the final cell product was harvested in 100 mL Composol (Fresenius) supplemented with 2.5% (w/v) HSA (Octapharma). Flow cytometric analyses were performed using the MACSQuant (Miltenyi Biotec). Following samples were analyzed: LP before enrichment, after CD4+/CD8+ enrichment, in-process control on day 5 and final product on day 12. The CAR T cells were timely independantly produced at the different sites using identical protocols.

Results:
In total 16 TCT processes were carried out at the different manufacturing sites. Flow analysis of the leukapheresis products revealed differences in the cellular composition: Frequencies of viable CD3+ cell varied from 8.9% to 67.4%. Upon combined CD4 and CD8 enrichment, the majority of T cells were obtained. The proportion of CD3+ cells varied from 89.5% to 99.6%. Of these, 1x108 CD3+ cells were used for subsequent activation.
Following results (mean values) were obtained from in-process control on day 5:
Miltenyi Biotec: 6x10E8 total CD3+ cell. Transduction efficiency (TE) was 42% resulting in 2.5x10E8 CD19 CAR T cells.
Erlangen: 8.3x10E8 total CD3+ cells. TE was 38.9% resulting in 3.2x10E8 CD19 CAR T cells.
Berlin: 5.8x10E8 total CD3+ cells. TE was 59.29% resulting in 3.1x10E8 CD19 CAR T cells.
Tübingen: 6.7x10E8 total CD3+ cells. TE was 40.1% resulting in 2.7x10E8 CD19 CAR T cells.
On day 12, the following results (mean values) were obtained from final cell product:
Miltenyi Biotec: 6.6x10E9 total CD3+ cells. TE was 46.5% resulting in 3.1x10E CD19 CAR T cells.
Erlangen: 5.0x10E9 total CD3+ cells. TE was 41.3% resulting in 2.1x10E9 CD19 CAR T cells.
Berlin: 3.9x10E9 total CD3+ cells. TE was 65.2% resulting in 2.4x10E9 CD19 CAR T cells.
Tübingen: 5.9x10E9 total CD3+ cells. TE was 41.3% resulting in 2.5x10E9 CD19 CAR T cells.

Conclusions:
The TCT process with the CliniMACS Prodigy has been shown to generate a sufficient number of CD19-specific CAR T cells for clinical application within 12 days at multiple manfacturing sites. CD19 CAR T production was successful in all runs in terms of meeting dossier specifications demonstrating that the robust CliniMACS Prodigy production plattform is feasible to provide appropriate products for our multicenter clinical trial. This decentralized manufacturing approach utilizing the CliniMACS Prodigy production platform combined with the MACSQuant for flow-based QC represents a promising alternative to existing CAR T manufacturing models by reducing complex logistics. Another advantage of decentralised point-of-care manufacturing is the flexibility in treatment as treatment sites can schedule their own manufacturing slots.

A22

Pre-clinical validation of ROR2-specific CAR T cells for the treatment of breast cancer

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Introduction: Chimeric antigen receptors (CAR) are synthetic receptors, which are able to redirect T cell functions to cells expressing a particular extracellular target structure in a MHC-independent manner. CAR T cells have shown profound clinical efficacy for a variety of hematological malignancies. However, results concerning the suitability of CAR T cell therapy to treat solid tumors have been controversial. Recently, we have proposed ROR2 (receptor tyrosine kinase-like orphan receptor 2) as a candidate for CAR T cell therapy. ROR2 is expressed during embryonic development and plays an important role for neural and skeletal development. The expression of ROR2 is downregulated during gestation and the protein is absent in most adult human tissues. Interestingly, human and murine ROR2 share 92% sequence identity and similar biological functionalities in vivo. Recurring ROR2 expression has been reported for a variety of solid tumors, such as breast cancer, ovarian cancer, stromal tumors and renal cell carcinoma. In the majority of cancer types, ROR2 expression correlates with rapid disease progression, tumor invasiveness and metastases. Methods: We validated the expression of human ROR2 (hROR2) and murine ROR2 (mROR2) in healthy tissues by qPCR using pooled tissue-specific cDNA extracts and Western Blotting. Additionally, we analyzed the ROR2 expression pattern of breast cancer (BC) cell lines by flow cytometry. We recently isolated a monoclonal antibody against hROR2 from a naive rabbit antibody library using phage display. The obtained antibody was used to generate antibody variants with different affinities for the same epitope of hROR2. The antibodies were characterized in vitro and three variants showing different binding affinities were chosen to generate 4-1BB-based second generation CAR constructs. Importantly, one of the three binders was found to have lost his cross-reactivity with mROR2. The three different ROR2 CARs were expressed by lentiviral transduction in CD4+ and CD8+ T cells isolated from the blood of healthy donors. CAR positive T cells were enriched and functionally characterized in vitro to evaluate antigen-dependent proliferation, cytokine secretion and cytotoxicity. Results: Database research, using the In Silico Transcriptomics database (Medisapiens Ltd., Helsinki, FIN), revealed low-level expression of hROR2 in some adult human tissues and recurring expression in different solid tumors, including a subset of BC cases. To confirm these findings, we analyzed BC cell lines for hROR2 expression using flow cytometry. We detected hROR2 on the cell surface of T-47D cells and found MDA MB231 to be hROR2 negative. We validated the expression pattern of ROR2 in healthy tissues by qPCR, using pooled, tissue-specific cDNA panels (Takara Bio Inc., Kusatsu, JPN). We detected ROR2 expression in human tissue samples derived from uterus and ovary and low expression levels on intestine, prostate, colon, testis and placenta. mROR2 showed similar expression patterns and levels. To establish a model to compare CAR affinity and cross-reactivity in more detail, we ectopically expressed full-length hROR2 and mROR2 on MDA-MB231 cells. The functional characterization of ROR2 CAR T cells revealed profound, ROR2-specific cytotoxicity, proliferation and cytokine secretion. All three constructs showed comparable cytolytic activity within a 48h time window. In order to identify more discreet differences, we performed a sequential killing assay and observed decreasing cytotoxicity after each round. This effect was significantly more pronounced for the low affinity construct. A similar trend was observed for antigen-dependent cytokine release and T cell proliferation. In general, higher binding affinity of the scFv utilized for each construct correlated with increased cytokine secretion and proliferation. As expected, the non-cross-reactive scFv was found to be non-functional against mROR2+ target cells in all assays. Conclusion and Outlook: Our data suggest hROR2 as a viable target for BC therapy. ROR2 is expressed on the cell surface of BC cell lines and can be targeted efficiently using ROR2 specific CAR T cells. CARs based on scFv fragments with higher affinity outperformed low affinity variants in all assays. Furthermore, we could demonstrate that both the expression pattern and expression level of hROR2 and mROR2 closely resemble each other. These findings, together with the availability of cross-reactive and hROR2-specific CAR T cells, will allow us to investigate on-target off-tumor effects in more detail using mice as an animal model.

A23

VEGFR2 as a target for CAR T cell therapy of Ewing sarcoma

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Metastatic Ewing sarcoma has a poor prognosis despite intensive multimodal therapy. Cellular targeting with T cells engineered to express chimeric antigen receptors (CARs) is a potential novel therapeutic option. The development of CAR T cells against Ewing sarcoma is limited by the lack of adequate surface antigens reliably expressed by this cancer, but not by indispensable normal tissues. In this project, we have investigated VEGFR2 expressed on tumor-associated blood vessels as a potential target structure for CAR T cells against Ewing sarcoma. Immunohistochemical analysis of 6 Ewing sarcoma tumor biopsies obtained from patients and of 4 murine Ewing sarcoma xenografts (2x VH-64 and 2x TC-71) demonstrated that Ewing sarcomas are strongly vascularized tumors. The tumor-associated endothelial cells in all samples strongly expressed VEGFR2, whereas the tumor cells were VEGFR2-negative. Among 19 Ewing sarcoma cell lines analyzed by flow cytometry, neither expressed VEGFR2 on the cell surface. To target VEGFR2 on the tumor endothelium in preclinical mouse models as well as in human patients, we engineered CARs with short (IgG1 hinge), medium (IgG1 hingeCH3) and long (IgG1 hingeCH2CH3) hinge domains against both human and murine VEGFR2 and expressed the CAR constructs in T cells from healthy donors by retroviral gene transfer. In functional in vitro assays, we compared the phenotypes and capacity of the CARs with their various hinge regions to activate T cells in response to cell-bound VEGFR2. Target cells for murine VEGFR2-specific CAR T cells were the murine endothelial cell lines bEND3, bEND5 or MS-1. Due to the lack of human endothelial cell lines consistently expressing VEGFR2 in in vitro culture, we transduced the human rhabdomyosarcoma cell line A204 and the human embryonic kidney cell line 293T with full-length human VEGFR2 as targets for human VEGFR2-specific CAR T cells. Anti-mouse and anti-human VEGFR2-specific CARs were effectively expressed in T cells from healthy donors with a median transduction efficiency of 37% and comparable proportions of central memory T cells among the three constructs for each species. In calcein cytotoxicity assays, all VEGFR2-specific CAR T cells, regardless of their hinge region and species specificity, specifically lysed VEGFR2 expressing target cells. In degranulation assays we found significantly higher proportions of CD107a-expressing cells in response to VEGFR2-positive target cells among VEGFR2 specific CAR T cells with short or medium long hinge regions from both species compared to CAR T cells with the long hinge region ($p < 0.05$). Anti-human VEGFR2-redirected CAR T cells released both TNF- α and IFN-γ in an antigen-specific manner. The proportion of cytokine-releasing cells was not significantly different among the three constructs. In a three-week proliferation assay in which anti-human VEGFR2 CAR T cells were co-cultured with irradiated VEGFR2 expressing target cells, the proliferation rates of CAR T cells tended to increase with a shorter hinge region. We conclude that VEGFR2 is expressed on endothelial cells of the tumor stroma of Ewing sarcomas and thus is a candidate target for CAR T cells against this cancer. A long hinge domain is not necessary and even unfavorable for optimal T cell activation responses of VEGFR2-specific CARs to antigen-expressing targets. In future experiments, we will investigate the potential of VEGFR2-specific CAR T cells to eradicate Ewing sarcomas in in vivo xenograft models.

Poster Session B

TCR-engineered T-Cell Therapy

B1

A high throughput approach for the parallel identification of TCRs recognizing multiple antigens with clinical relevance for the treatment of B-cell malignancies

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CAR T-cell therapies for the treatment of B-cell malignancies have shown great promise in clinical trials. However, antigen negative escape variants can cause disease relapse and therefore additional therapies are needed. To increase the number of potential targets we propose a TCR-based approach to target both intracellular and extracellular proteins. Illumina HT-12 microarray data was used to select 31 target genes expressed in B-cell malignancies but not in healthy tissues other than B-cells. To broaden to scope of TCR gene therapy beyond HLA-A*02, peptides presented in HLA-A*01, A*24,B*08 or B*35 were selected. Elution of HLA-presented peptides and mass spectrometry was used, which led to the identification of 19 target gene derived peptides presented in one of the HLA-alleles of interest. Peptide-HLA tetramers were used to single cell sort tetramer+ CD8+ T-cells from healthy donor PBMCs. Donors were negative for the HLA alleles of interest to allow identification of high affinity T-cells from the allo-HLA repertoire. In total 12.300 T-cells were single-cell sorted of which 62 T-cell clones recognized one of the candidate peptides. Of these 62 T-cell clones 23 were potent enough to recognize endogenously processed and presented peptide by target gene and HLA transduced k562 cells. Seven T-cell clones recognized primary B cell malignancy samples or B cell malignancy derived cell lines, these T cells recognize peptides derived from VPREB3 presented in HLA-A*24, RALGPS2 presented in HLA-B*08, FCRL5 presented in HLA-A*01 or POU2AF1 presented in HLA-B*35. For six T cell clones screenings for off target reactivity did not show any cross reactivity with other HLA-peptide combinations. One T cell clone recognizing a peptide from FCRL5 presented in HLA-A*01 additionally recognized a unknown peptide presented in HLA-B*08 which could cause off target cytotoxicity when used to treat an HLA-B*08+ patient. To test the potency of the 6 TCRs after TCR gene transfer, TCRs were sequenced and retroviral vectors were constructed. Healthy donor CD8+ T cells were retrovirally transduced and TCR transduced T cells showed avidity and recognition patterns comparable to the original T cell clones. In conclusion, tetramer based T cell isolation from the allo-HLA repertoire of healthy donors resulted in the isolation of safe and potent T cells recognizing malignant B cells. After TCR gene transfer recipient T cells showed comparable functionality to the original T cell clones confirming that these TCRs are very promising candidates for TCR gene therapy based treatment of patients with B cell malignancies.

B2

Adoptive cell transfer in metastatic melanoma patients at the NKI-AVL in Amsterdam; from autologous TIL towards neoantigen directed T cell therapies.

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Adoptive cell transfer (ACT) is an attractive treatment option for cancer patients. In the last few years, the NKI-AVL has successfully initiated three clinical trials with ACT for metastatic melanoma (MM) patients. In the first phase I/II trial, 10 MM patients were treated with autologous tumor infiltrating lymphocytes (TIL), preceded by non-myeloablative chemotherapy and followed by high dose bolus interleukin-2. Five out of these 10 patients showed a clinical response upon cell infusion, including two durable complete responders. We have previously shown that melanoma TILs are able to recognize patient specific antigens derived from expressed mutated genes, so-called neoantigens. Extensive immune monitoring in this trial showed that neoantigen-specific T cells were detectable in TIL infusion products from three out of three responding patients. In most cases, the neoantigen reactivity significantly increased in the peripheral blood compartment upon therapy and was detectable for up to three years after TIL reinfusion. This observation confirms in vivo expansion and long-term engraftment of neoantigen reactive T cells originating from the TIL infusion product. As a next step in the development of TIL therapy in melanoma, an international, multi-center, randomized controlled phase III clinical trial was initiated together with Herlev Hospital, Copenhagen, in which TIL treatment is compared to treatment with ipilimumab (anti-CTLA-4). Thus far, 69 MM patients have been treated in this trial, of which 34 received TIL therapy and 35 received ipilimumab. In this study, MM patients are treated in second-line after failure of anti PD-1 monotherapy. Since alternative treatment options for these patients are limited, this trial could provide an extra treatment option and is thus still very relevant and could possibly lead to future re-imbursement of TIL therapy. Beyond TIL therapy, the NKI-AVL has also executed a TCR gene therapy trial. The TCR used for T cell modification was specific for the HLA-A*0201 restricted MART-1 26-35 epitope, which is expressed on the majority of melanoma cells. Unique to this trial was the use of the combination of anti-CD3/CD28 beads for T cell activation plus IL-7/ IL-15 for subsequent culture and expansion. This is different from the more commonly used strategy, which utilizes the combination of anti-CD3 and IL-2 for expansion. The aim of this altered production strategy is to generate a 'less differentiated' T cell product that may have a better engraftment potential and anti-tumor activity. After treating 12 patients at different T cell dose levels, we conclude that MART-1 TCR-modified T cells produced by this method have high potential for engraftment and show in vivo activity at low cell doses infused (as few as 50xE6 transduced T cells). This suggests the potential value of this manufacturing protocol for TCR or CAR gene therapy for the treatment of cancer. In recent years, it has become evident that tumours with a higher mutational burden respond better to checkpoint inhibition and TIL therapy. It is likely that the neoantigen reactive T cell compartment is an important driver for this phenomenon, and therefore, the development of neoantigen directed immunotherapy is of great interest for future cancer treatment. Neon Therapeutics (Cambridge, MA), is developing a personal neoantigen T cell therapy called NEO-PTC-01 in collaboration with the NKI-AVL. For this ACT, we induce multiple neoantigen T cell populations against multiple personal neoantigens for each patient, using an ex-vivo induction protocol called NEO-STIMTM. NEO-STIM has been optimized to prime, activate and expand both memory and de-novo T cell responses from the CD8+ and CD4+compartments from PBMCs. Here we will show representative data generated using PBMCs from a patient with melanoma. We are currently completing GMP process development to progress this ACT approach into clinical development where we plan to evaluate this in the solid tumor setting.

B3

Adoptive therapy with TCR gene-engineered T cells to treat patients with MAGE-C2-positive melanoma and head and neck cancer

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Despite promising results of immune therapies of metastatic melanoma and head-and-neck squamous cell carcinoma (HNSSC) using immune checkpoint inhibition, there is still a need for additional therapies. We are scheduling a patient study, in which we will provide a combination therapy to patients with metastatic melanoma and HNSCC consisting of TCR-engineered T cells directed against the Cancer Germline Antigen MAGE-C2 (MC2) and epigenetic drugs. MC2 is highly expressed in, amongst others, melanoma, HNSSC, bladder, and triple-negative breast cancers but not in healthy adult tissues as evaluated by Q-PCR and in situ stainings. We isolated MC2-specific TCRs from melanoma patients who showed clinical responses following vaccination that were accompanied by significant frequencies of anti-MC2 CD8 T cells in blood and tumor without apparent side effects. Following extensive evaluation of in vitro anti-tumor and self-reactivities, we have selected a TCR that recognizes the ALK epitope in the context of HLA-A2 for clinical development. Furthermore, our preclinical studies showed that epigenetic pretreatment (5-azacitidine and valproic acid) of tumor cells, but not normal cells, up-regulated MC2 gene expression and resulted in enhanced recognition of MC2 by the selected TCR. In parallel to the above studies, we renewed our GMP protocol to process T cells, using stimulating antibodies (CD3 and CD28) and cytokines (IL-15 and IL-21), to generate T cells with a younger phenotype (CD45RA+, CD62L+, CD27+). In a phase I/II study, we will explore the safety and anti-tumor efficacy of T cells engineered with the selected TCR in patients with MC2-positive melanoma and HNSSC. The study contains the following unique elements: • CT antigen not targeted before by T cell therapy • a new T cell processing method to generate young T cells • pretreatment of patients with epigenetic drugs to increase MC2 expression in the tumor • no chemotherapy prior to T cell infusion All preparations, including production and certification of the clinical vector batch and the clinical scale process validations, have been successfully conducted. The license application (use of the vector) and permission for the clinical trial are under review at the regulatory authorities, and start of patient treatment is projected for Q4 2019.

B4

Effective NY-ESO-1-specific MHC II-restricted T cell receptors from antigen-negative hosts enhance tumor regression

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Adoptive transfer of T cell receptor (TCR)-engineered T cells is a promising approach in cancer therapy but needs improvement for more effective treatment of solid tumors. While most clinical approaches have focused on CD8 T cells, the importance of CD4 T cells in mediating tumor regression has become apparent. Regarding shared (self) tumor antigens, it is unclear, whether the human CD4 T cell repertoire has been shaped by tolerance mechanisms and lacks highly functional TCRs suitable for therapy. Here, TCRs against the tumor-associated antigen NY-ESO-1 were isolated either from human CD4 T cells or from mice that express a diverse human TCR repertoire with HLA-DRA/DRB1*0401 restriction and are NY-ESO-1-negative. NY-ESO-1-reactive TCRs from the mice showed superior recognition of tumor cells and higher functional activity compared to TCRs from humans. We identified a candidate TCR, TCR-3598_2, which was expressed in CD4 T cells and caused tumor regression in combination with NY-ESO-1-redirected CD8 T cells in a mouse model of adoptive T cell therapy. These data suggest that MHC II-restricted TCRs against NY-ESO-1 from a non-tolerant non-human host are of optimal affinity and that the combined use of MHC I- and II-restricted TCRs against NY-ESO-1 can make adoptive T cell therapy more effective.

B5

Effective re-routing of NK cell cytotoxicity against B-cell malignancies upon TCR gene transfer.

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T-cell receptor (TCR) gene transfer involves ex-vivo introduction of a tumour-reactive TCR into patient-derived CD8 T-cells enabling specific-targeting of tumour cells. Competition for expression with CD3 from the endogenous TCR and the potential for TCR mixed-dimer formation necessitate optimisation of cellular therapeutics with sustained potency and increased safety. NK-cells (CD3-CD56+) are potent short-lived effector cells that lyse abnormal or stressed cells independent of antigen. Efficacy and safety of adoptive NK therapy has been demonstrated in the treatment of haematological malignancies in both the autologous and allogeneic setting. Here, we aimed to exploit NK-cell cytotoxicity and redirect it toward antigen-specific recognition of tumours without the limitation of TCR mixed-dimer formation and competition for CD3. Peripheral blood derived NK-cells were expanded in vitro using K562 expressing membrane bound IL21 and 41BBL (K562-mbIL21-41BBL) in the presence of IL2 and IL15. On Day 3 post stimulation NK cells were retrovirally transduced with BOB1-specific TCRab, restricted to HLA-B*07:02, linked via T2A to CD8ab co-receptor. BOB1 is a B-cell restricted transcription factor, important for malignant B-cell survival. As a negative control NK cells were retrovirally transduced with CD8ab alone. Transduced NK cells were purified for CD8b expression by FACS and subsequently retrovirally transduced with CD3 ε, δ, γ and ζ chains to allow for cell surface expression of BOB1-TCR. NK cells were stimulated weekly with irradiated K562-mbIL21-41BBL. Between Day 16 and 21 purified NK cells were assessed for cell surface expression of introduced TCR by FACS and used in cytotoxcity assays against malignant B-cells with known HLA-B*07:02 and BOB1 antigen expression. Purified BOB1-TCR expressing NK-cells demonstrated antigen-specific binding of BOB1-specific pMHC-tetramer and proliferated upon co-culture with HLA-B*07:02 positive B-lymphoblastic cell lines (B-LCL) but not with HLA-B*07:02 negative B-LCL. Furthermore, BOB1-TCR expressing NK-cells demonstrated in vitro cytotoxicity against HLA-B*07:02 positive B-LCL, multiple myeloma and B-ALL cell lines. Conversely, these tumour cell lines remained resistant to NK-cell mediated lysis when co-cultured with mock transduced NK-cells. Finally, NK sensitive cell line K562 was comparably lysed by both BOB1-TCR or mock transduced NK-cells demonstrating retained NK-cell mediated activity. These data demonstrated that NK-cell cytotoxicity can be redirected toward antigen-specific recognition of tumours and is TCR-dependent. Retention of NK-cell function in genetically modified cells allows for a double-hit therapeutic approach which can offer advantages over current cellular approaches.

B6

Exploiting clonal tracking of WT1-specific T cells to generate a library of tumor-specific T cell receptors (TCR) for TCR gene editing of acute leukemia

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Recent encouraging clinical results obtained with engineered T lymphocytes have opened new opportunities for adoptive T cell therapy for cancer. Unfortunately, two main issues are still present: the difficulty in identifying appropriate tumor-specific antigens and the limited number of high avidity T cell receptors (TCRs) against shared oncogenic antigens. Here, we aim at the identification of a panel of novel tumor-specific TCRs, to be exploited by TCR gene transfer and TCR gene editing. We focused on Wilms' Tumor 1 (WT1), a tumor-associated antigen widely expressed on a variety of hematological and solid tumors, elected as a high priority antigen by the National Cancer Institute (NCI). We designed and implemented an innovative protocol for the rapid isolation of WT1-specific T cells and for the characterization of a library of tumor-specific TCRs restricted to different human leukocyte antigen (HLA) alleles. We repetitively stimulated T cells from healthy donors (HDs) with autologous antigen-presenting cells, including immortalized B cells, pulsed with overlapping peptides spanning the entire WT1 protein. T cell recognition was assessed by flow cytometry in terms of CD107a expression and IFNγ production. Recognized peptides were mapped by a deconvoluting grid and their HLA restriction assessed by using a panel of cell lines harboring the HLA alleles of interest. Tumor-specific TCRs were identified by TCR αβ sequencing. We achieved successful expansion of tumor-specific T cells from 14 consecutive HDs, with an average of 4 rounds of in vitro stimulation. Upon identification of the immunogenic epitopes, we assessed the ability of WT1-specific T cells to recognize naturally processed peptides and their on-target specificity upon co-culture with antigen-expressing targets, including primary leukemic blasts. TCR αβ sequencing at different time points enabled the longitudinal clonal tracking of the tumor-specific T cells and the correct pairing of TCR α and β chains, without the need of performing single cell analysis. We identified 20 clonotypes recognizing several tumor-associated peptides that were restricted by more than 5 HLA alleles, including HLA-A*02:01. Newly identified TCRs were then expressed as transgenes via genome editing. Briefly, simultaneous editing of endogenous TCR α and β chain genes was achieved by using CRISPR/Cas9 technology (editing efficiency >90%), followed by transduction of edited T cells with lentiviral vectors encoding WT1-specific TCRs (transduction efficiency >95% CD8+ T cells). Phenotypic characterization of engineered T lymphocytes showed a major enrichment of cells exhibiting a T stem cell memory phenotype. Functional validation of edited T cells is currently ongoing. Preliminary results of a 6-hour co-culture experiment show that TCR-edited T cells kill fresh WT1+ leukemic blasts harvested from HLA-matched patients with an efficiency up to 70%, at an effector to target cell ratio of 5 to 1, while no killing of controls is observed. Comparative analysis of both safety and efficacy profile of TCR edited T cells will be discussed.

