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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 by 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 patients[4] 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

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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] 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,[21] 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 model[24] expressing a membrane-bound ovalbumin (OVA) in the pancreas, kidney and in medullary thymus epithelial cells (mTEC). Transfer of high affinity OT-I 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 ($ID8^{OVA\text{-}Luc}$) 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,[25-27] 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 IFNγ 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 (T_{reg}) in the FoxP3-DTR model revealed that, independent of the antigen dose, T_{reg} 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 T_{reg} cells. Investigating the role of different TLRs in T_H compared to T_{reg} function, they found that at high but not at low doses, T_{reg} 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.[28-30] These results were reproduced in cancer patients with concomitant immunodeficiency syndromes.[31,32] The underlying mode of action as discovered by Kroemer and Zitvogel is dissected in the concept of immunogenic cell death and its consequences.[33] 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.[30,34] 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 T_{reg} 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).[35] 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.[36-38] 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.[39,40] 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 mutation-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.[41] Selecting a mutated form of isocitrate dehydrogenase type 1, *IDH1*R132H, previously shown to be highly prevalent in the majority of diffuse grade II and III gliomas but not in wild-type tissue[42] and being an early driver of tumorigenesis,[43,44] 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 *IDH1*$R132H_{123–142}$ revealed a mutation-specific, MHC class II-restricted T_H1 T cell response. The growth of pre-established *IDH1*$R132H^+$ sarcomas was reduced in A2.DR1 transgenic mice immunized with *IDH1*$R132H_{123–142}$ 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, *IDH1*P132H-specific, spontaneous MHC class II-restricted T cell as well as antibody responses were found in patients with *IDH1*P132H-mutated but not in patients with *IDH1* wild-type gliomas.[45] A clinical phase I study in grade III/IV glioma patients (NCT-2013–0216, EudraCT 2014–000503–27) will comprise the mutation-specific *IDH1*P132H 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,[46] and exhibiting an inflammatory TH2 phenotype had been found among tumor infiltrating leukocytes in melanoma patients.[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.[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.[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 with known anti-neoplastic properties by modulating DC and thus T cell polarization, for the treatment of a variety of solid cancers, also in combination with antigen-specific immunotherapy and checkpoint antibodies (NCT02423863,[52], among others). Finally, Bhardwaj proposed that a better understanding of innate immune mechanisms and specific modulation thereof may prove beneficial for treatment outcome, especially in combination with other treatment regimens.

Complementing Ugur Sahin's talk on early clinical results (Personalized Immunotherapy), Sebastian Kreiter (TRON-Translational Oncology, Mainz, Germany) presented the preclinical evaluation of a truly personalized RNA-based vaccination approach that targets individual neoantigens. Kreiter and colleagues demonstrated in 3 different tumor models that, unexpectedly, the majority of mutations was recognized by CD4+ T cells, although a subset of these mutations was selected based on a good MHC class I binding score.[12] Vaccination against CD4+ T cell targets led to profound tumor control and survival benefit, and depending on the tumor model, CD4+ T cells either exhibited a direct antitumoral effect or acted indirectly by CD40-CD40L-dependent induction of CD8+ T cells. Neoantigen-specific RNA vaccination induced tumor infiltration of CD4+ and CD8+ T cells, accompanied by a decrease of T_{regs} as well as myeloid derived suppressor cells (MDSCs). Addressing tumor heterogeneity and immune escape in the murine colon carcinoma, Kreiter and coworkers designed RNAs encoding 5 different mutated epitopes. Vaccination with such a pentatope RNA 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 polyneoepitope 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[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[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, Rüttinger 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[55] 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.[56] First results of 2 clinical trials launched in 2010 complemented Beckhove's preclinical study published in 2013[57] 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[62]: (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] Trastuzumab[64] 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, NCT02420 938), 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 Darafenib 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 Darafenib nor Trametinib had deleterious effects on T cells in vivo. Moreover, in BRAF V600–mutant melanoma cell lines, Trametinib reduced the expression of immunosupressive 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.[67]

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 chemotherapies (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 cells[71] 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 amount 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 Vemurafenib[73] and Dabrafenib[74] 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. Reponses 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 reprogression 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] 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 Nivolumab[83] and Pembrolizumab[80] 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.[84] 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 Lymphoma[86] 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 advanced[89] and untreated melanoma patients[90] 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), T_{reg}/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 year (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.

