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MEETING REPORT

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CIMT 2018: Pushing frontiers in cancer immunotherapy — Report on the 16th Annual Meeting of the Association for Cancer Immunotherapy

Jan Beck, Matthias Birtel, Daniel Reidenbach, Nadja Salomon, and Mustafa Diken

TRON-Translational Oncology at the University Medical Center of the Johannes Gutenberg University Mainz gGmbH, Mainz, Germany

ABSTRACT

The 16th Annual Meeting of the Association for Cancer Immunotherapy (CIMT), Europe's largest meeting series of its kind, took place in Mainz, Germany from 15–17 May, 2018. Cutting-edge advancements in cancer immunotherapy were discussed among more than 700 scientists under the motto "Pushing Frontiers in Cancer Immunotherapy". This meeting report is a summary of some of the CIMT 2018 highlights.

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Cellular therapy

T cells are one essential element of the immune system and a countermeasure against infections and cancerous cells. **Andrew Sewell (Cardiff University, Cardiff, UK)** explained that evolution of pathogens into T cell blind spots could only be prevented by the multi-specific character of the T cell receptor. Using decamer combinatorial peptide libraries, comprised of synthetic peptides in which one amino acid is fixed while all others are degenerate, a single TCR was identified that can recognize over a million peptides.[1] Unfortunately, the peptides identified in this manner lack physical and enzymatic stability for use in vaccination. To address this issue, stable D-amino acid-based peptides were tested, of which one triggered human Influenza-specific $CD8^+$ T cells *in vitro*.[2] The same peptide was also shown to generate protective immunity in humanized mice against challenge with Influenza viruses.

Sewell further argued that testing specificities of T cell clones originating from the blood of patients after successful therapy could lead to the identification of non-HLA-restricted, pan-cancer T cell targets for therapy. He closed his talk with data showing that simultaneous knock-out of endogenous TCRs using Crispr/Cas9 and introduction of TCRs using lentiviral vectors could significantly increase receptor expression and induce 5000-fold higher reactivities. This was demonstrated for αβ as well as γδ TCRs.[3]

Gwendolyn Binder (Adaptimmune, Philadelphia, USA) started her presentation with a closer look on the advances in TCR-based therapies.[4,5] She talked about experiences of Adaptimmune with NY-ESO-1-specific TCRs targeting synovial sarcoma with an example of overall responses in 50% of the patients in a high dose fluradabine/cyclophosphamide treatment cohort. Adaptimmune has a unique platform to generate Specific Peptide Enhanced Affinity Receptors (SPEAR) for the treatment of solid malignancies. Binder referenced recent data suggesting that chimeric antigens receptors (CARs) might be potent and quick killers, but that affinity matured TCRs can have superior sensitivity for antigen recognition. However, increasing the affinity of TCRs might also increase the sensitivity of the TCR to other off-target reactivities. To identify those, Adaptimmune uses X-scan based screenings, where in a given peptide each position is substituted by one of the natural amino acids.[6] Receptors passing this screen are further tested for alloreactivity against different HLAs in an allo panel screen and in primary cell line screenings. She also presented data showing that peak persistence of SPEAR T cells during therapy correlates with the clinical response. She and her colleagues also observed that $CD8^+$ T cells tend to persist longer than their $CD4^+$ counterparts, and that those persisting cells are still capable of killing *ex vivo*.[7] Those T cells originated from a self-renewing T stem cell memory (T_{SCM}) subset in the manufactured product. The NY-ESO-1 clinical programs are in the process of being transferred to Glaxo-Smith-Kline this year. Four SPEAR T cell clinical studies (multiple tumor indications for Mage-A4 and Mage-A10 expressing tumors, and AFP expressing hepatocellular carcinoma) are ongoing and initial readouts should be available later this year.

Rodabe Amaria (MD Anderson Cancer Center, Houston, USA) focused her talk on tumor infiltrating lymphocyte (TIL)-based therapy and provided insight into the practice of how TILs for therapy are generated. In her strategy, which involves a 4–6 week production, tumor biopsies are cultured with IL-2 to expand existing T cell infiltrates to at least 40 million cells. After this first expansion, cells are frozen for the following rapid expansion protocol (REP), in which cells are expanded over a two-week period before being infused into preconditioned patients. Amaria pointed out that, among the logistical challenges of TIL therapy, the time-consuming and costly GMP production is critical. Scientific challenges still lie in a lack of persistence and localization of T cells to the tumor as well as the immunosuppressive microenvironment. An

CONTACT Mustafa Diken mustafa.diken@tron-mainz.de

interesting side note was that in the era of checkpoint blockade the overall response rates of TIL therapy have dropped.

