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MEETING REPORT

CIMT 2023: report on the 20th Annual Meeting of the Association for Cancer Immunotherapy

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The Association for Cancer Immunotherapy (CIMT) celebrated the 20th anniversary of the CIMT Annual Meeting. CIMT2023 was held 3-5 May 2023 in Mainz, Germany. 1051 academic and clinical professionals from over 30 countries attended the meeting and discussed the latest advances in cancer immunology and immunotherapy research. This report summarizes the highlights of CIMT2023.

Key words: CIMT, cancer immunotherapy, immune escape, cellular therapy, tumor microenvironment, personalized therapy

INTRODUCTION

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IMPROVING IMMUNITY

The first session of the CIMT Annual Meeting 2023 was opened by Samra Turajlic (Francis Crick Institute, UK). She presented on the evolution of cell-intrinsic properties that can lead to immune evasion in melanoma and clear cell renal cell carcinoma (ccRCC). Both tumor types share similarities with regard to immunotherapy responsiveness but differ in their molecular makeup. Turajlic and colleagues investigated tumor cell—intrinsic mechanisms driving immune evasion in 573 post-mortem tumor biopsies from 14 melanoma patients treated with immune checkpoint blockade (ICB) (NCT03004755). Whole-genome doubling and reduction of expression of neoantigens occurred frequently but were not ubiquitous. Loss of components in the antigen-presenting machinery were frequently detected in all lesions of the same patient, suggesting that loss occurred early during tumor evolution. By leveraging mixed responses to compare ICB-responding and non-responding lesions, Turajlic and colleagues found that the target genes of MYC, which contributes to immune exclusion,[1,2] were highly correlated with resistance. She concluded that tumors were likely immune infiltrated at early stages and developed adaptive immune resistance, suggesting that early treatment reduces the risk of emerging immune resistance. Moving the focus to ccRCC, Turajlic highlighted this tumor type has a low mutational burden and is highly infiltrated by immune cells. By analyzing 100 tumor samples from 15 patients participating in a phase II trial investigating preoperative and post-operative programmed cell death protein 1 (PD-1) inhibition (NCT02446860), Turajlic and her group showed that genetic features in tumor cells do not correlate with response.[3] Instead, responders exhibited pre-existing immunity with expanded T-cell clones that were maintained during ICB. In contrast, pre-existing clones in non-responders were less expanded and were replaced under ICB treatment.

Metabolites in the tumor microenvironment can impair antitumor immunity by regulating the activity of immune cells. Guoliang Cui [HI-TRON and German Cancer Research Center (DKFZ), Germany] gave insights into how metabolic pathways govern regulatory T cells (Treg)s in the tumor microenvironment (TME). Unbiased analysis of 630 metabolites identified in the TME of three different mouse tumor models revealed significant enrichment of sphingolipid metabolism. Serine palmitoyltransferase long chain base subunit 2 (Sptlc2), an enzyme involved in the initial step of the pathway, was investigated deeper after up-regulation was identified in Tregs located in the tumors, but not in the spleen. Inoculating tumors in

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Sptlc2$^{Fl/Fl}$*Foxp3*$^{YFP\text{-}Cre}$ mice, where Sptlc2 is specifically deleted in Tregs, decreased their intratumoral accumulation, reduced PD-1 and T cell immunoreceptor with Ig and ITIM domains (TIGIT) expression on Tregs, and resulted in significantly retarded tumor growth. Feeding mice a diet lacking serine, a substrate of Sptlc2, resulted in reduced tumor growth and less infiltration of Tregs in the TME irrespective of the *Sptlc2* genotype, indicating that the presence of serine is essential for their activity. Mechanistic studies further revealed that the sphingolipid pathway is essential for the maintenance of FoxP3 and PD-1 expression in Tregs. Sphinganine, a downstream metabolite of the pathway, stabilizes c-Fos homodimers or heterodimers with additional partner proteins such as c-Jun and Nuclear factor of activated T-cells (NFAT) to enhance *Pdcd1* transcription, which in turn signals to induce *Foxp3* expression and thereby enhances the immunosuppressive Treg function.

