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MEETING REPORT

The right patient for the right therapy: 13th Annual Meeting of the Association for Cancer Immunotherapy (CIMT), Mainz, Germany, May 11–13, 2015

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Abbreviations

ACT	Adoptive cell transfer
ALL	Acute lymphoblastic leukemia
CAR	Chimeric antigen receptor
CIMT	Association for Cancer Immunotherapy
CLL	Chronic lymphocytic leukemia
CSF-1R	Colony stimulating factor 1 receptor
CTL	Cytotoxic T lymphocytes
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CTx	Chemotherapy
DC	Dendritic cells
EMA	European Medicines Agency
EORTC	European Organization for Research and Treatment of Cancer
FDA	Food and Drug Administration
GM-CSF	Granulocyte macrophage colony-stimulation factor
HLA	Human leukocyte antigen
HMGB-1	High-mobility group box-1
HNPCC	Hereditary nonpolyposis colorectal cancer
HPV	Human papilloma virus
HSCT	Hematopoietic stem cell transplantation
IDH1	Isocitrate dehydrogenase 1
IFN	Interferon
IHC	Immuno-histochemistry
IL	Interleukin
iNOS	Inducible nitric oxide synthetase
mAb	Monoclonal antibody
MDACC	MD Anderson Cancer Center
MDSC	Myeloid-derived suppressor cells
MEK	MAP kinase kinase
MHC	Major histocompatibility complex
MMP-2	Matrix metalloproteinase-2
MMR	Mismatch repair
MSI	Microsatellite instability
mTEC	Medullary thymic epithelial cells
NGS	Next-generation sequencing
NHL	Non-Hodgkin lymphoma
OS	Overall survival
OVA	Ovalbumin
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PD-1	Programmed death-1
PDAC	Pancreatic ductal adenocarcinoma
PFS	Progression-free survival
RIP	Rat insulin promoter
RNA	Ribonucleic acid
SLP	Synthetic long peptide
TAM	Tumor-associated macrophages
TCGA	The Cancer Genome Atlas
TCR	T cell receptor
T_{eff}	Effector T cell
TGF	Transforming growth factor
TIL	Tumor-infiltrating lymphocytes

This meeting report is a summary of presentations from the *Thirteenth Annual Meeting of the Association for Cancer Immunotherapy*, CIMT 2015, published together with a series of Focussed Research Reviews based on lectures given at the conference.

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TLR	Toll-like receptor
TNF	Tumor-necrosis factor
T_{reg}	Regulatory T cells

Introduction

The 2015 Annual Meeting of the Association for Cancer Immunotherapy (CIMT) maintained the trend of the last CIMT meetings and with 840 participants attracted more scientists than ever before. Altogether, 77 speakers presented their data and the diverse areas of research in the field of cancer immunotherapy were discussed after the talks or in front of well over 250 posters. The overall topic was the targeted use of cutting-edge immunotherapeutic approaches by preselecting patients for a given therapy leading to the title "The right patient for the right therapy." In this review, the highlights of the meeting are comprehensively summarized focusing on emerging trends in the field.

Personalized immunotherapy

The first session of this year's CIMT meeting with the title "Personalized Immunotherapy" perfectly underlined the theme of the meeting. Until now, only little success has been achieved in fighting all cancers with only one drug, it has been increasingly realized that owing to the high genetic heterogeneity, therapies are required that target a tumor in one given patient and that are not hampered by, but rather harness the individual tumor's characteristics. **Ton Schumacher** (The Netherlands Cancer Institute, Amsterdam, the Netherlands) presented impressive data showing that T cells specific for mutation-containing epitopes can be an important effector population in the anti-tumor immune response. When he and his group sequenced a melanoma patient's tumor exome, they identified sequences containing mutations that were predicted to translate into immunogenic peptides. For one putatively immunogenic sequence, the group could show via flow cytometry-based multimer analysis of cytotoxic T lymphocytes (CTL) that a pronounced population of mutation-specific T cells was detectable in a patient with stable disease. Remarkably, that CTL population was further expanded upon treatment of the patient with the checkpoint inhibitor ipilimumab, a monoclonal antibody directed against the membrane protein CTLA-4. These data indicate that mutation-specific T cells might benefit from the administration of checkpoint inhibitors. Similar populations of $CD8^+$ T cells with reactivities toward the individual patients' mutations were detected in 8 out of 10 patients, clearly demonstrating that individual genomic features can lead to the mounting of an immune response. Having focused on only the CTL compartment until then, Ton Schumacher in the next part of his talk expanded the analysis to the $CD4^+$ T cells. The group switched to a functional readout in which they used 31mer peptides with a non-silent mutation in the center, flanked by 15 wild-type amino acids. Cumulatively, the group detected $CD4^+$ T cell responses in 4 out of 5 patients. With the presented data so far, only the presence of mutation-specific T cells could be shown. The group thus tried to correlate the vast amount of potentially immunogenic mutations with the clinical response. Analyzing a panel of patients with non-small-cell lung cancer who received anti-PD-1 antibody treatment, they could correlate a high mutational load in a patient's tumor with the response rate toward anti-PD-1 treatment. Accordingly, Ton Schumacher speculated that a high number of mutations increases the likelihood that one mutation exhibits highly immunogenic features and can potentially drive the tumor response. Since these mutation-specific T cells are highly activated in the microenvironment, they are further likely to develop an exhausted phenotype and display a surface expression of PD-1. Thus, to harness a patient's mutation-specific T-cell response for efficient tumor therapy, a combination with checkpoint inhibitors might be required.