She mentioned that the success rate for TIL generation is about 60% in cutaneous, while only being 45% for uveal melanoma. Treatment for each and every patient is still not guaranteed, and the pre-REP phase can lead to patient loss. To overcome these hurdles, a modified pre-REP protocol was developed, combining all three signals required for optimal T cell activation. Using anti-CD3 and anti-41BB agonistic antibodies in combination with high dose IL-2, they were able to drastically increase cell yields in a "3-signal pre-REP" production process for several tumor entities.

As a side story, she and her colleagues observed that late-stage melanomas show elevated levels of TGF-β. To bypass the suppressive impact on T cell activity, a dominant-negative TGF-β receptor was designed and was retrovirally introduced in TILs during a novel manufacturing process.[8] Treatment of metastatic melanoma patients with these modified T cells are under way.

Chimeric antigen receptors by advanced therapies (CARAT)

Bruce Levine (University of Pennsylvania, Philadelphia, USA) summarized the scientific work paving the way for recent advances of the first CAR T cell therapies, which were approved by the FDA last year. After years of experience, larger patient populations have revealed that CTL019 T cell persistence is a correlate for therapy success.[9] In two of the first patients treated, the CAR T cells can be detected for at least 7 years. T cells from CLL patients exhibit low CD45RA expression, skewed CD4/CD8 ratios and CD3zeta defects during therapy. The proliferative and functional defects can be counteracted by the administration of Ibrutinib[10] (NCT02640209). CTL019 has shown remarkable efficacy in the treatment of refractory/relapsed (r/r) diffuse large B cell lymphomas (DLBCL) patients.[11] Moreover, therapeutic outcome of a non-responding "double-hit" cutaneous large B cell lymphoma (CLBCL) patient could be improved with a Pembrolizumab combination therapy.[12] One important message from Levine was that not every patient's T cell product is the same, and that cell quality is essential. Melenhorst and colleagues suggest that the infusion of $CD45RO^{-}CD27^{+}CD8^{+}$ T cells has a favorable impact on therapy outcome. In animal experiments, infusion of human CAR T cells depleted of the $CD27^{+}PD1^{-}CD8^{+}$ population into leukemic mice resulted in loss of therapeutic efficacy.[13] In line with this, Levine mentioned a NY-ESO1 TCR study where a patient's CAR product became exhausted over time.[14] CRISPR/Cas9-mediated TCR/PD-1 knock-out is a long-term goal for T cell therapies since this can potentially increase the safety and function of cellular products, as shown in animal experiments[15] and in the first clinical trial of CRISPR technology (NCT03399448) outside of China, with NY-ESO1 specific TCRs. Levine concluded with alternative CAR concepts[16] stating that targeting multiple targets at once will become more important in the future and that in-depth studies of a few patients could vastly increase the knowledge about any given treatment.

Martin Pule (University College London, London, UK) and his team generated a CD19-specific CAR (CAT-41BBz) using a scFv fragment with lower affinity than the FMC63 scFv fragment (UPEN) and a quicker off rate. CAT-41BBz T cells have higher mobility and elicit a higher repetitive killing capacity than UPEN. *In vivo*, these CAR T cells led to better engraftment and anti-tumoral effectivity than FMC63-based CARs. In a Phase I study treating pediatric ALL patients (CARPALL), transferred T cells persisted up to 90 days in patients, and only mild cytokine release syndrome was observed. The overall survival at the six month follow-up was 70%. Additional work on a 28zeta-based CAR carrying a humanized GD2 scFv for the treatment of neuroblastoma was also presented by Pule.[17] In the context of a clinical trial (CRUKD/15/001), CAR T cells could be manufactured for all patients. During therapy, no neurotoxicities were observed, while the first cytokine release syndrome (CRS) and tumor lysis syndrome were observed for solid tumors. Future trials will be performed with combination strategies as well as CAR T cells with modified cytokine profiles and TGF-β resistance. Finally, Pule presented a set of data on the treatment of peripheral T cell lymphomas (PTCL) using CAR T cells.[18] Both the TCR beta constant domain subtypes (TRBC1 and TRBAC2) can normally be found in equal parts while the ratio drastically shifts to one subtype during PTCL. His group showed that CARs with TRBC1-specific scFv fragments (JOVI-1) can target $TRBC1^{+}$ primary malignancies *in vitro* and can specifically kill $TRBC1^{+}$ Jurkat cells in a xenograft mouse model.