Niels Halama [German Cancer Research Center (DKFZ), Germany] dedicated the third talk of the session to immune-metabolic modulation in pancreatic cancer, a largely therapy-resistant cancer indication with high medical need. The current standard of care for metastatic pancreatic cancer is FOLFIRINOX (oxaliplatin, irinotecan, fluorouracil, and leucovorin), which has an objective response rate of 34% and comes with severe side effects.[4] Interestingly, pancreatic cancer can trigger diabetes through an unknown mechanism, and the associated decline in glycemic control as measured by a reduction in HbA1c levels is associated with treatment response. Likewise, treatment response is more common in patients with pre-existing type 2 diabetes mellitus. Studies in human tumor explant cultures[5] revealed that FOLFIRINOX induces a local Th1-signature immune response and reduces insulin secretion in the TME. Among the up-regulated cytokines was C-C motif chemokine ligand 27 (CCL27), which had not been investigated in pancreatic cancer before. Following up on this, Halama and team found that CCL27 is secreted by β cells in the Langerhans islets, which prompted their hypothesis that CCL27 could be linked to reduced glycemic control. In line with that, increased CCL27 during FOLFIRINOX treatment correlated with type 2 diabetes and improved overall survival. Additional studies in the explant culture system highlighted the importance of CCL27 in the induction of the local immune response given that blocking C-C chemokine receptor type 10 (CCR10), the receptor of CCL27, abrogated cytokine secretion. Screening potential inducers of CCL27 revealed that tumor necrosis factor α (TNF-α) and interleukin 1 β (IL-1β) trigger CCL27 release and simultaneously reduce the expression of insulin. In the last part of his talk, Halama illustrated that monocytes are the source of TNF-α and IL-1β, which is secreted in response to FOLFIRINOX in the presence of tumor cells.

TUMOR MICROENVIRONMENT

Interaction of immune cells and tumor organoids can be predictive for cancer immunotherapies but imaging those interactions is challenging. Anne Rios (Princess Máxima Center for Pediatric Oncology, The Netherlands) and her team developed the BEHAV3D platform, which combines single-cell multispectral three-dimensional imaging and transcriptomics to investigate tumor cell death dynamics and T-cell behavioral landscape in immune-organoid co-cultures.[6] Using $\alpha\beta$-T cells engineered to express $\gamma\delta$-T-cell receptors (TCR)s recognizing the tumor metabolome (called TEG) and organoids derived from breast cancer biopsies, Rios and her group found that organoids from the same patient can differ in their susceptibility to T-cell killing, reflecting intratumoral heterogeneity in treatment response. In addition, BEHAV3D enabled the identification of nine behavioral states of T cells based on differences in mobility and engagement with breast cancer organoids. In particular 'super engagers' were highly capable of serial killing. Single-cell transcriptomics revealed a novel gene signature associated with this state as well as dynamic transcriptome changes in TEGs during tumor targeting, and led to the finding that sensitive organoids secrete interferon (IFN)-β. Taking a closer look at immune cell interactions, Rios and colleagues found that CD4+ and CD8+ T cells differ in their behavioral state and communicate through direct interactions during targeting of tumor organoids. Concluding her talk, Rios further demonstrated that BEHAVE3D is a promising tool to understand the interaction of immunosuppressive macrophages and chimeric antigen receptor (CAR)-T cells in a diffuse midline glioma organoid model.

Miriam Merad (Mount Sinai Medical School, USA) works on strategies to reengineer the immune system to enhance tumor immunity with a focus on the interactions of T cells with dendritic cells (DC)s and macrophages. To better understand T cell—DC interactions, Merad and colleagues studied pre- and post-treatment biopsies from hepatocellular carcinoma patients treated with PD-1 blockade (NCT03916627).[7] Using single-cell RNA sequencing (scRNA-seq), the group identified high levels of PD-1^{high} CD8+ effector T cells, and CXCL13+ CD4+ T follicular helper-like cells in responders, while non-responders showed elevated levels of Tregs. CD8+ T cells appeared to undergo differentiation in the tumor, with non-responders showing an accumulation of terminally exhausted CD8+ T cells. Merad and her team then employed physically interacting cell sequencing (PICseq) and spatial transcriptomics to analyze the phenotypes of interacting cells and found that mregDCs, a specialized subset of mature DCs induced by uptake of tumor debris,[8] interact with CXCL13+ CD4+ T cells and stem-like progenitor CD8+ T cells.[9] These cells formed tight triads that resulted in differentiation of CD8+ T cells into effectors in responding patients and terminally exhausted cells in non-responders. Investigating potential mechanisms that determine the differential outcome of T-cell differentiation prompted Merad to look into cholesterol synthesis and transport, which are central for mregDC differentiation and up-regulation of associated receptors including IFN-γ receptor 1 (IFNγR1). Inhibition of cholesterol transport reduced the expression of IFNγR1 in splenic DCs as well as their capacity to stimulate OT-I

and OT-II T cells *ex vivo*. DC-specific knock-out of IFNγR1 in mice led to increased formation of lung metastases upon injection of KP tumor cells expressing Green Fluorescent Protein (GFP) and was associated with reduced IL-12 production by DCs, suggesting that mregDC maturation states determine the trajectory of T-cell differentiation.

