Connecting Basic, Translational and Clinical Science

JOINT **ASTCT + EBMT**

Basic and Translational

SCIENTIFIC MEETING

May 23-25, 2023 • Sitges, Spain

WELCOME

EBMT and the American Society for Transplantation and Cellular Therapy (ASTCT) are proud to announce the 2nd Joint ASTCT-EBMT Basic and Translational Scientific Meeting, taking place May 23 - 25, 2023.

The Basic and Translational Scientific Meeting will be a 2.5-day conference focused on cutting edge basic and translational biology in the field. The meeting will focus on fundamental themes and novel technologies with an emphasis on unpublished and innovative science. Attendance to the meeting will be limited to 120 attendees with afternoon breaks to allow for informal networking opportunities between junior and senior attendees. The meeting will host two days of poster sessions and a best oral abstract session.

Tuesday, 23 May 2023

09:00 – 09:15	**Welcome and Introduction**	
09:15 – 11:15	**Immune metabolism** **Session chair: Bruce Blazar**	
09:15 – 09:45	Metabolic Adaptation in GVHD T Cells	**Craig Byersdorfer**
09:45 – 10:15	Metabolic reprogramming of T cells to enhance GVL effect	**Robert Zeiser**
10:15 - 10:45	Obesity, Inflammation, and Cancer	**Jeffrey Rathmell**
10:45 – 10:55	Oral presentation: Graft-versus-host disease is locally maintained in target tissues by resident progenitor-like T cells	**Faruk Sacirbegovic**
10:55 – 11:05	Oral presentation: Combinatorial Adaptor-Mediated Targeting of Acute Myeloid Leukemia with CAR T-Cells	**Laura Volta**
11:05 – 11:15	Oral presentation: Paneth cell niche function is dispensable in experimental GVHD	**Viktor Arnhold**
11:10 – 11:45	**Coffee break**	

Bruce Blazar (United States, Minneapolis)

Dr. Bruce Blazar received a BS degree from Rensselaer Polytechnic Institute and MD degree from Albany Medical College following which he obtained clinical training in pediatrics and hematology/oncology/blood and marrow transplantation at the University of Minnesota. He is a Regents Professor and founding director of the Clinical and Translational Science Institute and the Center for Translational Medicine. He has directed preclinical basic and translational immunology and stem cell research and early phase clinical studies with particular emphasis in the blood and marrow transplantation immunobiology. His early contributions to preventing acute GVHD included preclinical modeling of commonly used immunosuppressant drugs as well as CTLA4-Ig that blocks the specific T cell immune response to host antigens and became the first U.S. Food and Drug Administration (FDA) drug for acute GVHD prophylaxis. In more current chronic GVHD studies, he discovered that donor germinal center B cells that produce anti-host antibodies can work in concert with macrophages to cause chronic GVHD. Preclinical supporting data have contributed to clinical studies of ibrutinib (BTK/ITK inhibitor), Belumosudil (Rock2 inhibitor), Ruxolitinib (Jak1/2), Bortezomib (proteosomal inhibitor), Entospletinib/Fostamatinib (pSyk), Axatilimab (anti-CSF1R mAb), and Pirfenidone (SMAD2/3) and Treg adoptive cell therapy.

Robert Zeiser (Germany, Freiburg)

Robert Zeiser is Full Professor of Medicine and Director of the Division of Tumor Immunology at the Department of Hematology, Oncology, and Stem-Cell Transplantation at the Medical Center-University of Freiburg in Freiburg, Germany. His work can be divided into clinical responsibilities, clinical and laboratory-based research, and teaching activities. Professor Zeiser's laboratory research is focused on graft-versus-host disease, tumor biology, in vivo imaging, and signaling. In particular, his research group has used different imaging techniques, including bioluminescence imaging, magnetic resonance imaging, fluorescence imaging, and positron emission tomography/computerized tomography, to monitor the fate of different cell types from living animals. His group has made major contributions to the field of pre-clinical and clinical research, with publications in New England Journal of Medicine, Nature Medicine, Journal of Experimental Medicine, Blood, Science Translational Medicine, Nature Communications, and Journal of Clinical Investigation, and has received several awards in Germany, Europe, and the USA and research grants from multiple funding agencies. Professor Zeiser has authored or co-authored > 250 peer-reviewed publications and 14 book chapters, and has served as a reviewer for Science, Nature Medicine, Nature Immunology, Nature Methods, Nature Reviews Immunology, Nature Reviews Cancer, New England Journal of Medicine, Lancet Haematology, Blood, and other journals. Professor Zeiser is Section Editor of Blood and an expert reviewer for research funding bodies in Germany, France, Israel, China, the Netherlands, Austria, the UK, Poland, Belgium, and Switzerland, as well as the European Union. He is Director of the Collaborative Research Center SFB 1479 and Director of the Division of Tumor Immunology at the University of Freiburg.

Jeffrey Rathmell (Nashville, United States)

Dr. Jeffrey Rathmell studies T cells in autoimmune or inflammatory diseases and cancer with a focus on metabolic mechanisms that regulate lymphocyte fate and function. He has an interdisciplinary research program using genetic and biochemical approaches to discover immunometabolic mechanisms that drive these immune-related diseases. He received his PhD in Immunology at Stanford University and performed postdoctoral studies at the University of Pennsylvania prior to beginning as faculty at Duke University and subsequently Vanderbilt University Medical Center. In this time, he showed that lymphocyte metabolism is dynamically regulated and his was the first group to show that each T cell subset adopts a specific metabolic program that can be targeted to modulate cell function and fate. These changes point to mechanisms of disease and offer new therapeutic targets in cancer and a variety of immune-related diseases. He joined Vanderbilt in 2015 to found and direct the Vanderbilt Center for Immunobiology and is leader for the Vanderbilt-Ingram Cancer Center Program in Host-Tumor Interactions. His awards include Scholar of the Leukemia & Lymphoma Society, Bernard Osher Fellow of the American Asthma Foundation, and William Paul Distinguished Innovator of the Lupus Research Alliance.

Graft-versus-host disease is locally maintained in target tissues by resident progenitor-like T cells

F. Sacirbegovic[1], *M. Günther*[2], *A. Greco*[2], *K. Quann*[1], *T. Höfer*[2], *W. Shlomchik*[1]
[1]University of Pittsburgh, Pittsburgh, United States, [2]German Cancer Research Center, Heidelberg, Germany

Topic: GVHD

Title: Graft-versus-host disease is locally maintained in target tissues by resident progenitor-like T cells

Short title: GVHD maintenance in target tissues by resident progenitor-like T cells

Background: Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (alloSCT). In GVHD, donor T cells recognize recipient tissues as non-self and mount a broad attack that results in multi-organ damage. While there has been some success in diminishing the incidence of GVHD, less progress has been made in treating established or steroid-refractory disease without severe global immunosuppression. This is in part due to a lack of understanding of the underlining mechanisms that sustain GVHD.

Methods: To address how GVHD is sustained in tissues over time, we used multiple approaches in mouse GVHD models including T cell clone tracking, parabiosis of GVHD mice as well as computational modeling.

Results: We showed that once GVHD is established, T cells within diseased tissues maintain GVHD locally, with only minor input from blood-derived T cells. Moreover, we fitted a mathematical model predicting that within each tissue a small number of progenitor T cells maintain a larger effector pool. Consistent with this, we identified a tissue-resident TCF-1$^+$ subpopulation in numerous tissues that preferentially engrafted, expanded, and differentiated into effectors upon adoptive transfer.

Conclusions: GVHD is sustained as a sum of locally maintained inflammatory T cell responses within each target tissue. TCF-1 $^+$ progenitor-like T cells are a likely candidate population required for this intra-tissue maintenance.

Disclosures: This work was supported by NIH R01 HL143349 (W.D.S.) and Marie Skłodowska-Curie Actions H2020-MSCA-ITN-2017 grant no. 764698 (T.H.)
W.D.S. is a cofounder, stockholder, and compensated advisor for BlueSphere Bio. He is also a compensated advisor for Orca Bio.

Combinatorial Adaptor-Mediated Targeting of Acute Myeloid Leukemia with CAR T-Cells

L. Volta[1], R. Myburgh[1], C. Pellegrino[1], F. Manfredi[1], A. Kaiser[1], M. Maurer[1], J. Müller[1], C.M. Wilk[1], M.M. Bühler[1], C.F. Magnani[1], D. Neri[2,3], M.G. Manz[1,4]
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Topic: Anti-leukemic strategies
Title: Combinatorial Adaptor-Mediated Targeting of Acute Myeloid Leukemia with CAR T-Cells
Short title: Combinatorial Adaptor-Mediated Targeting of Acute Myeloid Leukemia with CAR T-Cells
Background: Chimeric Antigen Receptor (CAR) T-cells have been clinically implemented to eradicate B-cell malignancies by targeting lineage specific cell-of-origin antigens, eliminating both tumor cells as well as healthy B- and plasma-cell counterparts, which can subsequently regenerate again from hematopoietic stem and progenitor cells (HSPCs). Similar on-target, off-tumor side effects would be detrimental in the context of HSPC-derived malignancies, such as acute myeloid leukemia (AML) or myelodysplastic neoplasia (MDS), where the heterogeneous overlapping antigen expression on tumor and HSPC would lead to terminal ablation of hematopoiesis.

