ESH EUROPEAN SCHOOL OF HAEMATOLOGY

2nd TRANSLATIONAL RESEARCH CONFERENCE

IMMUNE & CELLULAR THERAPIES:

FOCUS ON ADVANCED GENE-ENGINEERED IMMUNE CELLS

#ESHIMMUNE2022

BERLIN, GERMANY

SEPTEMBER **12-14**, 2022

Chairs: **Chiara Bonini, Michael Hudecek, Stan Riddell**

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UPCOMING ESH CONFERENCES

ESH plenary conference programmes are designed to place emphasis on leading-edge basic and clinical research and future perspectives. Ample time is deliberately provided for in-depth scientific discussion and interaction. Registered participants further benefit from a variety of opportunities for informal scientific interaction with the world's leading experts in their field, including during small Meet the Expert sessions and Mentored Posters Walks. ESH conference delegates are invited to submit abstracts. Selected abstracts are presented as brief oral communications or posters.

HOW TO DIAGNOSE AND TREAT CONFERENCES

→ emphasize clinical management, including future perspectives, and use case-based lectures to stimulate interaction.

TRANSLATIONAL RESEARCH CONFERENCES

→ focus on leading-edge basic, clinical and therapeutic research and future perspectives. The presentation of unpublished data is encouraged.

2022

October 7-9, 2022 – Mandelieu-La Napoule, France **#ESHMM2022**
6th Translational Research Conference
MULTIPLE MYELOMA
Chairs: **M.V. Mateos, P. Moreau, V. Rajkumar**

October 21-23, 2022 – Mandelieu-La Napoule, France **#ESHCML2022**
24th Annual John Goldman Conference on
CHRONIC MYELOID LEUKEMIA: Biology and Therapy
Chairs: **J. Cortes, T. P. Hughes, D. S. Krause**

November 4-6, 2022 – Paris, France **#ESHLYMPHOMA2022**
3rd How to Diagnose and Treat
LYMPHOMA
Chairs: **C. Buske, M. Crump, C. Thieblemont**

November 18-20, 2022 – Paris, France **#ESHBMFS2022**
2nd ESH-EBMT Translational Research Conference
BONE MARROW FAILURE SYNDROMES: From the cell to the cure of the disease
Chairs: **T. Brümmendorf, R. Peffault de Latour, C. Dufour, A. Risitano**

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2023

February 5-7, 2023 – London, UK
4th Scientific Workshop
THE HAEMATOLOGICAL TUMOUR MICROENVIRONMENT AND ITS THERAPEUTIC TARGETING
Chairs: **D. Bonnet, M. Konopleva, D. Krause, S. Mendez Ferrer**

#ESHMICRO2023

March 3-5, 2023 – Berlin, Germany
3rd How to Diagnose and Treat
CML / MPN
Chairs **C. Harrison, A. Hochhaus, R. Mesa**

#ESHCMLMPN2023

March 3-5, 2023 – Paris, France
3rd Translational Research Conference
ERYTHROPOIESIS CONTROL AND INEFFECTIVE ERYTROPOIESIS: FROM BENCH TO BEDSIDE
Chairs**: M-D. Cappellini, M. Fontenay, S. Orkin**

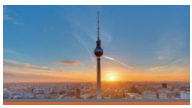
#ESHERYTHRO2023

April 14-16, 2023 – Berlin, Germany
4th How to Diagnose and Treat
MULTIPLE MYELOMA
Chairs: **H. Einsele, I. Ghobrial, M.-V. Mateos**

#ESHMM2023

May 19-21, 2023 – Berlin, Germany
3rd Translational Research Conference
ACUTE LYMPHOBLASTIC LEUKAEMIA
Chairs: **J. Cools, H. Dombret, A. Fielding, M. Litzow**

#ESHALL2023



September 29 – October 1, 2023 – Berlin, Germany
2nd How I manage
CAR-T THERAPIES AND BISPECIFIC ANTIBODIES FOR MY PATIENTS
Chairs: **U. Jäger, M.J. Kersten, L. Nastoupil**

#ESHCART2023

October 20-22, 2023 – Estoril, Portugal
7th Translational Research Conference
LYMPHOID MALIGNANCIES
Chairs: **M. Hallek, G. Lenz, L. Sehn, L. Staudt**

#ESHLYMPHOID2023

October 20-22, 2023 – Mandelieu-La Napoule, France
25th Annual John Goldman Conference on
CHRONIC MYELOID LEUKAEMIA: Biology and Therapy
Chairs: **J. Cortes, D. Krause, T. Hughes**

#ESHCML2023

October 29-31, 2023 – Estoril, Portugal
6th International Conference
ACUTE MYELOID LEUKAEMIA: "MOLECULAR AND TRANSLATIONAL": ADVANCES IN BIOLOGY AND TREATMENT
Chairs: **B. Löwenberg, H. Döhner, M. Tallman**

#ESHAML2023

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GENERAL INFORMATION:

Opening hours

Monday, September 12 – from 07:30 am CEST
Tuesday, September 13 – from 08:30 am CEST
Wednesday, September 14 – from 07:30 am CEST

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All participants, speakers and staff are expected to wear their name badges at all times in the breakout rooms, exhibition area and conference room.

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Presentation of data during ESH conferences does not constitute publication in the sense of patent law and the data presented therefore retains unpublished status.

Ask the faculty

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At this event, we want to make sure we address your most burning questions. Therefore, we will be using a simple audience interaction platform which allows you to submit your question online.
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1. Please take out your smartphones, tablets or laptop and connect to the WiFi (ESHIMMUNE2022)
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Please do not leave bags or suitcases unattended at any time, whether inside or outside session halls.

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The programme of the ESH 2nd Translational Research Conference: Immune & Cellular Therapies 2022 has been reviewed and approved for CME accreditation by the European Board for Accreditation in Haematology (EBAH). The EHA-CME Unit has approved this educational activity for a maximum number of 12 CME credits. Each physician should only claim credits for time that she/he actually spent in the educational activity.

The ESH 2nd Translational Research Conference: Immune & Cellular Therapies 2022 is committed to providing unbiased, balanced and objective educational and scientific programmes. In accordance with EBAH guidelines, all planners and speakers are asked to provide disclosures of their relevant financial relationships, which are available for audience review via a slide presentation. The CME provider has a mechanism in place to identify and resolve any conflicts of interest prior to the start of each presentation. Audience members will be asked to assess each presentation for the presence/absence of commercial bias.

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for supporting the Meet the Expert session:

Engineered NK cells for adoptive therapy

SCIENTIFIC PROGRAMME

2nd Translational Research Conference
IMMUNE & CELLULAR THERAPIES: Focus on Advanced Gene-Engineered Immune Cells
September 12-14, 2022 - Berlin, Germany
Chairpersons: Chiara Bonini (Milan), Michael Hudecek (Würzburg), Stan Riddell (Seattle)

Monday, September 12th, 2022

08:30–08:40	**Welcome**	Chiara Bonini (Milan) Michael Hudecek (Würzburg)
	SESSION I – Status of pre-clinical and clinical development of CAR-T cell therapy **Chairs: Michael Hudecek (Würzburg), Eliana Ruggiero (Milan)**	
08:40-08:45	Introduction	Michael Hudecek (Würzburg) Eliana Ruggiero (Milan)
08:45-09:05	CAR T cells: a road trip from the lab to clinic (and back)	Michael Hudecek (Würzburg)
09:05-09:10	*Discussion*	
09:10-09:30	Harnessing T memory stem cells for adoptive immunotherapy of cancer	Luca Gattinoni (Regensburg)
09:30-09:35	*Discussion*	
09:35-09:55	Tailoring adoptive cell therapy to the most promising tumor antigens in high-risk pediatric cancer	Sabine Heitzeneder (Stanford)
09:55-10:00	*Discussion*	
10:00-10:07	**Selected brief oral communication** *CSF1R blockade improves response to CAR T-cell in aggressive lymphoma*	Philipp Gödel (Cologne)
10:07-10:10	*Discussion*	
10:10-10:40	*Panel discussion*	
10:40-11:10	***Coffee Break***	
	SESSION II – Gene editing applied to engineered immune cell therapy **Chairs: Dirk Busch (Munich), Annette Künkele (Berlin)**	
11:10-11:15	Introduction	Dirk Busch (Munich) Annette Künkele (Berlin)
11:15-11:35	Pooled CRISPR Knockin Screens: Reprogramming Therapeutic T Cells	Franziska Blaeschke (San Francisco)
11:35-11:40	*Discussion*	

11:40-12:00	Engineering T lymphocytes with newly identified TCRs for the treatment of hematological and solid tumors	Eliana Ruggiero (Milan)
12:00-12:05	*Discussion*	
12:05-12:25	Advanced engineering of recombinant TCR expressing T-cells for adoptive immunotherapy	Dirk Busch (Munich)
12:25-12:30	*Discussion*	
12:30-12:37	**Selected brief oral communication** *EBAG9-silencing links cytolytic efficacy and memory formation in CD8+ T cells for improved cancer immunotherapy*	Anthea Wirges (Berlin)
12:37-12:40	*Discussion*	
12:40-13:10	*Panel discussion*	
13:10-14:50	***Lunch***	

SESSION III – Engineered immune cell therapy: Beyond T-cells
Chairs: Giulia Casorati (Milan), Jürgen Kuball (Utrecht)

14:50-14:55	Introduction	Giulia Casorati (Milan) Jürgen Kuball (Utrecht)
14:55-15:15	Adoptive immunotherapy of acute leukemia by iNKT cells retargeted against CD1c	Giulia Casorati (Milan)
15:15-15:20	*Discussion*	
15:20-15:40	Genetic engineering and bispecific formats inspired by γδT cells and their receptors	Jürgen Kuball (Utrecht)
15:40-15:45	*Discussion*	
15:45-15:52	**Selected brief oral communication** *Enhancement of CD33-CAR-NK Cell-Mediated Anti-Leukemic Cytotoxicity By Knockout of Immune Checkpoint NKG2A*	Evelyn Ullrich (Frankfurt)
15:52-15:55	*Discussion*	
15:55-16:02	**Selected brief oral communication** *Early cancer immune surveillance via Vg9Vd2 TCR T cells regulated by novel BTN3A1-linked protein network*	Astrid Cleven (Utrecht)
16:02-16:05	*Discussion*	
16:05-16:40	*Panel discussion*	
16:40-17:10	***Coffee break***	

SESSION IV – Engineered immune cell therapy: Beyond CARs
Chairs: Aude Chapuis (Seattle), Mirjam Heemskerk (Leiden)

17:10-17:15	Introduction	Aude Chapuis (Seattle) Mirjam Heemskerk (Leiden)
17:15-17:35	Tackling mechanisms of immune escape to TCR therapy	Aude Chapuis (Seattle)
17:35-17:40	*Discussion*	
17:40-18:00	Optimization of TCR gene therapy and preventing tumor escape	Mirjam Heemskerk (Leiden)
18:00-18:05	*Discussion*	
18:05-18:25	New mechanisms of T-cell recognition via dissection of successful cancer immunotherapy	Andrew Sewell (Cardiff)
18:25-18:30	*Discussion*	
18:30-18:37	**Selected brief oral communication** *Pre-clinical development and characterization of a decitabine-induced regulatory HLAG+CD4+-T cell-enriched cell product against GvHD*	Dionysia Kefala (Rio)
18:37-18:40	*Discussion*	
18:40-18:47	**Selected brief oral communication** *CAR-Tregs for Systemic Lupus Erythematosus*	Matteo Doglio (Milan)
18:47-18:50	*Discussion*	
18:50-19:20	*Panel discussion*	
19:20-20:30	**Mentored poster walk - Welcome get-together buffet dinner for all** Leaders (biology): Mirjam Heemskerk (Leiden), Luca Gattinoni (Regensburg) Leaders (clinical): Aude Chapuis (Seattle), Annette Künkele (Berlin)	

Tuesday, September 13th, 2022

09:00-10:00 **3 simultaneous Meet the Expert sessions**

1.	Engineering CAR-Ts for solid tumors	Michael Jensen (Seattle)
2.	Gene Transfer and gene editing for pediatric CAR-Ts	Annette Künkele (Berlin) Franziska Blaeschke (San Francisco)
3.	Development and genetic engineering of TCR-transgenic T cells	Dirk Busch (Munich)

10:00-10:30 ***Coffee break***

SESSION V – Synthetic biology in engineered immune cell therapy
Chairs: Yvonne Chen (Los Angeles), Chiara Bonini (Milan)

10:30-10:35	Introduction	Yvonne Chen (Los Angeles) Chiara Bonini (Milan)

10:35-10:55	Engineering next-generation CAR-T cell therapy for cancer	Yvonne Chen (Los Angeles)
10:55-11:00	*Discussion*	
11:00-11:20	Synthetic solutions for improving CAR-T solid tumor immunotherapy	Michael Jensen (Seattle)
11:20-11:25	*Discussion*	
11:25-11:45	Translation of new CAR-T cells into the clinic	Marcela Maus (Boston)
11:45-11:50	*Discussion*	
11:50-11:57	**Selected brief oral communication** *Improving bone marrow homing and rersistence of CD33. CAR-CIK cells by overexpression of a gain-of-function variant of CXCR4 to increase efficacy in acute myeloid leukemia*	Marta Biondi (Monza)
11:57-12:00	*Discussion*	
12:00-12:30	*Panel discussion*	
12:30-14:35	***Lunch***	

13:25-14:25 **SATELLITE SYMPOSIUM**
CAR-T in clinical practice: exploring the evidence for treatment of r/r FL and r/r DLBCL
Chair: Antonia Müller (Vienna)
Speakers: Christian Buske (Ulm), Roch Houot (Rennes), Annalisa Chiappella (Milan)

This symposium is organized and sponsored by Novartis

SESSION VI – Engineered immune cell therapy: B-cell tumors and beyond
Chairs: Michael Hudecek (Würzburg), Marcela Maus (Boston)

14:35-14:40	Introduction	Michael Hudecek (Würzburg) Marcela Maus (Boston)
14:40-15:00	Real life anti-CD19 CAR-T cell therapy in relapsed/refractory Lymphoma patients	Cristiana Carniti (Milan)
15:00-15:05	*Discussion*	
15:05-15:25	CAR-engineered NK cells - Empowering the first line of defense against cancer	Winfried Wels (Frankfurt)
15:25-15:30	*Discussion*	
15:30-15:50	Modification of GD2.car t-cells with a constitutively active IL7 receptor for the treatment of GD2 expressing malignancies	Cliona Rooney (Houston)
15:50-15:55	*Discussion*	
15:55-16:02	IF-BETTER-gated CAR T cells detect combinatorial target signatures and prevent antigen-low AML escape.	Sascha Haubner (New York)
16:02-16:05	*Discussion*	

16:05-16:12	**Selected brief oral communication** *Pharmacologically inhibiting MYCN improves L1CAM-directed CAR T cell efficacy against MYCN-amplified neuroblastoma*	Laura Grunewald (Berlin)
16:12-16:15	*Discussion*	
16:15-16:45	*Panel discussion*	
16:45-17:05	***Coffee break***	

SESSION VII – Going universal with engineered immune cells
Chairs: Maria Themeli (Amsterdam), Cliona Rooney (Houston)

17:05-17:10	Introduction	Maria Themeli (Amsterdam) Cliona Rooney (Houston)
17:10-17:30	The big bang of universal immune receptors	Daniel Powell (Philadelphia)
17:30-17:35	*Discussion*	
17:35-17:55	Switchable CAR-T for safe and efficient targeting of less differentiated antigens	Marc Cartellieri (Dresden)
17:55-18:00	*Discussion*	
18:00-18:20	iPSC-derived CAR-T and CAR-NK	Maria Themeli (Amsterdam)
18:20-18:25	*Discussion*	
18:25-18:32	**Selected brief oral communication** *Pluripotent STEM cell-derived CAR-neutrophils for immunotherapy of SOLID tumors*	Igor Slukvin (Madison)
18:32-18:35	*Discussion*	
18:35-19:05	*Panel discussion*	

Wednesday, September 14th, 2022

08:00-09:00 **3 simultaneous Meet the Expert sessions**

1. Engineered NK cells for adoptive therapy — Winfried Wels (Frankfurt)
2. Imaging of CAR & TCR art work — Johannes Huppa (Vienna)
3. Synthetic immunity concepts – logic gates and universal cells — Yvonne Chen (Los Angeles), Maria Themeli (Amsterdam)

SESSION VIII – Overcoming T-cell exhaustion and the immune-suppressive tumor microenvironment
Chairs: Chiara Bonini (Milan), Daniel Powell (Philadelphia)

09:10-09:15	Introduction	Chiara Bonini (Milan) Daniel Powell (Philadelphia)

09:15-09:35	Genome editing to overcome T-cell exhaustion	Chiara Bonini (Milan)
09:35-09:40	*Discussion*	
09:40-10:00	Mechanisms that adjust and control the T cell effector capacity	Dietmar Zehn (Freising)
10:00-10:05	*Discussion*	
10:05-10:25	Decoding the mode of action of CAR-T cells using intravital imaging	Philippe Bousso (Paris)
10:25-10:30	*Discussion*	
10:30-10:37	**Selected brief oral communication** *Joint forces - synergizing microbiome-derived metabolites and T cell engineering to combat solid tumors*	Maik Luu (Wüzburg)
10:37-10:40	*Discussion*	
10:40-11:10	*Panel discussion and/or controversial debates*	
11:10-11:40	***Coffee break***	

SESSION IX – Imaging engineered immune cells at the receptor, cell and organism level
Chairs: Johannes Huppa (Vienna), Michael Jensen (Seattle)

11:40-11:45	Introduction	Johannes Huppa (Vienna) Michael Jensen (Seattle)
11:45-12:05	Interrogating TCR & CAR function with high-end microscopy	Johannes Huppa (Vienna)
12:05-12:10	*Discussion*	
12:10-12:30	Super-resolution fluorescence imaging of plasma membrane receptors by dSTORM	Markus Sauer (Würzburg)
12:30-12:35	*Discussion*	
12:35-12:55	Imaging and monitoring engineered immune cells art work	Louisa von Baumgarten (Munich)
12:55-13:00	*Discussion*	
13:00-13:07	**Selected brief oral communication** *Superresolution microscopy reveals disparity in CAR expression and function of CAR T cell generated using distinct gene-transfer strategies*	Leon Gehrke (Wüzburg)
13:07-13:10	*Discussion*	
13:10-13:40	*Panel discussion*	
13:40-13:50	***Closing remarks***	Chiara Bonini (Milan) Michael Hudecek (Würzburg)

MEETING CLOSURE

LIST OF SPEAKERS

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SASCHA HAUBNER
Center for Cell Engineering
Memorial Sloan Kettering Cancer Center
New York
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SESSION I – STATUS OF PRE-CLINICAL AND CLINICAL DEVELOPMENT OF CAR-T CELL THERAPY

Chairs: Stan Riddell (Seattle), Eliana Ruggiero (Milan)

STANLEY RIDDELL (SEATTLE)

CAR T CELLS – A ROAD TRIP FROM THE CLINIC BACK TO THE LAB

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Adoptive cell therapy (ACT) using T cells modified by gene transfer to express a tumor reactive T cell receptor (TCR) or chimeric antigen receptor (CAR), can be effective in refractory malignancies[1-3]. The most dramatic results are in B cell malignancies where CAR T cells (CAR-T) specific for CD19, CD20, or CD22 have potent antitumor activity[4-9]. Work in our lab[10-13] provided the foundation for Liso-Cel, a CD19/4-1BB/CD3z CAR expressed in a defined composition of CD4/CD8 T cells that is FDA approved for treatment of refractory and relapsed diffuse large B cell lymphoma (DLBCL) [14,15]. We also advanced BCMA CAR-T cells to the clinic alone and combination with a gamma secretase inhibitor (GSI) for multiple myeloma[16]. These studies have resulted in impressive response rates in clinical trials however many patients either fail to respond or progress after an initial response. Several obstacles have been shown to limit the efficacy of T cells engineered with synthetic receptors including the escape of tumor cells that express low levels of antigen or lose antigen expression, and the inability transferred T cells to persist or remain functional[17]. I will discuss strategies being pursued in our lab to overcome these barriers.

CAR-T cells target tumor cell surface molecules in an MHC independent fashion. We and others have shown that CARs do not fully engage the TCR signaling machinery and require much higher Ag density on target cells than is necessary for TCR recognition of peptides bound to major histocompatibility complex (MHC) molecules[18-20]. To overcome this barrier and improve receptor sensitivity, we designed functional hybrid receptors (TCR/CARs) by fusing variable heavy (V_H) and variable light (V_L) chains of monoclonal antibodiees to TCR alpha and beta constant chains. Mispairing with endogenous TCR chains can be avoided by highly efficient knock-out of TRAC and TRBC genes using base editing. TCR/CARs retain MHC independent recognition, associate with all CD3 chains of the TCR complex, and upon antigen binding induce phosphorylation of ITAMs on all CD3 chains. Unlike some CARs that tonically signal in the absence of ligand binding and drive T cell dysfunction, TCR/CAR hybrid receptors lack tonic signaling and exhibit improved recognition of tumor cells expressing low levels of antigen.

The efficacy of CAR T cells in adoptive therapy also correlates with the presence of less differentiated subsets of T cells in the product[21,22]. Current approaches for producing CAR and TCR engineered T cells use stimulation with beads coated with anti CD3 to mimic signal 1 and anti CD28 to mimic signal 2 and culture in high concentrations of gamma chain cytokines (IL-2, IL-7 and IL-15). These conditions promote effector T cell differentiation, potentially limiting the ability of the cells to persist long term. We have studied alternative signaling pathways and designed protein ligands for activating these pathways in T cells to promote retention of memory differentiation and augment persistence and function of therapeutic T cells.

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LUCA GATTINONI (REGENSBURG)

HARNESSING T MEMORY STEM CELLS FOR ADOPTIVE IMMUNOTHERAPY OF CANCER

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Immunotherapies based on the adoptive transfer of T cells engineered with chimeric antigen receptors (CAR) are revolutionizing medical oncology[1]. While these treatment modalities have demonstrated remarkable activities against hematologic malignancies, a significant fraction of patients fails to respond or experiences tumor relapse within a year after CAR T cell transfer, underscoring the need to further improve these types of treatment[1]. Most of CAR T cells in the market and under clinical evaluation are manufactured from unselected T cell populations isolated from patients' or donors' peripheral blood, resulting in phenotypically and functionally heterogeneous cell products. It is now well established that the differentiation state of transferred T cells is a critical parameter influencing treatment efficacy[2-4]. Specifically, CAR products enriched in early memory T cells endowed with stem cell-like attributes have been associated with successful antitumor responses across several studies[5-7]. Thus far, the clinical exploitation of stem-like T cells has been hindered by their relative paucity in the circulation. We previously developed a clinical-grade platform for manufacturing CAR-modified T_{SCM} cells and showed that these cells mediate superior and long-lasting anti-leukemic activity compared to conventional CD19-CAR T cells against systemic acute lymphoblastic leukemia xenografts[8]. Here, I will discuss initial findings on a first in human trial employing donor-derived CD19-CAR (FMC63-28z) T_{SCM} cells as salvage therapy in patients with CD19+ malignancies relapsing after allogeneic stem cell transplantation (alloHSCT). No lymphodepletion chemotherapy or other therapies were administered. T cells were obtained from each recipient's alloHSCT donor. Eleven patients with $CD19^+$ malignancies refractory to alloHSCT were treated (dose range from 0.25 x 10^6/kg to 2 x 10^6/kg). Complete remission (CR) was observed for cell doses as low as 0.25 x 10^6/kg. Overall response rate for cell doses $\geq 10^6$/kg was 71% with 43% CRs. Treatment was well tolerated with a single patient developing a grade 3 cytokine release syndrome (CRS). No graft versus host disease nor immune effector cell-associated neurotoxicity syndrome (ICANS) were observed. Comparable doses of conventional CD19-CAR T cells engineered with the same CAR construct and administered to a similar subset of patients at the same Institution resulted in fewer objective responses (OR 28%; CR 14%) and higher rates of grade 3-4 toxicities[9]. CAR-modified T_{SCM} cells exhibited a delayed, but higher peak expansion, with lower production of inflammatory cytokines, and reduced inflammatory responses compared to conventional CAR cell T products. Preliminary observations using integration site analysis to track clonal expansion and differentiation trajectories in the circulation revealed distinct waves of clonal expansion. No evidence of aberrant clonal behavior was observed. Moreover, CAR T cell clones that persisted were preferentially derived from T_{SCM} cells that did not participate in the early expansion phase. Lastly, resistance to treatment was largely due to antigen escape, rather than poor persistence, and severe CRS was found to be driven primarily by tumor burden. These data suggest that CAR T_{SCM} cells display a higher therapeutic index compared with conventional CAR T cells.

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SABINE HEITZENEDER (STANFORD)

TAILORING ADOPTIVE CELL THERAPY TO THE MOST PROMISING TUMOR ANTIGENS IN HIGH-RISK PEDIATRIC CANCER

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No significant improvement in survival rates for children with the most high-risk solid tumors and brain tumors has been made in the last four decades due to the lack of potent and safe targeted therapies. Standard therapies continue to comprise multimodal regimens, including dose-intensive cytotoxic chemotherapy, resulting in substantial risks of lifelong late effects, including secondary malignancies. Still, primary refractory or recurrent disease remains universally lethal with very few treatment options. Engineered T cells (TCRs/CARs) are a promising treatment modality, capable of mediating impressive clinical anti-tumor activity with acceptable toxicity. Developing such synthetic immunotherapies for the treatment of childhood cancer is of particular significance because they can target non-mutated tumor antigens and unlike adult malignancies, pediatric cancer manifests with extremely low mutational burden(1). However, the development of potent and safe cell therapies relies on promising tumor-restricted target antigens, that are absent from any normal, vital tissue in the body. Conventional methods to detect tumor-antigens often fail to identify those and rather yield targets that are overexpressed in tumors but not truly tumor-specific, which has caused significant toxicity from on-target/off-tumor recognition of vital normal tissue in the body. Therefore, novel pipelines for rational target antigen discovery are critical for advancing synthetic immunotherapies for the treatment of the most aggressive childhood malignancies. To identify tumor antigens that meet the requirement for adoptive cellular immunotherapy, differential gene expression analysis of compiled transcriptomes from tumors and normal tissues incorporating bioinformatic cell-surface annotation filtering has proven to identify top differentially expressed targets best suited for CAR T cell approaches, such as the placental antigen PAPP-A in Ewings sarcoma(2) and the oncofetal antigen Glypican-2 (GPC2) in Neuroblastoma and Medulloblastoma(3). Once target antigens are identified, adoptive cell therapy approaches require elaborate tailoring for optimal preclinical efficacy and safety. For CAR T cells, antigen density has emerged as a major factor determining their potency and traditional designs (CD8a hinge-transmembrane, 41BB co-stimulatory domain) manifest a high antigen density threshold for full activation, increasing the risk of relapse with antigen low variants, as observed following CARs targeting CD22(4, 5). This clinical experience emphasizes the critical interplay between CAR design and antigen density threshold and highlights the need to measure accurate levels of target molecules/cell on patient's tumor cells and iteratively engineer CAR modules towards clinically relevant levels. Accurate antigen density quantification of GPC2 on clinical neuroblastoma tumor samples metastatic to the bone-marrow by Flow Cytometry found clinically relevant GPC2 site density to be ~5,000 molecules/cell. We developed GPC2 CAR T cells and achieved potent and durable tumor eradication in preclinical models of neuroblastoma expressing clinically relevant GPC2 antigen density, in the absence of toxicity by iterative engineering of transmembrane (TM) and co-stimulatory domains plus overexpression of c-Jun to lower the antigen density threshold of GPC2-CAR T cells(6). Ongoing work shows similar promising preclinical activity in orthotopic medulloblastoma models

upon locoregional CAR delivery. These efforts culminate in a planned phase 1 clinical trial of GPC2-CAR T cells for children and young adults with recurrent/refractory neuroblastoma and medulloblastoma at Stanford University in 2023, supported by the NCI Experimental Therapeutics (NExT) program.