B7

Identification of a neoantigen targeted by tumor-infiltrating lymphocytes in a patient with HER2+ breast cancer

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Introduction: During the last decade cancer-immunotherapy has become an encouraging new therapeutic option for patients with different malignancies. Especially targeting neoantigens with adoptive T-cell transfer or cancer vaccines appears to be a promising approach. Those neoantigens, which arise in the process of tumorigenesis, are tumor specific and lack of central tolerance. Consequently, they can elicit high avidity and tumor-specific T-cell responses. First studies showed promising results with checkpoint-inhibitors in breast cancer (BC) as well. For the more aggressive subtypes of BC such as HER2+ and Triple Negative Breast Cancer (TNBC), recent studies have demonstrated a strong association between the number of tumor infiltrating lymphocytes (TILs) and outcome as well as response to neoadjuvant chemotherapy. But the targets of those TILs remain unknown. Thus, it is the aim of our study to identify their targets with a main focus on tumor-specific mutations. Methods: TILs were expanded from BC biopsies at the timepoint of diagnosis by unspecific stimulation. For the identification of potential neoantigens tumor specific mutations were identified by comparing whole genome sequencing data from tumor and autologous blood cells as reference. All non-synonymous mutations were analyzed for RNA expression of the encoding genes to determine potential neoantigens. Finally, binding analysis to patient's specific HLA molecules was performed and potential neoantigens were synthesized. TILs were cocultured with potential neoantigens loaded onto autologous antigen presenting cells (APCs). After clonal expansion of all IFNγ producing cells, T-cell clones were tested for neoantigen specificity with the aim to identify neoantigen-specific T-cell clones. Results: In a patient with HER2+ BC we were able to identify three peptide-specific CD4+ T-cell clones. They were isolated from the core biopsy at the time point of diagnosis. All three T-cell clones recognized the same mutated peptide derived from RBMX protein without showing any reactivity against the wildtype counterpart. Interestingly, we could also isolate a T-cell clone recognizing the same neoantigen from the resected tumor tissue after neoadjuvant therapy. The sequencing data of the CDR3 region of the T-cell receptor (TCR) demonstrate the polyclonal nature of the T-cell response. All four T-cell clones exhibit different TCR Vb chains and though represent individual clones. Moreover, we confirmed the ability of processing and presenting of the respective protein. T-cell clones recognized the endogenously expressed mutated antigen presented by APCs. Moreover, transduction of the mutated RBMX into the MHC-II negative BC cell-line MCF-7 led to T-cell recognition after IFNγ induced MHC-II upregulation. EBV-LCLs loaded with cell lysates generated from those RBMX transduced MCF-7 could also stimulate the T-cell clones. This indicates that inflammation may lead to direct presentation of this neoantigen and indirect presentation is possible on surrounding APCs in the context of destroyed tumor cells. Additionally, we addressed the HLA restriction of this neoepitope. Interestingly, we could show that it is presented in two different HLA molecules of the patient each leading to individual T-cell responses. Three of the clones recognized the peptide presented in HLA-DPB1*0401 and one in HLA-DPB1*0201. These results further underline the immunogenicity of this neoantigen. Conclusion: In conclusion, our data demonstrate neoantigen-specificity of TILs in a patient with HER2+ BC. Furthermore, we show the feasibility to identify individual cancer specific T-cell targets in BC patients, which in future may contribute to the development of targeted patient-specific immunotherapies. * Authors contributed equally

B8

HA-1-specific T-cell receptors for the treatment of hematological malignancies

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Minor histocompatibility antigens like HA-1 were identified in the context of allogenic hematopoietic stem cell transplantation and it was shown that they can play an important role in the graft versus leukemia effect. As the expression of HA-1 is restricted to hematopoietic cells it is a promising target for the treatment of malignancies resulting from these cells. Theoretically, adoptive T-cell therapies targeting HA-1 can be used after allogenic hematopoietic stem cell transplantation either as a prophylaxis to prevent relapses or as a treatment after relapse, if donor and patient have the right mismatch in HA-1. To not be limited to preexisting T cells in the stem cell donor's repertoire, we aimed at isolating HA-1 specific T-cell receptors (TCRs) that can be used for adoptive T-cell therapy with gene-modified T cells. Using our TCR discovery platform, we efficiently generated HA-1 specific T-cell clones from different healthy blood donors and identified their TCR sequences. These sequences were transferred into CD8-positive T cells. The modified cells were subsequently analyzed for their efficacy and safety profile. T cells expressing our HA-1 candidate TCRs released cytokines and showed cytotoxicity in response to HA-1 positive but not to HA-1 negative target cells. Peptides that could be potentially cross-recognized by the HA-1 TCRs were predicted by Expitope 2.0 and analyzed in co-culture assays. Cross-recognized peptides were de-risked by using target cells expressing the corresponding transgene. To further reduce the risk of off-target effects by the HA-1 TCR transgenic T cells, the reactivity was analyzed against a set of lymphoblastoid cell lines covering the most common HLA types and a set of normal cells representing vital organs. In summary, we were able to efficiently isolate and characterize TCRs specific for the minor histocompatibility antigen HA-1, that are ready to be tested in a clinical trial.

B9

Redirecting CD4 and γdelta T cells by RNA electroporation of mRNAs encoding CD8 and an MHC class I-restricted Wilms Tumor 1-specific TCR.

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Redirecting CD8 T cell specificity towards tumor-associated antigens through TCR and CAR engineering has played a major role in cancer immunotherapy. However, there is growing interest on the role of CD4 and γ-δ (γδ) T cells. We studied the feasibility of redirecting the specificity of CD4 and γδ T cells via transfection of MHC class I-restricted Wilms' tumor 1 (WT1)-specific TCR mRNA and whether co-electroporation of human CD8αβ mRNA would improve tumor-specific recognition and cytotoxicity. Non- and pre-activated CD4 T cells were transfected in accordance to our in-house developed double sequential electroporation method, first with DsiRNA to suppress de novo expression of endogenous TCR followed by co-transfection of WT1_37-45 TCR mRNA (T37) with or without CD8αβ mRNA (CD8). Zoledronate-expanded γδ T cells were subjected to a single electroporation with T37 mRNA with or without CD8 mRNA. The transgenic TCR was highly expressed on activated CD4 or expanded γδ T cells after electroporation. High frequencies of CD8(+) cells were observed both in non- and pre-activated CD4 T cells and expanded γδ T cells. Subsequently, modified T cells were challenged with peptide-presenting tumor cells. Expression of activation markers, and cytokine and granzyme B secretion by T37+CD8 transfected pre-activated CD4 T cells and cytotoxic activity of γδ T cells indicate that they can specifically recognize the tumor cells. In summary, we confirmed that, similar to CD8 T cells, electroporation is also an effective transfection method to successfully engineer CD4 and γδ T cells with a transgenic TCR. Moreover, co-introduction of CD8 mRNA has a beneficial effect on T cell avidity and antigen recognition and suggests a possible redirection of CD4 and γδ T cells into effector-like T cells. This results show the potential of these lymphocytes for adoptive cancer immunotherapy.

B10

Revealing efficacy and toxicity of high affinity WT1-specific TCRs

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The Wilms' tumor gene 1 (WT1) is expressed in a broad range of cancers, especially in acute myeloid leukemia (AML), ovarian carcinomas and mesotheliomas. Using T-cell receptor (TCR)-based therapies, WT1-expressing tumor cells could be targeted. Several research groups have been searching for WT1-specific TCRs and the first clinical trials in AML patients are ongoing. However, commonly observed limitations are the low affinity and limited efficacy of the introduced TCRs. We aim to identify more potent high affinity T-cells by searching within the allo-HLA repertoire. Nonetheless, high affinity T-cells can also increase toxicity risks, since WT1 shows expression in several healthy tissues. Therefore, potential toxicities of WT1 as a target for TCR gene therapy need to be explored. In this study we have searched in PBMCs of healthy individuals for WT1-specific CD8+ T-cells, using 8 different WT1-specific peptide-MHC tetramers. These individuals were negative for our HLA alleles of interest, enabling us to search within the allo-HLA repertoire. The WT1 peptides were presented by HLA-A*01:01, HLA-A*02:01, HLA-A*03:01 or HLA-B*35:01 and were selected based on HLA-peptide elution data of primary AML and ovarian carcinoma material. Initially, over 7,000 tetramer positive CD8+ T-cell clones were collected via single-cell sorting. Specific recognition of peptide loaded target cells, as well as target cells transduced with the WT1 gene, were analysed. In total, 62 T-cell clones efficiently recognizing WT1 transduced target cells were selected for further screenings. Using cell panels consisting of different malignant cell types, efficacy screenings were conducted. Altogether, 10 high affinity WT1-specific T-cell clones were found for 6 different peptides, recognizing WT1-expressing tumor cell lines. Also primary AML samples expressing the right HLA alleles were recognized, only if they expressed WT1, as determined by qPCR. The TCR sequences of a first selection of T-cell clones were constructed and transduced into CD8+ T-cells using retroviral vectors. Similar recognition patterns of the WT1-specific TCR transduced CD8+ T-cells and the original T-cell clones could be demonstrated. In addition, to focus on the potential toxicities of WT1, expression levels were quantified by qPCR in primary tumor samples, tumor cell lines and non-malignant cells. High levels of WT1 were observed in primary AML and ovarian carcinoma samples, in contrast to no or limited expression in several non-malignant cells. However, publicly available RNAseq databases show WT1 expression in kidney, heart, adipose visceral omentum and reproductive organs, underscoring the need to focus on toxicity risks. Therefore, follow up safety screenings are planned, investigating reactivity of the WT1-specific TCRs against a broad panel of non-malignant cells with quantified WT1 gene expression levels. If corresponding non-malignant cells are not available, human Induced pluripotent stem cells (hiPSC) will be used. Preliminary data using hiPSC-derived cardiomyocytes demonstrated no recognition by our identified WT1-specific T-cell clones. In conclusion, WT1-specific TCRs efficiently recognize WT1-expressing primary AML samples and the first safety screenings show no toxicity risks.

B11

Targeting mutant p53 in spontaneous cancer by T cell receptor gene therapy

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Somatic mutations in cancer can result in neoantigens that serve as an attractive target for cancer immunotherapy. Many human cancers develop 'spontaneously' i.e. without known exposure to carcinogens and therefore have far fewer mutations than murine cancers induced with carcinogens. We, therefore, chose the murine fibrosarcoma Ag104A that spontaneously arose in an aging C3H mouse, as a model to analyze mutation-specific T cell receptor gene therapy under conditions that closely resemble the clinical situation. An Ag104A-specific TCR (M2/3) was isolated from T cells of a C3H mouse after immunization with the tumor cells. To determine the target of the M2/3 TCR, mutations in Ag104A were determined by whole-exome and RNA sequencing. All 77 potential neoantigens were expressed as tandem minigenes and transfected into target cells engineered with H-2k restriction elements. Subsequent analysis revealed that TCR M2/3 is directed against a H-2Kk-presented peptide derived from mutant Tp53, p53S256E (mp53). To determine if the mutation is seemingly present in all cells of the uncloned Ag104A tumor cell line, we generated 133 clones. All of them seem to harbor the same p53D256E mutation suggesting that most, if not all, Ag104A cancer cells express it. Since Ag104A cancer cells also harbor the wildtype p53 allele, the p53S238A mutation may represent a driver mutation that cannot be lost. We then used M2/3-engineered T cells for adoptive T cell therapy to treat established Ag104A tumors in C3HxRag2-/- mice. The transferred T cells expanded in an antigen-specific manner and caused growth arrest of this aggressive cancer. However, all tumors relapsed 4-5 weeks after treatment. Analysis of the re-isolated tumors suggests down-regulation of mp53, possibly regulated epigenetically under T cell pressure in vivo. However, none of the tumors that had relapsed lost the mp53 gene consistent with the idea that the p53D256E mutation is essential for cancer cells survival. Our analysis also suggests that T cell exhaustion was not the reason for treatment failure. Next, we will investigate the underlying mechanism of tumor escape. To support this, we will follow the behavior of the re-isolated and re-established tumor cell lines in vitro to examine whether and how the phenomenon can be reverted. We will also study the impact of mutant Tp53 in Ag104A on tumor cell growth and survival by restoring wildtype p53 expression using CRISPR/Cas9. The results will allow us to determine the parameters for effective mutation-specific TCR gene therapy using mp53 as a target. Supported by NIH grants R01-CA22677 and R01-CA37156, the Berlin Institute of Health and the Einstein-Stiftung Berlin.

B12

Analysis of potential epitopes from drug-selected mutations in chronic myeloid leukemia for T cell receptor gene therapy

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Presently, the first line treatment for chronic myeloid leukemia (CML) is tyrosine kinase inhibitors (TKIs). Although TKIs are effective, drug resistance is often observed due to drug-selected somatic mutations. Since drug-selected mutations can be potential neo-antigens expressed on cancer cells, T cell receptor (TCR) gene therapy becomes an ideal treatment to eliminate recurrent cancer cells. To identify potential epitopes for TCR gene therapy, the frequently occurring mutations after drug treatment in CML were filtrated by IEBD prediction program with stabilized matrix method. I tested two candidates with good prediction bindings to HLA-A*0201, ABL-E255V (IC50 = 25 nM) and ABL-T315I (IC50 = 103 nM), to determine their immunogenicity in transgenic ABabDII mice carrying the human TCR α, β gene loci and the human MHC I HLA-A*0201 gene. Although no specific immune response was elicited by ABL-T315I, a response to ABL-E255V was detected following immunization. Two different ABL-E255V specific TCRs, T9141 and T8922, were identified from each one immunized ABabDII mouse. My results prove that ABL-E255V was endogenously processed, since T9141- or T8922-transduced CD8+ T cells responded when co-cultured with ABL-E255V expressing cell lines. The T9141 with more sensitive peptide recognition was tested for off-target toxicity. No cross-reactivity of T9141 was detected in HLA-allotype assay and HLA-A*0201 restricted self-antigen library. In addition, a potential recognition motif of T9141 was identified by alanine scan. One human protein CB061 comprising the same motif elicited a response from T9141 transduced CD8+ T cells under high peptide concentration (≥ 10-4 M), and appeared not to be endogenously processed. For application, recognition of CML cells carrying ABL-E255V by T9141 needs to be analyzed.

B13

Selective inhibition of the transcription factor NFAT in mitigating graft-versus-host disease

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Bone marrow transplantation (BMT) is a very promising therapy for some forms of leukemia, aplastic anemia or immuno deficiency diseases, but due to immunological incompatibility between donor T cell and host tissues, graft-versus-host disease (GvHD) occurs. To protect patients, suffering from acute GvHD, cyclosporineA (CsA) or tacrolimus (FK506) are administered. These drugs are able to control inflammation but interfere with the graft-versus-leukemia (GvL) activity. In line, regulatory T cells (Tregs), which protect from GvHD, but promote GvL, are suppressed by CsA and FK506 like conventional T cells (Tcons). Both drugs inhibit calcineurin (CN), which at first dephosphorylates and thereby activates members of the NFAT (nuclear factor of activated T-cells) family of transcription factors. We found that absence of one or two NFAT family members in donor T cells prevents harmful GvHD. Moreover, absence of only one family member fully preserved the valuable GvL effect. Accordingly, number and function of Tregs was preserved (Vaeth et al., 2015). Therefore, we propose here to develop and evaluate treatment regimens that avoid broad immune suppression and instead specifically target NFAT, i.e. pro-inflammatory Tcons but not Tregs, during allo-BMT. NFAT-specific drug development will be based on the assessment of presumably selective inhibitors of the activation cascade. First focus will be on compounds that mimic the PxIxIT motif and compete with NFAT for binding to CN without interfering with the phosphatase activity of CN. Currently, we are evaluating KD-optimized PxIxIT peptides and small molecules that interfere with the docking of CN to NFATs. In a second aim we envision to mutate individual NFAT family members or isoforms by CRISPR/Cas9 ahead of allo-BMT. Inhibitor candidates as well as CRISPR/Cas9-mediated NFAT-ablated T cells will be tested in our routine GvHD/GvL mouse models, in engineered human skin as well as in xeno-GvHD/GvL models established in NSG mice with the perspective to initiate a future clinical pilot trial.

B14

Targeting FLT3 with T cell receptor-modified T cells for treatment of hematological malignancies: potential on target toxicity in clinical applications

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Acute myeloid leukemia (AML) is a disease with poor prognosis. Fsm-like tyrosine kinase 3 (FLT3) is a promising target because of its overexpression in AML cells. Efforts have been put to develop new therapeutics targeting FLT3 by small molecule inhibitors and most recently with chimeric antigen receptor (CAR) modified T cells. We developed HLA-A2-restricted, FLT3-specific T cell receptors (TCR) to target FLT3-positive AML and hematopoietic stem cells (HSCs) in an HLA-A2-mismatched allo-HSC transplantation. In our proposed set up, FLT3-specific TCRs would eliminate AML cells as well as HLA-A2-positive HSCs of the patient allowing engraftment of a healthy HLA-A2-negative hematopoietic system. FLT3 is a self-antigen, therefore, T cells bearing high-affinity TCRs against its epitopes are deleted in humans. To circumvent the tolerance, we immunized a transgenic mouse model expressing a diverse human TCR repertoire and HLA-A2 molecule (ABabDII) with an in silico predicted epitope of human FLT3 (GLL). We identified two TCRs that recognize GLL peptide when loaded on HLA-A2-positive cells. We could detect T-cell activation upon co-culture with FLT3-positive cell lines; however, IFN-δ release could not be detected by ELISA. This could be due to i) the sub-optimal affinities of the identified TCRs to the pMHC complex ii) low binding affinity of GLL epitope to HLA-A2 molecule resulting in a poor presentation on the cell surface. During the in vitro safety testing, we discovered high, intracellular FLT3 expression in the Purkinje cells of the human cerebellum. We have stopped our attempt to identify high-affinity FLT3-specific TCRs due to potential cerebellar toxicity. We believe FLT3 could still be a safe, valuable target for therapies other than TCR-modified T cells.

B15

TCR gene therapy of CD22-positive B cell malignancies

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CD22, a B-cell receptor regulating B cell interaction and signaling, is expressed on mature B cells and therefore a promising target for directed T cell receptor (TCR) gene therapy of B cell-derived lymphomas and leukemia. T cells are negatively selected against self-antigens in the thymus to avoid autoimmune disease. To generate a CD22-specific TCR, we used a mouse model that is transgenic for the human TCRα and TCRβ loci as well as transgenic for the human HLA-A*02:01 for proper epitope presentation. After vaccination with the non-homologous human CD22 cDNA, we achieved a specific immune response. Further evaluation of reactive T cells revealed their peptide specificities. The epitope that elicited the most frequent reactivity in vaccinated mice was previously described. After in vitro enrichment with specific peptide, T cells were sorted for IFNγ secretion and were used for the isolation of TCRα and TCRβ sequences. One of the identified TCRs showed in functional assays superior recognition and/or killing of CD22 expressing cells such as LCLs, B cell lines, primary healthy B cells and patient's cells. Also, a preliminary xenograft mouse experiment demonstrated the therapeutical potential of this TCR. Currently, we are investigating the safety of this TCR and the target antigen. We did not observe any allo-reactivity and identified the peptide recognition motif of the TCR. Peptides that share this recognition motif as well as around 100 self-peptides will be tested for recognition. CD22 expression in non-lymphoid tissue is currently investigated. We detected its expression in in-vitro differentiated macrophages and dendritic cells and are now evaluating the potential recognition by CD22 TCR T cells comparing it to CD22 CAR T cells. CAR therapy directed against CD22 was already tested in a clinical phase 1 study that did not reveal toxicity. A main failure of CAR T therapy is the downregulation of the antigen which is also the case in CD22 CAR treatment. As CD22 is only downregulated on the cell surface in these patients, a specific TCR can still recognize it by peptide/MHC presentation and therefore, we believe that a CD22 TCR T cell therapy is especially for patients that failed CAR therapy an attractive treatment option. After finishing last safety tests, we will apply for a clinical study phase I.

B16

With great power comes great vulnerability:Functional comparison of TCR and CAR transduced T cells targeting CD20

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With the rise of chimeric antigen receptor (CAR) T cells, the role & potential of T cell receptors (TCRs) targeting surface antigen derived peptides for gene transfer therapy needs to be reevaluated. While the non HLA-restricted CAR T cells have demonstrated remarkable clinical efficacy in hematological malignancies, severe toxicity in responders and non-responsiveness in a subset of patients remain major challenges. We hypothesized that TCR transduced T cells targeting tumor surface antigen derived peptides pose a valuable alternative to CAR T cells by maintaining a comparable efficacy while exhibiting a more tolerable toxicity profile, alongside higher resistance to activation induced cell death and exhaustion. Our group has previously identified a high affinity TCR targeting a CD20 derived peptide presented in the context of HLA-A2. Using a panel of primary human acute lymphoblastic leukemia cells differing in their CD20 expression level, we are functionally comparing our TCR with a set of CD20 specific CAR constructs incorporating different antigen recognition and costimulatory domains. Primary endpoints are effector functions (cytokine secretion and killing capacity), T cell phenotype (differentiation and exhaustion) and resilience (maintenance of phenotype during antigenic challenge). Finally, we aim to translate our findings into an in vivo model of acute lymphoblastic leukemia. In terms of effector functions, CAR transduced T cells show markedly elevated IFNγ secretion upon target cell encounter as compared to TCR transduced T cells. CAR transduced T cells also display higher killing efficacy of CD20 low and intermediate target cells than TCR transduced T cells, while specific killing of CD20 high expressing targets is comparable in both TCR and CAR transduced T cells. However, CAR transduced T cells show signs of tonic signaling, leading to progressive differentiation and upregulation of exhaustion markers even in the absence of antigen, while TCR transduced T cells phenotypically resemble Mock transduced T cells. Strikingly, in proliferation studies using physiologic E:T ratios, TCR transduced T cells expand in a CD20 expression sensitive manner, whereas CAR transduced T cells ultimately fail to maintain their population upon encounter of high levels of antigen, correlating with high expression of apoptotic markers in CAR transduced T cells. Taken together, our results indicate that CAR T cells show stronger immediate effector functions as compared to TCR transduced T cells. However, this appears to trade off against progressive differentiation and exhaustion, rendering CAR T cells highly susceptible to antigen induced cell death, potentially compromising clinical efficacy.

Poster Session C

Immunological Checkpoints

C1

1,25-dihydroxyvitamin-D3 levels measured at the time of allogeneic hematopoietic stem cell transplantation predict one-year survival

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25-hydroxyvitamin-D3 deficiency is a well-documented phenomenon during allogeneic hematopoietic stem cell transplantation and associated with chronic Graft-versus-Host disease (GvHD). Little is known about the role of 1,25-dihydroxyvitamin-D3 serum levels in this context. In a discovery cohort of 143 patients undergoing allogeneic hematopoietic stem cell transplantation (Tx) with concomitant high-dose vitamin D3 supplementation, 25-hydroxyvitamin-D3 and 1,25-dihydroxyvitamin-D3 serum levels were monitored before and after transplantation (day -16 to 100). We analyzed their association with transplant-related mortality within one year after transplantation (23 patients with Tx-related death). The findings of the discovery data were followed in a replication data consisting of 365 patients from three different sources of varying clinical settings including settings with high-dose vitamin D3 supplementation (as the discovery cohort), moderate or no supplementation. In the discovery cohort, lower 1,25-dihydroxyvitamin-D3 around Tx (day -2 to 7, peritransplant) was associated with higher risk of Tx-related death independent of severe acute GvHD (Cox-model unadjusted P=0.001, adjusted P=0.003) and peritransplant 25-hydroxyvitamin-D3 (Cox-model adjusted P=0.750). 25-hydroxyvitamin-D3 steadily increased after transplantation, however, late follow-up 25-hydroxyvitamin-D3 levels did not show association with Tx-related death independent of severe acute GvHD (Cox-model adjusted P=0.090). The optimal threshold for 1,25-dihydroxyvitamin-D3 to identify patients at high risk was 139.5 pM. This finding for 1,25-dihydroxyvitamin-D3 was replicated in 365 patients of the joint replication data (66 with Tx-related death, Cox-model unadjusted P=0.000, adjusted P=0.001). Patients with 1,25-dihydroxyvitamin-D3 below 139.5 pM compared to those with higher levels had a 3.3-fold increased risk of Tx-related death independent of GvHD. Our data highlight 1,25-dihydroxyvitamin-D3 at the time of transplantation as a novel and potent predictor of one-year survival. This suggests 1,25-dihydroxyvitamin-D3 as feasible biomarker for Tx-related adverse events and emphasizes its potentially under-acknowledged role compared to 25-hydroxyvitamin-D3.

