CIMT 2016: Mechanisms of efficacy in cancer immunotherapy — Report on the 14th Annual Meeting of the Association for Cancer Immunotherapy
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Introduction

Cancer immunotherapy at its golden age is now included as a standard of care treatment for cancer. Its well-established pre-clinical era has been flourishing in the recent decade with many patients benefiting from the therapeutic efficacy of cancer immunotherapy through checkpoint inhibitors, adoptive cell therapies as well as highly individualized immunotherapies. To present and discuss these “mechanisms of efficacy,” the city of Johannes Gutenberg, Mainz, Germany, welcomed this year more than 1,000 scientists specialized in various fields of cancer immunotherapy for the 14th Annual Meeting of the Association for Cancer Immunotherapy (CIMT) (May 10–12, 2016), the highlights of which are summarized in this report.

Tumor microenvironment

Mikael Pittet (Massachusetts General Hospital, Boston, MA, USA) initially summarized his contributions to our understanding of the cross-talk between tumor and immune cells and the origins and roles of myeloid cells in diseased tissues.1-4 His discoveries are often supported by high resolution in vivo imaging to reveal how and where immune cells act in live subjects.5,6 He then presented a recent publication from his laboratory on the relevance of subcapsular sinus macrophages (SCS Mph) in tumor-draining lymph nodes.7 These cells normally constitute a barrier that prevents infectious agents from spreading to the rest of the body.8,9 The new study found that SCS Mph can also suppress cancer growth by physically blocking the dissemination of tumor-derived extracellular vesicles (tEV). However, tumor progression undermines the SCS Mph barrier, which enables tEV to flow deep within lymph nodes, bind B cells, and elicit a tumor-promoting IgG response. In another recent study, the Pittet laboratory uncovered a new way to sensitize tumors to checkpoint therapy.10 The approach rationally identifies drug combinations that trigger relevant immunogenic phenotypes in tumors and induce tumor infiltration by CD8 T cells. The findings have exciting implications: They indicate that the proportion of cancers responding to checkpoint inhibition can be feasibly and substantially expanded. The study also spotlights the need for creating a rational selection guide to identify immuno-oncology therapies that most effectively stimulate antitumor immunity.

The presentation of Ellen Puré (University of Pennsylvania, PA, USA) focused on the role and heterogeneity of tumor stroma during tumor development. The new concept of “stromagenic switch” has been suggested for optimization of immunotherapy. Very desmoplasic tumors showed a strong increase of fibroblast activation protein (FAP)-expressing fibroblasts displaying strong tumor promoting properties as well as therapeutic resistance. FAP is a known biomarker for lung, colon, pancreatic and other cancers.12 In the most recent work of Puré and collaborators, they generated a FAP-specific chimeric antigen receptor (FAP-CAR), and transduced T cells demonstrated therapeutic efficiency in several murine tumor models.3 Depletion of FAP+ stroma cells in the tumor environment by these CAR-transduced T cells offers an attractive tool for complementary large-spectrum antitumor therapy against several cancer entities.

Improving immunity

Hergen Spits (Academic Medical Center and AIMM Therapeutics, Amsterdam, The Netherlands) focused on the discovery of tumor-specific antibodies for the treatment of acute myeloid leukemia (AML). Memory B cells immortalized via retroviral transduction of BCL6 and BCL-xl followed by treatment with IL-21 were used to generate antibody-secreting plasmablasts.4 Subsequent screening of antibodies from single plasmablasts isolated from AML patients showing a potent graft versus leukemia response after haematopoietic stem cell transplantation were then used to identify specific binders of AML cell lines. By analyzing 3
different AML patients, several IgG1 and IgG3 antibodies specific for sialylated epitopes or surface-exposed snRNP200 (a protein that is usually located in the nucleus of normal cells) were identified. One monoclonal antibody (mAb) was shown to specifically target the sialylated molecule CD43 on all 40 tested AML samples. Coupling the mAb to an anti-CD3 single-chain variable fragment (scFv) allowed the redirection of T cells to killing of leukemia cell lines. Moreover, a second mAb against snRNP200 which specifically recognized 20 out of 40 AML samples induced direct killing of AML cells in vitro and delayed tumor growth of an AML model in vivo. By extending the methodology to melanoma patients, a third antibody was discovered that was reactive to the tetraspanin CD9 which is known to be involved in tumor cell spreading. Spits concluded by suggesting the clinical testing of the identified tumor-specific antibodies, for example in combination with checkpoint inhibitors in an adjuvant setting.