More than 70% of the human proteome represent intracellular molecules, a target-pool that is accessible to engineered TCRs through presentation of peptides via MHC. True antigen landscapes for TCRs are determined by empiric immunopeptidome analysis and largely unknown in childhood cancer. To profile the immunopeptidome in Ewing Sarcoma, we have eluted peptides from MHC-class I of Ewing Sarcoma cells by immunoprecipitation with a pan-MHC-I antibody, followed by liquid chromatography and tandem mass spectrometry (LC-MS/MS). No neoantigenic peptides caused by common mutations in EWS or the EWS-Fli1 breakpoint region were found. Instead, incorporating this data into transcriptomic differential gene expression analysis to identify targets best suited for TCR-based therapies identified peptides from oncofetal and cancer-testis antigens for TCR-based treatment approaches. In conclusion, truly tumor-specific antigens are rare in pediatric solid tumors and brain tumors, however, they often manifest stalled fetal developmental programs and continue to express placental, oncofetal and cancer testis antigens as an attractive pool of effective and safe immune targets. Comprehensive analysis of the surfaceome and immunopeptidome has proven an efficient method to identify such candidate targets which hold tremendous potential for the development of adoptive cellular immunotherapies for childhood cancers, which are characterized by otherwise low immunogenicity.

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Sahaf B, Alag A, Fetahu IS, Hasselblatt M, Parker KR, Anbunathan H, Hwang J, Huang M, Sakamoto K, Lacayo NJ, Klysz DD, Theruvath J, Vilches-Moure JG, Satpathy AT, Chang HY, Lehner M, Taschner-Mandl S, Julien JP, Sorensen PH, Dimitrov DS, Maris JM, Mackall CL. GPC2-CAR T cells tuned for low antigen density mediate potent activity against neuroblastoma without toxicity. Cancer Cell. 2022;40(1):53-69 e9. Epub 2022/01/01. doi: 10.1016/j.ccell.2021.12.005. PubMed PMID: 34971569.

SESSION II – GENE EDITING APPLIED TO ENGINEERED IMMUNE CELL THERAPY

Chairs: Dirk Busch (Munich), Katy Rezvani (Houston)

FRANZISKA BLAESCHKE (SAN FRANCISCO)

POOLED CRISPR KNOCKIN SCREENS: REPROGRAMMING THERAPEUTIC T CELLS

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While genetically modified CAR (chimeric antigen receptor) T cells have emerged as powerful treatment options for some malignancies, T cell dysfunction through tonic signaling and repetitive antigen stimulation can limit potency of adoptive cell therapies. (Esensten et al., 2017; Fesnak et al., 2016; Fraietta et al., 2018; Gardner et al., 2017; June and Sadelain, 2018; Maude et al., 2018). Engineering therapeutic T cells with improved fitness in contexts that otherwise predispose T cells to dysfunction is a promising strategy to improve clinical responses.

Based on previous advancements in non-viral CRISPR/Cas9-based knockins (Roth et al., 2020; Roth et al., 2018), we have developed Modular Pooled Knockin (ModPoKI) to rapidly screen hundreds to thousands of DNA sequences combined with a clinically relevant TCR or CAR. We generated three ModPoKI libraries: a 100-member transcription factor (TF) library; a 129-member surface receptor (SR) library containing synthetic checkpoint/cytokine/death switch receptors, surface receptors, and chemokine receptors; and a ~10,000-member combinatorial TFxTF library. Using bead stimulation, target-cell stimulation, repetitive stimulation and tonic signaling assays, we identified naturally occurring transcription factors and surface receptors as well as synthetic genes that can improve TCR/CAR T cell fitness. In validation assays, a novel BATF and TFAP4 multi-gene knockin improved tumor control of tonic signaling GD2 CARs *in vitro* and *in vivo* while promoting a stem cell-like and central memory phenotype with high levels of CD25 expression.

In summary, these studies highlight large-scale modular pooled knockin screens as a powerful method to accelerate synthetic biology programming of cell states with enhanced durability and therapeutic functions.

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ELIANA RUGGIERO (MILAN)

ENGINEERING T LYMPHOCYTES WITH NEWLY IDENTIFIED TCRS FOR THE TREATMENT OF HEMATOLOGICAL AND SOLID TUMORS

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Empowering T cells with new T cell receptors (TCR) can effectively redirect some of the most potent players of our immune system against virtually all tumor antigens. Dressing T cells with novel identified tumor-specific TCRs provides patient's body with a highly specific and effective living drug harboring the ability to patrol for tumor recurrence. Even though TCRs have so far operated in the shadow of CARs, they harbor 2 main advantages: 1) the ability to target surface and intracellular epitopes and 2) the higher sensitivity. Furthermore, the therapeutic value of TCR-based approaches has proved promising in clinical trials for solid tumors, such as melanoma and, more recently, pancreatic cancer[1]. However, due to the low frequency of cancer-specific T cells, pinpointing the exact tumor-specific TCR remains the major bottleneck for the broad use of TCR-based therapies in clinical practice.

Hence, there is an emerging need to develop strategies for identifying novel TCRs specific for tumor antigens which can be used to genetically engineer T lymphocytes and increase their applicability as a cancer immunotherapeutic approach.

Recently, focusing on Wilms Tumor 1 (WT1), a tumor antigen expressed on a multitude of blood and solid malignancies, and by longitudinal monitoring T cell functionality and dynamics in 14 healthy donors, we designed and implemented a pipeline for the rapid isolation of WT1-specific T cells and for the thorough characterization of a library of tumor-specific TCRs. With this approach, we isolated 19 WT1-specific receptors recognizing peptides restricted by different human leukocyte antigens (HLA) and displaying a wide range of functional avidities, including some in the low nM range. By applying a funnel selection strategy, 2 high avidity TCRs were selected and inserted in T lymphocytes upon disruption of the endogenous TCR repertoire[2]. TCR targeted integration into the TRAC locus combined with TRBC knock-out, was successfully employed to generate a T cell product with an improved biosafety profile while preserving T cell fitness. A nearly complete redirection of T cell specificity was achieved also when circulating T cells harvested from AML patients were used as starting material, thus highlighting the compliance of the manufacturing protocol to clinical translational. Of note, one TCR showed antigen-specific responses in CD4 and CD8 T-cells, and efficiently eliminated patients' blasts and a glioblastoma cell line in vitro and in vivo in the absence of off-tumor toxicity[3]. T cells engineered to express this receptor are being tested in a clinical trial for AML immunotherapy. Stemming from these results, we have now built a library of 56 anti-tumor TCRs recognizing epitopes of antigens expressed by hematological and solid tumors (including cold malignancies) and restricted to HLA alleles common in the Caucasian population. Newly discovered TCRs are currently being functionally tested and prioritized with the final aim of rendering TCR-based immunotherapies an effective off-the-shelf treatment readily accessible to each tumor patient.

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DIRK BUSCH (MUNICH)

ADVANCED ENGINEERING OF RECOMBINANT TCR EXPRESSING T-CELLS FOR ADOPTIVE IMMUNOTHERAPY

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Effective antigen-specific T cell immune responses are usually characterized by the initial recruitment of polyclonal populations from the naïve compartment, consisting of a repertoire of different T cell receptors (TCRs) with varying affinities/avidities for cognate pMHC ligands. The underlying mechanisms of recruitment and maintenance of polyclonality, as well as its relevance for the quality of immunity during acute and chronic diseases, as well for immune memory are not well understood. This is mainly due to technical hurdles in studying functionally 'polyclonality' in preclinical experimental models as well as directly in humans.

By implementing and developing advanced technologies (like single cell TCR sequencing/identification, traceable TCR retrogenic T cells, Crispr-Cas9-mediated orthotopic TCR replacement, structural and functional TCR avidity measurement, high-throughput TCR reporter cell screening) we are now in a position to analyze T cell polyclonality in hitherto unmatched detail. First findings during infections and anti-tumor responses demonstrate that T cells with high-avidity antigen receptors are rare within the naïve repertoire and initially a much larger fraction of low-avidity T cells gets recruited until high-avidity T cells dominate the acute response. During chronic antigen-exposure, evolutionary changes occur and lower-avidity T cells can become the prevalent subset.

We believe that a better understanding of T cell polyclonality during normal physiological immune responses in health and disease will not only help to improve our understanding of immunity and immune pathology, this knowledge will also be relevant for the engineering of most effective T cell products (including CAR-T cells) for adoptive T cell therapy.

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SESSION III – ENGINEERED IMMUNE CELL THERAPY: BEYOND T-CELLS

Chairs: Giulia Casorati (Milan), Jürgen Kuball (Utrecht)

GIULIA CASORATI (MILAN)

ADOPTIVE IMMUNOTHERAPY OF ACUTE LEUKEMIA BY INKT CELLS RETARGETED AGAINST CD1C

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Adoptive immunotherapy with T cells engineered with TCRs specific for tumor antigens is a promising strategy for cancer treatment. The TCR recognition of peptides derived from tumor-associated antigens and presented by MHC molecules determines the target specificity of the approach. However, the elevate MHC polymorphism implies the necessity to isolate different tumor-specific TCRs, each restricted for the most frequent HLA alleles in order to treat as many patients as possible. Furthermore, the endogenous TCR repertoire expressed by the tumor-redirected T cells can react against allogeneic MHC alleles of the recipient, restricting its application to autologous clinical setting. Recent data show the existence also of tumor-specific T cells restricted by monomorphic antigen-presenting molecules, suggesting the possibility to use their TCRs to overcome this hurdle and treat all patients independently of their MHC. CD1 molecules are non-polymorphic MHC class I-related proteins that present lipid antigens to T cells and, being identical in all individuals, may fulfil the above requirement. Human CD1 molecules are classified into group 1 (CD1a, CD1b, CD1c), group 2 (CD1d) and group 3 (CD1e) and are mainly expressed on cells involved in antigen presentation. Group 1 CD1 molecules are exclusively expressed on mature myelomonocytic and B cells, implying their presence also in cancers of hematological origins, which are the malignant counterparts of the normal cells that express them. Indeed, we have shown that acute myeloid and lymphoblastic leukemia express CD1 molecules at high frequency, particularly CD1c that was found in 40% of Acute Myeloid Leukemia (AML), 70% of B cell Acute Lymphoblastic Leukemia (B-ALL), 23% of T cell Acute Lymphoblastic Leukemia (T-ALL) in adults, and 45% of AML, 26% B-ALL and 12% T-ALL in pediatric patients (1,2). We also described the isolation of a group of self-reactive T cell clones from healthy donors that directly recognize leukemia cell lines in a CD1c-dependent manner (1-3). These clones were found to recognize a novel self-lipid antigen (methyl lysophosphatidic acid, mLPA) that is highly enriched in primary AML and B-ALL blasts and leukemia cell lines, while it is very low in primary circulating monocytes and B cells, suggesting that mLPA is a leukemia-associated lipid antigen for CD1c-restricted T cells (1). We identified a lead mLPA-specific TCR (DN4.99) that was selected for its overall superior behavior on transduced T cells and highest reactivity against several targets, and we showed that primary T cells engineered with the DN4.99 TCR efficiently killed disparate CD1c-expressing leukemia cell lines and primary AML, B-ALL and T-ALL blasts in vitro, and significantly delayed the progression of CD1c-expressing leukemia xenografts in NSG mice (2). By contrast, DN4.99 TCR-T cells spared primary circulating CD1c-expressing monocytes, DCs and B cells (1,2), in line with their minimal mLPA content. The lack of polymorphism makes CD1c molecules universally targetable in any individual, across MHC barriers, while its absence in early hematopoietic precursors and parenchymatous cells provides a favourable safety profile for mLPA-specific TCR-engineered T cells in terms of on target/off tumor reactivity (1,2). To further

improve our therapeutic strategy, we investigated the possibility to harness the unique CD1d-restricted invariant NKT (iNKT) cells with our mLPA-specific TCR. Indeed we (3,4) and others (5-7) have shown that the iNKT cells can control tumor progression by restricting immunosuppressive myeloid populations infiltrating the microenvironment of solid or hematological malignancies. CD1d is also nonpolymorphic, permitting donor-unrestricted iNKT cell functions and, although it can be found expressed at low levels also on parenchymatous cells (hepatocytes, intestinal epithelium, keratinocytes, Schwann cells) (8), it does not trigger iNKT cell self-reactivity and toxicity in clinical trials, including adoptive immunotherapy with GD2 CAR-iNKT cells (9). We successfully engineered iNKT cells with mLPA- specific TCR generating a single immune effector (TCR-iNKT) endowed with the simultaneous ability to kill leukemia cells and restrict the suppressive TME. Our results highlight a novel approach for ACT of acute leukemia with T or iNKT cells engineered to recognize malignant cells by the transfer of a lipid-specific TCR that works across MHC-barriers like a CAR.

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JÜRGEN KUBALL (UTRECHT)

GENETIC ENGINEERING AND BISPECIFIC FORMATS INSPIRED BY γδT CELLS AND THEIR RECEPTORS

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Clinical responses to checkpoint inhibitors used for cancer immunotherapy seemingly require the presence of αβT cells that recognize tumour neoantigens, and are therefore primarily restricted to tumours with high mutational load. Approaches that could address this limitation by engineering αβT cells, such as chimeric antigen receptor T (CAR T) cells, are being investigated intensively, but these approaches have other issues, such as a scarcity of appropriate targets for CAR T cells in solid tumours. Consequently, there is renewed interest among translational researchers and commercial partners in the therapeutic use of γδT cells and their receptors. Overall, γδT cells display potent cytotoxicity, which usually does not depend on tumour-associated (neo)antigens, towards a large array of haematological and solid tumours, while preserving normal tissues. However, the precise mechanisms of tumour-specific γδT cells, as well as the mechanisms for self-recognition, remain poorly understood. In this Review, we discuss the challenges and opportunities for the clinical implementation of cancer immunotherapies based on γδT cells and their receptors. [1] We will discuss most recent insights how gdTCR diversity impacts gdTCR receptor hunt [2, 3], how to translate such findings into treatment of solid cancer and how imaging can assist in understanding diversity when abT cells are used as carriers [4]. Finally, we will discuss how to translate such concepts into the world of bispecifics [5].

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KATY REZVANI (HOUSTON)

NK CELLS: NEXT GENERATION CELL THERAPIES FOR CANCER

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Dr. Rezvani will discuss a new frontier in NK cell therapeutics: engineering NK cells with chimeric antigen receptors. She will discuss the opportunities and challenges of NK cell CAR engineering, and present pre-clinical and early phase clinical data on cord blood-derived NK cells expressing CD19 CAR and IL-15 to enhance their in vivo persistence in patients with relapsed or refractory blood cancers. In addition, she will discuss new data on targeting solid tumor with CAR NK cells as well strategies for the gene editing of CAR NK cells to enhance their function by targeting immune checkpoints. Finally, she will discuss the approach of precomplexing NK cells with an anti-CD16 bispecific antibody targeting cancer targets to redirect their specificity, thus providing a rapid approach to translate NK cells with CAR-like characteristics to the clinic

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SESSION IV – ENGINEERED IMMUNE CELL THERAPY: BEYOND CARS

Chairs: Aude Chapuis (Seattle), Eliana Ruggiero (Milan)

AUDE CHAPUIS (SEATTLE)

TACKLING MECHANISMS OF IMMUNE ESCAPE TO TCR THERAPY

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Introduction/short summary: Dr Aude Chapuis leads a laboratory that is working to improve T cell receptor gene-engineered T (TCR-T) cell therapy. Their translational pipeline spans target identification, generation of high-affinity TCRs, cell process methodologies to translate TCR-encoding constructs into effective products for patients, clinical trial development and execution, and sophisticated high dimensional immune-monitoring to maximize the information that can be derived from each treated patient. Based on challenges encountered and lessons learned from previous adoptive T cell therapy trials, the lab is developing strategies to increase tumor expression of the targeted human leukocyte antigen (HLA)/peptide complexes for better T cell recognition, and to engage TCR-tethered co-stimulatory signals in $CD4^+$ and $CD8^+$ T cells for maximizing T cell activation/proliferation/survival. The most recent strategies will be discussed, including those geared towards promoting localization and countering dysfunction of adoptively transferred TCR transgenic cells in vivo.

Targeting the over-expressed self-antigen WT1 with transgenic TCR-T cells. We targeted the $WT1_{126\text{-}134}$ HLA-A*0201-restricted epitope in acute myeloid leukemia (AML) with CD8 T cells expressing the optimized TCR_{C4}. In a first trial (NCT01640301), $CD8^+$ T cells were obtained from each AML patient's matched related or unrelated donor, engineered to express TCR_{C4} ($T_{TCR\text{-}C4}$), and infused into patients at high-risk of post-transplant relapse but not yet relapsed (Prophylactic Arm, n=12).[1] Relapse-free survival (RFS) was 100% at a median of 44 months following infusion, while a concurrent comparative group of 88 AML patients with similar risk features had 54% RFS (p=0.002 vs. the Prophylactic Arm). Transgenic T cells maintained TCR expression, were polyfunctional and expressed molecules associated with long-term survival. Persistence of the transgenic T cells was remarkable, at frequencies of up to 50% of circulating $CD8^+$ T cells for >2 years. Infusions were well tolerated, with no evidence of injury to normal tissues or induced graft-versus-host disease. However, $T_{TCR\text{-}C4}$ administered to patients who had already relapsed post-HCT (Treatment Arm, n=15) yielded disappointing results, as overall survival from the last relapse was not significantly different when compared to a matched comparative group of 17 patients treated at the same institution.

Exploration of the reasons for progression in this set of patients revealed **adaptation of the AML to evade the activity of $T_{TCR\text{-}C4}$.** One patient's AML switched proteasome enzyme composition from the immunoproteasome (expressed in the context of inflammation and the dominant isoform in cells of the hematopoietic system)[2,3] to the standard proteasome (expressed by somatic cells and many solid tumors), such that the $WT1_{126\text{-}134}$ epitope was not efficiently processed and presented despite abundant HLA and WT1 protein expression.[4] As a major cornerstone of a TCR-T cell approach is to target an epitope presented regardless of the state of the tumor (inflamed, 'cold', solid, liquid), this mechanism of escape prompted us to investigate how common epitopes are processed by one or the other proteasome. We used soluble HLA technology to elute antigenic peptides, [5] and screened for alternate HLA A*0201-restricted WT1 epitopes

that would not be reliant on a specific proteasome for their processing. We isolated a TCR specific for the $WT1_{37\text{-}45}$ epitope, which is processed by either the standard or immuno-proteasome and is presented by both solid and liquid tumors. This epitope is now being clinically targeted by our group and others.[4,6]

Another observed barrier to effective TCR-directed therapy was **limited T cell persistence in patients with high AML burdens at the time of infusion.** In select patients with both progression and T cell persistence, $T_{TCR\text{-}C4}$ expressed genes associated with cytokine/granzyme production and dysfunction but not proliferation, suggesting that they were encountering antigen but were unable to mount an effective immune response. To overcome this potential barrier in rapidly relapsing disease, one approach is to engage functional $CD4^+$ alongside $CD8^+$ T cells towards the same HLA Class I-restricted target.[4] Tumor-specific, HLA Class II-restricted $CD4^+$ T cells produce IL-2 and promote Class I-restricted $CD8^+$ T cell proliferation, survival and effector functions,[7] and $CD4^+$ T cells can rescue exhausted antigen-specific $CD8^+$ T cells in a chronic viral infection model.[8] Co-transferred tumor-reactive $CD4^+$ T cells can provide major clinical benefit, as shown with therapeutic CD19-CAR T cells.[9] Tumor recognition can be imparted to $CD4^+$ T cells by co-expressing Class I TCRs with the CD8αβ co-receptor in $CD4^+$ T cells.[4] Indeed, a combination of functional $CD4^+$ and $CD8^+$ $T_{TCR\text{-}C4}$ cells had the most effect in killing tumors in vitro and this strategy is now being investigated in the clinic.

TCR redirected therapy is an attractive modality for solid tumors due to the ability to target intracellular proteins. However, the hostile tumor environment is a therapeutic challenge.[10] To assess whether engaging functional $CD4^+$ T cells alongside $CD8^+$ T cells would control the cancer in a solid tumor model, we leveraged MISTRG "humanized" mice, which carry a human immune system and recapitulate the interface between human cancers and immune cells.[11,12] In this model, implanted tumor cell lines are infiltrated by innate human-derived immune cells that recapitulate the tumor environment found in human melanomas. We used TCR-T to specifically target the MAGE-A1 cancer testis antigen in this model. Transgenic $CD4^+$ and $CD8^+$ T cells preferentially reached the tumor, but they expressed surface markers associated with activation/dysfunction and were embedded within a thick mesh of fetal-derived, tumor-induced macrophages, suggesting that additional genetic engineering will be required to break the solid tumor immune barrier.

Blocking inhibitory pathways (e.g., PD-1, CTLA-4) can rescue some T cells from exhaustion, but is often insufficient to maintain T cell proliferation and function in the face of repeated antigen stimulation, as occurs at a tumor site.[14] For efficient activation and proliferation, T cells require TCR interactions with HLA/peptide (Signal 1) and costimulation (Signal 2). Signal 2 has been shown to be essential for the restoration of T cell function by PD-1-targeted therapies.[14] Unfortunately, most solid tumors exhibit limited expression of Signal 2 ligands, including CD80 or CD86 (CD28 binding) and/or 4-1BB ligand.[15] This suggests function and resistance to dysfunction of antigen-specific T cells may be enhanced if Signal 2 is triggered upon specific HLA/peptide recognition, regardless of the presence/absence of co-stimulatory ligands. A related strategy is employed in successful CAR T cell constructs that are dependent on a downstream co-stimulatory signal (CD28, 4-1BB) tethered to CD3ζ, with both signaling modules triggered when the surface antibody recognition structure encounters its target.[16] Recent murine models have shown efficacy with TCR co-stimulation provided through chimeric fusion receptors that ligate inhibitory

ligands in the tumor environment, but provide co-stimulatory rather than inhibitory signals.[17,18] To limit lentiviral vector size and reliance on the presence of inhibitory ligands, and to guarantee immediate proximity of co-stimulation to TCR signaling,[19] we further **engineered the CD8αβ coreceptor with intracellular CD28 to provide Signal 2, which showed promise in pre-clinical models**. Our goal is to determine if the most effective strategy will require a positive co- stimulatory signal plus the blocking of inhibitory pathways, to overcome immunotherapy barriers provided by the tumor environment.

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MIRJAM HEEMSKERK (LEIDEN)

OPTIMIZATION OF TCR GENE THERAPY AND PREVENTING TUMOR ESCAPE

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Multiple myeloma (MM), which accounts for approximately 10% of blood cancers, is a malignancy of plasma cells originating in the bone marrow. Increased understanding of disease biology over the years has resulted in improved treatment strategies, demonstrated by the emergence of novel therapeutics such as proteasome inhibitors, immunomodulatory drugs, and monoclonal antibodies. While these new therapeutics have resulted in better disease control, myeloma remains largely incurable. Drug resistance is inevitable and disease relapse remains a great clinical challenge.

Immunotherapy by allogeneic stem cell transplantation (allo-SCT) has demonstrated thus far to be the only curative strategy for myeloma by a profound graft versus myeloma effect. Multi-antigen targeting by alloreactive donor T cells was able to eliminate multiple myeloma, although frequently at the cost of a high risk of graft versus host disease and transplant related mortality and morbidity, and therefore, currently this treatment regimen is not frequently used anymore. It, however, illustrates that multiple myeloma is highly susceptible to T cell mediated immunotherapy. Gene transfer technologies provide the opportunity to specifically direct the therapeutic power of immunotherapy towards defined cancer antigens, and recent clinical studies have demonstrated the power of this technology. CD19 targeting chimeric antigen receptor (CAR) T-cell therapies have demonstrated impressive clinical results in the treatment of B-cell malignancies. B-cell maturation antigen (BCMA) expressed on the cell surface of MM cells is one of the most promising targets for CAR T-cell therapy of MM patients. Clinical studies with BCMA CAR T-cells demonstrated that patients with relapsed MM can achieve objective responses. However, due to heterogeneous expression of BCMA, antigen loss on targeted tumor cells caused relapses in treated patients demonstrating the importance of selection of antigens for the success of T-cell therapies.

Unlike CARs, T-cell receptors (TCR) can recognize antigens from both extracellular and intracellular derived proteins presented by HLA molecules. This extends the range of antigenic options and increases tumor-specificity via targeting of neoantigens, or intracellular proteins promoting oncogenesis, such as transcription factors. Furthermore, T-cell therapies directed against transcription factors that are essential for growth and survival will drastically reduce the chance of tumor escape, since shutting down of these essential transcription factors will result in growth retardation and death of tumor cells. The B-cell lineage specific B-cell Oct-binding protein 1 (BOB1) meets these important criteria. BOB1 is expressed at nearly all stages of B-cell development and differentiation, and is critical for the generation of circulating naïve B-cells, germinal center formation and antibody production. As a result of its lineage-specific expression, BOB1 is expressed in a vast array of B-cell malignancies. Several studies as well as our own data imply an important function for BOB1 in cell survival of B-cell neoplasia, especially in MM.

While BOB1, being an intracellular transcription factor, cannot be targeted by CAR T-cells, we have recently identified T-cell receptors (TCR) that recognize peptide sequences of BOB1 in the context of either HLA-B*07:02 or HLA-B*35:01[1, 2]. We established that BOB1-TCR modified CD8 T-cells were highly reactive against a panel of primary B-cell malignancies including primary samples from patients with multiple myeloma, B-cell chronic lymphocytic leukemia (B-CLL), B-cell acute lymphoblastic leukemia (B-ALL), and mantle cell lymphoma (MCL), while ignoring any non-B-cell types. Moreover, BOB1-TCR modified CD8 T-cells, resulted in rapid and reproducible targeting of established bone-marrow located human MM tumors in a preclinical mouse model. In addition, we have identified a library of TCRs directed against other B cell lineage specific genes and cancer associated antigens e.g. WT1, MAGE-A1, A3, and A9[3].

The TCR expression was optimized by simultaneous deletion of endogenous TCRαβ by CRISPR/Cas9 editing, and T cell receptor engineering of primary NK cells was performed to therapeutically target tumors and tumor immune evasion[4, 5]. BOB1-TCR gene therapeutic strategy will be explored in a phase I clinical study in which we will investigate the feasibility, safety, and first indication of clinical efficacy of BOB1-TCR gene therapy in multiple myeloma. This clinical study will focus on the BOB1 TCR that is restricted to HLA-B*07:02 (BOB1-B7 TCR), based on high population frequency of HLA-B*07:02 (28%) in the European population.

