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MEETING REPORT

"Tumor Immunology Meets Oncology (TIMO) XIV", May 24–26th 2018, Halle/Saale, Germany

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Abbreviations

Arg1	Arginase-1
bgn	Biglycan
ccRCC	Clear cell renal cell carcinoma
CPI	Checkpoint inhibitor
EBV	Epstein Barr virus
ECM	Extracellular matrix
EGLN3	Egl nine homolog 3
FCIS	Favorable combined immune signature
FGFR3	Fibroblast growth factor receptor 3
GLUT	Glucose transporter
HNSCC	Head and neck squamous cell cancer
MCT4	Monocarboxylate transporter 4
miRNA	MicroRNA
NAD	Nicotinamide adenine dinucleotide
NB	Neuroblastoma
NGS	Next generation sequencing
OXPHOS	Oxidative phosphorylation
PHD3	Prolyl hydroxylase 3
PMN	Polymorphonuclear
RCC	Renal cell carcinoma
SB	Sleeping beauty
SLC16A3	Solute carrier family 16 member 3
SLN	Sentinel lymph node
SLRP	Small leucine-rich proteoglycan
STING	Stimulator of interferon genes
TAN	Tumor-associated neutrophil
TCGA	The Cancer Genome Atlas
TLS	Tertiary lymphoid structure
TM	Target module
TME	Tumor microenvironment
Treg	Regulatory T cell
UCIS	Unfavorable combined immune signature

This meeting report is a summary of presentations from the *Fourteenth Workshop and Symposium "Tumor Immunology meets Oncology"* (TIMO XIV) published together with a series of Focussed Research Reviews based on lectures given at the conference.

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Introduction

This article summarizes the highlights of the presentations during the XIVth "Tumor Immunology Meets Oncology" (TIMO) symposium, which was held May 24–26th 2018 at the Martin-Luther-University Halle-Wittenberg in Halle/Saale, Germany. Basic scientists, translational researchers and clinicians provided talks on topics ranged from immune escape mechanisms, immune modulation, composition of the tumor microenvironment and immune metabolism to the design and optimization of immunotherapies.

This meeting attracted again participants from more than ten nations to discuss the major advances in basic tumor immunology and immunotherapies alone or in combination with other regimes thereby building up a close link between pre-clinical and clinical research.

The complex interactions between the immune system and tumor cells led to the identification of a number of molecules that governs these interactions. One major goal of immunotherapeutic strategies is to overcome the mechanisms developed by tumors to escape immune destruction. Despite immunotherapies have currently been successfully implemented in various solid and hematologic diseases, the clinical outcome of patients has still to be improved due to response rates around 20–40% and the development of resistance during treatment. The meeting provides information on

evasion of immune surveillance of tumors with focus on genomic heterogeneity and oncogenic pathways, the role of the tumor microenvironment for tumor–host interaction, the modulation of immune responses and the development of different immunotherapeutic strategies. Since treatment of advanced or relapsed tumors remains an unmet need, a better understanding of the relevant immunologic, metabolic and neoplastic features of tumors is required. Despite different immunotherapeutic approaches have a modest response rate, their combination with chemotherapy, radiation or other therapies results in increased response rates of tumor patients. However, it is noteworthy that an immunotherapy safety profile is advantageous, since a unique inflammatory toxicity known as immune-related adverse events can develop by boosting the immune system. This often leads to the discontinuation of therapy and administration of immune suppressive reagents. With this in view, the TIMO meeting covers various topics, which are summarized and discussed in this report. These include the molecular and basic mechanisms leading to immune evasion and reduced immune responses, the role of the tumor microenvironment (TME) in immune suppression, the design of more efficient immunotherapies with fewer side effects, avoidance of resistance by combination or sequence of treatments with conventional and targeted therapies as well as monitoring of immunotherapies and the development of biomarkers.