Thus, enhanced tumor cell selectivity and/or tunable effector activity is required. This might be achieved by Adaptor-mediated (Ad) CAR T-cell that do not directly recognize an antigen but bind to a specific tag placed on antigen-binding adaptors. Such tagged adaptors would then act as safety switch of the system, modulating CAR T-cell activation on-demand. In addition, combinatorial use of multiple adaptors with different tumor-antigen specificities might enhance tumor selectivity.
Methods: We engineered second generation CAR T-cells to display various anti-fluorescein binders, either in scFv format or with an artificial lipocalin scaffold. We further generated fluorescein-labelled adaptors in IgG or diabody format, directed against AML markers, determined by profiling patient-derived cells (i.e. CD117, CD33, CD371). Cytotoxicity elicited by adaptors was evaluated in vitro against various cell lines and primary patient samples.

Pharmacokinetic studies were performed in NSG mice to determine serum half-life of adaptor molecules in vivo and AML-xenograft mouse models were generated to assess on-tumor residence time of the adaptors as well as their therapeutic efficacy when administered with AdCAR T-cells.
Results: Site-specific conjugations of diabodies mediated the strongest AML killing over a broad range of concentrations, both against AML cell lines and primary blasts. Importantly, combinatorial use of adaptors led to higher adaptor-antigen recognition by CAR T-cells and resulted in significantly enhanced tumor cell lysis compared to equimolar concentrations of single adaptor *in vitro.*

Pharmacokinetic studies performed in NSG mice revealed short serum half-lives of of diabody adaptors upon i.v. and i.p. injection. I.p. injected diabodies in AML-engrafted mice exhibited surface labelling of leukemia cells as long as 12h *in vivo*, thus indicating the necessity for twice-daily applications to achieve efficient tumor-residence of the diabody.

Both, anti-CD117 and anti-CD33 diabodies in combination with AdFITC CAR T-cells led to bone marrow (BM) elimination of leukemia cells in a therapeutic xenograft model (~40% leukemia engraftment in the BM at start of therapy). Importantly, *in vivo* imaging as well as terminal analysis showed that AdFITC CAR T-cells in combination with adaptors were equally efficient as direct CAR T-cells.
Conclusions: We here provide proof-of-concept that AdFITC CAR T-cells and selective combinations of small antibody fragments as adaptors can be an efficient strategy for next-generation immuno-targeting, potentially allowing for individualized treatment and conditional cytotoxic activity, with the aim to recover healthy HSPCs or permit subsequent HSC transplantation.
Disclosures: Nothing to declare

Paneth cell niche function is dispensable in experimental GVHD

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Topic: GVHD
Title: Paneth cell niche function is dispensable in experimental GVHD
Short title: Epithelial stem cell niche in GI GVHD
Background: The intestinal stem cell (ISC) niche creates an environment that supports ISC maintenance and regeneration. In the small intestine (SI), Paneth cells (PC) provide essential growth factors, such as WNT3 and EGF, and are a major constituent of the niche. Whether other epithelial cells make up the niche and their contribution shifts following intestinal injury remains unclear.
Methods: Please see Results part.
Results:  We first evaluated PCs using transgenic *Lyz1^{DTR}* mice that undergo PC ablation upon diphtheria toxin (DT) treatment. Effectively PC-ablated tissue (Fig. 1A-C) demonstrated intact crypt-villus architecture with unaltered crypt and ISC frequencies (Fig. 1D-G). In addition, SI crypts from PC-ablated mice were still able to form organoids (Fig. 1H-I). These findings suggest PCs are an important, but incomplete, component of the niche.
In GVHD patients, PC loss has been associated with a poor prognosis. Surprisingly, we observed similar survival rates, clinical GVHD scores, and weight loss between PC-ablated mice and WT controls that underwent allo-BMT (Fig. 2A-C). On day +5, we did not detect any significant differences in GVHD pathology scores between PC-ablated mice and WT controls (Fig. 2D). Further examination revealed decreased SI crypt height in PC-ablated mice, suggesting an impaired regeneration 5 days post-BMT (Fig. 2E-F). However, SI crypt frequency remained unaffected, suggesting a comparable injury (Fig. 2G). We next examined growth factors in SI crypts on day +5 post-BMT. Unexpectedly, ISC growth factors remained detectable post-transplant despite PC ablation (Fig. 2H-K). While PC ablation led to a reduction in EGF concentration, WNT3 concentration as well as *Dll1* and *Dll4* expression remained entirely unaffected.
Since PC ablation did not worsen GVHD injury and growth factors persisted, we hypothesized that other epithelial cell populations express growth factors. Therefore, we re-analyzed a scRNA-seq dataset of SI epithelium from naïve WT mice. We found that *Egf* and *Wnt3* were predominantly expressed in PC (Fig. 3A-B), but also present in goblet (GC) and enteroendocrine cells (EEC). By performing FISH and IF staining for WGA and CHGA, for GC and EEC, respectively, we confirmed *Wnt3* and *Egf* expression in these populations (Fig. 3C). We further detected that 39.6% of crypt epithelial *Egf* and 28.4% of crypt epithelial *Wnt3* are localized in non-Paneth epithelial cells (non-PC) in naïve WT mice (Fig. 3D-E). After BMT, the contribution of PC as sources of *Wnt3* and *Egf* significantly declined (Fig. 3D-E). Concurrently, the frequencies of GC and EEC, and in particular *Egf*$^{+}$ GC and *Wnt3*$^{+}$ EEC, increased (Fig. 3D-I). Finally, we examined duodenal biopsy samples from BMT patients with and without GI GVHD, compared to samples from non-BMT patients (Fig. 3J-O). Human GC and EEC demonstrated distinct patterns of recovery from PC after hematopoietic transplantation.
Conclusions: Our findings suggest the epithelial ISC niche may encompass other epithelial cells in addition to PC such as GC and EEC.
Disclosures: Nothing to declare.

Tuesday, 23 May 2023

11:45 – 13:45	**Graft VS Host Disease** **Session chair: Warren Shlomchik**	
11:45 – 12:15	Target tissue: Tolerance and reminiscence	**Pavan Reddy**
12:15 – 12:45	T-cell exhaustion and GVHD/GVL	**Teshima Takanori**
12:45 – 13:15	Exploiting dual GVHD and GVL function of regulatory T cells	**Bruce Blazar**
13:15 – 13:25	Oral presentation: A novel high-throughput method for Identifying T-cell receptor: minor histocompatibility antigen interactions in mouse models of graft-vs-host disease	**Kevin Quann**
13:25 – 13:35	Oral presentation: Lipocalin-2 expression identifies an immuno-regulatory intestinal neutrophil population during GVHD	**Marie Czech**
13:35 – 13:45	Oral presentation: Tissue-specific landscape of the endogenous and allogeneic human T cell repertoire	**Jonathan Peled**
13:45 – 17:30	**Lunch break**	
17:30 – 18:30	**Poster tour + Networking hour**	

Pavan Reddy (Texas, United States)

Pavan Reddy, M.D., is the Director of the Dan L Duncan Comprehensive Cancer at Baylor College of Medicine in Houston, Texas. In addition to this role, he is Senior Associate Dean of Cancer Programs and holds an executive physician leadership role/ and leads all Oncology Services, research, and strategic growth at Baylor St. Luke's Medical Center. A physician-scientist who does both bench and translational research, his work is focused on understanding the role of immune cells in blood diseases, cancer and transplantation. His groundbreaking research is supported by funding from the National Institutes of Health, the Leukemia and Lymphoma Society, and other foundations. Dr. Reddy was born and raised in India. He earned his medical degree from Osmania University in Hyderabad in 1994 then came to the United States where he completed a residency in internal medicine at the University of Missouri in 1998. Following the completion of fellowships in hematology/oncology and bone and marrow transplantation at the University of Michigan, he joined the U-M faculty in 2002 and began to co-direct the Bone Marrow Transplant Program and the Hematologic Malignancies Program since 2011. He served as the Chief of the Division of Hematology/Oncology and Deputy Director of the Rogel Cancer Center at University of Michigan until September 2022, when he joined Baylor College of Medicine as the Director of the Dan L Duncan Comprehensive Cancer Center in Houston, Texas. He is the author or co-author of over 250 peer-reviewed journal articles and book chapters. Dr. Reddy has received many honors, including the Jerome Conn Award from department of medicine, awards from national societies including the election to honorary societies such as American Society of Clinical Investigation (ASCI), Association of American Physicians (AAP), and American Clinical and Climatological Association (ACCA). He holds leadership roles in multiple scientific committees and served as the immediate past-President of the American Society of Transplantation and Cellular Therapy (ASTCT), scientific committees of the American Society of Hematology (ASH), NIH panels. He is the Deputy Editor of JCI Insight, and Associate Editor for the journals Hematologica, Transplantation and Cellular Therapy. In the laboratory, Dr. Reddy focuses on the immunobiology of graft-versus-host disease (GVHD), a devastating immune response that can occur after a stem cell or bone marrow transplant in which the newly transplanted donor cells attack the transplant recipient's body. Dr. Reddy hopes to translate the knowledge he gains through his research into powerful, lifesaving treatments for patients everywhere. His research has been continuously funded by the NIH with multiple grants for over 20 years. Dr. Reddy and his wife Dr. Madhulata Reddy have two sons: Sidharth and Saahith.

