

MEETING REPORT



CIMT 2019: report on the 17th Annual Meeting of the Association for Cancer Immunotherapy

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Introduction

The 17th Annual Meeting of the Association for Cancer Immunotherapy (CIMT), Europe's cancer immunotherapy meeting, took place in Mainz, Germany from 21 to 23 May, 2019. Recent advancements in cancer immunotherapy were discussed among more than 800 participants. This meeting report summarizes the highlights of CIMT2019.

Counteracting immune escape

Christian Ottensmeier (University of Southampton, United Kingdom) set out by emphasizing the unprecedented clinical success of cancer immunotherapy. Yet, he also stressed that the majority of patients still do not respond to immune checkpoint blockade (ICB). A big issue in predicting response remains the limited capability of predictive tools; hence, Ottensmeier and his group drive the development of valuable biomarkers. To this end, they established a pipeline for the systematic evaluation of the immune environment in tumor biopsies by histology, flow cytometry and next-generation sequencing (NGS). Using this approach, Ottensmeier found that tumor-infiltrating CD8 T cells share similar RNA expression patterns across different tumor entities, whereas patterns vary from patient to patient.¹ A dominant factor correlating with interpatient variability is the presence of CD8 T cells exhibiting a tissue-resident memory (Trm) phenotype characterized by expression of CD103, which is associated with increased T cell infiltration and predicts a better survival outcome. A profound analysis of intratumoral CD8 T cells by single-cell transcriptome analysis led to the identification of five individual clusters within the CD103⁺ subset. A distinct cluster of cells expressing TIM-3 and IL-7R aroused certain interest as it is highly enriched for genes involved in cell division and cytotoxicity, suggesting that TIM-3 and IL-7R define a particularly effective subset of Trm cells. Underpinning this hypothesis, Ottensmeier showed that TIM-3⁺ IL-7R⁻ CD103⁺ CD8 T cells are enriched in lung cancer patients responding to PD-1 blockade and the majority of clonal expansion upon response occurs in this subset.

In the second talk of the session, Rafi Ahmed (Emory Vaccine Center, USA) revealed how he and his group shed light on the ill-defined state of T cell exhaustion by characterizing T cell subpopulations that emerge during chronic viral infection or cancer. Over the course of their studies, Ahmed and coworkers identified a subset of CD8 T cells, which is unique by expression of TCF-1 and PD-1.² These CD8 T cells have stem-like features, act as “resource cells” in order to maintain T cell immunity, and are characterized by a high proliferative potential, which is unleashed during ICB. Furthermore, stem-like CD8 T cells give rise to terminally differentiated effector cells with high expression of effector molecules. Given that this cell population was identified in mouse studies, Ahmed confirmed the presence of a population with similar gene expression profile in human lung and head and neck cancers, finally proposing that chronic antigen stimulation gives rise to stem-like CD8 T cells. Following on from the first part of his talk, Ahmed elucidated that also during anti-PD-1 and IL-2 to combination therapy, stem-like CD8 T cells, but not terminally differentiated cells, are the source of the proliferative burst.³ However, the accumulation of T cells in the circulation is considerably higher compared to ICB alone, and most strikingly, the final product of differentiating stem-like cells differs in terms of transcriptome and accessible chromatin sites as shown by single-cell transcriptome analysis and ATAC sequencing, giving novel insights into the mechanism behind the synergy of PD-1 blockade and IL-2.

Josef Penninger (University of British Columbia, Canada) presented creative ways to identify and manipulate novel pathways required for T cell activation. First, he showed that Cbl-b is involved in the control of T cell responses, since Cbl-b^{-/-} mice are prone to develop autoimmunity, owing to the fact that Cbl-b deficient T cells do not require a costimulus *via* CD28 for proper activation.^{4,5} This deficiency can be exploited to generate T cell responses during insufficient conditions for T cell priming, thus Cbl-b^{-/-} mice spontaneously reject TC-1 tumors and are more resistant to UVB-induced skin cancer compared to wild-type mice.⁶ In addition to that, Penninger found that NK-cell

