CIMT 2015: The right patient for the right therapy - Report on the 13th annual meeting of the Association for Cancer Immunotherapy

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Introduction

The 13th Annual Meeting of the Association for Cancer Immunotherapy (CIMT) brought together more than 800 scientists in Mainz, Germany, from May 11–13, 2015, to present and discuss current research on various fields of cancer immunotherapy. Special focus was set on personalized approaches, and independent of the specific therapeutic strategy, the exploitation of mutated neoantigens predominated all sessions - in line with the motto of this year’s meeting, “The right patient for the right therapy.”

Personalized Immunotherapy

Cancer is an extremely heterogeneous disease, and accumulating evidence in the field of tumor immunology suggests that a “one-for-all” therapy is likely to be inefficacious for the majority of patients. Although there is hope that immune checkpoint inhibitors such as Ipilimumab or PD-1/PD-L1 blocking antibodies serve as a universal weapon against cancer, it has become more and more clear that there are inherent rules that determine the therapeutic success or failure of such treatments. Understanding the peculiarities of a patient’s tumor may allow for specific targeting of “weak spots” with an individually tailored therapy which can still be combined with the aforementioned novel drugs.

Ton Schumacher (Netherlands Cancer Institute (NKI), Amsterdam, The Netherlands) kicked off the theme session by hypothesizing that mutation-specific T cell responses are the driving force in effective immunotherapies such as checkpoint blockade. For this reason, his group screened for CD8+ T cell responses against neoepitopes using labeled MHC multimers.1 A strong immune response against a mutated antigen identified via exome and RNA sequencing was detected in a melanoma patient that showed a partial clinical response after anti-CTLA-4 (Ipilimumab) treatment,2 and this pre-existing CD8+ T cell response was further increased by Ipilimumab administration. In a second patient showing a complete response after re-infusion of tumor-infiltrating lymphocytes, a more than 5,000-fold increase in 2 pre-existing neoepitope-specific CD8+ T cell responses was detected. In total, 8 out of 10 melanoma patients analyzed so far developed mutation-specific T cells. Wondering about the existence of neoepitope-specific CD4+ T cells, Schumacher and colleagues tested the recognition of 31-mer peptides that covered individual mutations in vitro expanded CD4+ T cells of melanoma patients.3 Again, neoantigen-specific T cell responses were detected in 4 out of 5 patients (against 8 mutations in total). Furthermore, Schumacher assumed that if neoantigen-specific T cells were crucial effectors in cancer immunotherapies, the number of mutations should correlate with clinical effects. Indeed, it was shown for anti-PD-1 antibody treatment of non-small cell lung cancer patients4 that the mutational load correlated with treatment success. Schumacher explained this outcome with a simple probability model: the more mutations, the higher the likelihood of a neoantigen-specific T cell response. These findings might explain why checkpoint blockade and tumor-infiltrating lymphocyte (TIL) therapy are especially successful in cancers with a high number of mutations such as melanoma and lung cancer, and point toward a combination with treatment modalities that induce novel or enhance pre-existing T cell responses against mutated antigens.

In this regard, Ugur Sahin (BioNTech AG, Mainz, Germany) presented an innovative next-generation sequencing (NGS)-based approach to target the cancer mutanome by personalized therapeutic RNA vaccination. Already in 2012, Sahin and coworkers presented a first proof of concept for personalized vaccination.5 Identification of mutated antigens in tumor biopsies is a challenging task as sequencing data can be biased by contamination of healthy tissue, necrotic cells and by tumor heterogeneity. As a
Consequence, Sahin’s team developed algorithms for the reliable and accurate identification of somatic mutations in NGS data outperforming existing methods (manuscript in preparation, 6-7). Once identified, mutations are prioritized according to parameters like MHC binding prediction as well as mRNA abundance and subsequently designed as pharmacologically optimized mRNA. 8-10 Therapeutic vaccination with mutation-encoding RNA was shown to elicit potent tumor control in mice (for details refer to Kreiter, Therapeutic Vaccination). Sahin presented 2 methods for the application of RNA currently tested in phase I clinical trials: (i) direct ultrasound-guided injection into lymph nodes (NCT020035956, 11), and (ii) intravenous injection of liposome-complexed RNA (NCT02410733, manuscript under submission). In both cases, the antigen-encoding RNA is internalized, translated and processed by dendritic cells that stimulate antigen-specific CD4+ and CD8+ T cells. Sahin concluded with exciting preliminary results from a first-in-men clinical trial in melanoma patients (NCT020035956), where 5 patients that had each received tailored intranodal RNA vaccination against 10 mutations revealed an immunogenicity of 48 % of all tested mutations.