Bruce Blazar (United States, Minneapolis)

Dr. Bruce Blazar received a BS degree from Rensselaer Polytechnic Institute and MD degree from Albany Medical College following which he obtained clinical training in pediatrics and hematology/oncology/blood and marrow transplantation at the University of Minnesota. He is a Regents Professor and founding director of the Clinical and Translational Science Institute and the Center for Translational Medicine. He has directed preclinical basic and translational immunology and stem cell research and early phase clinical studies with particular emphasis in the blood and marrow transplantation immunobiology. His early contributions to preventing acute GVHD included preclinical modeling of commonly used immunosuppressant drugs as well as CTLA4-Ig that blocks the specific T cell immune response to host antigens and became the first U.S. Food and Drug Administration (FDA) drug for acute GVHD prophylaxis. In more current chronic GVHD studies, he discovered that donor germinal center B cells that produce anti-host antibodies can work in concert with macrophages to cause chronic GVHD. Preclinical supporting data have contributed to clinical studies of ibrutinib (BTK/ITK inhibitor), Belumosudil (Rock2 inhibitor), Ruxolitinib (Jak1/2), Bortezomib (proteosomal inhibitor), Entospletinib/Fostamatinib (pSyk), Axatilimab (anti-CSF1R mAb), and Pirfenidone (SMAD2/3) and Treg adoptive cell therapy.

Tissue-specific landscape of the endogenous and allogeneic human T cell repertoire

S. DeWolf[1], Y. Elhanati[1], K. Nichols[1], O. Lyudovyk[1], N. Waters[1], D. Ponce[1], M.A. Perales[1], S. Giralt[1], B. Greenbaum[1], M. van den Brink[1,1], J. Peled[1]
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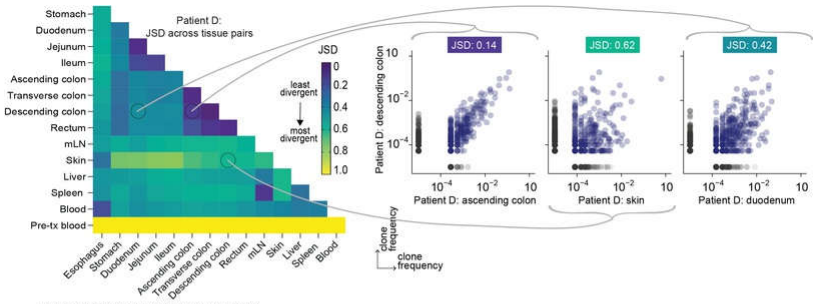
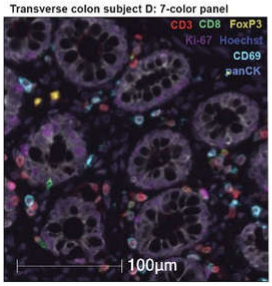
Topic: GVHD
Title: Tissue-specific landscape of the endogenous and allogeneic human T cell repertoire
Short title: TCR repertoire in GVHD autopsies

Background: Investigating T cell immunity in tissues is critical for understanding the immune responses at the site of pathology, such as in graft-versus-host disease (GVHD). While studies of peripheral blood have enabled detailed characterization of circulating lymphocytes following allogeneic hematopoietic cell transplantation (allo-HCT), knowledge of the post-transplant T cell compartment in tissues is limited.
Methods: We investigated the tissue-specificity of the post-transplant T cell repertoires in tissues in patients with GVHD from a prospectively collected autopsy series (5-18 tissue sites per patient), as well as from mouse models of this disease. In parallel, we studied tissues from comparator patients who did not undergo transplantation.
Results: T cell receptor (TCR) sequencing revealed greater repertoire overlap between anatomically similar regions, in particular within the gastrointestinal tract (figure, upper panels), independent of transplant history in patients. Top clones were distinct across tissue sites and the TCRs identified in blood and spleen in patients and mice did not reflect the most abundant clones in the tissues. Furthermore, motif-based clustering analysis revealed shared TCR signatures across patients, particularly in similar tissues, which was also evident in mouse studies. T cells in post-transplant tissues were of donor origin by single-cell chimerism analyses and had a tissue-resident phenotype (figure, lower panel).





Conclusions: Taken together, these data underscore the importance of studying tissue-resident T cells at the site of pathology rather than in peripheral blood assays and provides new insights into the site-specificity of both the endogenous and allogeneic T cell repertoire in tissues.

Disclosures:
DMP:Evive Biotechnology, Kadmon Corporation, CareDx, and Ceramedix. SAG: Miltenyi Biotec, Takeda Pharmaceutical Co., Celgene Corp., Amgen Inc., Sanofi, Johnson and Johnson, Inc., Actinium Pharmaceuticals, Inc., and is on the Advisory Boards for: Kite Pharmaceuticals, Inc., Celgene Corp., Sanofi, Novartis, Johnson and Johnson, Inc., Amgen Inc., Takeda Pharmaceutical Co., Jazz Pharmaceuticals, Inc., Actinium Pharmaceuticals, Inc. MAP:Abbvie, Astellas, Bristol-Myers Squibb, Celgene, Equilium, Incyte, Karyopharm, Kite/Gilead, Merck, Miltenyi Biotec, MorphoSys, Novartis, Nektar Therapeutics, Omeros, OrcaBio, Takeda, and VectivBio AG, Vor Biopharma. He serves on DSMBs for Cidara Therapeutics, Medigene, Sellas Life Sciences, and Servier, NexImmune. BG:Merck, Bristol Meyers Squibb, Chugai Pharmaceuticals, Merck, Darwin Health, PMV Pharma, Rome Therapeutics. MVDB: Seres Therapeutics Notch Therapeutics, Pluto Therapeutics, Thymofox, Wolters Kluwer, WindMIL Therapeutics, Rheos Medicines, Merck, Magenta Therapeutics, Frazier Healthcare Partners, Nektar Therapeutics, Notch Therapeutics, Forty Seven Inc., Ceramedix, Lygenesis, Pluto Therapeutics, GlaskoSmithKline, Da Volterra, Vor Biopharma, Novartis (Spouse), Synthekine (Spouse), and Beigene (Spouse), Juno Therapeutics; DKMS. JUP Seres Therapeutics, DaVolterra, CSL Behring, MaaT Pharma, Postbiotics Plus Research. Memorial Sloan Kettering Cancer Center: Seres Therapeutics.

Lipocalin-2 expression identifies an immuno-regulatory intestinal neutrophil population during GVHD

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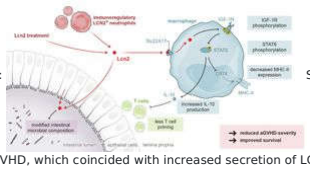
Topic: GVHD
Title: Lipocalin-2 expression identifies an immuno-regulatory intestinal neutrophil population during GVHD
Short title: Lipocalin-2 reduces acute GVHD