Peter Borchmann (University Medical Center Köln, Köln, Germany) gave clinical insight into patient treatment with CAR T cells. He clearly stated that conventional therapy approaches for non-Hodgkin lymphoma are often not potent enough to increase the overall survival of patients. Borchmann presented results from the SCHOLAR-1 meta-study, which clearly emphasized the need for effective treatments of r/r DLBCL.[19] In this study the CR rate was 7% with a median survival of 6.3 months. In contrast to this, the ZUMA-1 phase II trial (28zeta CAR) against aggressive non-Hodgkin lymphoma (NHL) led to CR rates of 47% with an overall 24-month survival of 52%[20] compared to 20% seen in SCHOLAR-1. The JULIET phase II trial (41BBzeta CAR) against r/r DLBCL resulted in CR rate of 40%. Interestingly, patients in CR at 3 months remained in CR 6 months after treatment. Borchmann said that the higher CR rates could also result in cure, which still has to be proven in the following years. He further discussed the critical pitfalls of CAR therapies, such as biased study results due to the lack of representative enrolled patients, failure in manufacturing and cell product quality. In line with Bruce Levine's comment that complex patient data could further improve therapeutic efficacy, Borchmann raised the question as to who would run and be responsible for such databases collected outside of clinical trials. He concluded by claiming that the use of cost-intensive cellular therapies might initiate a cost-benefit discussion in the near future.

Counteracting immune escape

Tania Watts (University of Toronto, Toronto, Canada) briefly outlined the three signals required for T cell priming. The presence of various TNF receptors (TNFRs) suggests, however, that adaptive immunity requires costimulatory signals beyond CD28 and cytokines. In aiming to illuminate the of the role TNFRs play in controlling adaptive immunity, Watts reported that TCR signaling transiently induces 4-1BB expression in acute influenza infection.[21] Conversely, 4-1BB expression persists during chronic infection and is required for the survival of infected mice. Interestingly, this is accompanied by TGF-β-dependent desensitizing of the 4-1BB costimulatory pathway.[22] Watts concluded that the immune system is able to affect the duration of an immune response through 4-1BB signaling and that the shutdown of signaling is eventually needed to avoid pathology. Watts then shifted the focus to GITR and explained that control of viral infection is decreased in *Gitr*$^{-/-}$ mice through impaired CD4^{+} T cell help.[23] Looking closer into expression patterns of TNFR ligands, Watts found that GITRL divides antigen-presenting cells (APC) into two subsets. GITRL is preferentially expressed by inflammatory APC that also bear several other costimulatory ligands but are low in CD80/86 and MHC class II.[24] These differential patterns are regulated by cell-specific responses to type I IFN. Watts suggested that conventional dendritic cells (cDC) have high priming ability through MHC class II and CD80/86 expression, while inflammatory APC deliver late costimulatory signals. Concordantly, Watts confirmed that GITR costimulation is a post-priming event, which confers viral control through increasing prosurvival molecules, such as CD25 and OX40, leading to increased numbers of CD4^{+} and CD8^{+} T cells. Based on her findings, Watts proposed TNFR ligands could be considered as signal 4 during T cell activation.

Melody Swartz (University of Chicago, Chicago, USA) devoted her talk to the role of lymphangiogenesis in cancer. Tumor-traversing lymphatic vessels are not just important for trafficking of immune activation signals to draining lymph nodes, but also promote the activation of immunosuppressive stromal cells.[25] For the latter reason, lymphangiogenesis is a marker for poor prognosis in human cancers. In accordance with this, B16-OVA (B16-OVA/VC) tumors expressing high levels of lymphangiogenic vascular endothelial growth factor (VEGF)-C grow more aggressively compared to B16-OVA.[26] Swartz showed that immunosuppressive cell types are enriched in lymphangiogenic tumors, while blockade of the VEGF-C receptor VEGFR3 reverses accelerated tumor growth and reduces infiltration of immunosuppressive subsets. On the contrary, blockade of VEGFR3 is disadvantageous when B16-OVA/VC tumors are treated with different immunotherapies. This indicates that the tumor-promoting role of lymphangiogenesis can be reversed to potentiate the efficacy of immunotherapy and to promote antigen spreading, which is beneficial for successful therapy. Mechanistically, VEGF-C induces CCL21, which then leads to recruitment of naïve T cells and DC into lymphangiogenic tumors, enabling priming in the TME during immunotherapy. Of relevance, survival of patients receiving anti-PD-1/anti-CTLA-4 combination checkpoint blockade is positively correlated with VEGF-C expression, strengthening the validity of the preclinical data.