References

1. Toebes M, Coccoris M, Bins A, Rodenko B, Gomez R, Nieuwkoop NJ, van de Kasteele W, Rimmelzwaan GF, Haanen JB, Ovaa H, et al. Design and use of conditional MHC class I ligands. Nat Med 2006; 12:246-51; PMID:16462803; http://dx.doi.org/10.1038/nm1360
2. Van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, van Dijk LJ, Behjati S, Hilkmann H, El Atmioui D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 2013; 31: e439-42; PMID:24043743; http://dx.doi.org/10.1200/JCO.2012.47.7521
3. Linnemann C, et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4 + T cells in human melanoma. Nat Med 2015; 21:1-7; PMID:25569538; http://dx.doi.org/10.1038/nm.3785
4. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science (80-.) 2015; 348:124-8; PMID:25765070; http://dx.doi.org/10.1126/science.aaa1348
5. Castle JC, Kreiter S, Diekmann J, Löwer M, van de Roemer N, de Graaf J, Selmi A, Diken M, Boegel S, Paret C, et al. Exploiting the mutanome for tumor vaccination. Cancer Res 2012; 72:1081-91; PMID:22237626; http://dx.doi.org/10.1158/0008-5472.CAN-11-3722
6. Löwer M, Renard BY, de Graaf J, Wagner M, Paret C, Kneip C, Türeci O, Diken M, Britten C, Kreiter S, et al. Confidence-based Somatic Mutation Evaluation and Prioritization. PLoS Comput Biol 2012; 8: e1002714; PMID:23028300
7. Castle JC, Loewer M, Boegel S, Tadmor AD, Boisguerin V, de Graaf J, Paret C, Diken M, Kreiter S, Türeci Ö, et al. Mutated tumor alleles are expressed according to their DNA frequency. Sci Rep 2014; 4:4743; PMID:24752137; http://dx.doi.org/10.1038/srep04743
8. Holtkamp S, Kreiter S, Selmi A, Simon P, Koslowski M, Huber C, Türeci O, Sahin U. Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. Blood 2006; 108:4009-17; PMID:16940422; http://dx.doi.org/10.1182/blood-2006-04-015024
9. Kreiter S, Selmi A, Diken M, Sebastian M, Osterloh P, Schild H, Huber C, Türeci O, Sahin U. Increased Antigen Presentation Efficiency by Coupling Antigens to MHC Class I Trafficking Signals. J Immunol 2008; 180:309-18; PMID:18097032; http://dx.doi.org/10.4049/jimmunol.180.1.309
10. Kuhn A, Diken M, Kreiter S, Selmi A, Kowalska J, Jemielity J, Darzynkiewicz E, Huber C, Türeci O, Sahin U. Phosphorothioate cap analogs increase stability and translational efficiency of RNA vaccines in immature dendritic cells and induce superior immune responses in vivo. Gene Ther 2010; 17:961-71; PMID:20410931; http://dx.doi.org/10.1038/gt.2010.52
11. Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, Türeci O, Sahin U. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. Cancer Res 2010; 70:9031-40; PMID:21045153; http://dx.doi.org/10.1158/0008-5472.CAN-10-0699
12. Kreiter S, Vormehr M, van de Roemer N, Diken M, Löwer M, Diekmann J, Boegel S, Schrörs B, Vascotto F, Castle JC, et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 2015; 520:692-6; PMID:25901682; http://dx.doi.org/10.1038/nature14426
13. Ivics Z, Izsvak Z, Minter A, Hackett PB. Identification of functional domains and evolution of Tc1-like transposable elements. Proc Natl Acad Sci U S A 1996; 93:5008-13; PMID:8643520; http://dx.doi.org/10.1073/pnas.93.10.5008
14. Singh H, Huls H, Kebriaei P, Cooper L J N. A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. Immunol Rev 2014; 257:181-90; PMID:24329797; http://dx.doi.org/10.1111/imr.12137
15. Kowolik CM, Topp MS, Gonzalez S, Pfeiffer T, Olivares S, Gonzalez N, Smith DD, Forman SJ, Jensen MC, Cooper LJ. CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. Cancer Res 2006; 66:10995-1004; PMID:17108138; http://dx.doi.org/10.1158/0008-5472.CAN-06-0160