Targeting the tryptophan degrading enzyme indoleamine 2,3-dioxygenase (IDO) which is critically involved in immune escape of tumors was the topic of the talk by George C Prendergast (Lankenau Institute for Medical Research, Wynnewood, PA, USA). IDO1 is an interferon response gene implicated in fetal tolerance and known to block tumor-specific T cell responses. Hence, inhibition of IDO (e.g. via 1-Methyltryptophan, 1-MT) is a treatment option of cancer, empowering “immunogenic” chemotherapy and anti-CTLA-4 checkpoint blockade.8 It has been proposed that IDO1 limits T cell immunity either in the lymph node during priming or during the effector phase in the tumor. Upregulation of IDO1 in tumors can be triggered via reduced expression of Bin1 which is a negative regulator of IDO1, or via NF-κB, Jak/STAT or Ras/Mek pathways. In addition to tryptophan starvation, IDO1 leads to translation of IL-6 and CCL2, IDO2 transcription as well as blockade of mTOR.9 IDO1 is essential for inflammatory carcinogenesis10 and for tumor angiogenesis as well as for lung metastasis of 4T1 tumors.11 The transcription of IDO2, the second IDO isoform, is linked to IDO1 expression and is the primary target of 1-MT. IDO2 has inflammatory roles distinct from IDO1 and is needed for IDO1-mediated induction of regulatory T cells (Tregs).12 Selective blockade of IDO2 can be achieved via chloroquine which was demonstrated to have an anti-tumoral activity in glioblastoma patients.13 Interestingly, IDO2 is especially overexpressed in pancreatic cancer patients harboring a polymorphism in the IDO2 gene, resulting in its loss of function and leading to an improved overall survival (OS). In summary, Prendergast stated that IDO pathways program inflammatory processes which determine adaptive immune responses and represent promising targets for combination cancer therapy.

The protective and therapeutic anti-cancer effect of physical exercise has been demonstrated in multiple studies. However, the underlying mechanisms are still poorly defined. Per thor Stratén (Copenhagen University Hospital, Herlev, Denmark) and colleagues demonstrated that mice exercising on a running wheel for 4 weeks prior and 2 weeks after inoculation of B16F10 melanoma cells developed a significantly reduced tumor burden.14 This finding was reproduced in a subcutaneously transplantable lewis lung cancer (LLC) model, a spontaneous Tg(Grm1)EPv melanoma model (transgenic mice overexpressing metabotropic glutamate receptor 1 (Grm1) under the Dopachrome tautomerase promoter that spontaneously produce melanocytic lesions) as well as in an N-nitrosodiethylamine-induced liver cancer. Pathway enrichment analysis of B16F10 tumors of exercising vs. control animals showed the differential regulation of a multitude of genes, mostly involved in immune function and inflammation such as IL-1β, IL-6, TNF-α, iNOS and the NK cell markers NKP46 and NKG2D for NK cells. Indeed, tumors of exercising mice were more strongly infiltrated by T cells, NK cells as well as DCs as shown by flow cytometry and immunohistochemistry. Subsequently, thor Stratén demonstrated that the therapeutic effect of exercise was completely abrogated only following depletion of NK cells and that exercise had significant impact on tumor burden in athymic mice that lack T cells but retain NK cells. Dissecting the cause of the exercise-mediated immune cell activation, thor Stratén and colleagues found that exercising mice had significantly higher epinephrine and nor-epinephrine levels than their inactive counterparts. Elevated numbers of NK cells in exercising mice, which express the β-adrenergic receptor binding to epinephrine and nor-epinephrine, were reduced to baseline levels by administration of the β-blocker propranolol. Furthermore, propranolol treatment abrogated the anti-tumoral effect of exercise which was mimicked at least in part by daily injections of low-dose epinephrine. Further studies will determine whether the mechanistic findings in the mouse tumor model B16F10, which is known to be susceptible to NK cells,15 will translate to human patients as well.