Immune modulation, microenvironment and metabolism

As important topics, immune modulation, immune escape and the tumor microenvironment were addressed during the first sessions of the meeting. **Andrea Schietinger** (Memorial Sloan Kettering Cancer Center in New York, USA) gave a talk on the decoding and reprogramming of the immune dysfunction in cancer using an autochthonous liver cancer model. She could demonstrate that tumor-specific T cells become dysfunctional early during tumorigenesis, which was due to different epigenetic programs. The phenotypic and functional properties were associated with distinct T cell differentiation stages, which were encoded by stage specific epigenetic changes and characterized by distinct transcription factor motives enriched in early and late T cell dysfunction. Thus, tumor-specific T cells were able to differentiate dysfunctionality via distinct chromatin states following antigen encounter. The discrete chromatin states upon therapeutic reprogrammability have been identified using different high-throughput technologies, which allowed distinguishing between reprogrammable and non-reprogrammable programmed death 1 receptor $(PD\text{-}1)^{high}$ $CD8^+$ T cells in heterogeneous murine and human tumor-infiltrating lymphocyte (TIL) populations. Moreover, novel transcription factors were identified that are uniquely expressed in dysfunctional tumor-specific T cells which appear to be induced through continuous T cell receptor (TCR) stimulation and nuclear factor of activated T cells signaling. In addition, there exist affinity effects during the differentiation of tumor-specific T cells in tumors suggesting signal strength defines the differentiation and dysfunction state dynamics of T cells in tumors.

Doriana Fruci (Onco-Immunology Laboratory of the Bambino Gesù Children Hospital in Rome, Italy) talked about the clinical and therapeutic relevance of the immune microenvironment of neuroblastoma (NB). NB is the most common type of cancer diagnosed in the 1st year of life and accounts for 15% of all childhood cancer deaths. It represents a complex and heterogeneous disease with unique features, including early age of onset, high frequency of metastatic disease at diagnosis (over 60% of patients) and spontaneous regression of tumors in infants without treatment, even if they are metastatic. Biological variables associated with poor prognosis are the amplification of the MYCN oncogene, gene mutations as well as loss and gain of various chromosomes. The survival rate of NB patients is below 35% demonstrating an urgent need to improve the treatment effectiveness of these patients. Since changes in the TME plays a key role in various cancers, Dr. Fruci determined the geographical and spatial distribution of T cells in NB and their prognostic value in this disease. The frequency of TIL was associated with an improved survival of patients with MYCN-amplified NB. The functional status of TILs was determined by studying the spatial distribution of proliferating T cells and the density of inhibitory immune checkpoints PD-1 and programmed death ligand 1 (PD-L1) using IHC. The density of $PD\text{-}1^+$ infiltrating lymphocytes was proportional to the density of TILs and tertiary lymphoid structures, while that of $PD\text{-}L1^+$ and HLA class I^+ tumor cells was associated with the clinical outcome of this disease. In addition, a murine transplantable mouse NB model was developed to analyze the TME in murine and patient-derived tumor spheroids including the recruitment of TILs. Thus, high-risk NB have a paucity of T cells. An immunoscore based on the presence of TILs might have a prognostic value for patients with MYCN-amplified and non-amplified NB. Furthermore, transplantable mouse NB models as well as patient-derived organotypic NB spheroids could be used as suitable tools for the evaluation of strategies to improve TILs.

The role of inflammatory and non-inflammatory TME was the topic of the talk of **Sven Brandau** (University Hospital in Essen, Germany) who demonstrated the importance of neutrophils in head and neck squamous cell carcinoma (HNSCC). The ratio of peripheral blood neutrophils to lymphocytes appears to be a prognostic marker in HNSCC and correlates with the survival of these patients. Neutrophils

are able to promote the migration of HNSCC cells due to activation of cortactin. Furthermore, neutrophil recruitment is involved in angiogenesis, metastasis, T cell suppression as well as priming of the pre-metastatic niche. Using digital pathology, spatial distribution of tumor-associated neutrophils (TAN) was determined and an interaction with T cells was demonstrated. These analyses revealed a high intratumoral heterogeneity of the HNSCC-TME and TAN or $CD3^-$ TIL densities in either tumor core areas or stromal regions, which differentially correlate with patients' survival. Next to the analyses of TAN an intense immunophenotyping of human circulating myeloid-derived suppressor cell (MDSC) subsets was performed demonstrating an induction of in particular polymorphonuclear (PMN)-MDSCs in cancer patients. Analyses of the suppressive activity of the three distinct MDSC subsets revealed high expression of arginase-1 (Arg1) and inducible nitric oxide species in PMN-MDSCs, while inhibitors partially restored T cell proliferation. $CD11b^+$/$CD16^+$/PMN-MDSCs displayed the highest suppressive capacity. In sum, there exists a bidirectional interaction of human TAN with tumor cells, which results in enhanced lymphatic metastases as a mechanism for tumor progression. Furthermore, new digital pathology tools allowed a better integration of spatial distribution of immune cells and intra-tumoral heterogeneity into biomarker profiles. A subset of $Arg1^{high}$PMN-MDSCs is the dominant and clinically most relevant MDSC subset in HNSCC patients and a high frequency of this subset is associated with a poor outcome of HNSCC patients.