C2

A screening for novel immune-checkpoints identifies a serine/threonine kinase to confer immune resistance in multiple myeloma

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Background: Multiple myeloma (MM) is a B-cell malignancy, characterized by the accumulation of plasma cell clones in the bone marrow. Despite tremendous progresses in cancer immunotherapy, a plethora of tumor patients is still refractory to current immunotherapeutic strategies. Several studies showed that by taking advantage of different immune-checkpoint molecules tumor cells can either dampen immune cell functionality or promote tumor cell resistance towards immune attack. Despite the encouraging results achieved by blocking CTLA-4 and the PD-1/PD-L1 axis in the treatment of various solid tumors and Hodgkin's lymphoma, targeting these checkpoints did not induce objective responses in Phase I/II trials in MM patients. Therefore, the identification of novel immune-checkpoints and defining the subsequent molecular mechanisms of inhibition is essential for further improvement. Materials and methods: As high-throughput (HT) RNAi screens offer the possibility to systemically search for immune-checkpoint molecules, we established a HT-screening system to discern candidate molecules and evaluate their use as potential targets for multiple myeloma immunotherapy. To this, we transfected stable luciferase expressing multiple myeloma cells (KMM-1-luc) with a siRNA library targeting 2887 genes (enriched for kinases and surface-associated molecules) and subsequently co-cultured the knocked down tumor cells with HLA-A2-matched patient-derived marrow-infiltrating lymphocytes (MILs). T cell- mediated killing of tumor cells was assessed by measuring the remaining luciferase activity of knocked down tumor cells. Candidate genes were validated in a secondary screen that allowed to distinguish between genes altering tumor resistance towards MIL-mediated killing and those impairing MILs activity. Luminex analyses were performed to investigate the underlying molecular pathways of candidate genes. Results: The HT-screening revealed 128 genes whose knockdown in tumor cells substantially increased T cell-mediated tumor cell death. Among the candidate immune-checkpoints the serine/threonine protein kinase CamV1 was selected for further validation and mechanistic analysis. We found that CamV1 supports the intrinsic tumor resistance against Fas receptor mediated apoptosis. Upon CamV1 activation through FasL induced calcium influx, CamV1 bound and phosphorylated the effector caspases 3, 6 and 7, thereby inhibiting their activation and activity resulting in markedly reduced tumor cell apoptosis after cytotoxic T cell attack. In addition, activation of CamV1 resulted in CREB-mediated transcription of antiapoptotic genes such as BCL2. These results obtained in a hematological malignancy were further confirmed in uveal melanoma emphasizing the relevant role of CamV1 in different tumor entities. Conclusions: Taken together, we generated a HT-discovery platform to unravel potential immune-checkpoint molecules as targets for immune-checkpoint blockade in multiple myeloma. Among the identified genes we demonstrate that CamV1 is a key modulator of tumor intrinsic resistance towards immune cell attack by blocking effector caspases.

C3

Alternative splicing of immune receptor SLAMF6 –a new regulatory mechanism with the potential to enhance T cell transfer

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Recently we characterized two isoforms of the human SLAMF6 gene: The 'canonical' sequence, composed of 8 exons, and SLAMF6Δ17-65 -missing part of exon-2, which constitutes the interacting V domain of the receptor. While the basic effect of canonical SLAMF6 engagement is to inhibit CD8 T cell function, the SLAMF6Δ17-65 short variant enhances T cell function, improving IFNg secretion and cytolytic activity against cancer. In a longitudinal study, an increase in the expression of the short variant of SLAMF6 was noted exceeding that of the canonical form. This trend was further increased in some melanoma patients undergoing treatment with PD-1 inhibitor for metastatic disease. Knocking down SLAMF6 improved T cell activation both in humans and in mice. Adoptive transfer using melanoma-specific CD8 T cells in melanoma-bearing mice showed improved cure rates when SLAMF6 was abolished. Lastly, growing T cells in a medium containing SLAMF6-ectodomain yielded cells of enhanced killing capacity and resistance to apoptosis. Adoptively transferred cells supported with IP injections of SLAMF6-ectodomain gave melanoma control in ratios comparable to those achieved with systemic IL-2 doses. The plasticity of SLAMF6 isoforms, and their opposite effects, suggests that alternative splicing may serve as a new level of regulation in the immune system. Exploiting the agonistic forms of the receptor, we managed to develop a platform to maintain tumor specific T cells, in order to improve immunotherapy and other T- cell based cell transfers.

C4

Patterns of immune checkpoint expression by primary tumor cells and tumor infiltrating lymphocytes across different tumor entities

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Immune-checkpoint inhibition (CKI) demonstrated breakthrough therapeutic efficacy in several kinds of cancer. These therapies are unique, as the primary target is not the tumor cell itself, but the crosstalk between immune cells and cancer cells within the tumor microenvironment. Hence, analysis of the respective protein in the tumor microenvironment only partially predicts response to the treatment. For example, recent publications demonstrated that efficacy of anti-PD(L)-1 treatment is not limited to patients with expression of the respective ligand on tumor cells and expression of PD-L1 on tumor-infiltrating lymphocytes (TILs) can be of similar importance. In this study, expression patterns of 30 described immune regulatory molecules were analyzed on T, B and NK cells in peripheral blood and single cell suspensions of 135 primary tumor samples of 10 different tumor entities using flow cytometry. Expression of the respective ligands and 10 key genes associated with antigen processing was assessed by NanoString technology. Finally, expression of selected ligands on primary tumor cells was assessed by immunohistochemistry (IHC) analysis of tissue microarrays. Our analyses revealed distinct patterns of TILs in the tumor microenvironment of different origins. While a fraction of lymphocytic subsets showed a large variety across the 10 entities (e.g. B cells), others were similarly increased across all included types of cancer (e.g. regulatory T cells). The majority of the analyzed immune regulatory molecules were overexpressed on at least one TIL subset (T cells, B cells or NK cells). For some molecules we also detected overexpression in PBMCs of cancer patients compared to PBMCs of healthy controls. In addition to their expression on TILs, we analyzed expression of immunoinhibitory ligands on tumor cells. Interestingly, the number of expressed immunoinhibitory ligands showed a large variety between the different tumor types. For example, the majority of colorectal cancer patients expressed more than 5 ligands simultaneously, while such co-expressions were rare in renal cell carcinoma samples. As an additional mechanism of immune escape, altered expression of genes associated with antigen processing and presentation was observed in many tumor samples. Taken together our data provides a comprehensive picture of immune escape across different tumor types. Our data clearly demonstrates that immune escape is a common feature of cancer, but shows remarkable differences between tumors from different primary locations. The expression patterns described in this study are of immediate translational relevance for ongoing and future immunotherapeutic trials. Keywords: Immune checkpoints, tumor-infiltrating lymphocytes, immunotherapy, human primary tumors

C5

In vivo studies of immunomodulatory antibody CP-870,893 in a humanized mouse model

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Recent findings in cancer immunotherapy have reinforced the hypothesis that the immune system is able to control most cancers. Immunomodulatory antibodies can enhance immune responses, having the potential to generate anti-cancer immunity. Most current studies addressing this question are performed in murine mouse model systems or use in vitro culture systems, which do not reflect the human in vivo situation, potentially leading to results that cannot be fully translated into human cancer therapy. Therefore, it is necessary to establish a new mouse model, which allows the study of cancer immunotherapy in the context of a human immune system. We focused on the establishment of a humanized mouse model, in which different immunomodulatory antibodies can be tested in the presence of a human immune system. First experiments concerning the suitability to test immunomodulatory antibodies in the humanized mouse model, revealed that effects of agonistic immunomodulatory antibody CP-870,893 were similar to the effects seen in patients of clinical studies. To analyse the anti-tumor activities of immunomodulatory antibodies in vivo we are establishing a human melanoma-like tumor model in humanized mice. This enables us to test the efficacy of immunomodulatory agonistic antibodies (such as CP-870,893) and checkpoint control antibodies (such as anti-CTLA-4) in eliminating a melanoma-like tumor. Furthermore, parameters like tumor infiltrating human cells und cytokine/chemokine production can be analysed.

C6

PD-L1 expression in oral leukoplakia: New player in prediction of malignant transformation and new starting point for treatment?

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Objective: In recent years cancer immunotherapy using antibodies targeting programmed cell death-ligand 1 (PD-L1) has become a promising approach to increase survival of patients suffering from OSCC. The main approaches for predicting the efficacy of PD1/PD-L1 blockade immunotherapy is the determination of the level of PD-L1 expression and of mutation load. PD-L1 overexpression in OSCC compared to healthy oral mucosa has already been shown. However, little is known about its expression in oral premalignancies, especially oral leukoplakia (OLP) and its role in malignant transformation. The aim of the study was to investigate the expression level of PD-L1 in order to check whether increased expression already occurs in OLP and whether there is an association to the risk of malignant transformation. Material and Method: In total 98 samples of OLP, consisting of 46 OLP with malignant transformation within 5 years in an OSCC (group 1), 52 samples of OLP without malignant transformation (group 2), 46 samples of OSCC corresponding to group 1 (group 3) and 35 samples of healthy oral mucosa (NOM, group 4) were included. The PD-L1 expression was analyzed by RT-qPCR and immunohistochemistry (IHC). In the IHC analyses, the presence of PD-L1-positive cells in the epithelium (E) and subepithelium (S) was additionally quantitatively evaluated separately. The expression rates between the groups were compared and the fold change and the statistical significance of altered expression were determined. In addition, the association between overexpression and diagnosis and malignant transformation was examined. Result The difference expression rates of PD-L1 in the comparison of group 4 to the group of total OLP (FCPCR=6.1; p=0.0001), the group of transforming (RQPCR=11.4; p=0.0001) and non-transforming OPL (RQPCR=3.7; p=0.0001) and tumor group (RQPCR=8.9; p=0.0001) was significant and associated with clinical diagnosis. These results could be confirmed by IHC ($p \leq 0.02$). There was no significant difference in the distribution of expression rates determined by RT qPCR between groups 1 and 2 (p=0.09). However, the expression levels between these groups were significantly increased (RQPCR =3.1). Therefore, though no significant difference was found, a trend an upregulation of PD-L1 expression in progressing OLP could be observed. Moreover, a significant overexpression of PD-L1 in the epithelial compartment of progressing OLP in comparison to stagnating OLP could be seen (p=0.007; FC=9.67). Conclusion The up-regulation of PD-L1 is associated with malignant disease. The strongly increased expression level of PD-L1 and the significant overexpression in the epithelial compartment of proceeding OLP may indicate that up-regulation of PD-L1 may be associated with malignant transformation and may represent a promising prognostic indicator in OLP. Moreover, overexpression of PD-L1 may represent a promising parameter for predicting the efficacy of PD1/PD-L1 blockade immunotherapy in order to avoid malignant transformation of OLP.

C7

Tumor microenvironment confers enhanced immune privilege of CLL cells by upregulation of PD-L1

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The discovery and delineation of immune checkpoints, such as PD-L1, in the past years has ushered in a new era of immune checkpoint-based therapies (ICBT). However, although first results were very promising, not all patients benefit from the ICBT showing complete non-responsiveness or suffering from relapse due to acquired therapy resistance. Among several discussed mechanisms of acquired therapy resistance is the over-expression of the respective immune checkpoints on the tumor cells out-competing the checkpoint antibodies and/or inhibitors. Yet, underlying mechanisms leading to immune checkpoint over-expression largely remain elusive. We could previously show that the interaction of chronic lymphocytic leukemia B-cells (CLL cells) with the bone marrow stromal niche leads to an up-regulation of MYC in the tumor cells. Recent literature has demonstrated that MYC represents one possible factor to induce the expression of PD-L1. Thus, we asked whether the stromal microenvironment is capable of enhancing the surface expression of PD-L1 on CLL cells leading to enhanced immune privilege. We compared primary CLL cells from patients to healthy donor-derived B-cells regarding their PD-L1 repertoire and immune competence. Furthermore, we analyzed the impact and underlying mechanisms of CLL-stroma interaction on the gene and surface expression of PD-L1 on CLL cells in an established in vitro co-culture approach. We found enhanced levels of PD-L1 expressed on CLL cells compared to their healthy control counterparts. This PD-L1 surface expression was further elevated on CLL cells in a contact-dependent manner via the Notch-MYC axis by mesenchymal stroma cells. We could confirm those findings by ex vivo analyses comparing recent stromal emigrants with long-time circulating CLL cells (based on their CD5/CXCR4 expression profile). Our findings strongly support the hypothesis that the tumor microenvironment enhances expression of immune checkpoints and possibly leads to ICBT resistance as CLL patients hardly benefit from PD-1/PD-L1 blockade. We furthermore elucidated several mechanistic target points to interfere with the CLL-stroma interaction to putatively break the therapy resistance and enhance treatment sensitivity.

C8

Regulation of the Immune Checkpoint Molecule PD-L1 in Cancer Cells

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Immune evasion has evolved as a new hallmark of cancer. It can be mediated by a plethora of mechanisms including the (enhanced) expression of immune checkpoint molecules like programmed death ligand-1 (PD-L1) on the surface of cancer cells. Upon binding its cognate receptor programmed cell death receptor 1 (PD-1), which is primarily expressed on antigen-stimulated T cells as well as on natural killer (NK) cells and monocytes, PD-L1 can exert inhibitory effects on T cells, e.g. inhibition of proliferation, differentiation, and cytokine production. Thereby, PD-L1 enables tumor cells to avoid destruction via T cell mediated immune responses and fosters tumor development and growth. This mechanism has been exploited in tumor therapies using anti-PD-L1 antibodies, which significantly increased overall survival times in some entities. Despite the unequivocal success, the majority of cancer patients (approximately 70 %) still do not respond to immune checkpoint inhibitors. Therefore it is crucial to elucidate the precise mechanisms, through which PD-L1 is upregulated on cancer cells, in order to interfere with its regulation and thereby improve the efficacy of cancer immunotherapy. In this project we sought out to investigate the potential metabolic regulation of PD-L1 in four cancer cell lines originating from different tissues. We could demonstrate that mimicking the inflammatory microenvironment often found in tumors via the pro-inflammatory cytokines IFNγ and TNFα leads to elevated levels of PD-L1 and that the activity of the JAK/STAT signalling pathway significantly contributes to this effect. Cancer cells are known to switch their metabolism towards a more glycolytic phenotype, thus we hypothesized that glucose metabolism could impact this upregulation. However, experiments using glycolysis inhibitors did not support this hypothesis. Nevertheless, the glucose analogue 2-deoxy-(D)-glucose (2DG) decreased PD-L1 levels significantly. In subsequent experiments we could delineate that this is due to a lack of N-glycosylation (and not glucose depletion) caused by 2DG. Next, we investigated several secondary malfunctions evoked by defective N-glycosylation, which revealed that neither increased ER stress nor increased autophagy significantly influences PD-L1 levels. In contrast to that, our data indicates a link between 2DG treatment and increased proteasomal degradation of PD-L1. Although many questions are still unanswered, our results emphasize the role of N-glycosylation in the regulation of PD-L1 and offer well-grounded preliminary data to further elucidate the complex regulatory network of this checkpoint molecule.

C9

Tumor immune microenvironment drives prognostic relevance correlating with bladder cancer subtypes

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Background: Muscle-invasive bladder cancer (MIBC) represents approximately two thirds of invasive urothelial bladder cancers (UBC) and has high morbidity and mortality. Despite intensive efforts to improve patient treatment and outcome, two thirds of patients with UBC will have a recurrence or disease progression within 5 years. We conducted this study to gain further insights in the immunological tumor microenvironment (TIME) Material and Methods: sTILs were scored continuously on HE slides in a cohort of 135 patients with MIBC treated by radical cystectomy (adjuvant chemotherapy n= 34) according to current recommendations (Salgado et al, 2015). In parallel, we assessed intrinsic subtypes by 21-gene Nanostring signature adapted from the MDACC-subtyping approach. Tertiary lymph structures were assessed by whole slide immunohistochemistry of CD3, CD8, CD68, and CD79a. Spatial immune profiling was carried out on regionally (tumor center, invasive margin) designed TMAs by CD3, CD8, CD56 (NK-Cells), CD68, PD-1 and PD-L1 and revealed spatial organized immune phenotypes. Results were validated in 407 MIBC of the TCGA cohort by hierarchical clustering analysis, immune cell population analysis via CIBERSORT and sTIL-scoring on digitalized HE-slides. Furthermore, tumor mutational burden, neoantigen load and mutational patterns as well as mutational signatures were correlated with immune phenotypes in the TCGA cohort. Results: We demonstrate that quantity and spatial distribution of stromal tumor infiltrating lymphocytes (sTILs) within the tumor immune microenvironment (TIME) predict stages of tumor inflammation, subtypes, patient survival and correlate with expression of immune checkpoints in an analysis of 542 MIBC. High sTILs indicate an inflamed subtype with 80% 5-year disease-specific survival. A lack of immune infiltrates identifies an uninflamed subtype with a survival rate of less than 25%. A separate immune evading phenotype with upregulated immune checkpoints associated with poor survival. Within the TIME are tertiary lymph node structures (TLS), which can mediate anti-tumor activity via active immune cells. High TLS amounts and close tumor distance correlated significantly with an inflamed phenotype and favorable survival. The uninflamed and evasion phenotypes showed lowest TLS numbers and farthest tumor distances and shortest survival. High inflammation also correlated with increased neoantigen load, high TMB and specific mutational patterns (TCGA-MSig1, TCGA-MSig3/4). Patients treated with adjuvant chemotherapy showed a favorable prognosis dependent on high sTILs. Conclusion: Determination of sTILs and tumor subtypes may stratify therapy success and patient survival. Considering sTILs can easily be quantified using simple morphological parameters, like hematoxylin-eosin, sTILs can be implemented for predicting patient survival and outcome after adjuvant platinum containing chemotherapy in a routine manner.

C10

Allergen and Rhinovirus (RV) regulation of PDL1 and IFNβin allergic asthma

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Rhinovirus (RV), a member of the picornaviridae family, is often detected in the airways of asthmatic children during symptomatic visits to the hospital because of an ongoing infection in the upper airways. The immune responses to rhinovirus comprise the upregulation of interferon gene pathways, especially type I and type III interferons were found associated with rhinovirus infection of the upper airways. Dysregulated interferon responses have been observed in non-controlled asthmatic patients during rhinovirus-induced exacerbations. We recently described that during acute rhinovirus infection, IFN type I was induced in serum of the cohort of pre-school children with asthma. Furthermore, in vitro, the RV infection upregulated Programmed cell death protein 1 ligand (PD-L1, CD274), a gene induced by IFN and implicated in the T cell exhaustion and Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4, CD152), a receptor present on lymphocytes which plays a critical role in the down-regulation of antigen-activated immune responses. Moreover it has been recently demonstrated that RV infects B cells. Thus in this study, we wanted to further investigate the influence of rhinovirus in immune responses in asthma. In line with the European study PreDicta in collaboration with the Children's Hospital in Erlangen, we isolated peripheral blood mononuclear cells (PBMCs) from the blood of pre-school children (age 4-6 years) with and without asthma and established a rhinovirus associated disease development. For the murine studies, we used a well-established OVA model of allergic asthma and infected lung cells with RV in vitro. In children, PD-L1 mRNA expression was found to be increased in total blood cells isolated from asthmatic children, and it further inversely correlates with their predicted FEV1%. By contrast IFN-β was found to directly correlate with their FEV1% indicating that worse asthma is associated with induced PD-L1 mRNA expression and reduced IFN-β levels in blood cells of children with asthma and RV in the airways. Similarly, in asthmatic mice, CD3+ PD-L1+ T cells were increased after in vitro exposure to RV. Further, we found that IFN-β is correlated with B cells expressing CTLA-4 in this mouse model. In summary, our results suggest that RV induces immunosuppression by activating PD-L1 in CD3+ T cells and CTLA4 in B cells in asthma associated with RV exacerbation. These studies will open new vaccination immune strategies for the therapy of RV induced asthma.

C11

Emerging Immuno-Oncology Targets for Glioma and Multiple Sclerosis

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Background & Question: Immune checkpoint molecules (ICMs) refer to the inhibitory and stimulatory receptor-ligand interactions that regulate self-tolerance and the amplitude of immune response. Dysregulation of ICM signaling is one of the immune evasion mechanisms exerted by many tumors such as glioblastoma. Although the tumor tissue is infiltrated with tumor specific T cells, glioma cells are able to suppress immune system for example by upregulating PD-L1 expression. There are recent ongoing immunotherapy clinical trials for gliomas, but when targeting the CNS tumors the main challenge is to balance immune response to prevent autoimmune reactions. The severest autoimmune disease of the central nervous system is Multiple Sclerosis (MS) and characterized by immune cell infiltrates into brain, demyelination and axonal degeneration. Like there are tumor reactive t cells in glioma, Myelin-specific cytotoxic CD8+ T cells were found in the brains of MS patients, which were indicated to play an important role in the immunopathology of MS. However, the cross-talk between autoreactive CD8+ T cells and oligodendrocytes needs to be investigated. We hypothesize that dysregulated ICMs signaling in oligodendrocytes can contribute to glioma immune evasion and may modulate autoreactive CD8+ T cell activity in MS patients. Our aim is to identify novel therapeutic targets on oligodendrocytes that could potentially play a role in the immunopathology of glioma and MS. Methods & Results: In order to model antigen-specific CD8+ T cell mediated oligodendrocyte killing, we developed an in-vitro luciferase based cytotoxicity assay where we co-culture human oligodendrocyte cell line MO3.13-A2-Luc with Flu antigen specific CD8+ T cells (FluT) isolated from PBMCs of HLA-A*02 healthy donors. Before co-culture, we pulse oligodendrocytes with Flu peptide to ensure FluT recognition. In order to identify ICMs involved in this mechanism we performed a high-throughput screen by co-culturing FluT with MO3.13-A2-Luc cells transfected with a siRNA library consisting 4160 genes. The impact of gene knockdown in target cells on T-cell cytotoxicity was measured using a luciferase readout system. Primary screen revealed 150 HITs that may modulate T cell mediated killing better than the positive control CCR9. Out of top 150 HITs we selected 56 surface HITs and performed a secondary screen both using oligodendrocyte cell line and primary glioblastoma cells. For these HITs we performed GO term analysis and the most prominent pathway is found to be cAMP pathway. Our previous data with melanoma cells and TILs showed that cAMP produced by tumor cells impairs Lck activity and shut downs TCR-associated signaling. We validated 7 overlapping HITs between 2 different cell types that increase T cell mediated killing upon knockdown in target cells. One of our HITs ANM1 (masked name) is a single-pass membrane protein and it is a ligand for a receptor tyrosine kinase, which is also identified as a HIT in the screen. It is important for migration, repulsion and adhesion during neuronal, vascular and epithelial development. ANM1 is expressed in human/mouse oligodendrocytes and significantly up-regulated in different GBM types according to TCGA dataset. Our 17-plex multiplex cytokine assay, ELISA and cytokine catch assays revealed that IFNγ secretion by T cells strongly decreased when they are co-cultured with ANM1 downregulated oligodendrocytes. Right now we are working further on the impact of ANM1 and its receptor on T cell functionality. Conclusions: We established an in-vitro co-culture model for MS and glioma to understand antigen-specific CD8+ T cell mediated glial cell killing and to identify novel ICM molecules that play a role in this mechanism. ANM1 is one of the candidate ICMs that can be used as a target in the immunotherapy of GBM and MS.