mediated control of metastases is improved in *Cbl-b*^{-/-} *Rag2*^{-/-} mice, making *Cbl-b* an exciting target for cancer immunotherapy.⁷ Furthermore, Penninger reported on GCH1, a hydrolase involved in the conversion of GTP into BH4 and initially found to affect pain perception. GCH1 is also upregulated by activated T cells, suggesting a multifaceted role of this gene.⁸ Looking deeper into the immunological role of the pathway, Penninger found that T cells deficient of GCH1 fail proliferation upon activation. Conversely, enforcing high GCH1 expression augments the proliferation of activated T cells. This prompted Penninger to alter the GCH1/BH4 pathway pharmacologically in both ways as means to treat autoimmunity or cancer. Treatment with sepiapterin, the product of GCH1-catalyzed conversion of GTP, markedly enhanced T cell proliferation, while the inhibition of the pathway downstream of GCH1 improved the clinical score in a mouse model of autoimmune colitis. Finally, Penninger closed his talk by confirming that the pathway plays the same role in human T cells, making it highly interesting for clinical translation.

Neoantigens and tumor evolution

Previous research provided evidence that personalized neoantigen-based cancer vaccines have the potential to cure cancers in mice as effective as ICB does⁹ and tumor-specific neoantigens recognized by CD8 T cells were the targets of cancer immunoediting.¹⁰ Besides CD8 T cells, Robert Schreiber (Washington University School of Medicine, St. Louis, US) highlighted the importance of CD4 T cells and MHC class II restricted neoantigens for progression of host-protective and cancer-specific immune responses. His group revealed elimination of T3 (an edited MCA sarcoma) sarcomas in not only CD8 but also CD4-dependent manner upon ICB, i.e. α PD-1 and α CTLA-4. As a result of analyzing of 700 nonsynonymous mutations in T3 tumor, a major MHC class I (mLama4) and class II (mItgb1) neoantigens were identified, respectively. Additionally, ectopic expression of MHC class I (mLama4), class II (mItgb1) or both neoantigens in oncogene driven KP (*Kras*^{G12D} *p53*^{-/-}) sarcoma model, which is poorly immunogenic and insensitive to ICB, in combination with α PD-1 and α CTLA-4 treatment resulted in tumor rejection only in the presence of both MHC class I and class II neoantigens. The rejection of KP tumors was shown to be dependent specifically on enforced expression of mItgb1 neoantigen but not on increased antigen load as the expression of two strong MHC class I antigens in the absence of mItgb1 revealed no tumor rejection following ICB. Thereby, his group showed the immune rejection required the expression of both MHC class I and class II epitopes within the tumor. He finalized his talk by showing data demonstrating that presence of MHC class II epitope in tumor microenvironment as well as in lymph nodes were required for effective CD8 T cell priming and maturation into CD8 cytotoxic T lymphocytes (CTLs) to facilitate tumor rejection highlighting the importance of MHC class II neoepitopes.

During the first part of his talk, George Coukos (Ludwig Institute for Cancer Research, Lausanne, Switzerland) focused on significance of tumor-infiltrating lymphocytes (TILs) in tumor islets and their impact on the progression and overall survival of ovarian cancer patients following chemotherapy.

Previous data revealed that patients with T cells in tumor islets lived longer compared to ones without infiltration of T cells.¹¹ Identification followed by TCR sequencing of tumor-associated antigen (TAA) specific TILs obtained from two different compartments, i.e. stroma and islet, via laser capture microdissection demonstrated that TAA specific T cells isolated from the tumors were mostly coming from the islets. The main theme of second part of his talk was neoepitope specific recognition of ovarian cancer which has low to medium mutational burden. His team revealed the presence of neoepitope specific CD8 T cells in most patients with ovarian cancer and recognition of a particular tumor neoepitope but not both by circulating T cells, i.e. PBLs, and TILs.¹² Even if circulating T cells were expected to have higher avidity than TILs because of their potential exhaustion, they observed higher functional avidity and higher predicted affinity of TCRs found in TILs, which might be the reason for the stronger neoepitope recognition of TILs compared to PBLs. Lastly, he introduced a whole-tumor antigen vaccination approach (OCDC) relying on dendritic cells (DCs) pulsed with oxidized autologous whole-tumor cell lysate.¹³ They found the amplification of preexisting neoepitope specific T cells upon OCDC vaccination in combination with bevacizumab and cyclophosphamide treatment as well as induction of high avidity CD8 T cells against tumor neoepitopes.