Jan P. Böttcher (Technical University of Munich, Munich, Germany) emphasized the importance of cDC1 for anti-tumor immunity and highlighted the role of prostaglandin E_2 (PGE_2) as an immunosuppressive mediator in the TME. In their most recent work, Böttcher and colleagues elucidated a previously unrecognized cross-talk between cDC1 and NK cells, which can lead to control of tumor growth but is suppressed by PGE_2.[27] Initially, Böttcher demonstrated that PGE_2 is required for the aggressive growth of BRAF^{V600E} tumors and the absence of PGE_2 in COX-deficient BRAF^{V600E} *Ptgs1/Ptgs2*$^{-/-}$ tumors favors cDC1-dependent growth control. His work demonstrated that not only are cDC1 are more abundant in BRAF^{V600E} *Ptgs1/Ptgs2*$^{-/-}$ tumors, but also a strong enrichment of NK cells co-localizing with cDC1 was evident. This raised the question whether accumulation of one cell type depends on the other. Indeed, Böttcher revealed that cDC1 recruitment to tumors depends on the production of the chemokines CCL5 and XCL1 by NK cells, which is directly inhibited by PGE_2. Unexpectedly, overexpression of CCL5 and XCL1 by tumor cells failed to rescue cDC1 recruitment in PGE_2-producing tumors, for the reason that cDC1 downregulate chemokine receptors in response to PGE_2, meaning that prostaglandin suppresses the NK cell/cDC1 axis on both ends. As a final point, Böttcher presented the analysis of human tumor biopsies, which established a close association of NK cells, chemokines and cDC1 in human tumors. Moreover, NK cell and cDC1 gene signatures positively correlate with patient survival in different cancer indications, underscoring a role for NK cell/cDC1 axis in cancer immune control in humans.

Tumor microenvironment

Although checkpoint inhibitors have achieved remarkable results treating different cancer entities in the clinic, there is a quest to expand the benefit of immunotherapy to a larger proportion of patients. **Andrea van Elsas (Aduro Biotech Inc., Oss, Netherlands)** reviewed the pathology of cancer biopsies associated with a lack of response to checkpoint inhibitors, consisting of a range of immune phenotypes including the complete absence of T cells, the presence of a non-functional immune response or exclusion of immune infiltrates.[28] In order to optimize cancer immunotherapy, the goal is to "heat up" "cold" tumors for T cell infiltration. Based on an understanding of the cGAS-STING pathway from different research collaborations, Aduro developed ADU-S100, a cyclic dinucleotide (CDN) and potent activator of the cGAS-STING (Stimulator of Interferon Genes) axis. The cGAS-STING pathway is naturally involved in cytosolic DNA sensing and upon activation, promotes the induction of type-I IFN gene response. Upon intratumoral injection, ADU-S100 led to potent rejection of B16 tumors,[29] superior to that seen on treatment with CpG 1668, Poly I:C and other TLR

agonists. The Gajewski lab demonstrated that the STING pathway is required for spontaneous rejection of immunogenic tumors, whereas the RIG-I and TLR pathways were dispensable.[30] Mode of action studies highlighted that ADU-S100 bridges innate and adaptive immune responses, inducing IFN-β and TNF-α secretion in tumor-resident immune cells, followed by enhanced presence of mature DC in local lymph nodes and generation of tumor antigen-specific $CD8^+$ T cells (data shown for the 4T1 and B16 tumor models), ultimately leading to systemic immunity. In collaboration with the Raulet lab (UC Berkeley), the efficacy of ADU-S100 was further assessed in MHC class I-negative tumors, revealing that ADU-S100 mobilizes a powerful anti-tumor response in the $\beta2m^{-/-}$ RMA, B16-F10-$\beta2m^{-/-}$ and CT26-$\beta2m^{-/-}$ models, being independent of $CD8^+$ T cells but relying on NK cells and $CD4^+$ T cells. Aduro and Novartis are currently investigating ADU-S100 as a single agent and in combination with anti-PD-1 (PDR001) or ipilimumab, as a first-in-human STING agonist.

Falk Nimmerjahn (University of Erlangen-Nürnberg, Erlangen, Germany) highlighted the importance of the mononuclear phagocytic system in cytotoxic IgG activity *in vivo*. Besides classical NK-mediated antibody-dependent cellular cytotoxicity (ADCC), tissue-resident or blood-derived monocytes and macrophages play an important role in target cell killing. In order to understand the contribution of tissue-resident versus bone marrow-derived macrophages for cytotoxic antibody activity (e.g. IgG-mediated B cell depletion in the liver),[31] he and colleagues designed a model system involving chimeric mice expressing organ-specific FcγR. When organ-resident $FcγR^{\pm}$ macrophages were depleted by clodronate liposomes, repopulation of the liver by Kupffer cells was observed to be bone marrow-dependent. FcγR expression on repopulating Kupffer cells was only observed in mice exhibiting FcγR+ bone marrow cells, indicating a central role of the organ environment for monocyte-macrophage differentiation and lesser to the origin of liver resident macrophages. Studies in B16 skin melanoma highlighted the dominant role of bone marrow-derived TAM for cytotoxic IgG activity, showing the crucial involvement of the CCR2-CCL2 axis to recruit inflammatory monocytes.[32] Furthermore, Nimmerjahn discussed the importance of CCR2-CCL2-recruited, bone marrow-derived monocytes for anti-CTLA-4 antibody-mediated intratumoral Treg depletion.