Therapeutic vaccination

Darrell J. Irvine (Massachusetts Institute of Technology, Cambridge, MA, USA) opened the session by presenting a vaccination approach that stimulates both adaptive and innate immunity in order to achieve tumor control. He and his colleagues designed lymph node-targeting amphiphilic peptide vaccines (amph-vaccine) consisting of an antigen and an adjuvant (CpG) linked to an albumin-binding tail as well as a polar chain that promotes solubilization.16 Based on the size-dependent physiology of lymphatic trafficking,17 vaccination of mice with this albumin-linked vaccine led to the accumulation in the spleen and increased antitumor immune responses in B16F10 melanoma and TC-1 cervical cancer syngeneic mouse models. These very potent peptide vaccines are being studied in combination with other immunomodulatory drugs to optimize anti-tumor responses against multiple tumor models.

Gerold Schuler (University Hospital Erlangen, Germany) presented an overview on phase I, II and III clinical trials of melanoma conducted in Erlangen reaching back to 1997 and utilizing yet another successful vaccination strategy, which is the adoptive transfer of DCs. Generated from monocytes in the presence of GM-CSF and IL-4, human immature DCs are matured with TNF-α, IL-1β, IL-6 and PGE2 and either loaded with tumor antigen-specific peptides or transfected with mRNA encoding specific tumor antigens. Key findings from these studies were that i) long term survival of patients correlated with presence of eosinophilia after the first series of vaccinations, ii) intravenous application of the DC vaccine was superior to the intracutaneous route, and that iii) long-term vaccination seemed to be critical to achieve long term survival. Immunogenicity of the DC vaccine was shown for shared antigens as well as neoepitopes. Despite the current dogma that a
high mutational load enhances the chances for a good prognosis, in patients vaccinated with DCs loaded with autologous tumor, melanoma-reactive T cells recognized not only neoantigens.\textsuperscript{18,19} In order to not restrict immune responses to next generation sequencing (NGS)-predicted neoepitopes or defined shared tumor antigens but take advantage of the total antigenic repertoire, DCs were transfected by the Erlangen group with PCR-amplified autologous total tumor RNA and are currently being tested in a randomized, multicenter phase III clinical trial of monosomy 3-positive uveal melanoma (NCT01983748), a highly metastatic disease with no effective standard therapy, few mutations and high resistance to checkpoint blockade. Patients with resected or destroyed primary ocular melanoma receive 8 intravenous vaccinations over a period of 2 years, and objectives are to prolong disease-free survival (DFS), OS and to induce immune responses.

Taking a closer look at tumor-associated antigens, Cornelis Melief (Leiden University Medical Center and ISA Pharmaceuticals, Leiden, The Netherlands) highlighted the potential of T cell epitopes that are associated with impaired peptide processing (TEIPPs). TEIPPs originate from universal housekeeping proteins, are processed independently of the peptide transporter TAP and are only presented by the residual MHC class I molecules on cancer cells with impaired TAP functions.\textsuperscript{20,21} Utilizing the lack of central tolerance, van Hall and colleagues demonstrated strong immunogenicity of these TEIPPs and induced TEIPP-specific T cells upon vaccination of TAP-deficient mice with synthetic long peptides (SLP).\textsuperscript{22} Immunization with TEIPP antigens may be beneficial especially in advanced, immune-edited tumors, where vaccination against neo-antigens may lose its effectiveness due to impaired TAP processing. In the second part of his talk, Melief focused on the devolement of oncogenic human papillomavirus (HPV)-specific SLP vaccines. In a pilot study of advanced HPV positive cervical cancer conducted to determine the effect of therapeutic SLP vaccination (ISA101) during chemotherapy with carboplatin (carboplatin and paclitaxel), reactivity of vaccine-induced T cells was sustained by chemotherapy-induced normalization of the number of circulating myeloid cells shown to be immunosuppressive.\textsuperscript{23} Preliminary data from a subsequent phase I/II trial for metastatic or recurrent HPV positive cervical cancer where patients were again treated with carboplatin before and during vaccination reveal increased T cell reactivities compared to the previous trial. In summary, the efficacy of vaccines in late stage cancer can be severely hampered by a strong immunosuppressive environment, but careful selection of the right neoadjuvant chemotherapy can help to overcome immune evasion and this way open the door for vaccine-induced T cell immunity.