Touching on the very same field, the next speaker in the session was **Ugur Sahin** (BioNTech AG and TRON Translational Cancer Center, Mainz, Germany). He expanded the discussion toward a RNA-based actively personalized cancer vaccine approach that exploits the cancer mutation spectrum (mutanome) of individual cancer patients. Ugur Sahin and his co-workers previously introduced a genomics-based blueprint approach for therapeutic exploitation of individual mutations and proved in preclinical models that therapeutic vaccinations with such neo-antigens can lead to effective tumor rejection. In parallel, a sophisticated process was developed that allows for the next-generation sequencing (NGS)-based identification of a given patient's tumor mutanome. The identified mutations are subjected to a bioinformatic analysis pipeline that prioritizes the mutations according to several parameters such as expression and the binding affinity toward the patient's HLA molecules. After the selection of the best-suited neoepitopes, these are incorporated into a RNA backbone that was optimized to exhibit potent pharmacological activity. Based on this concept, Ugur Sahin presented data from the first-in-man trial, in which RNA molecules coding for multiple patient-specific mutations are injected intranodaly into melanoma patients. The presented preliminary data from the established immune monitoring program showed the impressively strong induction of $CD4^+$ and $CD8^+$ T cell immune responses against multiple vaccine encoded neoepitopes. Having shown the effectiveness of the vaccine, Sahin next provided an outlook to the up-coming developments of BioNTech RNA Pharmaceuticals GmbH.

Thus, to take the introduced approach one step further, the next step is the clinical development of a liposome-based formulation for intravenous RNA vaccine application. **Robert Holt** (British Columbia Genome Sciences Center, Vancouver, Canada) completed the panel of speakers of this first plenary session. He analyzed the large amount of data that are available on the servers of The Cancer Genome Atlas (TCGA) and focused on the detection of T cell receptor (TCR) signatures and profiles in the sequence reads of tumor specimen. After the analysis of TCR profiles in situ, he drew the conclusion that by far the most T cells present in a given sample are not tumor-specific, but are rather infiltrating by chance into the malignant tissue. He next touched on studies that his group had performed in a model of murine ovarian cancer, the ID8 system. Here, the group could identify potentially immunogenic mutations in the sequence reads of the tumor's exome and assessed these for immunogenicity. An astonishingly high number of mutations (more than 70 %) were confirmed in vitro to potently induce an immune response. However, when these mutations were used in an adjuvant or therapeutic setting, none of them exhibited an anti-tumoral effect. Holt discussed these results and pointed out the prerequisite that for an efficient detection of tumor cells, the complex of the MHC and the respective peptide might not have been present in the animal model. He emphasized the necessity to thoroughly select and validate mutanome-based vaccines to assure that cognate peptide MHC complexes can be presented to effector cells in situ. The session was making a clear point that a personalization of tumor therapy is exhibiting substantial potential to improve the prognosis of cancer patients. At an astonishing pace, impressive data have been generated and it is exciting to think about the progress to be presented on the CIMT 2016.

Cellular therapy

The second plenary session of the meeting then switched gears and moved to the quality of effector cells that can be used to treat cancer. The first talk of the session started with a presentation on how to equip effector cells with new or modified receptors and thus antigen specificities such as chimeric antigen receptors (CARs) or TCRs. Currently, retroviruses are most commonly used for doing so. On behalf of Laurence Cooper, **Harjeet Singh** (Pediatrics, UT MD Anderson Cancer Center (MDACC), Houston, USA) presented an interesting alternative to this viral gene transfer that bears the risk of uncontrolled insertions into the DNA of cells. He introduced the so-called Sleeping Beauty system that was pioneered by Perry Hackett and which consists of two plasmids, one containing a specific transposon, the transgene of choice, and the second one the transposase both of which are co-transfected by nucleofection to allow integration of the gene of interest at specific dinucleotide sites into the target cell's genome. The group from MDACC used the Sleeping Beauty technology to generate effector cells for several clinical trials with a second generation anti-CD19 CAR. With an innovative strategy, they expanded CAR-transduced T cells by co-culturing them with K562-derived antigen presenting cells which resulted in preserved telomere length and a desired heterogeneous phenotype. 99.9 % of the Sleeping Beauty-mediated insertions occurred at the expected dinucleotides and the minority were intragenic. With that, an approximately 100-fold expansion of T cells was achieved within 28 days. Several patients were treated in different settings for non-Hodgkin lymphoma (NHL), acute lymphoblastic leukemia (ALL) or chronic lymphocytic leukemia (CLL), and tumor responses were observed, with the best results in adjuvant treatment after auto-hematopoietic stem cell transplantation (HSCT). Notably, for up to 20 days, high levels of transferred T cells were detectable by a CAR-specific PCR and after a decline, the transferred cells could still be found at low levels after 200 days. However, CAR-specific T cells could not be detected by flow cytometry indicating that expression and stability of the CAR in vivo may yet need to be optimized. To improve the persistence, the next generation of their CAR T cells will be co-transfected with IL-15 receptor alpha—IL-15 fusion protein to provide an additional third signal. This strategy has already resulted in increased persistence and in vivo anti-tumor activity in a xenogeneic mouse model. As a long-term perspective, the group is aiming at developing their approach toward an allogenic off the shelf therapy to enable faster and more wide-spread treatment of patients. The second speaker in this session was **Paul Robbins** (National Institutes of Health, Bethesda, USA) who talked about the identification of targets that can mediate effective cancer immunotherapy. His group works on cell therapy approaches with a focus on tumor-infiltrating lymphocytes (TIL). Next to studies assessing correlates of persistence of TIL, he and his group are interested about the quality of antigens that can mediate durable tumor regression. He mentioned that studies that used differentiation antigens as targets for melanoma show rather a limited anti-tumor efficacy, along with a varying level of toxicity. This toxicity is attributed to the fact that the antigen-specific T cells also attack antigen-positive healthy melanocytes. However, responses in adoptive cell transfer (ACT) trials with NY-ESO-1 as the harnessed target have returned promising results in several indications, showing that this class of antigens can elicit a potent anti-tumor response. However, the expression frequency of cancer-testis antigens in tumors is often low and the success of TIL and checkpoint inhibitors in conjunction with current studies, suggest that neo-antigens may be the most