C12

Immunomodulatory Effect of Vitamin D during Allogeneic Hematopoietic Stem Cell Transplantation

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Vitamin D deficiency has a high prevalence among hematopoietic transplant recipients which is an unpleasant side effect and might contribute to pathogenesis of Graft-versus-Host Disease (GvHD). Previous studies have shown a significant correlation between low vitamin D serum levels and an increased incidence of chronic GvHD. Vitamin D does not only affect the adaptive but also the innate immune system, since it acts in an immunosuppressive manner by fostering microbial defenses and the tumoricidal activity of macrophages. As mediators in antigen presentation, macrophages represent a pivotal factor in driving pathogenesis of GvHD. Conversely, tissue resident host macrophages were indicated to have an immunosuppressive function in mice. Given the close link of GvHD, immune cell regulation and vitamin D3, we searched for immune checkpoints regulated by vitamin D3 which might alleviate the destructive potential of alloreactive T cells. Since vitamin D is known to modulate the phagocytic activity of macrophages, the inhibitory CD47-SIRPα axis turned out to pose as a promising candidate. We observed that activation of human and mouse T cells led to overexpression of CD47 on their surface, while pretreatment with Vitamin D3 hampered this upregulation. The alteration of such 'don't eat me'- and 'eat me'-signals could be of tremendous importance for antibody dependent cell mediated cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) by macrophages in GvHD patients. T cell depleting antibodies, such as anti-thymocyte globulin (ATG), are currently applied in treatment of allograft rejection. In our phagocytosis assay, we used ATG to study the difference between inflammatory (GM-CSF-generated) and anti-inflammatory (M-CSF-generated) macrophages. We determined that anti-inflammatory macrophages display higher phagocytosis activity of allogeneic T cells than inflammatory ones. We also suggest that phagocytosis of T cells triggers a shift from a pro-inflammatory activity towards a tolerogenic state of macrophages. In summary, our data indicate that vitamin D could ameliorate GvHD by modulating checkpoint molecules which might trigger the phagocytosis of allogeneic T cells by macrophages as well.

C13

Testing human Fc-FcγR contributions to agonistic antibodies in a humanized mouse model system

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The finding that immunomodulatory monoclonal antibodies can enhance human immune responses has revolutionized the field of cancer research in recent years. Agonistic antibodies such as a-CD137 are one type of immunomodulatory antibodies which target co-stimulatory molecules on different immune cell subpopulations, in order to induce optimal anti-tumor responses. One of the big surprises concerning this topic was the observation that agonistic antibodies need to interact with FcγR in order to elicit their immunomodulatory activity. However, most of the existing knowledge in this research field comes from studies carried out in murine mouse model systems, where the presence of mouse innate immune effector cells with the capacity to recognize human antibodies via murine Fc-receptors can potentially lead to results that cannot be fully translated into human cancer therapy. Therefore, we have established a humanized mouse model which allows the study of cancer immunotherapies in the context of a human immune system. The particular feature of this model is that all immune cells lack functional mouse activating Fcγ-receptors, enabling us to solely study the role of human Fc-Fcγ-interactions for immunomodulatory antibodies. We focused on understanding how the immunomodulatory agonistic antibody a-CD137 works in the context of a human immune system and how its in vivo activity can be modulated by its Fc-part. We show that the injection of a-CD137 into humanized mice leads to a massive proliferation of mainly CD8+, CD4+CD8+ and CD4-CD8- T cells in the blood and organs of humanized mice. Moreover, treatment with the deglycosylated a-CD137 antibody showed a delayed T cell proliferation, suggesting a dependence on FcγR. Furthermore we could observe accumulation of liver T cells upon antibody injection. These findings show the potential of our humanized mouse model to conduct preclinical studies with therapeutic antibodies and specially focus on the role of Fc-FcγR interactions and the side effects of the antibodies of interest.

Poster Session D

Modulation of the Tumor-Immune-Microenvironment

D1

Accelerated glucose metabolism is associated with less T cell infiltration in human OSCC

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Introduction: The oral squamous cell carcinoma (OSCC) belongs to the group of head and neck cancers and is the sixth most prevalent malignant disease worldwide. It commonly but not exclusively affects older male individuals and is associated with extensive abuse of tobacco and/or alcohol, HPV or asbestos exposure. Our aim was to characterize the metabolic phenotype as well as the immune cell status of OSCC to achieve possible new treatment strategies for OSCC. Material and Methods: For this purpose we analyzed tumor tissue, mucosa and blood of 27 patients with OSCC. Flow cytometry was used to analyze tumor infiltrating immune cells (T and myeloid cells). Additionally, mRNA analyzes of lactate dehydrogenase A (LDHA), monocarboxylate transporter-1 (MCT-1) and glucose transporter-1 (GLUT-1) were performed via qPCR. Metabolic parameters, such as lactate, glucose, Lp(a), triglyzerides were analyzed in serum of patients and healthy individuals. Moreover, CD3, CD4, Foxp3, CD127 and GLUT-1 were analyzed in a tissue microarray of 249 OSCC patients. Results: We could clearly show that OSCC demonstrates a Warburg phenotype which is in line with the findings of other groups. Tumors exhibited a strong upregulation of glycolytic markers, like LDHA, GLUT-1 and MCT-1. While lymphocytes were decreased in peripheral blood of tumor patients, higher numbers of peripheral myeloid cells could be detected. Comparing tumor tissue with corresponding healthy mucosa, we could also observe a significant decrease of intra-tumoral lymphocytes while myeloid cells were increased. Tumor-infiltrating CD4+ T cells were mainly CD25+Foxp3+ and CD4+ levels in tumor lesions were associated with increased peripheral glucose levels. Conclusion: Enhanced glycolysis in OSCC is associated with less infiltration of anti-tumoral lymphocytes as well as an accumulation of tumor-promoting regulatory CD4+ T cells. Targeting the Warburg phenotype of tumor cells may therefore improve the efficacy of checkpoint therapy in human OSCC.

D2

Development of a murine, myc-driven lymphoma model expressing human CD22 enables testing of targeted therapies and their effects on tumor immune microenvironment

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Background: The tumor microenvironment (TME) is composed of several cell types like stromal cells, blood vessels and immune cells. All these cell types are in close interaction via cell-to-cell contacts and cytokines leading to immune cell inhibition, tumor promotion, prevention of tumor rejection, and protection against chemotherapy (Kumar and Xu, 2018, Front Oncol). In B-cell malignancies, myeloid-derived cells play a central role in supporting tumor growth and inhibiting immune cell function (Roussel et al., 2017, Cancer Immunol Immunother). Thus, the TME is increasingly recognized for its role in cancer immunotherapies including CAR-T cells, BiTEs, or immunotoxins. Despite the importance of a syngeneic TME, preclinical studies with these drugs have mainly been performed in immune compromised animals representing a major drawback of current animal models. Objective: We aimed to establish a syngeneic, murine lymphoma model expressing the human CD22 under physiologic promoter control to test CD22-targeted therapies and their effects on TME in an immune competent background. Methods & Results: To maintain the physiologic function of human CD22 in a murine background, a chimeric protein was designed (h/mCD22) which consists of an extracellular human and an intracellular murine domain. Transduction of murine B-cell lymphoma cells with h/mCD22 renders cells sensitive to the CD22-targeted immunotoxin Moxetumomab pasudotox (Moxe) in vitro confirming h/mCD22 as a functional target for in vivo therapy. Crossbreeding of h/mCD22 transgenic BL6 mice (BL6h/mCD22) and BL6 mice carrying a λ-myc translocation results in a strain that spontaneously develops h/mCD22 positive B-cell lymphoma. Three primary lymphoma subclones were isolated from distinct mice and serially transplanted into BL6h/mCD22. Stable engraftment and tumor cell growth of all three subclones was established after subcutaneous (sc) and intravenous (iv) injection. However, immunological characterization revealed that sc tumors were infiltrated by less than 1% immune cells, while myc-driven lymphoma in humans usually show substantial immune infiltration. In contrast to sc tumors, systemically growing lymphoma in the bone marrow (BM) are infiltrated by 30 % myeloid cells and 1 % T-cells and in the spleen by 10 % and 30 % respectively. Hence, Moxe activity in vivo was tested after systemic tumor injection. In accordance with studies in immune compromised models, we observed that Moxe is more active, the longer blood levels were maintained (Müller, 2016, CCR). After Moxe was given intraperitoneally 4 times a day for 3 days, a 3-fold to 100-fold reduction in tumor infiltration was achieved in all three lymphoma models. In the next steps, changes in tumor immune microenvironment were analyzed after treatment with Doxorubicin, a chemotherapeutic agent which is known to induce immunogenic cell death associated with anti-tumor immune responses (Galluzzi et al., 2012, Nat Rev Drug Discov.). In our model, we found a 1.5-fold increase of CD11b+ myeloid cells in spleens of Doxorubicin-treated mice compared to untreated tumor-bearing and healthy mice. Among these cells, Ly6G+ granulocytic cells increased most substantially. Conclusion: By cross-breeding h/mCD22-transgenic and λ-myc expressing mouse strains, we generated primary aggressive B-cell lymphoma expressing h/mCD22. These lymphoma cells stably engraft sc and systemically in syngeneic mice and are sensitive to CD22-targeted immunotoxin Moxe. Moreover, myeloid cell infiltration in systemically growing tumors resembles the phenotype of myc-positive lymphoma in patients and can be altered by Doxorubicin treatment. Therefore, our unique model provides a valuable platform to test the impact of novel treatment strategies targeting the human CD22 on TME in an immune competent background.

D3

Ex vivo hyperthermia of B16 melanoma and MCF-7 / MDA-MB-231 breast cancer cells by microwave irradiation compared to warm-water in a closed-loop system

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The treatment of some solid tumor entities by radiotherapy with additional hyperthermia is a promising approach. However, little is known about immunobiological effects of different heat sources such as warm-water or microwave irradiation, as well as the importance of various treatment parameters (e.g., temperature and time) and combined radiotherapy. In our self-designed closed-loop system, heat treatment of murine B16 melanoma and human MCF 7/MDA MB-231 breast cancer cells was performed using either a warm-water bath or a microwave (2.45 GHz) as heat source. In addition to treatment temperatures between 39 °C and 48 °C, the volume flow (2 or 5 mL/s) and the effective time of treatment was varied. Flow cytometry was used to determine the cell death forms by AnnexinV/PI staining and the expression of immune checkpoint molecules (IC) by antibody staining before and after (day 3 and 5) the respective treatments. In addition, danger signals such as heat shock protein 70 (HSP70) were quantified by ELISA. Using the commercial software COMSOL Multiphysics © 5.3b, theoretical temperature profiles of the two heat sources were numerically calculated based on (1) and compared. Circulation of tumor cells in the closed-loop system at 37 °C without heat treatment reduced the number of living cells only slightly (1 % to 4 %). At higher temperatures, the number of living B16 cells decreased under warm-water heating from a characteristic threshold temperature with increasing treatment time and reached a value of 73 % of living cells at 2 mL/s after 60 minutes and 44 °C. Under microwave heating, only 21 % of the B16 cells were alive at identical treatment parameters. The two breast cancer cell lines MCF-7 and MDA-MB-231 were more thermoresistant and showed a shift in the inactivation threshold of +2 °C to +3 °C. The quantitative determination of the danger signal HSP70 in the supernatant showed that in all tumor cell lines from 39 °C on, a significantly increased release takes place, whereby under microwave irradiation higher levels of HSP70 occurred in comparison to warm water heating. Under microwave irradiation, tumor cell inactivation thus occurs at lower temperatures and at higher rates in comparison to warm-water treatment. Using our self-designed closed-loop hyperthermia system (warm-water or microwave treatment) and the data on immunological parameters, clinically relevant inactivation conditions in the clinic can be better estimated in the future. Hyperthermia and radiation (2x5Gy or 5x2Gy) experiments are currently running. In addition, detailed analyzes of the expression of immune checkpoint molecules will be performed to optimize future radioimmunotherapy with hyperthermia. ACKNOWLEDGEMENT: This work is supported by the Bayerische Forschungsstiftung (Microthermia, AZ 1261-17).

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D4

Extracellular depletion of hydrogen peroxide leads to induction of regulatory dendritic cells capable of suppressing T cell proliferation

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Dendritic cells (DCs) are key players in the initiation and maintenance of immune responses against pathogens and malignant cells but their function is often impaired in the tumor microenvironment, also known as tumor immune-escape. Reactive oxygen-species (ROS) play a pivotal role in tumor immune escape as they have been demonstrated to impair immunological tumor-rejection resulting in uncontrolled tumor progression. However, tumors themselves express increased levels of antioxidant enzymes to deplete ROS and escape H2O2-mediated apoptosis and cell damage. In this study we demonstrate a link between ROS and DC maturation, with H2O2 as a critical molecule for function and differentiation of DCs. Incubation of human monocytes with the H2O2-depleting enzyme catalase during DC-differentiation and maturation (CAT-DCs) resulted in significantly impaired expression of common maturation markers and costimulatory molecules CD80/CD86. CAT-DCs proved to be functional in suppressing T cell proliferation in a contact-dependent, IDO-mediated manner. Moreover, CAT-DCs were able to skew T cell polarization to an immunosuppressive phenotype with increased IL-10 and IL-17 expression, which has been reported in AML patients previously. They exhibited an altered cytokine profile with increased expression of pro-inflammatory cytokines IL-6, IL-8, CCL2 and CCL5. Increased expression of CD163 and PAI-1 indicated a shift to a tumor-associated M2 macrophage phenotype. Interestingly, extracellular depletion of H2O2 resulted in a significant 61% increase of intracellular H2O2 production, increased glucose uptake and increased lactate production, highlighting the extensive consequences of extracellular H2O2-depletion on intracellular processes. Furthermore, CAT-DCs exhibited a significantly increased LDL and oxLDL uptake and inhibition of LDL uptake led to a 33 % reduced immunosuppressive phenotype, revealing a link between altered lipid metabolism and immunosuppressive properties of CAT-DCs. Targeting the tumor microenvironment presents a new strategy for enhancing the efficacy of existing anti-tumor therapies and developing new immunotherapeutic treatment options. The link between tumor metabolism, ROS and immunosuppressive dendritic cells uncovered by our research shows promising options for the development of future anti-cancer therapies.

D5

Multiple myeloma (MM) is considered a chronic and incurable disease due to its highly complex and heterogeneous molecular abnormalities. In recent years, integrating proteasome inhibitors and immunomodulatory drugs into MM frontline therapy has signi

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Multiple myeloma (MM) is considered a chronic and incurable disease due to its highly complex and heterogeneous molecular abnormalities. In recent years, integrating proteasome inhibitors and immunomodulatory drugs into MM frontline therapy has significantly improved treatment efficacy with the median survival being prolonged from 3-4 to 7 years. Despite this progress, patients' refractory to the aforementioned agent classes display a median overall survival of only 9 months. Thus, the clinical necessity for developing novel therapeutic alternative approaches is self-evident. Methylation of N6 adenosine (m6A) is known to be important for diverse biological processes including gene expression control, translation of protein, and messenger RNA (mRNA) splicing. m6A regulatory enzymes consist of 'writers' METTL3 and METTL14, 'readers' YTHDF1 and YTHDF2, and 'erasers' FTO and ALKBH5. However, the functions of m6A mRNA modification and the specific role of these enzymes in MM remain unknown. Here, we report that METTL3, a key component of the m6A methyltransferase complex, is highly expressed in MM cell lines and in isolated patient's MM cells. In contrast, we found no significant differences in the expression of the m6A demethylases FTO and ALKBH5. Accordingly, compared to plasma cells from healthy donors, global PolyA+ RNA showed a significant increase in m6A content in patient's MM plasma cells. In MM cell lines, global m6A profiling by methylated RNA-immunoprecipitation sequencing revealed m6A peaks near the stop codon in mRNAs of multiple oncogenes including MAF and CCND1. Cross-linking immunoprecipitation showed that METTL3 bound to the m6A peak within MAF and CCND1 mRNA. Depletion of METTL3 by shRNA had little effect on global mRNA levels, but specifically reduced protein levels of c-Maf and Cyclin D1. Moreover, downregulation of METTL3 in several MM cell lines results in cell cycle arrest and apoptosis. Together, these results describe a role for METTL3 in promoting translation of a subset of oncogenes in MM and identify this enzyme as a potential therapeutic target for multiple myeloma.

D6

PD-L1-specific immunocytokines augment functionality and antitumor activity of CAR-engineered NK cells

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Natural killer cells play an important role in cancer immunosurveillance, with their cytotoxicity triggered rapidly upon stimulation through germline-encoded cell surface receptors. In addition, NK cells modulate adaptive antitumor immunity by maintaining the quality of dendritic cells and improving presentation of tumor antigens. Genetic engineering of NK cells with chimeric antigen receptors (CARs) can enhance specific recognition and selective elimination of tumor cells. Nevertheless, expression of programmed death receptor-ligand 1 (PD-L1) by tumor cells may dampen the CAR NK cells' direct and indirect antitumor activity. To overcome immunosuppressive effects, we aim to develop advanced CAR NK cells, which secrete PD-L1-specific antibody-cytokine fusions that carry IL-12 or IL-15. These immunocytokines can be retained in the tumor microenvironment by binding to PD-L1 on the tumor cell surface, and may simultaneously block the PD-1/PD-L1 immune checkpoint and activate innate and adaptive bystander immune cells. For initial testing of their functionality, antibody-cytokine fusion proteins were expressed in HEK293 cells and purified from culture supernatants. Binding of the recombinant proteins to PD-L1 and activity of their IL-12 and IL-15 domains were verified by flow cytometry and in bioactivity assays. The immunocytokines enhanced T-cell activation in mixed lymphocyte reactions and increased cytotoxicity of NK cells against tumor cells. Subsequently, PD-L1-specific antibody-cytokine fusions were introduced into ErbB2 (HER2)-specific CAR NK-92 cells by lentiviral transduction. The ectopically expressed immunocytokines activated the respective cytokine signaling pathways in the producer cells, in the case of anti-PD-L1-IL-15 resulting in cell growth and activity becoming independent from exogenous IL-2. Furthermore, in transwell assays stimulatory effects on different types of co-cultured immune cells were observed. Our results show that PD-L1-specific IL-12 and IL-15 immunocytokines are functional, and can be expressed in CAR NK cells thereby modulating the cells' growth and direct antitumor activity as well as their stimulatory activity towards bystander immune cells.

D7

PKC-βdependent changes in the metabolism of the bone marrow niche after CLL contact

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Bone marrow stromal cells (BMSCs) play an important role for microenvironment mediated survival of CLL cells. Importantly, interactions between CLL cells and BMSCs not only protect leukemic B cells from spontaneous, but also from drug-induced apoptosis. Therefore, understanding the molecular mechanisms of CLL-stroma interactions may offer new therapeutic options and help in eradicating CLL cells from the bone marrow niche. To study the communication between CLL cells and there microenvironment, we established a coculture system of primary CLL cells and the stromal cell line EL08-1D2. This cell line is derived from murine embryonic livers (E11) and supports long-term cultures of hematopoietic stem cells and also CLL survival ex vivo. We already showed that primary CLL cells induce the expression of the Proteinkinase C-β (PKC-β) in stromal cells. This PKC-β induction is essential for CLL survival also in vivo. The fact that the CLL-dependent induction of PKC-β in the microenvironment was neither species restricted nor limited to a subtype of BMSCs, suggests that the upregulation of PKC-β may be related to metabolic alterations. Additionally, it has been reported that members of the PKC family play key regulatory roles in glucose transport. In order to evaluate the impact of stromal PKC-β on metabolic alterations upon CLL contact, we want to analyze the BMSCs and the CLL cells before and after coculture conditions. Coculturing CLL cells on PKC-β-/- BMSCs clearly enhance the lactate production. In line with this observation, we detect an increased glucose uptake in these CLL cells. Interestingly, we can measure an altered expression of glucose transporters after CLL contact and upon glucose addition. Remarkably, the enhanced CLL cell death on PKC-β-/- BMSCs can be rescued using high-glucose conditions. We can confirm that PKC-β negatively modulates the ATP level in BMSCs after CLL contact. Notably, a subgroup of CLL cells expressing low ATP levels in monoculture show increased ATP production under PKC-β-/- BMSCs coculture conditions, which could be a sign of chemoresistance. Additionally, we can detect PKC-β dependent changes in ROS species regulation and expression levels of key glycolytic enzymes in BMSCs upon CLL contact. Conclusively, we describe PKC-β dependent metabolic changes during communication between CLL cells and the BMSCs. Interference with the PKC-β pathway activated in the leukemia microenvironment in combination with glucose metabolism treatment may offer new therapeutic options to fully eradicate malignant B cells from bone marrow niches.

D8

Reduction of CLL-induced immunosuppression by DUSP1/6 inhibition via BCI

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Chronic lymphocytic leukemia (CLL) is characterized by a clonal expansion of neoplastic B cells that carry auto- and polyreactive B cell receptors (BCRs). CLL is the leukemia with the highest incidence among adults in the Western world. Immunodeficiency can be observed at early stages of the disease and influences the clinical course. It is at least partially mediated by cell populations with immunosuppressive capacity, namely regulatory T cells (Tregs) as well as myeloid-derived suppressor cells (MDSCs). Tregs are important regulators for the immunological homeostasis and the high frequencies in cancers promote tumor immune escape and therefore tumor progression. Monocytic MDSCs are able to suppress T cell activation, T cell proliferation and are involved in Treg induction. The interaction of suppressive immune cells with the malignant tumor cells leads to defective immune response in CLL patients. Targeting BCR signaling pathway for treating B cell malignancies is used by different therapeutic approaches. Despite the striking success of some strategies, so far, none of them are curative and relapses are challenging to treat. Here, we test a novel strategy for simultaneously targeting the malignant cells and reverting the CLL-suppressed immune response by inducing a hyperactivation of BCR signaling pathway via negative feedback inhibition. The phosphatases DUSP1 and DUSP6 dephosphorylate ERK1/2 and therefore downregulate the activation of ERK1/2. Using a small molecule inhibitor for DUSP1 and DUSP6, BCI, we observed that hyperactivation of the ERK signaling cascade is followed by induction of cell death in CLL. We observed a reduced viability in primary CLL cells, in the CLL-like cell lines MEC-1 and EHEB and in murine CLL cells derived from the Tcl1-mouse model. Interestingly, BCI treatment-mediated cell death was significantly less pronounced in other malignant B cell lymphoma cells or healthy donor-derived B cells. Beside this direct cytotoxic effect on CLL cells, our data indicate that BCI affects several aspects involved in CLL-induced immunosuppression. First, BCI treatment of CLL patient-derived PBMCs resulted in selective enrichment of cytotoxic T cells and may therefore directly influence suppressed T cell signaling. Furthermore, we observe specific features of immunogenic cell death in CLL cells upon treatment with BCI: In vitro cultivation of CLL cells with ovalbumine followed by BCI treatment increased the proliferation of OT-I CD8 T cells in the presence of bone marrow-derived dendritic cells. In addition to the enhanced antigen-specific T cell proliferation, we observed a significant increase in the release of HMGB1 upon BCI treatment, which acts as a danger associated molecular pattern. Finally, further in vitro experiments showed a reduction of CLL-induced immunosuppressive cells upon BCI treatment. Co-culturing CLL cells for 5 days with healthy donor-derived PBMCs confirmed an induction of suppressive cell populations in the PBMCs. Treatment of primary CLL cells and CLL cell lines with sublethal doses of BCI, followed by a washing step reduced both, frequencies of MDSCs as well as Tregs as compared to the untreated CLL cell control. In conclusion, our data suggest that BCI treatment targets CLL cells by induction of immunogenic cell death and modulates the tumor immune microenvironment to revert the CLL-induced dysfunction of the immune system. We propose that inhibition of DUSP1/6 is a promising approach for CLL treatment.