**Cellular therapy**

Chiara Bonini (Università Vita-Salute San Raffaele, Milano, Italy) opened her talk by pointing out that TCR-engineering is a means to overcome limited tumor-specific T cell numbers in patients. Although so far not observed in the clinic, in mice the introduced TCRs mispair with endogenous TCR chains leading to recognition of neoantigens and hence, autoreactivity.\textsuperscript{24} Using genome editing via Zinc finger nucleases, her lab knocked-out one or both endogenous TCR chains, resulting in either single (SE) or completely edited (CE) T cells, respectively.\textsuperscript{25} SE, compared to CE, requires shorter T cell incubation times, but can already drastically reduce alloreactivity. SE prior to TCR transduction results in high expression of the introduced TCR and antigen-specific effector function as opposed to unedited or CE T cells in vitro. In vivo, SE led to remarkable tumor reactivity in an NSG-based U266 mouse tumor model. Unedited T cells showed similar anti-tumor activity against NY-ESO-1, but induced signs of GvHD (Mastaglio et al submitted). Bonini further underlined the importance to study T cell subtypes suited for adoptive cell transfer (ACT). In long-living leukemia patients that had received haematopoietic stem cell transplantation (HSCT) and T cells modified retrovirally with suicidal thymidine kinase, persistence of T cells was checked after 2–14 years. Long-lived T cell clones were identified by their viral integration sites and originated preferentially from the stem cell memory (TSCM) subtype. The last part of her talk had a strong focus on immune-inhibitory mechanisms. In comparison with patients in complete remission, recipients of HLA-matched HSCT with relapsing disease showed an early upregulation of more than one inhibitory receptor (PD-1, 2B4, TIM-3) on leukemia specific T cells. Bonini argued that this upregulation might be used as a predictive marker for therapy success, and that the blockage of inhibitory receptors might rescue T cell function.

Robert Hawkins (University of Manchester and The Christie, UK) addressed the potential toxicity of engineered T cells. Treatment of colorectal cancer patients by his group with carcinoembryonic antigen (CEA)-specific 1st generation CARs resulted in on-target/off-tumor toxicity in the lung, hypothesized to originate from basal target expression after IFN-gamma secretion by CAR T cells. Hawkins highlighted the progress in TIL therapy and reported that in his laboratory 22 patients were treated with TILs in non-/cutaneous melanoma. These T cell products resulted in no (autoimmune) toxicity or treatment-related deaths, while rapid recovery of lymphocytes was observable after lymphodepleting regimens, leading to global CR rates of 10–25% with durable responses. ACT of T cells specific for an immunoglobulin idiotype, an archetypical tumor antigen, resulted, in contrast to an anti-idiotypic vaccine, in reduction of lymphoma burden in mice.\textsuperscript{27} In the clinical setting, enrichment of T cells specific for mutated Erbb2IP promoted regression of metastatic epithelial cancer. Hawkins remarked that mutational burden may not always be associated with immunogenicity when looking at different tumor entities. Low mutational burden in combination with constant costimulatory signals, for example via CD27/CD70 interaction in clear renal cell carcinoma, might lead to proliferative exhaustion of T cells.\textsuperscript{28} The success of TIL therapy inspired Hawkins’ lab to further investigate TIL isolation (Bridgeman et al submitted: IL-2 independent expansion), T cell phenotypes, stimulatory factors, homing as well as persistence of cells. He concluded that T cells engineered with high affinity receptors can be very effective for cancer therapy, but pose the risk of off-tumor toxicity. Personalized TIL therapy benefits from the fact that it targets multiple, also mutation-specific antigens simultaneously, still the key is to engineer truly specific cells.