Combination therapies

After anti-PD-L1 antibody therapy (Atezolizumab), anti-cancer responses in urothelial bladder cancer vary among patients showing complete response, stable disease or progressive disease.[33] The reason why some patients respond to anti-PD-L1 and others not, comprises the central question addressed by **Shannon Turley (Genentech, San Francisco, United States)**. In order to expand the depth and duration of response, as well as to convert non-responders to responders, data from 429 patients with advanced metastatic urothelial bladder cancer were assessed regarding PD-L1 status, $CD8^+$ T cell localization, whole exome and transcriptome sequencing. Whereas tumor mutational burden, predicted neoantigens, pre-existing $CD8^+$ T cell effector signature and PD-L1 expression on tumor-infiltrating immune cells clearly associated with response to atezolizumab, TGF-β and TGF-βR expression were found to be negative correlates of response.[34] In addition, tumor sections were analyzed regarding the localization of T cells, classifying sections on the basis of the immune phenotypes T cell inflamed, T cell excluded and T cell desert.[35] In-depth analysis revealed that TGF-β and TGF-βR expression significantly differed in responders versus non-responders within the T cell excluded immune phenotype. Turley pointed out that in immune-excluded tumors, $CD8^+$ T cells reside in the stroma making direct contact with cancer-associated fibroblasts (CAFs) and collagen fibers. Using the orthotopic EMT6 mouse tumor model, exhibiting a T cell excluded phenotype, Turley and colleagues showed that therapeutic anti-TGF-β, together with anti-PD-L1, led to complete tumor regression.[34] Observed anti-tumoral effects clearly relied on effector $CD8^+$ T cells, which were able to infiltrate the tumor bed. In detail, therapeutic anti-TGF-β reduced SMAD2/3 phosphorylation in non-immune cells, reducing the fibroblast TGF-β response signature, preventing fibroblast differentiation, proliferation, deposition and cross-linking of extracellular matrix components. Mass spectrometry analysis of tumor collagen validated experimental findings, indicating a lower percentage of mature crosslinks after anti-TGF-β and anti-PD-L1 combination therapy. Mechanistically, dual therapeutic blockade serves to switch the tumor immune phenotype from an immune excluded one to that of an immune inflamed tumor.

Sandra Demaria (Weill Cornell Medical College, New York, United States) provided an in-depth peek into the immune adjuvant effect of radiotherapy. Whereas immune checkpoint inhibitors act on pre-existing immune responses, radiation possesses the ability to render immunologically cold tumors hot, by causing cell death and local inflammation.[36] Radiotherapy alone, however, is seldom able to act as an *in situ* vaccine and elicit anti-tumor immune responses that mediate systemic tumor rejection. Such responses manifest as regression of tumors outside of the irradiated field, known as abscopal responses. Abscopal responses have been more frequently observed when radiotherapy is combined with checkpoint blockade such as anti-CTLA-4, in patients otherwise not responsive to checkpoint blockade. In preclinical studies, Demaria and colleagues investigated the mechanisms whereby tumor-targeted radiotherapy promotes the activation of tumor-specific $CD8^+$ T cells, and found that the radiation dose and fractionation schema were critical for immune activation.[37] Systemic anti-tumor responses were achieved when anti-CTLA-4 was combined with single 8 Gy radiation doses repeated in 3 consecutive days but not with single high doses of 20 or 30 Gy.[38] Furthermore, cancer cells treated with 8 Gy x 3 accumulated cytosolic DNA that was sensed by cGAS, resulting in activation of STING and downstream production of IFN, which is required for the recruitment and activation of $BATF3^+$ DC. Depletion studies highlighted the crucial role of $CD8^+$ T cells, $BATF3^+$ DC and host IFNAR in the induction of abscopal responses by radiotherapy and checkpoint blockade. Moreover, they showed that high dose radiotherapy was unable to induce the production of type I IFN due to upregulation of Trex1, an enzyme responsible for degrading

cytoplasmic DNA, and thus preventing cGAS activation. In recent studies, currently in press, they also found that tumor-derived exosomes (TEX) transport TREX1-sensitive, IFN-stimulatory dsDNA from irradiated cancer cells to DC. In prophylactic vaccination studies, TEX derived from irradiated cancer cells induced protective anti-tumor immunity against the parent tumor cells, suggesting that TEX produced by irradiated cancer cells may contribute to the induction of anti-tumor immunity.