Matthias Schwab (Department of Clinical Pharmacology, University Hospital Tübingen and Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology in Stuttgart, Germany) gave a talk about the interaction of the cellular metabolism and immunology using renal cell carcinoma (RCC) as a model. There exists high tumor heterogeneity in RCC associated with an altered gene expression pattern associated in part with altered patients' survival. For example, the expression of the lactate transporter MCT4 and methylation of solute carrier family 16 member 3 (SLC16A3) in clear cell RCC (ccRCC) and/or metastases is predictive for patients' survival. In addition, a number of potential anti-cancer drugs could be used, which target metabolic processes. HLA ligandomics established by the Rammensee group at the University Tübingen was applied to identify tumor-specific peptides in ccRCC patients. Employing a unique combined approach of ligandomics and pathway enrichment analyses of transcriptomics data a large number of pathways have been identified and 179 candidate genes were validated using The Cancer Genome Atlas data (TCGA). This approach led to the identification of potential targets for RCC vaccination and selected candidate genes were further analyzed for their regulation and functionality. Interestingly, the HIF polyl hydroxylase 3 (PHD3) encoded by the Egl nine homolog 3 (EGLN3) gene was expressed in ccRCC lesions and its expression was directly correlated to the patients' survival. PHD3 had an impact on the replication and apoptosis of RCC cells, enhances proliferation, inhibits apoptosis and supports glycolysis while inhibiting mitochondrial activity. HLA ligandomics analysis led to the identification of presented peptides from genes involved in extracellular matrix (ECM), lipid metabolism and transport, oxygen sensing and blood vessel development. Thus, peptide vaccination using the target genes identified might be an important factor to prevent rapid peptide loss and consequential resistance.

In addition, **Ramon Klein Geltink** (Max-Planck-Institute of Immunology and Epigenetics in Freiburg, Germany) demonstrated a complex interplay between T cell metabolism and function. T cell responses to infections require a metabolic remodeling and plasticity with changes of the metabolic flux. Little information exists how these changes were regulated. It is known that CD28 signaling enhances glucose uptake and effector function and preserves mitochondrial flexibility after activation. The presence of CD28 increased interferon (IFN)-γ production, glycolysis and oxidative phosphorylation (OXPHOS) resulting in tumor clearance, while in the absence of CD28 IFN-γ production is reduced and glycolysis and OXPHOS diminished leading to tumor recurrence. High-throughput analyses of glycolytic T effector cells versus OXPHOS T memory cells demonstrated distinct expression patterns of metabolites, which occur early after TCR signaling. Furthermore, an overlap between transcriptomics and phosphoproteomics after early T cell activation was found. Since the central carbon metabolism enzymes might be differentially expressed under these conditions, Ramon Klein Geltink analyzed the expression of the metabolic enzymes throughout T cell activation and differentiation into memory T cells. Glucose transporter (GLUT)-1, but not GLUT-3 protein expression was associated with glucose import. Currently, it is analyzed whether glucose depletion alters the redox state in T cells and higher GLUT-1 expression leads to an accumulation of nicotinamide adenine dinucleotide (NAD) during glucose depletion in T cells and whether this NAD allows upstream glycolysis to rapidly respond.

Barbara Seliger (Institute of Medical Immunology of the Martin-Luther-University Halle-Wittenberg, Halle, Germany) gave insights into novel mechanisms leading to HLA class I-mediated tumor immune escape including the role of ECM and microRNAs (miRNAs) in this process. Alterations in the expression of HLA class I surface antigens and components of the antigen processing machinery (APM) frequently occur in tumors of distinct origin, which can be associated with a lower overall survival (OS) of tumor patients. Furthermore, loss or downregulation of HLA class I APM components appear to be associated with the development