**Novel technologies for immune assessment**

Tregs modulate the immune system, maintain tolerance to self-antigens and prevent autoimmune disease. Although adaptive
Treg therapy is an attractive option for controlling aberrant immune responses in autoimmunity and transplantation, technical limitations have restricted a comprehensive characterization of the Treg compartment to accurately define the most-suited subpopulation. Timothy Tree (King’s College, London, UK) presented a combination of tools used for clinically monitoring Treg phenotype, function and specificity. For phenotypic characterization, he and his colleagues employed an extensive panel of 26 lineage-specific surface markers to determine the phenotypic composition of the Treg compartment at the single-cell level by mass cytometry.\(^\text{31}\) A tool called viSNE was utilized to project high-dimension data sets derived from mass cytometry of PBMC-isolated Tregs on a biaxial plot based on their similarity across all parameters simultaneously, while maintaining single-cell resolution and both the multidimensionality and complex parameter relationships of the data. Parameter expression profiles generated from viSNE projections revealed excellent separation of distinct Treg subpopulations based on individual markers. Automated cluster analysis in an unsupervised manner using the FLOCK tool which takes all analyzed parameters into account, was performed to identify 22 clusters or subpopulations, demonstrating a so far unrecognized high degree of phenotypical complexity and heterogeneity within the Treg compartment. Tree postulated that this method might accelerate the understanding of Treg subset dynamics during disease and help define the optimal subpopulation(s) for clinical application. Following this technique for phenotypic assessment, Tree presented the in vitro coculture \(\mu\)-suppression assay, which is based on sorted reactive (CD127\(^{hi}\), CD25\(^{lo}\)) and regulatory T cells (CD127\(^{lo}\), CD25\(^{hi}\)) as well as antigen presenting cells (APCs, CD14\(^{hi}\) or CD19\(^{hi}\)) to determine Treg suppressive function. This assay requires only low cell numbers available from small sample volumes and accelerates sample throughput, while, similarly to conventional suppression assays, suppression of proliferation is Treg dose-dependent and depends on the strength of stimulation. Eventually, he presented a collection of publications on key molecules predictive of Treg antigen specificity that do not require knowledge of epitope specificities. According to these data, antigen-specific Tregs are characterized by high FoxP3, HELIOS, GARP and 4-1BB, and low or no expression of CD26 and CD40L. Tree emphasized the complexity and heterogeneity in human Tregs, and that the quality of mechanistic assays is key to build on success and understand failure of immunotherapy trials.

Quantification of antigen-responsive T cells has become an integral part of assessing complex immune recognition. With limited unmanipulated patient material available, the current strategy of combinatorial encoding of peptide-MHC multimers coupled to fluorescence tags is limited to the detection of maximum 120 distinct T cell populations per sample.\(^\text{32,33}\) Sine Reker Hadrup (Technical University of Denmark, Frederiksborg C, Denmark) highlighted that there was an unmet need for technical improvements able to cope with the vast complexity of the human T cell repertoire, and able to combine epitope prediction based on deep sequencing with T cell responsiveness. The introduced technique is based on DNA barcode labeled pMHC multimers (barcode complexity 10\(^8\)) which can be prepared in mixtures of >1,000 differently loaded and barcoded pMHC with bulk PBMCs or tumor infiltrating leukocytes (TILs). T cells recognizing their cognate peptide can be sorted via flow cytometry, and amplification of the DNA barcode via deep sequencing reveals the identity and the magnitude of distinct T cell specificities (submitted for publication). With several examples from healthy donors as well as melanoma patients, she demonstrated that mixtures of >1,000 different pMHC complexes can be used to simultaneously analyze T cell recognition in a single sample, that the detection limit is similar to combinatorial encoding (i.e. combinations of different fluorescence-only labeled pMHC multimers), and that low avidity T cells can potentially be better detected. By sorting of effector-cytokine positive T cells determined from intracellular staining of patient PBMCs or TILs and adding barcoded pMHC multimers, functional T cell analysis and epitope recognition may be coupled. Utilizing its capacity for genome-wide coverage, Hadrup presented how barcoded multimers can be used to identify CD8 T cell reactivities to clonal neoantigens,\(^\text{34}\) benefiting from the ability to screen larger samples sizes (e.g., PBMC samples) due to the wide capacity of the technology, i.e., the ability to screen for the full neo-epitope library in a single tube, and benefiting from increased detection capabilities of neoantigen specificities compared to combinatorial encoding. In conclusion, this technique may be of high interest for high-throughput epitope mapping, biomarker discovery, functional and phenotypic analysis of antigen-specific T cells, and TCR fingerprinting, especially when limited biological sample is available.