Background: Success of allogeneic hematopoietic cell transplantation (allo-HCT) remains limited by acute graft-versus-host disease (aGVHD). In previous studies, we demonstrated that neutrophil granulocytes enhance early aGVHD by releasing reactive oxygen species. Additionally, they can act as antigen-presenting cells and migrate to mesenteric lymph nodes, presenting antigen to donor T cells. Here, we identified a neutrophil population in the gastrointestinal tract of allo-HCT recipients that was characterized by expression of Lipocalin-2 (LCN2). LCN2 is an antimicrobial peptide that has been demonstrated to have a protective function in inflammatory bowel disease, making it an interesting target to study during aGVHD.
Methods: To characterize neutrophils infiltrating the gastrointestinal tract, we implemented single-cell RNA sequencing, leading to the definition of a new neutrophil subset. We confirmed these initial findings by using major mismatch models of allo-HCT, where mice were subjected to treatment regiments or adoptive transfer of neutrophils or primary macrophages. For mechanistic analyses, we performed 16S rRNA gene sequencing, microarray and kinase array, the latter of which were confirmed with Western blot and qPCR. Finally, we used biopsies, serum and stool samples from aGVHD patients to translate murine findings to the human system.
Results:  Single-cell RNA sequencing identified a donor-derived neutrophil population, characterized by expression of the antimicrobial peptide LCN2. We confirmed the presence of LCN2+ neutrophils during GVHD, which coincided with increased secretion of LCN2 into the intestinal lumen. Loss of hematopoietic LCN2 led to aggravated disease, as well as reduction of protective bacteria in the stool and increased infiltration of immunogenic immune cells into intestinal tissues. Interestingly, we did not only find a protective effect of LCN2-overexpressing neutrophils during aGVHD, but also found similar effects by treatment with recombinant LCN2. Treatment did also modify the immune cell composition of the GI tract, as we found less immunogenic and more tolerogenic cell types. To decipher the mechanism of this effect, we screened for expression of a LCN2 receptor (*Slc22a17*), and found high expression in macrophages. Treatment with LCN2 decreased the expression of MHC class II in macrophages and adoptive transfer of LCN2-primed tolerogenic macrophages promoted reduced disease activity. Additionally, we found increased expression of *Il10* in LCN2-treated macrophages $IL10^{-/-}$ macrophages did not respond to LCN2 treatment with decreased MHC-II expression, strengthening our hypothesis of LCN2 being IL10-dependent in a treatment setting. Indeed, loss of IL10 abrogated the protective effect of LCN2 in an *in vivo* model of aGVHD. To further delineate the effect of LCN2 on macrophages, we performed a kinase array and found insulin-like growth factor receptor 1 (IGF1R) to be activated upon LCN2 treatment. Additionally, we found that loss of epithelial LCN2 in the gastrointestinal tract aggravates GVHD, strengthening its position as protective disease modulator. Finally, we used samples from four different patient cohorts and could demonstrate increased expression of LCN2 in allo-HCT patients that developed GVHD, which correlated with expression of IL10 and serum LCN2.
Conclusions: We identified a protective LCN2-expressing neutrophil population during aGVHD, which led us to the identification of LCN2 as a potential treatment option for patients suffering from aGVHD.
Disclosures: Potential conflict of interest: RZ received honoraria from Novartis, INcyte, MNK and Sanofi outside of this study. This study was supported by the Deutsche Forschungsgemeinschaft, Germany, SFB-1479 – Project ID: 441891347 (P01 to RZ, S1 to MB, P03 to NK), Project-ID 259373024 – TRR 167 (Z01 to M.B.), Germany's Excellence Strategy – (CIBSS - EXC 2189 Project ID: 390939984), DFG individual grant 872/4-2 to R.Z., SFB1160 (Project ID 256073931 - TP B09 to R.Z. and Z02 to M.B.), CRC1453 (Project ID 431984000-S1, M.B.), the European Union: GVHDCure (ERC consolidator grant to R.Z.), the Deutsche Krebshilfe (grant number 70113473), the Jose-Carreras Leukemia Foundation (grant number DJCLS 01R/2019) (R.Z.), by the Max Planck Society (D.G.), and by the German Research Foundation (DFG) (322977937/GRK2344 MeInBio) (D.G.). We also acknowledge funding from the German Federal Ministry of Education and Research (BMBF) within the Medical Informatics Funding Scheme MIRACUM FKZ 01ZZ1801B (M B) and EkoEstMed FKZ 01ZZ2015 (G A)

A novel high-throughput method for identifying T-cell receptor: minor histocompatibility antigen interactions in mouse models of graft-vs-host disease.

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[1]University of Pittsburgh, Pittsburgh, United States

Topic: GVHD

Title: A novel high-throughput method for identifying T-cell receptor: minor histocompatibility antigen interactions in mouse models of graft-vs-host disease.

Short title: Method for identifying TCR:miHA interactions in GVHD.

Background: Graft-vs-host disease is a complication of allogeneic stem cell transplant (alloSCT) wherein donor T cells target alloantigens on healthy recipient tissues. In major-histocompatibility matched alloSCT, CD8-mediated GVHD is caused by T cell receptor (TCR) allorecognition of class-I presented minor histocompatibility antigens (miHAs). The vast majority of miHAs remain unknown between any given donor-recipient pairing. However, determining the identities of these miHAs and their cognate TCRs would prove useful towards the development of allografts that minimize GVHD yet preserve the therapeutic benefits of alloSCT. Here, we implement a high-throughput TCR cloning method, TCXpress, to screen alloreactive TCRs from a mouse model of MHC-matched alloSCT against putative miHA targets determined informatically.

Methods: 129 (H-2^b) mice, which express the immunodominant miHA H60, were lethally irradiated and reconstituted with bone marrow and $CD4^+$ and $CD8^+$ T cells from B6 (H-2^b) donors. At day +10, recipient spleens were harvested and $CD44^+PD\text{-}1^+$ donor $CD8^+$ cells were single-cell sorted according to H-2Kb:H60 tetramer (Tet^{H60}) staining into unknown miHA-reactive (Tet^{H60-}) and control H60-reactive (Tet^{H60+}) cohorts for TCR cloning using the TCXpress platform (BlueSphere Bio). Clonotyping was performed by sequencing the CDR3 region of TCRs. Putative miHAs were identified informatically by filtering non-synonymous variants between B6 and 129 reference exomes on predicted class I binding affinities. To interrogate TCR target specificity, Jurkat reporter cells, which express GFP and CD69 upon TCR activation, were transduced with selected alloreactive TCRs and reacted in a multiplexed fashion against a panel of B6WT3 antigen-presenting cells (H-2^b) transduced with tandem minigenes (TMGs) of predicted miHAs. To test TCR reactivity against hematopoietically-expressed miHAs independent of our calling strategy, Jurkat reporters were also reacted against bone-marrow derived dendritic cells (BMDCs) from control B6 and 129 mice.

Results: From 3 recipient mice, 966 alloreactive TCRs were cloned and sequenced (735 Tet^{H60-} and 231 Tet^{H60+}) corresponding to 553 unique clonotypes (437 Tet^{H60-} and 116 Tet^{H60+}). Sixty-seven percent (N=668) of TCRs sequenced had clonotype occurrences of ≥ 2, indicating clonal expansion post-alloSCT, whereas there were no repeated clonotypes among donor B6 $CD8^+$ cells pre-transplant (N=173 TCRs). A total of 62 Tet^{H60-} TCRs (47 clonotypes) and 4 Tet^{H60+} control TCRs (3 clonotypes) were selected for transduction into Jurkat reporters and screened against a limited TMG library of 158 putative B6→129 miHAs, as well as H60. Expectedly, all TCR clonotypes isolated from the Tet^{H60+} sort gate reacted against H60-expressing B6WT3 cells and 129 BMDCs. From the Tet^{H60-} TCRs, 2 (1 clonotype) were validated against another documented 129 miHA, H4, while an additional 6 were found to react against novel B6→129 miHAs. Interestingly, 3 TCRs (2 clonotypes) were autoreactive against B6 tissues (B6WT3 cells and BMDCs), but not 129 BMDCs. The identities of these miHAs and their MHC restrictions are currently being deconvolved in a validation screen.

Conclusions: Here, we demonstrate the capacity of a novel TCR cloning platform to validate informatically-predicted miHAs and their cognate TCRs in a mouse model of GVHD. This approach may be readily adapted to develop new TCR:miHA therapeutics and gain a deeper understanding of GVH responses targeting miHAs.

Disclosures: W.S. and M.S. are co-founders of BlueSphere Bio and serve on its scientific advisory board.

Tuesday, 23 May 2023

18:30 – 20:30 **Dinner session**
Chair session: Pavan Reddy
Keynote presentation: leveraging mixed hematopoietic chimerism to achieve transplantation tolerance **Megan Sykes**
Networking dinner

Pavan Reddy (Texas, United States)

Pavan Reddy, M.D., is the Director of the Dan L Duncan Comprehensive Cancer at Baylor College of Medicine in Houston, Texas. In addition to this role, he is Senior Associate Dean of Cancer Programs and holds an executive physician leadership role/ and leads all Oncology Services, research, and strategic growth at Baylor St. Luke's Medical Center. A physician-scientist who does both bench and translational research, his work is focused on understanding the role of immune cells in blood diseases, cancer and transplantation. His groundbreaking research is supported by funding from the National Institutes of Health, the Leukemia and Lymphoma Society, and other foundations. Dr. Reddy was born and raised in India. He earned his medical degree from Osmania University in Hyderabad in 1994 then came to the United States where he completed a residency in internal medicine at the University of Missouri in 1998. Following the completion of fellowships in hematology/oncology and bone and marrow transplantation at the University of Michigan, he joined the U-M faculty in 2002 and began to co-direct the Bone Marrow Transplant Program and the Hematologic Malignancies Program since 2011. He served as the Chief of the Division of Hematology/Oncology and Deputy Director of the Rogel Cancer Center at University of Michigan until September 2022, when he joined Baylor College of Medicine as the Director of the Dan L Duncan Comprehensive Cancer Center in Houston, Texas. He is the author or co-author of over 250 peer-reviewed journal articles and book chapters. Dr. Reddy has received many honors, including the Jerome Conn Award from department of medicine, awards from national societies including the election to honorary societies such as American Society of Clinical Investigation (ASCI), Association of American Physicians (AAP), and American Clinical and Climatological Association (ACCA). He holds leadership roles in multiple scientific committees and served as the immediate past-President of the American Society of Transplantation and Cellular Therapy (ASTCT), scientific committees of the American Society of Hematology (ASH), NIH panels. He is the Deputy Editor of JCI Insight, and Associate Editor for the journals Hematologica, Transplantation and Cellular Therapy. In the laboratory, Dr. Reddy focuses on the immunobiology of graft-versus-host disease (GVHD), a devastating immune response that can occur after a stem cell or bone marrow transplant in which the newly transplanted donor cells attack the transplant recipient's body. Dr. Reddy hopes to translate the knowledge he gains through his research into powerful, lifesaving treatments for patients everywhere. His research has been continuously funded by the NIH with multiple grants for over 20 years. Dr. Reddy and his wife Dr. Madhulata Reddy have two sons: Sidharth and Saahith.