As has been illustrated by the effectiveness of multi antigen targeting in allogeneic stem cell transplantation, we anticipate that in the future combination therapies with CARs and TCR engineered T cells have to be developed as effective treatment for patients suffering from multiple myeloma and other B-cell malignancies. To investigate whether combination therapy would be beneficial we established MM xenograft models in which escape due to heterogeneous expression of tumor targets, using CRISPR/Cas9 editing systems, could be investigated. We have demonstrated that effective population control of these heterogeneous MM tumors demonstrated that simultaneous treatment with TCR as well as CAR T cells was required, while single treatment eventually led to outgrowth of tumor cells.

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SESSION V – SYNTHETIC BIOLOGY IN ENGINEERED IMMUNE CELL THERAPY

Chairs: Yvonne Chen (Los Angeles), Chiara Bonini (Milan)

YVONNE CHEN (LOS ANGELES)

ENGINEERING NEXT-GENERATION T CELLS FOR CANCER IMMUNOTHERAPY

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The adoptive transfer of T cells expressing chimeric antigen receptors (CARs) has demonstrated clinical efficacy in the treatment of advanced cancers, with anti-CD19 CAR-T cells achieving up to 90% complete remission among patients with relapsed B-cell malignancies. However, challenges such as antigen escape and immunosuppression limit the long-term efficacy of adoptive T-cell therapy. Here, I will discuss the development of and clinical data on next-generation T cells that can target multiple cancer antigens and resist antigen escape. I will also present recent work on tuning CAR signaling activities via rational protein design to achieve greater *in vivo* anti-tumor efficacy. This presentation will highlight the potential of synthetic biology in generating novel mammalian cell systems with multifunctional outputs for therapeutic applications.

SESSION VI – ENGINEERED IMMUNE CELL THERAPY: B-CELL TUMORS AND BEYOND

Chairs: Michael Hudecek (Würzburg), Marcela Maus (Boston)

PAOLO CORRADINI (MILAN)

REAL LIFE ANTI-CD19 CAR-T CELL THERAPY IN RELAPSED/REFRACTORY LYMPHOMA PATIENTS: IMPACT OF THE PHENOTYPIC COMPOSITION OF TISA-CEL AND AXI-CEL INFUSION PRODUCTS AND OF THE LEUKAPHERESIS CELLULAR MATERIAL IN MEDIATING IN VIVO EXPANSION AND DISEASE RESPONSE

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Background: Following promising results in pivotal trials, two novel CD19-targeted chimeric antigen receptor (CAR)-T cell products, namely tisagenlecleucel (Tisa-cel) and axicabtagene ciloleucel (Axi-cel), have been approved for the treatment of relapsed/refractory diffuse large B cell lymphomas, high grade lymphomas, primary mediastinal B cell lymphomas and transformed follicular lymphomas.

Despite the promising results reported in clinical trials and in real life settings, the determinants of response are not well characterized. A number of pre-treatment patient clinical features including mainly parameters of lymphoma burden and levels of inflammatory cytokines were used to predict clinical response to CAR-T cell therapy (Vercellino L, Blood Adv 2020) but were not able to discriminate the outcome at the single patient level. On the other hand, in vivo CAR-T cell proliferation after infusion was found of central importance to clinical response and toxicities in acute lymphoblastic leukemia (ALL) (Ghorashian S., Nat Med 2019) and within clinical trials (Awasthi R, Blood Adv 2020, Ayuk FA., Blood Adv 2021) but has been less investigated in lymphoma patients receiving Tisa-cel and Axi-cel as standard-of-care therapy. Additionally there are few data correlating properties of Tisa-cel and Axi-cel infusion bag content and CAR T cell expansion.

Recent evidence suggest that cellular and molecular features of infused CAR-T cell products are major factors accounting for the variability in efficacy among Axi-cel treated lymphoma patients of the ZUMA-1 trial (Deng et al., Nat Med 2020) and among chronic lymphocytic leukemia (CLL) patients receiving CTL019 therapy (Fraietta JA, Nat Med 2018). Of note, these data have been generated mainly as part of exploratory analysis of clinical trials using comprehensive and challenging methodological approaches to identify predictive biomarkers in patients treated with specific products. Real life evaluations assessing the phenotypic composition of the CAR-T cell infusion products and analyzing how these features affect CAR-T cell fate in vivo and outcome are scanty. Besides, no studies directly comparing Tisa-cel and Axi-cel characteristics and their impact on expansion kinetics have been reported yet.

Aims: In a single centre prospective study enrolling all consecutive patients who received standard-of-care Tisa-cel or Axi-cel between December 2019 and January 2022 at the Hematology Division of the Fondazione IRCCS Istituto Nazionale dei Tumori (Milano, Italy), we aimed at identifying key phenotypic Tisa-cel and Axi-cel product characteristics associated with improved in vivo CAR-T cell expansion and therefore tumor responses. Additionally, as it is likely that the characteristics of the source material influence the manufacturing process and therefore affect the immune cell composition and T cell phenotypic features of the final CAR-T cell product, we analyzed autologous cells collected at

leukapheresis to identify specifications of starting material ultimately affecting the safety and efficacy of CAR-T therapy.

Results: Residual cells obtained after washing 61 CAR-T product bags [29 Tisa-cel (47.5%) and 32 Axi-cel (52.5%)] were analyzed by flow cytometry (FCM). When comparing the two different products, Tisa-cel was characterized by a lower fraction of CAR+ and of CAR+/CD8+ cells as compared to Axi-cel bags (median CAR+ cells: 25.37% vs 72.87%, P<0.0001; median CAR+/CD8+ cells: 25.06% vs 40.85%, P<0.005). The presence of different CAR+ cell differentiation subsets [T central memory, T_{CM} (CD45RO+/CD197+/CD62L+); T naïve-like, $T_{N\text{-}like}$ (CD45RO−/CD197+/CD62L+); T effector, T_E (CD45RO-/CD197-/CD62L-) and T effector memory, T_{EM} (CD45RO+/CD197-/CD62L−)] in infusion products was then assessed. Collectively CAR-T infusion products were largely composed of differentiated T-cell subsets such as CD4+T_{EM} (21.9%), CD8+T_{EM} (12.4%) and of CD4+T_{CM} (18.4%), CD8+T_{CM} (11.4%), whereas T_N-like and T_E cells were less represented (CD4+T_E 0.01%, CD8+T_E 0.03%, CD4+T_N-like 0.04%, CD8+T_N-like 0.09%) with few differences between Tisa-cel and Axi-cel products.

To evaluate whether quantitative or qualitative features of CAR-T infusion products could affect *in vivo* CAR-T cell fate, expansion kinetics data [including concentration of CAR+ cells at day 7 (C_7) and at day 10 after infusion (C_{10}), time to maximal expansion (T_{max}), concentration of CAR+ cells at T_{max} (C_{max}) and the magnitude of expansion up to 30 days (calculated as the area under the curve, $AUC_{0\text{-}30}$)] were analyzed.Early expansion kinetics were similar for Tisa-cel and Axi-cel and maximum CAR-T cell expansion was achieved on day 10 for both products. Additionally as the $AUC_{0\text{-}30}$ and C_{10} parameters were strongly correlated [r=0.88 (95%CI 0.79-0.92, p<0.001)], the C_{10} was selected as the surrogate measure to quantify CAR-T cell expansion and the median C_{10} (24.5 CAR-T cells/µl) was used as a cut-off to dichotomize "expanders" (patients with a $C_{10}\geq$ 24.5 CAR-T cells/µl) and "poor-expanders" (patients with a $C_{10}<$ 24.5 CAR-T cells/µl). When looking for possible differences in the infusion products administered to these two groups, we established that expanders had received infusion products significantly enriched in CAR+CD8+ cells with a central memory phenotype as compared to poor-expanders (median CAR+/CD8+T_{CM}: 13.8% in expanders versus 4.5% in poor-expanders; P<0.005) thus suggesting that CAR+/CD8+ T_{CM} have the highest ability to proliferate. Additionally, expanders by C_{10} had higher chance to respond by day 90 (OR=5.600; Chi-squared test 95%CI range 1.681-18.65; P<0.005) and this association remained significant even after correcting for other parameters commonly linked to outcome in a multiple logistic regression analysis (C_{10} OR=1.268; 95% CI range 1,062 to 1,676; p<0.05). Consistently, expanders had significantly longer survival rates when compared to poor-expanders (PFS median: not reached versus 3.7 months, respectively; P<0.05).

Taken together these data suggest that despite the fact that Tisa-cel and Axi-cel infusion products are significantly different, Tisa-cel and Axi-cel expansion kinetics are similar and are mediated by the presence of CAR-T cells with central memory phenotypes in infusion products (Monfrini C et al, Clinical Cancer Research in press 2022).

We then wanted to test the hypothesis that features of autologous cells collected at leukapheresis could affectTisa-cel or Axi-cel infusion product characteristics and ultimately the efficacy of CAR-T therapy. Flow cytometry was used to compare the T cells composition of the apheresis and the CAR-T cell infusion products in 48 patients.

The presence of less differentiated phenotypes in the apheresis products was predictive of CAR-T cell

expansion *in vivo*. Additionally among the analysed T cell populations, a moderate correlation between CD8+ naive T cells, CD8+CD62L+ T_N cells and CD8+ stem cell memory T cells (T_{SCM}, CCR7+CD45RO- CD62L+CD95+) in the leukapheresis and CAR+ cells with a CD8+ T_{CM} phenotype in infusion products was detected (r=0.43 p=0.0026, r=0.43 p=0.0021, r=0.41 p=0.0040, respectively). Additionally, when dividing patients according to the products they received, in the Tisa-cel cohort, only the presence of CD8+ T_{SCM} cellsin the leukapheresis was positively associated with CAR+CD8+ T_{CM} cells in the infusion products (r=0.63 p=0.0026). On the contrary, in the 28 patients receiving Axi-cel, both CD8+ T_N and CD8+CD62L+ T_N cells in the leukapheresis, moderately correlated with CD8+ T_{CM} cells in infusion products (respectively r=0.44 p=0.0193, r=0.47 p=0.0126).

These results suggest that the T cell composition of the leukapheresis influences the CAR-T cell phenotypes of the infusion product. More specifically, the enrichment of less-differentiated T lymphocytes in the leukapheresis positively affects the amount of T cells with central memory phenotype in the infusion product, a relevant population for CAR-T cell in vivo expansion and clinical efficacy, as we have demonstrated (Monfrini C et al, Clinical Cancer Research in press 2022).

Further studies on larger populations are warranted to validate our results, but collectively they support the concept that the presence of T cells phenotype in the apheresis product could be used to predict the fitness of the infusion products and potentially allow a pre-treatment stratification of patients or at least a closer monitoring of their *in vivo* expansion.

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CLIONA ROONEY (HOUSTON)

MODIFICATION OF GD2.CAR T-CELLS WITH A CONSTITUTIVELY ACTIVE IL7 RECEPTOR FOR THE TREATMENT OF GD2 EXPRESSING MALIGNANCIES

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While CAR T-cells have had spectacular success for the treatment of B-cell malignancies, similar success against other tumors has not been achieved. Barriers include the complex immunosuppressive tumor microenvironment and tumor location outside the hematopoietic circulation. Multiple strategies to overcome these limitations have been evaluated, but perhaps the most basic of these is the provision of cytokines, the third essential signal for T-cell activation, persistence and function; signals 1 and 2, antigen and costimulation respectively, being provided by the CAR. Several groups have introduced cytokines into CAR T-cells, however, secreted cytokines may promote the expansion of undesirable bystander cells, such as T regulatory cells, that may have inhibitory effects, or contribute to cytokine release syndrome (CRS) that is a severe side effect of CAR T-cells.

We are clinically evaluating a constitutively active IL7 receptor (C7R), that not only provides signal 3, but also promotes the expression of anti-apoptotic molecules, and since it is not secreted, it should not affect bystander cells or contribute to CRS. In preclinical studies, C7R increased the efficacy of GD2.CAR T-cells in murine xenograft models of neuroblastoma, and while it increased the proliferation and function of antigen-stimulated CAR T-cells it did not induce autonomous growth in the absence of signals 1 and 2. Our clinical trial uses C7R-modified T-cells expressing a GD2.CAR containing a 4-1BB costimulatory endodomain, for the treatment of patients with GD2+ solid tumors (i.e. neuroblastoma and osteosarcoma). A separate companion trial uses the same product to treat pediatric patients with GD2 expressing brain tumors. The solid tumor trial has 2 arms each dose escalating from 1×10^7 CARTs per m^2, to 1×10^8 for patients with and without lung metastases. Dose level 0 was infused without lymphodepletion, while dose levels 1 to 3 were infused after lymphodepletion with Cytoxan and fludarabine.

Manufacture: To generate C7R-GD2.CAR T-cells, PBMCs were stimulated using CD3 and CD28 antibody coated plates with IL7 and IL15, then transduced sequentially on days 2 and 3, with retroviral vectors expressing the GD2.BB.CAR and the C7R with a CD34 ectodomain allowing phenotypic detection and preventing transduced T-cells from acting as a sink for IL7. Transduced cells were cryopreserved for infusion between days 7 and 10. Release criterion included sterilities, HLA typing for identify, transduction efficiency, vector copy number per cell and killing of GD2+ tumor cells in 5-hour cytotoxicity assays.

Mean transduction efficiencies were 76.3% for the GD2.CAR, 66.7% for C7R and 58.9% for C7R/GD2.CAR double transduced cells. Vector copy number per cell ranged from 2.2 to 10.9 (mean 5.5) and mean killing efficiency was 62.3% at an E:T ratio of 20:1.

Clinical activity:

Solid tumors: 13 patients have been treated to date, two without lymphodepletion (LD) and eleven with standard LD (cytoxan and fludarabine). Of these, 12 patients received a single infusion and one received two infusions. While no toxicity was observed without LD, the combination of standard LD with C7R-GD2.CARTs resulted in monocyte activation and significant cytokine release syndrome in one patient at dose level 1 and one patient at dose level 2. Implementation of early treatment with anakinra resulted in rapid resolution of symptoms and prevented more severe CRS. None of the patients developed neurological toxicities. Two of three treated patients with neuroblastoma achieved a partial response, including one subject who had resolution of tumor on CT imaging after two infusions and did not require additional therapy for 8 months. Six of eight osteosarcoma patients treated with LD experienced stable disease with a decrease in size of pulmonary metastases in three of these patients, while two others experienced progressive disease. The frequency of circulating gene-marked T-cells at dose levels 0 and 1 was similar, showing that the C7R was able to induce post infusion expansion without lymphodepletion. Based on these data we are now testing additional dose levels without lymphodepletion; treatment on these dose levels is ongoing.

Brain tumors: All three patients treated with GD2.CARTs without C7R experienced clinical improvement; however, the benefit was temporary, lasting less than 4 weeks with subsequent tumor progression. More recently, we have treated three children with C7R.GD2.CAR T-cells. Side effects observed include mild fever and transient symptoms associated with tumor inflammation. The first patient experienced improved balance and muscle strength that persisted for 2 months. The second patient with a thalamic glioma had improvement in their abnormal eye moments with sustained improvement for more than 6 months after two infusions. The third patient, treated for DIPG, experienced resolution of her eye lid drooping (ptosis) and regained her ability to climb stairs. Her clinical improvement is ongoing and has been sustained: she remains completely asymptomatic 6 months after treatment.

SESSION VII – GOING UNIVERSAL WITH ENGINEERED IMMUNE CELLS

Chairs: Maria Themeli (Amsterdam), Cliona Rooney (Houston)

DANIEL POWELL (PHILADELPHIA)

THE BIG BANG OF UNIVERSAL IMMUNE RECEPTORS

Daniel J Powell
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Chimeric antigen receptor (CAR) T cells have shown great success in the treatment of CD19+ and BCMA+ hematological malignancies, leading to recent approvals by the FDA as a new cancer treatment modality. However, their broad use is limited since a CAR conventionally targets a single tumor associated antigen (TAA), which is not effective against tumors with heterogeneous TAA expression or emerging antigen loss variants. Moreover, the microenvironment of solid tumors harbors physical cellular barriers and immunosuppressive cells that block or dampen CAR T cell activity. Beyonf these challenges, stably engineered CAR T cells can continually and uncontrollably proliferate and activate in response to antigen, potentially causing long-term immunocompromise, fatal on-target off-tumor toxicity, cytokine release syndrome, or neurotoxicity without a method of control or elimination. To address these issues, our lab and others have developed various adaptable universal immune receptors (UIRs) that allow for targeting of multiple TAAs by T cells expressing a single receptor. UIRs function through the binding of an extracellular adapter domain which acts as a bridge between intracellular T cell signaling domains and a soluble tumor antigen targeting ligand (TL). The dissociation of TAA targeting and T cell signaling confers many advantages over standard CAR therapy, such as dose control of T cell effector function, the ability to simultaneously or sequentially target multiple TAAs, and control of immunologic synapse geometry. In general, there are currently four UIR platform types: ADCC-mediating Fc-binding immune receptors, bispecific protein engaging immune receptors, natural binding partner immune receptors, and anti-tag CARs. These UIRs all allow for potential benefits over standard CARs, but also bring unique engineering challenges that have to be addressed to achieve maximal efficacy and safety in the clinic. Still, UIRs present an exciting new avenue for adoptive T cell transfer therapies and could lead to their expanded use in areas which current CAR therapies have failed. Here, I will review the development of UIR platform types and their unique functional benefits, detail some of the potential hurdles that may need to be overcome for continued clinical translation, and describe a new UIR platform that 1) combines the use of a fully humanized UIR construct with picomolar affinity and repurposed use of TLs with an established safety profile in patients, and 2) easily adapts to the use of integrated diagnostic imaging, and image guided treatment and monitoring.

MARC CARTELLIERI (DRESDEN)

SWITCHABLE CAR-T FOR SAFE AND EFFICIENT TARGETING OF LESS DIFFERENTIATED ANTIGENS

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Chimeric antigen receptor (CAR) T-cell therapy has demonstrated significant efficacy in B cell malignancies like ALL, B-cell lymphoma, and Multiple Myeloma. While on-target/off-tumor toxicity with CAR-T therapies targeting CD19 and BCMA has been clinically manageable, severe cytokine release and neurotoxicity remain a clinical challenge.[1,2] Breakthrough of conventional CAR-T technology beyond B-cell malignancies has also been hampered by lack of suitable target antigens. For example, CD123 is an interesting target for treatment of a number hematologic malignancies, as it is expressed on approximately 80% of AML patients and it is also frequently expressed on other hematologic and lymphatic malignancies[3], but it is also present on normal hematopoietic progenitor cells posing a risk for continued aplasia.[4,5] While targeting of CD123 by conventional CAR-T cells achieved clinically meaningful remissions, patients not undergoing subsequent allogeneic hematopoietic cell transplantation suffered from long-lasting myelosuppression.[6,7] Thus, innovative approaches putting the power of CAR-T technology under the control of reliable and fast-acting on/off switches to avoid and/or abrogate acute and long-term side effects are required.

To overcome these challenges, the concept of switchable CAR-T was introduced by several groups.[8] We developed a modular, universal two component CAR-T platform. The first component is a universal CAR-T cell with a CAR that by itself does not recognize any human surface antigen, but a peptide motif included in the second component, a soluble adaptor called targeting module (TM). The TM confers specificity against the cancer antigen of choice and due to the high flexibility of the tumor binding domain multiple antigens in solid tumors and hematologic malignancies, including AML, can be targeted.[9,10] Our preclinical data obtained with a CD123-specific TM (TM123) showed, that the small-sized TM efficiently penetrate and accumulate in bone marrow and solid tumor tissue.[9,11] Crucially, this TMs has a short half-life, enabling a rapid switch-off of the UniCAR system by TM withdrawal. We hypothesized that treatment-related toxicities could be mitigated by withholding the administration of TM which will rapidly disappear from the circulation due to its short half-life. To produce autologous patient products, a closed, largely automated production process based on the CliniMACS Prodigy Platform was developed and stage-appropriate validated.

Based on our pre-clinical data a FIH study was started with the objective to evaluate the safety and tolerability of our switchable CAR-T platform in combination with TM123 (UniCAR02-T-CD123, NCT04230265) and to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) for subsequent clinical trials. We have recently reported first results for the first three patients, who all have shown a clinical response, with one patient showing a partial remission and two patients a complete remission with incomplete hematologic recovery.[12] The study is on-going, and fourteen patients were treated so far, of which 12 completed treatment and 2 dropped out during treatment due to disease

progression or DLT. Treatment was generally well tolerated with limited treatment-related AEs. Importantly, treatment-related toxicity could be mitigated by interruption of TM administration resulting in rapid resolution of symptoms in all patients. The obtained clinical evidence provides a proof-of-concept for the possibility of controlling the potency of CAR-T cells in patients and preventing severe side effects. An up-date on the status of the clinical trial as well as on the experience with the production of autologous CAR-T products from the starting material of the severely pre-damaged patients will be presented at the conference.

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MARIA THEMELI (AMSTERDAM)

IPSC-DERIVED CAR-T AND CAR-NK

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Both T cells as well as NK cells have been proven robust therapeutic agents and several approaches to obtain anti-tumor therapeutic lymphocytes have been proposed. The introduction of genetic engineering with chimeric antigen receptors (CAR) boosted the applicability and efficacy of adoptive cell immunotherapy.

Unfortunately, the rapid development of CAR-T/NK cell therapy has also revealed important challenges that limit the feasibility of cost-effective, easier and broader application. For instance, current manufacturing is complicated and time-consuming, which is not ideal since the required vein-to-vein processing time (3-5 weeks) is critical for the patient's health in case of rapid disease progression. In addition, the quality of the autologous, patient-derived, apheresis product is highly variable, as it depends on the type and stage of the disease, previous therapies and immune cell composition. Differences in the quality of the apheresis product lead to a high degree of variability in the clinical safety and efficacy. **The use of allogeneic healthy donor-derived T cells** has been suggested as an alternative, readily available source of CAR-T cells. However, their use **requires substantial genome engineering**; knocking out of T cell receptor (TCR)[1] and HLA genes **to avoid graft-versus-host disease (GvHD) and graft rejection**. Other specific genetic modifications in CAR-T cells have been shown to improve their efficacy and performance, including multi-targeting (dual CAR-Ts) and co-expression of cytokines or receptors. Unfortunately, genetic engineering of primary autologous or allogeneic T cells in a multiplex manner is very challenging, as it currently results in a) **reduced production yield** (ex. 20-60% of double knockout efficiency)[2]; b) **genotoxicity** due to undesired off-target effects and gene translocations[3]; c) an **exhausted T cell phenotype and product** due to the requirement of extended *ex vivo* expansion[4]; and d) **prohibitive production costs and effort** for safety release testing if attempted in the autologous setting. Therefore, new technical solutions are urgently needed.

The technology of induced pluripotent stem cells (iPSC) offers new perspectives for the production of immunotherapeutic cellular products due to two major characteristics. First, similar to embryonic stem cells, iPSCs can be cultured unlimitedly in vitro and be successfully differentiated towards the lymphoid lineage [5]. Having access to constant and continuous production of T and/or NK lymphocytes offers solution to cell number and doses limitations due to restricted availability or expansion of primary cells. Second, iPSCs can be easily amenable to genetic transformations in vitro and thus, can generate immune effectors, which may eventually be genetically modified to augment their applicability, potency and persistence. While the potential for multiplex gene editing is limited in primary cells, iPSC can theoretically bear unlimited genetic changes. In contrast to primary cells [1], genetic engineering of iPSCs results in fully modified clonal lines, which could be extensively evaluated resulting in a stable and safe source. Especially

the rise of gene editing technologies, such as CRISPR/Cas9 and TALEN technologies, offers new perspectives for the multiplex modification of iPSC.
The use of CARs endows iPSC-derived T/NK cells with HLA-independent, customizable antigen recognition as scFv domains of different specificity can be used giving the potential for a wider applicability range. Similar to conventional CAR-T/NK cells, iPSC can be also further genetically armed with cytokines, receptors and other regulatory molecules in order to provide their derivatives with optimal immunotherapeutic properties such as enhanced proliferation and reduced exhaustion[6, 7]. Previous work on iPSC that are engineered to constitutively express a CAR has shown that these cells give rise mainly to CD8αα CAR-T cells with an expression profile and function similar to innate T cells, which lack some key features for therapeutic potency, such as the long-term memory and in vivo persistence[8]. Recent data show that timely and calibrated CAR expression can allow the generation of mature adaptive CD8αβ CAR T cells.

The major barrier limiting the applicability of allogeneic iPSC-derived products is the HLA-disparity between the effector T cells and the host, which may lead to graft rejection or graft-versus-host (GvH) reaction. Using matched previously banked iPSCs from HLA-homozygous donors as a starting material has been previously proposed. However, the establishment of “universal” iPSC lines is considered to provide a “true” solution as they would provide widely applicable cellular products without the need for HLA-matching. Hypoimmunogenic, histocompatible pluripotent stem cell lines can be generated by elimination of HLA class I and II expression by disruption of β2m and CIITA gene respectively[9]. Allogeneic cells, which lack "self" class I HLA molecules can however be rejected by host NK cells. Introduction of HLA-E, HLA-G or of patient-specific HLA-C have been shown to reduce NK mediated rejection of iPSC-derived cells.

There are still multiple roadblocks associated with the first wave of iPSC technology such as:
a) the genotoxicity and complexity of genetic engineering to generate master-producer iPSC banks;
b) the suboptimal function and developmental maturity of iPSC-derived in comparison to ‘primary’ CAR-T cells;
c) the lack of robust, scalable, and exportable manufacturing protocols for clinical-grade iPSC-derived CAR-T cells;
d) the absence of clear regulation for the product quality assessment and release for clinical use;
e) the lacking acceptance of key stakeholders, most importantly patients, towards iPSC technology.

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SESSION VIII – OVERCOMING T-CELL EXHAUSTION AND THE IMMUNE-SUPPRESSIVE TUMOR MICROENVIRONMENT

Chairs: Chiara Bonini (Milan), Daniel Powell (Philadelphia)

PHILIPPE BOUSSO (PARIS)

DECODING THE MODE OF ACTION OF TUMOR IMMUNOTHERAPIES USING INTRAVITAL IMAGING

Philippe Bousso
Dynamics of Immune Responses, Institut Pasteur and Inserm, Paris, France

Understanding complex interactions between the immune system and the tumor microenvironment is an essential step towards the rational development and optimization of immunotherapies. Several experimental approaches are available to tackle this complexity but most are not designed to address the dynamic features of immune reactions, including cell migration, cellular interactions, and transient signaling events. By providing a unique means to access these precious parameters, intravital imaging offers a fresh look at intratumoral immune responses at the single-cell level. We will illustrate how in vivo imaging sheds light on fundamental aspects of tumor immunity and helps elucidate modes of action of immunotherapies such as CAR T cell therapy.