Despite recently approved immunotherapies and targeted therapies, there is an unmet clinical need for melanoma patients with local recurrence. Giuliano Elia (Philochem, Zurich, Switzerland) presented preclinical and clinical data on the intralesional treatment with Daromun, a combination of the immunocytokines L19-IL2 (Darleukin) and L19-TNF (Fibromun). These agents represent targeted forms of IL-2 and TNF, individually coupled to the L19 antibody directed against extracellular domain B of fibronectin (EDB), a marker of angiogenesis. L19 efficiently targets EDB in different human tumors, with a long residence time in the lesion. Preclinical data revealed strong synergistic effects dependent on T cell activity.\(^\text{35}\) Stage III melanoma patients rapidly progress to stage IV which is almost always incurable. Aiming at blocking the disease at stage III and preventing or delaying progression to stage IV, a phase II monotherapy study with L12-IL2 in stage IIIB/C melanoma patients (EudraCT No. 2009-014799-23) revealed an improved time-to-stage IV compared to historical controls, but not all lesions responded.\(^\text{36}\) In another phase II study, the combined treatment with L19-IL2 and L19-TNF in stage III/IVA melanoma patients (EudraCT No. 2012-001991-13) led to a systemic immune response, i.e. non-injected lesions (neighboring or distant) shrank or disappeared upon intralesional injection (bystander effect), and decelerated progression to stage IV melanoma.\(^\text{37}\) Quality of life was improved as the treatment is less disfiguring than surgery and does not suffer from margin problems. A randomized, controlled phase III trial for intralesional application of Daromun followed by surgery in patients with fully resectable stage IIIB/C melanoma and injectable cutaneous or lymph node has started, the primary endpoint being recurrence-free survival at one year post randomization. Elia concluded that Daromun showed excellent tolerability and
Monoclonal antibodies can achieve their therapeutic effect through direct (i.e., neutralization) and indirect (i.e. antibody dependent cellular cytoxicity (ADCC)) mechanisms, which are largely determined by the chosen immunoglobulin framework which is critical for their differential engagement of Fc-receptors. As highlighted by Rony Dahan (Rockefeller University, New York, NY, USA), Fcγ-receptors profoundly modulate the anti-tumor activity of antibodies against the well-known immunomodulatory targets PD-1, PD-L1, CTLA-4 and CD40. By utilizing the tumor model MC38, he found that the efficacy of PD-L1 targeting mAbs critically depended on ADCC-mediated depletion of PD-L1-expressing cells of myeloid origin within the tumor-microenvironment (TME) and not simply by blockade of the receptor/ligand interaction.38 In line with Dahan’s finding, Simpson et al reported that the activity of CTLA-4 targeting antibodies depended on the presence of Fcγ-receptor expressing macrophages in the TME and regulatory T cell depletion mediated by engagement of the activatory receptor FcγRIIIA, signifying that such Fc variants may serve as the optimal immunoglobulin framework for agonistic CD40 antibodies and other members of the tumor necrosis factor receptor (TNFR) family (OX40, 4-1BB). Here, engagement of FcγRIIB by a mouse IgG1 resulted in strong agonistic activity, presumably as a result of forced receptor crosslinking. However, given the fact that the human IgG repertoire lacks an analog with comparable Fcγ binding properties, clinical translation and identification of the ideal human isotype remains challenging. Addressing this issue, Ann White (University of Southampton, UK) reported that human IgG2 showed unexpected agonistic, Fcγ receptor-independent activity, which was attributed to its unique arrangement of hinge region disulfide bonds.40 Dahan described the development of a mouse model fully humanized for its FcγRs and CD40. Using this model, they were able to appropriately assess the activity of human CD40 agonistic Abs by considering both its Fab- and Fc-mediated mechanisms.43 They discovered that the antitumor activity of the human IgG, including the IgG2 subclass, depends on FcγRIIB-engagement. Moreover, by engineering Fc variants with selective enhancement to FcγRIIB but not to FcγRIIA, significantly improved antitumor immunity was observed, indicating that such Fc variants may serve as the optimal immunoglobulin framework for agonistic CD40 antibodies. Taken together, the findings of Dahan and White underline that careful selection of the antibody isotype is a crucial determinant of therapeutic efficiency.