promising overall targets. Although mutation-derived neoantigens are usually not shared between different patients, Paul Robbins and his group are exploring ways how to harness a mutation-derived target for the treatment of several patients. Such strategies can include cellular therapy or vaccine-based approaches. This presentation was bridging to the session personalized immunotherapy. Suitable targets can be identified, and the respective specific receptors used to generate high numbers of effector cells. Combining multiple approaches in order to optimize existing treatments was a topic that was present throughout the entire conference underlining the necessity to combine efforts and forces to develop novel potent therapies. The last speaker of this plenary session was **Stephen Schoenberger** (La Jolla Institute for Allergy and Immunology, La Jolla, USA) who presented about the issue of off-tumor but on-target autoimmunity in the context of adoptive cell therapies. In this respect, he and his group used flow cytometry-based sorting of multimer-stained SIINFEKL-specific T cells that they isolated from a RIP-mOVA model (OT-3). Here, due to the expression of the transgene OVA in the medullary thymus epithelial cells (mTEC), central tolerance mechanisms are in place and thus only low-affinity TCRs toward SIINFEKL pass the selection, whereas OT-1 T cells exhibit a higher-affine TCR. To investigate how the TCR affinity toward a given target impacts the anti-tumor and off-tumor response, Schoenberger and his group used a transplantable mouse model (ID8-OVA-luc). They transplanted luciferase-transduced murine ovarian cancer cells into RIP-mOVA animals that, in addition to mTEC, exhibit an expression of the OVA antigen in the pancreas and the kidneys. Accordingly, high-affine OVA-specific T cells induced a diabetes type I-like phenotype in addition to the anti-tumor effect. However, the low-affinity TCRs derived from the RIP-mOVA model still lead to a transient tumor control without the induction of diabetes-like symptoms. Based on these data, Stephen Schoenberger concluded that depending on the scenario, it can be a useful tool to fine-tune the effect of ACT by adjusting the affinity of the harnessed TCRs.

Improving immunity

After the discussions of highly personalized immunotherapeutic approaches and how to ideally select its effector cell population, the third plenary session now moved on to another topic. After a thorough selection of the antigen and the effector cells, there is still a need to exploit the effector potential in an ideal way. To address possible drawbacks that could hamper an efficient anti-tumor response upon immunotherapeutic intervention, three speakers presented their ideas about how to further optimize therapeutic approaches in order to improve immunity. The first speaker in this session was **Guido Kroemer** (Gustave Roussy Cancer Campus, Paris, France) who contributed substantially to the development of the concept of immunogenic cell death. The concept he presented states that tumor cells that are killed by distinct chemotherapeutics can elicit a potent immune response. This idea is in contrast to the long-held theory that apoptosis is immunologically silent. Accordingly, he proposed that an effective chemotherapy in large parts is dependent on its stimulation of the immune system. He based this hypothesis on several preclinical models as well as patient data, showing that immunodeficient or immunocompromised individuals do not benefit to the same degree from chemotherapy compared to individuals whose functionality of the immune system is unhampered. Key players in the induction of this immunogenic cell death are several soluble factors released by the tumor cells upon induction of the immunogenic cell death such as certain chemokines like CXCL8, HMGB1 that binds to TLR4 and ATP that binds to P2RX7. He further shared some recent data showing that autophagy is crucial for the induced release of ATP since autolysosomes are orchestrating the recycling of ATP, which in turn is important for immune cell recruitment to the site of tumor cell death. Importantly, he showed that knock out cell lines for ATG5, a key component of the autophagy pathway, exhibit an accelerated in vivo growth. This effect is due to the HIF-1-induced expression of ENTPD1 (CD39), a prominent exonucleotidase. The hydrolysis of ATP or likewise a defective release of the nucleotide by defects in the autophagy system subsequently locally recruits regulatory T cells (T_{reg}), hampering an efficient recognition and destruction of the tumor cells by other immune effector cells. He finished his presentation with patient data from a breast cancer cohort. Importantly, the group found that the amount of autophagic spots as detected by LC3B punctae correlated with patient prognosis and negatively with T_{reg} numbers. The need to identify means to improve therapeutic outcomes by a thorough understanding of intracellular pathways was impressively stressed in this presentation. The second speaker of this session was **Bruno Kyewski** (German Cancer Research Center, Heidelberg, Germany). He presented parts of his work on the mechanisms of central thymic tolerance. His talk addressed the need for a detailed understanding of how tolerance is induced and he raised interesting issues about the selection of antigens for tumor vaccination. Bruno Kyewski was largely involved in the discovery of AIRE-mediated promiscuous gene expression in medullary thymic epithelial cells (mTEC) and mentioned that, with few exceptions such as sensory genes, for example, basically all genes such as fetal antigens or even pregnancy-related antigens are expressed in mTEC and thus induce central tolerance either by negative selection of highly affine TCRs or induction of T regulatory cells. These