16. Huls MH, Figliola MJ, Dawson MJ, Olivares S, Kebriaei P, Shpall EJ, Champlin RE, Singh H, Cooper LJ. Clinical application of Sleeping Beauty and artificial antigen presenting cells to genetically modify T cells from peripheral and umbilical cord blood. J Vis Exp 2013; e50070; PMID:23407473; http://dx.doi.org/10.3791/50070
17. Dudley ME, Wunderlich JR, Shelton TE, Even J, Rosenberg SA. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. J Immunother 2003; 26:332-42; PMID:12843795; http://dx.doi.org/10.1097/00002371-200307000-00005
18. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP,

Robbins PF, Wunderlich JR, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011; 17:4550-7; PMID:21498393; http://dx.doi.org/10.1158/1078-0432.CCR-11-0116
19. Rosenberg SA, Dudley ME. Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr Opin Immunol 2009; 21:233-40; PMID:19304471; http://dx.doi.org/10.1016/j.coi.2009.03.002
20. Baldan V, Griffiths R, Hawkins R, Gilham D. Efficient and reproducible generation of tumour-infiltrating lymphocytes for renal cell carcinoma. Br J Cancer 2015; 112:1510-8; PMID:25867267; http://dx.doi.org/10.1038/bjc.2015.96
21. Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, Lin JC, Teer JK, Cliften P, Tycksen E, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med 2013; 19:747-52; PMID:23644516; http://dx.doi.org/10.1038/nm.3161
22. Rodenko B, Toebes M, Hadrup SR, van Esch WJ, Molenaar AM, Schumacher TN, Ovaa H. Generation of peptide-MHC class I complexes through UV-mediated ligand exchange. Nat Protoc 2006; 1:1120-32; PMID:17406393; http://dx.doi.org/10.1038/nprot.2006.121
23. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014; 344:641-5; PMID:24812403; http://dx.doi.org/10.1126/science.1251102
24. Kurts C, Heath WR, Carbone FR, Allison J, Miller JF, Kosaka H. Constitutive class I-restricted exogenous presentation of self antigens in vivo. J Exp Med 1996; 184:923-30; PMID:9064352; http://dx.doi.org/10.1084/jem.184.3.923
25. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 1998; 393:480-3; PMID:9624005; http://dx.doi.org/10.1038/31002
26. Feau S, Arens R, Togher S, Schoenberger SP. Autocrine IL-2 is required for secondary population expansion of CD8(+) memory T cells. Nat Immunol 2011; 12:908-13; PMID:21804558; http://dx.doi.org/10.1038/ni.2079
27. Feau S, Garcia Z, Arens R, Yagita H, Borst J, Schoenberger SP. The CD4+ T-cell help signal is transmitted from APC to CD8+ T-cells via CD27–CD70 interactions. Nat Commun 2012; 3:948; PMID:22781761; http://dx.doi.org/10.1038/ncomms1948
28. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, Schmitt E, Hamai A, Hervas-Stubbs S, Obeid M, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med 2005; 202:1691-701; PMID:16365148; http://dx.doi.org/10.1084/jem.20050915
29. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007; 13:1050-9; PMID:17704786; http://dx.doi.org/10.1038/nm1622
30. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, Shen S, Kepp O, Scoazec M, Mignot G, et al. Autophagy-Dependent Anticancer Immune Responses Induced by Chemotherapeutic Agents in Mice. Science (80-.). 2011; 334:1573-7; PMID:22174255; http://dx.doi.org/10.1126/science.1208347
31. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 2007; 13:54-61; PMID:17187072; http://dx.doi.org/10.1038/nm1523
32. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot DP, Adam J, Vitale I, Goubar A, Baracco EE, Remédios C, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 2014; 20:1301-09; PMID:25344738; http://dx.doi.org/10.1038/nm.3708
33. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol 2013; 31:51-72; PMID:23157435; http://dx.doi.org/10.1146/annurev-immunol-032712-100008