Further analyzing neo-antigen-specific T cell responses, Robert Holt (British Columbia Cancer Agency, Vancouver, Canada) dissected the plethora of sequencing data collected by The Cancer Genome Atlas. Investigation of the intratumoral landscape of TCR sequences revealed that the TCR diversity correlates with HLA class II expression and that the bulk of tumor-associated T cells is most probably bystanders and not tumor-specific. In the second half of his talk, Holt presented data on the immunogenicity of mutations identified via exome and RNA Sequencing. In accordance to recent work by Kreiter et al., 12 a high number of predicted mutations (15/21) from the mouse ID8 ovarian epithelial tumor was immunogenic and, despite prediction for MHC class I binding, generated CD4+ T cell responses after peptide vaccination. Unfortunately, none of the induced T cell responses exhibited antitumoral effects in prophylactic or therapeutic settings. Holt suggested that this might be due to the lack of cognate peptide:MHC complexes (pMHC) and pointed out that there is a need to match TCRs to their cognate pMHC for an effective mutanome-based vaccine approach.

**Cellular Therapy**

Constitutive expression of genetic information in human cells is still a safety issue for molecular and cell therapy approaches. Harjeet Singh (MD Anderson Cancer Center, Houston, TX, USA) and his colleagues have been intensively working on improving the Sleeping Beauty (SB) gene transfer system 13,14 as well as CD19-specific second generation chimeric antigen receptors (CAR). 15 Using artificial APCs, 16 Singh and his colleagues generated almost 100 %-expressing CAR+ patient T cell populations within 28 d of manufacture, which showed no reduction in telomere length. Comparing expression levels of hundreds of genes in T cells of patients before and after SB modification revealed differential regulation in only 37 genes. The minority of transgene insertions occurred intragenically and when this was the case, the CAR was integrated predominantly inside introns. As appears typical for T cells expressing CARs that activate via CD28 and CD3ζ, the persistence of the infused product lasted about 28 d. This may be due to the CAR design and the apparent lack of supportive cytokines in the recipients. To address this issue, a membrane-bound IL15R-IL15 fusion protein (mIL-15) was coexpressed with the CAR, which led to an increase of in vivo persistence in immunocompromised mice, even in the absence of antigen-expressing tumors. mIL-15-modified CAR-expressing T cells exhibited a superior in vivo performance against CD19+ tumors compared to T cells expressing the CAR alone. Concluding his talk, Singh emphasized that the results of his group present the non-viral SB gene transfer system as a cheap and safe method for cellular immunotherapy.

While working on genetic modification of infused T cells, the laboratory of Paul F. Robbins (Surgery Branch, National Cancer Institute, Bethesda, MD, USA) has also been extensively studying the use of TILs in the clinical setting, trying to decipher the key characteristics of T cells important for a successful infusion and persistence in human patients. It has previously been shown that isolation, expansion and infusion of TILs for adoptive cell therapy (ACT) can be very beneficial in melanoma patients, independent of treatment history. 17,19 Besides some experimental data for renal cell carcinoma. 20,21 however, such strong effects have so far not been seen in other types of cancer. Robbins and his group used whole exome sequencing approaches coupled with tandem minigenes coding for non-synonymous mutations, 22 and a high-throughput pMHC screening method 22 to identify CD4+ TIL subpopulations specific for neoantigens of Erbb2IP in a patient with cholangiocarcinoma. 23 When TILs encompassing 25 % antigen-specific CD4+ T cells were infused, tumor regression of 30 % starting after one month, and a stable tumor burden for around 13 months after infusion was observed. In a second treatment cycle, a 95 % pure antigen-specific T cell population led to an even earlier onset of tumor mass decrease and a continuous shrinkage of lesions. Robbins concluded with presenting the future challenges for ACT, ranging from improvement of tumor-reactive T cell isolation, development of new engineering techniques of tumor-reactive TCR+ T cells, strategies to target tumor heterogeneity and development of vaccination approaches, to targeting of cancer neoantigens.