Megan Sykes (New York, United States)

Dr. Sykes is the Michael J. Friedlander Professor of Medicine and Professor of Microbiology & Immunology and Surgical Sciences (in Surgery), Columbia University. She is the founding Director of the Columbia Center for Translational Immunology and serves as Director of Research for the Transplant Initiative and as Director of Bone Marrow Transplantation Research at Columbia. Dr. Sykes joined Columbia University in April, 2010 from Massachusetts General Hospital/Harvard Medical School, where she was the Harold and Ellen Danser Professor of Surgery and Professor of Medicine (Immunology) and Associate Director of the Transplantation Biology Research Center. Dr. Sykes has over 37 years' experience in transplantation biology and Type 1diabetes research, including translational research from animals to clinical trials and mechanistic studies of human transplant recipients. She is currently President of the Federation of Clinical Immunology Societies (FOCIS). Dr. Sykes received numerous honors and awards, including the Medawar Prize 2018 and is a member of the National Academy of Medicine and of the Association of American Physicians.

Wednesday, 24 May 2023

09:00 – 11:00	**Stem cell biology** **Session chair: Markus Manz**	
09:00 – 09:35	The Microbiome-IL-1 axis drives HSC inflamm-aging and clonal hematopoiesis	**Markus Manz**
09:35 – 10:10	Harnessing the microenvironmental regulation for improved outcomes of HSCT	**Simon F. Mendez**
10:10 – 10:45	Targeted Anti-CD117 Antibody-Based Conditioning in Hematopoietic Stem Cell Transplantation	**Judith Shizuru**
10:45 – 11:00	Oral presentation: Pre-transplant leukemic stem cells is more Predictive for early relapses than conventional flow MRD in AML Patients undergoing allogeneic stem cell transplantation in 1st CR	**Radwan Massoud**
11:00 – 11:30	**Coffee break**	

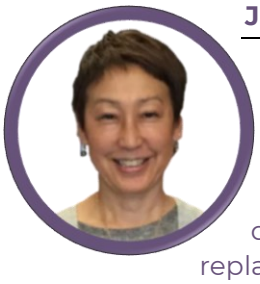
Markus Manz (Zurich, Switzerland)

M.G. Manz studied medicine, obtained his doctoral degree and completed his medical training in internal medicine, hematology and oncology at the University of Tubingen in Germany. He conducted postdoctoral research at Stanford University Medical School, and subsequently established his own research group at the Institute for Research in Biomedicine in Bellinzona, Switzerland. In parallel, he worked as an attending physician at the Oncology Institute of Southern Switzerland. Since 2009, M.G. Manz is Professor of Hematology at the Medical Faculty of the University of Zurich and director of the division of hematology at the University Hospital Zurich. In 2017 he became director of the department for Medical Oncology and Hematology at the University Hospital of Zurich. He also chairs the Leukemia-, Lymhoma-, and Myeloma-Center. Since 2020 he is chair of the Comprehensive Cancer Center Zurich. Currently he is also acting president of the Swiss Society of Hematology. His clinically and laboratory research focuses on healthy hematopoiesis in the context of aging and inflammation, as well as on hematopoietic malignancies and the improvement of respective treatment options, with a special focus on novel immunotherapeutic approaches. M.G. Manz received several prestigious research awards, and he advises on national and international organizations for the promotion of cancer research.

Simon F. Mendez (Cambridge, United Kingdom)

My multidisciplinary training in neuroscience, physiology and stem cells has allowed me to develop an interphase research field. I gained my PhD in 2004 from The Department of Medical Physiology at the University of Seville in Spain. My Thesis characterised properties of the carotid body of potential interest for neuroregenerative strategies, which were translated into clinical studies in Parkinson's disease. From 2004-9, I worked as a post-doc at New York Medical College and Mount Sinai School of Medicine, where I trained in stem cells in the cardiovascular and haematopoietic systems and discovered a connection between the bone marrow, the brain and other systemic signals, which regulate the behaviour of blood stem cells and immune cells. I found that the sympathetic nervous system regulates daily oscillations in stem cells, with could impact the yields available for regenerative medicine. These findings contributed to the emerging fields of neuroimmunology and inter-organ communication. My subsequent work identified self-renewing mesenchymal stem cells that relay signals from the nervous system and have a crucial role in the haematopoietic stem cell niche. I moved to Cambridge in 2015 as Reader at the Department of Hematology (University of Cambridge), Group Leader at the Wellcome-MRC Cambridge Stem Cell Institute and PI at NHS Blood and Transplant. Blood stem cells reside in specialised

niches, which allows them to self-renew, proliferate, differentiate and migrate according to the organism's requirements. My research has revealed multisystem regulatory mechanisms by which the haematopoietic stem cell niche fulfils these complex functions and how the deregulation of these mechanisms contributes to haematological disorders. This work has been recognised with numerous invited presentations, international awards (ASH Scholar Award, Joanne Levy Memorial Award, HHMI International Early Career Scientist, ERC consolidator grant, CRUK Programme Foundation Award), international publications in reference journals and 3 patent applications. My translational research led to two Phase-II multicenter clinical studies testing the redeployment of drugs to modulate the bone marrow stem-cell niche in myeloproliferative neoplasms. Ongoing efforts in my lab are targeting the microenvironment to improve bone marrow transplantation procedures and as a complementary therapeutic target for the treatment of myeloid malignancies.



Judith Shizuru (Stanford, United States)

Dr. Shizuru is a Professor of Medicine and of Pediatrics at Stanford Medicine, where she is member of the Blood and Marrow Transplantation faculty, the Stanford Immunology Program and the Institute of Stem Cell Biology and Regenerative Medicine. Her current clinical efforts and basic research focus on improving the safety and efficacy of hematopoietic cell transplantation (HCT). Her research lab has developed novel ways to achieve engraftment of blood forming stem cells, aiming to replace chemotherapy and radiation, and developed the tools and methods that will enable engrafting of pure blood forming stem cells, with the goal to eliminate potentially harmful passenger cells contained in a blood stem cell graft.

Pre-transplant leukemic stem cells is more predictive for early relapses than conventional flow MRD in AML patients undergoing allogeneic stem cell transplantation in 1st CR

R. Massoud[1], *E. Klyuchnikov*[1], *A. Badbaran*[1], *P. Freiberger*[1], *C. Wolschke*[1], *D. Janson*[1], *F. Ayuk*[1], *U. Bacher*[1], *N. Kröger*[1]
[1]Department for Stem Cell Transplantation, University Cancer Center Hamburg-Eppendorf, Hamburg, Germany

Topic: Stem cell biology

Title: Pre-transplant leukemic stem cells is more predictive for early relapses than conventional flow MRD in AML patients undergoing allogeneic stem cell transplantation in 1st CR

Short title: leukemic stem cells is more predictive for early relapses than conventional flow MRD

Background: Several studies have shown that the leukemic stem cells (LSCs: $CD34^+CD38^-CD45RA^+$/Combi-6^+) plays a crucial role in the development of relapses in AML patients (pts). LSCs are less sensitive to conventional therapy; moreover their phenotypical characteristics may allow immune evasion promoting relapses after allo-SCT. Recent ELN guidelines recommend to include the LSCs into MRD assessment, but this approach has not yet been using routinely. In this study we investigated the impact of pre-transplant LSCs assessment on post-transplant outcomes in AML patients and correlated it with conventional flow-MRD.

Methods: 52 pts (male, n=26; median, 60.5 years, 20-74) with AML in CR and available pre-transplant MRD data (multicolored flow cytometry, “different from normal” approach, according to ELN guidelines), who received allografts (matched, n=45; mismatched, n=7) during 2019-2022 years at the Department of Stem Cell Transplantation University Medical Centre Hamburg were included. The MRD assessment in all patients included pre-transplant LSCs detection (Zeijlemaker et al. 2019). Both, MRD method and LSCs detection were investigated solely and combined for their prognostic impact.

Results: Majority of pts had de novo AML (73%), normal cytogenetics (65%), intermediate ELN risk (67%), received matched grafts (related, n=14, 27%; unrelated, n=31, 60%) and MAC regimen (n=33, 63%). ATG was GvHD prophylaxis in 43 pts (83%). Fifteen pts (29%) received venetoclax-based therapy immediately pre-SCT. There were 22 MRD- (42%) and 30 MRD+ (58%) pts.

LSCs were detected in 29 (56%) pts (21 (70%) MRD+ and eight (36%) MRD-, p=0.016). The median proportion of LSCs were 0.01% (0.005-0.37%) of WBC. There was no correlation between LSCs proportion and MRD status. We observed higher rate of LSCs negativity among pts who received venetoclax-based therapy (5/15, 33% vs 24/37, 65%, p=0.039). Pts with iCR tended to have higher levels of LSCs comparing to those in CR (7/11, 64% vs 5/18, 28%, p=0.065) as well as pts with abnormal cytogenetics at diagnosis (7/11, 64% vs 5/17, 29%, p=0.081).

The 1-year OS and LFS were significantly higher in LSCs negative patients: 100% vs 75% (55-88%), p=0.021; and 92% (64-99%) vs 41% (21-64%), p=0.001, due to a higher relapse rate at 1-year: 42% (22-65%) vs 8% (1-36%), p=0.02. The median time to relapse was 165 days (40-395). The difference in NRM was not significant. The area under the ROC curve for relapses was 0.75 (0.62-0.89, p=0.005).