mTEC largely randomly express an average of 1–3 % of all ubiquitously expressed genes per cell and thus in their entire pool cover all of these genes. Using single-cell RNA sequencing, his team discovered that only 300 randomly sorted cells jointly cover 80 % of all expressed genes. Interestingly, mTEC are described to not properly initiate the transcription for MART-1, for example, leading to defective negative selection. Accordingly, since no negative selection is established in the thymus for non-translated T cell epitopes, highly affine TCRs pass through selection and get released into the periphery. Findings like these should be considered when selecting antigens for potent tumor vaccines. Yet, despite the importance of this field for tumor immunotherapy, many open issues still require an in-depth analysis. Here, Kyewski mentioned that, for example, non-coding RNAs seem to be involved in differential splicing patterns in mTEC but little is known about the mechanism. Ongoing efforts might shed more light onto how the release of effector cells is fine-tuned and can thus potentially identify "suitable" or "unsuitable" target structures for immunotherapy. The last speaker in this session was **Jolanda de Vries** (Radboud University Medical Center, Nijmegen, Netherlands). She presented her work on mutation-derived neo-antigens. She focused on hereditary nonpolyposis colorectal cancer (HNPCC), which due to inherited or acquired defects in mismatch repair (MMR) genes and the resulting microsatellite instability (MSI) frequently exhibit frame-shift mutations, often resulting in neo-antigens. This disease, also called Lynch syndrome, constitutes around 5 % of all colorectal carcinomas. During her presentation, she explained that in PBMC of Lynch syndrome patients, immune reactivity toward at least one mutated antigen was observed. These data impressively show that errors in the DNA repair machinery and the resulting mutations can generate epitopes toward which immune responses can be mounted. She next expanded her talk on how to target these mutations using DC loaded with peptides encoding mutations from TGF-beta II receptor and caspase 5 resulting in elevated frequencies of antigen-specific T cells. The presented data nicely complemented the previous discussions further pointing out that an efficient treatment of cancer requires a thorough analysis of the patient's disease and a tailored therapy.

Tumor vaccination

Integrating several aspects from other sessions of this year's CIMT meeting, the talks in this plenary session summarized important recent findings using different vaccination approaches to treat cancer. **Michael Platten** (German Cancer Research Center, Heidelberg, Germany) started by emphasizing once more the suitability of mutation-derived neo-antigens for tumor immunotherapy. Being truly new to the patient's immune system, the immunogenicity of those antigens is believed to be in the range of viral antigens. In line with this hypothesis, Michael Platten presented the work of his group on the isocitrate dehydrogenase-1 (IDH1) mutation IDH1R132H and the attempt to treat glioma patients with an innovative vaccination approach. The acquisition of this mutation constitutes an early and driving event in the genesis of the majority of diffuse gliomas. Owing to the extreme heterogeneity of gliomas, few general targets exist besides IDH1R132H which is expressed in more than 80 % of grade II and III gliomas. Further, escape mutants might not occur as frequently as for instance when vaccinating against mutations in EGFRvIII, due to the early acquisition of the mutation rendering it a very suitable immune target. Analyzing immune reactions against epitopes carrying the IDH1R132H mutation, he and his group detected a prominent, mutation-specific MHC class II-restricted T_H1-type response in MHC-humanized A2.DR1 transgenic mice carrying IDH1R132H-positive sarcoma lines. Further, they found significant reductions in the tumor burden of animals, when they vaccinated with peptides against this epitope. Compared to a classical $CD8^+$ T cell-restricted immune reaction, the tumors did not disappear but presumably entered a state of senescence. Bridging these preclinical findings to the clinics, Michael Platten discussed findings in which IDH1 mutation-specific MHC class II-restricted T cell and antibody responses were found in patients with IDH1R132H-mutated glioma. Based on these exciting data, a phase I trial was set up to investigate the efficacy of peptide vaccinations targeting this mutation. Platten emphasized in the end the importance of the selection of a suitable target since escape mechanisms will efficiently prevent a sustained anti-tumor effect if not a crucial structure is vaccinated against. **Nina Bhardwaj** (Mount Sinai School of Medicine, New York, USA) illustrated strategies how to target innate immune cells in cancer immunotherapy. She first pointed out a novel role of matrix metalloproteinase-2 (MMP-2) in tumor immunology. Her group found that melanoma patients have MMP-2-specific TIL showing a T_H2 phenotype. This could be explained by an effect of MMP-2 on dendritic cells: MMP-2 cleaves type I IFN receptor and binds TLR2, leading to reduced IL-12 production, OX40L up-regulation, and induction of TNF-alpha and IL-6. This T_H2 skewing, implying a negative prognosis, was abrogated in TLR2-deficient mice. Nina Bhardwaj suggested that this MMP-2-mediated immune dysregulation might be attenuated, for example, by clinically impactful listeria vectors, which are potent activators of dendritic cells. Second, she focused on NK cells, which often show signs of exhaustion in melanoma, such as down-regulation of cytokine receptors (IL-2R, IL-15R, IL-18R), changes in transcription factors like eomesodermin