In the last talk of the session, Sjoerd van der Burg (Leiden University Medical Center, The Netherlands) presented how spatial transcriptomics can be deployed to elucidate states in the TME that are associated with complete response (CR) to immunotherapy. Synthetic long peptide vaccination can induce responses in human papilloma virus (HPV) 16-positive vulvar intraepithelial neoplasia patients as shown in several clinical trials.[10-12] However, previous work showed that the strength of vaccine-induced T cell is insufficient to robustly stratify complete, partial, and non-responders and instead implied a role of the pre-treatment TME.[13] The pre-treatment TME in patients experiencing CR resembled that of healthy vulva and harbored pre-existing, coordinated lymphoid and myeloid responses. Vaccine-induced T-cell responses alone were not able to trigger inflammation in cold TMEs. Deeper TME characterization by single-cell transcriptomics led to the identification of six distinct epithelial cell subtypes that are differentially prevalent in responders versus non-responders. In addition, the proportions of several immune subtypes varied between complete, partial, and non-responders. Using spatial analysis, van der Burg and colleagues found that CR-signature cells outnumbered non-supportive immune cells in tumors from complete responders and frequently interacted. Finally, distinct hot and cold tumor areas were identified in a non-responding patient, including the potential explanation for these.

KEYNOTE LECTURE

In his keynote lecture, Rafi Ahmed (Emory University, USA) presented how CD8+ T cells adapt to chronic antigen stimulation and deduced the implications for cancer immunotherapy. In mice chronically infected with lymphocytic choriomeningitis virus (LCMV), three PD-1+ CD8+ T-cell subsets specific for the dominant viral antigen epitopes gp33 and gp276 can be found. Ahmed defined their relationship as a linear trajectory. Stem-like Tcf-1+ CD8+ T cells are the least differentiated subset and give rise to transitory effector CD8+ T cells expressing T-bet, TIM-3, various effector molecules, and CX3CR1, which finally develop into terminally differentiated exhausted CD8+ T cells. Stem-like CD8+ T cells depend on the transcription factors Tcf-1 and Tox and emerge within the first weeks of infection. They are resident cells settling in characteristic niches of secondary lymphoid organs. Importantly, stem-like CD8+ T cells have the capacity to self-renew. This enables them to sustain the T-cell response and is the reason Ahmed refers to them as the 'resource cell' during chronic infection. Stem-like CD8+ T cells express minimal to no effector molecules and inhibitor receptors, but exhibit strong expression of costimulatory molecules, chemokines such as XCL1, and chemokine receptors that enable close interaction with DCs. In contrast to the stem-like subset, killing of infected cells is the main duty of transitory effector CD8+ T cells, which are circulating and express higher levels of effector molecules. They eventually mature into terminally differentiated CD8+ T cells, which are retained at sites of infection in both lymphoid and non-lymphoid tissues. Upon terminal differentiation, the T cells lose their costimulatory molecules, effector function and proliferative capacity, and quickly undergo apoptosis.

Ahmed continued his talk by translating these findings from mice to human cancers. He explained that blocking PD-1 triggers a proliferative burst originating exclusively from the stem-like population in a CD28 co-stimulation-dependent manner.[14] In line with that, all three T-cell subsets were also identified in human cancers,[15-18] and Ahmed and his colleagues found that stem-like cells from human non-small-cell lung cancer (NSCLC) indeed express high levels of CD28. Tracking down their location in NSCLC, they found that stem-like cells reside primarily in tertiary lymphoid structures. Exploiting the presence of a strong tumor-specific antigen to analyze tumor antigen-specific CD8+ T cells, Ahmed and his team conducted a study of 17 patients with HPV+ head and neck squamous cell carcinoma.[19] Tetramer staining readily enabled the detection of HPV-specific CD8+ T cells in primary tumors and metastatic lymph nodes at high frequencies [up to 5%-10% of the CD8+ tumor-infiltrating leukocytes (TIL)s], but their frequencies in peripheral blood were very low (<0.02%). scRNA-seq of sorted HPV-specific CD8+ T cells confirmed that they consist of a stem-like, a transitionary and a terminally differentiated population. The stem-like subset located in the lymphocyte-rich tumor stroma proliferated upon *ex vivo* stimulation with HPV-derived peptide, and differentiated into effector-like cells. Ahmed concluded that the presence of stem-like cells as bona fide PD-1 blockade responders together with a virus-derived tumor-specific antigen warrants the combination of therapeutic vaccination and PD-1 checkpoint inhibition in this tumor indication.