Within the tumor, one could also observe heterogeneity referred to as intratumoral heterogeneity, the presence of multiple sub-clones of tumor cells within a single tumor mass.¹⁴ This heterogeneity within the tumor might be of use to explore the evolution of the tumor as well as initiating events and their change over time. Starting from this point, Nicholas McGranahan (UCL Cancer Institute, London, United Kingdom) mentioned while some of the tumors had a relatively simpler evolutionary history, others were evolutionarily more complex both at point mutation level as well as copy number level.^{15,16} His team also showed the presence of a diversity at the immune microenvironment besides heterogeneity of the tumors at the genomic level as well as a direct correspondence between genomic and immune microenvironment similarity.¹⁷ Lung squamous cell carcinoma and lung adenocarcinoma patients with high levels of neoantigens possessed high levels of immune infiltrate within them. He emphasized as well that not total neoantigens identified but clonal neoantigens can be prognostically predictive. He spent the rest of his talk to address how tumor cells could evade the immune system, one of the major questions in cancer immunotherapy. Using loss of heterozygosity in human leukocyte antigen (LOOHLA) approach, they revealed that almost 30% of lung adenocarcinomas and 60% of lung squamous cell carcinomas experience loss of one of the HLA antigens¹⁸ which seemed to happen more frequently as a subclonal event and mainly occur in metastatic samples.¹⁹ Therefore, he supported the idea that loss of heterozygosity (LOH) may facilitate tumor evolution as it leads to the accumulation of mutations, which are no longer being presented to the immune system. He also shared data to show there is a negative selection against neoantigens for instance through copy-number loss at the DNA level.¹⁷ He proposed that grouping of tumors with low and high immune evasion might give insights for how these patients would progress.

Chemical immunology

Ferry Ossendorp (Leiden University Medical Center, Leiden, Netherlands) drew attention to TLR-ligand conjugated synthetic peptide cancer vaccines. He showed that chemically defined T cell vaccines by conjugating TLR – ligands and peptides can be a promising tool. He pointed out synthetic TLR ligands (Pam3CysSK4 (TLR2 agonist)), CpG (TLR9 agonist, Hydroxyadenine (TLR7 agonist), Lipid A (TLR 4 agonist)), which could be conjugated to tumor-specific synthetic long peptide (SLP). TLR ligand-peptide conjugates showed effective MHC I cross presentation, as well as an enhanced uptake *in vitro* and *in vivo*, preserved activity of TLR stimulation. Due to the essential importance of TLR activation for T cell priming *in vivo*, Ossendorp (in collaboration with Dmitri Filippov) improved the binding of Pam3CSK4 in the TLR pocket by synthesizing a Pam3Cys analog called UPam (trade name Amplivant®).²⁰ Amplivant improved immunogenicity and tumor control in *in vivo* models and exhibited an increased level of DC maturation as well as augmented CD8 T cell responses.²¹ In addition to that, Ferry Ossendorp also presented promising results in combination therapy with Amplivant conjugate HPV vaccines, which are tested in a phase I/II clinical trial. Such a vaccine design with HPV16 E6 peptides was well tolerated and induced strong IFN γ responses in PBMC of cervical cancer (CxCa) patients as well as T cell proliferation. Ferry Ossendorp's team is evaluating several TLR and NLR ligands as single and dual conjugates.