A prime example on how deep scientific understanding can stimulate innovation was outlined by Paul Parren (Gennab, Utrecht, the Netherlands) who presented one of Gennab’s proprietary antibody technologies. Driven by the observation that intermolecular Fc:Fc interactions can be tuned using specific Fc mutations,44,45 they developed the HexaBody platform. HexaBody molecules represent an improved antibody format with superior complement activation due to increased hexamerization and enhanced C1q binding upon antigen recognition while retaining characteristics of the wild-type IgG1 molecule, such as stability, serum compatibility, pharmacokinetics and manufacturability. Further innovative antibody designs were presented by Maria Kreuzberg (Ganymed Pharmaceuticals, Mainz, Germany) who summarized the development of Mertansine (DM1) and Monomethyl auristatin E (MMAE) antibody-drug conjugates of Ganymed’s claudin-6-specific antibody IMAB027. Both IMAB027 antibody-drug conjugates demonstrated excellent anti-tumor activity against claudin-6 positive xenograft tumors and were well tolerated in mice. Following the hypothesis that anti-PD-L1 therapy might be enhanced through a combination approach, Kin-Ming Lo and colleagues (EMD Serono, Rockland, MA, USA) developed a fusion protein comprising an anti-PD-L1 moiety and soluble TGFβ receptor II. Conceptually this “Antibody-Cytokine Trap” combines the blockade of cell intrinsic and extrinsic pathways. Lo noted that dosing regimens resulting in optimal PD-L1 target occupancy in turn led to efficient trapping of bioactive TGFβ. The treatment of EMT-6 and MC38 tumors with the novel Antibody-Cytokine Trap format resulted in a superior outcome compared to anti-PD-L1 blockade or TGFβ trap alone. Antibody-based, selective depletion of immune effector cell subsets showed that both CD8 cytotoxic T cells and NK cells were required for tumor rejection.