D9

Stroma Cells Promote a S100A8/A9high-Subset of AML Blasts with Distinct Metabolic Features in a Jak/STAT3-Dependent Manner

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Introduction: It is well established that the bone marrow stromal niche can serve as a protective environment in hematological malignancies such as AML by multiple cell contact-dependent and independent mechanisms. Intensive research of the bidirectional interactions between leukemic cells and mesenchymal stromal cells already highlighted numerous modes-of-action how malignant cells are capable of hijacking or altering their surroundings to their own favor. However, the entirety of underlying mechanisms is still incompletely understood. We found two small intracellular calcium-sensing molecules, S100A8 and S100A9, among the top upregulated genes in primary AML cells upon stromal contact. S100A8/A9 are members of the S100 protein family that, by functioning both as intracellular Ca2+ sensors and as extracellular mediators, can modulate cellular responses such as proliferation, migration, inflammation, and differentiation. Dysregulation of S100 protein expression is described as a common feature in several human cancers. Specifically in AML expression of S100A8 in leukemic cells predicts poor survival in de novo AML patients. Thus, we aimed to elucidate the underlying mechanisms of stroma-mediated S100A8/A9 upregulation as well as the consequences, and characterized S100A8/A9high AML cells in comparison to their S100A8/A9low counterparts in terms of gene expression pattern, differentiation, metabolic profiles, and chemoresistance. Methods: We co-cultured both AML cell lines and primary AML blasts in a contact-dependent and -independent manner with human bone marrow stromal cells. After co-culture AML cells were re-purified and analyzed by RNA sequencing, flow cytometry and quantitative real-time PCR. Results: We found S100A8 and S100A9 among the top upregulated genes in an unbiased transcriptome analysis of primary AML cells cultured in the presence of HS-5 cells compared to the controls. Upregulation of S100A8/A9 could be confirmed in AML cell lines and was shown to be reversible. We could demonstrate that S100A8/A9 upregulation is mediated by soluble factors as cell-to-cell contact was not necessary and exosome-free conditioned medium from HS-5 cells did not induce S100A8/A9 gene expression. We found the Jak/STAT3 signaling being one major responsible pathway. The S100A8/A9high population was characterized by increased surface levels of maturation markers (such as CD14 and CD11b) as well as altered metabolically important transporters (e.g. for glucose, fatty and amino acids). Finally, we could demonstrate an increased chemoresistance of the S100A8/A9high cells. Conclusion: We could delineate bone marrow stroma-induced S100A8/A9 upregulation in AML cells is mediated by soluble factors activating the Jak/STAT3 pathway. S100A8/A9 leads to metabolic alterations and increased differentiation of AML cells conferring enhanced chemoresistance and thus represents a potential therapeutic target against AML.

D10

Whole tumor cell-based vaccines generated with high hydrostatic pressure act synergistically with radiotherapy by generating a favorable immune microenvironment

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Radiotherapy (RT) is known to be capable of turning tumors into in situ cancer vaccines through the induction of immunogenic cell death and a favorable tumor microenvironment. However, long-lasting anti-tumor immune responses often do not result from RT alone. Whole tumor cell vaccines generated with high hydrostatic pressure (HHP) bear the potential to further boost RT-induced immune responses. So far, it is not known how RT and HHP-vaccines synergize to generate a tumor microenvironment fostering tumor growth retardation. Tumor cells were treated with HHP between 100MPa and 500MPa and subsequently cell cycle and the induction of cell death was analyzed. Furthermore, selected HHP-killed tumor cells were used in phagocytosis assays with peritoneal macrophages and bone marrow-derived dendritic cells (DCs). For in vivo experiments C57Bl/6 and Balb/c mice were injected s.c. with syngeneic B16 (melanoma) and CT26 (colon carcinoma) tumor cells, respectively. Established tumors were locally irradiated with 2x5Gy and mice were vaccinated once with HHP-vaccines generated with 200MPa. Tumor growth and survival of the mice was monitored. Tumor-infiltrating leukocytes were analyzed via multi-color flow cytometry. The treatment of tumor cells with a pressure of 200MPa and higher predominantly resulted in necrotic cell death and degraded DNA. Furthermore, those cells were completely inactivated and lost the potential to form colonies in vitro or tumors in vivo. Tumor cells treated with HHP were better phagocytosed by macrophages. Although the phagocytosis of HHP-killed melanoma cells by dendritic cells was decreased, it resulted in significantly increased expression of activation markers CD40 and CD86 on these phagocytosing cells. In vivo combined treatment of local irradiation with 2x5Gy and HHP vaccination resulted in both tumor models (B16 and CT26) in significantly retarded tumor growth and prolonged survival of the mice. The amount of cells per gram tumor of NK cells, monocytes/macrophages, CD4+ T cells and NKT cells was only significantly increased in B16 tumors treated with RT plus HHP vaccination, while the amount of B cells was significantly decreased. In irradiated tumors the majority of tumor-infiltrating T cells was positive for PD-1 expression. HHP treatment generates inactivated and immunogenic tumor cells suitable for autologous whole tumor cell-based vaccines. Together with RT, HHP vaccines synergize to retard tumor growth by creating a favorable immune microenvironment. High expression of PD-1 on tumor-infiltrating T cells suggests that the treatment might profit from a triple combination with immune checkpoint inhibition. Funding: This work was supported by the research training group of the SFB643 and by research training group GRK1660 of the German Research Foundation (DFG), by the German Federal Ministry of Education and Research (BMBF; GREWIS and GREWISα, 02NUK017G and 02NUK050E), and by the European Commission (DoReMi, European Network of Excellence).

Poster Session E

Immune Effector Cells (T-Cells, NK-Cells)

E1

Adoptive Transfer of CMV- and EBV- Specific Peptide-Stimulated T Cells after Allogeneic Stem Cell Transplantation: Update to the Phase I/IIa Clinical Trial [MULTIVIR-01]

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Reactivation of CMV and EBV negatively impacts on outcome after allogeneic stem cell transplantation (aSCT). Specific antiviral therapy is only available for CMV. With the exception of ganciclovir all drugs are being used off-label. 40-50% of patients reactivate CMV following aSCT. For the 20-30% of patients reactivating EBV, only rituximab is available to control EBV. Rituximab leads to long term B-cell depletion requiring frequent administration of immunoglobulins. To cover the unmet medical need of CMV and EBV control after aSCT, we investigate a somatic cell therapy approach by means of CMV- and EBV-specific peptide-stimulated T cells. We have set up a prospective randomized controlled phase I/IIa multi-center clinical trial to evaluate the preventive and preemptive adoptive transfer of this ATMP in patients after aSCT (EudraCT number 2012-004240-30). The multi-center trial is currently recruiting. For manufacturing of the cell product two peptide pools (CMV and EBV) each covering 17 well-defined HLA class I and class II epitopes for stimulation of donor derived PBMC are used. PBMC collected by leukapheresis of mobilized or non-mobilized donors can be used as starting material. To avoid a second leukapheresis of the donor, CMV- and EBV-specific T cells are preferentially expanded from a small fraction of the stem cell graft. A strong expansion of virus-specific T cells could be observed in the cell products as determined by HLA class I multimer analysis. Reconstitution and cell counts of leukocytes after aSCT are monitored for both treatment and control group. Study design: After recruitment patients are randomized in intervention or control group. Patients of the intervention group receive three applications of virus specific T cells (5x10e4/kg bodyweight) starting the first adoptive transfer 30 days after allogeneic stem cell transplantation. Cells are transferred as preventive, preemptive, or also as therapeutic treatment. Patients are monitored for occurrence of GvHD, for viral load, as well as for immune reconstitution, especially of virus-specific T cells. So far, 31 patients have been randomized. The reconstitution of virus-specific T cells of treated patients looks encouraging after transfer. The immunomonitoring of 20 included patients is completed. Our first observations show promising results regarding feasibility and safety of our approach under clinical trial conditions.

E2

A novel mouse model for superagonistic anti-CD28 monoclonal antibody-induced cytokine release syndrome in humans

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Superagonistic anti-CD28 monoclonal antibodies (CD28-SA) do not require parallel stimulation of the T cell receptor complex to activate T cells. While CD28-SA treatment of mice with autoimmune/inflammatory diseases led to CD4+ Foxp3+ regulatory T cell (Treg)-mediated protection, healthy human volunteers injected with saturating amounts of CD28-SA developed a severe cytokine release syndrome (CRS) due to activation of effector/ memory CD4+ T cells. Apart from the relatively increased suppressive potency of Treg from mice compared to humans, the lack of a true memory CD4+ T cell compartment in cleanly housed mice best explains the failure of mouse models to predict CD28-SA-induced CRS in humans. We, therefore, transferred in vitro differentiated TCR-transgenic OT-II Th1 cells into diphtheria toxin-treated wild-type or DEREG-C57BL/6 recipient mice. Injection of saturating amounts of CD28-SA into Treg-depleted recipient mice activated the transferred OT-II and concomitantly endogenous Th1 cells leading to the release of high amounts of IFNδ into the circulation. The comparison of Treg-sufficient and -deficient animals confirmed that in mice Treg are very potent in limiting effector/ memory CD4+ T cell activation in vivo. Transfer of Th1 cells and other T helper cell subsets into cleanly housed recipient mice will, thus, facilitate pre-clinical testing of immunomodulatory reagents. It will, further, help to better understand the molecular requirements for CD28-SA-mediated effector/memory CD4+ T cell activation in vivo.

E3

AML immune evasion is mediated by soluble factors interfering with CD8+ T cell metabolism

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults with a poor 5-year-survival rate of less than 20%. The only curative therapy for a majority of patients consists of allogeneic hematopoietic cell transplantation (allo-HCT). Relapse after transplantation is common with about 40% leaving the affected patients with a poor prognosis. One strategy to increase the intrinsic battle of the new immune system against the recurring malignancy, the Graft-versus-leukemia (GvL) effect, is donor lymphocyte infusions (DLIs). Consecutive infusions of donor derived lymphocytes contain significant amounts of CD3+ T cells which fight and eliminate residual cancer cells. DLIs have been proven to be efficient in chronic myeloid leukemia (CML) with ~80% remission rate, whereas only a minority – less than 20% - of AML patients profit from DLIs. Cells transforming to malignant cancer cells undergo massive changes including alterations in metabolism. Aerobic glycolysis or Warburg effect describes the fermentation of pyruvate to lactate without feeding into the tricarboxylic acid cycle. This process enables cancer cells to ingest vast amounts of glucose depriving the microenvironment of nutrients and acidifying it which results in an impaired immune response. This hallmark of cancer has been primarily investigated in solid tumor entities. The exact mechanisms in leukemia are unknown. We investigated the metabolic phenotype of CD8+ T cells isolated from AML patients at primary diagnosis, during remission and relapse after allo-HCT. Despite low T cell frequencies in the blood of AML patients we observed a robust metabolic activity at primary diagnosis comparable with healthy controls. Besides, we detected a strong production of IFNγ and other effector molecules. In contrast, T cells isolated from patients at relapse after allo-HCT exhibited a dramatically reduced metabolic phenotype compared to a time point during remission. Analysis of the immune activity revealed impaired cytokine production and cytotoxic activity. A cohort consisting of patients after allo-HCT with continuing remission showed stable metabolic and immune activity of the T cells over time emphasizing the specific changes occurring during relapse. Metabolomics of the patient sera at all three disease states disclosed major changes in metabolite concentrations at relapse including depletion of arginine, while lactate was increased. In order to further investigate the mechanism we used our established GvL model and found reduced metabolic activity of T cells isolated from mice with leukemia burden compared to controls. When culturing murine T cells with allogeneic AML cells their metabolism was greatly impaired. The effect was still observed when we only administered the pre-conditioned supernatant which hints towards soluble factors being responsible. Pre-activation of the T cells prior to co-culture showed that the AML supernatant interfered with the pivotal metabolic reprogramming in the T cell activation phase. The observed effect was tumor cell specific since benign myeloid cells did not induce metabolic changes in the T cells. Transcriptional analysis confirmed a strong impairment of the major metabolic pathways such as glycolysis and oxidative phosphorylation. When transplanting the T cells pre-incubated with AML cell supernatant into tumor bearing mice we monitored an impaired GvL activity with lower survival rates and increased tumor burden. NMR spectroscopy of cell culture supernatants for quantitative determination of metabolite concentrations revealed major changes in key molecules, some also found in the human metabolome analysis. We excluded classic nutrient deprivation by administering nutrients such as glucose which did not rescue the phenotype. In order to boost T cell metabolism we incubated T cells with modulators of mitochondrial architecture, Mdivi-1 and M1. The treatment led to an increased memory phenotype which has been shown to be favorable for the GvL reaction. The improved anti-tumor activity was verified in the murine model. The use of metabolic stimuli in DLIs could enhance anti-tumor immunity ultimately improving the success of DLIs and other adoptive T cell transfer strategies in the treatment of AML relapse.

E4

Evolution of a GMP-compliant allogeneic EBV peptide-specific T cell bank and the use of multi-parameter flow cytometry for product analysis.

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Adoptive immunotherapy with allogeneic Epstein-Barr virus (EBV)-specific T cells has shown significant benefits in patients with EBV-related malignancies such as post-transplant lymphoproliferative disease. In our centre we have released over 100 products to patients with PTLD. Improved clinical manufacture of T cells for therapy requires a rapid, scalable, cost-effective process that conforms to GMP regulations. This compliance is ensured by use of GMP-grade reagents and protocols and validated analyses for quality management and product release. We report the development and implementation of a new allogeneic EBV-specific T cell bank generated by expansion of interferon-γ selected virus-specific T cells (VST). Pre-screening of prospective donors by seropositivity and EBV peptide-induced cytokine expression analysis confirmed that seropositivity alone was insufficient to ensure a virus-specific T cell response in vitro. Virus-specific T cells were isolated from leukapheresis using an IFN-γ based cytokine capture system followed by co-culture with irradiated autologous leukocytes in GMP-compliant medium supplemented with IL-2. Cells were cultured in closed process culture flasks (G-Rex M100CS, Wilson Wolf) over 19-21 days then harvested and cryopreserved at therapeutic dose (1.5x10^8 T cells per dose, 4 doses per treatment). Flow cytometric analysis for surface phenotype and intracellular functional markers was used as an in-process control to assess VST content, expansion and differentiation throughout culture. Consolidation of multi-parameter flow data using dimensionality reduction (t-SNE, FlowJo) proved a powerful tool for VST product characterization and comparison with the previous VST bank. We generated 8 VST products in pre-production and further products after Process Validation. The pre-production process generated a mean of 1.27x10^10 VST from a starting isolate of 5.6x10^7 IFN-γ positive cells (expansion of approximately 3000 fold). The protocol reduced processing time from 3 months (with our previous EBV-VST bank) to 20 days, generating an average of 96.8 ± 16.8 adult doses per single production run, offering a massive improvement over standard lymphoblastic cell line-based VST culture in terms of quantity and cost. The use of fully GMP-compliant reagents, closed process manufacturing and suitable quality management has offered an effective approach to clinical manufacture of EBV-specific T cells in terms of speed, cost and complexity, and determination of functional quality through flow cytometry.

E5

New whole genome association scanning approach for the discovery of HLA class I-restricted minor histocompatibility antigens

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Patients that undergo allogeneic stem cell transplantation (SCT) as treatment for haematological diseases face the risk of Graft-versus-Host Disease (GvHD) as well as relapse. GvHD as well as the favourable Graft-versus-Leukemia effect are mediated by donor T cells. These T cells recognise minor histocompatibility antigens: polymorphic peptides, which are presented on the cell surface by HLA molecules and are caused by genetic differences in single nucleotide polymorphisms (SNPs) between patient and donor. Identification of minor histocompatibility antigens has been a laborious process in the past. Therefore, whole genome association scanning was developed in our laboratory. However, this was restricted to two HLAs and the screening of one million of SNPs. In an optimized approach, donor T-cells isolated from patients after allogeneic SCT are now tested for recognition of a panel of 191 B-cell lines, which are sequenced in the 1000 Genome Project. This standardized panel enables the inclusion of seven common HLAs and increases SNP coverage to 12 million (MAF > 0.01). SNPs that strongly associate with T-cell recognition are subsequently validated to encode minor histocompatibility antigens. This approach for whole genome association scanning has been successfully applied and 17 novel minor histocompatibility antigens have been found. Besides conventional antigens, cryptic peptides are included as well as peptides restricted to HLAs that were not primarily targeted, showing the potential of this panel. Furthermore, this standardized panel can be extended to other HLAs of interest. The aim is to use the optimized method to identify the dominant repertoire of HLA class I-restricted minor histocompatibility antigens, since knowledge about mismatched antigens may enable a more directed donor search as well as better prediction and measurement of the Graft-versus-Leukemia effect and GvHD for personalized treatment after transplantation, thereby contributing to a better outcome for patients treated with allogeneic SCT.

E6

NF-kappaB activation triggers NK-cell stimulation by monocyte-derived dendritic cells

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(GS, NS, and JD share senior authorship) Immunotherapy of cancer is currently experiencing a renaissance. Because dendritic cells (DCs) are potent stimulators of innate and adaptive immunity, they are used for therapeutic cancer vaccination. Several trials have shown that monocyte-derived cytokine-matured DCs can induce specific T-cell responses. Previously, we have shown that triggering the NF-κB pathway in such DCs by transfection of mRNA encoding constitutively active IKKβ(caIKKβ) led to IL-12p70 secretion and improved the DCs' capability to activate and expand memory-like cytotoxic T cells (CTLs). As the importance of activating innate immunity next to adaptive responses actually emerges, we examined whether the caIKKβ-transfected DCs were in addition capable of activating natural killer (NK) cells. Those are crucial in innate anti-tumor immunity, because they act as helper cells for the induction of a CTL response, recruit other immune cells, and are able to directly kill the tumor. We could show that cytokine-matured monocyte-derived DCs, transfected with caIKKβ RNA efficiently induced the expression of the activation markers CD54, CD69, and CD25 on autologous NK cells. They also triggered cytokine secretion and expansion of NK cells, which were then capable of lysing the classical NK target cell line K562. This capacity of caIKKβ DCs to activate both the adaptive and innate immune response indicates an enhanced potential for their clinical efficacy.

E7

Oncogene inactivating drug combined with irradiation and vaccination breaks tolerance against autochthonous tumors

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Tumor-specific T cells are often tolerized or exhausted due to aberrant activation or chronic tumor antigen encounter, respectively. We exploited a murine autochthonous tumor model in which the expression of the cancer-driving antigenic oncogene SV40 large T (Tag) can be conditionally controlled. Neonatal Tag expression resulted in autochthonous tumor development that was accompanied by tolerance against Tag. Deprivation of Tag in established tumors resulted in tumor regression but persistence of dormant tumor cells that regrew unimpeded after cessation of the oncogene-inactivating drug, indicating also the absence of effective immune responses against potential neo-antigens expressed by the tumors. In an attempt to break anti-tumor tolerance, we depleted the tolerant/exhausted T cell repertoire in mice with minimal residual diseases to promote the egress of naive T cells with full effector capacity. Indeed, vaccination with live Tag+ tumor cells four weeks after lymphodepletion provoke a profound Tag peptide IV specific immune response which was able to eradicate residual tumor cells and prevented mice from tumor regrowth after therapy cessation. Furthermore, depletion of CD4+ T cell from splenocytes of Tag-tolerant hosts before transfer into Rag-ko mice similarly broke Tag tolerance, suggesting that CD4+ T cells play a vital role in tolerance maintenance in our model. Together, our data suggest that vaccination under lymphopenic conditions at a stage of minimal residual disease can reestablish immunity against tumor antigens and might be exploit in the clinic for cancer patients with high mutational load.

E8

Metabolic stress related immune alterations with impact on the GvL effect and GvHD in allo-HSCT

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Therapeutic success of allogeneic hematopoietic stem cell transplantation (allo-HSCT), the only curative option for a range of hematological diseases such as leukemia, relies on the potency of transplanted donor T cells to destroy residual malignant host cells – termed the Graft-versus-Leukemia (GvL) effect. Oxidative stress, primarily caused by reactive oxygen species (ROS), has a detrimental impact on immune cells and is known to severely affect T-cell biology and function. Beyond that, upon allo-HSCT, antecedent conditioning regimes can lead to increased ROS production. Since allogeneic donor T-cells are the primary effectors and regulators of GvL and of the severe side effect Graft-versus-Host Disease (GvHD), we hypothesized that oxidative stress interferes with proper allo-T-cell effector function and might predispose for GvHD following allo-HSCT. In consequence, this study states an in-depth ex vivo analysis of allo-HSCT samples focused on patients' redox balance. Upon allo-HSCT, we observed prevalence of systemic oxidative stress as determined by increased levels of oxidized proteins, lipids and nucleic acids which also can be transferred into single T-cell level. Elevated ROS levels positively correlated with the activation status and markers for Glucose metabolism – indicating a link between oxidative stress, metabolic stress and T-cell function after allo-HSCT. Collectively, in this study we have shown that presence of oxidative stress upon allo-HSCT is associated with an altered immunophenotype that potentially triggers insufficient T-cell effector functions. With the objective to identify novel biomarkers for GvL/GvHD prediction, in the next step, clinical parameters will be included and analyzed retrospectively regarding the clinical outcome.

E9

Two distinct NK cells subsets differ in cytokine expression without effect on cytotoxic potential

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NK cells, as key players in anti-tumor response, are used in many clinical trials for the treatment of cancer. Key factors involved in success of NK cells therapy are cell dose and activation status. NK cells are activated and expanded ex vivo by various protocols using different types of cytokines (IL-2, IL-15, IL-12), media and feeder cells in order to affect their properties, like increase of activation receptors expression, enhancement of memory-like immunophenotype or proliferative capacity. We have developed expansion protocol based on combination of feeder cells (mononuclear cells) and IL-2, which allows rapid increase of CD25, NKp44 and NKG2D receptors and high cell proliferation rate. We tested NK cells from healthy donors and patients after allogeneic stem cells transplantation and studied expression of activation receptors and proliferation. The aim of the study was to compare cytokines/chemokines and functional proteins expression, cytotoxic activity and proliferation potential of CD25pos/NKp44neg or CD25neg/NKp44pos populations. NK cells were isolated from mononuclear cells using NK cells isolation kit (Miltenyi) and cultured with presence of irradiated mononuclear cells (25Gy) and IL-2 (Proleukin, 1000IU/ml) in SCGM medium (CellGenix) with 5% FBS (Gibco). Then CD25 or NKp44 single positive cell fractions were sorted using FACS ARIA Fusion sorter and co-cultured with K562 cells for 3hrs. The part of cells was used for detection of chosen cytokines/chemokines/functional proteins: CCL3, CCL5, GM-CSF, IFN-γ, IL-10, TNFα, TNFβ, perforin, granzyme B and FasL using RT-PCR. The correlation of cytotoxicity and expression of CD25/NKp44 on NK cells (unsorted NK cells) was determined also in killing assays using NK resistant KG1a cell line and primary AML cells. Dynamics of expression of CD25 and NKp44 was individual without any association with origin of the cells (donors or patients). Both CD25 and NKp44 cells populations have shown similar cytotoxic potential. The expression of proliferation marker Ki67 did not correlate with expression of CD25 either NKp44. We detected variabilities in 6 tested analytes. CD25 expressing cells had higher expression of CCL3, TNFβ, GM-CSF, IFN-γ in contrast with NKp44 where CCL5 and TNFα were elevated. The functional proteins (FasL, perforin, granzyme B) were without any association with expression of CD25/NKp44. We did not observe any correlation between cytotoxic potential against AML cells and expression of followed markers. Our study confirmed the presence of two distinct NK cells subsets with different expression of cytokine/chemokines but without any association with cytotoxic potential. These differences could be probably more important for interactions with other immune cells or microenvironment and require further investigation. The future follow-up study will address multiple roles of these subpopulations within the immune system and will focus on their migratory capacity and ability to travel to the tumor site. The study was funded by the Ministry of Health of the Czech Republic - Czech health research council (project no. 15-30661A).