of resistances to immunotherapies including adoptive cell therapy (ACT). The impaired HLA class I APM component expression could be due to either structural abnormalities, which are rare, transcriptional, epigenetic or post-transcriptional regulation. Recently, small leucine-rich proteoglycans (SLRP), in particular biglycan (bgn) has been identified to be downregulated in murine and human tumor cells and to regulate MHC class I surface expression. Overexpression of bgn in bgn-deficient cells as well as recombinant bgn increased MHC class I surface expression, while shRNA mediated bgn downregulation had adverse effects. Furthermore, crosstalk between bgn and transforming growth factor (TGF)-β exists with a bgn-mediated downregulation of TGF-β receptors as well as its ligands. These in vitro results were further confirmed by in vivo data demonstrating a reduced tumor incidence of bgn overexpressing cells, which was accompanied by an increased immune cell infiltration. TCGA data analyses also demonstrated a role of bgn in the prognosis of many cancers. Next to these different mechanisms, miRNAs appear also to modulate the expression of MHC class I APM components and HLA-G, a non-classical immune suppressive HLA class I surface antigen, known to inhibit T cell and NK cell responses and to be overexpressed in tumors of distinct origin. The miRNAs were identified by a combination of miTRAP analyses with RNA sequencing and could either downregulate or enhance MHC class I surface expression or HLA-G. These miRNAs might be used as biomarkers or therapeutic targets. Thus, different modes of rescue of the MHC class I-mediated immune escape phenotype are possible, which not only include cytokines, but also SLRPs and miRNAs.

The keynote lecture from **Thomas Gajewski** (University of Chicago, Chicago, USA) was about tumor and host factors regulating the anti-tumor immunity and immunotherapeutic efficacy. Inhibitory factors such as regulatory T cells (Treg), PD-L1 and indolamine-2,3-deoxygenase expression, were associated with $CD8^+$ T cell infiltration. Reactivation of $CD8^+$ T cells in the tumor results in an altered TME composition, which could be categorized into a T cell inflamed and non-inflamed TME. The molecular mechanisms underlying the T cell inflamed versus non-inflamed TME could be due to somatic differences at the level of tumor cells, genetic differences at the level of the host as well as of the environment. While the T cell-inflamed TME exhibits high levels of chemokines, $CD8^+$ T cells and type I IFN signature, its immune escape is mediated by inhibitory pathways. The non-T cell-inflamed TME demonstrated a low inflammatory signature, the absence of intra-tumoral $CD8^+$ T cells leading to an immune escape by T cell exclusion, which is in accordance with an association of an increased activity of anti-PD1 antibody with immune cell infiltration. Lymphocyte-activation gene 3 $(LAG\text{-}3)^+$ and $4\text{-}1BB^+$ antigen-specific $CD8^+$ TIL in the TME exhibit a reduced interleukin (IL)-2 and tumor necrosis factor (TNF)-α production, yet retain in vivo proliferation and chemokine production as well as ex vivo lysis. A high frequency of non-T cell-inflamed melanoma exhibits an active β-catenin signaling. Furthermore, adoptive transfer of tumor-specific T cells failed to control the β-catenin expressing tumors and to recruit antigen-specific TCR transgenic effector T cells in a genetically engineered mouse model. Stimulator of interferon gene (STING) agonists promote T cell infiltration and tumor control in multiple mouse tumor models, including β-catenin expressing genetically engineered models. The immune signature is also associated with a $CD8^+$ T cell infiltration in bladder cancer and alterations of the fibroblast growth factor receptor 3 are unique to non-T cell-inflamed bladder cancers. Secondary recurrence in melanoma was associated with an upregulation of β-catenin in one case, and deletion of PTEN in a second case, both associated with loss of an immune signature. Commensal bacteria shape anti-tumor immunity. Direct administration of certain Bifidobacterium strains to tumor-bearing recipients improved tumor-specific immunity and response to anti-PD-L1 treatment. Interestingly, mice from the Jackson and Taconic laboratory exhibit distinct levels of anti-tumor T cell responses due to differences by their housing and bacterial exposure. In sum, Thomas Gajewski suggested that T cell-inflamed TME serves as a predictive biomarker for response to immunotherapies, such as checkpoint inhibitors (CPI). Functional TILs express a number of regulatory receptors, which appear to represent potential tools and targets for combination therapies. Non-inflamed tumors might be manageable by de novo immune priming with innate immune pathway agonists. T cell exclusion from the TME mediated by β-catenin identifies important pathways for drug development. Multi-dimensional genomic-based analyses might identify individualized molecular correlates of response versus resistance in patients including commensal microbiota, which also may lead to novel therapeutics.