Lutz Nuhn (Max Planck Institute for Polymer Research, Mainz, Germany) and his team generated pH-degradable polymeric nanogels for local and systemic cancer immunotherapy. Lutz Nuhn highlighted the importance of nanogels as macromolecular therapeutics, which could be used as a toolbox for immune-pharmacologic tumor therapies. He and his team generated nanogels for targeting tumor-associated macrophages (TAMs) by binding to mannose macrophage receptor (MMR/CD206) on immunosuppressive TAMs.²² These polymeric nanogels are composed of pH degradable polymer chains and showed a lymph node focusing accumulation after subcutaneous injection.²³ Furthermore, the nanogels can acquire immunomodulatory properties by conjugation of imidazoquinolines²⁴. With this synthetic agonist for TLR7/8 signaling pathway, Lutz Nuhn and this group demonstrated immune activation in the draining lymph node in the form of tumor-specific CTLs and could achieve tumor growth inhibition. Nanogel delivery could also modulate antigen-specific T cell responses as well as promoted DCs activation.²⁵

Li Tang (Institute of Bioengineering – EPFL, Switzerland) focused his talk on the major challenge in cancer vaccine development, the vaccine delivery. He and his group developed a strategy to counteract vaccine delivery by the usage of a carrier-free nanogel delivery system, which are composed of neopeptide and adjuvant. Nanogel vaccines demonstrated a highly efficient lymph node targeting and DC internalization *in vitro* and *in vivo*. Li Tang also provided a responsive release of antigen *in vitro* and endosomal escape of antigen with the nanogel system.²⁶ He proposed the nanogel delivery system as

a versatile platform for neoantigen vaccines for clinical use thanks to facile manufacturing. The technology can be also exploited for improving adoptive T cell therapy by responsive cytokine nanogels containing human IL-15 which is in phase I clinical trials for solid tumors and hematologic cancers.

Immunoguiding

The Immunoguiding session this year looked not only at how immune cells behave in tissues (“monitoring”) but also at how to guide the cells to where we need them. Evan Newell (Fred Hutchinson Cancer Research Center, Seattle, USA), opened the session by showing us impressive data generated using CyTOF (single-cell mass spectrometry). This enables the simultaneous use of over 40 different markers on a single cell based on which heavy metal is conjugated to the antibody. Using CyTOF, Newell demonstrated how lymphocyte populations differ in various human tissues.²⁷ By combining those markers with unique heavy metal barcodes, Newell's group then focused on antigen-specific T cells. Employing data from various human tissues, he illustrated how heterogeneous the different cell populations are both within a patient as well as between different patients.²⁸ The painstaking work done by his team to analyze over 140 tumor samples exemplifies this across various tumor types as well. Using their barcoding system to detect antigen-specific cells, they could show that TILs are not only tumor specific, but that a substantial number of cancer-unrelated antigen-specific T cells are also present in tumors. These consisted mostly of cells specific for virus infections such as EBV, HCMV or Influenza. These cells often expressed CD69 & CD103, whereas tumor-specific T cells were found to robustly express CD39. CD39 as a marker for tumor-specific T cells was also recently published elsewhere.²⁹ Virus-specific T cells populate tumors and can also be exploited for immunotherapy by treating tumors with virus-specific peptides.³⁰

Moving on from single-cell mass spectrometry, Thorbald van Hall (LUMC, Leiden, the Netherlands) presented his findings on NKG2A, an inhibitory molecule on NK and T cells. Specifically, the talk started on HLA-E, a highly conserved HLA type, which presents essentially the same peptide across a wide range of mammalian species. The peptide is presented by HLA-E is part of the nascent MHC-I chain, and as such, it serves a role in the steady-state signaling: as long as MHC-I is expressed by, HLA-E presents its peptide to NKG2A receptors on CD8 T cells and inhibits T cell action. This system is highly expressed in immune privileged sites such as testis and placenta. In cancer, HLA-E expression serves as a biomarker, where high HLA-E expression correlates with poorer prognosis in renal cell carcinoma. The receptor NKG2A is overexpressed in cytolytic TILs such as CD8 T cells and NK cells. Van Hall inadvertently managed to connect back to the talk given by Newell in that he detected the strongest NKG2A expression on tissue resident effectors (CD103, and presumably CD39, expressing cells). Vaccination increased the expression of NKG2A receptors on CD8 T cells.³¹ Blocking NKG2A conversely enhances the efficacy of vaccines in tumor settings, as illustrated by the treatment of TC-1, B16, and RMA tumors. Interestingly, NK cells did not play a major role in NKG2A-blockade – the effect was mainly dependent on boosted CD8 T cell infiltration.