The first part of Stephen Schoenberger’s (La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA) talk focused on the balance between anti-self vs. anti-tumor activity in the context of ACT. His group has been conducting research with the Rip-mOVA mouse model24 expressing a membrane-bound ovalbumin (OVA) in the pancreas, kidney and in medullary thymus epithelial cells (mTEC). Transfer of high affinity OT-1 CD8+ T cells induced type 1-like diabetes in these mice. Mice infused with a low affinity OVA-specific CD8+ T cell population isolated from Rip-mOva mice (OT-3) also developed diabetes-like symptoms when systemically encountering OVA-expressing Listeria monocytogenes (Lm-OVA). Transplanted OVA+ ovarian carcinoma (ID8OVA-Luc) induced proliferation and an effector phenotype
of transferred OT-3 T cells in Rip-mOVA mice, and OT-I cells reduced tumor burden only slightly better as the low affinity OT-3 cells. Interestingly, infusion of high affinity OT-I T cells but not OT-3 cells into tumor-bearing mice resulted in diabetes-like symptoms, indicating that functional avidity as well as local activation may be exploited to avoid autoimmunity while retaining antitumor efficacy.

In line with previous data published by his group, Schoenberger presented mechanisms of CD4-independent CD8+ T cell generation in the second part of his talk. He demonstrated that the requirement of CD4+ T cell help for CD8+ T cell priming was dependent on antigen dose, as CD4+ T cells were only necessary for OVA-specific CD8+ T cell expansion in response to low doses of Lm-OVA, and the absence of CD4+ T cells even increased the CD8+ T cell response at high doses. In the presence of low but not high antigen doses, blockade of the CD40/CD40L axis inhibited IFN-γ secretion by OVA-specific CD8+ T cells, emphasizing the dual role of CD4+ T cells as helpers and regulators. Whereas the depletion of FoxP3+ CD4+ regulatory T cells (Treg) in the FoxP3-DTR model revealed that, independent of the antigen dose, Treg cells constrain the primary CD8+ T cell response, CD4+ T cell help was only required at low doses to maintain CD8+ T cell responses in the absence of Treg cells. Investigating the role of different TLRs in Treg cells, they found that at high but not at low doses, Treg cells depend on TLR9 signaling for efficient expansion. Schoenberger summarized his data by emphasizing the antigen dose dependency of CD4+ T cell help for CD8+ T cell priming and the dual role of CD4+ T cells in modulating this process, also in a time-dependent manner.

Improving Immunity

Guido Kroemer (Center de Recherche des Cordoliers, Paris, France) opened the session with the statement that effective cancer therapies are de facto immunotherapies, setting the tone for his talk. Based on his and the work of collaborators, he showed that the antitumoral effect of several chemotherapies relies on immune-dependent effects missing in immune-deficient mice. These results were reproduced in cancer patients with concomitant immunodeficiency syndromes. The underlying mode of action as discovered by Kroemer and Zitvogel is dissected in the concept of immunogenic cell death and its consequences. One major step in the course of events leading to antigen release and DC activation is autophagy in the tumor cell. Autophagy is primarily a mechanism for the sequestration and lysosomal degradation of damaged organelles and invading microorganisms, but also for the promotion of survival by recycling of cellular components. Utilizing knock-out models for an essential autophagy gene (ATG5), Kroemer and co-workers demonstrated the relevance of ATG5 for the natural and chemotherapy-induced immunosurveillance in tumor models. In the second half of his talk, he presented concepts aiming at harnessing this mechanism therapeutically by autophagy induction. Starting from molecular pathway analyses of autophagy regulation, the experimental validation of several caloric restriction mimetics (CRM) inducing autophagy demonstrated improved immunosurveillance of cancer in mice. Bridging to the human setting, Kroemer showed that in breast cancer patients, a negative correlation exists between autophagy in the tumor and Treg infiltration. The clinical translation of the discovered principles during the coming years will hopefully add further therapeutic options to cancer therapy.