34. Rao S, Yang H, Penninger JM, Kroemer G. Autophagy in non-small cell lung carcinogenesis: A positive regulator of antitumor immunosurveillance. Autophagy 2014; 10:529-31; PMID:24413089; http://dx.doi.org/10.4161/auto.27643
35. Giardiello FM, Allen JI, Axilbund JE, Boland CR, Burke CA, Burt RW, Church JM, Dominitz JA, Johnson DA, Kaltenbach T, et al. Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. Gastroenterology 2014; 147:502-26; PMID:25043945; http://dx.doi.org/10.1053/j.gastro.2014.04.001
36. Saeterdal I, Bjørheim J, Lislerud K, Gjertsen MK, Bukholm IK, Olsen OC, Nesland JM, Eriksen JA, Møller M, Lindblom A, et al. Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer. Proc Natl Acad Sci U S A 2001; 98:13255-60; PMID:11687624; http://dx.doi.org/10.1073/pnas.231326898
37. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, Tariverdian M, Benner A, von Knebel Doeberitz M. Immune Response Against Frameshift-Induced Neopeptides in HNPCC Patients and Healthy HNPCC Mutation Carriers. Gastroenterology 2008; 134:988-97; PMID:18395080; http://dx.doi.org/10.1053/j.gastro.2008.01.015
38. Schwitalle Y, Linnebacher M, Ripberger E, Gebert J, von Knebel Doeberitz M. Immunogenic peptides generated by frameshift mutations in DNA mismatch repair-deficient cancer cells. Cancer Immun [electronic Resour. a J. Acad. Cancer Immunol. 2004; 4:14; PMID:15563124
39. Willemen Y, Van den Bergh JM, Lion E, Anguille S, Roelandts VA, Van Acker HH, Heynderickx SD, Stein BM, Peeters M, Figdor CG, et al. Engineering monocyte-derived dendritic cells to secrete interferon-α enhances their ability to promote adaptive and innate anti-tumor immune effector functions. Cancer Immunol Immunother 2015; 64(7):831-42; PMID:25863943; http://dx.doi.org/10.1007/s00262-015-1688-2
40. Van Tendeloo VF, Van de Velde A, Van Driessche A, Cools N, Anguille S, Ladell K, Gostick E, Vermeulen K, Pieters K, Nijs G, et al. Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. Proc Natl Acad Sci U S A 2010; 107:13824-9; PMID:20631300; http://dx.doi.org/10.1073/pnas.1008051107
41. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 2014; 344:1396-401; PMID:24925914; http://dx.doi.org/10.1126/science.1254257
42. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, et al. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: A study of 1,010 diffuse gliomas. Acta Neuropathol 2009; 118:469-74; PMID:19554337; http://dx.doi.org/10.1007/s00401-009-0561-9
43. Cairns RA, Mak TW. Oncogenic isocitrate dehydrogenase mutations: Mechanisms, models, and clinical opportunities. Cancer Discov 2013; 3:730-41; PMID:23796461; http://dx.doi.org/10.1158/2159-8290.CD-13-0083
44. Suzuki H, Aoki K, Chiba K, Sato Y, Shiozawa Y, Shiraishi Y, Shimamura T, Niida A, Motomura K, Ohka F, et al. Mutational landscape and clonal architecture in grade II and III gliomas. Nat Genet 2015; 47:458-68; PMID:25848751; http://dx.doi.org/10.1038/ng.3273
45. Schumacher T, Bunse L, Pusch S, Sahm F, Wiestler B, Quandt J, Menn O, Osswald M, Oezen I, Ott M, et al. A vaccine targeting mutant IDH1 induces antitumour immunity. Nature 2014; 512:324-7; PMID:25043048; http://dx.doi.org/10.1038/nature13387
46. Kessenbrock K, Plaks V, Werb Z. Matrix Metalloproteinases: Regulators of the Tumor Microenvironment. Cell 2010; 141:52-67; PMID:20371345; http://dx.doi.org/10.1016/j.cell.2010.03.015
47. Godefroy E, Moreau-Aubry A, Diez E, Dreno B, Jotereau F, Guilloux Y. α v beta3-dependent cross-presentation of matrix metalloproteinase-2 by melanoma cells gives rise to a new tumor antigen. J Exp Med 2005; 202:61-72; PMID:15998788; http://dx.doi.org/10.1084/jem.20042138