E10

Successful ex vivo expansion of BK-virus-specific T-cells from immunosuppressed patients after kidney transplantation indicates potential for adoptive T-cell therapy

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Background: Patients after kidney transplantation are continuously immunosuppressed to avoid graft rejection. As a consequence, patients are highly susceptible to polyomavirus BK (BKV) infections, which cause BKV-associated nephropathy with graft failure in up to 30% of cases. Therapeutic options are limited and mainly based on reduction of immunosuppression, which, however, leads to acute graft failure in about 10% of patients. Recently, an association between reduced BK viral load and reconstitution of BKV-specific T-cells (BKV-T-cells) were seen in patients after kidney transplantation, emphasizing the importance of BKV-specific immunity. We aimed to verify whether BKV-T-cells derived from immunosuppressed transplanted patients with or without acute BKV viremia can be expanded ex vivo as a prerequisite for the adoptive transfer of BKV-T-cells. Methods: For the ex vivo short-term expansion (STE), isolated mononuclear cells of 10 healthy donors (HD), 10 patients without (BKV-) and 10 patients with BK viral load (BKV+) were stimulated at day 0 and 6 with BKV-specific peptide pools and with interleukin 15 at day 9. After 12 days, cell products were characterized via IFN-δ-ELISpot and intracellular staining of activation markers (such as IFN-δ and TNF-α) including detailed analyses of T-cell phenotypes via flow cytometry. Furthermore, cytolytic activity and potential alloreactivity of expanded BKV-T-cells against autologous and allogeneic target cells were assessed via flow cytometry. Results: STE revealed highly specific BKV-T-cell spot forming colonies (SFC)/105 cells) from healthy donors (497±31/105 cells), patients without (428±19/105 cells) and with BK viral load (485±21/105 cells) without significant differences between these groups. Generally in all groups, predominantly CD4+ T-cells were expanded (HD: 58%±2,7 vs BKV+: 60%±4,3 vs BKV-: 65%±3,2) compared to CD8+ T-cells (HD: 32%±2,5 vs BKV+: 33%±3,4 vs BKV-: 27%±3,1) among CD3. In all 3 groups, CD8+ and CD4+ T-cells showed an effector-memory (CD62L-/CD45RA-) and central-memory (CD62L+/CD45RA-) phenotype, known to induce fast and long-term immunity, respectively. Expanded CD8+ as well as CD4+ BKV-T-cells were 'polyfunctional' as determined by secretion of different cytokines such as IFN-δand TNF-α. Conclusion: Successful ex vivo expansion and characterisation of patient-derived BKV-specific T-cells is a prerequisite for a potential follow-up study treating patients with BKV viremia.

E11

The importance of NFATc1 for anti-tumoral immune responses during the development of lung cancer

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NFATc1 (nuclear factor of activated T cells 1) is a transcription factor activated by TCR (T cell receptor) and Ca2+-signaling that affects T cell activation along with effector and cytotoxic T cell functions. Tumor-specific cytotoxic T cells are essential for successful anti-tumoral immune responses and the elimination of cancer cells. However, in most of the established tumors, inhibitory checkpoint receptors like PD-1 (programmed cell death protein 1) contribute to the functional impairment of T cells a process that is called T cell exhaustion which leads to cancer immune evasion. A promising immunotherapeutic approach is the inhibition of such inhibitory checkpoint receptors by using e.g. anti-PD-1 antibodies. PD 1 is one of the most successful checkpoint targets in different cancer types including non-small cell lung cancer (NSCLC). Nevertheless, only ~30% of NSCLC patients are responsive to this therapy, and there is a need to find alternate regulators to improve currently applied immunotherapies. As NFATc1 is important for T cell activation we asked whether this transcription factor could be crucial for the reactivation of exhausted T cells during the development of NSCLC. Therefore, we analyzed NFATc1 and its interplay with PD-1 in a human NSCLC patient cohort and in a murine model of lung adenocarcinoma. In this study, we report a progressive decrease of NFATc1 in lung tumor tissue and in tumor-infiltrating lymphocytes (TIL) of patients suffering from advanced-stage NSCLC. Mice harboring conditionally inactivated NFATc1 in T cells (NFATc1ΔCD4) showed increased lung tumor growth associated with impaired T cell activation and function. Furthermore, in the absence of NFATc1, reduced IL2 influenced the development of memory CD8+ T cells. Specifically, we found a reduction of effector memory and CD103+ tissue-resident memory (TRM) CD8+ T cells in the lung of tumor-bearing NFATc1ΔCD4 mice, underlining an impaired cytotoxic T cell response and a reduced TRM tissue-homing capacity. In addition, targeting PD-1 induces NFATc1 in CD4+ and CD8+ T cells of lung tumor-bearing wild-type mice and was associated with increased anti-tumor cytotoxic functions. These results indicate that anti-PD-1 antibodies induce NFATc1 in TILs resulting in T cell activation and improved effector functions which promote the functional restoration of exhausted T cells. Together, this study reveals a multi-faceted role of NFATc1 in the activation and function of T cells underlining the importance of this transcription factor for successful anti-tumor immune responses especially in the setting of lung cancer.

E12

The NK cell compartment in multiple myeloma patients –a phenotypic and functional analysis

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Multiple myeloma is a neoplastic plasma cell disorder and despite recent therapeutic advances still considered an incurable disease. NK cells are promising candidates for cellular-based anti-myeloma therapy, due to their genetically encoded cytotoxic activity against malignantly transformed cells. Using CyTOF technology a comprehensive phenotypic analysis of the NK cell compartment was done in patients with newly diagnosed multiple myeloma. We thereby observed changes within the expression of activating and inhibitory receptors within the NK cell compartment compared to non-infiltrated bone marrow specimens. Surprisingly, we did not detected enhanced expression of checkpoint receptors on bone marrow-derived NK cells. In addition, we performed functional analysis on the degranulation capacity of NK cells as well as their intracellular signaling cascade upon CD16 engagement using fluorescent cell barcoding and phospho-epitope flow cytometry. Degranulation capacity and phospho-epitope staining upon CD16 stimulation were similar between bone marrow-derived NK cells from multiple myeloma patients and non-infiltrated bone marrow specimens. We provide new information on the biology of bone marrow-derived NK cells of newly diagnosed multiple myeloma patients and we will further investigate the nature of our findings.

E13

TNF-αbased adoptive T cell therapy

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The extent of T cell infiltrates in the primary tumor is one of the most important prognostic factors in many cancer entities. Still, how exactly the T cell infiltrates influence the prolonged patient survival remains unclear. To answer this question we have studied the presence and functional activity of tumor infiltrating lymphocytes (TIL) together with their specificity in colorectal cancer (CRC) patients and correlated the obtained results to long term survival. With the help of intracytoplasmic cytokine staining, in conjunction with human leukocyte antigen (HLA)- multimers loaded with peptides derived from tumor antigen (TA), as well as antigen specific cytokine secretion assays, we have demonstrated that tumor necrosis factor α (TNF-α) expression characterizes a population of TA specific, in situ active cytotoxic T cells. We have observed that the presence of these TNF-α expressing tumor infiltrating T cells correlates with increased TNF-α concentration in CRC tumors. Based on these observations we concluded that intratumoral TNF-α can be a direct indicator of the in situ functionality of tumor specific effector T cells in CRC. Furthermore in a retrospective multivariate analysis of a cohort of 102 CRC patients involving various other immune parameters, such as CD4+, CD8+ T cell infiltration, and the presence of regulatory T cells or mast cells we have determined that the intratumoral TNF-α concentration was an independent long term prognostic factor in CRC. Based on these observations, we have hypothesized that TNF-α-secreting T cells possess therapeutic potential and we could utilize these tumor specific TNF-α secreting cells for adoptive T cell therapy. We are currently investigating the presence of TNF-α-secreting T cells in other tumor entities such as head and neck squamous cell carcinoma (HNSCC) and we are optimizing protocols for their isolation and expansion. Furthermore we want to understand the factors that influence the expansion and proliferation capability of these cells. We are aiming to utilize a prognostically relevant and functionally effective subset of T cells, which are highly reactive to broad spectrum of tumor antigens and their use for adoptive transfer carries a lower potential risk for unspecific reactivity and escape variants, thus making them a powerful tool toward efficient T cell based immunotherapy.

E14

Tumor-specific immune responses following Microwave Ablation in patients with Hepatocellular Carcinoma

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Thermal ablative therapies, such as microwave ablation (MWA) are standard treatments for localized hepatocellular carcinoma (HCC). In addition to local tumor destruction, tumor regression in distant lesions has been described (abscopal effect), which might be mediated by systemic anti-tumor immune response. This study provides detailed analyses of immune-related effects of MWA in HCC patients. In our prospective investigation, we analyzed longitudinal samples of peripheral blood mononuclear cells (PBMC) from 23 HCC patients undergoing MWA. In a retrospective investigation, we analyzed PBMC samples of 13 patients with early relapse (within 1 year), 17 patients with long-term remission (longer than 1 year) and tumor resection specimens from 18 historical patients who underwent combined ablation and surgical resection. While flow cytometric analyses of prospective patients revealed only moderate effects of MWA on circulating immune cell subsets, Fluorospot analyses of IFN-γ and Interleukin-5 against 9 cancer testis antigens commonly expressed in HCC, revealed de-novo or enhanced tumor-specific immune responses in 6/20 patients (30%). Anti-tumor immune response was related to tumor control as patients with a long-time remission after MWA showed more antigen-specific T cell responses than patients suffering from an early relapse (8/16 vs. 0/12 patients). Digital image analysis revealed superior survival of patients with high T cell infiltration (CD3+ cells/mm2 in immunohistochemistry tumor sections). Taken together, our data demonstrates remarkable immune-related effects of MWA in our cohort of HCC patients and provides additional evidence for a combination of local ablation and immunotherapy in this challenging disease.

E15

Immune cell infiltration predicts malignant transformation of precursor lesions of oral cancer

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Background Objectives:An association of malignancy parameters and prognosis of patients with oral squamous cell carcinoma OSCC) with immunological parameters is already known. Tumor-associated macrophages play a key role in this process. Most OSCC originate from oral leukoplakia (OLP). To date, it is not known whether immunological changes in the tumor microenvironment occur during tumor progression or precede malignant transformation. The present study should clarify if OLP transforming into cancer within 5 years differ regarding macrophage infiltration and macrophage polarization.Materials and Methods:50 samples of OLP with malignant transformation into OSCC within 5 years (transforming OLP, group 1), 50 samples of OLP without malignant transformation (non-transforming OLP, group 2), 50 samples of OSCC corresponding to group 1 (invasive squamous cell carcinoma, group 3) and 50 samples of healthy oral mucosa (group 4) were immunohistochemically investigated. The infiltration of CD68, CD11c and CD163 positive macrophages in the epithelial and subepithelial compartment was quantitatively evaluated.Results:OLP with malignant transformation showed significantly ($p<0.05$) increased macrophage infiltration (CD 68, CD163 and CD11c expression). Furthermore, transforming OLP revealed a significantly increased M2 polarization of macrophages (CD163/CD68 and CD163/CD11c ratio) compared to OLP without malignant transformation.Conclusion:OLP with malignant transformation show significantly enhanced macrophage infiltration and significantly enhanced M2 polarization compared to OLP without malignant transformation. These data suggest that immunological changes precede malignant transformation of OLP. Increased local macrophage-mediated immunotolerance could promote malignant transformation. This shows the potential of cellular immunotherapies even for precursor lesions of oral cancer.

E16

A database of computationally predicted T cell epitopes from overexpressed proteins in cutaneous melanoma

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Therapeutic anti-cancer vaccination has been adapted as an immunotherapy in several solid tumors. However, the selection of promising candidates from the total quantity of possible epitopes poses a challenge, and very few epitopes have been tested in experimental or clinical settings to validate their efficacy. Here, we present a comprehensive database of predicted non-mutated peptide epitopes derived from genes which are overly expressed in melanoma biopsies compared to healthy tissues. They were filtered against expression in a curated list of survival-critical tissues. We hypothesize that these 'self-tolerant' epitopes have two desirable properties: they do not depend on mutations, being immediately applicable to a large patient collective, and they potentially cause fewer auto-immune reactions. To support epitope selection, we provide an aggregated score of expected therapeutic efficiency as a shortlist mechanism. The database has applications in facilitating epitope selection and trial design.

E17

Cellular and molecular changes in the anti-tumor immune response after therapeutic vaccination with CpG and α

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CD8+ cytotoxic T lymphocytes (CTL) play an essential role in anti-tumor immune responses. They are activated by professional antigen-presenting cells, usually dendritic cells (DC). To efficiently prime CTL and induce their cytotoxic function, DCs need to be licensed by CD4+ T helper cells (TH). The licensing process can be enhanced by CpG, a TLR9 ligand that promotes DC activation and induces the production of chemokines such as CCL3, CCL4 and CCL5 to recruit CCR5+CD8+ T cells. This process is called classical, TH mediated cross-priming. Next to classical cross-priming an alternative mechanism, initiated by the synthetic glycolipid α-GalCer (αGC) and natural killer T cells (NKT), exists. There, DCs present αGC via CD1d to invariant-NKT cells (iNKT), both cells become highly activated and produce CCL17 and CCL22 to recruit CCR4+CD8+ T cells. Recently, we have shown that the combination of both licensing processes markedly improves anti-tumor immune responses. Therefore, a better understanding of the cellular and molecular changes is beneficial. Our results confirm that the combination of the two adjuvants results in a higher number of tumor-infiltrating antigen-specific CTLs, reduced tumor growth and prolonged survival. Additionally, we detected increased numbers of pro-inflammatory tumor associated macrophages (TAM) with concurrent reduction of anti-inflammatory TAMs. We found that the tumor-infiltrating CTLs, generated by the combination therapy, release more Granzyme-B and produce more IFN-γ and TNF-α, than their single-treatment counterparts. These CTLs show a reduced expression of PD-1, TIM-3 and LAG-3, common exhaustion markers which induces the suppression of CTL effector functions. Despite reduced PD-1 expression, a further delay in tumor growth and prolonged survival could be achieved by combining our vaccination approach with additional inhibition of PD-1. We propose that the combination of the two adjuvants may be useful as therapeutic vaccination in tumor treatment.

E18

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

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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 two pancreatic cancer cell xenograft models. 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.

E19

Development of an off-the-shelf cell product for antigen-dependent cytokine secretion to increase anti-cancer immune responses

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Tumor infiltration is considered a favorable prognostic marker for solid malignancies. To stimulate priming of an endogenous T cell response against cancer neoantigens, we equipped the NK-92 cell line with the previously described synthetic Notch (synNotch) receptor circuit to induce local expression of immune cell chemoattracting CCL4 and dendritic cell maturation factor GM-CSF in an antigen-dependent manner. The synNotch receptor contains a single chain variable antibody fragment (scFv) for recognition of L1CAM (CD171), an adhesion molecule aberrantly expressed by several solid tumors. Receptor engagement upon antigen contact results in proteolytical release of an intracellular transcriptional activator domain that binds to its response element, enabling the customized expression of downstream genes. We will perform in vitro analysis of receptor function in regards to cytokine secretion using ELISA following co-cultivation with different neuroblastoma and HNSCC cell lines that differ in L1CAM surface expression. We screened 18 HNSCC cell lines and selected 6 with negative, low, medium and high antigen expression. The broad cytotoxic activity of NK-92 cells towards the mentioned cell lines was confirmed in a degranulation assay. Analysis of GM-CSF and CCL4 expression in HNSCC and NB revealed that GM-CSF is differentially expressed in HNSCC lines and absent in NB, while CCL4 was only expressed upon co-cultivation with untransduced NK-92 cells. Secreted CCL4 levels were 10 – 100-fold lower than minimal concentrations reported to be effective to induce chemotaxis. The influence of irradiation (10 Gy) on the functionality of the cellular product, which renders it safe for in vivo introduction as an off-the-shelf therapeutic, will be determined. Although NK-92 cells require IL-2 addition for survival in culture, we established that it was not essential to extend the survival period after irradiation. The migratory capacity of CD4 and CD8 T cells, as well as DCs and NK cells in response to a CCL4 chemokine gradient will be explored in vitro via transwell assays. In vivo, the migration of NK-92 cells towards an established tumor and the benefits of combination therapy with immune checkpoint inhibitors will be examined.

E20

Diet-induced Vitamin D insufficiency alters immune cell composition of Balb/c mice

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Vitamin D deficiency is prevalent among patients undergoing allo-HSCT and a possible link between vitamin D status and the pathogenesis of GvHD has been established. Vitamin D has several biological effects on the body that exceed its well-established role in bone metabolism and homeostasis. Increasing evidence demonstrates that Vitamin D forces an anti-inflammatory environment. This is amongst others driven by the generation of tolerogenic dendritic cells and regulatory T cells (Tregs). As the Vitamin D receptor is expressed in immune cells, it is not surprising that Vitamin D is capable of modulating both innate and adaptive immune responses. Furthermore, Vitamin D is known to influence hematopoiesis and the proper development of different immune cell populations. In the present study we aimed to systematically investigate the effect of diet-induced Vitamin D insufficiency on the immune cell composition of healthy Balb/c mice. 3-week-old female Balb/c mice were fed with a diet containing 1500 IU Vitamin D3 per kg or a diet with low Vitamin D3 for 14 weeks. At this time point, the animals were sacrificed, the serum 25(OH)D levels were determined and the immune cell populations present in the blood, spleen and bone marrow were analysed by means of flow cytometry. Analysing serum levels of 25(OH)D we confirmed that the low Vitamin D3 diet decreases 25(OH)D serum levels compared to the diet containing 1500 IU Vitamin D3 (19,2 nM vs. 41,6 nM, respectively). Furthermore, FACS analysis revealed that the immune cell composition of Balb/c mice is altered by Vitamin D serum levels: Lower serum Vitamin D levels led to a decreased amount of myeloid cells (CD11b+) in the bone marrow. No differences were found in the amount of B cells. Also, T cells seem to be modulated by vitamin D levels, since mice with lower serum Vitamin D levels possessed a decreased amount of CD3+ cells in the bone marrow. CD3+CD4-CD8- (double negative) T cells, a population known to have immunoregulatory activity, were also found to be decreased both in spleen and bone marrow in mice fed with low levels of Vitamin D3 compared to mice fed with 1500 IU Vitamin D3. Furthermore, we detected vitamin D-induced differences in the composition of the intestinal microbiome. Our data demonstrate that Vitamin D levels are not only important for the proper anti-inflammatory function of immune cells but also for the immune cell composition and the intestinal microbiome under steady state conditions. A situation which might promote the development of different disease states.

E21

Specific PRMT5 inhibitors suppress human CD8+ T cells by upregulation of p53 and impairment of the AKT pathway similar to the tumor metabolite MTA

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Genetic alterations in tumor cells provide promising targets for anti-tumor therapy. Recently, loss of methylthioadenosine phosphorylase (MTAP), a deletion frequently occurring in cancer, has been shown to create vulnerability to the inhibition of the protein arginine methyltransferase 5 (PRMT5). MTAP deficiency leads to accumulation of methylthioadenosine (MTA), which reduces PRMT5 activity, and thus sensitizes the tumor cells to further specific PRMT5 inhibitors (PRMT5i). PRMT5i are investigated as new strategy to selectively kill MTAP-deficient cells by blocking residual PRMT5 activity, but also to treat PRMT5-overexpressing cancer. Though many studies investigated the role of PRMT5 in cancer, only little data about the effect of PRMT5 inhibition on immune cells exists. As we could show that the tumor metabolite MTA suppresses T cells, we asked if specific PRMT5 inhibition is detrimental for T cell immune responses. Therefore, we examined the effect of the synthetic PRMT5 inhibitor EPZ015666 on human CD8+ T cells in direct comparison to the natural occurring PRMT5-inhbiting molecule MTA. Both compounds reduced T cell proliferation, viability and functionality. In addition, we found T cell metabolism to be impaired upon PRMT5 inhibition. These effects coincided with the induction of p53 expression and reduced AKT/mTOR signaling. In summary, we found PRMT5 activity to be involved in various cellular processes of human CD8+ T cells associated with T cell function. Therefore, PRMT5 inhibitors might critically influence anti-tumor immune responses and hence therapy success. This emphasizes the importance of considering side effects on the immune system when developing new strategies to specifically target not only MTAP-deficient tumors.

E22

LDHB overexpression alters mitochondrial metabolism in human CD4+ T cells

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Lactic acid inhibits the effector functions of human T cells, NK cells and monocytes. In line, accelerated tumor glycolysis correlated with resistance towards adoptive T cell transfer therapy in melanoma patients. We hypothesized that overexpressing the lactate-metabolizing LDH-subunit LDHB would lead to an increased tolerance towards lactic acid in T cells. Using a retroviral transduction system, we overexpressed a HIS-tagged LDHB in human CD4+ T cells. T cell metabolism and function under treatment with lactic acid was characterized by assessing viability, proliferation, cytokine production and mitochondrial metabolism. Retroviral transduction led to a 50 % overexpression of LDHB in CD4+ T cells as determined FACS analysis, Western Blot analyses and LDH zymogram. T cells overexpressing LDHB were characterized by higher oxygen consumption under incubation with lactic acid at equal mitochondrial content, indicating that the overexpressed LDHB was functional. Analyzing cytokine production under lactic acid, we found that LDHB-overexpressing HIS+ cells had a higher percentage of intracellular IFNγ+ and TNFα+ cells, but this was not reflected in the amount of secreted effector cytokines. Induction of apoptosis by lactic acid in T cells was not reduced by LDHB overexpression. Our experiments demonstrate that overexpression of LDHB changes the mitochondrial metabolism and cytokine expression in human CD4+ T cells, but does not rescue viability upon lactic acid treatment.

E23

Strategies for enhancing VγVδT-cell functions against cancer

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Among other promising concepts of cellular therapy, γ-δ (γδ) T-cells could become a valuable resource for the treatment of cancer. This idea is supported by data from a variety of models in vitro and in vivo as well as proof of relevant anti-tumor effects in early phase clinical trials. Especially the subset of Vγ9Vδ2 T-cells, the most abundant γδ T-cell subpopulation in the human blood, possess favorable anti-tumor characteristics and can be specifically and efficiently activated in vitro and in vivo. Vγ9Vδ2 T-cells have the ability to recognize malignant cells, infiltrate tumors and they depict strong cytotoxic and pro-inflammatory activity. Furthermore, previous clinical studies consistently documented a favorable safety profile of γδ T-cell based therapies [1]. Here we compare several recent pre-clinical strategies for the enhancement of Vγ9Vδ2 T-cell anti-tumor functions. We also discuss their translational perspective, current limitations, promising new developments and identify future research need. This includes concepts for the sensitization of cancer cells, the improvement of cytotoxic effector mechanisms and combination immunotherapies. Specific aspects are the combination with monoclonal antibodies and antibody constructs, the importance of immune checkpoint molecules and strategies that target the tumor cell metabolism [1, 2]. If we succeed in advancing such promising approaches to more valid models and clinical studies it will help to improve γδ T-cell based anti-cancer therapies in the future.

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2. Hoeres, T., et al., PD-1 signaling modulates interferon-γ production by Gamma Delta (γδ) T-Cells in response to leukemia. OncoImmunology, 2018: p. 1-11.

Poster Session F

Antigen Presenting Cells

F1

A phase I/II vaccine clinical trial in metastatic malignant melanoma patients with step by step optimized mRNA-electroporated dendritic cells: an increase in survival correlates with eosinophilia and upregulation of PEBP1

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Therapeutic cancer vaccination aims to treat cancer-patients by immunizing them against their tumor. Dendritic cells (DCs) are specialized in initiating immune responses and can be generated in large numbers from autologous blood monocytes (moDC) for vaccination purposes, a technique we established and improved over several years. To optimize our vaccine approach, we i) changed the antigen loading (from peptides to mRNAs) and ii) tuned the maturation procedure of moDCs. We performed a phase I/II clinical trial with three cohorts including 82 patients using moDCs, that were electroporated with mRNA encoding the melanoma defined antigens MelanA, Mage-A3, and Survivin (MMS-RNA). In the 1st cohort, the DCs were matured with a cytokine cocktail of IL-1ß, IL-6, TNF, and PGE2 (IITP), and electroporated with MMS-RNA. We loaded the moDCs of every 2nd patient with KLH and injected the vaccine intradermally. The mean survival was 12 months, and no influence of the KLH emerged. For the 2nd cohort i) electroporation was improved, resulting in 2x higher antigen expression and ii) in addition to MMS-RNA a functional recombinant E/L-selectin (ELS) was co-electroporated in every 2nd patient to permit DC migration from blood also into the lymph nodes, making intravenous (i.v.) infusion theoretically more effective. The intravenous DC administration resulted in improved mean survival of 18 months, but to our surprise, there was no difference between patients receiving moDC with or without ELS-expression. In the 3rd cohort, we continued to administer DCs intravenously, but we expressed activating proteins inside the DCs. Half of the patients were vaccinated with IITP-cocktail-matured moDCs, electroporated with MMS-RNA and an optimized CD40L-RNA, while the others received moDCs transfected with MMS-RNA (at the immature stage) in combination with 'Trimix'-RNA (developed by Thielemans et al.) encoding constitutively active TLR4 to mediate maturation, CD40L, and CD70. The outcome of the described modifications resulted in a mean survival of 36.5 months in CD40L-DC-treated patients, while that of Trimix-DC-treated ones was 19 months. Specific immune responses to the vaccination antigens were frequently observed, with the highest increase in those patients, who are still alive today. Remarkably, as in our previous trials we found that patients with eosinophilia, notably if it emerged upon vaccination showed a better survival. Long-term survivors also had a significantly more favorable immunoscore in the tumor than short-term survivors before vaccination. We could also confirm the value of the early predictive biomarker PEBP1 (phosphatidylethanolamine binding protein 1 [PEBP1]/Raf Kinase inhibitory protein [RKIP]), that increased or maintained expression early during vaccination in patients with long survival, while a decrease occurred in all short term survivors. The insights from this trial point to the maturation stimulus and delivery route which achieve the best survival, and also support the usefulness of two 'early on' biomarkers we described earlier. This information will be useful to maximize vaccine effects in upcoming trials against melanoma and other cancers.