and T-bet, and over-expression of exhaustion markers. She presented Tim-3 as exhaustion molecule on NK cells. Its up-regulation is partly mediated by CEACAM-1, which is co-expressed and dimerizes with Tim-3. Anti-Tim-3 antibodies were able to partially reverse NK cell exhaustion, restoring activation markers like NKG2D. Surprisingly, also ipilimumab, an anti-CTLA-4 antibody, reversed NK cell exhaustion. In the third part of her presentation, she reported on trials of intratumoral poly-IC:LC, one of the best inducers of IL-12 and IFN from human dendritic cells. For example, studies led by Dr. Joshua Brody at Mt Sinai found that intratumoral FLT3 ligand for dendritic cell attraction, followed by poly-IC:LC for dendritic cell activation, resulted in tumor remission and loss of circulating tumor cells in some cases. Intratumoral poly-IC:LC is now being tested in trials involving several solid cancers. Finally, she reported on trials testing the usefulness of several adjuvants and combinations thereof upon therapeutic vaccination of resected melanoma patients with an NY-ESO-1 protein vaccine. With imiquimod, weak antibody and CD4 responses, but no CD8 responses were induced. CpG led to strong antibody and CD4 responses, and to CD8 responses in 50 % of cases. Resiquimod combined with Montanide led to antibody responses, polyfunctional T cell responses (IFN-gamma, IL-2, TNF-alpha), and a T cell response with a CD8 component. By contrast, Montanide alone led to a weaker antibody response and less polyfunctional T cell responses, leaving CD8 T cells unaffected. This compares well with another trial involving 4 long peptides from NY-ESO-1, combined with either Montanide alone or Montanide plus poly-I:C, the latter leading to more CD8 responses. Various trials are ongoing in the adjuvant setting in melanoma, combining checkpoint inhibitors with vaccines, to elucidate efficacy and safety of protein versus long peptide vaccines and to compare different adjuvants. **Sebastian Kreiter** (TRON, Mainz, Germany) expanded on one of the former presentations by Ugur Sahin and presented detailed insights into studies on RNA-based vaccination approaches targeting the tumor's mutanome. He and his group used RNA molecules coding for mutations that were detected in B16F10 melanoma tumors in a preclinical model using next-generation sequencing (NGS). Surprisingly, 20–30 % of all mutations tested in the B16F10 model were detected by CD4 T cells, while only 4–10 % were targeted by CD8 T cells. Up to 75 % were not recognized by T cells at all. RNA vaccination against a mutated MHC class II-restricted epitope in Kif18b led to functional tumor-infiltrating T cells and significantly improved the survival of the tumor-bearing animals. The tumor infiltration of CD4 and CD8 T cells was increased, while the numbers of myeloid-derived suppressor cells (MDSC) and T_{reg} were reduced. Remarkably, only the in vivo depletion of $CD4^+$ but not of $CD8^+$ T cells abrogated the anti-tumoral effects of the vaccine. Likewise, in the CT26 model, RNA vaccination against a mutation in the aldehyde dehydrogenase led to strong anti-tumoral effects mediated by CD4 T cells. Both mutations addressed in these two models were in non-functional parts of the respective proteins and thus probably no driver mutations. Owing to the tumor heterogeneity and immune editing, Sebastian Kreiter suggested the use of multiple mutations in the form of RNA pentatopes. These contain mutated epitopes which are connected by non-immunogenic linkers and inserted into an invariant RNA vaccination cassette, allowing for translation and optimal antigen processing. He showed that vaccinating with two pentatopes was highly efficient in the CT26 model with respect to prolonging the survival and increasing the tumor infiltration by CD4 and $CD8^+$ T cells. Remarkably, pentatopes led to synergistic effects as compared to monotopes regarding the reduction in lung metastases after vaccination. Notably, he could show that in the CT26 tumor model, the CD4 T cell-mediated anti-tumor effect critically depended on CD8 T cells, and that this was mediated via the CD40-CD40L axis. Epitope spreading of CTL epitopes could be observed within $CD8^+$ T cells isolated from spleens of tumor-bearing vaccinated mice. Finally, he showed that in silico pentatope selection for use in vivo should include selection for high expression of the antigen in tumors, as well as good MHC binding of the potential epitope.

Keynote address

This year's keynote address was given by **Alexander Eggermon**t (Institut Gustave Roussy, Villejuif, France). The director of the Institut Gustave Roussy in his presentation put together an impressive and comprehensive story of how the treatment of cancer in general and malignant melanoma in particular developed in the last few years. Several drugs have been successfully developed for melanoma, many of which rely on the mutation-prone nature of this disease. He started his talk with the development of targeted tyrosine kinase inhibitors such as vemurafenib. This agent targets a prominent mutation in the *BRAF* gene, namely the V600E mutant that is present in around 60 % of melanomas. Selecting patients who harbor the respective mutation is a very prominent example of how this year's CIMT meeting moto can lead to significant benefits in clinical applications. Yet, despite increasing the progression-free survival (PFS) as well as the overall survival (OS), these targeted agents only show transient effects. However, the delineation of the molecular pathways led to the clinical development of a combinatorial approach. The combination with MEK inhibitors that target the MEK kinase downstream of BRAF further

improved the survival of melanoma patients nicely showing the often-cited transition from bench to bedside. Eggermont made a clear point about the necessity to dissect molecular interplays and that combinatorial therapies are clearly able to improve the prognosis. The next wave of cancer therapeutics that only recently entered routine application in the clinics is the tumor immunotherapeutics. Eggermont guided through the developments that finally led to the breakthrough status for this novel concept of cancer treatment. The success story of tumor immunotherapy started with the development of ipilimumab, a monoclonal anti-CTLA-4 antibody. In a physiological context, the receptor CTLA-4 dampens immune responses, also the responses toward tumor tissue. The application of the antibody leads to a less pronounced inhibition of T cells and thus pen ultimately to their activation. First studies showed that this inhibition of the inhibitor is a potent mean to achieve major clinical success in the treatment of late-stage malignant melanoma in terms of OS. Further, as data from a large phase III trial of the European Organization for Research and Treatment of Cancer (EORTC) showed, ipilimumab also significantly prolonged the RFS upon treatment of completely resected stage III melanoma patients yet at the cost of serious side effects. Having wrapped up the story of ipilimumab, Eggermont introduced the antibodies nivolumab and pembrolizumab which target another checkpoint receptor, namely PD-1. Physiologically, CTLA-4 is a key player in negatively regulating the activation of T effector cells in secondary lymphoid tissue, whereas PD-1 inhibition is largely affecting T cell activation and proliferation in situ. Owing to the nature of the receptor, far less severe side effects were observed in clinical trials using anti-PD-1 antibodies. Most notable, however, is the largely increased OS of 16.8 months and an almost 50 % PFS rate at 6 months when administered biweekly. This new class of antibodies provided a treatment option for patients who are $BRAF^{V600E}$ negative, regardless of the expression of the most prominent ligand PD-L1, in situ. These results led to an increase in the interest in tumor immunotherapy and leading to the entitlement of drugs of the year for nivolumab and pembrolizumab. Several scientists have then tried to expand this success story in the treatment of melanoma to other entities as well and succeeded. Several investigations in non-small-cell lung cancer, gastric cancer and refractory renal cancer—to name only three of them—showed that the suppression of tumor-specific T cells via the PD-1—PD-1 ligand axis seems to be an indication-independent immune escape mechanism. Accordingly, the prognosis of cancer patients could be improved in those trials. At the end of his talk, Eggermont gave an outlook on what will be important to address in future studies. He made a clear point that combination therapies can change the way cancer will be treated in the future. So a potentially efficient vaccination schedule, for example, can benefit from accompanying checkpoint inhibition, the depletion of regulatory cells such as regulatory T cells or MDSC can further improve the impact of immunotherapeutic interventions. A precise understanding of molecular pathways, the interplay of immune cells and an understanding of the tumor microenvironment can thus lead to the design of efficient therapeutic interventions.