SESSION IX – IMAGING ENGINEERED IMMUNE CELLS AT THE RECEPTOR, CELL AND ORGANISM LEVEL

Chairs: Johannes Huppa (Vienna), Michael Jensen (Seattle)

JOHANNES HUPPA (VIENNA)

INTERROGATING TCR & CAR FUNCTION WITH HIGH-END MICROSCOPY

Johannes Huppa
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T-cells can detect the presence of even a single antigenic pMHC among thousands of endogenous pMHCs presented on the surface of APCs or target cells [1, 2, 3]. How they achieve this, especially in view of rather low affinity TCR-pMHC interactions, is not well understood despite intense research activities over the past 30+ years. We think this gap in understanding results in large part from the complexity of membrane biophysics, the highly transient nature of biochemical and structural changes that are associated with T-cell antigen recognition and also the limitations of conventional biochemical methods, which fail to account for synapse-specific context [4]. To meet these challenges and arrive at a deeper understanding, we continue to devise non-invasive live cell imaging modalities that allow us to draw quantitative conclusions at the molecular level with millisecond temporal resolution. In this talk, I will outline our experimental strategies and what they have so far taught us about how T-cells typically engage their antigens [5, 6, 7, 8, 9, 10, 11, 12, 13]. I will then showcase our strides into the field of human and clinical immunology, where we wish to advance our understanding of T-cell antigen recognition in settings of tumor immunology, autoimmunity and transplant immunology with benefits for the patient [14, 15].

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MARKUS SAUER (WÜRZBURG)

SUPER-RESOLUTION FLUORESCENCE IMAGING OF PLASMA MEMBRANE RECEPTORS BY *D*STORM

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Imaging technologies are central platforms that drive fundamental research in virtually all disciplines across the biological and medical sciences. Visualising cells and tissues by light and electron microscopy has led to more discoveries than any other technology in biological research. By seeing how a system looks, function can be understood and comparison made between healthy and unhealthy systems such as cells, tissue, and whole organism or population, in plants, animals, and humans. In biology, nearly non-invasive fluorescence imaging techniques played a pivotal role in improving our understanding of cellular function. In medicine, different powerful techniques such as positron emission tomography (PET), x-ray computed tomography (CT), and magnetic resonance imaging (MRI) has provided insight into function and metabolism of organisms. This allows the investigation of the effectiveness of new targeted therapies down to the cellular level. However, our knowledge about the quantitative structural organization and interaction patterns of basic molecules and molecular machines indispensable to life is incomplete. Since detailed quantitative molecular information about targets of drugs and the biological consequences of their action is invaluable for designing new drugs and therapies new refined imaging technologies for quantitative imaging with virtually molecular resolution are urgently required. However, obtaining high-resolution information about the architecture of cellular structures and their interrelations is hindered by the diffraction barrier of classical fluorescence microscopy.

Super-resolution microscopy methods now can provide optical resolution that is well below the diffraction limit approaching virtually molecular resolution. They can be applied to biological samples and provide new and exciting views on the structural organization of cells and the dynamics of biomolecular assemblies on wide timescales. Especially, single-molecule based super-resolution fluorescence microscopy has the potential to fundamentally revolutionize our understanding of how nature encodes brain function at the molecular level. Among all powerful, high-resolution imaging techniques introduced in recent years single-molecule localization microscopy methods such as *direct* Stochastic Optical Microscopy (*d*STORM)[1,2] excel because they deliver single-molecule information about molecular distributions, even giving absolute numbers of proteins present in subcellular compartments.[3] This provides insight into biological systems at a molecular level that can yield direct experimental feedback for modeling the complexity of biological interactions.

Thus, single-molecule localization microscopy methods such as *d*STORM provide new techniques to visualize the distribution and quantify the absolute number of tumor-associated antigens on healthy and tumor cells. A paradigm shift is currently taking place in cancer medicine (hematology & oncology). It has been realized and clinical-proof-of concept has been obtained that immunologic anti-cancer treatments with antibodies and tumor-specific T cells are highly effective and indeed capable of curing advanced, end-stage malignancies that are resistant to conventional treatments. The spectrum of immune-based cancer treatments ranges from antibodies that bind to surface antigens on tumor cells via bi-specific antibodies

that possess two binding moieties, one for a tumor antigen and one that engages T cells or other immune cells to clear the tumor to designer T cells that are modified by gene transfer to express a synthetic chimeric antigen receptor (CAR) that binds to a surface antigen on tumor cells and is coupled to the killing machinery of the T cell. Several attributes make immunologic cancer treatments highly attractive, including their exquisite specificity for tumor cells due to recognition of antigens that are present on malignant but not on healthy tissues, thus sparing the toxic side effects of chemoradiotherapy. Moreover, an attraction of immunotherapy with CAR- and TCR-modified T cells is that a single administration may already confer tumor eradication and life-long protection from relapse, because T cells are "living drugs" that persist and proliferate until tumor clearance has been accomplished and form memory thereafter.

Another important attribute of immune based anti-cancer treatments is their extraordinary sensitivity. The detection threshold of CARs is in the order of less than 100 molecules of antigen per tumor cell.[4] A critical challenge that arises in both research and clinical practice is that the sensitivity of antibodies and T cells is much higher than the sensitivity of detection methods such as immunohistochmistry (IHC) and flow cytometry (FACS) that are available to actually screen the patient's tumor for expression of the respective antigen. Here, single-molecule localization microscopy by *d*STORM can be used advantageously to quantitatively map tumor-associated membrane receptors, ideally the entire tumor receptome, using commercially available fluorescently labeled antibodies.[4-7] This provides important information about possible tumor-specific surface targets for antibodies and T cells. That is, *d*STORM imaging of the receptome of primary tumor cells enables the detection and selection of single or combinations of tumor antigens, and based on molecule density per cell, the selection of the optimal treatment modality for an individual patient.

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SELECTED ABSTRACTS FOR AN ORAL PRESENTATION

CSF1R BLOCKADE IMPROVES RESPONSE TO CAR T-CELL IN AGGRESSIVE LYMPHOMA

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Introduction: Notwithstanding remarkable changes in the therapeutic landscape of aggressive large B-cell lymphoma (LBL) curative options remain limited in the relapse or refractory (r/r) setting. CD19-directed CAR T-cell (CART) therapy enables long-lasting remissions. However, this curative potential is restricted to only a fraction of 40 to 50 % of patients. Especially in case of high baseline tumor burden, outcomes remain disappointing. Recently, high metabolic tumor burden could be linked to increased serologic and transcriptional macrophage signatures in patients undergoing CART therapy for r/r LBL (Jain et al, Blood 2021). Colony stimulating factor 1 receptor (CSF1R) inhibition was shown to modulate tumor associated macrophages (TAM) promoting a proinflammatory phenotype in mouse models of solid cancers (Pyonteck et al, Nat Med, 2013).

Methods & Results: Following a reverse translational approach using metabolic tumor volume (TV) data obtained in PET/CT scans and longitudinally taken biopsies of patients undergoing CART therapy we could confirm negative correlation of high baseline TV with lack of response. Moreover, we detected a shift of tumor-associated macrophages (TAM) towards an immunosuppressive transcriptional signature comparing matched pair baseline and relapse biopsies using bulk 3'RNA sequencing and CIBERSORTx analysis (Fig 1).

To leverage the modification of TAM we applied CD19-targeting CART therapy to a fully immunocompetent mouse model of aggressive LBL (Flümann et al, Blood Cancer Discov, 2021). We could show that (1) responses to CART monotherapy were worse in a high TV setting reflecting the clinical dilemma and (2) this lack of response in the high tumor burden setting (spleen tumor volume > 500 µl) could be significantly reverted by limited-time inhibition of CSF1R using a monoclonal antibody. Importantly, the responses were durable (Fig. 2). Results of immune phenotyping data as well as a validation cohort using a small molecule inhibitor will be presented.

Conclusion: Modifying tumor-associated macrophages through CSF1R pathway modulation represents an attractive strategy to enhance responses of CART therapy in aggressive B-cell lymphoma especially in the clinically challenging high tumor burden setting.

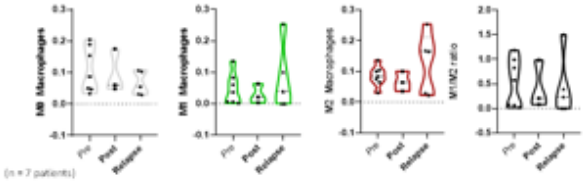

Figure 1

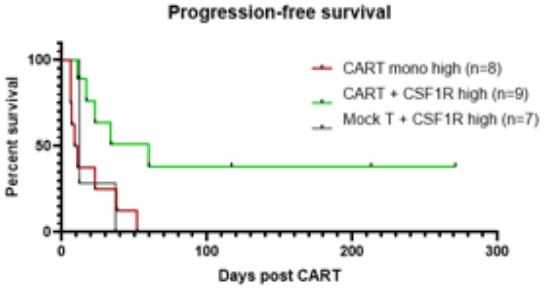

Figure 2

EBAG9-SILENCING LINKS CYTOLYTIC EFFICACY AND MEMORY FORMATION IN CD8$^+$ T CELLS FOR IMPROVED CANCER IMMUNOTHERAPY

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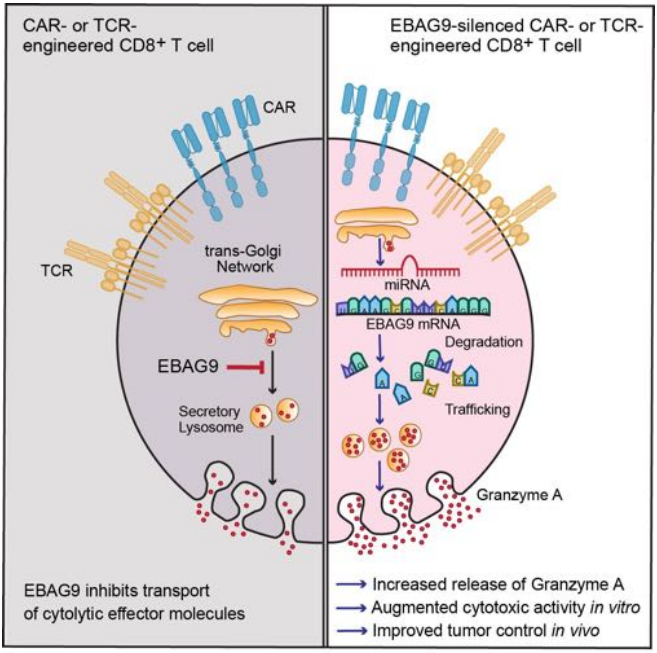
Background: The efficiency of adoptive immunotherapy correlates with the ability of the transferred T cells to release effector molecules. Furthermore, T cell memory formation is important to ensure a long lasting anti-tumor effect and thus, to prevent tumor relapse. Here, we optimized the efficacy of adoptive immunotherapy by silencing EBAG9, a negative regulator of cytolytic enzyme release from CD8$^+$ T cells.

Methods: Using a retroviral vector that includes an intronically located EBAG9-targeting miRNA, we equipped T cells with defined TCR or CAR specificities and, simultaneously, knocked down EBAG9. In vitro, cytotoxicity as well as cytokine release assays were employed. Kinetics of tumor cell killing and the sensitivity of CAR activation were determined by microscopy-based methods. Knockout mice, cytotoxicity assays and xenotransplantation models served as tools to study EBAG9 functionality in vivo. We studied T cell memory formation, *in vivo* target cell killing and tumor growth after adoptive transfer of engineered T cells.

Results: EBAG9 knockdown in mouse CTLs resulted in a significant increase of antigen-specific *in vivo* killing activity. Engineered human TCR and CAR T cells with silenced EBAG9 revealed that granzyme A release was increased from CD8$^+$ T cells, while cytokine secretion was not altered. Enhanced granzyme release translated into strongly improved killing capability. Enhanced cytolytic activity did not lead to faster exhaustion. Adoptively transferred EBAG9-silenced BCMA CAR T cells exhibited an extended control of multiple myeloma growth, even at low effector cell numbers. Upon leukemia challenge of *Ebag9*$^{-/-}$ mice, the enhanced CD8$^+$-mediated cytotoxicity was associated with increased commitment to the CD8$^+$ T cell memory lineage. Single cell RNA sequencing revealed a preferential expression of the memory-related transcription factors *Zeb1, Id3*, and *Stat3* as well as anti-apoptotic genes like *Traf1* in *Ebag9*$^{-/-}$ CD8$^+$ cells.

Conclusion: Targeting EBAG9 has a promising therapeutic potential when applied to adoptive immunotherapy of leukemia and multiple myeloma. We suggest a strategy to boost the efficacy of engineered TCR and CAR T cells that, concomitantly, facilitates an optimized manufacturing combined with the need for a lower therapeutic dose. From Ebag9-KO mice we infer that an enhanced primary immune response leads to improved memory formation and, thus, lowers the risk for tumor relapse. Gain of cytolytic efficacy by EBAG9 silencing does not come at the expense of adverse effects, such as faster exhaustion or release of inflammatory cytokines. Collectively, EBAG9-targeting is a platform technology that can be favorably combined with variable CAR or TCR specificities.

Disclosures: A.W., M.B., U.E.H. and A.R. have filed patent applications on the BCMA CAR and on the therapeutic application of EBAG9 modulation, respectively. This work was funded by grants form Deutsche Forschungsgemeinschaft (DFG), SFB/TR36 (Project B12), RE 1103/6-1; and from the MDC PreGoBio funding program (all to A.R. and U.E.H.).



EARLY CANCER IMMUNE SURVEILLANCE VIA VG9VD2 TCR T CELLS REGULATED BY NOVEL BTN3A1-LINKED PROTEIN NETWORK

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Objectives: Although tumor infiltrating Vγ9Vδ2T cells often have a good prognostic value, their role in cancer immune surveillance remains to be defined.

Methods: We employed a human genetically engineered step-wise mutagenesis organoid model of colorectal cancer and examined reactivity of Vγ9Vδ2TCR T cells.
With proximity-dependent labeling we investigated the interactome of butyrophilin-3 isoform A1 (BTN3A1), crucial for activation of Vγ9Vδ2TCR T cells.

Results: We demonstrate that a single oncogenic mutation introduced into healthy organoids derived from the colon is sufficient to activate T cells through a Vγ9Vδ2TCR. During early mutagenesis we observed changes in spatial dynamics of BTN2A1 which allowed, after transformation binding of soluble Vγ9Vδ2TCR and re-localization and an increased expression of the small GTPase RhoB. We identified PHLDB2, SYNJ2 and CARMIL1 as key players in modulating spatial orchestration during early transformation by acting directly on BTN3A1 and indirectly on BTN2A1. This mode of action depended on phosphorylation of BTN3A1 and allowed tumor control in vitro and in vivo.

Conclusions: In this study we established a detailed interactome of BTN3A1-related proteins using various protein proximity techniques that resolved spatial interaction hierarchies among the studied proteins. Our results emphasize the crucial role of these molecules from early mutagenesis to advanced cancer stages and the therapeutic potential of a Vγ9Vδ2TCR.
These findings do not only shed light in the role of Vg9Vd2T cells during early cancer immune surveillance but have also great implication for all Vγ9Vδ2TCR cell based immune therapies.

PRE-CLINICAL DEVELOPMENT AND CHARACTERIZATION OF A DECITABINE-INDUCED REGULATORY HLAG$^+$CD4$^+$-T CELL-ENRICHED CELL PRODUCT AGAINST GVHD

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Background: Graft-vs-host-disease (GvHD) is a life-threatening complication of allogeneic hematopoietic cell transplantation with inadequate approved therapies. HLA-G is an immunosuppressive molecule playing a central role during pregnancy preventing the rejection of the semi-allogeneic fetus, and its expression is epigenetically repressed during adulthood. We have previously reported the *ex vivo* generation of decitabine-induced HLAG$^+$CD4$^+$FOXP3$^-$ regulatory T-cells exerting potent suppressive function *in vitro* (Cytotherapy 2017;19:521). Herein, we aimed to further elucidate the molecular mechanisms of HLAG$^+$CD4$^+$ T cell-mediated immunosuppression, produce and characterize a clinical scale product enriched for HLA-G$^+$ immunosuppressive cells (iG-Tregs).

Methods: Peripheral Blood Mononuclear Cells from healthy donors were enriched for T-cells through monocyte depletion, activated 3 days with CD3/CD28-beads, followed by a 3-day decitabine treatment (small-scale: n=20, clinical-scale: n=13). The final clinical product (iG-Treg) was assessed *in vitro* and *in vivo* (NSG mice) and validated under GMP conditions(n=2). FACS-sorted HLAG$^+$CD4$^+$ T-cells were further characterized by RNA-seq (Illumina) and single-cellRNA-seq (scRNA-seq, 10X Genomics).

Results: The iG-Tregs contained median(Δm) 94%(89.35%-98.6%) CD3$^+$cells, predominantly CD4$^+$[Δm CD4:CD8 ratio: 2.1(0.81-3.39)]. Compared to untreated controls(PBS), iG-Tregs were enriched for HLAG$^+$ cells within the total CD3$^+$ population[Δm 15.8%(7.2-23.8%)] and within the CD4$^+$[Δm 22.5%(6.5-34.2%) and CD8$^+$[Δm 17.7%(7.5-26.9%)] subpopulations. Consequent immunophenotype on iG-Tregs revealed the expression of TIM3 and LAG3 [iG-Tregs:Untreated ΔmMFI LAG3: 4(3-4.5), TIM3: 1.5(1.1-1.8)] with significant enrichment within the CD8$^+$ population [ΔmMFI CD8:CD4 ratio LAG3: 2.29(1.81-4.65), TIM3: 1.46(1.23-2.49)] and the HLAG$^+$ population [ΔmMFI HLAG$^+$:HLAG$^-$ ratio LAG3: 3.6(1.7-4.2), TIM3: 1.7(1.4-2.8)]. iG-Tregs demonstrated a favorable safety profile vs untreated cells *in vitro,* according to their diminished alloreactivity against allogeneic PHA blasts (1.8±1% lysis *vs* 49±4, p<0.0001) and their hyporesponsiveness after restimulation via intracellular reduction of effector cytokines (IL-2,p=0.02, IFNγ,p=0.008, IL-17a,p=0.04). Except IL-13 (p=0.05) no other Th1/Th2 cytokines were increased in iG-Treg supernatants. Respectively, the GMP products were comparable with pre-clinical iG-Tregs regarding the HLA-G+ enrichment, immunophenotype, and alloreactivity. The diminished alloreactivity of iG-Tregs over untreated controls was confirmed *in vivo* after infusion in NSG mice; 6/9(67%) iG-Treg-treated mice survived until sacrifice whereas all 7 PBS-control-treated mice developed GvHD from day 21 and succumbed by day 35 (Δm). iG-Tregs ability in preventing GvHD was tested by their co-infusion with T-cells; while T-cell infusion resulted in lethal GvHD by day 36 in all 3 control mice, the co-administration of iG-Tregs delayed (5/6 mice died by day 66) or even prevented(1/6) GvHD onset. RNA-seq showed that FACS-sorted decitabine-induced HLAG$^+$CD4$^+$ cells had a distinct and homogenous expression profile compared to their HLAG$^-$CD4$^+$ counterparts. HLAG$^+$CD4$^+$ cells differentially expressed regulatory genes (e.g.CCL17, CCL22, CXCL9) and interestingly, the predominantly myeloid suppressor gene IDO-1, a finding which was validated at mRNA and protein level. The prominent regulatory signature of the HLAG$^+$CD4$^+$ cells was confirmed by scRNA-seq analysis.

Conclusions: iG-Tregs can be consistently produced through a short-protocol constituting a well-characterized and safe product. Besides HLA-G, the possible contribution of IDO-1, LAG3, TIM3, IL-13 and other regulatory genes, in the immunosuppressive function of $HLAG^{+}CD4^{+}$ cells, remains to be assessed in future functional assays. Concluding, iG-Tregs will be evaluated in the upcoming phase I-II clinical trial for the prevention of GvHD (EUDRACT-2021-006367-26).

IMPROVING BONE MARROW HOMING AND PERSISTENCE OF CD33.CAR-CIK CELLS BY OVEREXPRESSION OF A GAIN-OF-FUNCTION VARIANT OF CXCR4 TO INCREASE EFFICACY IN ACUTE MYELOID LEUKEMIA

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Chimeric Antigen Receptor (CAR) Cytokine-Induced Killer (CIK) cell therapy is a rising treatment for Acute Myeloid Leukemia (AML). Nevertheless, it is hampered by dampened CAR-CIK cells infiltration ability in the bone marrow (BM) niche, leukemic stem cells' preferential location. Noticeably, CAR-CIK cells *ex vivo* manipulation affects the expression of several chemokine receptors, specifically CXCR4, and may influence the capacity of therapeutic cells to respond to CXCL12 gradient, generated by mesenchymal stromal cells (MSCs) within the niche. Thus, combining the expression of CD33.CAR and CXCR4 might promote CAR-CIK cells homing to the BM and consecutive leukemia eradication. In particular, the gain-of-function variant of CXCR4 (R334X), described in WHIM syndrome and linked with leukocytes retention in the BM, may provide *in situ* persistence of CAR-CIK cells.

Two different 2A peptide-based bicistronic Sleeping Beauty transposon vectors were designed to vehiculate the concomitant expression of CD33.CAR and $CXCR4^{WT}$ or $CXCR4^{MUT}$. The monocistronic CD33.CAR was used as control. *In vitro* effector functions were compared performing cytotoxicity, cytokines release and proliferation assays. *In vitro* migration toward recombinant CXCL12 (rhCXCL12) or MSC supernatants was tested in transwell migration assay. *In vivo* BM homing ability was assessed in NSG mice, evaluating CAR-CIK cells engraftment in BM, blood and spleen after 7, 10 and 14 days from CAR-CIK cells infusion.

Overexpression of CXCR4, both in wild-type and in mutated variants, was maintained during culture on CD33.CAR-CIK cells transfected with bicistronic vectors, whereas it was consistently downregulated on the control. CD33.CAR was comparably expressed among the three different constructs. CIK cells engineered either with CD33.CAR, CD33.CAR-$CXCR4^{WT}$ or CD33.CAR-$CXCR4^{MUT}$ preserved the expression of their phenotypic markers, and no differences were noticed in terms of memory phenotype. Next, it was evaluated CAR-CIK cells chemotactic ability in a transwell migration assay. To assess the contribution of $CXCR4^{WT}$ or $CXCR4^{MUT}$ molecules on CD33.CAR-CIK cells functionality, we selected CAR^+-CIK cells by immunomagnetic sorting. Both $CD33.CAR^+$-$CXCR4^{WT}$ and $CD33.CAR^+$-$CXCR4^{MUT}$ demonstrated improved chemotaxis toward rhCXCL12, and stromal cells conditioned medium, compared to control $CD33.CAR^+$-CIK cells. To establish if CXCR4 overexpression may have an impact on CAR-related effector functions, we tested $CD33.CAR^+$-, $CD33.CAR^+$-$CXCR4^{WT}$ and $CD33.CAR^+$-$CXCR4^{MUT}$-CIK cells against $CD33^+$ AML target cell line KG-1. Comparable cytotoxic activity, proliferative response and IFN-γ or IL-2 secretion levels were observed. Furthermore, to assess if CXCR4-overexpressing CD33.CAR-CIK cells acquire superior BM homing *in vivo*, we infused NSG mice with either $CD33.CAR^+$-$CXCR4^{WT}$-, $CD33.CAR^+$-$CXCR4^{MUT}$- or $CD33.CAR^+$-CIK cells. The frequency and absolute number of $hCD45^+$ cells retrieved from BM, peripheral blood, and spleen was determined by FACS analysis 7, 10 or 14 days after transplant. Notably, CXCR4-overexpressing $CD33.CAR^+$-CIK cells showed enhanced BM homing *in vivo*, associated with a prolonged retention in the case of $CXCR4^{MUT}$.

Overall, engineering CD33.CAR-CIK cells to overexpress CXCR4, in its wild-type or hyperactive mutant form, improves CAR-CIK cells *in vitro* migration toward CXCL12 and *in vivo* homing ability to the BM while retaining CAR-related functionalities. Therefore, arming anti-AML CAR-CIK cells with a chemokine receptor involved in the leukemia-niche interplay may represent a promising strategy to increase their therapeutic efficacy for AML.

PHARMACOLOGICALLY INHIBITING MYCN IMPROVES L1CAM-DIRECTED CAR T CELL EFFICACY AGAINST *MYCN*-AMPLIFIED NEUROBLASTOMA

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Objectives: Current treatment protocols have only limited success in pediatric patients with high-risk neuroblastomas harboring amplifications of the central oncogene *MYCN*. Adoptive T cell therapy presents an innovative strategy to improve cure rates, however, L1CAM-targeting CAR T cells achieved only limited response against refractory/relapsed neuroblastoma in an ongoing clinical trial. One possible factor limiting success in trials might be *MYCN* amplification, that is always associated with a "cold" tumor microenvironment and repression of the immune response. Here we investigated how oncogenic MYCN levels influence tumor cell response to CAR T cells with the aim to illuminate mechanisms inhibiting CAR T cell success against neuroblastoma.

Methods: High MYCN levels were determined in the SK-N-AS neuroblastoma cell line harboring the normal diploid *MYCN* complement using a tetracycline-inducible system to allow comparison of differential MYCN expression in the same cell background. The neuroblastoma cell model with the different *MYCN* expression was cocultured with L1CAM-CAR T cells harboring either the CD28 or 4-1BB costimulatory domain. L1CAM-CAR T cell function was assessed in assays for activation/inhibitory receptor expression, cytokine release and tumor cell cytotoxicity. The cell line was further characterized using RNA sequencing and our data was compared to publicly available RNA and proteomic data sets of neuroblastoma patients. ChIP-sequencing data was used to determine transcriptional regulation by MYCN on *L1CAM* using public data sets. Combination of CAR T cells with the indirect MYCN inhibitor, MLN8237, was assessed *in vitro* via combinatorial cytotoxic effects and synergism calculation and *in vivo* using an immunocompromised mouse model.

Results: MYCN induction impaired L1CAM-CAR T cell activation, tumor cytotoxicity and cytokine release by 14-fold *in vitro*. Interestingly, MYCN induction caused a significant downregulation of L1CAM expression on tumor cells. We confirmed diminished L1CAM mRNA and protein expression in different cohorts of primary neuroblastomas with high *MYCN* expression. Inverse correlation of MYCN and L1CAM can be caused by transcriptional repression by MYCN on *L1CAM*. By indirectly inhibiting MYCN *in vitro* using the aurora a kinase inhibitor, MLN8237, increased L1CAM expression on the neuroblastoma cells and restored L1CAM-CAR T cell effector function. MLN8237 synergistically enhanced L1CAM-CAR T cell-directed killing of neuroblastoma cells overexpressing MYCN.

Conclusion: We shed new light on a possible resistance mechanism of neuroblastoma controlled by *MYCN* against L1CAM-CAR T cell therapy by downregulation of the target antigen. The data suggest that combining L1CAM-CAR T cell therapy with pharmacological MYCN inhibition may benefit patients with high-risk neuroblastomas harboring *MYCN* amplifications.