Since preventive vaccines against infectious diseases are one of the most successful measures in medicine, the development of a preventive cancer vaccine is desirable but difficult to translate. Jolanda de Vries (Radboud University Medical Center, Nijmegen, The Netherlands) reported an innovative approach to harness adaptive immune responses against predicted mutations in hereditary cancer through vaccination with antigen-loaded blood-derived DCs. Conceptually she focuses on the Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC), an inherited disease that increases the risk of many types of cancer, particularly colorectal carcinoma (CRC). Causative for the disorder are germline mutations in mismatch repair genes which lead to microsatellite instability (MSI) and frame-shift mutations. It has already been known for some years that patients with HNPCC have a higher T cell infiltration into the tumor tissue as compared to sporadic CRC which is assumed to be the result of neoepitopes created by frame shifts. Genes with known coding microsatellites (cMS) provide a predictable source of tumor-specific neoepitopes that can be used for vaccination. The team developed a clinical trial concept based on the prior experience with DC vaccines focusing on HLA-A*02:01 positive patients. The first phase of clinical testing was performed in Lynch carriers suffering from CRC, and further clinical trials will be started to expand this approach.

Tumor Vaccination

Michael Platten (German Cancer Research Institute and the National Center for Tumor Diseases, Heidelberg, Germany) opened the session by presenting translational research on a mutatioan-based glioma/glioblastoma immunotherapy. The failure of current approaches may be mainly due to a high phenotypic, epi-genetic and spatio-temporal heterogeneity between tumor subtypes and especially within the same tumor consisting of a mixture of individual cells corresponding to different glioma subtypes that form based on the presence or absence of specific mutations. Selecting a mutated form of isocitrate dehydrogenase type 1, IDH1R132H, previously shown to be highly prevalent in the majority of diffuse grade II and III gliomas but not in wild-type tissue and being an early driver of tumorigenesis, Platten and colleagues identified an immunogenic epitope encompassing the mutated amino acid. Vaccination of MHC-humanized A2.DR1 transgenic mice with the long 20-mer peptide IDH1R132H123–142 revealed a mutation-specific, MHC class II-restricted T141 T cell response. The growth of pre-established IDH1R132H+ sarcomas was reduced in A2.DR1 transgenic mice immunized with IDH1R132H123–142 but not with
the wild-type peptide in a CD4+ T cell-dependent manner, while loss of IDH1/R132H expression in response to vaccination led to tumor escape. Importantly, IDH1P132H-specific, spontaneous MHC class II-restricted T cell as well as antibody responses were found in patients with IDH1P132H-mutated but not in patients with IDH1 wild-type gliomas.\textsuperscript{45} A clinical phase I study in grade III/IV glioma patients (NCT-2013–0216, EudraCT 2014–000503–27) will comprise the mutation-specific IDH1P132H peptide vaccine in combination with radiotherapy and/or the chemotherapeutic agent temozolomide. Platten concluded that targeting early occurring and thus widely expressed driver mutations may prevent immune escape, and emphasized the need for patient-specific concepts supported by modulators of T cell exhaustion and tumor microenvironment for future glioma immunotherapy.

Taking a closer look at innate immune mechanisms to improve immunity, Nina Bhardwaj (The Icahn School of Medicine at Mt Sinai, New York, NY, USA) presented dysregulated innate immune cell functions and their role in forming a protumorigenic tumor microenvironment. CD4+ T cells specific for MMP-2, a matrix metalloproteinase with described tumor promoting and immunosuppressive properties,\textsuperscript{46} and exhibiting for MMP-2, a matrix metalloproteinase with described tumor promoting and immunosuppressive properties,\textsuperscript{46} and exhibiting an inflammatory TH2 phenotype had been found among tumor infiltrating leukocytes in melanoma patients.\textsuperscript{47–49} Bhardwaj and colleagues demonstrated that MMP-2 causes DCs to lower IL12p70 expression while upregulating OX40L, thereby acting as an endogenous TH2 conditioner for other melanoma antigen-specific CD4+ T cells losing IFNγ and IL-2 expression in favor of IL-4 and IL-13 expression. MMP-2 was found to directly interact with TLR2, resulting in the expression of OX40L and IL-6 and the formation of a pro-tumorigenic TH2 T cell phenotype.\textsuperscript{50} Another observation in melanoma patients is the progressive exhaustion of blood NK cells whose capacity to produce IFNγ, proliferate and exert cytotoxic functions ceases over time. TIM-3 was discovered to be upregulated on these NK cells in patients with poor prognostic parameters and blockade of TIM-3 was able to partially reverse the exhausted phenotype and to restore activation receptors NKG2D and CD16 as well as IL2Rα and γ expression.\textsuperscript{51} In patients treated with Ipilimumab, the clinical response correlated with a reversal of NK cell function including increased levels of NKG2D and Nkp46. In order to reverse innate immunosuppression in the tumor microenvironment, Bhardwaj and colleagues are currently testing intratumoral application of immune modulator poly IC:LC, a TLR3 ligand thereof. Kreiter and coworkers designed RNAs encoding 5 different mutated epitopes. Vaccination with such a pentatope RNA vaccine revealed a strong antitumoral effect whereas vaccination with a combination of the corresponding single mutated epitopes was only moderately active. Encouraged by these findings, a polymorphic epitope RNA vaccine was designed based solely on expression levels and favorable MHC class II binding prediction. Without prior confirmation and immunogenicity testing, the RNA vaccine induced potent tumor control and complete rejection of established tumors. Kreiter concluded that highly reliable bioinformatic epitope prediction based on the identified non-synonymous mutations for each cancer may be one key for rapid, tailored vaccine production and just-in-time tumor therapy.