In the last part of his talk, Ahmed focused on the combination of PD-1 blockade and IL-2, which synergizes in reinvigorating the T-cell response in mice with chronic LCMV infection.[20] Elucidating the mechanism behind the synergy, Ahmed and co-workers found that the combination fundamentally modifies the exhaustion program in stem-like CD8+ T cells, demonstrating that they are indeed not fate-locked.[21] scRNA-seq and chromatin analysis revealed that adding IL-2 to PD-1 blockade generates a transcriptionally and epigenetically distinct 'better effector' subset. This is characterized by high IL-18 receptor expression and stronger effector and weaker exhaustion gene expression signatures, therefore resembling a phenotype usually associated with acute LCMV infection. Notably, programmed death-ligand 1 (PD-L1) blockade and IL-2 lead to up-regulation of the trimeric high-affinity IL-2 receptor (IL-2R) on LCMV-specific CD8+ T cells. Specific blockade of the IL-2Rα (CD25) subunit ablated the synergistic effect of the combination, indicating that the inherent bias of IL-2 towards

antigen-specific CD8+ T cells underlies the synergistic effect. In line with that, substituting wild-type IL-2 with a 'non-alpha' IL-2 variant (IL-2v) that binds IL-2Rβ/γ but not IL-2Rα failed to control the infection. Instead, IL-2v promoted the proliferation of unrelated CD8+ T cells. Ahmed concluded his talk by highlighting the importance of this finding as a possible explanation for the recent failure of three clinical trials (NCT03729245; NCT03435640; NCT04914897) investigating non-alpha IL-2vs. He proposed that targeting IL-2v to the right cell population may overcome this limitation and can be achieved by fusing IL-2v to a PD-1-binding antibody.[22]

NOVEL TARGETS

To identify novel tumor antigens, Sebastian Amigorena (Institut Curie, France) and his team investigate how epigenetic defects in cancer cause the aberrant expression of non-coding parts of the genome, which they call the 'dark genome'. They focus on cancer-related epigenetic derepression of transposable elements and alterations in the splicing machinery of tumor cells that lead to aberrant junctions between exons and transposable elements (JET)s, serving as a potential source of antigenic peptides.[23] A novel bioinformatics pipeline allowed the identification of multiple JETs specific to different mouse tumor models. Mining of immunopeptidomic datasets revealed that JET-derived peptides are presented on major histocompatibility complex (MHC) class I molecules. T cells specific for these antigens were identified in tumor-bearing mice. Vaccinating mice against them significantly impaired tumor growth, highlighting that the immune system is indeed able to 'see' the dark genome. Furthermore, enforcing JET expression by tumor cells through knock-out of the histone methyltransferase Setdb1, which leads to open chromatin structures and enables JET expression, rendered B16-Ova cells more responsive to PD-L1 blockade.[24] Taking a closer look at human tumors, Amigorena and colleagues found recurrent expression of JETs that were either associated with certain tumor types or tumor specific.[25] JET-derived peptides were presented by tumor cells and cognate CD8+ T cells could be enriched through *ex vivo* TIL expansion. Together with MNEMO Therapeutics, Amigorena seeks to exploit the translational potential of JETs by developing vaccination or tumor targeting (for example) through bispecific T-cell engagers or CARs directed against JET-derived peptide/MHC complexes.

In the second talk of the session, Ali Salanti (University of Copenhagen, Denmark) presented intriguing malaria research that resulted in the identification of an actionable tumor target. To avoid circulation and splenic clearance, erythrocytes infected with *Plasmodium falciparum* express adhesion proteins that anchor them to host endothelial receptors.[26] One of these proteins is VAR2CSA, which binds to a unique type of chondroitin sulfate (CS) found exclusively in the placenta during fetal development.[27] This unique CS type serves as a cytokine-binding site and enables the placenta to grow rapidly and as an immune modulator of this privileged site. Since this key feature between placenta and tumor cells, Salanti and his team investigated whether malignant cells exploit this molecule. Selective expression by tumor cells was confirmed since recombinant VAR2CSA bound to various tumor cells and tissues, but not to healthy cells found outside of the placenta, hence classifying this CS type as an oncofetal (of) tumor antigen.[28] Phage display enabled the identification of antibodies binding both human and murine ofCS, which did not exhibit any cross-reactivity to CS in healthy tissues. One of the antibodies showed promising efficacy in different formats. An antibody—drug conjugate containing the tubulin inhibitor monomethyl auristatin E induced full tumor regression in different syngeneic mouse tumor models as well as in a patient-derived xenograft model of prostate cancer. As a bispecific T-cell engager, it was effective against orthotopic 4T1 breast tumors.[29] A clinical phase 0 study of this antibody is underway to determine dosing and pharmacokinetic parameters before proceeding to a phase I trial.