PLURIPOTENT STEM CELL-DERIVED CAR-NEUTROPHILS FOR IMMUNOTHERAPY OF SOLID TUMORS

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CAR-T cell and CAR-natural killer (NK) cell therapies have already demonstrated tremendous success in the eradication of lymphoid malignancies. However, many challenges remain in the applying CAR therapies for solid tumors, and responses with CAR-T cells have been limited to isolated exceptional cases. Given the known capacity of neutrophils to infiltrate and reside within solid tumors, opportunities exist for new immunotherapies for specific targeting of solid tumors using CAR-weaponized neutrophils which are capable of cytotoxicity and migration into solid tumors. However, generation of CAR neutrophils from peripheral blood represent a significant challenge due to their very short life-span. Human pluripotent stem cells (hPSCs) are a logical alternative for large-scale production of CAR neutrophils due to their renewability and uniform quality. In our study, we generated hPSCs with GD2-CARs integrated into *AAVS1* locus and differentiated into neutrophils using serum- and xeno-free differentiation system based on modified *ETV2* mRNA. Neutrophils generated from GD2-CAR iPSCs, as compared to wild type (WT), demonstrated superior cytotoxicity *in vitro* against $GD2^+$ WM266-4 melanoma and CHLA20 neuroblastoma, while no differences of cytotoxicity were observed against GD2-negative SKOV3 ovarian and SK-BR3 breast cancer cells, indicating the specificity of anti-tumor therapeutic effect of CAR neutrophils. To assess *in vivo* potential of GD2-CAR neutrophils, NCG and NSG mice were inoculated intraperitoneally (IP) with $3x10^5$ Luc2-$eGFP^+$ WM266-4 melanoma cells and engraftment was assessed by IVIS bioluminescent imaging. On day 4 post WM266-4 injection, mice were either treated with 10^7 WT or GD2-CAR neutrophils via IP injection every 7 days. Upon assessment over 30 days, GD2 CAR neutrophil-treated mice showed reduced tumor burden and prolonged survival as compared to WT neutrophil-treated or untreated mice. Overall, our studies demonstrate a feasibility of using hPSC-derived CAR-neutrophils for immunotherapies against solid tumor cancers.

JOINT FORCES - SYNERGIZING MICROBIOME-DERIVED METABOLITES AND T CELL ENGINEERING TO COMBAT SOLID TUMORS

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Background: The intestinal microbiota has been identified as a crucial factor in influencing the efficacy of cancer immunotherapy. Recent studies have demonstrated that members of the gut microbiota, such as *Akkermansia muciniphila*, are able to improve immune checkpoint inhibition (ICI), thereby enhancing immune cell-mediated anti-tumor responses. Notably, the impact of soluble microbial molecules and metabolites such as short-chain fatty acids (SCFAs) on the outcome of cellular cancer immunotherapy remains poorly investigated.

Methods: In this study, we compared 14 abundant bacterial species representing proportionally the most common phyla in the human intestine and performed GC-MS analysis to assess their SCFAs production profile. Intracellular cytokine and phospho-stainings were used to analyse the phenotype of murine and human T cells which were differentiated in vitro in presence of SCFAs. The functional characterization of SCFA-treated antigen-specific T cells and engineered chimeric antigen-receptor (CAR) T cells was performed by cytotoxicity assays and adoptive transfer experiments in syngeneic solid tumor models.

Results: Recently, we identified the SCFA pentanoate as a metabolite produced by the low-abundant commensal *Megasphaera massiliensis* and analysed its effect on $CD8^+$ cytotoxic T lymphocyte (CTL)-mediated anti-tumor immunity. Pentanoate induced the metabolic reprogramming of murine and human CTLs towards elevated glycolysis by increasing the activity of the mTOR complex. Furthermore, the enhanced cytotoxic capacity of CTLs was achieved by SCFA-mediated inhibition of class I histone deacetylases (HDACs) promoting production of effector molecules such as perforin, granzyme B, TNF-a and IFN-g.

Pentanoate treatment led to histone hyperacetylation at the promoter regions of the master transcription factors EOMES and T-bet in $CD8^+$ T cells, highlighting that epigenetic modifications are mechanistically involved in inducing the CTL-associated phenotype. Importantly, the SCFA treatment of antigen-specific CTLs and $CD8^+$ chimeric-antigen receptor (CAR) T cells augmented their capacity to reduce tumor progression in syngeneic solid tumor models. Our current findings show that pentanoate also confers CTL characteristics on $CD4^+$ CAR T cells. In this regard, we observe that secretion of effector molecules is accompanied by enhanced IL-2 and CAR expression which proffers support for both endogenous and $CD8^+$ CAR T cells. Moreover, we examine the effects of SCFAs on tumor-intrinsic features to find novel targets for synergistic therapy approaches.

Conclusions: Collectively, our work demonstrates that SCFAs as commensal-derived epigenetic and metabolic modifiers have a therapeutic potential in the context of adoptive cancer immunotherapy. Our data provide new insights into the microbiome-CAR T cell interface and might be utilized to improve the efficacy of engineered CAR T cell products.

SUPERRESOLUTION MICROSCOPY REVEALS DISPARITY IN CAR EXPRESSION AND FUNCTION OF CAR T CELL GENERATED USING DISTINCT GENE-TRANSFER STRATEGIES

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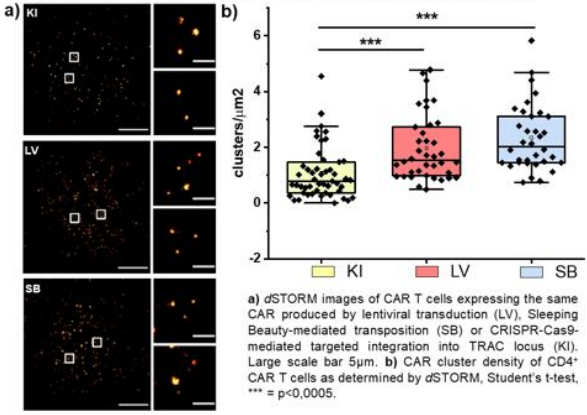
Objectives: For optimization of chimeric antigen receptor (CAR) T cell products towards both, maximal safety and efficacy, it is critical to understand how distinct gene transfer strategies affect CAR expression and organization on the T cell surface and modulate T cell effector functions.

Methods: We therefore established a test platform in which we are able to compare CAR constructs covering a range of different target and epitope specificities and graded affinity that are engineered by either lentiviral transduction (LV), Sleeping Beauty (SB) retrotransposon-based gene-transfer or CRISPR-Cas-mediated targeted CAR insertion (KI) into the TRAC locus.
Using *d*STORM super-resolution microscopy, we analyzed CAR surface expression (using flow cytometry as a comparison) as well as localization and clustering of CAR receptors on the T cell surface; in addition, we assessed the influence of endogenous vs. exogenous promoters on the CAR expression and determined variations in CAR copy numbers by droplet digital (dd)PCR.
Utilizing target cells with distinct, graded antigen densities, the generated CAR T cells were functionally characterized regarding short vs. long-term cytolytic activity, cytokine secretion and proliferation capacity *in vitro*. We employed whole-cell avidity assays to link CAR expression level and cell-cell interaction strength. Furthermore, we assessed the expression of exhaustion markers as well as the rate of antigen-induced cell death (AICD) by flow cytometry.

Results: We were able to detect pronounced differences in dynamics of CAR expression and absolute numbers, spatial distribution and clustering of CAR receptors during sequential stimulation campaigns. On the surface of LV/SB CAR T cells, we detected a >2-fold higher CAR density compared with TRAC knock-in T cells (Fig. 1).
The observed range of CAR expression was more concise for targeted insertion with endogenous promoters compared to random integration-based techniques in both, $CD4^+$ and $CD8^+$ T cells.
In functional experiments, LV/SB CAR T cells exhibited higher cytokine production and conferred stronger cytolytic activity compared with KI CAR T cells in short-term lysis assays (< 20 hours, multiple E:T ratios) depending on antigen density. In contrast, at a longer follow-up (> 20 hours) LV/SB CAR T cells and KI CAR T cells were equally effective. Relative avidity was distinct between gene transfer methods and independent of CAR expression. Intriguingly, we observed a significantly lower extent of antigen-induced cell death (AICD) in KI CAR T-cells, suggesting that a lower number and density of CAR receptors provide better protection from overstimulation. Extended analyses *in vitro* and in pre-clinical *in vivo* models are ongoing.

Conclusions: Taken together, these data indicate that non-targeted (LV/SB) and targeted (KI) CAR insertion result in distinct patterns of CAR expression, organization and regulation, which translate into differential anti-tumor reactivity. Our data suggest that adapting gene-transfer method depending on the target antigen levels in a particular tumor entity may provide benefits for CAR T cell performance.

Fig. 1: CHIMERIC ANTIGEN RECEPTOR SURFACE EXPRESSION



a) *d*STORM images of CAR T cells expressing the same CAR produced by lentiviral transduction (LV), Sleeping Beauty-mediated transposition (SB) or CRISPR-Cas9-mediated targeted integration into TRAC locus (KI). Large scale bar 5µm. **b)** CAR cluster density of CD4+ CAR T cells as determined by *d*STORM, Student's t-test, *** = p<0,0005.

SELECTED ABSTRACTS FOR A POSTER PRESENTATION

BIOLOGY POSTERS

ABSTRACTS SELECTED AS POSTERS
IN ALPHABETIC ORDER ACCORDING TO THE NAME OF THE FIRST AUTHOR

BIOLOGY POSTERS

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Miriam Alb, Hermann Einsele, Peter Loskill, Andries van der Meer, Katherina Sewald, Kristin Reiche, Ulrike Köhl, Michael Hudecek

Poster 2: **ENHANCEMENT OF CD33-CAR-NK CELL-MEDIATED ANTI-LEUKEMIC CYTOTOXICITY BY KNOCKOUT OF IMMUNE CHECKPOINT NKG2A**
Nawid Albinger, Tobias Bexte, Leon Buchinger, Philipp Wendel, Ahmad Al-Ajami, Alec Gessner, Vinzenz Särchen, Jamal Alzubi, Sarah Mertlitz, Olaf Penack, Raj Bhayadia, Maike Vogler, Dirk Heckl, Jan-Henning Klusmann, Nina Möker, Toni Cathomen, Michael A. Rieger, Katharina Imkeller, Evelyn Ullrich

Poster 3: **SHED L1CAM IMPAIRS L1CAM-DIRECTED CAR-T CELL EFFICACY AGAINST NEUROBLASTOMA**
Lena Andersch, Anika Klaus, Silke Schwiebert, Michael Jensen, Angelika Eggert, Kathleen Anders, Annette Künkele

Poster 4: **IMMUNOTHERAPY AGAINST CYTOMEGALOVIRUS USING CHIMERIC ANTIGEN RECEPTORS**
Christopher Bednar

Poster 5: **PUTTING ONCOLYTIC VIRUS VACCINES IN THE DRIVER'S SEAT: EVALUATING THE CAR AS A UNIVERSAL BOOSTING PLATFORM IN ADOPTIVE T CELL THERAPIES**
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Poster 6: **PLASMA CELL ENGINEERING FOR A GRAFTED ADAPTIVE IMMUNOTHERAPY**
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NOVEL STRATEGIES TO ENHANCE THE SAFETY OF CAR T CELL IMMUNOTHERAPY: THE IMSAVAR PROJECT

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Introduction: Adoptive immunotherapy with CAR T cells is a transformative treatment in hematology but can be associated with significant toxicity from e.g. cytokine release syndrome (CRS). Non-clinical testing to assess CRS is not standardized and there is a strong medical need to establish algorithms assessing the propensity of novel CAR T cell products inducing CRS and other toxicities. imSAVAR – immune safety avatar – is an EU Innovative Medicines Initiative project that tackles this challenge in a joint academia-industry consortium seeking to establish a platform for assessing the utility of innovative non-clinical models and endpoints for enhancing the safety assessment of e.g. CAR T cells.

Methods: A key deliverable is the development of a conceptual map of CRS pathogenesis as an immune-related adverse outcome pathway (irAOP) comprising key molecular and cellular events identified through a systematic literature review on pathophysiology and clinical occurrence of CRS after CAR T cell therapy. To each event existing and emerging non-clinical assays were allocated. imSAVAR is engaging all stakeholders in CAR-T immunotherapy, including clinicians, scientists, model developers, bioinformaticians, data analysts as well as patients through surveys and workshops for patients and health care professionals.

Results: We established a roadmap for studies establishing a new set of non-clinical assays and endpoints that will be validated incorporating the feedback from multi-stakeholder workshops to help mitigate clinical safety concerns. To this end, an experimental campaign was conducted with n=3 CAR T cell products in n=5 test systems from within the consortium. Test systems available in imSAVAR include *in vitro* co-culture assays, *in vivo* models, genomic approaches, and organ-on-a-chip models. All data will be correlated with clinical data to identify optimal non-clinical assays for enhancing assessment and prediction of CRS.

Conclusions: imSAVAR established an irAOP that will enable the development and validation of novel non-clinical assays that aim to enhance the characterization of CAR T cell associated CRS during non-clinical development. This effort is ultimately anticipated to enhance the safety assessment of therapeutic CAR T cell products, thus potentially accelerating patient access to CAR T cell products.

POSTER 1

ENHANCEMENT OF CD33-CAR-NK CELL-MEDIATED ANTI-LEUKEMIC CYTOTOXICITY BY KNOCKOUT OF IMMUNE CHECKPOINT NKG2A

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Background: Application of allogeneic chimeric-antigen-receptor (CAR) NK cells has proven safety and efficacy in first clinical trials. However, therapy of acute myeloid leukemia (AML) remains challenging. CAR-NK cell function can be impaired by upregulation of inhibitory receptors NKG2A (natural killer group 2A). By applying a CRISPR/Cas9 knockout (KO) we showed improved CAR-NK cell functionality *in vitro* and *in vivo*.

Methods: CD33 targeting CAR-NK cells were generated by lentiviral transduction. KO of the NKG2A-encoding *killer cell lectin like receptor C1* (*KLRC1*) locus was performed using CRISPR-Cas9. The CD33-CAR- and NKG2A-expression as well as cytotoxicity were analysed using flow cytometry after feeder cell-free, IL15/IL2-based expansion. The *in vivo*-efficacy was evaluated in OCI-AML2 (GFP^+, Luc^+) xenografted NSG-SGM3 mouse models.

Results: Lentiviral transduction resulted in up to 60% CD33-CAR positive NK cells, while gene *KLRC1* disruption resulted in 50% reduction of NKG2A expression. CD33-CAR-NKG2A-KO-NK cells showed significantly higher elimination of $CD33^+$/HLA-E^+ OCI-AML2 cells in *in vitro* cytotoxicity assays compared to NKG2A-KO- or CD33-CAR-NK cells. Cite-Seq and qPCR analysis revealed upregulation of CXCR4, CD16 and CD70 in CAR- and CAR-KO-NK cells. Furthermore, in the AML-xenografted mouse model, a significant reduction of leukemic burden was observed *in vivo* following a single injection of a low dose ($3x10^6$ cells) of CD33-CAR-NKG2A-KO-NK cells compared to NKG2A-KO or CAR-NK cell-treatment. A double injection led to a complete tumor elimination in the bone marrow of mice treated with CD33-CAR-NKG2A-KO NK cells, which was confirmed by bone marrow re-engraftment analysis.

Conclusion: Removing an inhibitory receptor in CAR-NK cells showed a synergistic effect. This double genetic modification has the potential to enable NK cells to bypass the suppressive effect not only in the AML tumor microenvironment but also in a broad range of other tumor identities.

POSTER 2

SHED L1CAM IMPAIRS L1CAM-DIRECTED CAR-T CELL EFFICACY AGAINST NEUROBLASTOMA

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Objectives: Target antigen expression by tumor cells and the hostile immunosuppressive microenvironment play crucial roles for CAR-T cell therapy success against solid tumors. L1CAM is overexpressed in neuroblastoma, and the cancer-specific glycosylation of the CE7 epitope makes it a suitable target antigen. Constitutive cleavage of the L1CAM extracellular domain by the ADAM metalloprotease sheds the CE7 epitope-containing L1CAM ectodomain into blood and ascites. The RGD motif in soluble L1CAM can also bind integrins in tissues. We hypothesized that soluble L1CAM in the blood or tissue matrix could neutralize CAR-T cells before they reach the tumor site or exhaust CAR-T cells more quickly through excessive CE7 CAR domain binding.

Methods: L1CAM shedding was analyzed in serum of neuroblastoma xenograft-bearing mice and neuroblastoma patients using ELISA assay. L1CAM CAR-T cells harboring the short or long extracellular spacer were incubated with fluorescent-labeled recombinant L1CAM protein to analyze L1CAM binding and subsequent activation of CAR-T cells via flow cytometry. The efficacy of CAR-T cells pre-incubated with plate-bound or soluble L1CAM against neuroblastoma cells was analyzed by cytokine release assay or flow cytometric detection of cell surface markers, killing ability was assessed by biophotonic cytotoxicity assay. Re-binding of recombinant L1CAM to tumor cells was assessed by incubation of L1CAM-KO SK-N-BE(2) neuroblastoma cells with fluorescent-labeled recombinant L1CAM for 24h and subsequent analysis of L1CAM cell surface expression via flow cytometry.

Results: Soluble L1CAM levels were elevated in serum from mice harboring neuroblastoma xenografts and patients with neuroblastoma, confirming *in vivo* L1CAM shedding. L1CAM-coated plates activated L1CAM CAR-T cells *in vitro*, but soluble recombinant L1CAM concentrations comparable with concentrations detected in patient samples did not. Interestingly, CAR-T cells with the long spacer bound L1CAM at lower concentrations and showed stronger upregulation of the activation marker CD137 than CAR-T cells harboring the short spacer. Pre-exposing L1CAM CAR-T cells to plate-bound L1CAM reduced *in vitro* cytotoxic potential against neuroblastoma cells. Tumor cells lacking L1CAM expression, but pre-exposed to soluble L1CAM concentrations found in patients, lead to L1CAM binding to tumor cells, presumably via integrin-binding. CAR-T cells exposed to L1CAM bound to tumor cells upregulated INFG levels to a comparable extent as when exposed to parental neuroblastoma cells.

Conclusion: We provide evidence for L1CAM shedding in neuroblastoma and that soluble L1CAM is present in serum from neuroblastoma-bearing mice and neuroblastoma patients. While pre-exposition to soluble L1CAM does not affect CAR T cell efficacy, bound L1CAM impairs cytotoxicity *in vitro*. Shed L1CAM can bind to integrins expressed on non-target cells and we provide evidence that bound L1CAM on non-target cells can activate L1CAM CAR-T cells. Our findings suggest that the cleaved L1CAM ectodomain sequestered in extracellular matrix or bound to integrins on non-target cells can neutralize L1CAM CAR-T cells before they reach the tumor site. To circumvent early activation of CAR-T cells we will equip T cells with a GD2-SynNotch-inducible L1CAM CAR construct that dr ives L1CAM CAR

expression on the T cells only after they encounter the GD2 glycoprotein, a second antigen expressed on neuroblastoma.

IMMUNOTHERAPY AGAINST CYTOMEGALOVIRUS USING CHIMERIC ANTIGEN RECEPTORS

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Introduction: The infection or reactivation of the human cytomegalovirus (HCMV) is a great risk for immunosuppressed patients (i.e. during an allogeneic stem cell transplantation) as well as for immunocompromised people. In immunocompetent individuals, the immune system suppresses the spread of HCMV during an infection, resulting in an asymptomatic or mild course of the disease. A potent T cell response is crucial for an efficient control of the viral infection, however, HCMV is able to deter it via various mechanisms. In particular, downregulation of the surface expression of the major histocompatibility complex (MHC) by viral effector proteins renders CMV-infected cells unrecognisable for immune effector cells.
This work investigates the effect of a potential immunotherapy with cytotoxic T cells expressing chimeric antigen receptors (CARs) on murine cytomegalovirus (MCMV) infection in the mouse model. The CARs consist of a single chain variable fragment (scFv), here derived from antibodies recognising viral glycoproteins (gB, gH, gN) on the surface of CMV-infected cells, coupled with a costimulatory domain (CD28 or 4-1BB) and the zeta-chain of the murine T cell receptor (CD3ζ) as activating domain. By means of the HLA-independent recognition via the scFv, CAR^+ T cells bind viral glycoproteins on MCMV-infected cells, leading to an activation of the T cells, which then lyse infected cells. In a previous project, Full et al. generated a HCMV-gB specific CAR and showed its efficacy in *in vitro* studies.

Objectives & Methods: In this project, various CAR constructs directed against different MCMV-specific glycoproteins were generated and expressed on effector T cells. Tests on expression, activation and cytotoxic effects against reporter cell lines were conducted in human and murine cell lines. Finally, the most potent CAR constructs from the *in vitro* studies will be transduced into cytotoxic T cells, which will be administered to immunodeficient $RAG^{-/-}$ mice infected with a luciferase-expressing MCMV-reporter strain (MCMVm157luc). Tests on the optimal efficacy dose, the ideal administration time, the combination of T cells carrying different CARs, as well as the administration of human T cells carrying human CARs will be conducted.

Results: Retrovirally transduced murine T cells showed a strong surface expression of MCMV-specific CARs. CAR^+ T cell killing assays were performed on newly generated cell lines expressing MCMV-glycoproteins and reporter genes, for general cytotoxic effects as well as specifically for granzyme B activity in the target cells. Murine CAR^+ T cells showed a strong activation and cytotoxic effects when co-cultered with gB-expressing reporter cells, but little to no activity against reporter cells without surface gB.

Conclusions. Newly generated reporter cell lines are a reliable option to indicate T cell cytotoxicity as well as granzyme B activity. Murine CAR^+ T cells targeting MCMV-gB efficiently kill gB-expressing target cells and can be further evaluated in the animal model as an approach to an adoptive immunotherapy against complications of a cytomegalovirus infection.

PUTTING ONCOLYTIC VIRUS VACCINES IN THE DRIVER'S SEAT: EVALUATING THE CAR AS A UNIVERSAL BOOSTING PLATFORM IN ADOPTIVE T CELL THERAPIES

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Introduction/objectives: Adoptive T cell therapies (ACT) have shown good clinical success in the treatment of hematological malignancies and melanomas. However, solid tumours remain a therapeutic challenge, in part because T cells often fail to engraft, expand, or persist enough to mediate an effective anti-tumour response. Oncolytic virus vaccines (OVV), wherein tumour-associated antigens are encoded in oncolytic viruses, drive robust expansion of both adoptively transferred and endogenous tumour-specific T cells. Currently, ACT/OVV combined therapy relies on *a priori* knowledge of targetable tumour markers and methods for expanding antigen-specific T cells. Heterogeneity in cancer antigen expression means that OVVs would need to be developed on a patient-by-patient basis, and the cost of this personalized approach severely limits clinical feasibility. Alternatively, bulk populations of polyclonal T cells can be directed against a single target through engineering with chimeric antigen receptors (CARs). We propose to evaluate a "universal" approach where tumour-specific T cells are engineered with a defined CAR and subsequently boosted with an OVV expressing the CAR target. This novel boosting strategy could be applied off-the-shelf to any tumour-specific T cell.

Methods: As proof-of-concept, we engineered murine TCR-transgenic T cells with boosting CARs against a defined antigen for studies in immunocompetent hosts. We optimized the T cell manufacturing protocol to allow for high CAR expression whilst maintaining a less-differentiated Tcm/Tscm phenotype. Following adoptive transfer, tumour-bearing mice were vaccinated with an OVV expressing the defined antigen. Expansion of the transferred cells in the blood was evaluated at early and late timepoints post-vaccination by flow cytometry. Implanted tumours were monitored using caliper measurements.

Results: The CAR-T cells showed robust proliferation upon CAR stimulation and efficient TCR-mediated cytotoxicity against tumour targets *in vitro*, demonstrating intact dual-functionality. Adoptive transfer of the CAR-T cells followed by vaccination with paired recombinant rhabdovirus led to robust *in vivo* expansion of tumour-specific CD8+ T cells, tumour regression, and prolonged survival in the aggressive B16.F10-gp33 syngeneic melanoma model. However, persistence of the boosted CAR-T cells appears to be limited. Ongoing work seeks to enhance this combination therapy and further capitalize on T cell engineering through addition of pro-survival STAT5 transgenes to the T cell product, evaluation of alternative OV platforms, and investigation into the interplay between the OVVs and CAR-T cells *in vivo*.

Conclusions: These data provide proof-of-concept for a novel and clinically feasible approach to enhancing ACT. Further work with this model will allow us to characterize the complex dynamics of ACT/OVV combination therapy and contribute to the basic understanding of CAR-T cell biology in the context of solid tumours.
This work was supported by the Terry Fox Foundation, the Canadian Institutes for Health Research, and the Canada Research Chairs program.

POSTER 5

PLASMA CELL ENGINEERING FOR A GRAFTED ADAPTIVE IMMUNOTHERAPY

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B cells play an essential role in humoral adaptive immunity by the secretion of immunoglobulins (Ig), also known as antibodies. They leave the bone marrow as naive cells to join the secondary lymphoid organs where they are activated by encounter with a specific antigen. During this activation, B cells increase their affinity for the antigen (bacteria, virus, parasite, etc.) by hyper- mutating their immunoglobulin genes: the IgL (L for light) and IgH (H for Heavy) locus. Subsequently, these cells undergo clonal expansion and further differentiation either into memory B cells or into plasma cells. The latter, is characterized by its capacity to secrete very large quantities of Ig.

Ig are composed of two identical heavy chains, which determine the Ig class (Gamma = G, Mu = M, epsilon = E, Alpha = A and Delta = D), and two identical light chains that can be kappa or lambda. This enormous diversity of antibodies allows the recognition of a wide range of pathogens. Additionally, antibodies have a double function in different regions of their structure. Their Fab fragment can bind an antigen and the Fc region has effector functions such as antibody-dependent cell-mediated cytotoxicity, opsonization (promoting phagocytosis), cytophilia towards neutrophils, NK, monocytes, etc... The main purpose of these functions is to effectively eliminate foreign substances or pathogens. Currently, immunotherapy with monoclonal antibodies is carried out by their direct injection. The major constraint of this approach lies in the short half-life of antibodies, which is of only 21 days at the most. Being capable of producing these antibodies continuously, over the long term and in vivo is a major challenge. We seek to use the large-scale antibody production capacity of plasma cells by forcing them to secrete a given therapeutic antibody. To this purpose, we use CRISPR genome editing technique to introduce a gene coding for an antibody in a specific way. In parallel, we are studying the survival capacity of human primary B cells as well as their ability to secrete antibodies in a mouse xenograft model. The objective is to establish a cell therapy process based on the genetic modification of B cells with the capacity to be “auto-grafted”, to survive and to produce in the long-term specific antibodies. Thus, chronic diseases requiring lifelong treatment would benefit from an optimal and stable specific immune response.

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CAR-TREGS FOR SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by an abnormal inflammatory response against nuclear antigens with consequent tissue damage. Autoreactive B cells and auto-antibodies have a fundamental role in SLE pathogenesis. Lupus nephritis (LN) has a great impact on patients' survival and quality of life and still represents and unmet clinical need due to the lack of specific treatments and to the poor response to conventional immunosuppression. Regulatory T cells (Tregs) physiologically maintain the immune tolerance and are impaired in SLE. Polyclonal Treg transfer obtained unsatisfactory results due to the low number of disease-relevant antigen-specific cells. Chimeric Antigen Receptors (CARs) are molecules capable of redirecting T cell specificity. CAR-Tregs proved effective in pre-clinical mouse models of autoimmunity.

Aim: We aimed at developing a CAR-Treg based product to be employed in SLE in particular in LN.

Results: We isolated Tregs from Healthy Donors Peripheral Blood Mononuclear Cells (PBMCs) and expanded them with IL-2 and rapamycin. We transduced Tregs with a Lentiviral Vector encoding for a second-generation anti-CD19 CAR, considering the relevant role of autoreactive B cells and autoantibodies in SLE. Engineered cells retained their immune suppressive capabilities upon polyclonal stimulation. Noticeably, they acquired new antigen-specific suppressive capacities, being able to block autologous B cell proliferation. We set up a humanized mouse model of SLE. In vivo, CAR-Tregs delayed the occurrence of B cell lymphopenia, producing immunomodulatory cytokines and without showing toxicity or reprogramming towards Th17 pro-inflammatory cells. In inflamed organs, CAR-Tregs restored the normal composition of the immune system.