**Tumor Microenvironment**

The tumor microenvironment is constantly moving closer into focus. Being able to promote epithelial-mesenchymal transition, angiogenesis or the development of drug-resistance while dysregulating interactions between cancer cells and antitumoral immune cells via chemokines, cytokines, nutrient starvation or inhibitory pathways, the tumor microenvironment has been recognized as a key player for cancer progression. Tumor-associated macrophages (TAM) and myeloid cells are frequent invaders in the tumor microenvironment, hence displaying ideal targets for immunotherapy and the development of novel treatment strategies.

Dominik Rüttinger (Roche Innovation Center, Penzberg, Germany) presented recently published results\textsuperscript{53} of a phase I first-in-human trial of RG7155/ Emactuzumab in diffuse type giant cell tumor (PVNS) and solid malignancies (NCT01494688). Emactuzumab targets exclusively the activation and thus the survival of M2 macrophages. Rüttinger showed that Emactuzumab induces apoptosis in CSFR1+CD163+ immunosuppressive macrophages, hereby decreasing CSFR1+ TAMs across different tumor types. Already after one treatment-cycle with Emactuzumab, circulating suppressive monocytes as well as macrophages were significantly reduced in various solid tumors. An increased CD8/CD4 T cell ratio as well as durable responses associated with profound clinical benefit in PVNS patients\textsuperscript{54} was shown. The reduction of CSFR1+ cells in PVNS patients is dose-dependent and goes along with only minor side effects, with more serious adverse events only rarely observed. Concluding that the beneficial effects of Emactuzumab
can be nicely combined with other treatment strategies, Rüttiger highlighted 2 recently launched clinical trials of this type using Emactuzumab in combination with anti-PD-L1 (NCT02323191) and paclitaxel (NCT01494688).

Niels Halama (National Center for Tumor Diseases (NCT, Heidelberg, Germany) described a platform of whole-slide immune cell quantification for the screening of tumor samples for T cells as well as cytokine and chemokine expression by immunohistochemistry (IHC). Using multi-agent modeling to analyze and extrapolate information gained via quantification of patient material avoids the extensive use of animal models. Besides enabling the characterization and localization of immune cells in tumor material, this method helps to understand how cancer cells initiate immune escape strategies and how clinical applications can play a role in cancer therapy.

The session was closed by a report on several clinical trials aiming at modulating the tumor microenvironment by Philipp Beckhove (Regensburg Center for Interventional Immunology, Regensburg, formerly German Cancer Research Center (DKFZ), Heidelberg, Germany). He showed that TILs are the main producers of vast amounts of TNF-α in the tumor tissue of CRC patients, and TNF-α expression can be used as a new prognostic biomarker in UICC stage III CRC patients.66 First results of 2 clinical trials launched in 2010 complemented Beckhove’s preclinical study published in 2013 using RIP-Tag-5 mice as a model of spontaneous insulinoma. In this work, the team showed the potency of local low dose irradiation as a therapeutic regimen to trigger repolarization of macrophages. The clinical trials now revealed a beneficial T cell infiltration after neo-adjuvant local low dose radiotherapy in locally advanced operable pancreatic cancer as well as operable liver metastases of colorectal cancer. Beckhove concluded by summarizing the recent publication of Khandelwal and colleagues,58 who used a very elegant high-throughput RNAi-based screening technique to discover new targets, i.e. inhibitory (e.g. CCR9) or stimulatory immunomodulators, that mediate tumor resistance to cytotoxic T cells.