Batya Isaacson (Lautenberg Center for Immunology at the Hebrew University Medical School, Jerusalem, Israel) demonstrated that NK cells control metastasis by the NKp40 receptor and that IFN-γ can cause structural editing of primary tumors. The activity of NK cells that can kill virus infected or tumor cells depends on activating and inhibiting receptors. One activating human NK receptor is NKp40. In the absence of murine analog Ncr1 the in vivo growth of lymphoma tumors is enhanced, while in human and murine melanoma cell lines NKp40 and Ncr1 mediates the cell lysis through NK-mediated cell recognition. Injection of B16 murine melanoma cells into Ncr1 knockout mice resulted in a much higher amount of metastases after 14 days when compared to wild-type mice, but the tumor volume remained unchanged. Since the survival of the mice was also identical, Ncr1 controls the growth of the metastasis, but not of the

primary tumor. Both mice strains have the same amount of cytotoxic NK cells and the expression of the Ncr1 ligand is at similar level. Furthermore, identical biochemical properties were found for the Ncr1 ligand under enzymatic digestion. In addition, the infiltration of NK cells into the tumor is equal in Ncr1 and Ncr1-diminished tumors. Since IFN-γ and TNF-α secretion was decreased in Ncr1-deficient mice, the tumor growth was analyzed in IFN-γ- and TNF-α-deficient mice. Here, only IFN-γ-deficient cells showed a similar reaction to the Ncr1-deficient mice with an increased amount of metastasis, while the growth of the primary tumor was unchanged suggesting an IFN-γ dependent effect of Ncr1 on metastasis. The use of the reflectance confocal microscopy imaging, which has an improved lateral resolution and sensitivity and is able to visualize tumor architecture, allowed to detect structural differences such as an increased formation of cerebriform nests in Ncr1- and IFN-γ-deficient tumors.

Diana Dudziak (Department of Dermatology of the University Hospital in Erlangen, Germany) extended the topic from NK cells to dendritic cells (DC), since her research focuses on DC functions and DC-based immunotherapeutic approaches. In her talk, she introduced the concept of antigen targeting via antigen-coupled antibodies directed against endocytic receptors on DC subsets. Her previous data demonstrated that the delivery of antigens to murine DCs initiated primary T cell immune responses in vivo, which were either $CD4^+$ or $CD8^+$ T cell responses and were subsequently depending on the presenting DC subset. Due to its strong potency to protect from murine melanoma growth, antigen-targeting approaches might be relevant for human immunotherapy. To transfer the antigen-targeting approach to the human system, the lab started to characterize primary DC subsets from various lymphohematopoietic and non-lymphohematopoietic tissues to identify useful endocytic receptors on DC subpopulations. Diana Dudziak classified DC subpopulations by combining surface marker expression and transcription factor expression. To define DC subsets and their functions high-throughput analyses from the different DC subsets from various human organs obtained from healthy donors were performed applying typical DC markers. Bioinformatics clustering of the data revealed cell type-specific contributions to the overall transcriptome of DCs. Potential transcriptional regulators upstream of co-regulated genes were found indicating the ontogenic origin of each of the human DC subsets. Most importantly, the data of this study imply that the non-lymphohematopoietic tissue microenvironment harbored a higher contribution to the overall transcriptome compared to the transcriptome of DCs isolated from lymphohematopoietic tissues. Furthermore, human DC activation upon stimulation by toll-like receptor ligands was determined exhibiting DC subset-specific cytokine secretion panels. Overall, the data presented by Diana Dudziak might lead to better understanding of DC functionality and thus to the development of optimized cancer vaccines.

Novel immunotherapeutic strategies

The second major topic of the symposium was based on different immunotherapeutic strategies using ACT and chimeric antigen receptor (CAR) therapies, immune CPI and DC vaccinations alone or in combination as well as immunomonitoring to identify biomarkers. **Dirk Busch** (Institute of Medical Microbiology and Immunology, Technische Universität München, Munich, Germany) reported about the choice of optimal T cell subsets and implementation of safeguards for adoptive T cell therapy. Since T cell specificity is determined by the TCR, activated and expanded T cell populations are extremely complex with a variety of TCR repertoires. The MHC-epitope complex as a natural ligand can identify T cell populations specific for this epitope, but the affinity of the TCR-MHC to the epitope is very low due to high dissociation rates. To increase this interaction, tetrameric MHC-epitope complexes can be used in flow cytometry, which allows highly specific detection and isolation of epitope-specific T cell populations ex vivo. In recent years, the generation of MHC class I multimers have been well established and characterized. Tetrameric MHC class I reagents with epitopes derived from various pathogens e.g., the bacterium *Listeria monocytogenes* or different viruses such as HIV, CMV, Epstein Barr virus (EBV), HCV and TAA have been generated for experimental and clinical studies. Following staining and dissociation at low temperatures, T cells are phenotypically and functionally indistinguishable from untreated cells. Thus, this T cell staining procedure maintains the specificity and sensitivity of the MHC multimer staining, while preserving the functional status of T lymphocytes and thus could be employed for ex vivo studies of T cell functions.