F2

Antigen targeting of Fc receptors induces strong and functional relevant T cell responses in vivo independent of ITAM signaling but dependent on dendritic cell subsets

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Dendritic cells (DCs) are important antigen presenting cells (APCs) and induce immune responses, but also preserve peripheral tolerance. We showed the preferential induction of either CD4+ or CD8+ T cell responses by DC subpopulations in vivo by targeting antigens to endocytic C-type lectin receptors. The also highly endocytic active Fc receptors (FcRs) enable APCs to take up antigens in form of immune complexes. As they are expressed on various APCs, we aimed to identify responsible APCs for primary and secondary immune responses by using our antigen delivery by recombinant antibodies to activating and inhibitory FcRs. This targeting induced CD4+ and CD8+ T cell responses independent the receptor's type. Moreover and in contrast to DEC205 and DCIR2 targeting, especially antigen delivery to FcγRIV was superior in inducing simultaneously CD4+ and CD8+ T cell responses., not only in a transgenic setting, but also in naive mice. As FcγRIV is expressed on both splenic cDC subsets, we used it to verify the subset intrinsic preferences to trigger either CD4+ or CD8+ T cell responses. Thereby we could clearly show the induction of CD4+ T cell responses by splenic CD8- DCs, whereas the CD8+ DCs induced CD8+ T cell responses. The naive CD8+ T cell responses were of functional relevance, as we demonstrated the effective dose-dependent killing of peptide loaded target cells in vivo. Therefore, we suggest antigen targeting to FcRs as useful tool to induce de novo as well as the modulation of immune responses for future therapeutic applications. Additionally, we could demonstrate the responses to be effective in a murine melanoma model (in a preventive as well as a therapeutic setting). We now further investigate, which mechanisms play a role after antigen targeting to CD11b+CD8- DCs, which adjuvant is most promising, and if the concomitant induction of a CD4+ T cell response is beneficial to the anti-tumor CD8+ T cell response in our system. This project was partly funded by the DFG (RTG1660, RTG1962, CRC1181-TPA7, CRC1054-TPA6, and SO1149/1-1), BayGENE, and the Emerging Fields Initiative (BIG-THERA). This work was further supported by intramural funding (IZKF-J54, IZKF-A65, and IZKF-A68).

F3

Characterizing the immunogenicity of DM-sensitive and DM-resistant antigens

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Introduction: The separation of graft-versus-leukemia (GvL) effect from graft-versus-host-disease (GvHD) is a major objective after allogeneic stem cell transplantation. We recently described two types of endogenous HLA class II restricted antigens depending on their behavior towards HLA-DM. While DM-resistant antigens are presented in the presence of HLA-DM, presentation of DM-sensitive antigens rely on co-expression of HLA-DO – the natural inhibitor of HLA-DM. Since the expression of HLA-DO is not upregulated by inflammatory cytokines and restricted to B-cells, dendritic cells and thymic epithelial cells, DM-sensitive antigens cannot be presented on non-hematopoietic tissues. Therefore, usage of CD4 T-cells directed against DM-sensitive antigens might allow separation of GvL from GvHD. However, it remains elusive whether immunogenicity and anti-tumorigenic potential of DM-sensitive and DM-resistant antigens have comparable properties in vivo. Methods: Therefore, we sought to create an in vivo system using a DM-sensitive and a DM-resistant variant of the same model antigen. First, we generated murine cell lines overexpressing either H2-M or H2-O (murine HLA-DM or HLA-DO, respectively) to allocate the two model antigens ovalbumin (OVA) and murine Y-chromosome antigen DBY to their category. Furthermore, we introduced one to three amino acid substitutions within the MHC II restricted T-cell epitopes of the two antigens and tested DM-sensitivity or DM-resistance by T-cell activation using proliferation and IFN-δ secretion as read-out in vitro. Finally, we vaccinated B6 mice with the generated epitope variants and measured expansion, phenotype and reactivity of OVA- or DBY-specific CD4 T-cells in vivo.. Results: By testing T-cell recognition of OVA or DBY on murine B-cell lines overexpressing H2-M and H2-O, respectively, we could show that OVA leads to a more potent T-cell activation in the presence of H2-O demonstrating its DM-sensitive character. In contrast the wildtype epitope of DBY does not rely on H2-O expression for strong T-cell activation and was therefore assessed as DM-resistant antigen. By introducing one to three amino acid substitutions within the T-cell epitope we could generate one further DM-sensitive variant of OVA but also two DM-resistant counterparts. Likewise, we designed both DM-resistant and DM-sensitive epitope variants of murine DBY. To assess T-cell receptor avidity to our epitope variants presented on natural antigen presenting cells, titration of DM-sensitive and DM-resistant variants of the same antigen on untreated splenocytes from OVA or DBY T-cell receptor transgenic mice, respectively, were performed. We observed comparable activation of the same T-cell clone activated by either variant of the epitope as measured by proliferation and IFN-δ secretion. Furthermore, upon vaccination of B6 mice with either variant of the epitope we could measured comparable expansion, phenotype, and reactivity of OVA- and DBY-specific T-cells both in vivo and ex vivo. Conclusion: We successfully generated DM-sensitive and DM-resistant variants of the same epitope for the two model antigens OVA and murine DBY. With this tool we could demonstrate that DM-sensitive antigens are not inferior to their DM-resistant counterpart. Therefore, targeting DM-sensitive antigens after allogenic stem cell transplantation might be an interesting tool to improve the GvL effect with only limited GvHD.

F4

Dendritic cell vaccination in metastatic uveal melanoma as compassionate treatment - immunological and clinical responses

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Other than cutaneous melanoma, metastatic uveal melanoma (UM) is minimally responsive to immune checkpoint inhibitors. The prognosis remains very poor with mortality rates nearly unchanged over the last decades. After metastases develop, overall survival (OS) is less than 12 months. The recently growing insight that immunotherapy significantly improves outcomes for cancer patients led to a re-emergence of vaccines including dendritic cell (DC) vaccines for specific immune induction. This is of particular interest in non-immunogenic solid malignancies, such as UM. We vaccinated an UM patient with liver and multiple skin metastases in a compassionate use setting. Before start of vaccination liver metastasis was resected and checkpoint blockade with anti-CTLA-4 (ipilimumab) was started. The first vaccination was in September 2013. In April 2014 the patient showed progression in the liver. We continued DC vaccination in shorter intervals and adapted antigen loading (with mRNA coding for an individual GNAQ driver mutation) accompanied by a further cycle of ipilimumab. The latter had to be stopped due to the development of autoimmune colitis and arthritis. Therapy resulted in complete remission of liver metastases, but the patient developed new skin metastases scattered over the whole body. Again the loading of DC was adapted (peptides of passenger mutations predicted after Next-Generation Sequencing (NGS) of skin metastasis) and infusions with pembrolizumab were started. Pathology from some regressing lesions showed a massive T cell infiltration and in parallel GNAQ mutation-specific T cells could be found in the patient's blood. Skin metastases regressed and no new metastases developed. The patient is now free of any detectable tumor after 65 months of continuous vaccinations. Side effects included the development of vitiligo in 2015, pronounced eosinophilia of up to 36% and grade 2 fever. Immune monitoring in the patient's blood showed a vaccine-induced functional T cell response (as represented by IFN γ and TNF production and degranulation) against the QNAQ-driver mutation and one of the passenger mutations and a preexistent T cell response against another passenger mutation which was increased by vaccination. Based on this positive experience we performed individual compassionate treatments in other patients using DC loaded by peptide pulsing and/or mRNA transfection (autologous tumor RNA or RNA coding for tumor antigens). Three of four patients are still alive, one in complete remission under DC vaccination in combination with pembrolizumab, two of them showing measurable disease (one stable, one progressive), and one deceased disease related after 28 months, resulting in a median OS of the five patients of 36.4 months. The two patients with measurable disease were treated with adjuvant DC vaccination alone (after resection and local therapy for liver metastases) until progression (24 and 16 months). Immune monitoring in one of those patients showed an unequivocal CD4+ and CD8+ INF-γ T cell response against the autologous tumor RNA vaccine after four vaccinations. No severe (grade 3 or 4) toxicity occurred. The vaccine-related side effects observed were grade 1 fatigue (1 patient), flu-like symptoms (2 patients), vitiligo (1 patient), headache (2 patients), and injection site reactions (5 patients). Interestingly, one responding patient regularly develops symptoms of a grade 1 cytokine release syndrome with transiently increased serum IL-6 several hours after administration of the vaccine. Four of the patients developed an eosinophilia after DC vaccination, an effect shown to correlate with improved OS in several other tumor entities. The observed prolonged median OS and the fact that 2/5 patients remain disease-free is definitely encouraging. Vaccination immunotherapy with antigen-laden DC is a potential therapeutic option for patients with metastatic uveal melanoma. Combinations with checkpoint inhibitors proved promising, and should be further evaluated in clinical trials.

F5

Induction of anti-tumor responses via antigen targeting of dendritic cells in vivo

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Dendritic cells (DCs) are the most potent antigen presenting cells (APCs) in mice and men. They are crucial for the induction of immune responses against invading pathogens as well as tumor cells. This feature and their additional capacity to induce regulatory T cells and to dampen immune responses against self-antigens renders them as interesting target cells for immune therapies. Multiple groups have shown that antigen targeting by using recombinant antigen-conjugated antibodies against different endocytic receptors specifically expressed on DCs is an excellent method to trigger and modulate immune responses in vivo. By taking advantage of the subset-specifically expressed endocytic C-type lectin receptors DEC205 present on splenic CD8+ DCs or DCIR2 on CD11c+CD8- DCs, it is possible to predominantly trigger antigen-specific CD8+ or CD4+ T cell responses, respectively. By this targeting approach the survival of tumor challenged mice is prolonged accompanied by a reduced tumor growth. Recently, we demonstrated FcγRIV, present on both classical DC subsets in murine spleen, to be effective in the induction of concomitant CD8+ and CD4+ T cell responses. In this project, we focus on two central aspects: 1. Potential of FcγRIV targeting: Even though this receptor is expressed on an array of immune cells, the induction of immune responses by targeting of FcγRIV is solely dependent on DCs. Since this receptor is found on both, CD8+ and CD8- DCs, and induced potent T cell responses, we set out to determine, if the targeting of this receptor is superior compared to targeting approaches via DEC205 and DCIR2 in murine tumor models in vivo. 2. Mechanism of tumor protection by DCIR2 targeting: Antigen delivery via DEC205 induces CD8+ T cells capable of killing tumor cells directly. In contrast, DCIR2 targeting triggers only minor T cell responses in naive mice. In the current project, we want to obtain mechanistic insights in the anti-tumor responses induced by DCIR2 targeting. We hypothesize that the minor induction of CD8+ T cells by DCIR2 targeting is sufficient to kill a small number of tumor cells. As a consequence, other tumor specific antigens are released and trigger de novo immune responses (CD8+ T cells and/or antibodies), which can in turn explain the observed protection. To investigate this, we are using several tumor cell lines to study cross-reactivity and the tumor-associated-epitope spreading after the initial immune response.

F6

Randomized, open-label Phase III multi-center study to evaluate the adjuvant vaccination with tumor RNA-loaded autologous dendritic cells versus observation of patients with resected monosomy 3 uveal melanoma

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The induction of T cells recognizing mutated and non-mutated cancer antigens is crucial for successful cancer immunotherapy. Our group has systematically developed cancer vaccination by adoptive transfer of monocyte-derived dendritic cells (DCs) loaded with tumor antigens in the form of peptides or RNA. In lately secluded trials, unequivocal immunogenicity and long-term clinical benefit correlating with certain 'early on' biomarkers in blood (development of eosinophilia, an increase of PEBP1) became evident. Using autologous tumor RNA to load DCs aims to utilize not only the patient's unique mutated neo-antigens (identifiable by next generation sequencing and epitope prediction) or shared non-mutated antigens, but to include the total antigenic repertoire by transfection of total tumor RNA (containing also RNAs generated by abnormal splicing or processing, and also some of the so-called cryptic peptides). This approach seems particularly promising in low mutated tumors such as uveal melanoma notably as mass spectrometry analyses have recently shown that mutated peptides, in general, unexpectedly represent only about 1% of the tumor MHC class I ligandome. Following preclinical work and evidence for immunological and clinical efficacy in metastatic melanoma, we have started a randomized multi-center phase III trial (NCT01983748) in high risk (monosomy 3) uveal melanoma patients using autologous total tumor RNA-transfected DCs in order to retard or prevent metastases after enucleation of the affected eye or resection of the primary tumor. A total of 200 patients will get randomized into arm A (DC vaccination) or arm B (observation as the standard of care). Twenty million mature, monocyte-derived DCs loaded with autologous tumor RNA are administered respectively at 8 vaccination time-points over two years. Goals are 1) to prolong disease-free and 2) overall survival and 3) to correlate them with immune responses and other parameters in blood. The trial was started in 2014 and is currently performed in cooperation with Departments of Ophthalmology at 9 University Hospitals in Germany (Dessau, Erlangen, Essen, Hamburg Eppendorf, Homburg/Saar, Köln, Lübeck, Tübingen, and Würzburg). Autologous tumor RNA of 165 patients screened for monosomy 3 (M3) has been extracted so far, with the confirmation of monosomy 3 in 95 patients. In 5 patients the quantity or quality of RNA did not allow trial inclusion, and in 3 patients metastases were detected at the time point of diagnosis of the primary tumor and thus prevented inclusion. In total, 81 patients have been included so far (6 patients in the screening process). So far 40 patients got randomized to the vaccination arm A, 41 patients to control arm B. Primary objectives of the trial will be overall survival, disease-free survival, and induction of immune responses portrayed at the single cell level (T cell receptor and gene expression analysis). A pre-defined interim safety analysis after 20 vaccinated patients revealed no severe side-effects (i.e., grade 3 and 4) and evidenced the feasibility of this treatment strategy. The next interim analyses will be performed after the death of 67 and / or progression of 69 patients.

F7

The generation of GMP-compliant human monocyte-derived dendritic cells by using the Quantum® hollow fiber bioreactor system

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First described by Ralph M. Steinman in the 1970s, dendritic cells (DCs) were identified as crucial players in inducing and directing immune responses. The ex-vivo generation of autologous DCs allows their maturation and loading with, e.g. tumor-specific antigens for cancer immunotherapy, and the adoptive transfer of such DCs represents a promising vaccine strategy with documented immunogenicity and clinical efficacy. Over the course of time, the generation, loading and application of DCs was continuously improved resulting in a safe and well-tolerated therapeutic approach to e.g. vaccinate cancer patients. A major obstacle, however, is still the need for cost intensive high-level class A and B cleanroom GMP facilities and associated trained personnel for generation of such DC vaccines. The Quantum® hollow fiber bioreactor system offered by Terumo BCT represents a new elegant platform integrating GMP-compliant manufacturing steps in a closed system for automated cultivation of different cellular products, e.g. bone marrow-derived mesenchymal stem cells or neural stem cells. A previous report using the Quantum® standard protocol - which involves fibronectin-coating of the hollow-fibers and trypsin digestion to harvest cells – to generate monocyte-derived DCs (Mo-DCs) proved unsatisfactory as despite its complexity only one tenth of an apheresis product could be processed. Our goal was to systematically optimize the DC generation in the Quantum® in order to i) avoid the fibronectin and trypsin steps and ii) to allow processing of a whole apheresis product. Leukapheresis was performed from healthy blood donors and monocytes were enriched by using the Elutra® cell separation system. Next, monocytes were differentiated over 6 days into immature DC in the presence of GM-CSF + IL-4, and then exposed to a maturation cocktail to generate mature Mo-DCs. This was performed in parallel either by using the Quantum® bioreactor or by our in-house established standard protocol of culturing in cell culture bags. Phenotype, antigen presentation, and functionality of these Mo-DCs were then analyzed and compared. After getting acquainted with the new Quantum® system intitial tests were performed to get a hint how the initial fribronectin coating and trypsin digestion for harvesting of cells could be avoided. Five leukaphereses were then processed to systematically adapt step-by step the Quantum® approach for DC-generation. This required optimization of the media exchange rate, cytokine concentration and cytokine addition into the extracapillary-site as well as the intracapillary-site of the Quantum® hollow fiber bioreactor system. Following these pilot experiments, we performed the standardized experiments (runs six, seven, and eight) to test the reproducibility of the optimized settings. Cells cultured in the Quantum® system resulted in a yield of 27.8% mature DCs (related to input monocytes) with CD83 expression in 92.0% of these cells. Total yield of mature Mo-DCs was slightly higher when the in-house established standard protocol was used. Survival of Mo-DCs analyzed in the washout test (24 hour culture in medium without cytokines) was comparable with both methods. The phenotype of DCs generated by our standard protocol indicated a trend towards a more mature phenotype as the cell surface expression of CD80, CD83, CD86, and HLA-DR was in general higher. In the next step, Mo-DCs were electroporated with mRNA encoding for green fluorescence protein (GFP). GFP was expressed 4 hours after electroporation in Mo-DCs generated by either Quantum® or the in-house established standard protocol. Electroporated Mo-DCs generated by Quantum® showed a trend towards higher GFP-expression as well as towards higher T-cell stimulation and proliferation in the primary allogeneic mixed lymphocyte reaction-assay. Taken together, we have adapted the Quantum® hollow-fiber bioreactor system so that one complete apheresis product can be processed at once to yield a large number of mature monocyte-derived DCs without any need for fibronectin coating or trypsin digestions. This new protocol circumvents the cumbersome manual processing of apheresis products in expensive GMP cleanroom facilities and nevertheless yields DCs which are equivalent with respect to yield, phenotype and T cell stimulatory capacity to standard Mo-DCs generated from enriched monocytes in culture bags in a semi-closed system. Thus, the Quantum® system overcomes the major hurdle in applying dendritic cells as cancer vaccines, and thus shows potential for the use in the growing interest of individualized DC-based immunotherapy.

F8

Automated closed-system generation of monocyte-derived dendritic cells by CliniMACS Prodigy® for the use in cancer immunotherapy

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Following the recent success of checkpoint-blockade antibodies in, e.g. metastatic melanoma patients, interest in cancer vaccines is emerging again due to the opportunity to induce antigen-specific T cells to personalized mutated as well as non-mutated antigens. Especially dendritic cell (DC)-based immunotherapy has safely and successfully been applied to treat patients with different cancers for over two decades. However, a major obstacle for DC-based vaccination therapies remains the need for high-level clean-room facilities, related infrastructure, and trained personnel for the generation of sufficient quantities of DCs, particularly for a larger number of patients in clinical trials. In this context, the CliniMACS Prodigy® by Miltenyi Biotech offers a platform integrating all GMP-compliant manufacturing steps in a closed-system for automated production of various cellular products, without the need of high-level clean-room facilities. We systematically tested CliniMACS Prodigy® for its suitability to produce human mature monocyte-derived DCs (Mo-DCs), and optimized it by comparing this approach to our standard production of Mo-DCs from elutriated monocytes (Elutra®) in cell culture bags. First, nine leukaphereses were performed to obtain monocytes. In the next step for monocyte enrichment, material of four aphereses was further processed by CliniMACS Prodigy® and material of five aphereses was further processed by Elutra®. All nine monocyte isolation runs, by CliniMACS Prodigy® or by Elutra® were performed without errors, which could have affected the safety or sterility of the cell product. After step-by-step optimization of the CliniMACS Prodigy® system and identification of the maximum effective cell concentration to generate Mo-DCs, the total yield (% of input CD14+ monocytes), phenotype, and functionality of mature Mo-DCs were equivalent to those generated by our standard protocol. Technician's labor time was comparable for both methods, but the CliniMACS Prodigy® approach significantly reduced hands-on time and high-level clean-room resources. However, the maximum amount of generated DC was limited as the cultivation volume of the CliniMACS Prodigy® CentriCult culture chamber was limited to 200 ml and thus, only one fourth of the monocytes contained in a standard apheresis could be used for cultivation and differentiation. In conclusion, a complete closed-system generation of mature Mo-DCs was possible by using the CliniMACS Prodigy® system after some adjustments regarding cell density. The major drawback of CliniMACS Prodigy® is, however, the limited capacity of the culture chamber, which can to be further optimized. Nevertheless, CliniMACS Prodigy® shows great potential for the use in the growing interest of individualized DC-based immunotherapy.

F9

Blood eosinophilia is an on-treatment biomarker in patients undergoing vaccination with dendritic cells (DC) that correlates with long-term patient outcome

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Background: The approvals of checkpoint blockade and the growing recognition that immunotherapy significantly improves outcomes for cancer patients led to a re-emergence of cancer vaccines including dendritic cell (DC)-based immunotherapy. Blood and tissue biomarkers to identify responders and long-term survivors early on and to optimize cost and cost-effectiveness of treatment are desperately needed. Eosinophilia has been associated with a better outcome for Dendritic Cell vaccination (reported for the Provenge^{TM} vaccine, and our peptide-loaded DCs) and checkpoint inhibitors. We wanted to investigate in more detail whether eosinophilia is a biomarker marker for patients receiving vaccinations with DCs loaded with autologous tumor-RNA. Methods: In total, 67 patients with metastatic solid tumors (n = 34 with cutaneous melanoma; n = 13 with uveal melanoma; n=4 with colon cancer; n=3 with neuroendocrine tumors; n = 3 with mucosal melanoma; n=2 with urothelial carcinoma; n=2 with renal cell carcinoma; n=1 with glioblastoma; n=1 with pleomorphic xanthoastrocytoma; n=1 with ovarian cancer; n=1 with pancreatic cancer; n=1 with prostate cancer; n=1 with leiomyosarcoma), who received autologous monocyte-derived DCs transfected with tumor RNA, were serially analyzed for eosinophil counts and survival over the course of up to 14 years. Eosinophilic count was detected by peripheral blood smear. We performed an intention-to-treat (ITT) analysis with all 67 patients and a per-protocol (PP) analysis with 43 patients that received at least four DC vaccines. Results: Pre-vaccination eosinophil counts did not associate with survival. 87% of the patients treated with DC-based immunotherapy experienced at least once an eosinophilia of 5% after initiation of therapy; 61 % reached levels of 10% eosinophils and 13% of patients showed eosinophil counts of 20% and above. DC-vaccinated patients who developed eosinophilia to a level of ≥ 20% at any point during the course of vaccinations showed a clear trend toward longer survival (p=0.03). In those patients (n=9) survival was prolonged with a median of 58 months (range 2-111 months) as compared with a median of 20 months (range 0-119 months). Regarding the time point of the development of eosinophilia the analysis showed, that eosinophilic count after the fourth vaccination does only predict survival, when it exceeds 20%. In 41 patients we could evaluate the early change in eosinophil count between the first and the second DC vaccine. Interestingly, in 12% of these patients we detected an immediate increase in eosinophil count ≥ 10% which appeared to correlate with survival (65 vs 24 months; p=0.06). This suggests that even a very early-on increase of eosinophils upon start of DC vaccination has a predictive value. This phenomenon is also reminiscent of our observation that the early post-vaccination increase in PEBP1-mRNA in blood correlates with later long survival (doi: 10.18632/oncotarget.18698). Conclusion: Survival of patients under DC vaccination correlates with blood eosinophilia: 1) An association with longer survival is found after an early and strong (≥ 10%) increase in eosinophil count immediately after the first DC vaccination. 2) Patients who develop blood eosinophilia above 20% at any point after DC vaccination tend to survive longer. Mechanistic studies about the role of eosinophils in cancer and vaccination are essential.