Sabrina Kirchleitner (Ludwig-Maximilians-University (LMU), Munich, Germany) and Christina Engel (University Hospital Bonn, Germany) presented evidence derived from pancreatic and melanoma tumor models, respectively, that RIG-I activation modulates the tumor microenvironment by polarizing suppressive innate immune cells and rescuing an effective adaptive immune response. Kirchleitner showed that immunogenic cell death of pancreatic tumor cells and reprogramming of MDSCs by RIG-I activation reduces the suppression of T cells by intratumoral MDSCs.59,60 In addition, Engel focused on the modulation of the hypoxic intratumoral environment in B16 melanomas via RIG-I-pathway activation by 5’ triphosphate RNA (3pRNA). Her data nicely demonstrated that hypoxic conditions induce epithelial-mesenchymal transition (EMT) in vitro and loss of differentiation antigens in tumor cells. These effects are partially rescued by intratumoral RIG-I therapy and potentiated when combined with vitamin C treatment.

In summary, all speakers in this session emphasized that future immunotherapies need to implement targeting the tumor microenvironment and support local adaptive as well as innate immune responses.

**Combination Therapies**

Combination therapies display great potential for innovative oncology and several clinical studies exploring the applicability of multimodal therapy regimes in immunotherapy already displayed prolonged survival in patients with metastatic melanoma.61 The concept to combine cancer immunotherapy with other cancer therapies is driven by 3 main scientific hypotheses: (i) complementary response kinetics, (ii) synergistic effects by activation of innate and adaptive antitumor immunity, and (iii) modulation of the suppressive tumor microenvironment.

Based on the observation that expression of CD137 (also known as 4–1BB) on NK cells increases significantly following FcRIII receptor-engagement, Holbrook Kohrt (Stanford Cancer Institute, Stanford, CA, USA) reported that agonistic antibody targeting of CD137 improves antibody-dependent cell-mediated cytotoxicity (ADCC), enhancing the effect of tumor-directed monoclonal antibodies (mAb) such as Cetuximab,63 Trastuzumab64 and Rituximab. Kohrt’s finding is of outstanding interest since, besides modulating NK cell activity, CD137 activation also amplifies CD8+ T cell effector function.65 Therefore, combination therapy may transmute a short-term effect elicited by tumor-directed mAbs into durable antitumor immunity. Currently, several clinical investigations are ongoing to evaluate the change of CD137 expression on NK cells in response to Rituximab, Cetuximab or Trastuzumab therapy (NCT01114256), as well as to the combination of an agonistic CD137 antibody (Urelumab) and Rituximab in B cell non-Hodgkin’s lymphoma and chronic lymphocytic leukemia (NCT01775631, NCT02420938), Cetuximab in head and neck as well as CRC (NCT02110082), and the CS1 antibody in multiple myeloma (NCT02252263). Patrick Mayes (GlaxoSmithKline Oncology, Collegeville, PA, USA) emphasized that combining inhibitors of the MAPK pathway, such as Daraferib and Trametinib, with checkpoint-blockade inhibitors, creates a synergistic effect in preclinical models, although earlier reports raised the caveat that these compounds may act immunosuppressive due to inhibition of ERK phosphorylation in T cells and DCs.66 In contrast to in vitro studies, Mayes and colleagues found that neither Daraferib nor Trametinib had deleterious effects on T cells in vivo. Moreover, in BRAF V600–mutant melanoma cell lines, Trametinib reduced the expression of immunosuppressive factors (e.g., PD-L1, VEGF-A, NT5E), increased the expression of HLA class I molecules regardless of IFNγ exposure, and boosted tumor antigen expression (NY-ESO-1, BAGE, TRP1, gp100) in a dose dependent manner. Subsequently, the combination of Trametinib with immunomodulatory antibodies anti-PD-1, anti-PD-L1 and anti-CTLA4 was evaluated in a syngeneic CT26 tumor model harboring the KRAS G12D mutation as well as MAPK1 and MET amplifications. Trametinib monotherapy significantly increased tumor-infiltrating CD4+ T cells. Dual therapy with Trametinib and anti-PD-1 resulted in a significant increase of CD8+ TILs improving the antitumoral response, providing strong scientific justification to further evaluate the combination of BRAF/MEK inhibitors with immunomodulators.57
To better understand the effect of pre- and postoperative checkpoint blockade inhibition of CD96 and PD-1 in pancreatic cancer, Engin Gürlevik (Hanover Medical School, Hanover, Germany) developed a resectable, non-transplanted transgenic pancreatic ductal adenocarcinoma (PDAC) mouse model (rTMM) to simulate the postoperative situation of PDAC patients in murine studies. Strikingly, he found that anti-PD-1/Gemcitabine therapy in an adjuvant setting did not result in improved survival rates, whereas neoadjuvant anti-PD-1/Gemcitabine therapy markedly decreased local recurrence of rTMM tumors and induced Lama4 (G1254V)68 –reactive CD8+ T cells. By depletion experiments, Gürlevik demonstrated that CD8+ T cells and NK cells prevented tumor recurrence at the resection site. However, recurrence at distant sites seemed to be mainly dependent on NK cells. Due to the expression of CD155 on pancreatic tumors of rTMM and humans, the inhibitory receptor CD96 was tested as therapeutic target on NK cells to prevent distant metastases.69 Indeed, checkpoint inhibition of CD96 in an adjuvant setting in combination with Gemcitabine resulted in control of distant metastases. The study of Gürlevik highlights that therapeutic regimens may act differently against primary tumors, local recurrence and distant metastases, and provides a rationale for using anti-PD-1 therapy in a neoadjuvant and anti-Cd96 in an adjuvant setting in combination with Gemcitabine.