Tumor microenvironment

Most of the talks given at this year's CIMT were focusing on identifying suitable targets for immunotherapy and were intensively discussing how to select or generate potent effector cells for anti-tumor therapy. Yet, the effector cell constitutes only one component in the decision if the tumor immunotherapy of choice is efficient or not. Often tumors establish an immunosuppressive environment, for example, by recruiting or re-differentiating immunosuppressive or effector cells, respectively. This plenary session was focusing on how to modulate this environment in order to improve the efficacy of immune effector cells. **Dominik Rüttinger** (Roche Innovation Center, Penzberg, Germany) presented data about a phase I first-in-human trial of Emactuzumab (RG7155), an antibody directed against the CSF-1R that can be found on tumor-associated macrophages (TAM). By binding to CSF-1R, this mAb potently abrogates the monocyte and macrophage recruitment to the tumor site and decreases the survival of M2 TAM. Macrophages of the M1-phenotype, which is known to exhibit anti-tumor effector functions, are increased in numbers owing to the fact that their differentiation is triggered by GM-CSF and not by CSF-1 signaling that is blocked by Emactuzumab. The trial with the identifier NCT01494688 Dominik Rüttinger was discussing is currently recruiting patients with pigmented villonodular synovialitis (PVNS) and other solid malignancies and will evaluate safety and tolerability of the mAb, and the response according to RECIST criteria is evaluated. To date, a total of 29 patients have been recruited that proved the ability of Emactuzumab to induce durable responses leading to symptomatic and functional improvements in the treated patients with facial edemas being the most frequently adverse events observed. Notably, the numbers of $CSFR1^{+}$ and $CD163^{+}$ M2-like TAM were significantly decreased in 35 out of 37 patients, with various solid malignancies as well as breast or colorectal cancer proving the mode of action of the mAb. Summarizing the observed effects upon treatment with Emactuzumab, Rüttinger delineated that the investigators could find (1) a clear, dose-dependent reduction in CSF-1R-positive cells, (2) a decrease in the circulating $CD14^{dim}$ $CD16^{bright}$ monocyte

population and (3) as detected by IHC, a reduction in macrophages in surrogate skin tissue as detected by CD68/CD163 stainings. Concluding his presentation, Rüttinger made a point about possible combination strategies involving Emactuzumab. Since the mAb treatment leads to a decrease in tumor-resident macrophages and alters the CD8/CD4 T cell ratio in favor of the $CD8^+$ T cells, combinations, for example, with checkpoint inhibitors, may potentially lead to a synergistic effect and thus to improved tumor control. The second speaker of the session was **Niels Halama** (National Center for Tumor Diseases (NCT), Heidelberg, Germany) who presented data on the question if T cell infiltration in colorectal cancer can control the development of distant metastasis in the liver. He and his group could show that the relevant T effector cell (T_{eff}) population is often not properly infiltrating into the center of the metastasis but rather is located at the invasive tumor margin in the respective sections although there is substantial heterogeneity to be found between individual patients. Correlating these infiltration data with the clinical outcome, Niels Halama found that a high density of margin-resident T cells is predictive of the response toward chemotherapy (CTx) with only 5 % of the T cells being in close contact with the tumor epithelium. Importantly, he and his team could show that a differential chemokine regulation leads to the accumulation of effector T cells at the invasive tumor margin prior to their infiltration into the tumor tissue. Halama then switched gears and asked the question of if this T cell trapping is relevant for the prognosis of colorectal carcinoma patients. In a phase I trial with an undisclosed immunomodulatory drug, he could show for five patients that have not responded to CTx before that there was a response to CTx detectable after the chemokine trap has been interfered with. These data prove the importance of targeting mechanisms established in the tumor microenvironment that can hinder successful tumor immunotherapy. The third and last speaker of the microenvironment session putting an emphasis on tumor necrosis factor (TNF) signaling was **Philipp Beckhove** (German Cancer Research Center, Heidelberg, Germany). In line with the previous speaker, he started his presentation by emphasizing the importance of a targeted alteration of the tumor microenvironment toward a less immune suppressive state to foster T cell responses and thus improve the efficacy of immunotherapies. He was speculating on the correlation of T cell infiltration and the patient's prognosis and mentioned that an improved prognosis could be due to the infiltration of tumor antigen-specific T_{eff} or the activation of these T cells in situ. Since in colorectal carcinoma, the expression of TNF is virtually restricted to antigen-stimulated TIL, the TNF positivity can be harnessed as a marker for tumor-specific, activated T_{eff}. However, TNF-expressing cells are scarce in the tumor microenvironment and most frequently, they belong rather to the myeloid lineage like macrophages. Beckhove next was bridging these ideas to a clinical setting and showed that only patients that had a history of a systemic tumor-specific T cell response show TNF expression in situ. He further mentioned that from the point of view of a clinician, TNF is the most promising prognostic marker as evaluated from a cohort of 102 colorectal carcinoma patients. However, Beckhove was pointing out to the issue of defective infiltration of antigen-specific T cells, for example, by vascular anergy that can effectively prevent a potent anti-tumor immune response. He continued to show data on the normalization of disrupted vessel structures by local low-dose irradiation. Notably, he and his group could demonstrate that macrophages are causative in mediating the irradiation effect, since depletion of those effectively prevented the beneficial effects of the irradiation. Further, they could link the vessel normalization to the inducible form of the nitric oxide synthetase (iNOS) that was up-regulated in macrophages upon irradiation leading to the normalization of the vessel structure and subsequently to an improved infiltration of antigen-specific T effector cells. In the second part of his presentation, he showed data on a high-throughput screening approach in which he and his colleagues tried to identify novel immunomodulatory ligands. In a luciferase-based in vitro killing assay, they could identify that CCR9, a chemokine-receptor with expression on several tumor entities with unknown function as a putative novel checkpoint inhibitor. Thus, they could demonstrate that the blockade of CCR9 could induce a more pronounced TNF secretion, STAT activation and granzyme B secretion from T_{eff}. Further, screening the cell lines from several entities, Beckhove could demonstrate that receptors, potentially serving as checkpoint inhibitors, exhibit only very little overlap between different entities. Accordingly, different pathways might have to get identified and harnessed for different entities. In this session, a clear picture was drawn that successful immunotherapy requires the targeting of multiple players. As outlined by this year's key note lecturer, Alexander Eggermont, combinatorial approaches will drive the next wave of tumor immunotherapeutics. A more and more precise understanding of basic molecular pathways and the interplay of various cell types in the microenvironment will be key to the development of tailored combinatorial approaches.