Vinod Balachandran (Memorial Sloan Kettering Cancer Center, USA) and his team are studying pancreatic ductal adenocarcinoma (PDAC), a type of cancer that is largely resistant to current treatment options. Using a model that ranks the quality of neoantigens in PDAC cells according to the degree of 'selfness' versus 'non-selfness', Balachandran and his team found that the presence of high-quality neoantigens is associated with improved patient survival,[30,31] and that high-quality neoantigens underlie immunoediting.[32] These findings indicate that neoantigen-directed immunity plays a role in PDAC. Therapeutic targeting of PDAC specifically might be possible given that the majority of PDACs harbor neoantigens of varying quality.[31,33] Balachandran and his colleagues aimed to test if an individualized mRNA vaccine (autogene cevumeran) can induce *de novo* immune responses in a phase I clinical study of 19 PDAC patients (NCT04161755).[34] Patients received a single dose of the anti-PD-L1 antibody atezolizumab after surgery, followed by eight immunizations. Patients were afterwards treated with 12 cycles of chemotherapy (modified FOLFIRINOX, comprising folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin) and a subsequent booster vaccination. Despite disruptions caused by the coronavirus disease 2019 (COVID-19) pandemic, patients on average received autogene cevumeran within 3 days of benchmarked cases. In 50% of patients who received the vaccines robust durable and polyclonal T-cell responses were observed, and responders had a longer median recurrence-free survival compared to non-responders. Using CloneTrack, a novel mathematical approach for monitoring T-cell clones, Balachandran and colleagues found that vaccine-expanded T cells comprised up to 10% of peripheral T cells, re-expanded after booster vaccination, and included long-lasting polyfunctional effector CD8+ T cells specific for vaccine targets. Baseline immune fitness did not influence the response to the vaccine since non-responders mounted equivalent immune responses to a COVID-19 mRNA vaccine. However,

responders had more clonal mutations in their tumors and immunogenic vaccine neoantigens had higher quality. Evidence of efficacy was collected in one patient, who presented with a suspicious liver lesion that was found to be free of tumor cells upon histologic examination but harbored a dense lymphoid aggregate containing neoantigen-specific CD8+ T-cell clones.

ORCHESTRATING IMMUNITY

Circadian rhythms are key regulators of many physiological systems.[35] During the first talk of the Orchestrating Immunity session, Christoph Scheiermann (University of Geneva, Switzerland/Ludwig-Maximilians-Universität, Germany) presented how the circadian clock governs cancer immunity. Analyses of adaptive immunity previously revealed time-of-day-dependent trafficking of DCs into lymphatic organs, which affects the strength of T-cell responses provoked by vaccination.[36] Moving on to investigate how circadian rhythms dictate tumor immunity, Scheiermann and his team found that tumor growth kinetics oscillate in a time-of-day-dependent manner in different mouse tumor models.[37] Tumors injected in the afternoon [zeitgeber time 9 (ZT9); 9 h after light onset] were inhibited, whereas tumors injected in the early morning (ZT21) progressed aggressively. Depletion of CD8+ T cells abrogated the time-of-day-dependent differences in tumor growth, which indicated that this effect is immune mediated. Concordantly, gene expression patterns in DCs isolated from tumor-draining lymph nodes exhibited significant time-of-day-dependent oscillation, with genes related to T-cell activation being highly expressed at ZT21. In line with that, DCs exhibited a strong capacity to prime OT-I T cells *ex vivo* in a time-of-day-dependent manner. DC-specific deletion of *Bmal1*, a key regulator of circadian rhythms, abolished these differences.