During the median follow up of 9 months (2-22), six of 21 (29%) MRD+LSCs+ pts developed relapse and four (19%) an NRM event; all MRD+/LSCs- pts (n=9) survived without relapses; three of eight (38%) MRD-LSCs+ pts developed relapses; one of 14 (7%) MRD-LSCs+ developed relapse.

Conclusions: Pre-transplant detection of LSCs has a stronger predictive value for early post-transplant relapses in AML patients than conventional flow MRD.

Disclosures: Nothing to disclose

Wednesday, 24 May 2023

11:30 – 13:30	**Cell engineering** **Session chair: Claire Roddie**	
11:30 – 12:05	CAR T-cells for brain tumours	**Crystal Mackall**
12:05 – 12:40	The UCL CAR-T programme: Better Targets, Better Targeting	**Claire Roddie**
12:40 – 13:15	Modulating T-cell memory and tumour glycosylation to improve CAR-T cell therapy	**Monica Casucci**
13:15 – 13:30	Oral presentation: Multiple Myeloma with reduction or loss of BCMA expression can be effectively controlled using novel BCMA and CD229 Dual-Targeted CAR T Cells	**Oriol Cardús**
13:30 – 17:30	**Lunch break**	
17:30 – 18:30	**Meet-the-professor**	

Claire Roddie (London, United Kingdom)

Claire is a Consultant Haematologist at UCLH and Associate Professor in Haematology at UCL with a particular interest in adoptive cell therapies. She completed an Immunotherapy PhD at UCL with Karl Peggs and subsequently undertook a clinician scientist role with Martin Pule to develop the UCL CAR-T program. Claire's current role involves pre-clinical development of novel cell therapy projects, GMP manufacture and clinical trial design. She is also responsible for the advanced therapies clinical service at UCLH.

Crystal Mackall (Stanford, United States)

Crystal Mackall is the Ernest and Amelia Gallo Family Professor of Pediatrics and Medicine at Stanford University. She serves as Founding Director of the Stanford Center for Cancer Cell Therapy, Associate Director of Stanford Cancer Institute and Director of the Parker Institute for Cancer Immunotherapy at Stanford. She leads an internationally recognized translational research program with a major focus on engineered cell therapies and children's cancers. Her work has advanced understanding of fundamental immunology and has translated this understanding for the treatment of human disease. She serves in numerous national leadership positions, including co-Leader of the NCI U54 Pediatric Immunotherapy Discovery and Development Network, and is the recipient of numerous awards, including the Richard V Smalley Award from the Society for Immunotherapy of Cancer and the AACR-St. Baldrick's Award for Outstanding Achievement in Pediatric Cancer Research. She is a member of the National Academy of Medicine and is a Fellow of the American Association for Cancer Research and is Board Certified in Pediatrics, Pediatric Hematology-Oncology and Internal Medicine.

Monica Casucci (Milan, Italy)

Dr. Monica Casucci is an active researcher in the field of CAR-T cell therapy of cancer. Biotechnologist by training, she obtained a PhD in cellular and molecular biology in 2012 (Open University, London/ San Raffaele University, Milan). In 2010, she visited Gianpietro Dotti's laboratory at the Baylor College of Medicine in Houston to learn about this innovative technology. During her PhD and postdoc, she developed a new CAR-based strategy for tackling different types of tumors and participated to the development of the first mouse model recapitulating cytokine release syndrome/neurotoxicity. She is currently leading the Innovative Immunotherapies Unit at the IRCCS San Raffaele Scientific Institute (Milan, Italy). She is crucially involved in several Italian and European Consortia aimed at promoting the bench-to-bedside translation of CAR-T cell therapies in Europe and this year she was awarded with an ERC-Starting grant. Main activities of her lab include development of strategies to boost efficacy against solid tumors through glycosylation inhibition, development of novel CAR specificities, improvement of CAR-T cell fitness, and exploitation of advanced mouse models to study CAR-related toxicities.

Wednesday, 24 May 2023

18:30 – 20:30 **Dinner session**
Session chair: Crystal Mackall
Keynote presentation: Genetic engineering of tumour-associated macrophages to reprogram the microenvironment towards development of anti-cancer immunity **Luigi Naldini**
Networking dinner

Crystal Mackall (Stanford, United States)

Crystal Mackall is the Ernest and Amelia Gallo Family Professor of Pediatrics and Medicine at Stanford University. She serves as Founding Director of the Stanford Center for Cancer Cell Therapy, Associate Director of Stanford Cancer Institute and Director of the Parker Institute for Cancer Immunotherapy at Stanford. She leads an internationally recognized translational research program with a major focus on engineered cell therapies and children's cancers. Her work has advanced understanding of fundamental immunology and has translated this understanding for the treatment of human disease. She serves in numerous national leadership positions, including co-Leader of the NCI U54 Pediatric Immunotherapy Discovery and Development Network, and is the recipient of numerous awards, including the Richard V Smalley Award from the Society for Immunotherapy of Cancer and the AACR-St. Baldrick's Award for Outstanding Achievement in Pediatric Cancer Research. She is a member of the National Academy of Medicine and is a Fellow of the American Association for Cancer Research and is Board Certified in Pediatrics, Pediatric Hematology-Oncology and Internal Medicine.

Multiple Myeloma with reduction or loss of BCMA expression can be effectively controlled using novel BCMA and CD229 Dual-Targeted CAR T Cells

O. Cardús Granell[1], L.G. Rodríguez Lobato[1,2], J. Mañé Pujol[1], A. M Battram[1], L. Perez-Amill[1], H. Calderón[1], B. Martín-Antonio[3], A. Oliver-Caldés[1,2], E. Lozano[1], D. F. Moreno[1,2], V. Ortiz-Maldonado[1,2], M. Queralt-Salas[1,2], A. de Daniel i Bisbe[2], N. Tovar[1,2], R. Jiménez[1,2], M.T. Cibeira[1,2], L. Rosiñol[1,2], J. Bladé[1,2], M. Juan[1,2], Á. Urbano-Ispizua[1,2], P. Engel[1,4], C. Fernández de Larrea[1,2]

[1]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, [2]Hospital Clinic de Barcelona, Barcelona, Spain, [3]Instituto de Investigación Sanitaria, Fundación Jiménez Díaz, Madrid, Spain, [4]Faculty of Medicine and Medical Sciences, University of Barcelona, Barcelona, Spain

Topic: Cell engineering

Title: **Multiple Myeloma with reduction or loss of BCMA expression can be effectively controlled using novel BCMA and CD229 Dual-Targeted CAR T Cells**

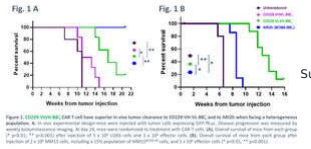
Short title: Bicistronic CAR-T cells to control Multiple Myeloma with BCMA reduction

Background: Despite showing promising results in relapsed/refractory multiple myeloma (RRMM), patients treated with Chimeric Antigen Receptor (CAR) T cells against BCMA still relapse. MM cells with negative/low BCMA expression can appear after the immune pressure exerted by CARTs and are implicated as a reservoir preceding relapse. Several approaches are currently being explored to overcome these relapses, such as manufacturing CARTs against two antigens simultaneously or targeting novel myeloma antigens. We have developed an academic BCMA-BBz CART product (ARI2h; NCT04309981) for RRMM patients with encouraging results. Also, we have developed antibodies against CD229, which is homogenously expressed on MM cells, is essential for the MM cell survival and could be expressed on myeloma precursor cells, which are believed to be responsible for relapses. Our objective is to develop a monospecific CD229 CART cell product with the subsequent generation of a bicistronic BCMA/CD229 CART cell to avoid relapses due to reduction or loss of BCMA.

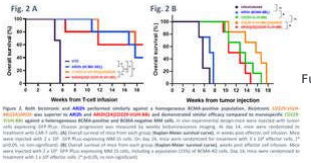
Methods: -

Results: Two monoclonal antibodies against the first and second domain of CD229 were obtained from hybridoma clones. Afterwards, two different CAR receptors were created with each antibody using the heavy and light variable regions to obtain single chain variable domains. The two CARs targeting the 1st domain were not expressed on the cell surface, so they had to be discarded. We compared our two remaining CD229 CART cell products (CD229-VHVL-BBz and CD229-VLVH-BBz) with ARI2h (BCMA-BBz).

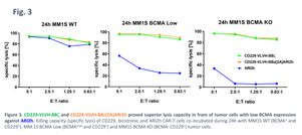
We confirmed tumor cell lysis, cytokine secretion and proliferation in response to myeloma cell lines that expressed BCMA $^{+}$ and CD229^{+} on both CD229 CARTs. A xenograft model with NSG mice and U266^{wt} cells was used to verify the *in vivo* activity of the CARs. A better tumor control and survival advantage using CD229-VLVH-BBz instead of CD229-VHVL-BBz was observed. To recreate the presence of a heterogeneous disease, a mix of MM1S^{wt} cells with 15% MM1S BCMA CRISPR KO (MM1S^{BCMAKO}) cells was administered to the mice in a subsequent experiment where the CD229-VLVH-BBz group showed a better survival compared to ARI2h (Fig. 1).