Conclusions: In conclusion, we efficiently generated anti-CD19 CAR-Tregs and proved their efficacy both in vitro and in an in vivo humanized mouse model of lupus.

DUAL TARGETING OF CAR-NK CELLS TO PD-L1 AND ERBB2 FACILITATES SPECIFIC ELIMINATION OF CANCER CELLS OF SOLID TUMOR ORIGIN AND OVERCOMES IMMUNE ESCAPE BY ANTIGEN LOSS

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Introduction: Retargeting of natural killer (NK) cells with chimeric antigen receptors (CARs) can be a powerful approach to overcome NK-cell resistance of tumor cells. However, targeting a single tumor-associated antigen may be insufficient for some tumors to trigger effective NK-cell activation or result in the selection of antigen-loss variants and tumor immune escape.

Methods: To overcome this hurdle, here we generated CAR-NK cells carrying two CARs that target the tumor-associated antigens PD-L1 and ErbB2 (HER2), respectively (Figure 1A). NK-92 cells were transduced with lentiviral CAR constructs, and their cytotoxicity against cancer cell lines of different solid tumor origins was compared to that of parental NK-92 and corresponding single-target CAR variants.

Results: Dual targeting significantly increased *in vitro* cytotoxicity against PD-L1 and ErbB2 double-positive tumor cell lines including breast, ovarian, pancreatic and gastric cancer cells when compared to single-target CAR variants (Figure 1 B, C). These results were also confirmed with 3D spheroid tumor models. Off-target cytotoxicity was not observed. On a molecular level, this enhanced cell killing may be explained by synergistic activation of PLCγ and MAPK pathways. Incubation of cancer cells with IFN-γ further improved killing efficacy due to upregulation of PD-L1 expression. Furthermore, blocking experiments revealed that dual PD-L1/ErbB2-CAR NK-92 cells can overcome immune escape based on loss or inacessibility of a single target antigen.

Conclusions: Altogether, we showed that dual targeting of PD-L1 and ErbB2 improves efficacy of CAR-NK cells against otherwise difficult to treat tumors, and counteracts potential resistance and immune escape mechanisms of cancer cells.

POSTER 8

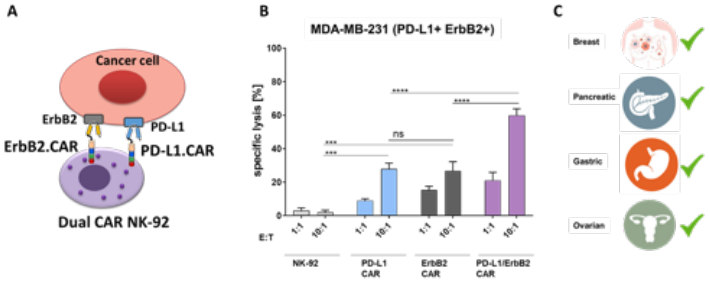

Figure1: A) schematic representation of Dual CAR NK-92 targeting ErbB2 and PD-L1 molecules on human cancer cell. **B)** Functional killing assay against human breast carcinoma cell line MDA-MB-231. **C)** Specific cytotoxicity of PD-L1/ErbB2.CAR NK-92 was confirmed against cancer cell lines of different origin.

TUMOR TAILORED LYMPHODEPLETION AND CAR T-CELL FUNCTIONALITY IN ADVANCED OVARIAN CANCER

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Introduction: Ovarian cancer is a common female malignancy. We have recently reported ovarian tumor reductions in mouse model using Mesothelin (MSLN) directed chimeric antigen receptor (CAR) T cell therapy. However, CAR T cell efficacy is limited in solid tumors. Accordingly, cytostatics conditioning may play a role in remodeling the tumor microenvironment and debulking the tumor mass.

Objectives: To investigate the potential *in vitro* interactions between MSLN expression on ovarian cancer cell lines and treosulfan/fludarabine killing efficacy in different conditions and the effect of the residual cytostatics concentrations on MSLN CAR T cells functions.

Methods: The ovarian cancer cell lines OVCAR4 and SKOV3 (either untransduced or transduced to induce MSLN overexpression) were incubated with either treosulfan and/or fludarabine at several concentrations for 48h at 37°C followed by survival assessment using WST1 assay. In order to mimic the tumor microenvironment, these experiments were repeated in hypoxic conditions (2% oxygen) and using tumor spheroids. Each experiment was conducted in triplicates and each experiment was repeated 3-5 times independently.

In an independent experiment, MSLN+ ovarian cancer cells were incubated with either treosulfan or fludarabine at a concentration corresponding to half of IC_{50} and MSLN expression was measured upon starting the incubation and after 48h on surviving cells.

After determining the residual cytostatics concentrations, MSLN CAR T cells were incubated with fludarabine or treosulfan for 24h, followed by intracellular cytokine staining and mitochondrial function assay to determine CAR T cells function. The study was performed by using three second-generation anti-MSLN CAR T cells: CD28-CD3ζ (M28z), 4-1BB-CD3ζ (MBBz) and CD28-CD3ζ-1XX (M1XX, a modified CD3z that contains a single immunoreceptor tyrosine-based activation motif).

Results: Both cytostatics exerted an antitumor effect on SKOV3 and OVCAR4 cells under normoxia and hypoxia. MSLN expression was stable during the cytostatics exposure, however, high MSLN expression at normoxia as well as tumor spheroids increased the cells resistance to cytostatics.

Both cytostatics induced T cell apoptosis in a dose- and time-dependent manner, determining the safe residual cytostatics concentrations for T cells. No significant differences were found in CAR T cell mitochondrial and functional profiles after exposure to the cytostatics residual concentrations. However, the cytokine production and degranulation were highest in M1XX CAR T cells showing enhanced effector functions after exposure to fludarabine.

Conclusions: To the best of our knowledge, this is the first study reporting the effect of treosulfan and fludarabine on SKOV3 and OVCAR4 cells, with a focus on the relevance to MSLN CAR T cell therapy.

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Both cytostatics killed the tumor cells with variable efficacy according to the MSLN expression levels, the oxygen levels and spheroids formation. Interestingly, MSLN surface expression was consistent during cytostatics exposure. Moreover, cytostatics residual concentrations did not alter CAR T cell functionality with M1XX being most functional. These findings are the basis for upcoming animal experiments and clinical trials. It is important to measure the cytostatics concentrations and to determine the time interval between the cytostatics conditioning and the start of CAR T cells infusion in order to improve the treatment efficacy and the clinical outcome.

COMBINATION OF GD2-DIRECTED BISPECIFIC TRIFUNCTIONAL ANTIBODY THERAPY WITH PD-1 IMMUNE CHECKPOINT BLOCKADE INDUCES ANTI-NEUROBLASTOMA IMMUNITY IN A SYNGENEIC MOUSE MODEL

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Objectives: Despite advances in treating high-risk neuroblastoma, 50-60% of patients still suffer relapse, necessitating new treatment options. Bispecific trifunctional antibodies (trAbs) are a promising new class of immunotherapy. TrAbs are heterodimeric IgG-like molecules that bind CD3 and a tumor-associated antigen simultaneously, whereby inducing a TCR-independent anti-cancer T cell response. Moreover, via their functional Fc region they recruit and activate cells of the innate immune system like antigen-presenting cells potentially enhancing induction of adaptive tumor-specific immune responses. Therefore, we set out to investigate whether cellular vaccination combined with SUREK trAb can effectively induce an endogenous adaptive anti-tumor immune response in a syngeneic neuroblastoma mouse model.

Methods: We used the SUREK trAb, which is bispecific for the neuroblastoma antigen GD2 and murine Cd3. Tumor-blind trAb and the monoclonal GD2 antibody ch14.18 were used as controls. A co-culture model of murine dendritic cells, T cells and a neuroblastoma cell line was established to evaluate the cytotoxic effect and the T cell effector function *in vitro*. Expression of immune checkpoint molecules on tumor-infiltrating T cells and the induction of an anti-neuroblastoma immune response using a combination of whole cell vaccination and trAb therapy were investigated in a syngeneic immunocompetent neuroblastoma mouse model. Finally, vaccinated mice were assessed for the presence of neuroblastoma-directed antibodies.

Results: We show that SUREK trAb-mediated effective killing of NXS2 cells *in vitro* was strictly dependent on the combined presence of dendritic cells and T cells. Using a syngeneic neuroblastoma mouse model, we showed that vaccination with irradiated tumor cells combined with SUREK trAb treatment significantly prolonged survival of tumor challenged mice and partially prevent tumor outgrowth compared to tumor vaccination alone. Prevention of tumor outgrowth was associated with a NXS2-directed humoral immune response. Treatment led to upregulation of programmed cell death protein 1 (Pd-1) on tumor infiltrating T cells and combination with anti-Pd-1 checkpoint inhibition enhanced the NXS2-directed humoral immune response.

Conclusion: Here, we provide first preclinical evidence that tumor vaccination combined with SUREK trAb therapy induces an endogenous anti-neuroblastoma immune response reducing tumor recurrence. Furthermore, a combination with anti-Pd-1 immune checkpoint blockade might even further improve this promising immunotherapeutic concept in order to prevent relapse in high-risk neuroblastoma patients.

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PERSONALIZED CRISPR/CAS9 CYTOKINE GENE THERAPY TO ALTER THE NEUROBLASTOMA TUMOR MICROENVIRONMENT AND ENHANCE SUBSEQUENT ADOPTIVE T CELL THERAPY.

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Introduction: Therapy-resistant solid tumors represent a growing global challenge. Neuroblastomas are among the most common malignant solid tumors in children and account for 15% of malignancy-associated childhood deaths. One therapeutic approach could be immunotherapy, e.g. chimeric antigen receptor (CAR) T cell therapy. CAR T cells show outstanding treatment successes against B cell malignancies but often fail in patients with solid tumors. A major reason for immunotherapy failure in solid tumors is an immunosuppressive tumor microenvironment. Previous studies show that solid tumors with higher immune cell infiltration and chemokine concentrations have a better prognosis and improved susceptibility to immunotherapies.

Objectives: In this proof-of-concept preclinical study, we aim to develop a personalized in vivo gene therapy that influences the microenvironment of immunosuppressive solid tumors in a favorable way to increase the infiltration of CAR T cells into the tumor. In this project, personalized cytokine gene therapy is demonstrated in neuroblastoma but will be extendable to other solid tumors with an immunosuppressive tumor microenvironment as well.

Methods and Results: The developed gene therapy aims to modify the tumor cells themselves to express tumor microenvironment altering transgenes. To that end, CRISPR/Cas9-HDR-dependent knock-in is used to introduce transgenes encoding for the T-cell-attracting chemokine CXCL10 or the immunostimulatory cytokine IFNG at tumor-specific genetic target sites. For proof of concept, tumor-specific CRISPR/Cas9 targets in two neuroblastoma cell lines were identified through a self-designed multistep selection process which integrates exomic- and transcriptomic data with CRISPR prediction algorithms. We identified mutation-specific CRISPR-Cas9 target sites in different neuroblastoma cell lines. The targets were confirmed via PCR amplification and Sanger sequencing. Co-electroporation of synthetic Cas protein, synthetic gRNA and a donor template (cytokine transgene (CXCL10) & reporter surface antigen (Q8) under a self-designed EF1 alpha-derived promoter (EF1a(s)) were performed to achieve stable and cell line-specific knock-in. Successful gene targeting was confirmed 28 days after treatment via (digital droplet) PCR and flow cytometry. Subsequently, cytokine secretion was measured via ELISA. Trans-well migration assays confirmed enhanced CAR T cell migration.

Conclusion: In our endeavor to develop a tumor microenvironment altering gene therapy using tumor-specific CRISPR/Cas9 targets we were able to deliver a proof of concept for targeted non-viral transgene integration and generation of stable transgenic tumor cell lines expressing relevant levels of

biologically active chemokines. For clinical translation, a targeted lipid nanoparticle delivery system will be developed to preferentially deliver the gene therapy to the tumor cells in vivo.

CONSTRUCTION AND EVALUATION OF MCMV-TARGETING BISPECIFIC T CELL ENGAGERS

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Objective: Cytomegalovirus (CMV) has a seroprevalence of 50 to 90% depending on the population and usually causes no complications in the latent state. However, either de-novo infection or reactivation can have severe if not life-threatening consequences for high-risk patients such as immunocompromised stem cell transplant recipients.

There are several therapeutic alternatives to treat CMV infection, however, they either suffer from toxicity, side effects or resistance (antiviral drugs), while cellular therapies are time and cost intensive (*ex vivo* expanded T cells) and/or require an HLA-matching of donor CMV-specific T cells.

In search of MHC-independent and universally applicable options, Bispecific T cell engager (BiTE®) incorporate both features, with potentially better safety effect profile caused by their high first-pass-effect.
The accepted animal model for CMV infection is the murine Cytomegalovirus (MCMV), which we chose to evaluate the efficacy of anti-MCMV BiTE®s.

BiTE®s consist of two single chain variable fragments derived from target-specific antibodies, one targeting either murine or human CD3ε and one targeting viral glycoproteins of MCMV, namely gB, gN and gH. The BiTE® functions as a connecting activating adapter between T cells and virus-infected cells, enabling an MHC-independent recognition of infected target cells.

Methods: In my project, I created 16 different bispecific constructs and transfected HEK293T cells for BiTE® production. Furthermore, half-life extended versions of these BiTE®s are currently cloned to lower the number of BiTE® injection necessary due to the short *in vivo* half-life of canonical BiTE®s. A mutated Fc-domain lacking effector functions is introduced, enlarging the protein. Those modified BiTE®s are then compared to canonical BiTE®s to assess their usability for *in vivo* testing.

Stable cell lines expressing the different glycoproteins of MCMV were created to evaluate efficacy of the BiTE®s. By this we can assess target specificity without inhibition of T cell activity by additional viral effector proteins. The glycoprotein-expressing cell lines additionally express an nLuc reporter for general assessment of cytotoxicity (nLuc-release). To assess the role of cytokine response, both TNF-α and IFN-γ will be measured in the supernatant using ELISA. Further analysis with respect to secreted factors includes the transfer of the assay supernatant onto untreated cells without effector cells and evaluation of cytotoxicity. The experiments are then to be repeated with MCMV-infected cells. As both negative and positive control, we have chosen two CD19-directed BiTE® (coupled to an scFv against murine and human CD3ε, respectively) similar to the FDA approved drug (Blinatumomab®)

Results: First experiments showed significant increase in RLU when applying CD19muCD3-BiTE® and CD19huCD3-BiTE® to HEK 293T expressing CD19 target and nLuc reporter (293T-nLuc-CD19) in combination with either murine or human effector T cells.

Conclusion: In conclusion, I am optimistic that my constructs will show *in vitro* efficacy and give further insight into both therapeutic potential of BiTE® application for viral infections and the role of cytokine-related immune response in the control of MCMV. They will provide an easy-to-use and off-the-shelf therapy option with low risk of resistance development or viral evasion.

CXCR4 HAS A DUAL ROLE IN IMPROVING THE EFFICACY OF BCMA-SPECIFIC CHIMERIC ANTIGEN RECEPTOR NK CELLS IN MULTIPLE MYELOMA

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Introduction: Multiple Myeloma (MM) therapy has experienced tremendous advances through improved pharmacological intervention, but even more strikingly, through improved immunotherapies. Chimeric antigen receptor (CAR) T cell therapy has emerged as a treatment option for relapsed cases, but despite excellent response rates, progression-free survival is limited. Various mechanisms of disease recurrence exist including antigen escape upon CAR T cell-mediated immune pressure and effector cell failure to traffic to the tumor site. While exhibiting comparable effector functions, CAR NK cells offer advantages over T cells, foremost their i) low risk of alloreactivity as an off-the-shelf product, ii) lack of CRS and neurotoxicity development, and iii) multifaceted immune cell function.

Methods: Primary NK (pNK) and NK-92 cells were retrovirally transduced with a high-affinity BCMA-CAR-encoding retroviral vector equipped with a CXCR4 chemokine receptor sequence. Migration and signaling assays were performed to screen for CAR and CXCR4 activity. CXCR4 signaling mutants were cloned. Tumor cell killing was measured in FACS-based killing and degranulation assays. Formation of the immunological synapse (IS) was studied by microscopic imaging. Adoptive transfer into NSG mice was performed.

Results: Less than 20% of primary NK (pNK) cells express the chemokine receptor CXCR4, which is deemed important for bone marrow invasion. Transduction of pNK and NK-92 cells with retroviruses encoding for CXCR4 and the BCMA-CAR endowed both cell types with enhanced migratory capacity toward CXCL12. CXCL12 has no additive pro-proliferative effect on pNK and NK-92 cells when cultured in the presence of IL-2. When challenged with Raji-BCMA+ target cells, CXCR4-equipped BCMA-CAR NK-92 cells exhibited significantly stronger cytolytic efficacy. This process was independent from CXCL12-mediated CXCR4 engagement. Mechanistically, recruitment of pZAP-70 into the IS occurred much faster. Finally, co-expression of CXCR4 in NK-92 cells delayed endocytosis of the BCMA-CAR.

Conclusions: We show that BCMA-CAR/CXCR4 co-expression endows NK cells with higher cytolytic efficacy and improved migratory capacity toward bone marrow-associated CXCL12. Faster ZAP-70 phosphorylation together with its rapid recruitment into the IS might indicate that the signaling threshold for our BCMA-CAR can be lowered when CXCR4 is present. These observations will guide us to determine and tune the antigen threshold of BCMA-CAR NK cells for optimized CAR sensitivity and activity towards low antigen densities, a feature of MM cells exposed to immunoselection.

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UNIVERSAL MANUFACTURING PLATFORM FOR GMP-COMPLIANT CAR-T MANUFACTURING BASED ON SYNTHETICALLY PREPARED LINEAR PIGGYBAC TRANSPOSON DNA

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Objectives: The manufacturing of clinical-grade CAR-T cells via lentiviral or retroviral vectors is limited by the slow production of viral vectors and their high costs; these production bottlenecks can be solved by non-viral approaches utilizing transposons such as piggyBac. Gene engineering is typically performed via electroporation of plasmid containing the transposon sequence and an mRNA encoding the transposase enzyme, randomly integrating the transposon DNA into the genome. However, using plasmid DNA creates several issues, such as the presence of bacterial DNA in the plasmid backbone or the complicated process of production of GMP-grade plasmids. To simplify the transposon-based gene engineering, we developed a rapid and GMP-compliant CAR-T manufacturing method based on synthetically prepared linear piggyBac transposon DNA.

Methods: The DNA and mRNA vectors for electroporation were produced abiotically via the enzymatic synthesis in vitro. The transposon is prepared via large-scale PCR as a linear DNA of approx. 5kb size, and the transposase mRNA is prepared via in vitro transcription. Both linear DNA transposon and mRNA are co-electroporated into PBMCs, and transfected T cells are expanded in the presence of IL-21 to preserve their stem-cell memory phenotype.

Results: To demonstrate the feasibility of this technique, we produced CD19-specific and CD123-specific CAR T cells (CAR19 and CAR123) and evaluated their functions. The production is highly effective as approx. 1 µg of linear DNA yields approx. 5x10e7 CAR-T cells per one electroporation of 1x10e7 PBMCs. We have observed that linear DNA gets integrated more efficiently than circular plasmids resulting in the vector copy number within the acceptable range (<3). CAR19 and CAR123 displayed expected cytotoxicity in vitro and in vivo in immunodeficient mice transplanted with an AML cell line.

Conclusions: The linear DNA transposon platform enables a straightforward cGMP certification and is especially suitable for the cost-effective production of highly experimental, early-phase CAR-T cell products. We are developing GMP-certified CAR123 T cells for a clinical trial of patients with CD123+ acute myeloid leukemia.

TYROSINE KINASE INHBITORS AND BLINATUMOMAB: FRIENDS, OR ENEMIES?

Adam Obr

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Blinatumomab and tyrosine kinase inhibitors: friends, or enemies?

Objectives: Blinatumomab (Blincyto®) is a bi-specific T cell engager therapeutic antibody (BiTE), which works by connecting the CD19 antigen in malignant cells with the CD3 antigen of cytotoxic T cells, allowing for effective T cell killing. Tyrosine kinase inhibitors (TKIs) are used mainly in the therapy of chronic myeloid leukemia (CML), where they prove invaluable by targeting the BCR-ABL oncogene. TKIs of the second and third generation (dasatinib, nilotinib, and ponatinib) target SRC family kinases (SFK) alongside the BCR-ABL oncogene. Recently, several clinical trials combined blinatumomab and TKIs in the treatment of B-ALL, and with immense success [1] — although theoretically, the combination of blinatumomab with TKIs should not be effective due to the inhibition of SRC kinases required for T cell activation [2]. L-selectin (CD62L) is a cell adhesion molecule that facilitates the entry of lymphocytes into secondary lymphoid organs from the blood stream and lymphocyte rolling during inflammation. Loss of CD62L is often used as a marker of activated T cells. However, T cells expressing a form of CD62L that is maintained upon cell activation are more efficient in cancer immunotherapy [3].

Methods & results: Pre-treatment of isolated PBMCs with dasatinib or ponatinib interferes with the activation of the cells (induced by blinatumomab or anti-CD3, measured by CD69 expression). Indeed, the cytotoxic effects of these cells on B-ALL cell line RAJI is not affected with lowest clinically relevant concentrations of both TKIs (2-10 nM), but is greatly diminished by the highest clinically achievable concentrations (~100 nM). Interestingly, both dasatinib and ponatinib upregulate the presence of CD62L on B-ALL (target) cells. In contrast, healthy CD19+ (but not CD4+ or CD8+) PBMCs treated with anti-CD3 activating antibody showed a reduction in the expression of CD62L. The effect is, however, abolished with when dasatinib is present.

Conclusions: We show that blinatumomab—induced cytotoxic effects are affected by TKI concentration *in vitro*, suggesting an effective "therapeutic window" in which the action of cytotoxic T cells is modulated by the TKI. Additionally, we show a potential role of a well-known cell adhesion molecule, CD62L, in TKI modulation of cytotoxic T cell effectivity.

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POSTER 15

IL3-ZETAKINE COMBINED WITH A CD33 COSTIMULATORY RECEPTOR AS A DUAL CAR APPROACH FOR SAFER AND SELECTIVE TARGETING OF AML

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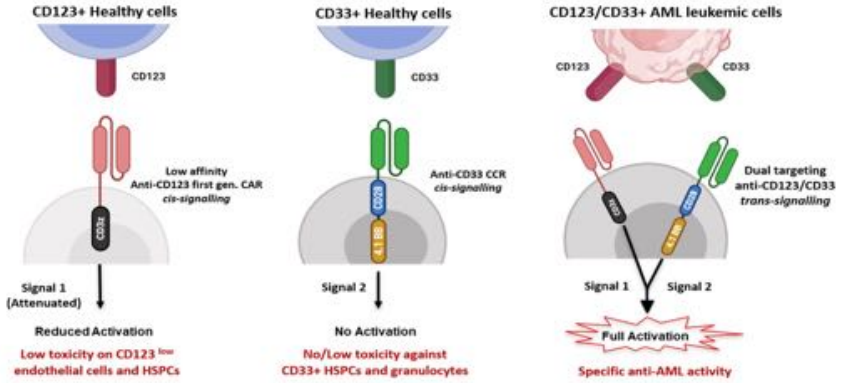
Objectives: Acute Myeloid Leukemia (AML) still represents an unmet clinical need for adult and pediatric patients. Adoptive cell therapy by chimeric antigen receptor (CAR)-engineered T cells demonstrated a high therapeutic potential, but further development is required to ensure a safe and durable disease remission in AML, especially in elderly patients. To date, translation of CAR-T cell therapy in AML is limited by the absence of an ideal tumor-specific antigen. CD123 and CD33 are the two most widely overexpressed LSCs biomarkers but their shared expression with endothelial and hematopoietic stem progenitor cells (HSPCs) increases the risk of undesired vascular and hematologic toxicities. To counteract this issue, we established a balanced Dual CAR strategy aimed at reducing off-target toxicities while retaining full functionality against AML (Figure 1).

Methods: Cytokine Induced Killer (CIK) cells were genetically modified co-expressing a first-generation anti-CD123 IL-3 zetakine (IL3z.CAR) and an anti-CD33 as costimulatory receptor without activation signaling domains (CD33.CCR). In vitro short- and long- term cytotoxicity and cytokine production assays were assessed upon challenge with different CD123/CD33 positive AML cell lines (THP-1 and KG-1), AML primary cells or with a CD123 positive human endothelial cell line (TIME). To assess the myelotoxicity against HSPCs, CD34+ cells were sorted after 24h co-culture with CAR-CIK cells and mixed with methylcellulose-based semisolid medium to evaluate Colony Forming Units (CFU) after 14 days through an automated imaging and counting system for hematopoietic colonies (Stemvision). The in vivo anti-leukemic activity was evaluated by monitoring luciferase-expressing KG1 AML cell line growth over time in NSG mice treated with 3 infusions of all different CAR-CIK conditions.

Results: Dual CAR-CIK cells (IL-3z.CAR/CD33.CCR) display a potent and specific in vitro anti-leukemic efficacy against AML CD123+/CD33+ cell lines and primary cells from AML patients, compared to single targeting IL-3z.CAR and non-transduced CIK cells. To further minimize on-target/off-tumor toxicity, predicted low affinity IL3z.CAR were screened by molecular dynamics to identify the IL3z.CAR mutant with the lowest binding affinity to CD123. This low affinity IL3z.CAR was then generated by site-directed mutagenesis and cloned within the dual CAR transgene, for efficacy and toxicity assays as compared to wild type (wt) dual CAR-CIK cells. Low affinity dual CAR-CIK cells show irrelevant cytotoxicity against the TIME endothelial cell line, comparable to non-transduced CIK cells, while preserving the efficacy against THP1 and KG1 AML cell lines. Low affinity dual CAR-CIK cells also preserve the in vitro CFU-E, CFU-GM and CFU-GEMM colony forming capacity as compared to wt dual CAR-CIK and wt IL-3z.CAR-CIK cells, highlighting the possibility to find a therapeutic window to minimize toxicity on healthy cells. The anti-leukemic activity of low affinity dual CAR-CIK cells has been

confirmed also in vivo, with a significant suppression of KG1 cell line leukemic growth. This trans-signalling model was further improved by optimizing the hinge/transmembrane regions, to avoid any possible homodimerization between CAR and CCR.

Conclusions: Low affinity Dual CAR with trans-acting costimulation provides improved efficacy and high specificity for CD123 and CD33 recognition on leukemic cells whilst substantially reducing the risk of life-threatening toxicities.



CAR T CELLS TARGETING *ASPERGILLUS FUMIGATUS* ARE EFFECTIVE AT TREATING INVASIVE PULMONARY ASPERGILLOSIS

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Background: *Aspergillus fumigatus* is a ubiquitous mold that can cause severe infections in immunocompromised patients, typically manifesting as invasive pulmonary aspergillosis (IPA). Despite the availability of anti-fungal drugs, the mortality rate due to IPA is still significant. Adoptive transfer of *Aspergillus*-specific T cells has been shown to reduce the burden of IPA. Such T cells are infrequent and cannot be consistently isolated and expanded for adoptive immunotherapy. Thus, CAR T cells present an attractive alternative.