In addition to DCs, macrophages are essential regulators of adaptive immune responses. Due to their substantial diversity, Julie Helft (Institut Cochin, France) aims for a deeper understanding of their role in cancer immunity. Tumor-associated macrophages (TAM)s can either promote or inhibit antitumor T-cell responses. Helft initially outlined her hypothesis that the origin of TAMs might determine their function. Single-cell RNA evaluation of human breast cancer tissue, which is characterized by a dense TAM infiltrate, revealed two transcriptionally distinct TAM populations characterized by either high Triggering receptor expressed on myeloid cells 2 (TREM2) or high Folate Receptor Beta (FOLR2) expression.[38] Hierarchical gene clustering indicated a high similarity between TREM2+ macrophages and monocytes, while FOLR2+ macrophages clustered separately from these populations. Comparative analysis of healthy breast and tumor tissues from humans as well as from mouse mammary tumor virus-polyoma middle tumor-antigen (MMTV-PyMT) tumor-bearing mice uncovered that FOLR2+ macrophages are located predominantly in healthy tissue. Their tissue frequency decreased as tumor progressed, supporting the hypothesis that FOLR2+ macrophages represent the tissue-resident population. On the other hand, TREM2+ macrophages appear to originate from recruited monocytes. Spatial analysis using tumor tissue microarrays showed that TREM2+ macrophages are strongly associated with the tumor margins and infiltrate the tumor bed. FOLR2+ macrophages were restricted to the tumor stroma, where they were located around tumor vessels and engaged with CD8+ T cells. Further characterization of both macrophage populations highlighted that TREM2 expression contributes to the development of an immunosuppressive phenotype,[39] whereas FOLR2+ macrophages were associated with a significantly improved survival of patients and generally higher immune infiltration.

In the last talk of the session, Eynav Klechevsky (Washington University School of Medicine St. Louis, USA) again shifted the focus towards DCs as key initiators of antitumor T-cell responses and gave insights on how the field developed over the past years. Her laboratory is especially interested in cutaneous DCs and previously found that a subset of Langerhans cells from the epidermis and migratory dermal DCs are characterized by distinct CD5 expression. Upon inflammatory skin disease, CD5+ DCs are enriched in the skin,[40] which indicates these cells might play a central role in promoting T-cell responses. Klechevsky and her team moved on by investigating the role of CD5+ DCs in cancer by subjecting DCs sorted from involved and uninvolved lymph nodes from a melanoma patient to scRNA-seq.[41] CD5 expression was restricted to the DC2 lineage and only detected in uninvolved lymph nodes. CD5+ DCs induced proliferation and cytokine secretion in allogeneic CD4+ and CD8+ T cells *ex vivo*, and CD5 expression on DCs correlated with improved survival in patients. This indicated that CD5+ DCs also play an important role in cancer. To confirm this hypothesis, Klechevsky and her colleagues employed the MCA1956 sarcoma and MC38 colon adenocarcinoma mouse models, where CD5 expression occurred mainly on resident DC1 and DC2 subsets. Tumors showed a robust response upon treatment with PD-1-blocking antibodies, which was prevented when the tumors were transplanted into mice that lacked CD5 expression specifically in DCs. Interestingly, CD5 expression levels on T cells correlated with CD5 expression on DCs, and T-cell-specific CD5 depletion likewise reduced the efficacy of PD-1 blockade.

IMMUNE ESCAPE

Natural killer (NK) cells are potential effector cells against tumors that reduce the expression of MHC class I to escape T-cell recognition. Adelheid Cerwenka (Heidelberg University, Medical Faculty Mannheim, Germany) is focusing on overcoming NK-cell dysfunction in the hostile TME.[42] Using scRNA-seq of NK cells isolated from RMA-S tumors, Cerwenka and her team found that NK cells up-regulate the hypoxia-inducible transcription factor HIF-1α.[43] Analysis of NK-cell functionality after culture in hypoxic conditions revealed reduced effector function, which prompted Cerwenka and her colleagues to investigate the role of HIF-1α in NK cells. RMA-S tumor growth was significantly reduced

in Hif1a$^{f/f}$Ncr1^{iCreTg} mice that lack NK-cell -specific HIF-1α expression. NK cells isolated from these mice had higher expression of activation markers and effector molecules compared to wild-type mice. NK cells exhibited a strong enrichment in the IL-18 signaling pathway, and blockade of IL-18 lead to accelerated tumor progression. Translating their observations to the human setting, the group illustrated that the HIF-1α inhibitor KC7F2 increased human NK-cell responses under hypoxic conditions. In addition, NK cells co-cultured with a resistant colon cancer patient-derived organoids showed enhanced killing capacity after knock-out of HIF-1α, which also improved the cytotoxic function of NK cells equipped with a CAR.