48. Godefroy E, Manches O, Dréno B, Hochman T, Rolnitzky L, Labarrière N, Guilloux Y, Goldberg J, Jotereau F, Bhardwaj N. Matrix Metalloproteinase-2 Conditions Human Dendritic Cells to Prime Inflammatory TH2 Cells via an IL-12- and OX40L-Dependent Pathway. Cancer Cell 2011; 19:333-46; PMID:21397857; http://dx.doi.org/10.1016/j.ccr.2011.01.037
49. Godefroy E, Bhardwaj N. Dysregulation of anti-tumor immunity by the matrix metalloproteinase-2. Oncoimmunology 2012; 1:109-11; PMID:22720227; http://dx.doi.org/10.4161/onci.1.1.17994
50. Godefroy E, Gallois A, Idoyaga J, Merad M, Tung N, Monu N, Saenger Y, Fu Y, Ravindran R, Pulendran B, et al. Activation of Toll-like Receptor-2 by Endogenous Matrix Metalloproteinase-2 Modulates Dendritic-Cell-Mediated Inflammatory Responses. Cell Rep 2014; 9:1856-70; PMID:25466255; http://dx.doi.org/10.1016/j.celrep.2014.10.067
51. Da Silva IP, Gallois A, Jimenez-Baranda S, Khan S, Anderson AC, Kuchroo VK, Osman I, Bhardwaj N. Reversal of NK-Cell Exhaustion in Advanced Melanoma by Tim-3 Blockade. Cancer Immunol Res 2014; 2:410-22; PMID:24795354; http://dx.doi.org/10.1158/2326-6066.CIR-13-0171
52. Salazar AM, Erlich RB, Mark A, Bhardwaj N, Herberman RB. Therapeutic In-Situ Autovaccination against Solid Cancers with Intratumoral Poly-ICLC: Case report, Hypothesis, and Clinical Trial. Cancer Immunol Res 2014; PMID:24801836; http://dx.doi.org/10.1158/2326-6066.CIR-14-0024
53. Cassier P, et al. CSF1R Inhibition with Emactuzumab in Locally Advanced Diffuse-type Tenosynovial Giant Cell Tumors of the soft tissue: a phase 1, dose escalation and expansion study. Lancet Oncol in press, 2015
54. Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, Rey-Giraud F, Pradel LP, Feuerhake F, Klaman I, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 2014; 25:846-59; PMID:24898549; http://dx.doi.org/10.1016/j.ccr.2014.05.016
55. Timke C, Winnenthal HS, Klug F, Roeder FF, Bonertz A, Reissfelder C, Rochet N, Koch M, Tjaden C, Buechler MW, et al. Randomized controlled phase I/II study to investigate immune stimulatory effects by low dose radiotherapy in primarily operable pancreatic cancer. BMC Cancer 2011; 11:134; http://dx.doi.org/10.1186/1471-2407-11-134
56. Reissfelder C, Stamova S, Gossmann C, Braun M, Bonertz A, Walliczek U, Grimm M, Rahbari NN, Koch M, Saadati M, et al. Tumor-specific cytotoxic T lymphocyte activity determines colorectal cancer patient

prognosis. J Clin Invest 2014; 125:739-51; PMID:25562322; http://dx.doi.org/10.1172/JCI74894
57. Klug F, Prakash H, Huber PE, Seibel T, Bender N, Halama N, Pfirschke C, Voss RH, Timke C, Umansky L, et al. Low-Dose Irradiation Programs Macrophage Differentiation to an iNOS+/M1 Phenotype that Orchestrates Effective T Cell Immunotherapy. Cancer Cell 2013; 24:589-602; PMID:24209604; http://dx.doi.org/10.1016/j.ccr.2013.09.014
58. Khandelwal N, Breinig M, Speck T, Michels T, Kreutzer C, Sorrentino A, Sharma AK, Umansky L, Conrad H, Poschke I, et al. A high-throughput RNAi screen for detection of immune-checkpoint molecules that mediate tumor resistance to cytotoxic T lymphocytes. EMBO Mol Med 2015; 7(4):450-63
59. Duewell P, Steger A, Lohr H, Bourhis H, Hoelz H, Kirchleitner SV, Stieg MR, Grassmann S, Kobold S, Siveke JT, et al. RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells and sensitize tumors toward killing by CD8(+) T cells. Cell Death Differ 2014; 21:1825-37; PMID:25012502; http://dx.doi.org/10.1038/cdd.2014.96
60. Schnurr M, Duewell P. Induction of immunogenic cell death by targeting RIG-I-like helicases in pancreatic cancer. Oncoimmunology 2014; 3, e955687; PMID:25941620; http://dx.doi.org/10.4161/21624011.2014.955687