Aims: Engineering CAR T cells targeting *Aspergillus fumigatus (Af*).

Methods: To specifically target *Af* hyphae, we designed an scFv derived from an antibody directed against Hyphal cell wall. We constructed a second-generation CAR (Af-CAR) containing the scFv fused to the extracellular IgG4-Fc spacer domain, followed by CD28 and CD3-ζ signaling domains. Using the sleeping beauty transposition system, we expressed the CAR on the surface of primary human $CD8^+$ T cells. We evaluated the anti-fungal activity of Af-CAR T cells in pre-clinical models of IPA *in vitro* and *in vivo*.

Results: Af-CAR T cells are specific for *Af* with no cross-reactivity with other Aspergilli or healthy human lung tissue. When activated, $CD8^+$ Af-CAR T cells mainly secreted Th1-cytokines (IFN-γ, TNFα, IL-8, and GM-CSF) and chemokines (mainly CCL3 and CCL4). Upon binding to the target, the cytolytic machinery of $CD8^+$ Af-CAR T cells was activated, leading to the release of perforin and granzyme B and up to 45% hyphal damage. Furthermore, in cooperation with macrophages, hyphal damage reached up to 80%. *In vivo*, $CD8^+$ Af-CAR T-cells localized to the site of infection engage innate immune cells and reduce the fungal burden. Moreover, mice treated with $CD8^+$ Af-CAR T-cells survived IPA better than untreated mice.

Conclusion: Our study illustrates the potential of gene-engineered T-cells to tackle aggressive infectious diseases that are difficult to control with conventional antimicrobial therapy.

ENHANCEMENT OF ANTI-TUMOR FUNCTIONS OF WT1-SPECIFIC TCR TRANSGENIC T CELLS VIA NOVEL CAR-BASED RECOMBINANT RECEPTOR

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Objectives: Adoptive T-cell therapies via transgenic T-cell receptor-expressing T cells (TCR-T) enable selective targeting of intracellular tumor-specific antigens in an HLA-dependent manner. However, the efficiency of TCR-T cells primarily depends on the avidity of recombinant TCR against HLA/peptide antigenic complex. Unfortunately, tumor tolerance mechanisms eliminate the best functioning high-avidity TCRs from the organisms of cancer patients. Therefore, we studied mechanisms to improve TCR-T cells using recombinant TCR specific to HLA A2/RMFNAPYL complex from WT1 tumor antigen (WT1-TCR), a well-described target for acute myeloid leukemia.

Methods: The piggyBac transposon was used to generate dual TCR-CAR T cells that 1) constitutively express WT1-TCR and 2) express GM-CSF ligand-based chimeric antigenic receptor (GM-CAR) under NFAT-inducible promoter - the receptor for GM-CSF (GM-CSFR) is another known AML target and is commonly expressed by AML cells. A third gene, the EGFR-based safety switch, was additionally inserted into the transposon so that the total size reached approx. 6.5kB. The anti-tumor functions of both types of WT1 TCR-T (i.e., enhanced with NFAT GM CAR or non-enhanced) were tested in vitro by co-culture with HLA A2+WT1+ AML cell lines (THP-1, SKM-1) and with primary AML cells obtained from patients.

Results: We observed that the generated NFAT GM-CAR WT1-TCR T cells displayed significantly enhanced anti-leukemic activity against AML lines as well as against primary AML cells compared to non-enhanced WT1 TCR-T. Activation of NFAT GM-CAR WT1-TCR T cells by antigen increased GM-CAR expression, which further boosted the T-cell activation via a positive feedback loop and led to their enhanced proliferation and maintenance of strong cytotoxicity. Significantly, the cytotoxicity of NFAT GM-CAR WT1-TCR T cells was mediated via TCR and not by GM-CAR since they inefficiently killed HLA A2 negative/GM-CSFR positive targets (K562 cell line) or AML cells from HLA A2 negative patients. Furthermore, the dependency of GM-CAR expression on NFAT signaling made the cytotoxicity of NFAT GM-CAR WT1-TCR T cells susceptible to cyclosporin and could be thus pharmacologically regulated.

Conclusions: In summary, we present a novel method to enhance the cytotoxicity of TCR-T cells by inducibly co-expressed CAR. The described mechanism can be used to improve the efficiency of adoptive T-cell therapies of AML via WT1-specific TCR-T cells, especially in non-HLA A2 patients.

BOOSTING T CELL THERAPY FOR GASTROINTESTINAL TUMORS BY MULTIPLEX GENOME EDITING WITH THE BE4MAX BASE EDITOR

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Objective: Gastrointestinal cancers are among the leading causes of cancer-related deaths worldwide. In the treatment of solid tumors, a major obstacle is represented by the tumor microenvironment (TME), which fuels inhibitory signals impeding the proper functionality of tumor-infiltrating immune cells. Adoptive cell therapy (ACT) with T cell receptor (TCR) - transgenic T cells is a promising therapeutic strategy for patients with advanced-stage cancers. The goal of this project is to develop a genetically engineered tumor-specific T cell product endowed with the ability to counteract the immunosuppressive TME.

Methods: We combined the analysis of published datasets with the multidimensional investigation of primary and metastatic patient-derived CRC and PDAC samples. We used these data to select a set of tumor-associated antigens (TAAs) to be targeted by ACT and key molecules involved in hindering T cells functions. To retrieve novel tumor-specific TCRs, we repetitively stimulated healthy donors' and cancer patients' peripheral blood mononuclear cells with immunogenic peptides belonging to the selected TAAs. We longitudinally monitored the TCR repertoire of T cells by TCR-sequencing, to identify the alpha and beta pairs enabling us to reconstruct the TCR, that we cloned into a bidirectional lentiviral vector. We coupled lentiviral transduction of TAA-specific TCR with conventional CRISPR/Cas9 technologies and base editor to generate a cellular product with redirected T cell specificity and devoid of a key inhibitory molecule.

Results: Our combined analysis identified 19 relevant TAAs and TIGIT as a major immunosuppressive druggable molecule. By clonal tracking of TCR repertoire of T cells stimulated with the selected antigens, we isolated 6 TCRs specific for different TAAs, including HER2 and Mesothelin. We generated genetically engineered T cells with a redirected antigen specificity and disrupted in TIGIT. We optimized the genome editing approach by relying on conventional CRISPR/Cas9 and on a cytosine base editor, BE4max. Both editing procedures proved highly efficient (median 78.5% and 97% of TRAC/TRBC disruption and 88% and 75% of TIGIT disruption with CRISPR/Cas9 and BE4max respectively) and permissive for LV transduction of a $HER2_{369\text{-}377}$-specific TCR (82.8% and 87.3%). In addition, BE4max efficiently mediated gene disruption without introducing bystander indels. TIGIT knockout did not affect memory phenotype differentiation and expansion abilities of T cells. Noticeably, TIGIT knockout endowed $HER2_{369\text{-}377}$-redirected T cells with superior abilities to kill PANC1 and BxPC3 PDAC cancer cell lines and CRC patient-derived organoids.

Conclusions: Our work showed that TCR-transgenic TIGIT-knockout T cells can be efficiently generated by CRISPR/Cas9 and BE4max and represent promising therapeutic tools for gastrointestinal tumors.

POSTER 19

CAR-T CELLS TARGETING CD19 AND GP350 PRODUCED VIA CRISPR/CAS GENE KNOCK-IN AS AN OFF-THE-SHELF PRODUCT AGAINST AGGRESSIVE EBV$^+$ BURKITT LYMPHOMA

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Burkitt lymphoma (BL) is a fast-growing B-cell malignancy frequently associated with Epstein-Barr Virus (EBV) infection. Advanced stage BL disease with spinal cord and bone marrow involvement shows poor responses with standard therapies. Administration of chimeric antigen receptor (CAR) T-cell therapy to effectively fight against BL would rely on a pre-manufactured allogeneic off-the-shelf product. Thus, we developed a method for GMP up-scalable CRISPR/Cas9 knock-in of CD19-CD28z-CAR into the T cell receptor alpha chain (*TRAC*) locus with the MaxCyte electroporation device. In addition, we produced gp350-CAR targeting the EBV envelope protein gp350. For both CARs, the percentage of CAR$^+$TCR$^-$ T cells at 14 days post gene editing was approximately 20%. Both types of edited CAR-T cells recognized and killed EBV-1$^+$ Daudi/GFP-fLuc and EBV-2$^+$ Jijoye/GFP-fLuc cell lines *in vitro* and secreted IFN-g. Cryopreserved and thawed gene-edited CAR-T cells maintained the identity and potency characteristics. For development of *in vivo* potency models, Nod.Rag.Gamma (NRG) mice were injected i.v. with 2x10^5 Daudi/GFP-fLuc or Jijoye/GFP-fLuc cells. Both models showed fast BL outgrowth and high morbidity and mortality within 4 weeks. Optical imaging analyses demonstrated BL spread in bones (femurs, spinal cord, ribs) with conspicuous infiltration of GFP$^+$CD20$^+$CD19$^+$gp350$^+$ blasts in the bone marrow and lymph nodes. Analyses of bone marrow smears confirmed the morphology of BL. Therapeutic administration of allogeneic cryopreserved 1x10^6 TCR$^-$CD19-CAR-T cells into mice demonstrated persistency of CD8$^+$ CD19-CAR-T cells, reduced BL dissemination and decreased EBV DNA load. Thus, allogeneic edited TCR$^-$CD19-CAR is a promising off-the-shelf product against aggressive BL. TCR$^-$gp350-CAR-T cells did not control BL *in vivo*, but targeting gp350 in combination with CD19 will be a future strategy for effectively eradicating virus-associated lymphomas.

POSTER 20

NK CELLS RECOVERY AND FUNCTION AFTER CHEMOTHERAPY

Jana Szabová[1], Marek Jedlička[1,2], Lucie Sládková[1,2], Eva Mašínová[1,2], Veronika Švubová[1,2] and Jan Frič[1,3]

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Despite improvements in new drugs, multiple treatment strategies and stem cell transplantation, AML relapse remains a severe problem responsible for mortality of AML patients. Recently, cellular immunotherapy provides new candidate treatment options including therapies base on adoptive transfer of NK cells. Natural killer (NK) cells show good promise in the treatment of various tumors, as they exhibit antitumor effects through their cytotoxic capacity.

Here we selected 4 cohorts of patients as suitable candidate for future clinical studies with NK cells. To these cohorts belong patients A) who showed primary resistance to classical "3+7" chemotherapy, B) continued to be MRD-positive after stem cell transplantation, C) who's AML relapsed after remission and D) who have primary resistance on classical "3+7" and also "FLAIDA" chemotherapy. Here we designed experimental in vitro preclinical assessment where we explore the effectiveness of NK cells cytotoxicity after adoptive infusion and exposure to various chemotherapy approaches and use of inhibitors including and thereby determine the best strategy for clinical applications of NK cells. The main goals of our research are therefore:
(1) to evaluate when this possible transfer to patients will be most efficient in context of used therapy, where chemotherapy will be combined with donor-derived in vitro activated NK cells for various exposure time.
(2) How the given NK cells and leukemic target cells are affected by chemotherapy, in order to evaluate the effectivity of cytotoxic properties of NK cells and their phenotype, or degradation of the tumor cells, which become more prone to their action. These findings will provide data allowing to better design future clinical studies especially in the timing of the administration of NK cells to the patient in relation to current stage of therapy.

PERIPHERAL BLOOD CYTOTOXIC T CELLS SHOW EARLY EXHAUSTED FEATURES IN MYELOFIBROSIS PATIENTS

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Objectives: Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN) characterized by megakaryocyte hyperplasia, bone marrow fibrosis and extramedullary hematopoiesis. Compelling evidence are suggesting that persistent antigen stimulation in the tumor microenvironment can differentiate T effector cells into terminally exhausted T cells, a functional state characterized by decrease in proliferation, cytotoxicity, and cytokine production. Cutting-edge therapies are focused on the reversion of exhausted state through immune checkpoint inhibition to recover the immune response against the tumor. Despite MPNs are myeloid neoplasms, T cells are showing dysfunctional features. To assess if immune checkpoint inhibition could be a new effective therapy in combination with those currently approved, we investigated T cell exhaustion in MF.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from 41 MF patients and 22 healthy donors (HD). The expression profile of the following inhibitory receptors (IRs): PD1, CD244, CD160, TIM3, LAG3, CTLA4 was characterized by flow cytometry in CD3+CD8+ T cells. Results were correlated with clinical features such as Dynamic International Prognostic Scoring System (DIPSS) classification, splenomegaly, hemoglobin level and other negative prognostic factors. For T cell activation assays we stimulated PBMCs overnight in the presence of coated anti-CD3, anti-CD28 and Brefeldin A, whilst cytokine production was then assessed by intracellular flow cytometry staining. Chi-squared and Mann Whitney tests were used for statistical analysis. Total cellular RNA was isolated from peripheral blood granulocytes of 141 primary and secondary MF patients and 28 HDs, and we performed Gene expression profiling (GEPs) by microarray platform.

Results: We reported expansion of PD1+CD244+, PD1+CD160+, PD1+CD57- cytotoxic T cell populations from MF patients, together with increased expression of all the IRs assessed on both total CD3+CD8+ T cells and PD1+CD57- T cells fraction. Furthermore, we observed decreased secretion of IFNɣ and TNFα by CD3+CD8+ T cells of MF patients, while significant reduction of Granzyme B production was observed mainly in patients with expansion of PD1+CD57- T cell population. GEPs analysis highlighted increased expression of IR-ligands like CD274, CEACAM1, CD48 on granulocytes from MF patients compared to HDs. The correlation analysis of IRs expression with patients' clinical features showed that higher levels of PD1, CD244, CD160, CTLA4, LAG3 or TIM3 associates with detrimental features like splenomegaly, low hemoglobin levels, HMR mutation number and more severe DIPSS classification.

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Conclusions: Our data evidenced in MF patients the presence of an impaired population of peripheral blood cytotoxic T cells expressing multiple IRs and with reduced cytokine production after *in vitro* activation. Moreover, granulocytes from MF patients display higher level of IR-ligands transcripts hinting an immunosuppressive interplay between the myeloid neoplastic clone and T cells. Lastly, clinical correlations suggest a more severe disease in patients with higher IRs expression on cytotoxic T cells. Taken together this data highlights an early exhausted state likely implicated in immune escape which will be investigated for immune checkpoint inhibition.

GENETIC ENGINEERING OF B CELLS FOR ADOPTIVE IMMUNOTHERAPY

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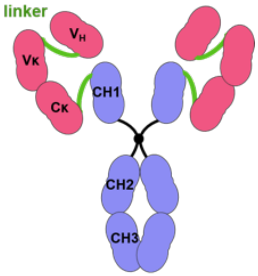
Objectives: Cell therapy, using the patient's own immune system, has hugely developed for the treatment of different cancers. CAR-T cells are currently at the frontline of such therapies, but other cell types can also be considered. B lymphocytes are interesting candidates, as they are not only able to establish an immune memory, but also to secrete large amounts of antibodies once differentiated into plasma cells. Gene edited B cells could survive in the patient's body for a long time patrolling for relapses, and secrete specific antibodies when needed. This project aims at establishing a proof of concept of a B cell-based cell therapy.

Methods: A classical IgG is a combination of heavy and light chains, which expression requires the modification of two loci. As gene editing of primary B cells remains difficult, we have designed an original format of a single-chain immunoglobulin (Ig), the "scFull-Ig", to make the edition easier, allowing expression of a full Ig after a single gene knock-in. We have chosen to introduce the expression cassette in the Ig heavy chain (IgH) locus, between the VDJ region and the enhancer μ, in order to allow the expressed scFull-Ig to undergo natural maturation mechanisms such as class switch recombination or somatic hypermutation. CRISPR was used to ensure a precise gene edition.

Results: The scFull-Ig format structure was validated and is functional. Target binding and capacity to induce ADCC (Antibody-Dependent Cell-mediated Cytotoxicity) was validated using two targets, HER2 and CD20. Then, gene edition was first optimized using a lymphoma cell line. Knock-in of a reporter cassette yielded more than 30% of positive cells. Although first inefficient, gene edition in primary B cells was progressively improved using enhancers and donor DNA with modified arms, until routinely yielding on average 3% of scFull-Ig expressing cells.

Conclusions: We have designed a new format of a single-chain Ig, the scFull-Ig, and have validated its expression. Conditions of gene edition of primary B cells were then optimized, to perform a knock-in of scFull-Ig expressing vector using CRISPR. Engineered primary B cells successfully expressed the scFull-Ig on cell surface. The precise insertion at the IgH locus allows the expressed scFull-Ig to undergo natural maturation mechanisms such as somatic hypermutation, and could be membrane-bound or secreted depending on the RNA splicing. Although letting space for further optimization, these results give confidence about the possibility of a B cell-based immunotherapy scheme in a near future.



scFull-Ig

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RESISTANCE MECHANISMS IN GD2-DIRECTED BISPECIFIC TRIFUNCTIONAL ANTIBODY THERAPY AGAINST NEUROBLASTOMA

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Objectives: Neuroblastoma is the most common extracranial solid tumor of childhood. Patients with high-risk disease undergo extremely aggressive therapy and nonetheless have cure rates below 50%, necessitating new therapeutic options. Bispecific trifunctional antibodies (trAbs) are IgG-like molecules directed against T cells and cancer surface antigens, redirecting T cells (via their CD3 specificity) and accessory immune cells (via their functioning Fc-fragment) towards tumor cells. Treatment with the GD2-directed bispecific trifunctional antibody EKTOMUN/SUREK demonstrated high efficacy in a preclinical neuroblastoma model. However, therapy resistance in trAb therapy in not yet well understood. We aimed to preclinically evaluate possible resistance mechanisms against neuroblastoma-specific trAb in a syngeneic immunocompetent neuroblastoma mouse model. Combination therapies targeting these mechanisms of resistance might further improve trAb efficacy to treat patients with high-risk or relapsed neuroblastoma.

Methods: Two different bispecific trifunctional antibody constructs were used in experiments, EKTOMUN, which is directed against GD2 and human CD3, and SUREK, which is directed against GD2 and murine Cd3. We established a co-culture model with both human peripheral blood mononuclear cells (PBMCs) and neuroblastoma cell lines to evaluate effector cell response to trAb therapy *in vitro* using the flow cytometric assessment of PBMC surface expression of inhibitory receptors. SUREK activity was also assessed in a syngeneic immunocompetent neuroblastoma mouse model for experimental metastases. A/J mice intravenously received 5 x 10^5 NXS2 cells, a GD2-expressing hybrid murine neuroblastoma cell line. SUREK was intraperitoneally administered at a suboptimal treatment regimen on days +1, +5, and +10, and tumor growth as well as tumor infiltrating T cell profiles were analyzed.

Results: The EKTOMUN bispecific trifunctional antibody mediated a strong upregulation of inhibitory receptors on T cells *in vitro*. SUREK administration *in vivo* led to a significant survival benefit, yet mice were not completely cured. Outgrowing tumors showed a significant amount of $GD2^-$ antigen loss variants. Expression levels of HLA class I molecules and the surface marker Pd-l1 on tumor cells did not differ between treatment groups. The amount of $CD3^+$ Tumor infiltrating T cells (TIT cells) and their Cd4/Cd8 ratio was not altered. However, tumor-infiltrating $Cd4^+$ but not Cd8+ T cells showed a significantly higher surface expression of the immune checkpoint molecules Pd-1 and Ctla-4.

Summary: Here we provide the first insights on resistance mechanisms of neuroblastoma upon treatment with GD2-directed trAb. Combination with immune-checkpoint inhibition might further enhance trAb efficacy.

SELECTED ABSTRACTS FOR A POSTER PRESENTATION

CLINICAL POSTERS

ABSTRACTS SELECTED AS POSTERS
IN ALPHABETIC ORDER ACCORDING TO THE NAME OF THE FIRST AUTHOR

CLINICAL POSTERS

Poster 25: **MAKE YOUR GUESTS FEEL AT HOME: FLUDARABINE EXPOSURE INFLUENCES THE 'HOST ENVIRONMENT' ASSOCIATING WITH CLINICAL OUTCOME IN CHILDREN AND YOUNG ADULTS TREATED WITH CAR T-CELLS FOR ALL**

Noel Dautzenberg, Linde Dekker, Joyce Meesters, Coco de Koning, Caroline Lindemans, Friso Calkoen, Stefan Nierkens

Poster 26: **TIMELY LYMPHOCYTE-APHERESIS AND TYPE OF DISEASE MAY INFLUENCE T CELL FITNESS FOR CAR-T MANUFACTURING**

Mirko Farina, Marco Chiarini, Camillo Almici, Eugenia Accorsi Buttini, Anna Galvagni, Alessandro Leoni, Federica Colnaghi, Vera Radici, Emilio Ferrari, Simona Bernardi, Federica Re, Katia Bosio, Nicola Polverelli, Enrico Morello, Chiara Cattaneo, Duilio Brugnoni, Michele Malagola, Alessandro Re, Domenico Russo

Poster 27: **PRIMARY IMMUNE THROMBOCYTOPENIA IN ADULTS MANAGEMENT AND LONG-TERM OUTCOME OF 162 CASES**

Imen Frikha, Hanen Charfi, Ines Ben Rekaya, Moez Medhaffar, Elloumi Moez

Poster 28: **CHEMO-IMMUNOTHERAPY FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA: MULTICENTRIC EXPERIENCE**

Imen Frikha, Karima Kacem, Haifa Regaieg, Sarra Boukhriss, Dorra Jaber, Ines Safra, Nour Louati, Nawel Ben Salah, Sami Zriba, Moez Medhaffar

Poster 29: **DEVELOPING AFFORDABLE AND SAFE ANTI-CD19 CAR-T CELL THERAPY: A PHASE-I CLINICAL TRIAL EXPERIENCE FROM INDIA.**

Hasmukh Jain, Atharva Karulkar, Neha Sharma, Manju Sengar, Ankesh Kumar Jaiswal, Shreshtha Shah, Aalia Khan, Afrin Firfiray, Sweety Asija, Prashant Suvasia, Priyanshi Suvasia, Juber Pendhari, Devanshi Kalra, Smrithi Ravikumar, Gaurav Narula, Shripad Banavali, Minal Poojary, Sumathi Hiregoudar, Lingaraj Nayak, Bhausaheb Bagal, Prashant Tembhare, Epari Sridhar, Navin Khattry, Nikhil Patkar, Sumeet Gujral, Tanuja Shet, Papagudi Subramanian, Jayashree Thorat, Sachin Punatar, Anant Gokarn, Kinjalka Ghosh, Archi Agarwal, Preeti Desai, Shashank Ojha, Subhash Yadav, Shil Pushp Bhosale, Sunil Rajadhyaksha, Anisha Navkudkar, Siddhartha Laskar, Sumeet Mirgh, Jayant Sastri, Albeena Nisar, Deepali Pandit, Rahul Purwar

Poster 30: **ENHANCING SLAMF7 CAR T CELL PRODUCTION BY PREVENTION OF FRATRICIDE**

Verena Konetzki, Kersten Heyer, Michael Reiser, Halvard Boenig, Hermann Einsele, Michael Hudecek, Sabrina Prommersberger

Poster 31: **T2EVOLVE STANDARDIZATION OF PRE-CLINICAL AND CLINICAL DEVELOPMENT OF ENGINEERED T CELL THERAPY IN EUROPE; A CROSS FUNCTIONAL MULTI STAKEHOLDER INITIATIVE**
Maik Luu, Carmen Sanges

Poster 32: **SEROLOGICAL PROFILE OF HEPATITIS B AND ITS IMPACT ON THE MANAGEMENT OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA**
Wafa Miled, Dorra Jabr, Raoudha Mansouri, Malek Sayedi, Karima Kacem, Raihane Ben Lakhal

MAKE YOUR GUESTS FEEL AT HOME: FLUDARABINE EXPOSURE INFLUENCES THE 'HOST ENVIRONMENT' ASSOCIATING WITH CLINICAL OUTCOME IN CHILDREN AND YOUNG ADULTS TREATED WITH CAR T-CELLS FOR ALL

Noel Dautzenberg[1], Linde Dekker[1], Joyce Meesters[1], Coco de Koning[1,2], Caroline Lindemans[1], **Friso Calkoen**[1] and Stefan Nierkens[1,2]

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Objectives: Chimeric antigen receptor (CAR) T-cell therapy has enormous clinical potential in children with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) to improve survival and reduce long-term treatment-related morbidity. Nevertheless, besides antigen-downregulation and/or outgrowth of CD19-negative subclones, CD19-positive relapse due to loss of CAR T cells occurs in approximately 50% of treated children. The most critical question at this time therefore remains largely unanswered: why do half of the CAR T-treated children with BCP-ALL fail current therapy despite the use of an identical CAR construct?

Methods: we performed OLINK proteomics (prior to and after CAR T cell infusion) and fludarabine measurements in patients treated with tisagenlecleucel in a real-world setting between April 2019 and April 2021 in the Princess Maxima Center for pediatric oncology. Data were analyzed using unsupervised clustering and dimension reduction approaches (e.g. PCA or UMAP) to build prediction models of event-free survival, B-cell aplasia and day +28 response based on preselected key discriminative biomarkers.

Results: we recently showed (Dekker, Blood Adv, 2022) that low fludarabine exposure (<14 mg*h/L) associates with a higher incidence of CD19-positive relapses and early B-cell recovery. We now show that these clinical parameters coincide with a diminished expansion of CAR T-cells, while non-CAR T cell subsets reconstitute faster. Higher fludarabine exposure induced deeper T-cell lymphopenia (mean 39 T-cells/ul versus mean 126 T-cells/ul; *p=0.03)*, and correlated with particular proteomic profiles (among others IL-7 and IL-15) prior to CAR T-cell infusion, and associated with accelerated immune reconstitution. Moreover, proteomic patterns associated with response and cytokine release syndrome (among others as previously reported IL-6 and IL-8) were defined.

Conclusions: these data confirm that fludarabine exposures influences the CAR T cell expansion and clinical efficacy. The fact that non-CAR T-cells expanded less, might be related to the actual fludarabine exposure, whereas the CAR T-cells have not been exposed to fludarabine. Optimizing fludarabine exposure and applying T-cell stimulatory strategies may have a relevant impact on leukemia-free survival, which needs to be validated in prospective clinical trials.

TIMELY LYMPHOCYTE-APHERESIS AND TYPE OF DISEASE MAY INFLUENCE T CELL FITNESS FOR CAR-T MANUFACTURING

Mirko Farina[1], Marco Chiarini[2], Camillo Almici[3], Eugenia Accorsi Buttini[1], Anna Galvagni[2], Alessandro Leoni[1], Federica Colnaghi[1], Vera Radici[1], Emilio Ferrari[3], Simona Bernardi[4], Federica Re[4], Katia Bosio[4], Nicola Polverelli[1], Enrico Morello[1], Chiara Cattaneo[5], Duilio Brugnoni[2], Michele Malagola[1], Alessandro Re[5] and Domenico Russo[1],

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Background: Chimeric antigen receptor T (CAR-T) cells are effective therapy in Diffuse large B-cell Lymphoma (DLBCL), Primary Mediastinal B-cell Lymphoma (PMBCL) and Acute Lymphoblastic Leukemia (ALL). CAR-T cells from T-lymphocytes (T-Ly) enriched for early lineage T-cells have shown higher replicative potential and better response. In the *BioCART BS* study, we assessed the T-cell repertoire at Ly-apheresis for evaluating the impact of previous treatments and type of disease.