Furthermore, **Inge Marie Svane** (Department of Oncology, Herlev Hospital, Herlev, Denmark) demonstrated the implementation of TIL therapy in melanoma patients in combination with lympho-depleting chemotherapy. The CCIT-DK-Center treated 25 patients, which progressed on high dose IL-2 and ipilimumab. Using this approach tumor regression was obtained in approximately 40% of the patients and could be directly associated with tumor reactivity of infused TILs. Relapse was directly associated with loss of the expression of HLA class I APM components. Furthermore, Dr. Svane analyzed T cell responses in patients with melanoma resistance to anti-PD1 immunotherapy demonstrating a preserved tumor reactivity of expanded TILs. In a randomized phase III trial comparing TIL-based ACT to standard ipilimumab treatment in metastatic melanoma, more than 50 patients have been currently included. Clinical

response to TIL therapy was observed also in patients previously progressing on anti-PD-1 therapy. Thus, T cell therapy using lympho-depleting high-dose chemotherapy and IL-2 is in general safe and the toxicity manageable. A complete and long-lasting response has been obtained and a clinical benefit of T cell therapy has been demonstrated also after progressive disease on other immunotherapies. Thus, the combination of TIL therapy with CPI could potentially increase its efficacy.

Wolfgang Uckert (Max-Delbrück-Center of Molecular Medicine in Berlin, Germany) described a TCR isolation platform, which constitutes of (1) T cell expansion to endogenously processed antigens (2) the use of a MHC cell library for screening of T cell responses followed by T cell sorting (3) TCR analyses by next generation sequencing (NGS) (4) identification of antigen-specific TCR (5) pre-clinical analyses including epitope mapping, tumor cell recognition and tumor rejection and last not least (6) the design of a clinical trial to determine the safety and efficacy of TCR-engineered T cells. The sources for TCR isolation could be either peripheral blood lymphocytes, TILs, autologous or allogeneic-stimulated T cells, transgenic mice or autologous T cell stimulation/ MHC class I cell library. Despite Dr. Uckert identified restriction elements using a K562 cell library, a successful TCR-mediated gene therapy requires a three component interaction. First, the epitope is presented by one out of six MHC molecules. Although MHC alleles are distributed at different frequencies so far, clinical trials of TCR gene therapy has only used HLA-A*02:01. Thus, there is an urgent need to isolate TCR for other MHC, since 53–82% of the population are HLA-A*02:01 negative. TCR analysis by NGS and the gene-specific TCR approaches identified TCRs recognizing CMV, HPV, EBV and tumor-associated antigens (TAA). However, the time from the discovery of target epitopes to mutation-specific TCR gene therapy currently takes more than 18 months due to regulatory requirements including good medical practice production of TCR retroviruses, leading to a delay of TCR gene therapy. TCR transfer by non-viral transposon-mediated transfection diminishes this time. Using the sleeping beauty (SB) transposon-based gene transfer system improved this approach by enhancing the efficacy of TCR gene transfer and increasing the generation and expansion of engineered T cells without selection. Stable expression of SB-transfected TCR and CAR in human T cells was obtained. In addition, an improved T cell functionality to mini-circles encoding miRNAs and optimized TCR genes was obtained. In sum, the transfection of DNA into human T cells results in a decreased viability and the delayed response to stimulation, while transfection of SB transposons encoding RNA T cell viability is retained and the procedure is shorter. In addition, transposon mini-circle vectors exhibit an increased transfection rate. Furthermore, the enhanced TCR expression by silencing of endogenous TCR and TCR gene optimization improves T cell functionality.