Jack Pollard (Sanofi Oncology, Cambridge, United States) presented preclinical and clinical studies using the anti-TGF-β antibody SAR439459 in combination with anti-PD-1 to block immunosuppression and increase therapeutic effectivity of immune checkpoint blockade. Using patient data mining, Pollard and colleagues identified mechanisms of innate anti-PD-1 resistance,[39] suggesting a role for TGF-β activation to pre-existing or acquired resistance to anti-PD-1 in melanoma patients.[40] Hypothesis testing of anti-TGF-β and anti-PD-1 combination therapy to increase therapeutic responses was performed by treating TGF-β-expressing MC38 murine colon carcinomas. Whereas 25% of tumor-bearing mice treated with SAR439459 alone exhibited complete tumor regression, combination therapy lead to tumor regression in 87% of mice. Mode of action studies confirmed that SAR439459 in combination with anti-PD-1 led to an increase of MIP2 and KC/GRO, both of which are chemokines involved in neutrophil and NK cell recruitment. SAR439459 as a single agent thereby increased cytotoxic T cells and NK cells in the tumor, and restored NK cell cytokine release and proliferation. Preclinical findings supported the launch of first-in-human studies applying SAR439459 as a monotherapy post-PD-1 failure and in combination with anti-PD-1 antibody, REGN2810, in patients with advanced solid tumors.

Immunomonitoring

Günter J Hämmerling (German Cancer Research Center, Heidelberg, Germany) drew attention to how ineffective current immunotherapy approaches are, since they work only in a subset of patients. Insufficient T cell infiltration into tumors is a major problem as it correlates with decreased patient survival and therapeutic success.[41,42] Hämmerling's lab is interested in how the immunosuppressive tumor microenvironment can be modulated in order to overcome patient-specific restriction of immunotherapy. He proposed a feedback amplification model in which macrophage-derived nitric oxide (NO) can be used to promote T cell infiltration into tumors. In this model, polarization of macrophages into the M1 phenotype through danger signals such as low dose 2 Gy radiation or TLR triggering leads to macrophage-mediated release of NO and vasculature normalization. This enhances expression of cell adhesion molecules on blood vessels, thereby facilitating transmigration of T cells into the tumor. The incoming T cells produce IFN-γ, leading to more polarization of M1 macrophages, thereby establishing a positive feedback loop. This process is accompanied by a decrease in tumor hypoxia, resulting in higher T cell activity.[43,44] This model is supported by patient data from various cancers, showing that a higher intratumoral presence of M1-skewed $CD68^+$ $HLA\text{-}DR^+$ $iNOS^+$ macrophages is correlated with clinical outcome.[45–47] Based on these results and observations that successful immunotherapy usually correlates with M1 macrophage skewing, Hämmerling asserted that M1 macrophage polarization acts as a master switch for T cell mediated tumor rejection and is an important target for successful immunotherapy. He further focused on the hitherto unknown effect of tumor eosinophilia. Co-transfer of eosinophils with $CD8^+$ T cells led to rejection of tumors, which was not reached with either $CD8^+$ T cells or eosinophils alone. Mechanistically, eosinophils increased T cell infiltration by chemokine production, vasculature normalization and reduced hypoxia, showing their potential for clinical application.[48] Similar to eosinophils, basophils were also found to enhance T cell infiltration.[49]

About 10 years ago, the role of complement in cancer was merely considered as auxiliary to antitumor antibodies, but **Dimitrios Mastellos (National Center for Scientific Research 'Demokritos', Athens, Greece)** showed that this is a rather more intricate relationship. Indeed, he showed data illustrating that complement can potentiate cancer therapy. This is either based on augmenting antibody-mediated cytotoxicity, via highly ordered hexameric antibody aggregates, or T cell immunotherapy.[50–52] On the other hand, imbalanced complement activation can lead to initiation of inflammation and tumorigenesis by inducing an immunosuppressive microenvironment.[52,53] Mastellos elucidated one specific example, where complement factor C5a was shown to mediate high fat diet-induced intestinal inflammation and neoplasia. Reduction of these adverse effects was reached by complement inhibition (C5aR blockade), showing the potential of complement-targeting therapeutics.[54,55] Another complement factor, C3, was also found to be required for homing of effector T cells to tumors and tumor suppression.[51] Furthermore, radiotherapy induced local complement activation in the tumor, which enhanced T cell infiltration, thereby potentiating anti-tumor immunity.[56] Focusing on clinical applications, Mastellos presented that complement modulation by inhibition of C3aR or C5aR together with immune checkpoint blockade can reverse tumor immunosuppression and effectively restrain tumor progression.[57–59]

Genomic instability is one of the hallmarks of cancer and leads to an increased mutation rate, which can shape the evolution of the cancer genome and lead to immune escape.[60,61] **Nicholas McGranahan (Cancer Research UK Lung Cancer Centre of Excellence, London, United Kingdom)** presented preliminary results from the TRACERx consortium, which is a multi-center UK-wide prospective study, including multi-region sequencing of primary non-small cell lung cancer (NSCLC) tumors, from diagnosis to relapse. Within this study, McGranahan showed that intratumoral heterogeneity (ITH) is evident in early stage NSCLC and occurs at both mutational and copy number level. Based on these results, he asked whether clonal architecture could be used as a biomarker. Results from the TRACERx study confirmed that high copy number ITH is associated with significantly reduced disease-free survival in early stage NSCLC.[62] In previous studies, neoantigen burden was identified as another marker.[63] McGranahan presented data suggesting sensitivity to checkpoint blockade was enhanced in tumors enriched for clonal neoantigens.[63] Finally, he

presented LOHHLA, a tool to decipher allele specific HLA copy number, and demonstrated that HLA loss of heterozygosity is a common mechanism of immune escape in lung cancer evolution.[64]