F10

CD86+ antigen-presenting B cells are increased in solid cancers and induce tumor antigen-specific T cell responses

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B cell effector functions do not only include secretion of antibodies, but also presentation of antigen to T cells. Recently, a physiological B cell subset with strong immunostimulatory properties was described in humans. These antigen-presenting B cells (BACP) are characterized by a high expression of CD86 and downregulation of CD21. BACP are expanded following vaccination or under inflammatory conditions. We analyzed seven different tumor entities for the presence of BACP by flow cytometry and found increased percentages in lung adenocarcinomas, head and neck squamous cell carcinomas, hepatocellular carcinoma, breast adenocarcinoma, urethral carcinoma, colorectal cancer and esophageal-gastric cancers. These increased percentages of BAPC were correlated with increased numbers of tertiary lymphoid structures in the tumor microenvironment. Tumor antigen-specific B cells isolated from tumor-draining lymph nodes of cancer patients showed increased percentages of BACP. Furthermore, we demonstrated a strong induction of tumor-specific T cell responses by autologous BAPC using an antigen-specific fluorospot assay. Our results highlight the relevance of BACP as professional antigen-presenting cells in cancer.

F11

Dendritic cell vaccination of a type III gastric Neuroendocrine Tumor and a microsatellite-stable Colorectal Carcinoma patient: immunological and clinical responses

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Immunotherapy with checkpoint inhibitors is an effective treatment option for several tumor entities, including metastatic melanoma. Its use in microsatellite-stable colorectal carcinoma (msCRC), however, proved futile in clinical trials, whereas its role for most neuroendocrine tumors (NETs) is unclear at present. Here we present two heavily pretreated progressive cancer patients who obviously benefited from dendritic cell (DC)-vaccination performed in a compassionate setting. A 64-year-old male patient, diagnosed with gastric NET (type III, stage IVB) in 2010, showed widespread disease (including peritoneal metastases) progressing despite multiple surgeries, radiation therapy, several lines of combination-chemotherapies and immunotherapy with CTLA-4 blockade and IFN-α. In August 2013, we started vaccination therapy with autologous tumor RNA-loaded DCs as a monotherapy for 13 months, and the rate of progression declined. Due to renewed progression after 13 months double checkpoint inhibitor therapy with anti-PD1/L1 and anti-CTLA-4 was started under a continuation of DC vaccination. In 2015, we broadened the antigenic repertoire of our vaccine by peptides comprising individual mutations as predicted by next-generation sequencing (NGS). In 2016 and 2017 new metastases developed, which could be resected. NGS of those tumors led to a new set of peptides subsequently utilized for further vaccinations. Since then, the patient has been stable for approximately two years with his tumor now almost disappeared. Immune monitoring showed a de novo induction of functional (IFN-γ/ TNF α producing) T cell responses against 10 of the 24 peptides used for vaccination. Of note, the vaccine-specific T cells also became detectable in the resected metastases (2016 and 2017). The de novo responses against one of the peptides were robust, with ex vivo frequencies of up to 30% of the CD8+ T cells in the peripheral blood, a frequency so far never reported with any other vaccine. Encouraged by the positive course of disease in this patient, we started DC vaccination of a 40-year-old female upon consultation in 2014. The patient had initially been diagnosed with microsatellite-stable colorectal carcinoma in 2010 (CRC of the colon ascendens, G3, pT4a, pN2a, mucinous) and showed progression with metastases into the ovary and peritoneum despite surgery, multiple lines of chemotherapy and hyperthermic intraperitoneal chemotherapy. Autologous total tumor RNA-loaded DC vaccination therapy in combination with the angiogenesis-inhibiting anti-VEGF-A antibody bevacizumab was started in February 2014 as an adjuvant treatment. After a relapse with hepatic, splenic and peritoneal metastases in December 2015 anti-PD-1 therapy was added. Subsequently, we adapted our vaccine and loaded DC with RNA encoding the NRAS Q61R mutation, which was present in the patient's tumor, as well as with peptides derived from individual mutations based on the NGS. In 2017 the patient presented with new PET positive peritoneal lesions and elevated tumor markers. However, putative metastases removed in an exploratory laparoscopy some weeks later proved to be granulomas. Since then the patient is in complete remission under ongoing DC-vaccination in combination with anti-PD-1 therapy. These two cancer patients who progressed despite all available standard therapies but stabilized and then completely regressed under DC-vaccination combined with checkpoint blockade again encourage formal clinical studies using personalized DC vaccines. The two case reports also underscore our observation that patients vaccinated with DCs frequently experience stabilization of disease over the years, as shown by our group for a larger cohort of metastatic melanoma patients (Gross and Erdmann et al. 2017 JCI Insight) and for metastatic uveal melanoma (see abstract and poster 147, Moreira and Gross et al.). Thus, DC vaccination alone or in combination with checkpoint inhibitors may be a promising treatment option for patients even with advanced stage solid tumors refractory or insufficiently responsive to checkpoint blockade.

F12

Potent target cell effects make Dendritic Cell (DC)-derived extracellular vesicles (EV) an attractive tool in cellular therapy

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Objective: To determine the effects of Dendritic Cell (DC)-derived extracellular vesicles (EV) on target cells. Methods: Multi-antigen mapping and quantification after EV-injection into mice, FACS analysis. Introduction: Dendritic cells produce significant amounts of extracellular vesicles (EV) or exosomes for reasons that are not entirely clear. Notably, these vesicles contain a whole array of cytokines, chemokine and soluble factors (CCF). So far EV are considered to be a rich source of biomarkers. Only few studies explored their potential as therapeutic tools. Results: Here we demonstrate that extracellular vesicles (EV) secreted by mature human DC (maDC), differentiate peripheral monocytes into immature DC (imDC), expressing a unique marker pattern, including 6-sulfo-LacNAc (slan), Zbtb46, CD64 and CD14. In addition we find that maDC-EV are preferentially ingested by NK cells. In both cases the target cells started to proliferate. Upon a maturation stimulus maDC-EV-produced precursors developed into T cell activating and IL12p70-secreting maDC. NK cells that ingested DC-EV secreted IFN-γ and upregulated NKG2A. When injected into mouse skin, murine maDC-EV attracted immune cells including monocytes that developed activation markers typical for inflammatory cells. Importantly, skin- and i.v. injected EV also reached lymph nodes, causing a similar immune cell infiltration. Conclusion: We conclude that DC-derived EV could be used to target and activate the innate immune system (e.g. in lymph nodes) in order to support cancer therapy.

F13

Tissue derived non-classical monocyte derived host macrophages protect against murine intestinal acute graft-versus-host disease

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Acute graft versus host disease (aGVHD) remains a major complication in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT), the only curative treatment for many malignant hematologic diseases. After initial priming in secondary lymphoid organs, alloreactive donor T cells efficiently migrate to the intestinal tract, liver and skin. We observed that alloreactive effector T cells infiltrating and attacking the lamina propria of the small and large intestines closely interact with intestinal myeloid cells of host origin. Here we asked whether these intimate interactions regulate alloreactive effector T cell responses and how they impact intestinal aGvHD. To address these questions, we employed non-invasive bioluminescence imaging, fluorescence and confocal microscopy, clinical and histopathologic scoring, flow cytometry and single cell RNA sequencing in murine models of myeloablative, MHC-mismatched allo-HSCT. In the intestinal lamina propria, we observed that allogeneic T cells closely interacted with CD11b+CD11c+CD103- radio-resistant host type hematopoietic myeloid antigen presenting cells. Selective depletion of intestinal CD11chi or CX3CR1+CD11chi host cells 3 or 8 days after allo-HSCT accelerated alloreactive T cell infiltration, increased T cell mediated inflammatory cytokine production and exacerbated tissue damage resulting in hyperacute lethal aGvHD. These results suggested a strong immunoregulatory effect of these intestinal host-type myeloid cells. Single cell RNA-Seq analysis and flow cytometry (e.g. MHC II, CD11c, F4/80, CD26, CD64, CCR2, CX3CR1), lineage reporter- and defined knockout mice determined these cells as non-classical monocyte derived macrophages as the development and differentiation of these cells did not depend on Flt3, Zbtb46, and CCR2 but rather on CSF-1R and CX3CR1. Adoptive transfer, bone marrow chimeras and parabiosis experiments revealed that these non-classical monocyte derived macrophages differentiated from non-circulating non-classical monocytic precursors. Finally, PD-L1 expression on these intestinal host non-classical monocyte derived macrophages but not on stroma or other hematopoietic cells regulated alloreactive T cell responses in the intestinal tract. Based on these findings we postulate that a specialized and persistent subpopulation of host non-classical monocyte derived macrophages can potently suppress alloreactive T cells in the lamina propria of the intestinal tract. Fostering the differentiation and function of these tissue resident cells may represent an attractive therapeutic strategy to prevent intestinal aGvHD.

F14

Transendothelial migration of monocytes as potential therapeutical target for acute Graft-Versus-Host-Disease of the liver

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Acute Graft-Versus-Host-Disease (aGvHD) displays a severe complication with high morbidity and mortality in patients undergoing allogenic stem-cell transplantation urging for the use of strong immunosuppressive treatment strategies. Unfortunately, this approach may also undermine the favourable Graft-versus-Leukaemia effect potentially leading to relapse/progress of the underlying malignant disease. Therefore, more recent studies focus on preventing transendothelial accumulation of effector cells (i.e. Vedulizumab) to overcome these limitations. Prior to this study we analysed transendothelial accumulation of monocytic cells in inflammatory liver disease. For this purpose, we applied a unique transmigration assay (so called flow-based adhesion assays) equipped with primary hepatic sinusoidal endothelial cells (HSECs). Noteworthy, this assay is capable of completely visualising the transendothelial migration cascade of immune-cells (attachment to the apical side of the endothelial monolayer, the actual process of diapedesis, and finalisation of diapedesis where cells appear at the basolateral side). Using this method, we found that, despite similar rates of attachment to the apical side of HSECs, monocytes featured a rather enhanced potential for transendothelial accumulation across HSECs as compared to their immunosuppressive counterpart, so called myeloid-derived suppressor cells. This finding prompted as to investigate if this also holds true for aGvHD. In a first step, we compared monocytes from patients suffering from aGVHD to monocytes from healthy individuals showing an enhanced transmigratory potential in case of aGvHD. Moreover, to analyse as to whether this finding is specific for monocytes in aGvHD, we matched monocytes from stem cell recipients with or without clinical signs of aGvHD with a monocytic cell line (THP-1). Herein, we detected that, irrespective of aGvHD, monocytes obtained from stem-cell-recipients showed a more pronounced capacity to migrate across hepatic sinusoidal endothelium as compared to THP-1. However, when comparing monocytes obtained from aGvHD patients vs. non-aGvHD patients no significant differences was found, except for the rate of cells which have finalised transmigration (3,2-fold change in aGvHD-patients as compared to non-GvHD-patients using unstimulated endothelium and 5,4-fold change using TNFα-stimulated endothelium). Based on these findings, we speculated that an interaction with donor lymphocytes (DLI) might be the cause of the enhanced migratory capacity of monocytes in stem cell recipients. To test this hypothesis we analysed the transmigratory capacity of monocytes from healthy individuals following coculture with DLI. Using this approach on unstimulated endothelium monocytes showed a 4,9-fold enhanced progress of transmigration and an 2.7-fold change of transmigrated cells, as compared to their matched control. Moreover on stimulated endothelium, monocytes showed a 2,4-fold enhanced progress of transmigration and an 9.7-fold change of transmigrated cells. This preliminary data indicates that interaction of DLIs with monocytes leads to an enhanced accumulation of monocytes in the hepatic compartment but does not necessarily correlate with development of aGvHD. However, accumulation of monocytes might be the first trigger for the initiation of aGvHD, i.e. by stimulating quiescent T-cells, and therefore a potential therapeutical target, to prevent aGVHD in its beginnings.

Poster Session G

Suppressor Cells
(Treg-Cells, MDSCs, MSCs)

G1

AML exosomes promote TLR2-mediated induction of glycolytic MDSCs

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Acute myeloid leukemia (AML) is a heterogeneous hematologic disorder characterized by an expansion of immature myeloid blasts. In recent years myeloid-derived suppressor cells (MDSCs) have emerged as an important mediator of tumor-associated immune suppression. MDSCs are of myeloid origin, possess immune regulatory properties, and accumulate in various malignant entities including AML. Monocytic CD14+ cells with low levels of surface HLA-DR represent the most prominent MDSC subset in cancer patients. The mechanisms that induce the expansion of MDSCs, their metabolic changes and suppression feature of them in acute myeloid leukemia (AML) have not been well described. Here, we have demonstrated that AML-derived exosomes mediate conversion of monocytes into glycolytic MDSCs (CD14+HLA-DRlow/neg cells). We have shown that an interaction between AML-derived exosomes and monocytes determines the suppressive activity of the MDSCs via activation of MTOR pathway. In addition, Toll like receptor 2 (TLR2) was subsequently identified as the critical driver of exosome-mediated MDSC induction. TLR2 activation induces MTOR-dependent CREB phosphorylation that induces IL-10 secretion. This study aims to reveal possible mechanisms of MDSCs induction, and investigate important construct that can be targeted in MDSCs, thereby reducing tumor burden via blocking MDSCs formation and MDSCs-related suppression on bystander cells simultaneously.

G2

Exposing TNF receptor super family members as therapeutic targets for pancreatic cancer

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Patients suffering from pancreatic ductal adenocarcinoma have a dismal prognosis with a median survival of less than 9 months. Recently, it has become evident that pancreatic tumors are infiltrated with immune cells including regulatory T cells (Tregs). Tregs in pancreatic cancer patients are associated with poor prognosis and in preclinical animal models prevent efficient tumor-immune surveillance. Previously, we and others demonstrated the importance of the TNF receptor super family (TNFRSF) for the regulation of Tregs. Here we investigated potential TNFRSF targets on Tregs to apply new immunotherapeutic strategies against pancreatic cancer. To this end, we investigated tumor-infiltrating Tregs and conventional T cells in an immunocompetent mouse model of orthotopic pancreatic ductal adenocarcinoma. Tumors arise from Panc02 cells injected into the tail of the pancreas of C57Bl/6 mice. Employing bioluminescence imaging, we characterized the kinetics of luciferase expressing Treg-infiltration. Based on the observed dynamic changes, we investigated the expression of TNFRSF members, activation markers and immunomodulatory receptors with flow cytometry. Starting at day 4 after tumor-cell injection, Tregs infiltrated the pancreas and thereafter remained highly abundant in the tumor microenvironment. By day 14, rapid tumor progression was accompanied with increasing Treg numbers infiltrating the pancreas and even expanding in the spleens of tumor-bearing mice. Notably, no elevated Treg numbers could be detected in the peripheral blood, while Tregs in the tumor-draining pancreatic lymph nodes slightly increased. Inside the pancreatic tumors FoxP3+ Tregs constituted 33±7 % of all CD4+ T cells and almost outnumbered cytotoxic CD8+ T cells. All tumor-infiltrating conventional and regulatory T cell subsets displayed an activated phenotype expressing markers such as CD69, CD44, ICOS and ICAM-1. Importantly, tumor-infiltrating Tregs expressed high levels tumor of necrosis factor receptor 2 (TNFR2). Furthermore, TNFR2high Tregs were highly proliferative (Ki-67high) and comprised the suppressive CTLA-4high and CD73high Treg population. Tumor-infiltrating Tregs expressed further costimulatory members of the TNFRSF, but also immunomodulatory receptors of other families, notably PD-1 and TIGIT, yet were low for Lag-3. In our preclinical study, we demonstrate the dominance of Tregs in the tumor microenvironment of pancreatic cancer. We propose TNFR2 as highly attractive for therapeutically targeting Tregs in patients suffering from pancreatic cancer.

G3

Human double-negative regulatory T cells modulate functionality of conventional T cells via inhibition of mTOR signaling and metabolic reprogramming

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The recently discovered subpopulation of TCRαβ+ CD4-/CD8- (double-negative, DN) T cells are highly potent suppressor cells in mice and human. In murine transplantation models, adoptive transfer of DN T cells specifically inhibits alloreactive T cells and prevents development of transplant rejection or Graft-versus-Host-disease (GvHD). Interestingly, clinical studies in patients who underwent stem cell transplantation reveal an inverse correlation between the frequency of circulating DN T cells and the severity of GvHD, suggesting a therapeutic potential of human DN T cells. However, their exact mode of action has not been elucidated yet. Investigating the impact of DN T cells on conventional T cells, we show that DN T cells selectively inhibit the metabolic key regulator mTOR but not activation of mitogen-activated protein kinases. Enforced activation of the mTOR pathway by a chemical activator rendered conventional T cells unsusceptible to DN T cell-mediated suppression, confirming the critical function of mTOR signaling. Consistent with this finding, DN T cells diminished upregulation of glycolytic machinery, expression of glucose transporters and glucose uptake. In contrast, the uptake of fatty acids was not modified, indicating that DN T cells induced a metabolic switch in conventional T cells. Given that both, mTOR activity and cell metabolism are crucial in determining T cell fate, suppressed conventional T cell displayed an altered expression of differentiation markers, transcription factors and homing receptors. In addition, migratory capacity of conventional T cells towards inflammatory conditions was impaired after DN T cell contact. In contrast, expression of central memory-cell associated cell surface markers and transcription factors were increased by DN T-cells. Further analyses demonstrated that conventional T cells failed to produce effector cytokines IL-17 and IFN-y after coculture with DN T cells, whereas IL-2 secretion was further amplified. The selective modulation was induced by a direct cell-cell contact dependent mechanism between conventional and DN T cells and not due to competition for nutrients or growth factors. Taken together DN T cells impaired metabolic reprogramming of conventional T cells by abrogating mTOR signaling, thereby inducing a quiescent phenotype. These results reveal new and various targets of DN T cell mediated-suppression, pointing out that DN T-cells could serve as cell-based therapy to limit alloreactive immune response.

G4

T cell receptor repertoires and gene expression profiles of regulatory T cells after polyclonal or allo-specific in vitro expansion

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Allogeneic stem cell transplantation (SCT) is the treatment of choice for a variety of hematologic diseases but carries the inherent risk of acute graft-versus-host disease (aGVHD) caused by alloreactive donor T cells. We previously showed that the adoptive transfer of donor CD4+CD25+ regulatory T cells (Treg) prevents lethal aGVHD even in complete MHC-mismatched SCT models, a strategy that has already been confirmed in first clinical trials. More recently, we demonstrated the efficacy of donor Treg infusions as cellular therapy of established aGVHD. Since Treg cells are rare in peripheral blood (PB), in vitro expansion and production of pure cell products with a stable phenotype is a prerequisite for clinical applications. To investigate the T cell receptor (TCR) repertoire and gene expression profile of Treg after in vitro expansion, we isolated CD4+CD25+CD62L+Foxp3+ T cells from C57BL/6 Foxp3-gfp reporter mice and cultured them either in the presence of CD3/CD28-coated beads (polyclonal expansion, poly-expansion) or CD11c+ dendritic cells from MHC-mismatched BALB/c mice (allo-specific expansion, allo-expansion) with high-dose IL-2 for 14 d. Samples before and after in vitro expansion were collected, RNA was extracted and analysed by subsequent TCR repertoire sequencing and RNA sequencing. After poly-expansion of Treg for up to 14 days, more than 3K genes were found either down- or upregulated compared to freshly isolated Treg. Downregulated genes were associated with lymphocyte differentiation, while upregulated genes were associated with cell cycle, reflecting the expansion stimulus of the in vitro culture. Comparing both expansion procedures, genes upregulated in allo-expanded Treg were associated with chemotaxis, suggesting a possibly higher migratory potential. Poly expanded Treg at day 14 specifically lost genes associated with mitotic cell cycle, in line with the strongly reduced capacity of C57BL/6 Treg to expand beyond d7 of polyclonal activation. In accordance with protein expression data obtained by FACS analysis, allo- and poly-expanded Treg displayed comparable profiles across gene panels crucial for Treg function (transcription and other factors/chemokine receptors and integrins). TCR repertoire analyses of allo- and poly-expanded Treg cultures showed that clonotype diversity is maintained during poly-expansion, while a clear narrowing of the TCR repertoire was observed in allo-expanded Treg. Taken together, these results demonstrate that allo-specific expansion of Treg is a feasible and less finite alternative to polyclonal in vitro stimulation since Treg retained their regulatory phenotype in both expansion protocols. Additionally, allo-expansion results in the narrowing of the TCR repertoire, potentially enhancing Treg suppressive capacity in the aGvHD therapy setting.

G5

Definition of inflamed, immune-excluded and immune desert head and neck squamous cell carcinomas based on intratumoral cytotoxic and regulatory T-cells

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Background: The model of inflamed, immune excluded and immune desert tumors has been proposed to predict treatment response to immune checkpoint inhibitors. There exists no valid method for distinguishing these tumor types in cancer patients. The hypothesis of this analysis is that these tree types of cancer immune response can be distinguished based on intratumoral cytotoxic and regulatory T-cells. Methods: Tumor tissues of 280 patients with head and neck squamous cell carcinoma (HNSCC) treated with either neoadjuvant, definitive or adjuvant chemoradiotherapy were analyzed. Immunohistochemistry of cytotoxic T-cells (CD8+) and regulatory T-cells (FoxP3+) was performed as double staining. Cell density was analyzed with an image analyzing software separately in the intraepithelial and stromal compartment. Results: In the entire patient cohort high CD8 correlated with longer overall survival both in the stromal and the intraepithelial compartment. High Fox P3 correlated with longer OS in the stromal compartment but had no impact on prognosis in the intraepithelial compartment. Based on overall survival data the best cut off value for immune desert tumors was stromal CD8 less than 50 cells/mm². The best cut off value for distinguishing immune excluded from inflamed tumors was intraepithelial CD8 500cells/mm². This resulted in the following median cell densities (CD8/FoxP3): desert intraepithelial 104.3/0.0cells/mm², desert stromal 0.0/18.8, excluded intraepithelial 153.2/59.9, excluded stromal 316.0/228.0, inflamed intraepithelial 1083.5/153.2, inflamed stromal 314.5/156.8. In the defined immune desert group, stromal FoxP3 had no impact on prognosis, but intraepithelial high FoxP3 correlated with worse prognosis. In immune excluded tumors high stromal FoxP3 correlated with good prognosis, whereas intraepithelial it correlated with worse prognosis. In inflamed tumors high FoxP3 correlated with good prognosis both in the stromal and the intraepithelial compartment. Conclusion: Double staining of CD8 and FoxP3 helps to distinguish immune desert, immune excluded and inflamed HNSCC. The lacking prognostic value of intraepithelial FoxP3 becomes visible when the subgroups inflamed, immune excluded and immune desert are analyzed separately. Inflamed tumors with low FoxP3 density will probably show the best response to immune checkpoint inhibitors.

G6

Functional Conversion of cytotoxic T cells into iTreg in breast cancer patients.

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Regulatory T cells (Treg) have a detrimental effect in cancer-immunity, as they hinder tumor-eradication by effector T cells, directly promote cancer progression and their increased accumulation in cancer patients correlates with poor prognosis. Based on animal studies, Treg enrichment results predominantly from natural Treg expansion but also through the conversion of conventional T cells (Tconv) into induced Treg (iTreg) under tumor-suppressive conditions. However, it remains unknown whether tumor-specific iTreg generation occurs in humans and to which extent it contributes to the peripheral Treg pool. Therefore, we compared the TCRβ repertoire of total Treg and tumor-reactive Teff or total Tconv in peripheral blood of breast cancer patients or healthy control individuals, to identify overlapping TCRs, indicating iTreg generation. Blood-derived Treg and tumor-reactive Teff or total Tconv of patients and healthy individuals showed no major overlap, arguing against the existence of iTreg in the blood. However, single-cell Laser Microdissection and gDNA TCRβ sequencing revealed that tumor-derived Treg and Tconv share highly-expanded clones, representing 10-65% of tumor-infiltrating Treg. Thus, within the tumor expanding Tconv may convert to iTreg with a major impact on the intratumoral Treg pool. To prove this, we performed single-cell transcriptome analysis combined with TCRαβ sequencing of tumor-infiltrating Treg and Tconv from freshly resected breast tumors. Tumor-infiltrating CD4+ T cells accumulated into distinct transcriptome clusters consistent with a cytotoxic phenotype of Tconv and an immune-suppressive profile of Treg. Importantly, the cytotoxic and the immune-suppressive cluster shared single-cells with identical TCRαβ chains, demonstrating conversion of Tconv into iTreg in cancer patients. Interestingly, conversion associated with distinct genetic programs indicating potential mechanisms of T cell reprogramming in tumors.

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