The last talk of the session was held by Jan Kisielow (ETH Zurich, Zurich, Switzerland) who presented a novel method to determine T cell reactivities for clinical use. Tumors mutate regularly and therefore harbor a set of peptides, neoantigens, of potential clinical interest. However, the detection of neoantigens and conversion into therapy remains difficult. Kisielow and colleagues set out to identify peptide specificities of tumor-infiltrating T cells (TILs) to guide effective therapy. Their approach is based on monitoring interactions between TCRs and peptide-MHC complexes from the side of antigen-presenting cells, using a reporter cell line carrying novel chimeric molecules, deemed MCRs (for MHC+TCR). Recognition of the peptide-presenting MCRs by antigen-specific T cells leads to a reporter signal. This allows the isolation of reporter cells carrying peptides recognized by the T cells of interest, from a library of reporter cells carrying different peptides. In practice, peptide-MCR libraries were iteratively co-cultured with T cell clones derived from tumors and activated reporter cells were sorted. After several cycles, peptides displayed by the reporter cells were identified by sequencing. Using this method to screen a whole tumor transcriptome in an unbiased manner, the team managed to find a novel tumor-specific antigen recognized by a high frequency of TILs. In addition, targets of several influenza- and LCMV-specific T cell clones, including alternative peptide ligands, were efficiently identified. The platform can also be used to screen for SNPs recognized by TILs.³² Furthermore, a systematic MCR screening enabled TCR cross-reactivity mapping and supports the idea that TCRs can recognize multiple epitopes.³³ This might allow more efficient screening for off-target reactivities of TCRs prepared for clinical use, especially if these TCRs are being mutagenized.

Tumor microenvironment

Karin de Visser (Netherlands Cancer Institute, Oncode Institute, Amsterdam, Netherlands) conceptually focused on tumor-induced systemic inflammation, investigating the role of the immune system in breast cancer metastasis formation. De Visser and her team impressively demonstrated that elevated blood neutrophil levels – associated with poor prognosis in patients – are a result of a systemic inflammatory cascade, triggered by IL-1 β production by TAMs, which activates $\gamma\delta$ T-cells to secrete IL-17, resulting in systemic, G-CSF-dependent activation and expansion of neutrophils.^{34,35} Aiming to address inter-patient heterogeneity in systemic immune parameters, de Visser's team turned to dissect the impact of the tumor-genetic make-up on systemic inflammation and metastasis formation. Analyzing mammary tumors from 16 unique genetically engineered mouse models (GEMM), elevated neutrophil levels were predominantly identified in mice bearing mammary tumors that were Trp53.^{-/-36} When culturing macrophages with conditioned media from p53^{+/+} and p53^{-/-} breast cancer cells, macrophage IL-1 β production was elevated when encountering media from p53^{-/-} cancer cells. Performing RNAseq on tumor-bearing GEMMs, de Visser and colleagues established a link between Trp53^{-/-} cancers and activated Wnt signaling. Wnt-ligand production by Trp53^{-/-} deficient cancer cells thereby activates IL-1 β production in macrophages and dictates pro-metastatic inflammation. The administration of

LGK974, a porcupine inhibitor, reduced the secretion of IL-1 β by macrophages exposed to conditioned medium from p53^{-/-} cancer cells and reduced neutrophil counts and metastasis in mice bearing p53-deficient tumors. De Visser and team established a causative link between Trp53 status and Wnt-dependent signaling in breast cancer, making a large leap toward the understanding of systemic pro-metastatic inflammation.