Therapeutic vaccination

Jolanda de Vries (Radboud University Medical Center, Nijmegen, the Netherlands) asked the question whether tumor antigen-loaded natural DCs are a powerful next generation vaccine. Initially, she presented results from a retrospective study, which showed favorable overall survival in stage III melanoma patients after monocyte-derived adjuvant DC vaccination.[65] Moreover, in the first approved myeloid DC (mDC)-based vaccination study for prostate cancer, median survival benefit of 4.1 months was observed, showing that this approach is feasible to induce an immune response in cancer patients.[66] de Vries then drew attention to plasmacytoid DC (pDC) vaccination as an alternative approach. Since preventative vaccines are GMP-quality products activating cells via TLRs, they are used for improved maturation of pDCs. Commercial FSME-IMMUN vaccine mediated improved maturation of pDCs derived from patients, led to upregulation of MHC class I and II as well as activation markers CD80, CD83 and CD86 on these cells. Moreover, IFNα production was induced. Employing these tumor-specific neoantigen-loaded pDCs as a vaccine led to a better overall survival and furthermore, 50% of patients in this group survived two years.[67] Finally, de Vries presented an approach of combined mDC and pDC vaccination after improved maturation. This approach resulted in a robust IFN response and increased number and magnitude of tumor antigen-specific T cells without causing severe side effects. In order to further characterize this approach, a multicenter randomized phase II/III melanoma combined pDC and mDC vaccination trial with 210 patients is currently running.

Özlem Türeci (Biontech, Mainz, Germany) presented an individualized RNA-based cancer vaccine approach. Her team mapped the mutanomes of several syngeneic mouse tumors, determined all mutations with predicted binding to MHC class I and systematically tested a comprehensive fraction of these as vaccines against the respective tumor. A large portion of mutations was found to induce immune responses and also mediate rejection of the mouse tumors they were identified in, establishing mutations as a rich source for cancer antigens.[68] Surprisingly, the vast majority of neoepitopes were recognized by $INF\gamma^+$ CD4 rather than by CD8 T cells.[69] Based on these data, her team developed an RNA vaccine containing two pentatopes with mutated epitopes recognized by CD8 and CD4 T cells. In mouse tumor models vaccination with this multi-antigen vaccine led to disease control and survival benefit and reshaped the tumor microenvironment towards an inflammatory phenotype. Prediction of neoepitope MHC II binding affinity and RNA expression level was implemented in a pipeline for vaccine design, which was translated into the clinic for individualized cancer mutanome vaccination.[70] In the first-in-human study of this concept in patients with resected or metastatic Stage III and IV melanoma, intranodal administration of this pharmaco-immunologically optimized mRNA vaccination induced immune response against multiple mutations in each and every patient including a frequent induction of CD4 T cells and memory formation.[71] Moreover, significant reduction of cumulative metastatic recurrence was observed. Türeci also pointed out various challenges neoepitope vaccination is facing which include knowledge-driven optimization of computational pipelines for prediction and ranking of neo-epitopess, determination of the most suitable clinical setting and synergistic combinations as well as shorter vaccine manufacturing turn-around time. She closed her presentation by showing further progress of the RNA-based vaccination approach: systemic targeting of lymphatic compartments via physico-chemically optimized formulation, combination with checkpoint blockade and with non-HLA dependent immune-oncology drugs.[72,73]

Since checkpoint blockade is only effective in a subset of cancer patients, vaccination is one possible strategy to enhance its therapeutic efficacy. Vaccine protection and specific site delivery are critical parameters to consider in product formulation. **Yared Hailemichael (MD Anderson Cancer Center, Houston, United States)** called attention to his work showing that formulation dictates synergy of vaccination with checkpoint blockade therapy. Investigating a combination of ipilimumab and gp100 peptide, formulated with incomplete Freund's adjuvant (IFA), vaccination exhibited lower overall survival than checkpoint blockade alone.[74] Hailemichael and his team observed that this effect is formulation-dependent.[75] In combination with anti-CTLA-4 treatment, effector T cells are sequestered at vaccination sites and undergo apoptosis, resulting in loss of tumor control. Effector T cell sequestration involves a feed-forward loop mediated by effector T cells, inflammatory monocytes, ICAM-1 and CXCR3/CCL2 chemotactic pathways. As a result, very few primed T cells actually reach the tumor. He then asked the question whether a non-persistent formulation would synergize with checkpoint blockade therapy. He showed that this is the case, when combining vesicular stomatitis virus encoding gp100 and CTLA-4 or PD-L1 blockade after early treatment and in the setting of primary resistance. In the absence of a persistently inflamed vaccination site, effector T cells are free to localize to the tumor site.[76]