Sjoerd van der Burg (Leiden University Medical Center, Leiden, The Netherlands) explored the effect of synthetic long peptide (SLP) vaccination in combination with chemotherapy. The investigation of potential SLP vaccination combination partners in the preclinical TC-1 tumor model revealed that none of the tested chemotherapeutics (Oxaliplatin, Doxorubicin, Paclitaxel, Cisplatin, Carboplatin/Gemcitabine, Gemcitabine) impaired the impact of SLP vaccination, and in particular Cisplatin in combination with SLP vaccination induced profound responses.70 Having shown that chemotherapy and SLP vaccination can act synergistic, further exploration of the mechanisms underlying this synergism uncovered that SLP-induced tumor regression required particular types of intratumoral myeloid cells71 and that a number of the tested chemotherapeutic agents normalized the pathophysiological altered composition of these cells in TC-1 tumor-bearing mice, a circumstance which also holds true in cervical cancer patients (Welters et al., submitted). The change in myeloid cell composition was significantly related to improved T cell responses after SLP vaccination.

Keynote Address

At this year’s CIMT meeting, checkpoint blockade was once more the predominant topic across all sessions. In his keynote address, Alexander Eggermont (Gustave Roussy Cancer Campus Grand Paris, Villejuif, France) nicely formed out of the vast addresses, Alexander Eggermont (Gustave Roussy Cancer Campus Grand Paris, Villejuif, France) nicely formed out of the vast number of accumulating clinical data, a stunning success story. He started out by introducing recent year’s breakthrough activities regarding stage IV melanoma patients. From a non-treatable disease, metastatic melanoma has now become the paradigm tumor for both mutation-driven drug development as well as for immunomodulatory drug development.72 The success is based on mutation-driven drug development where targeting mutated BRAF by Vemurafenib73 and Dabrafenib74 showed a positive impact on progression free survival (PFS) about 5–6 months and on overall survival (OS) of about 3–4 months in metastatic melanoma patients in comparison to Dacarbazine. Responses were observed in 50 % of patients with a BRAF mutation (about 50 % of the total melanoma population), but they were short-lived and hence the impact on OS was quite limited. Heterogeneity, innate and acquired resistance all play an important role. Results can be improved by the intra-pathway blocking combination of BRAF inhibitor + MEK inhibitor, which resulted in about a 70 % response rate and a further improvement of PFS by 4 months and OS of about 2–3 months.75 Eggermont appealed to address nodes of convergence of molecular pathways,76 and to explore the complexity of cross-talk between these to avoid resistance and increase the therapy success rate.77 Targeted therapy combinations indicate incremental improvements but still a lack of long lasting tumor responses and a high progression rate. In contrast the current revolution in immunotherapy is typified by long lasting responses, and although response rates may be lower than with targeted agents, the impact on overall survival seems more prominent. Breaking tolerance by “inhibiting the inhibitor” has proven essential, and more important than “activating the activator.” By now several studies have shown the potential of anti-CTLA-4 antibody (Ipilimumab) treatment to enhance long-term survival, with about 20 % of advanced melanoma patients alive 3 up to 10 y after treatment.78,79 In connection to this promising data, Eggermont presented results of a European Organization for Research and Treatment of Cancer (EORTC) phase III trial in patients with completely resected stage III melanoma receiving Ipilimumab. Ipilimumab significantly improved relapse-free survival (RFS), but was associated with non-trivial side effects, mostly colitis and endocrinopathies. Five patients died of drug-related causes (1.1 % compared to 0 % in the placebo group).8 In where in prior EORTC adjuvant trials with IFNα an impact on RFS and OS was only observed in patients with microscopic nodal involvement and ulcerated primary tumors, the impact of Ipilimumab was present across all subgroups.81,82