Subsequently, two dual-targeted bicistronic CAR constructs against BCMA and CD229 were developed (CD229-VLVH-BBz[2A]ARI2h and ARI2h[2A]CD229-VLVH-BBz). Both CARs expression was confirmed by flow cytometry, as well as their capacity to proliferate, secrete cytokines and lyse the tumor cells in response to myeloma cell lines that expressed only BCMA, only CD229 or both antigens. Two xenograph mouse models were analyzed, one with a homogeneous population (MM1S^{wt}) where both bicistronic CARTs performed similar to ARI2h, and another with heterogeneous population (MM1S ^{wt} 85% + MM1S$^{BCMA\ KO}$ 15%) where CD229-VLVH-BBz[2A]ARI2h showed superior tumor contro and survival than ARI2h and ARI2h[2A]CD229-VLVH-BBz) (Fig. 2).



Furthermore, in response to a low BCMA expression line (MM1S $^{BCMA\ Low}$), both bicistronics proved to have superior tumor cell lysis capacity against ARI2h (Fig. 3).



Conclusions: This is the first bispecific BCMA/CD229 CART cell that adequately controls BCMA-expressing and non-expressing myeloma cells. This approach could also mitigate escape due to downregulation of BCMA expression or biallelic loss of BCMA.

Disclosures: Luis Gerardo Rodríguez Lobato declares speaker honoraria and travel grants from Janssen and Amgen.
Carlos Fernández de Larrea declares advisory boards: Janssen, BMS, Amgen, Pfizer, Sanofi; honoraria: Amgen, Janssen, BMS, GSK, Sanofi; grants: BMS, Janssen, Amgen
The remaining authors have no conflicts of interest to declare

Thursday, 25 May 2023

09:00 – 11:00	**Anti-leukemic Strategies and NK Cells I** **Session chair: Nicolaus Kröger**	
09:00 – 09:30	Factors determining the transition from clonal hematopoiesis to myeloproliferative neoplasms and progression to myelofibrosis	**Radek Skoda**
09:30 – 10:00	Off-The-Shelf Targeted NK Cell Therapies to Treat Leukemia	**Jeffrey Miller**
10:00 – 10:30	Effect of pre-leukemic mutations on hematopoietic stem cell and their progenies	**Dominique Bonnet**
10:30 – 11:00	TCR-T cell therapy for leukemia post-transplantation	**Marie Bleakley**
11:00 – 11:30	**Coffee break**	

Nicolaus Kröger (Hamburg, Germany)

Dr. Nicolaus Kröger is Professor of Medicine and Medical Director of the Department of Stem Cell Transplantation at the University Medical Center Hamburg-Eppendorf, Germany. Prof. Kröger is board certified in Hematology-Oncology and Internal Medicine. From 2018 to 2022 he was President of the European Society of Blood and Marrow Transplantation (EBMT) and from 2012 to 2018 Chairman of the Chronic Malignancy Working Party of EBMT and from 2014 to 2018 Scientific Council Chair of EBMT. He served also as chairman of the German Stem Cell Working Group (DAG-KBT) from 2014-2020 and is member of several editorial boards such as Blood, Haematologica, Bone Marrow Transplantation and Biology of Blood and Marrow Transplantation. He is Co-Editor of the EBMT Handbook and the EBMT/EHA CAR-T Cell Handbook and also member of numerous Scientific Committees such as ASH, EHA, and ESH. He has received several awards for his work to date including the prestigious EBMT van Bekkum Award in 2015. In 2020 he was awarded as Doctor Honoris Causa from the University Belgrade. Prof. Kröger has published extensively in his area of expertise and has contributed to more than 800 publications in peer-reviewed journals such as NEJM, Lancet, JCO, JNCI, PNAS, Blood, and Leukemia.

Radek Skoda (Basel, Switzerland)

Radek Skoda, M.D., is Professor of Molecular Medicine at the Department of Biomedicine, University Hospital Basel and University of Basel in Basel, Switzerland. His research is focused on the molecular pathogenesis of myeloproliferative neoplasms (MPN), to which he contributed by describing the recurrent somatic JAK2-V617F mutation in patients with MPN, dissecting the factors that contribute to the clonal evolution of MPN and discovering mutations in the THPO gene in familial thrombocythemia and EPO gene in familial erythrocytosis. His current research is focused on defining the factors that determine the early expansion of the MPN clone and risk factors for disease progression to myelofibrosis and acute leukemia. He received several prizes and awards including the Ham-Wasserman Lecture Award of the American Society of Hematology in 2007, and the David Grimwade Award of the European Hematology Association 2020. In 2013 he has been elected to the Swiss Academy of Medical Sciences. He is a member of ASH and EHA, and participates in the EHA Scientific Working Group on MPN.

Jeffrey Miller (Minneapolis, United States)

Jeffrey S. Miller, MD, received a Bachelor of Science degree from Northwestern University in Evanston, Illinois, and received his MD from Northwestern University School of Medicine. He completed an internship and residency in Internal Medicine at the University of Iowa in Iowa City. After completing a post-doctoral fellowship in Hematology, Oncology and Transplantation at the University of Minnesota, he joined the faculty in 1991. Dr. Miller is currently a Professor of Medicine at the University of

Minnesota. He is the Deputy Director of the University of Minnesota Masonic Comprehensive Cancer Center. He has more than 25 years of experience studying the biology of NK cells and other immune effector cells and their use in clinical immunotherapy with over 300 peer-reviewed publications. He is a member of numerous societies such as the American Society of Hematology, the American Association of Immunologists, a member of the American Society of Clinical Investigation since 1999. He serves on the editorial board for the Journal of Immunology and Transplantation and Cellular Therapy and is a reviewer for a number of journals and NIH grants. He was the recent recipient of the National Cancer Institute Outstanding Investigator Award for 2015.

Dominique Bonnet (London, United Kingdom)

Dominique Bonnet obtained her PhD degree at University of Paris VII. She then joined the group of Prof. John Dick's laboratory in Toronto, Canada for her post-doctoral training there. Four years later, she accepted a position as Group Leader at the Coriell Institute for Medical Research, in New Jersey and became Assistant Professor, University of Medicine and Dentistry of New Jersey. In 2001, she moved to the Cancer Research UK, London Research Institute where she became a Senior Group Leader in 2006. Since August 2012, she is also Professor, at the University College of London, division of Biosciences, and a Senior Lecturer at the Institute of Child Health. In 2016, her group move to the new Francis Crick Institute. Her group is investigating the molecular program that regulate human normal blood stem cells and how oncogenic events impede the normal development both directly and via the stem cell microenvironment. More recently, she developed humanised niche model to further study the interaction of human HSC/LSC with the BM niche.

Marie Bleakley (Seattle, United States)

Dr. Bleakley is a transplantation and cellular therapy physician scientist. She completed medical school, residency and a fellowship in pediatric oncology and BMT in Australia before moving to Fred Hutchinson Cancer Center where she completed a second fellowship and research training in Dr. Stan Riddell's lab. Since 2011 Dr. Bleakley has led her own lab and clinical research team, which focuses on the development and evaluation of T cell immunotherapy and graft engineering for hematologic disorders and pediatric cancers. In collaboration with Dr. Warren Shlomchik, Dr. Bleakley developed strategies for engineering stem cell grafts to reduce GVHD after allogeneic hematopoietic stem cell transplantation (HCT) and completed three single-arm clinical trials to evaluate the depletion of naïve T cells from peripheral blood stem cell grafts, observing very low rates of chronic GVHD and overall favorable outcomes (JCO 2022 PMID 35007144). She has now initiated two randomized controlled trials of TN-depleted PBSC HCT. Although HCT reduces relapse of acute leukemia compared to chemotherapy alone, relapse after HCT still occurs in approximately 30% of HCT recipients and is generally fatal. This means that the graft-versus-leukemia (GVL) effect mediated by donor T cells transferred to recipients in stem cell grafts is frequently inadequate and that a better understanding of GVL and strategies for augmenting it are required. T cells targeting leukemia-associated minor histocompatibility (H) antigens to prevent or treat relapse are being developed and evaluated in clinical trials, such as HA-1 TCR T cell therapy developed by Dr. Bleakley's team (PMID: 29051183). Dr. Bleakley will discuss TCR-T cell therapy for the management of post-HCT relapse and share some preliminary data from their phase 1 clinical trial of HA-1 TCR T cell therapy for the treatment of post-HCT relapse (NCT03326921).

Thursday, 25 May 2023

11:30 – 12:30	**Anti-leukemic Strategies and NK Cells II** **Session chair: Harry Dolstra**	
11:30 – 12:00	Training NK Cells for Enhanced Leukemia Attack	**Todd Fehniger**
12:00 – 12:30	Editing of T and HASC for Improved CART Function	**John DiPersio**
12:30 – 12:40	Case presentation: Identification & Characterisation of Alloreactive Antigens and Cognate T Cell Responses in Acute Myeloid Leukemia	**Gerda Mickute**
12:40 – 12:45	**Closing remarks**	

Todd Fehniger (Saint Louis, United States)

Todd A. Fehniger, MD/PhD is a Professor of Medicine at Washington University School of Medicine in St. Louis. As a physician-scientist, his laboratory program focuses on mechanisms of natural killer (NK) cells development and function, and translating basic findings into novel NK cell immunotherapies. He serves as the Director of the Biological Therapy Shared Resource at the Siteman Cancer Center, and Laboratory Director of the Center for Gene and Cellular Immunotherapy.