Robert Manguso (Massachusetts General Hospital Cancer Center, USA) focused his talk on the identification of resistance mechanisms of cancer to immunotherapy. To this end, Manguso and team developed the Tumor Immunotherapy Discovery Engine (TIDE), which comprises the compiled data from eight genome-wide and seven sub-genome CRISPR screens conducted in nine independent mouse tumor models.[44] Using TIDE, they found that loss of type I or type II IFN pathways or MHC class I antigen presentation pathways sensitized most of the tumor models to immune control, which was surprising due to the essential role of these pathways in antitumor immunity. Further interrogation demonstrated that IFNs required MHC class I expression to mediate immune resistance. NK cells were responsible for depleting tumor cell clones that lacked IFN pathway components, but several genes related to antigen presentation still sensitized tumors in the absence of NK cells. Among these was H2-T23 encoding Qa-1 (Human Leukocyte Antigen E; HLA-E), a non-classical MHC class I molecule engaging the inhibitory receptor NKG2A/CD94 expressed by T and NK cells. scRNA-seq of TILs from ICB-treated KPC tumors showed that cytotoxic CD8+ T cells express NKG2A, potentially inhibiting their antitumoral function. In line with that, knock-out of H2-T23 sensitized KPC tumors to PD-1 blockade, which was dependent on CD8+ T cells. Manguso concluded his talk by presenting that a 'Goldilocks Zone' for IFN sensing exists, which is occupied by inflamed tumors and exhibits just the right amount of IFN sensing for tumors to up-regulate inhibitory receptors without affecting growth and fosters recruitment of suppressive cells.

For an anticancer T-cell response to lead to effective killing of cancer cells, a series of events must be initiated and amplified. Ira Mellman (Genentech, USA) started his talk by depicting these events in the cancer-immunity cycle.[45] He outlined how immune checkpoint inhibitors fit into this model and focused on TIGIT, a checkpoint expressed by CD8 T cells that inhibits co-stimulation through CD226 by competing for ligand binding.[46] The combination of TIGIT blockade with PD-L1 blockade already showed beneficial effects on overall survival in a randomized phase II study of patients with NSCLC (NCT04294810); phase III overall survival data are pending. Mechanistic studies in an E0771 mouse tumor model showed that the combination treatment enhanced CD226 expression and decreased expression of Tox, a central transcription factor driving exhaustion, in tumor antigen-specific CD8+ TILs. scRNA-seq of CD8+ T cells revealed that the combination treatment induced clonal expansion of antigen-specific T cells in blood, tumor, and tumor-draining lymph node, and had profound effects on their trafficking between these compartments. Combined treatment expanded stem-like T cells in the draining lymph node, enhanced trafficking of antigen-specific progeny T cells via the blood to the tumor site and favored differentiation into effector and effector memory cells over the exhaustion trajectory in the TME. Mellman proposed that the suppression of CD28 and CD226 co-stimulation by PD-1 and TIGIT is an important driver of the exhaustion pathway. Interestingly, the Fc domain of the anti-TIGIT antibody appears to contribute by activating cytokine secretion by myeloid cells through engagement of Fc receptors. In the last part of his talk, Mellman presented Skin Tumor Array by Micro-Poration (STAMP), a preclinical high-throughput screening platform that allows the monitoring of thousands of tumors and their immune phenotypes *in vivo*.[47]

CELLULAR THERAPY

John Haanen (The Netherlands Cancer Institute, The Netherlands) started the session by providing an overview about developments in the field of TIL therapy over the past years. Before 2022, clinical efficacy was observed in a range of phase I/II trials including in heavily pre-treated melanoma patients (NCT02360579,[48] NCT02360579[49]). Haanen then reported on the outcome of his recently concluded seminal phase III study of 168 patients with unresectable stage IIIC-IV melanoma patients (NCT02278887).[50] Adoptive TIL therapy yielded an overall response rate that was superior to ICB with ipilimumab and translated into significantly better progression-free survival. While searching for the 'active ingredient' in the TIL product, Haanen focused on tumor mutational burden, which has previously been identified as a biomarker for clinical response to TIL therapy.[51] Detection of gene signatures characteristic for neoantigen-specific TILs[52,53] through scRNA-Seq allowed the identification of neoantigen-specific TCRs from both CD4+ and CD8+ T cells that were shared between fresh tumor digest and infusion product. While neoantigen-reactive CD8+ TILs were retained during TIL production, CD4+ TCRs identified in fresh digest frequently disappeared. Neoantigen-reactive CD8+ TIL clones showed considerable variability in expansion and contraction, hence the frequency of neoantigen-specific T cells in TIL products was highly variable. Haanen finished his talk by discussing the possibility of tackling the heterogeneity of TIL products through selection of patient-specific TCRs reactive for clonal neoantigens for personalized, autologous TCR therapy.