CAR-modified T cells have been also shown to give impressive clinical results, but the cytokine release syndrome and on target off tumor reactions are the major concerns. **Michael Bachmann** (Helmholtz-Centre Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research in Dresden, Germany) has developed a novel CAR platform termed UniCARs, which consists of CAR-modified T cells and tumor-specific target modules (TMs) to improve the safety of CAR T cell therapy. He employed a monovalent nanobody-based anti-EGFR TM. Pharmacokinetic studies in immune-deficient mice revealed that these TMs can be released from UniCAR TM complexes, which suggest an on and off switchable UniCAR system. The efficacy of the anti-EGFR TM was improved by increasing their binding activity generating a novel bi-specific anti-EGFR TM. This construct was able to mediate EGFR signaling effects and anti-tumor efficacy. The binding capacity of the TM in combination with the density of the EGFR expression on tumor cells is responsible for the attack of UniCAR T cells to target cells.

Rolf Kiessling (Cancer Center Karolinska, Stockholm, Sweden) presented patient data that the combination of ACT with DC vaccination could overcome the resistance to checkpoint blockade. Although the 1 year survival rate of metastatic melanoma patients was increased in the past few years with the combined therapy of nivolumab and ipilimumab to 73%, the majority of treated patients do not survive 5 years. Here, the challenge for checkpoint inhibition is the lack of predictive biomarkers. To increase the knowledge in this field, the melanoma unit at the Karolinska established a biomarker and immune monitoring program: blood and tumor samples from patients with malignant melanoma and ongoing CPI treatment are collected at different time points and analyzed by functional T and NK cell assays, IHC, xenograft models, NGS and established tumor cell lines or with multi-color flow cytometry (both sample types). As an example, the frequency of $CD14^+$ monocytic MDSC with low levels of HLA-DR was higher in patients with a progressive disease and was associated with a significant lower OS. The generation of highly efficient TILs from melanoma patients who have failed on checkpoint therapy was feasible. Administration of these TILs to preconditioned patients also receiving low-dose IL-2 resulted in complete and long-lasting clinical responses in several patients. This was particularly evident, when adoptive transfer of TILs was combined with a DC vaccine expressing tumor antigens from the autologous tumor. TIL clones could be detected for more than 1 year after therapy in the blood of treated patients indicating a long-term clinical response.

Mary L. Disis (UW Medicine Cancer Vaccine Institute in Seattle, USA) described in her talk how vaccines could target oncogenic proteins. Since the first signal in T cell

activation is antigen recognition, this mechanism is of high medical interest and might be modulated by an immunogenic and non-immunogenic TME. Despite the latter is common in many patients, vaccines to stimulate antigen-specific $CD4^+$ T cells are developed and tested. One example is a HER-2/neu Th epitope-based vaccine, which prevented the recurrence in patients with HER-2/neu-positive breast cancer. While the Th2 epitopes inhibit the anti-tumor effect of Th1, vaccines containing both Th1 and Th2 epitopes are not effective. Other candidate antigens in breast cancer can be associated with epithelial to mesenchymal transition or cancer stem cells. In common, the dose of the vaccine and boosting increases incidence, magnitude and broadness of response. Furthermore, multi-antigen vaccines can increase responses to much higher levels of T cells than single-antigen vaccines. In conclusion, vaccines targeting oncogenic proteins can induce de novo Th1 tumor-specific T cell responses without self-regulation and lead to an amplification of an existing response or to an increased diversity of the tumor-specific response.

Tanja de Gruijl (Dept. Medical Oncology at VU University Medical Center in Amsterdam, The Netherlands) presented therapeutic tools in early stages of melanoma to prime and activate the sentinel lymph node (SLN) through the use of CpG-B or anti-CTLA-4. This method is applied in early stages of the disease (clinically stage I and II) with 10-year survival rates varying from 15–40%. SLN is the first LN to contain metastases and show decreased maturation of migratory and LN-resident DC subsets. Immune modulators were injected i.d. at the primary tumor excision site, one week prior to the surgical removal of the SLN. After standard prognostic procedures, SLN were used for isolation of viable cells and immune status analysis. Local delivery of CpG oligonucleotides enhanced local and systemic anti-melanoma T cell responses and increased the recurrence- and metastasis-free survival. In SLN from breast cancer patients, combined ex vivo treatment with CpG and STAT3 inhibition induced Th1 skewing and facilitated T cell activation to mammaglobin, while CD83 on LN-resident DCs was upregulated. In cervix carcinoma-draining LN, $CD8^+CD25^+FoxP3^+PD\text{-}1^+$ T cells specifically correlated with the efficacy of PD-1 blockade in vitro. Interestingly, these T cells were previously linked with a much better prognosis, since they produce more IL-2 and IFN-γ.