In addition to anti-CTLA-4, Eggermont continued with a summary of the success story of PD-1/PD-L1 axis blocking antibodies (Nivolumab, Pembrolizumab; both anti-PD-1), entitled the drugs of the year 2013. In contrast to anti-CTLA-4, mainly functioning centrally in lymphoid tissues, the checkpoint blockade of PD-1/PD-L1 addresses T and tumor cell function peripherally in the tumor microenvironment, thereby leading to much less toxicity than anti-CTLA-4 drugs. To put into numbers, treatment of advanced melanoma patients with Nivolumab83 and Pembrolizumab80 resulted in 16.8 months of median OS and 47.3 % estimated 6 months PFS rate (Q2W), respectively. Auspicious, however, was the durable and persistent response after drug discontinuation and decrease in treatment-related adverse events. Furthermore, it was shown that PD-L1 expression on tumor tissue is beneficial but not a prerequisite for response, and
that anti-PD-1 drugs are highly effective also in BRAF mutation-negative patients. In the latter study, the median overall survival (MOS) of 18 months was not even reached.

Very important is that these checkpoint blockade inhibitors have a transversal impact across many tumor types, as investigations in several other cancer entities prove. Examples are remarkable outcomes of Pembrolizumab (phase I trial)85 and Nivolumab (phase III trial, FDA announcement January 2015) in the treatment of non-small-cell lung cancer as well as Pembrolizumab in mesothelioma (Evan W. Alley, AACR Annual Meeting 2015), gastric cancer (Kei Muro, Gastrointestinal Cancers Symposium 2015), Hodgkin’s Lymphoma86 and Nivolumab in refractory renal cancer.87,88 The value of check-point inhibitor drugs for reaching superior response in cancer patients can be even further increased by combination of those, as studies in advanced89 and untreated melanoma patients90 show: The combination of Nivolumab and Ipilimumab was able to impressively increase the survival rates compared to monotherapy but also worsened their safety profile.

Concluding his talk, Eggermont stated that breaking tolerance is essential to open the door to the effective use of activating molecules or vaccines. Possible future immunotherapy combination strategies could include checkpoint inhibitor plus cytokine treatment, adoptive T cell therapy (TCRs, CARs), Treg/MDSC depletion, antibody conjugates or vaccination strategies with DCs, RNA or DNA, while combination with radio- or chemotherapy needs to be carefully considered in the context of choosing schedules and agents that lead to immunogenic cell death.33 Supported by a better understanding of the target pathways and mechanisms of resistance, immunotherapy and its combinations will dominate the scene for years, and has the potential to break tolerance and give hope to “clinically cure” metastatic melanoma and potentially many other cancer types.91

Conclusion

In addition to the fast growing field of neoepitope prediction and personalized therapy, the power of checkpoint blockade and especially targeting the PD-1/PD-L1 axis was again impressively demonstrated at this meeting. Unlocking the potential of intelligent combination of personalized immunotherapies targeting individual, tumor-specific neoepitopes with immuno- and/or tumor modulating antibodies may eventually shift the progressing imbalance between immune system and tumor in favor of the former. Cancer immunotherapy has at last been recognized not just as an alternative way of treating cancer but a way of great promise that might supersede suboptimal conventional therapies for more successful treatment outcomes. Great advancements have been made since the last CIMT meeting in 2014, and the performance of immunotherapies currently under clinical investigation will hopefully undermine the grand possibilities of cancer immunotherapy, to be reflected on during the CRI-CIMT-EATI-AACR Inaugural International Cancer Immunotherapy Conference this September (16–19, 2015 in New York) and next year at CIMT2016 (May 10–12, 2016 in Mainz.)

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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