George Coukos (Ludwig Institute for Cancer, Switzerland) shared how insights into the TME can help to create synthetic immunity and improve adoptive TIL therapy. In a clinical trial of TIL therapy in pre-treated melanoma patients (NCT03475134), tumor biopsies from responders, as

opposed to non-responders, were highly inflamed and enriched for PD-1+ and granzyme B+ CD8+ T cells that exhibited a CD28 co-stimulation signature. To achieve higher numbers of such cells in the TIL product, Coukos and team developed the NeoTIL protocol in which patient-derived TILs are stimulated with antigen-presenting cells pulsed with patient-derived neoantigen peptides.[54] NeoTIL therapy was associated with responses in melanoma patients. Multiplexed immunofluorescence microscopy of pre-treatment tumors revealed a busy cross-talk between TILs and DCs in responding lesions, whereas resistant lesions were silent. CD28, a central signal mediator in the immunological synapse, was expressed by TILs exhibiting an effector and precursor effector signature along with an IL-2 signaling signature. To endow TILs with properties that would make them independent of this cross-talk, Coukos and his team developed the genetic engineering for enhanced performance (GEEP) T-cell therapy, in which T cells are engineered to express a non-alpha IL-2 variant, IL-33, and anti-PD-L1.[55] In OT-I T cells, this modification enabled a synthetic effector state that comprised a profound effector signature, absence of exhaustion, and superior metabolic fitness.

Therapeutic TCRs used to genetically modify immune cells can address a large target space comprising several target categories. Johanna Olweus (Oslo University Hospital, Norway) summarized previously published clinical TCR-T-cell trials, which have focused on tumor-associated antigens (TAA) including cancer testis or oncofetal antigens,[56,57] private neoantigens,[58] or viral antigens.[59] These categories bear promise but also several challenges. TAAs are shared across multiple cancer types but are often heterogenous with low expression. Private neoantigens are promising targets, but are often expressed at low levels, have low immunogenicity, and 99% are unique to an individual patient. Olweus suggested several new target categories that might be able to overcome such disadvantages. One example is intracellular, cell-type-specific antigens that are transiently expressed during normal differentiation. In this regard, Olweus and her team showed that terminal deoxynucleotidyl transferase (TdT) is a promising immunotherapy target, representing a cell-type-specific target that is only transiently expressed during differentiation of lymphoid cells but overexpressed in acute lymphoblastic leukemia (ALL) of T-cell and B-cell type. T cells modified with TdT-specific TCR identified using an alloreactivity mechanism to overcome self-tolerance eliminated ALL cells without affecting normal T or B cells, human thymocytes, or normal hematopoiesis in humanized mouse models.[60] Another potential new target category is shared neoantigens. However, identification of reactive TCRs has proven challenging in cancer patients. In the naïve T-cell compartment of healthy donors, Olweus and her team identified a highly affine TCR specific for the shared D835Y driver mutation in the tyrosine kinase FLT3.[61] $FLT3^{D835Y}$-specific TCR-T cells eradicated acute myeloid leukemia (AML) cells in patient-derived xenograft models and eliminated leukemia cells that propagate AML cells (*Nat Cancer*, in press). Shared neoantigens can moreover occur at the RNA level through epigenetic dysregulation of transposable elements, leading to tumor-specific aberrant MHC class I-presented peptides, potentially widening the target space.[24,25] Finally, dysfunctional protein translation can result in aberrant HLA class I-presented peptides generated by cancer cells in a situation with IFN-γ-induced, IDO-1-mediated tryptophan depletion, and Olweus and colleagues have demonstrated that such peptides can induce CD8+ T-cell responses.[62,63] Summarizing her talk, Olweus highlighted that highly affine TCRs targeting highly and homogeneously expressed antigens are essential for efficacy. To enable safe and effective TCR therapy in the future, Olweus and team are working on a preclinical safety pipeline for the early detection of off-target reactivity of TCR-T cells.

CONCLUSION

A record number of nearly 300 posters were presented at the 20th CIMT Annual Meeting and all abstracts are available at https://www.meeting.cimt.eu/call-for-abstracts.

The German immunologist Hans-Georg Rammensee received the 2023 CIMT Lifetime Achievement Award for his significant contributions to cancer immunotherapy research. We are excited to discuss more advances in the field of cancer immunotherapy at the 21st Annual CIMT Meeting 2024 (Mainz, Germany).

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