The search for prognostic/predictive biomarkers as tools for predicting high responsiveness to vaccination trials was the topic of the talk from **Constantin N. Baxevanis** (Cancer Immunology and Immunotherapy Center at the St. Savas Cancer Hospital in Athens, Greece). Vaccination of prostate cancer patients with the peptide AE37 stimulates peptide-specific T cells, but not all patients' respond in the predicted pattern. High responders showed the HLA alleles DR11, A24 or a combination of both while the HLA allele A2 was found in patients, who developed moderate responses to the AE37 vaccine. Under treatment with AE37 vaccination for 6 months and boosting 1 year after starting the vaccination HLA-A2 non-responders had a higher amount of $CD4^+$ Treg at all monitored time points, while IFN-γ secretion was much higher in HLA-A2-negative prostate cancer patients. Therefore, the survival of patients with high HLA-A2 expression is worse than of HLA-A24. Interestingly, HLA-A2 patients had preexisting immunity to a series of TAA including PSA, PSMA, TERT, survivin and HER-2/neu suggesting that their reduced survival could be due to acquired immune resistance mechanisms. In invasive non-metastatic ductal mammary carcinoma differential densities of CD8 and CD163 in the invasive margin and tumor center (high CD8 in the tumor center combined with low CD163 vs low CD8 in the invasive margin combined with high CD163) are suited as prognostic biomarkers leading to a much higher overall and distant metastasis-free survival (favorable combined immune signature; FCIS) than patients with the inverse combination (unfavorable combined immune signature; UCIS). A correlation between tertiary lymphoid structures (TLS) and the outcome was also present in breast cancer patients: the presence of peri-tumoral (adjacent and distal) TLS has an unfavorable prognostic role, whereas their absence or presence at lower numbers in the case of adjacent TLS was correlated with an extended progression-free survival and overall survival. The combined FCIS/TLS signature has improved prognostic value. Furthermore, several miRNAs, such as miR-23 and miR-181, are associated with immune infiltration and thus might represent useable prognostic markers for the clinical outcome.

Sjoerd H. van der Burg (Department of Medical Oncology, Leiden University Medical Center, Leiden, The Netherlands) showed data demonstrating that HPV-induced oropharyngeal squamous cell cancer has a significant improved survival compared to the HPV-negative counterparts. This may be due to virally derived tumor-specific antigens, since survival was clearly linked to the presence of an intratumoral type 1 T cell immune response to these antigens. In the tissue of HPV-positive patients, a higher number of tumor-infiltrating immune cells with elevated frequencies of $CD161^+$ $CD4^+$ Th1/Th17 effector-memory cells were observed when the T cells responded to HPV16 E6 and/or E7 peptide antigens. Patients with an HPV-specific T cell response had a 38-fold higher chance of achieving a response to therapy, which was highly significant over all stages of this disease. In HPV-induced cervical cancer, no correlation with the intra-tumoral presence of HPV16-specific $CD4^+$ T cells and survival was found. This was explained by the fact that the overall $CD4^+$ T cell infiltration, and in particular that of $CD161^+CD4^+$ T cells, was lower in HPV-induced cervical cancers when compared to HPV-induced oropharyngeal cancers. To overcome the lack of a strong HPV-specific Th1

response in these patients, vaccination with HPV-specific T cell epitopes can be used to improve the clinical outcome of cancer patients, but only when combined with compounds removing immune suppressive hurdles installed by myeloid cells or checkpoint expression.

Awards

The three poster prizes were awarded to:

1. Poster prize: Susann Albert (Technische Universität Dresden).
2. Poster prize: Naveen Shridhar (Universitätshautklinik Magdeburg).
3. Poster prize: Jürgen Bukur (Martin-Luther-Universität Halle-Wittenberg).

Conclusions

In conclusion, the TIMO symposium and workshop 2018 highlights the importance of in-depth analyses of the TME, liquid biopsies and tumors to characterize the immune escape mechanisms and the role of tumor and immune cell metabolism for mounting an immune escape. In addition, the design of different immunotherapies, their challenges and limitations were discussed. This demonstrates an urgent need to develop novel strategies to improve the outcome of tumor patients and to search for biomarkers, which allow predicting therapy response and outcome. There is increasing evidence that next to CPI therapy, ACT, CAR therapy as well as vaccination are promising strategies alone or in combination even with conventional therapies. However, to increase anti-tumoral immune responses, a better understanding of the molecular processes leading to immune suppression at all levels, e.g., the tumor, TML- and peripheral blood is urgently required.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. Barbara Seliger is the organizer of the conference.