Methods: Between April 2021 and July 2022, 20 patients (pts) underwent Ly-apheresis (14 DLBCL, 5 PMBCL and 1 ALL). 7 out of the 14 DLBCL pts with poor prognostic risk factor (refractory to I line of treatment; PET positivity before ASCT; first complete response less than 12 months) were enrolled in an early/pre-emptive Tisa-cel Ly-apheresis program, scheduling leukapheresis as soon as possible, before ASCT. Combinations of monoclonal antibodies were used by Flow Cytometry to evaluate the CD4+/CD8+ T-ly subsets: T-naïve (CD45RA+CCR7+); central memory (CM, CD45RA-CCR7+); effector memory (EM, CD45RA-CCR7-); terminally differentiated (TD; CD45RA+CCR7-), and T-reg (CD4+CD25+CD127low/-).

Results: 7 out of the 14 (50%) DLBCL pts (median age: 59 ys) underwent Tisa-cel pre-emptive Ly-apheresis before ASCT, while the other 7 underwent Ly-apheresis following standard indications (3 Tisa-cel; 4 Axi-cel). At the time of Ly-apheresis, no differences were observed between pts who previously underwent ASCT and those who did not, except for age. ASCT induced a more “exhausted” T-cells profile (Figure 1). Pts who underwent ASCT had lower CD4+/CD8+ ratio as well as lower levels of CD4+ Naïve (p 0.03) and a trend of lower CD8+ Naïve T-Ly (p 0.06). They also had lower prevalence of CD4+ Naïve T cells (p = 0.004), as well as higher prevalence of CD4+ EM T-Ly (p 0.04). In addition, pts in the pre-emptive group had higher levels of T-reg Ly (p 0.01).

The 5 PMBCL pts (median age: 38 ys) showed a Ly-profile more similar to the DLBCL-standard group pts, having an even more exhausted T-Ly profile (Figure 1), characterized by lower levels of CD4+ and CD8+ CM T-Ly compared to the DLBCL-standard group pts. When compared with the DLBCL-pre-emptive group, PMBCL pts presented lower CD4+ and CD8+ Naïve T cells, as well as higher percentage of CD4+ EM and TD T cells.

Notably, 5 out of 7 (71%) pre-emptive DLBCL pts had already activated a CAR-T cells program.

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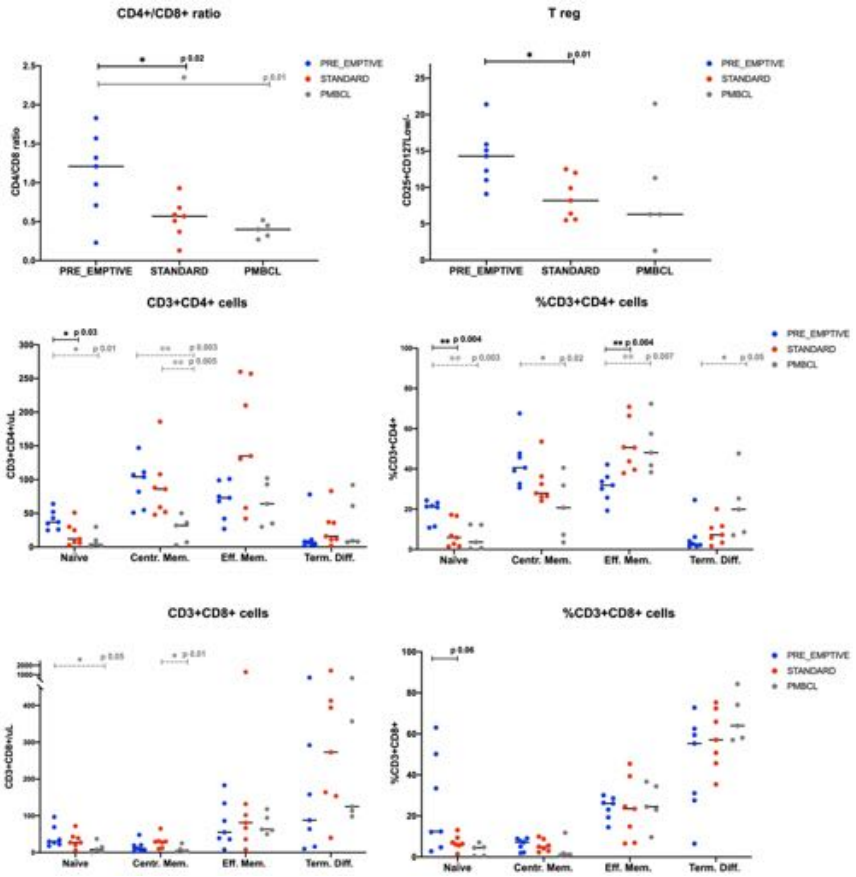
Conclusion: ASCT before Ly-apheresis may result in a more “exhausted” T-Ly repertoire. In high-risk DLBCL pts, timely pre-ASCT Ly-apheresis would be considered. In addition, type of disease may also influence Ly-repertoire. Indeed, PMBCL pts had lower levels of “Fit” Ly compared to DLBCL pts.



PRIMARY IMMUNE THROMBOCYTOPENIA IN ADULTS MANAGEMENT AND LONG-TERM OUTCOME OF 162 CASES

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Introduction: Primary immune thrombocytopenia (ITP) is an acquired disease characterized by immunological peripheral platelet destruction. ITP occur at any age and had a more chronic evolution in adult. We report the therapeutic results and outcomes of adult ITP.

Patients and Methods: Between January 2010 and December 2020, we retrospectively analyzed the data of 162 adults with ITP diagnoses. The first treatment was Glucocorticoïds: Prednisone 1 mg/kg/day for 3-4 weeks followed by a degression dose, and duration was adapted to the elderly and the diabetics. The evaluation on day 28 is complete response (CR) if the platelet count is higher than 100 G/L, partial response (PR) if the platelet count is higher than 30 G/L, and failure if the platelet count is lower than 30 G/L. We classified our patients according to disease duration as acute (12 months), persistent (3 to 12 months), or chronic (>12 months). For patients with chronic evolution, we evaluated various therapeutic lines such as high dose of glucocorticoïds (bolus of methylprednisolone), Rituximab®, Cyclophosphamide®, Danatrol®, and splenectomy.

Results: One hundred sixty-two adults (108 women and 54 men) with a median age of 44.5 years (16 to 94 years) were followed over eleven years. All Newly diagnosed ITP patients are managed with corticosteroids. One hundred twenty-one (80%) patients achieved a good response (CR+PR) to prednisone®. Forty-six (36%) patients relapsed. Our patients are classified into persistent ITP for 19 patients and chronic ITP for 54 patients. Among 19 patients with persistent ITP, 7 received again corticosteroid with a good response in 6 cases, a bolus of solumedrol in one case with a good response, Cyclophosphamide in 3 cases with a good response in all cases, and Rituximab in 2 cases with a good response. Six patients underwent splenectomy after a median follow-up of 7 months, with a good response in all cases. For patients with chronic ITP evolution, the treatment is based on corticotherapy for 30 patients with good responses of 63%, Cyclophosphamide in 4 cases with a good response for 2 patients, Danatrol in 2 cases with a good response in all cases and, 2 patients received Rituximab without any responses. Eleven patients underwent Splenectomy after a median follow-up of 24 months with a response rate was 82%. Finally, we have noted one death related to severe bleeding.

Conclusion: Glucocorticoïds is the first-choice treatment for ITP resulting in a response in 84% as reported studies. However, relapse occurs in a third of cases. Several treatments can be used for persistent and particularly chronic ITP with less level of response. Splenectomy still became the best treatment for those patients. However, it was indicated after the failure of all other treatments such as Rituximab and TPO-RAs. Recently in our local guidelines, TPO-RAs constitute the major treatment for persistent and chronic ITP after 6 months of evolution.

CHEMO-IMMUNOTHERAPY FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA: MULTICENTRIC EXPERIENCE

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(2) Aziza Othmena Hospital, Tunis, Tunisia
(3) Farhat Hached Hospital, Sousse, Tunisia
(4) Fattouma Bourguiba Hospital, Monastir, Tunisia
(5) Pasteur Institute, Tuins, Tunisia
(6) Regional Blood Transfusion center, Sfax, Tunisia
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(8) Military Hospital, Tunis, Tunisia
(9) Hematology Department at Hedi Chaker Hospital, sfax, Tunisia

Introduction: Chemo-immunotherapy (CIT) is still in the last decade the gold standard treatment of chronic lymphocytic leukemia (CLL) without TP53 abnormality. A combination of Rituximab (R) with other agents such as Fludarabine (F) Cyclophosphamide (C), Bendamustine (B), and Chlorambucil (CLB) has shown improvement in the overall response rate and progression-free survival. We report the results of first-line CIT protocols in the treatment of CLL patients.

Patients and methods: Between January 2016 to December 2020, we included CLL patients without 17 p deletion, treated in first-line therapy with FCR, BR, or R CLB protocol, in 5 hematologic departments in Tunisia: Aziza Othmena Hospital, Military Hospital, Farhat Hached Hospital, Fattouma Bourguiba Hospital, and Hedi Chaker Hospital. According to the Tunisian guidelines, the treatment of CLL patients was based on the association of Rituximab with chemotherapy such as FC, Bendamustine, or CLB regardless of Fitness and 17p deletion. Response criteria were assessed according to National Cancer Institute Criteria (NCI). Then we report relapse rate, response duration, and overall survival (OS).

Results: Among 132 treated CLL patients in the study period, 91 received CIT as first-line therapy: FCR regimen 50 patients, BR regimen 3 patients, and R CLB regimen 38 patients. Overall rate response was 80% (FCR regimen= 80%, BR regimen=100%, and R CLB regimen=60%). Death was noted in two Patients in the FCR group, and one patient in the R CLB group. Failure treatment was noted in 5 cases (10%) in the FCR group, and 6 cases (16%) in the R CLB group. Richter transformation was noted in one case of the FCR and one case of the R CLB groups (5% and 4% respectively). The relapse rate was 27% of patients: 25% in the FCR regimen and 30% in the R CLB regimen. We have not noted any relapse in the BR regimen. The median time of relapse was respectively 24 months and 12 months for the FCR and R CLB groups. With a follow-up of 40 months, OS at 3 years was 43.5%: 45% for the FCR group and 40% for the R CLB group (p=0.289)

Conclusion: Chemoimmunotherapy has become the standard of care for CLL patients and offers good response and higher PFS compared with those treated with chemotherapy alone. This result was observed especially for CLL patients without TP53 abnormality or Unmutated IGHV.

Recently, in our national guidelines, we recommended the analysis of TP53 abnormality and mutational profile of IGHV for better selection of CLL patients to receive CIT. patients with unfavorable cytogenetic and molecular abnormalities should be treated actually with new targeted therapies such as BCL2 inhibitors and BTK inhibitors.

DEVELOPING AFFORDABLE AND SAFE ANTI-CD19 CAR-T CELL THERAPY: A PHASE-I CLINICAL TRIAL EXPERIENCE FROM INDIA.

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Background: Chimeric antigen receptor T-cell (CAR T-cell) therapy is highly effective in CD19-positive hematologic malignancies. However, toxicities and prohibitive cost make CAR-T cell therapy inaccessible in low-resource settings. We developed a novel humanized anti-CD19 CAR-T cell (HCAR19) therapy that showed favourable balance of efficacy and toxicity in the preclinical studies. Here, we report the phase-I clinical trial experience of HCAR19 in relapsed/refractory (r/r) B-cell lymphomas.

Methods: Patients with r/r DLBCL, PMBCL and transformed follicular lymphoma (as per SCHOLAR-1 criteria) without any CNS involvement were eligible. The clinical trial (CTRI/2021/04/032727) was conducted at Tata Memorial Center, Mumbai upon regulatory approval. The CAR-T cells were manufactured at cGMP facility of IIT Bombay. A lymphodepleting conditioning regimen of Fludarabine at 30mg/m2/day (D1-D3) and cyclophosphamide at 500 mg/m2/day (D1-D2, n=5 and after an amendment on D1-3, n=5) was given to the patients 5 days prior to infusion. The CAR-T cells ($1x10^7$-$5x10^9$) were infused in a split dose, 10% on day 0, 30% on day 1 and 60% on day 2 respectively. The patients remained hospitalised for 7 days or till resolution of toxicities to grade ≤1. They were monitored weekly in the first month, monthly till 6 months, 3-monthly till completion of two years. A response assessment PET-CT scan was performed on day 90 (D28 for last 5 patients).

Results: A total of 14 patients were enrolled between May 2021 till June 2022, manufacturing feasibility was passed in 13 patients (92%). HCAR19 cells were manufactured with transduction efficiency of 31.9 ± 4.86% (Mean ± SEM) and the required HCAR19 dose was achieved within 8 days of culture. Total 10 patients (r/r DLBCL (n=7), r/r PMBCL (n=3)) received HCAR19 and 3 patients were withdrawn prior to the intervention (Figure 1). Four out of seven (57%) patients of r/r DLBCL patients showed a response (3 complete and 1 partial response). However, 3/7 (42%) r/r DLBCL patients and 3/3 (100%) r/r PMBCL did not respond to HCAR19 therapy. An association was noted between response and CAR-T cell dose (11.3 ± 3.3 X 10^6 T cells/kg vs 5.2 ± 2.1 X 10^6 T cells/kg; Mean ± SEM), number of lines of therapy (</=2 vs >2 lines) and peak CAR-T cell *in vivo* expansion. Immunophenotyping of the HCAR19 final product in responders and non-responders revealed no difference in T-cell subsets. Importantly, only 5/10 patients receiving HCAR19 products had Grade I/II CRS within 7-14 days, which responded to a single dose of tocilizumab. More importantly, none of the patients had any neurological events (ICANS) and required ICU admissions. There were no treatment-related deaths. The total costs of end-to-end CAR-T manufacturing per patient is estimated approximately $30,000 including logistics, lentiviral vector cost and quality control.

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Conclusions: HCAR19 is safe over a wide range of doses, with promising early signs of activity. This therapy is easy to deliver on an outpatient basis, cost-effective and will improve access in developing countries.

ENHANCING SLAMF7 CAR T CELL PRODUCTION BY PREVENTION OF FRATRICIDE

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Background: The SLAMF7 antigen is highly and uniformly expressed on malignant plasma cells and under investigation as a target for antibody-based and cellular immunotherapy in multiple myeloma. We are pursuing the clinical development of SLAMF7 CAR T cell therapy in an ongoing phase I/IIa trial (CARAMBA EudraCT: 2019-001264-30). A particular challenge with generating SLAMF7 CAR T cells is the physiologic expression of SLAMF7 on T cells which induces fratricide and submaximal SLAMF7 CAR T cell expansion during manufacturing. Here, we determined the effect of using an anti-SLAMF7 antibody (aSF7-mAb) to shield SLAMF7 on T cells on the yield and potency of SLAMF7 CAR T cells in preclinical campaigns.

Methods: We generated SLAMF7 CAR T cells following the cGMP-compliant manufacturing protocol of the CARAMBA trial. Different concentrations of aSF7-mAb were added to the culture medium after SLAMF7 CAR gene-transfer, replenished every 2nd day and removed either mid-way or at the end of the manufacturing process. We performed several manufacturing and release campaigns with extensive phenotyping and functional testing.

Results: We found that the addition of aSF7-mAb had a profound and consistent positive effect on SLAMF7 CAR T cell manufacturing. At the end of the manufacturing campaign, the total expansion factor of T cells treated with aSF7-mAB was highly increased and both methods resulted in comparable gene transfer rates. aSF7-ab addition exerted the strongest effect (in preventing fratricide) immediately after gene-transfer, evidenced by a higher proportion of viable T cells. At the end of manufacturing, SLAMF7 CAR T cells had a SLAMF7-negative phenotype, suggesting that fratricide had still occurred but at a pace and extent that was compatible with productive proliferation and expansion. Phenotypic analysis showed that a sizeable fraction of SLAMF7 CAR+ T cells had lower expression of PD-1, TIM-3 and LAG-3 after treatment with aSF7-mAb. Of particular note, we observed strong anti-myeloma efficacy of SLAMF7 CAR T cells that had been treated with aSF7-mAb: In particular, we found greater proliferation of SLAMF7 CAR T cells after stimulation with multiple myeloma target cell lines, indicating substantially improved T cell fitness.

Conclusion: Taken together, the data show that the addition of aSF7-mAb to shield SLAMF7 on T cells substantially facilitates the manufacturing of SLAMF7 CAR T cells for adoptive therapy of multiple myeloma. The prevention (or rather: the steering of controlled fratricide) leads to increased yield, augmented phenotype and anti-myeloma function in pre-clinical models. These data suggest that SLAMF7 CAR T cells produced with this improved protocol will also confer superior anti-myeloma efficacy in a clinical setting. The aSF7-mAb is available in pharmaceutical grade and be seamlessly incorporated into cGMP manufacturing processes for the next generation of trials under the CARAMBA IND.

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T2EVOLVE STANDARDIZATION OF PRE-CLINICAL AND CLINICAL DEVELOPMENT OF ENGINEERED T CELL THERAPY IN EUROPE; A CROSS FUNCTIONAL MULTI STAKEHOLDER INITIATIVE

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(2) BioSci Consulting, Maasmechelen, Belgium
(3) Institut de Recherches Internationales Servier, Suresnes, France

Objectives: T2EVOLVE is a breakthrough alliance of academic and industry leaders in cancer immunotherapy that started in 2021, under the European Union's Innovative Medicines Initiative (IMI). The key objective of T2EVOLVE is to accelerate the development of engineered T-cell therapies and increase their access for patients.

Methods: T2EVOLVE screened preclinical models to evaluate safety and efficacy of engineered T-cell therapies and identified unmet needs and gaps that will define the consortium's research roadmap. The joint forces of key opinion leaders in engineered T-cell therapy field aim to close these gaps by developing innovative *ex vivo* models, approaching regulatory authorities as well as standardizing pre-clinical and clinical development procedures.

Two committees have been established to begin developing standards for analytical methods; the Standard Technical Committee and the Committee of European National Engineered T-cell therapy practices, consisting of external key EU leaders in the field. Both bodies are involved in the definition of a survey intended to collect information on starting material, release criteria and analytical methods for immunomonitoring.
Through engagement of patient and carers, the consortium aims to collect first-hand information from those directly experienced with receiving engineered T cell therapy in order to identify educational needs, and information gaps. Furthermore, two dedicated workshops were hosted in 2021 with a Working Group set up by T2EVOLVE composed of patients, carers and patient organization representatives, to introduce this EU-funded initiative.

Results: Using innovative technologies such as organotypic tissue slices, microphysiologic 3D-tumor models and advanced microscopy T2EVOLVE is working on developing new validated preclinical models and test parameters that recapitulate what observed in patients with higher predictive value for clinical safety and efficacy. While being in close dialogue with the EMA's Innovation Task Force, the consortium has the ambition to set new standards for pre-clinical development, immunomonitoring assays and GMP processes.

The results of the analytical methods survey (launched at the 4th EBMT-EHA CAR T-cell meeting) will be published by the end of 2022 and used as the foundation to develop new analytical standards. A second survey, that will be launched late 2022, will gather patients' experiences on receiving engineered T-cell therapies and carers' experiences on supporting patients as well as long-term quality of life parameters.

Conclusion: T2EVOLVE is developing new tools and standards that will be validated by the consortium partners with the ultimate goal to provide investigators and clinicians with innovative assays and guidelines for improved pre-clinical development, immunomonitoring and GMP manufacturing. Moreover, patient involvement will ensure that the perspectives of cancer patients are listened and incorporated, reflected in educational materials and in the research setting.

SEROLOGICAL PROFILE OF HEPATITIS B AND ITS IMPACT ON THE MANAGEMENT OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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(2) Department of clinical haematology, Aziza Othmana Hospital, Tunis, Tunisia

Introduction: In Tunisia, Hepatitis B represents a major public health problem because of its high morbidity and mortality rates. Rituximab is the cornerstone of the treatment of diffuse large cell B lymphoma (DLBCL). HBV reactivation remains a fatal complication which can be prevented by preemptive therapy.

Patients and methods: We performed a retrospective study involving patients with DLBCL treated, in the clinical hematology department of Aziza Othmana Hospital,in Tunisia between 2013 and 2020.
The aim of this study was to investigate HBV infection frequency, serological profile and therapeutic approach.

Results: Three hundred and nineteen patients were included. 303 patients had complete serology test :209 patients had negative serology,68 had a resolved infection profile, 14 were vaccinated, 5 patients had chronic hepatitis B, and 7 patients had acute hepatitis B initially. Sixteen patients had an incomplete serological profile limited to a negative Hepatitis B surface antigen (HbsAg).

The prevalence of HbsAg was 3.7%. 80 patients had contact with HBV (26%). The median age of this population was 61 years [16-86] with sex ratio of 1.27.

70% of patients received two weeks of preemptive therapy before starting Rituximab. Four patients received Rituximab without preemptive therapy. Seven patients received curative therapy. Fifteen patients did not receive it due to gastroenterologist refusal.

75% of patients in contact with HBV received Rituximab. 52% of patients treated with Rituximab received it with delay compared to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone). Waiting for serology results and gastroenterologists' agreement to initiate preemptive therapy were the main reasons for this delay.
Two HBV reactivations had occured.

Conclusion: The prevalence of HbsAg in DLBCL is higher compared to the general population. Late HBV reactivations were observed and indicated a longer duration of preemptive treatment.

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DISCLOSURES

ESH 2nd Translational Research E-Conference
IMMUNE & CELLULAR THERAPIES: Focus on Advanced Gene-Engineered Immune Cells
Berlin, Germany - September 12-14, 2022

DISCLOSURES

F.	Blaeschke	Gilead/Kite	Research Support, Consultant
		BMS	Other (Award)
C.	Bonini	Intellia Therapeutics	Research Support, Scientific Advisory Board
		Novartis	Consultant, Scientific Advisory Board
		Janssen	Consultant, Scientific Advisory Board
		Quelltx	Consultant, Scientific Advisory Board
		Miltenyi Biotec	Consultant
		Genyo	Scientific Advisory Board
P.	Bousso	No affiliation	/
D.	Busch	BMS	Research Support, Consultant
		Immatics	Scientific Advisory Board
M.	Cartellieri	AvenCell Europe GmbH	Employee
		AvenCell Inc.	Other (Stock options)
G.	Casorati	No affiliation	/
A.	Chapuis	Affini-T	Scientific Advisory Board
		BioNTech	Scientific Advisory Board
		Tscan	Scientific Advisory Board
		Signal One	Scientific Advisory Board
		Cullinan	Research Support
		Amazon	Research Support
		Lonza	Research Support
		Metagenomi	Scientific Advisory Board
Y.	Chen	ImmPACT Bio	Consultant, Major Stockholder, Scientific Advisory Board
		Catamaran Bio	Scientific Advisory Board
		Prime Medicine	Speakers' Bureau, Scientific Advisory Board
		Pluto Immunotherapeutics	Scientific Advisory Board
		Notch Therapeutics	Scientific Advisory Board
P.	Corradini	Abbvie	Consultant, Scientific Advisory Board, Other (Lecturer)
		ADC Therapeutics	Consultant, Scientific Advisory Board, Other (Lecturer)
		Amgen	Consultant, Scientific Advisory Board, Other (Lecturer)
		BeiGene	Consultant, Scientific Advisory Board, Other (Lecturer)
		Celgene	Consultant, Scientific Advisory Board, Other (Lecturer)
		Daiichi Sankyo	Consultant, Scientific Advisory Board, Other (Lecturer)
		Gilead/Kite	Consultant, Scientific Advisory Board, Other (Lecturer)
		GSK	Consultant, Scientific Advisory Board, Other (Lecturer)
		Incyte	Consultant, Scientific Advisory Board, Other (Lecturer)
		Janssen	Consultant, Scientific Advisory Board, Other (Lecturer)
		KyowaKirin	Consultant, Scientific Advisory Board, Other (Lecturer)
		Nerviano Medical Science	Consultant, Scientific Advisory Board, Other (Lecturer)
		Novartis	Consultant, Scientific Advisory Board, Other (Lecturer)
		Roche	Consultant, Scientific Advisory Board, Other (Lecturer)
		Sanofi	Consultant, Scientific Advisory Board, Other (Lecturer)
		Takeda	Consultant, Scientific Advisory Board, Other (Lecturer)
L.	Gattinoni	Lit Regensburg	Employee
		Poseida	Scientific Advisory Board
		Advaxis	Consultant
		Kiromic BioPharma	Scientific Advisory Board
		Lyell	Consultant
S.	Haubner	Takeda	Research Support
M.	Heemskerk	Miltenyi Biotec	Research Support
S.	Heitzeneder	No affiliation	/
M.	Hudecek	Novartis	Other (Speaker)
		Gilead/Kite	Other
		T-Curx	Major Stockholder

J.	Huppa	Boehringer Ingelheim	Research Support
M.	Jensen	No affiliation	/
J.	Kuball	Novartis	Research Support
		Miltenyi Biotec	Research Support
		Gadeta	Research Support, Major Stockholder
A.	Künkele	No affiliation	/
M.	Maus	Adapt immune	Consultant
		Agenus	Consultant
		Arcellx	Consultant
		Astellas	Consultant
		AstraZeneca	Consultant
		Atara	Consultant
		Bayer	Consultant
		BMS	Consultant
		Cabaletta Bio	Consultant
		Century Therapeutics	Research Support
		CRISPR Therapeutics	Research Support, Consultant, Major Stockholder
		In8bio	Consultant
		Intellia	Consultant
		GSK	Consultant
		Kite Pharma	Research Support, Consultant
		Micromedicine	Consultant
		Novartis	Research Support, Consultant
		Sanofi	Consultant
		TCR2	Consultant, Major Stockholder
		Tmunity	Consultant
		Torque	Consultant
		WindMIL	Consultant
		Servier	Research Support
		2Seventy Bio	Major Stockholder, Scientific Advisory Board, Other
D.	Powell	AstraZeneca	Scientific Advisory Board
		Bellicum Pharmaceuticals	Scientific Advisory Board
		Incyte	Research Support
		InsTIL	Research Support, Scientific Advisory Board
		Tentarix	Scientific Advisory Board
K.	Rezvani	Takeda	Other
		Affimed	Other
		Avenge Bio	Scientific Advisory Board
		Kiadis	Consultant
S.	Riddell	Lyell Immunopharma	Research Support, Consultant, Major Stockholder
		BMS	Research Support
C.	Rooney	Allovir	Major Stockholder
		Marker Therapeutics	Major Stockholder
		Bellicum Pharmaceuticals	Other
		Tessa Therapeutics	Other
E.	Ruggiero	Intellia Therapeutics	Research Support
M.	Sauer	No affiliation	/
A.	Sewell	Adaptimmune	Scientific Advisory Board
		Enara Bio	Research Support, Consultant, Scientific Advisory Board
M.	Themeli	Fate Therapeutics	Other (Royalties)
L.	von Baumgarten	EUSA Pharma	Scientific Advisory Board
		Bayer	Scientific Advisory Board
D.	Zehn	No affiliation	/

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