61. Larkin J, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015; http://dx.doi.org/10.1056/NEJMoa1504030
62. Diken M, Attig S, Grunwitz C, Kranz L, Simon P, van de Roemer N, Vascotto F, Kreiter S. CIMT 2013: Advancing targeted therapies-report on the 11th Annual Meeting of the Association for Cancer Immunotherapy, May 14-16 2013, Mainz, Germany. Hum Vaccin Immunother 2013; 9:2025-32; PMID:23877042; http://dx.doi.org/10.4161/hv.25768
63. Houot R, Kohrt H. CD137 stimulation enhances the vaccinal effect of anti-tumor antibodies. Oncoimmunology 2014; 3, e941740; PMID:25610724; http://dx.doi.org/10.4161/21624011.2014.941740
64. Kohrt HE, Houot R, Weiskopf K, Goldstein MJ, Scheeren F, Czerwinski D, Colevas AD, Weng WK, Clarke MF, Carlson RW, et al. Stimulation of natural killer cells with a CD137-specific antibody enhances trastuzumab efficacy in xenotransplant models of breast cancer. J Clin Invest 2012; 122:1066-75; PMID:22326955; http://dx.doi.org/10.1172/JCI61226
65. Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellström KE, Mittler RS, Chen L. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat. Med. 1997; 3:682-5; PMID:9176498; http://dx.doi.org/10.1038/nm0697-682
66. Vella LJ, Pasam A, Dimopoulos N, Andrews M, Knights A, Puaux AL, Louahed J, Chen W, Woods K, Cebon JS. MEK Inhibition, Alone or in Combination with BRAF Inhibition, Affects Multiple Functions of Isolated Normal Human Lymphocytes and Dendritic Cells. Cancer Immunol Res 2014; 2:351-60; PMID:24764582; http://dx.doi.org/10.1158/2326-6066.CIR-13-0181
67. Liu L, Mayes PA, Eastman S, Shi H, Yadavilli S, Zhang T, Yang J, Seestaller-Wehr L, Zhang SY, Hopson C, et al. The BRAF and MEK Inhibitors Dabrafenib and Trametinib: Effects on Immune Function and in Combination with Immunomodulatory Antibodies Targeting PD1, PD-L1 and CTLA-4. Clin Cancer Res 2015; 21:1639-51; PMID:25589619; http://dx.doi.org/10.1158/1078-0432.CCR-14-2339
68. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014; 515:577-81; PMID:25428507; http://dx.doi.org/10.1038/nature13988
69. Chan CJ, Martinet L, Gilfillan S, Souza-Fonseca-Guimaraes F, Chow MT, Town L, Ritchie DS, Colonna M, Andrews DM, Smyth MJ. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. Nat Immunol 2014; 15:431-8; PMID:24658051; http://dx.doi.org/10.1038/ni.2850
70. Van der Sluis TC, van Duikeren S, Huppelschoten S, Jordanova ES, Beyranvand Nejad E, Sloots A, Boon L, Smit VT, Welters MJ, Ossendorp F, et al. Vaccine-induced tumor necrosis factor-producing T cells synergize with cisplatin to promote tumor cell death. Clin Cancer Res 2015; 21:781-94; PMID:25501579; http://dx.doi.org/10.1158/1078-0432.CCR-14-2142
71. Van der Sluis TC, Sluijter M, van Duikeren S, West BL, Melief CJ, Arens R, van der Burg SH, van Hall T. Therapeutic peptide vaccine-induced CD8 T cells strongly modulate intratumoral macrophages required for tumor regression. Cancer Immunol Res 2015; PMID:25888578; http://dx.doi.org/10.1158/2326-6066.CIR-15-0052
72. Eggermont AMM, Spatz A, Robert C. Cutaneous melanoma. Lancet 2014; 383:816-27; PMID:24054424; http://dx.doi.org/10.1016/S0140-6736(13)60802-8
73. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011; 364:2507-16; PMID:21639808; http://dx.doi.org/10.1056/NEJMoa1103782
74. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH Jr, Kaempgen E, et al. Dabrafenib in BRAF-mutated metastatic melanoma: A multicentre, open-label, phase 3 randomised controlled trial. Lancet 2012; 380:358-65; PMID:22735384; http://dx.doi.org/10.1016/S0140-6736(12)60868-X
75. Robert C, et al. Improved Overall Survival in Melanoma with Combined Dabrafenib and Trametinib. N Engl J Med 2014; http://dx.doi.org/10.1056/NEJMoa1412690