Keynote lecture

Tremendous efforts have been invested in order to illuminate the dynamic system of cellular interactions in immunology. Nonetheless, wide areas of cellular communication remain cryptic. Considering that immune cells comprise a very minor population in large tissues, how are the appropriate cells able to find each other in such complex environments? **Ronald Germain (NIAD, NIH, Bethesda, United States)** dedicated his keynote lecture to the principle that "seeing is believing" and demonstrated how he makes use of highly elaborate imaging techniques to address this fundamental question directly *in vivo*. His perception is that cellular interactions happening just by chance might be too rare to drive functional immune responses. Consequently, rules must exist that determine cellular contacts. In this regard, Germain and his group employed two-photon excitation-based intravital

microscopy to elucidate dynamic interactions of innate and adaptive immune cells and provided examples for the aforementioned interactions.

Breaching of the epidermis, which constitutes the first defense layer of many multi-cellular hosts, triggers an innate immune response. By imaging the skin after epidermal breach, Germain elucidated the trafficking of neutrophils and macrophages that sequentially home to the site of tissue destruction in order to re-establish the barrier function of the epidermis, clear the damaged tissue and ultimately resolve the inflammation.[77,78] His videos showed quick reaction and particularly directed migration of the cells in the complex tissue environment, suggesting that obscure signals must exist to guide immune cells. The proinflammatory response, marked by extensive neutrophil swarming, eventually faded, but was reinvigorated by induction of novel tissue damage. This observation raised new questions of how signals are integrated in order to shape the time course of the immune response from onset to decay. Adding further complexity, tissue damage does not trigger a proinflammatory response *per se*. In fact, the damage intensity controls the strength of proinflammatory signaling since small lesions are cloaked by tissue-resident macrophages, preventing the spread of damage signals. The physiological reason for this might lie in the fact that an inflammatory response causes further tissue damage before it is resolved, which is not favorable when microlesions – comprising only a few cells – need to be cleared.

Moving on to the third defense layer, adaptive immunity, Germain explained that priming of an adaptive response must be more sophisticated than the accepted idea of T cells wandering around in lymph nodes until they meet an APC that happened to present their cognate antigen. The challenge of the immune system is to get relevant T cells, B cells and APC together in time and space. Lymph nodes are organized by stromal structures that are different in T cell and B cell zones. More video evidence showed associated lymphocytes and APC migrating along the same stromal network, which facilitates relevant cellular contacts. In addition, the movements are directed by chemokine gradients. Hence, the cells are more likely to meet their appropriate interaction partners. This is crucial, since $CD4^+$ and $CD8^+$ T cells are primed by different DC subsets.[79,80]

Altogether, two-photon intravital microscopy provides precious insights into cellular interactions of the immune system, yet the method is restricted in terms of color spectrum and the missing possibility to perform whole-organ imaging. To overcome this limitation, Germain and his group developed an analytical microscopy method called histo-cytometry, which enables the staining of up to 14 different markers in whole tissue sections.[81] After having revealed dynamic interactions of APC and T cells, the scope of analysis was expanded to anatomical localization of the cells in the lymph nodes. Germain elucidated the asymmetric distribution of DC and T cell subsets within the lymph node, where the location of $CD4^+$ and $CD8^+$ T cells matches the position of their respective DC interaction partners.[82]

Nevertheless, a lot of information is left uncaptured by using thin tissue sections for staining. In order to extend the boundaries of the possible even more, Germain and his group keep on improving imaging techniques. To investigate the location of cells by histo-cytometry in whole organs, they developed the Ce3D method for tissue clarification, which preserves the cellular and tissue structures and allows for high multiplex antibody staining.[83] Implementation of further improvements will eventually enable the three-dimensional visualization of an unprecedented multitude of parameters in complex, intact tissues. There is no doubt that future advances in the field of imaging will be valuable to untangle the complex interaction of immune cells during acute inflammation, pathology and immunotherapy.

Conclusion

Advances covered at CIMT2018 and summarized in this report clearly indicate how the field has matured in the last years and that cancer immunotherapy is now regarded as a potent therapy against cancer. We anticipate that new frontiers will be set for the coming years, from which we will hear at the fourth CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference (September 30–October 3, 2018 in New York City, USA) as well as 17th Annual CIMT Meeting (May 21–23 2019, Mainz, Germany)

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed

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