Sergio A. Quezada (University College London, London, United Kingdom) presented recent data from the TRACERx consortium, deciphering CD4 and CD8 T cell evolution in non-small cell lung cancer (NSCLC). In his talk, Quezada focused on the link between tumor mutational burden (TMB) and CD8 and CD4 T cell differentiation in NSCLC (unpublished data). Performing high-dimensional flow cytometry analysis, Quezada and colleagues describe 15 clusters of intratumoral CD8 and 9 clusters of intratumoral CD4 T cells in NSCLC. In the CD8 compartment, tumor mutational burden (TMB) correlated with an increase in Tdys CD8 T cells (CCR7⁻CD45RA⁻CD57⁻PD-1^{hi}), a cluster of PD-1^{hi} T_{rm} cells, exhibiting molecular features of dysfunction. An enrichment of Tdys was especially present in tumors possessing a high neoantigens load and antigen presentation defects. In the CD4 compartment, early differentiated CD4 T cells declined with TMB, whereas two distinct PD-1⁺ dysfunctional subsets increased: a checkpoint high expressing (Tdys) and CD57⁺Eomes⁺ terminally differentiated effector (TDE) population. As Quezada points out, the acquisition of dysfunctional phenotypes and loss of early differentiated CD4 population may be associated with Treg abundance although this needs validation in a larger and independent cohort. In essence, TMB seems to be linked with T cell differentiation toward a dysfunctional/exhausted T cell phenotype (high PD-1, low Tcf7) in NSCLC. Furthermore, immune evasion and regulatory T cell infiltration seem to positively correlate with the accumulation of dysfunctional CD8 and CD4 T cell “early/progenitor” pool in NSCLC patients.

Pablo Umaña (Roche, Schlieren, Switzerland) presented recent advances in developing next-generation bispecific antibodies and targeted co-stimulators to re-direct T cells for cancer immunotherapy. Umaña presented the design of CD20-TCB, a novel “2:1” T-cell engaging bispecific antibody, composed of two B-cell binding CD20 domains and a single T cell engaging CD3 domain. In a phase I study, treating relapsed/refractory B-cell non-Hodgkin Lymphoma, complete remission could be achieved with CD20-TCB showing a tolerable safety profile with obinutuzumab pre-treatment mitigating CRS-associated toxicity. Obinutuzumab pretreatment reduced on-target, systemic cytokine release of CD20-TCB, while maintaining anti-tumoral efficacy in preclinical studies. Umaña also highlighted challenges in developing an agonistic anti-4-1BB, facing Fc γ R-mediated hepatic CD8 T cell activation and thus toxicity within the liver and underlined the importance of designing new generation 4-1BBL specific antibodies in a bispecific format to overcome these limitations.

Improving immunity

According to Ignacio Melero (Clinica Universidad de Navarra, Pamplona, Spain), translational research is key for successful cancer treatment. He proposed that ICB has broad pan-tumor

potential. However, there is a need for reliable biomarkers, fitting combinatorial approaches and the next breakthrough. In this context, he showed that elevated IL-8 serum levels correlate with poor outcomes in various cancer entities after anti-PD-1 treatment.³⁷ RNA sequencing data from these patients revealed that there is a positive correlation between the expression of IL-8 and monocyte as well as neutrophil abundance and a negative correlation with T cell and IFN- γ presence. Besides being a potential biomarker, IL-8 could also be target in cancer therapy, since it furthermore induces NETosis in human neutrophils and granulocytic MDSCs.³⁸ In mice, treatment with anti-IL-8 monoclonal antibody, pertussis toxin or reparixin led to reduction of NETosis. In the following, Melero examined the potential of a combinatorial approach for checkpoint inhibitor therapy. TGF- β blockade enhances radiotherapy mediated abscopal effects in combination with anti-CD137 and anti-PD-1 monoclonal antibodies in 4T1 breast and MC38 colorectal cancer models.³⁹ He closed his talk, showing that Nivolumab and Ipilimumab treatment is efficient against advanced melanoma, but can lead to immune-related adverse events in these patients.^{40,41} As a solution, he presented a prophylactic treatment with clinically available TNF inhibitors which led to less immune-related adverse events after CTLA-4 and PD-1 monoclonal antibody treatment in human colon cancer xenograft mice, while retaining the anti-tumoral effect.⁴²

Ugur Sahin (TRON – Translational Oncology, and BioNTech SE, Mainz, Germany) opened his talk asking whether tumor antigens derived from mutations (neoantigens) or shared non-mutated tumor antigens are more suitable for the design of a therapeutic vaccine. Based on sequencing techniques, neoantigens can be easily identified by analyzing patient tumors, but only 1–2% are spontaneously immunogenic. However, this percentage could be increased by vaccination. As a vaccine, mRNA can be a strong and versatile tool.^{43,44} For an individualized neoantigen vaccine approach (IVAC mutanome), patient material is sequenced and epitopes are predicted leading to a mRNA vaccine encoding for multiple epitopes. He demonstrated that after the start of vaccination the cumulative rate of metastatic events was highly significantly reduced and resulted in a sustained progress-free survival.⁴⁵ Looking ahead, he pointed out that machine and deep learning approaches could meet the need of better neoantigen prediction.