John DiPersio (Saint Louis, United States)

Dr. John F. DiPersio, Professor of Medicine and Pathology & Immunology, Director, Center for Gene and Cellular Immunotherapy at Washington University School of Medicine in St. Louis and the Virginia E. and Samuel J. Golman Professor of Medicine. His research focuses on mechanistic and translational aspects of leukemia and stem cell biology. He has played a key role in the clinical development of plerixafor as a mobilizing agent for stem cell transplantation. His recent studies have focused on the development of novel methods of targeting the hematopoietic niche through the development of highly active small molecule inhibitors of CXCR4 and VLA-4, and agonists of CXCR2, for both stem cell mobilization and chemosensitization. He was the first to implicate the role of JAK1/2 signaling in GvHD pathogenesis which led to FDA approval of Ruxolitinib for the treatment of steroid refractory acute GvHD. His recent studies have uncovered the mechanisms by which JAK inhibitors alter T cell biology, and have led to the identification of 'best-in-class" JAK inhibitors for the prevention and treatment of GvHD in humans. DiPersio has played a key leadership role in the team-science work at Washington University that has defined the genetic and epigenetic factors that contribute to clonal evolution and relapse in AML. His group was the first to use whole genome sequencing to define clonal evolution at relapse resulting from the expansion of very small genetically defined AML subclones. Recently, this group showed that epigenetic downregulation of HLA Class II antigens on AML blasts is associated with immune escape, often leading to relapse after allogeneic transplantation. Together, these studies have changed our understanding of AML relapse after chemotherapy and/or transplantation. His group has recently developed a novel conditioning regimen for successfully engrafting donor cells across major allogeneic barriers, using chemotherapy- and radiation-free conditioning regimens which may significantly influence how patients are prepared for gene therapy for inherited diseases, such as sickle cell anemia. Finally, his lab has developed the first off-the-shelf, fratricide-resistant CAR-T cells for the treatment of patients with relapsed CD7+ T-ALL and have found ways to enhance the expansion, persistence and anti-tumor efficacy of CAR-T cells for multiple cancers using analogues of IL-7 and IL-15. DiPersio is an internationally recognized leader in hematopoietic stem cell transplantation and acute leukemia. He has served in leadership roles for the American Society of Hematology (ASH), multiple NIH, CIRM, LLS, and CPRIT Study Sections, and has served on the Board of

Scientific Counselors. He is an elected member of ASCI and AAP, and past president of the American Society of Transplantation and Cellular Therapy (2019). He has received the AACR Joseph H. Burchenal Memorial Award for Outstanding Achievement in Clinical Cancer Research in 2014, the ASH Mentor Award for Clinical Investigation in 2014, the 2022 American Italian Cancer Foundation Prize for Scientific Excellence in Medicine, 2022 American College of Physicians Harriet P. Dustan Award for Science as Related to Medicine and an NCI R35 Outstanding Investigator Award in 2017. His work has resulted in more than 450 publications, more than 20 patents, and the co-founding of two companies (Magenta Therapeutics, Cambridge MA and WUGEN, St Louis MO). Dr. DiPersio was the Chief of the Division of Oncology and Deputy Director of the NCI-CCC Siteman Cancer Center at Washington University School of Medicine from 1994-2022.

Identification & Characterisation of Alloreactive Antigens and Cognate T Cell Responses in Acute Myeloid Leukemia

G. Mickute[1], *C. Sweeney*[2], *M.C. Barbanti*[1], *B. Tseu*[1], *K. Gupta*[1], *M. Metzner*[1], *B. Usukhbayar*[1], *R. Chakraverty*[1], *P. Borrow*[3], *P. Vyas*[1]
[1]Weatherall Institute of Molecular Medicine, Oxford, United Kingdom, [2]Department of Haematology, Cancer and Haematology Centre, Oxford, United Kingdom, [3]Nuffield Department of Medicine, Oxford, United Kingdom

Topic: Anti-leukemic strategies

Title: Identification & Characterisation of Alloreactive Antigens and Cognate T Cell Responses in Acute Myeloid Leukemia

Short title: Harnessing alloreactive T cell responses in Graft-versus-Leukaemia

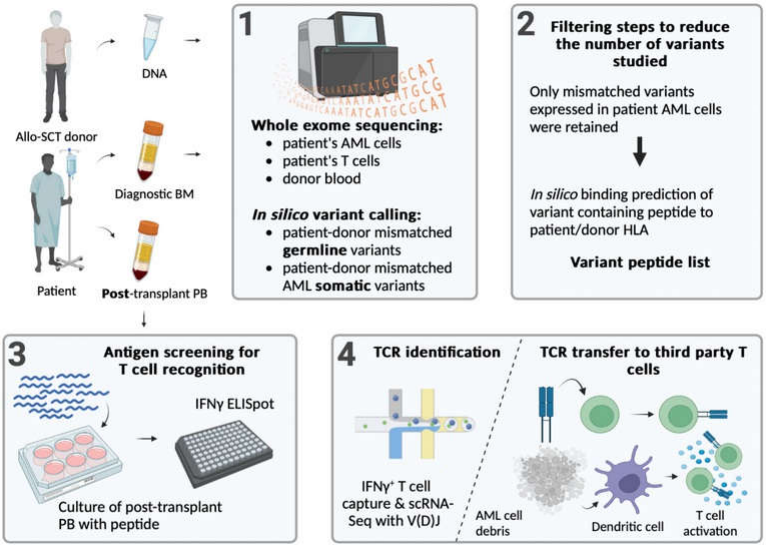
Background: Allogeneic stem cell transplantation (allo-SCT) is the most established, curative T cell cancer immunotherapy. The curative effect of allo-SCT relies on a graft-versus-leukaemia (GvL) effect; but this can be difficult to dissociate from graft-versus-host disease (GvHD). For decades, the key unsolved challenge has been to identify common alloreactive peptide-human leukocyte antigen (HLA) complexes targeted by T cells that mediate GvL but not GvHD.

Methods: We developed a workflow (Figure 1) for identification of alloreactive antigens, analysis of alloreactive T cells and their T cell receptors (TCR). We studied bone marrow and peripheral blood samples, collected over 15 years, from 12 high-risk acute myeloid leukaemia (AML) patients who remain in continuous complete remission following allo-SCT (>2 years). All patients had relapsed at least once pre-allo-SCT and/or were AML flow cytometry minimal residual disease positive pre-allo-SCT. Seven had received donor lymphocyte infusions. No patients developed >1 grade GvHD.

Results: Whole exome sequencing of patient AML, healthy cells and matched donor samples allowed the identification of somatic and germline protein-encoding mismatched variants. 98.7% of the mismatched variants were germline. To determine likely GvL antigens, we applied two sequential filtering steps: (i) mismatched variants were restricted to those expressed in patient AML by RNA-Seq, and (ii) only variants with high affinity HLA-I or HLA-II binding prediction were retained (NetMHCpan 4.0 and NetMHCIIpan 3.0).

We screened for alloreactive T cell responses to the filtered list of peptides (n=5300) using patient peripheral blood collected 2-7 years after allo-SCT. Using IFNγ ELISpot, we identified responses to 22 germline variants, whose allele frequency varies from 0.5% to 72% in the Caucasian population. HLA restriction was determined using IFNγ ELISpot with HLA antibody blockade, and the T cells were phenotyped with flow cytometry-based intracellular cytokine staining. Of the 22 alloreactive peptide responses,17 were $CD4^+$ HLA-II restricted and 3 were $CD8^+$ HLA-I. Epitope-specific $CD4^+$ T cells produced Th1 cytokines (IFNγ, TNFα) and degranulated (CD107a expression) in response to peptide stimulation. Next, we performed single cell 5'RNA-Seq with VDJ enrichment of epitope-restricted $IFN\gamma^+$ T cells. It confirmed $CD4^+$ T cell cytokine-producing and cytotoxic effector potential by expression of genes encoding factors such as MIP-1α and MIP-1β, IFNγ, granzyme B and FasL.

For a single $CD4^+$ HLA-DP-restricted T cell response to a haematopoietic protein, top enriched VDJ sequences were cloned into a lentiviral-based TCR vector for production of transgenic third-party primary T cells. T cell co-culture with antigen-expressing cell lines showed TCR specificity to the germline variant as well as restriction to the presenting HLA-DP allele. Target protein expression was also found in primary AML samples and the antigen could be presented by monocyte-derived dendritic cells to induce transgenic T cell activation.



Conclusions: Our approach dissects the biology of alloreactive anti-AML responses and enables the identification of TCRs for therapeutic purposes. The high proportion of $CD4^+$ T cell responses identified here is concordant with a known evasion mechanism of HLA-II loss in AML post-allo-SCT. Further *in vitro* and *in vivo* assays to establish TCR efficacy and toxicity are in progress.

Disclosures: Nothing to declare.

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