**Keynote**

It was back in 1969 when Wolf Hervé Fridman (Centre de Recherche des Cordeliers, INSERM, Paris, France) published first results on the stimulation of lymphocytes by autologous leukemic cells in a patient with acute leukemia.46 This discovery contributed profoundly to the cancer immunology field and the development of immune-based tools for efficient prognosis and therapy of cancer. In his keynote address, he focused on the key elements of the immunoscore complemented by expression analysis, emphasizing the impact of the immune landscape on the clinical outcome and on parameters which identify high-risk cancer patients. The prognostic evaluation of patients takes place via the assessment of type, density and location of the natural in situ immune reaction within distinct tumor areas. If the immune reaction is readily infiltrating into the invasive margin of a tumor the chance of tumor control increases. Tumors are kept in check by infiltrating CD8 memory T cells and immune cells with a Th1-phenotype (good prognosis), whereas infiltrating Th2, Th17 and Treg cells are often associated with a poor prognosis. However, besides the evaluation of the phenotype of infiltrating cells, the place where the endogenous immune reaction is shaped and infiltrating immune cells are educated needs to be determined. Histological analysis on the structure and topology of colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) revealed that the tumors consist of various structures such as epithelium, tumor cell zones, invasive margin and lymphoid islets. The latter, also described as tertiary lymphoid structures
(TLS), are located outside the tumor margin and have a major impact on the local education of CD8 T cells. TLS display highly organized lymph node-like immune cell aggregates consisting of mature DCs (DC-Lamp+), B cell follicular zones and T cell zones served by high endothelial venules (HEVs), and are thought to be the place where priming of T and B cells occurs, thus potentially essential for the antitumor immune response.58 Activation of infiltrating immune cells of the Th1 phenotype occurs via DC-Lamp+ cells, whose presence correlates with a better prognosis.59 The presence of follicular B cells in TLS also correlates with a good prognosis in NSCLC patients,50 as they can infiltrate into the tumor margin after the priming process and secrete tumor antigen specific IgG or IgA. Accordingly, poor prognosis can be concluded upon lacking immune cell infiltration by tumoral deletion events, as shown for CXCL13 or IL-15 in CRC patients.51,52 Moreover, upregulation of immune checkpoint inhibitors like Lag3 or PD-1 on infiltrating T cells in clear-cell renal cell carcinoma (ccRCC) patients also leads to poor prognosis.53 Besides the expression pattern of tumor and immune cells, also the special distribution of cells plays a major role. In ccRCC patients with poor outcome, most of the DC-Lamp+ are located outside the TLS in contrast to NSCLC patients, which show DC-Lamp+ only within the TLS. Those non-TLS located DCs (NTLS-DCs) were found within the tumor margin, close to CD34+ blood vessels and had an immature phenotype. Fridman concluded that what matters most is the location of mature DCs, rather than pure quantity of potent CD8 T cells. DC-Lamp+ with a mature phenotype educate T cells in TLS and are associated with a good prognosis, whereas tolerogenic DCs with an immature phenotype are associated with a poor prognosis. TLS, he emphasized, facilitate the adaptive intra-tumor and systemic immune responses against cancer.55 In the last part of his talk Fridman focused on categorizing patients by means of their molecular signatures of their immune cell infiltrates. By immunoprofiling of CRC patients, 4 molecular groups (consensus molecular subtypes, CMS) were identified: i) hyper-mutated (14%), ii) canonical (37%), iii) metabolic (13%) and iv) mesenchymal (23%) (TCGA Cancer Genome Atlas Network, 55–57). Depending on the categorization into these different molecular subgroups, individualized therapies can be applied. For instance, if the molecular signature is low within the lymphocytic pattern, a CAR therapy may be beneficial. In contrast, a high myeloid and fibrotic signature may be treated with checkpoint inhibitors to modify the tumor microenvironment.58 Further unveiling the cell type(s) responsible for an inflammatory tumor microenvironment, a gene signature of purified cells showed an increased inflammatory signature in monocytic cells, fibroblasts and endothelial cells.57 Prognostic molecular subgroups were also shown for ccRCC.58 They found that RCC cell lines overexpress genes involved in inflammation, angiogenesis and immunosuppression compared to CRC cell lines, thus explaining the rather poor prognosis. Fridman excellently illustrated the complex and delicate interactions between malignant stromal cells and beneficial immune cells in the tumor microenvironment, leading him to his final statement that emphasis should be put on better characterization of these interactions in order to intervene more precisely and turn a poor prognosis into a good one.

Conclusion

The field of cancer therapy benefited from the first (check point inhibitors) and second (adoptive cell therapy with receptor gene modified T cells) waves of cancer immunotherapy which are now closely followed by individualized immunotherapies as the next level of innovation. It is of great importance to elucidate the mechanisms of efficacy for these therapeutic breakthroughs in order to create next waves, to develop potent combination therapies and improve clinical benefit. CIMT 2016 delivered state-of-the art updates spanning the entire cancer immunotherapy field and promoted the clinical implementation of our increased understanding for mechanisms of efficacy. Further recent developments in this field will be introduced during the 2nd CRI-CIMT-EATI-AACR Inaugural International Cancer Immunotherapy Conference (September 25–28, 2016, New York, USA).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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