Focusing on refractory tumor types like colorectal cancer (CRC) Dirk Jäger (National Center for Tumor Diseases, Heidelberg, Germany) asked the question which patients might respond to checkpoint inhibitor therapy. He pointed out that T cell infiltration could be a promising biomarker for survival benefit. Accordingly, it was shown that localization and density of immune cells in the invasive margin of human CRC liver metastases is prognostic for response to chemotherapy.^{46,47} An in-depth analysis of the microenvironment revealed that T cell low tumor regions showed more macrophage-related markers, in contrast to high T cell infiltrated areas, which showed more chemotactic signaling.⁴⁸ In this context, Jäger highlighted CXCL9/CXCL10 produced by myeloid cells as important factors. Furthermore, he demonstrated that CD4⁺ and CD8 T lymphocytes could have a tumor-promoting role, mediated by the CCL5 – CCR5 axis. This mechanism can be targeted in human cancer patients by blocking CCR5, which led to anti-

tumoral repolarization of macrophages.⁴⁸ Jäger closed his talk by presenting an organotypic human tumor explant model. For its generation, tumor and adjacent tissue is taken from a patient and cultivated in a bioreactor. This culture is stable, fully human and immunocompetent, which allows short-term exploiting of treatment mechanisms and resistance for different tumor entities.

Cellular therapy

This year's cellular therapy session was opened by Carl June (University of Pennsylvania, USA), who summarized the original ideas and the progress of CAR design.^{49–52} The first clinical application of a first-generation CAR was in the context of HIV, with a reported cell half-life of over 17 years. In cancer, a first-generation TAG-72 specific CAR was used, but the transferred T cells persisted only in the short term in patients due to CAR T cell rejection and receptor design.⁵³ With CD19 specific second-generation CARs, persistence has been vastly improved. June stated that 28 ζ cells persist in patients only about a month, potentially due to exhaustion and AICD, while BB ζ T cells can be found up to 8 ½ years.⁵⁴ The “living drug” expands with a doubling time of 0.78 days, a maximum at 5–10 days, before it contracts with persisting memory cells.⁵⁵ June proceeded with CD19 CAR successes in pediatric patients with r/r ALL, characterized by its poor prognosis. CAR T cells lead to 80% CR rates in patients, but responses can be accompanied by cytokine release syndrome (CRS) and high fevers, which are controlled with IL6 antagonists. Neurological toxicities are a second side effect.⁵⁶ Unpublished single-cell RNA sequencing data from mouse and human brain stroma identified CD19 transcripts in brain pericytes, a potential reason for CAR-mediated CNS toxicity. In mouse models, CD19 CAR T cells induced permeability of the blood–brain barrier, which was stronger for 28 ζ CARs. Nevertheless, June underlined the high clinical safety of modified T cells, that shorter manufacturing processes will further improve CAR T-cell responses⁵⁷ and also reduce product costs.