Combination therapies

An emerging topic that was touched in almost every talk and discussion so far was the necessity to create synergistic effects by combining several strategies. This last plenary session was dedicated to this topic. The session was started by **Holbrook Kohrt** (Stanford School of Medicine, Stanford, USA) who talked about alternative immunotherapy

combinations, a popular topic at this year's CIMT. He mentioned that the overall survival rates of cancer patients treated with anti-CTLA-4 and anti-PD-1 with approximately 88 % 2-year overall survival are very high. However, the therapies with >400,000 dollars per course are expensive and can induce severe toxicity. Holbrook Kohrt argued that one thus should continue to investigate alternative therapies and presented a concept in which he used an anti-4-1BB (CD137) antibody to stimulate NK cells. For his studies, he used an ADCC system in which cetuximab, an anti-EGFR antibody, causes NK cell activation toward EGFR-positive tumors. In vitro, the specific lysis of the tumor cells was massively increased when cetuximab treatment was used in combination with the anti-CD137 antibody. Intriguingly, in mouse models, the number of $CD137^{+}$ NK cells rose exponentially after anti-CD137 treatment when combined with monoclonal antibody treatments such as rituximab, trastuzumab or cetuximab. Clinical trials using the anti-CD137 antibody urelumab against NHL have shown a more prominent NK cell infiltration into the tumor. Further, a reduced state of T cell activation was detected although also a CD4 and CD8 memory response was expanded. Holbrook Kohrt mentioned that other phase I trials are currently ongoing and that in general NK cell-based therapies harbor the advantage of MHC class I independence and could thus complement T cell-based strategies. Next in line was **Patrick Mayes** (GlaxoSmithKline (GSK), Collegeville, USA) who continued the combination therapy theme by presenting GSK data on the combination of the BRAF inhibitor diabrafenib and the MEK inhibitor trametinib. He showed impressive data, proving the superiority of a combined usage of the two drugs in a phase III trial in patients exhibiting the $BRAF^{V600E}$ mutation. Used together, dabrafenib and trametinib showed a survival benefit in melanoma patients over the current standard care. After the treatment, TIL were found to express higher levels of the exhaustion markers PD-1 and TIM-3, while also secreting IFN-gamma. The MAP kinase chain of signaling that is inhibited by dabrafenib and trametinib stays silenced by the treatment; however, the PD-L1 expression in the tumor increases after 10 days or more of treatment. These data show that, rather than from the inhibited MEK pathway, the PD-L1 expression actually comes from other signaling pathways. As a matter of fact, the acute exposure of the tumor to dabrafenib and trametinib causes PD-L1 downregulation, but only chronic exposure leads to PD-L1 up-regulation, resulting subsequently in T cell exhaustion. In preclinical models, the addition of anti-PD-1 antibodies likewise improved the treatment efficacy, especially when dabrafenib and trametinib were added first, followed later by the anti-PD-1 mAb. Clinical trials addressing this combination are ongoing. Once more it became clear that combination therapies can and should not only target one pathway, but need to be designed based on a fundamental knowledge to target escape variants right away. Along the line of the previous speakers in this session and as a matter of fact of the conference, **Engin Gürlevik** (Hannover Medical School, Hannover, Germany) stressed the importance of combining synergistic therapies. In his presentation, he discussed pancreatic ductal adenocarcinoma (PDAC), an entity that with a 10-year survival rate of 1.1 % is one of the cancers with the worst prognosis. Currently, a complete resection upon early detection is the only efficient treatment modality with adjuvant gemcitabine helping to increase progression-free survival times. Since also completely resected patients are very likely to relapse, new treatment modalities are desperately needed. Engin Gürlevik presented his results derived from a new mouse model mimicking resectable PDAC. In these animals the postoperative situation can be evaluated in vivo. In this model, he found that when gemcitabine was combined with an anti-PD-1 therapy in an adjuvant setting postsurgery, the survival time of the mice was actually decreased, but when the same combination was administered prior to surgery, the survival of the animals could be greatly improved, with local tumor recurrences being decreased. Gürlevik could correlate this improved prognosis with a mutation-specific CD8 T cell response toward the $Lama4^{G1254V}$ mutant. Remarkably, the control of metastatic disease was found to be largely performed by NK cells, since depletion of these cells led to an increase in lesions in distant sites. This control required the combination of gemcitabine with the blockade of the NK cell checkpoint receptor CD96 to prevent binding to its ligand CD155 on the PDAC cells. Concluding his presentation, Engin Gürlevik emphasized that combinations of agents do not only improve the outcome of therapy of one lesion, but might further be required to target local and distant lesions simultaneously, and that a proper schedule might need to be investigated to generate the highest efficacy. The last speaker of this session and of the conference was **Sjoerd van der Burg** (Leiden University Medical Center, Leiden, the Netherlands) who explored synergisms between a vaccination with synthetic long peptides (SLPs) and several chemotherapy regimens. To do so, he used the HPV-16 E7-positive lung cancer cell line TC-1 in a preclinical transplantation model. The SLPs targeting the E7 protein *per se* are effective in stimulating an immune response, and van der Burg tried to combine the vaccine with several classical chemotherapeutic agents. Of those, especially the combination with cisplatin led to a significantly better tumor control. The underlying mechanisms were a T cell-derived TNFa-enhanced apoptosis of tumor cells, when treated with cisplatin, as well as a change in the intratumoral myeloid cell compartment toward a more pro-inflammatory environment. Van der Burg and his team found that the SLP vaccine-driven tumor regression

required a population of pro-inflammatory intratumoral macrophages, prohibiting the use of currently developed macrophage depleting agents instead of chemotherapy.