76. Boussemart L, Malka-Mahieu H, Girault I, Allard D, Hemmingsson O, Tomasic G, Thomas M, Basmadjian C, Ribeiro N, Thuaud F, et al. eIF4F is a nexus of resistance to anti-BRAF and anti-MEK cancer therapies. Nature 2014; 513:105-9; PMID:25079330; http://dx.doi.org/10.1038/nature13572
77. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF (V600E) inhibition through feedback activation of EGFR. Nature 2012; 483:100-3; PMID:22281684; http://dx.doi.org/10.1038/nature10868
78. Hodi S, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010; 363:711-23; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
79. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen TT, Berman DM, Wolchok JD. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. J Clin Oncol [Epub ahead of print] 2015; 33(17):1889-94; PMID:25667295; http://dx.doi.org/10.1200/JCO.2014.56.2736
80. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 150419053123009 2015; 372(26):2521-32; http://dx.doi.org/10.1056/NEJMoa1503093
81. Eggermont AMM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol 2015; 16(5):522-30; http://dx.doi.org/10.1016/S1470-2045(15)70122-1
82. Eggermont AMM, Suciu S, Testori A, Kruit WH, Marsden J, Punt CJ, Santinami M, Salès F, Schadendorf D, Patel P, et al. Ulceration and stage are predictive of interferon efficacy in melanoma: Results of the phase III adjuvant trials EORTC 18952 and EORTC 18991. Eur J Cancer 2012; 48:218-25; PMID:22056637; http://dx.doi.org/10.1016/j.ejca.2011.09.028
83. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, Brahmer JR, Lawrence DP, Atkins MB, Powderly JD, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014; 32:1020-30; PMID:24590637; http://dx.doi.org/10.1200/JCO.2013.53.0105
84. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, et al. Nivolumab in Previously Untreated Melanoma without BRAF Mutation. N Engl J Med 2014; 372:320-30; PMID:25399552; http://dx.doi.org/10.1056/NEJMoa1412082
85. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, et al. Pembrolizumab for the Treatment of Non-Small-Cell Lung Cancer. N Engl J Med 2015; 372:2018-28; PMID:25891174; http://dx.doi.org/10.1056/NEJMoa1501824
86. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, et al. PD-1 Blockade with Nivolumab in Relapsed or Refractory Hodgkin's Lymphoma. N Engl J Med 2015; 372:311-9; PMID:25482239; http://dx.doi.org/10.1056/NEJMoa1411087
87. McDermott DF, Drake CG, Sznol M, Choueiri TK, Powderly JD, Smith DC, Brahmer JR, Carvajal RD, Hammers HJ, Puzanov I, et al. Survival, Durable Response, and Long-Term Safety in Patients With Previously Treated Advanced Renal Cell Carcinoma Receiving Nivolumab. J Clin Oncol 2015; 33(18):2013-20; http://dx.doi.org/10.1200/JCO.2014.58.1041
88. Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, Vaishampayan UN, Drabkin HA, George S, Logan TF, et al. Nivolumab for metastatic renal cell carcinoma (mRCC): Results of a randomized, dose-ranging phase II trial [abstract]. J Clin Oncol 2015; 33:1430-7; PMID:25452452; http://dx.doi.org/10.1200/JCO.2014.59.0703
89. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013; 369:122-33; PMID:23724867; http://dx.doi.org/10.1056/NEJMoa1302369
90. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, et al. Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. N Engl J Med 2015; 372:2006-17; PMID:25891304; http://dx.doi.org/10.1056/NEJMoa1414428
91. Eggermont AMM, Maio M, Robert C. Immune Checkpoint Inhibitors in Melanoma Provide the Cornerstones for Curative Therapies. Semin Oncol 2015; 42:429-35; PMID:25965361; http://dx.doi.org/10.1053/j.seminoncol.2015.02.010