Michael Hudecek (University Würzburg, Germany) introduced the CAR target FLT3, which is highly and uniformly expressed on AML blasts. Mutations in its kinase domains increase blast survival, and decrease the probability of target loss. CAR *in vivo* functionality was presented, and could be increased in combination with a FLT3 inhibitor forcing target surface upregulation.⁵⁸ Another antigen, SLAMF7, is expressed on multiple myeloma and also promotes cell survival. A humanized Luc63 scFv was fused into 28 ζ and BB ζ CARs with adjusted spacers.⁵⁹ In comparison to different BCMA specific CARs, SLAMF7 CAR T-cells completely eradicated myeloma cells in the marrow of xenograft mouse models.⁶⁰ A clinical trial with a 28 ζ CAR (CARAMBA) is in preparation and will use the sleeping beauty transposase system in combination with minicircle DNA.^{61,62} Hudecek emphasized the potential to lower manufacturing costs and the high genomic safety profile of this system. As mentioned by June, IL6 blockade and immunosuppressive treatments reduce CRS. But to directly control infused CAR T cells, Hudecek and colleagues fine-tuned receptor signaling with the Lck inhibitor Dasatinib, which resulted in titratable and reversible inhibition of CAR T cell signaling and killing.⁶³ The inhibitor can put CAR T cells into an OFF-mode *in vivo*, which was released by clearance of the compound from

the body. By this means, CRS dependent toxicities were controlled in a humanized mouse model, which might be transferable also to human patients.

Hyam Levitsky (Century therapeutics, Philadelphia, USA) proposed that manipulation of cells beyond what is achievable with autologous cells could solve problems seen for the cellular therapies of solid tumors. Three challenges for autologous cell products can be identified: (i) variability in patient lymphocyte function used to make product, leading to inconsistent product quality, as illustrated when patient CAR T-cells were infused into tumor-bearing NSG mice, where T cells from responder patients out-perform non-responder T cells.⁵⁴ (ii) tumor homing, exhaustion, suppressive host factors, and hypoxia are obstacles encountered by transferred T-cells, which may be addressed via multiple gene editing steps that are not easily accomplished at the population level using autologous cells. (iii) But tools for gene editing are imprecise, and can induce genomic toxicities. A clonal, well-defined off-the-shelf product could solve this issue. For this, nonrenewable cell sources such as mature T cells from healthy donors allow quicker availability of cell products, but extensive expansion to maximize the number of doses generated from a manufacturing run induces differentiation and exhaustion, requiring iterative recreation of the therapeutic product from different donors. In contrast, in-scale renewable products like induced pluripotent stem cells (iPSCs) derived T-cells are not yet available, but are tested in the field of NK cells.⁶⁴ However, both nonrenewable donor T cell-derived allogeneic products as well as iPSCs-derived products may be targets for rejection by host versus graft reactivities. Recent preclinical evidence has demonstrated engineered resistance to immune rejection when iPSCs had MHC knocked out, while also providing “don’t eat me” signals to the host innate immune system.⁶⁵ Levitsky pointed out that besides the risk of genetic rearrangements, genetic modifications of iPSCs can also interfere with the differentiation into the final product which may require regulatable expression systems. He closed the session and argued that off-the-shelf cell products could in the future reduce costs, increase availability, quality, and consistency of cell products, while also addressing the shortcomings of current autologous cell therapies.

Keynote lecture

In his keynote lecture, Mark Davis (Stanford University, Stanford, USA) highlighted new strategies which prove that human immunology is an ideal landscape for a systems approach. In this regard, he summarizes such tools for T cell specificity and repertoire in cancer he provided evidence for de novo antigen identification of tumor-infiltrating CD8 T cells in colorectal cancer. Some of the identified TCRs shared specificity with a non-mutated self-antigen implying that the MCH-bound peptide contains enough information to predICB sequences of unrelated peptide targets and that identification of tumor antigens through unbiased screening is feasible.⁶⁶ His group also developed an algorithm called GLIPH (grouping of lymphocyte interactions by paratope hotspots) which can be used to analyze large numbers of TCR sequences and define TCR specificity groups shared by TCRs and individuals. The motifs identified by this algorithm were sufficient to ensure shard antigen

recognition among specificity groups.⁶⁷ Mark Davis also underlined the importance of longitudinal studies including twins to further assess the systems biology of the human immune system using such high throughput analysis to evaluate T cell specificity and function.

Conclusion

Wolf-Herman Fridman (Cordeliers Research Center, France) received CIMT Lifetime Achievement Award for his outstanding contribution to a deeper understanding of cancer immunology and the tumor environment. We anticipate to hear more advances from the field of cancer immunotherapy at the 18th Annual CIMT Meeting (May 5–7 2020, Mainz, Germany)

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