CIMT Immunoguiding Program (CIP) session: computational tools for flow analysis

Many of the present scientists, to a varying degree, are using flow cytometric analysis to work on their respective topics. Owing to the abundant use of this method in tumor immunotherapeutic investigations, the CIP session, in which novel tools for the automated analysis of flow cytometry data are presented, evolved as an important and well-received workshop of the CIMT meeting. A welcome address of this CIP symposium was given by **Pia Kvistborg** (The Netherlands Cancer Institute, Amsterdam, the Netherlands) and **Marij Welters** (Leiden University Medical Center, Leiden, the Netherlands), who also reviewed the activities and the progress of the CIP in the last year. They pointed out to the need for the harmonization of immunological assay protocols. The inter-institute harmonization has been a primary goal of the CIP, and this year the NK cells and MDSC have been highlighted. Another area that has substantially moved on is the use of TCR-engineered reference samples to standardize multicenter clinical trials. Further, a consensus on a minimally required marker set for the detection of T_{reg} by flow cytometry has been established in the last year. Having focused on assay standardization, it was discussed that the next year will be switching gears and that automated flow cytometry gating will face important novel developments. **Ryan Brinkman** (British Columbia Cancer Agency, Vancouver, Canada) presented the wide variety of open-access online computational tools that are available for the automated analysis for flow cytometric data. These programs, such as OpenCyto and flowdensity, yield a similar gating quality as when manual analyses are performed to flow cytometry data. Further, FlowCAP has been comprehensively testing these tools and has evaluated the available software tools. Yet, the complexity of many of those tools is big and the training to use these often takes around 1 year for an average immunologist, so bioinformaticians will be required to perform automated flow cytometry analysis in the future. Similar to FlowCAP, EuroFlow is focusing on automated gating tools for the analysis of flow cytometric data. **Tomas Kalina** (Charles University Prague, Prague, Czech Republic) here emphasized that EuroFlow aims at standardizing flow cytometry in order to allow for a subsequent automated computational analysis. The standardization of analysis by implementing, for example, general standard operating procedures or the application of bead-based cytometer setups can further improve the accuracy and precision of assays, meaning that data can be compared to other time points, study centers or even clinical studies.

Voice of the patients

For the first time in history of the annual CIMT meeting, a patient reported to the scientific community his experiences as a participant in a clinical trial. The possibility to receive feedback from a former trial patient was well received, and his story and clinical course were presented in an interview-like format. The trial participant provided invaluable insights into a clinical trial from a patient's view and emphasized that study-relevant information in the patient information sheet is hard to understand for a layman and that it would be appreciated if these forms were presented in a more understandable way. Further, he mentioned that online databases indicating what trials are currently recruiting could facilitate decision making for individual patients and could lead to a tight cooperation and interaction of the treating physician and the patient.

CIMT Endeavour

The concept of CIMT Endeavour is to organize and build up a unique and focused education and networking platform that addresses the translation of cancer immunotherapies from preclinical proof of concept to products for patients. Excellent science and technology sets the foundation for a successful commercialization, but specific knowledge about the intellectual property situation, how to raise money and how to compose a complete team are equally important. This year, two scientists from the CIMT community had the opportunity to share their research and in return receive feedback from the experts **Ute Fink** (Projektträger Jülich, Jülich, Germany), **Thomas Hanke** (Evotec AG, Hamburg, Germany), **Michael Kring** (High Tech Corporate Services GmbH, München, Germany), **Ioannis Sapountzis** (Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein, Germany) and **Rainer Wessel** (CI3 Clustermanagement, Mainz, Germany) with regards to the prospects of future commercialization and product development. Furthermore, **Dolores J. Schendel** (Medigene AG, Martinsried, Germany), **Øyvind Arnesen** (Ultimovacs AS, Oslo, Norway) and **Claus Kremoser** (Phenex Pharmaceuticals AG, Ludwigshafen, Germany) reported on their experiences and what they have learned in translating a scientific concept into a company (Trianta Immunotherapies GmbH, Ultimovacs AS, and Phenex Pharmaceuticals AG, respectively).

Conclusions

Throughout large parts of the meeting, the harnessing of mutation-derived neo-antigens as well as combinatorial approaches, mostly involving checkpoint inhibitors, was in the center of attention. Impressive successes have been achieved since last year's CIMT meeting, and the potential synergisms that can be unlocked by releasing the brake of the effector cells together with the identification of highly immunogenic targets are exciting. Two years after the Science magazine granted cancer immunotherapy the "Breakthrough of the year status", more and more pieces of data pile up and emphasize the clinical relevance of harnessing the patient's immune system to control malignant diseases. The effect sizes that are possible led to the approval of several agents such as the anti-PD-1 antibodies by the FDA and EMA and allowed tumor immunotherapy to be applied as a standard-of-care in advanced diseases. The continuous increase in participants of the CIMT meeting further reflects the attention the strategies pursued in the field are having, and it is fascinating to think about the up-to-come achievements to be presented on the CIMT 2016 in Mainz from May 10 to 12.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. Björn-Philipp Kloke has been co-organizer of the CIMT Endeavour workshop.