Human Vaccines & Immunotherapeutics 9:9, 2025–2032; September 2013; © 2013 Landes Bioscience

CIMT 2013

Advancing targeted therapies—report on the 11th Annual Meeting of the Association for Cancer Immunotherapy, May 14–16 2013, Mainz, Germany

Mustafa Diken[1,*], Sebastian Attig[2], Christian Grunwitz[1,2], Lena Kranz[1,2], Petra Simon[1], Niels van de Roemer[1,2], Fulvia Vascotto[1], and Sebastian Kreiter[1]

[1]TRON—Translational Oncology at the University Medical Center of Johannes Gutenberg University; Mainz, Germany; [2]Medical Department of University Medical Center; Johannes Gutenberg University; Mainz, Germany

Keywords: cancer immunotherapy, tumor vaccination, cellular therapy, combination, immunomonitoring, CIMT, tumor microenvironment

The 11th Annual Meeting of Association for Cancer Immunotherapy (CIMT) welcomed more than 700 scientists around the world to Mainz, Germany and continued to be the largest immunotherapy meeting in Europe. Renowned speakers from various fields of cancer immunotherapy gave lectures under CIMT2013's tag: "Advancing targeted therapies" the highlights of which are summarized in this meeting report.

Opening Lecture: Oncology Meets Immunology

In his opening lecture, Ira Mellman (Genentech, San Francisco, USA) introduced the current landscape of immunotherapeutic strategies comprising of checkpoint inhibitors, reversing tumor immunosuppression, vaccines and adoptive T cell therapies (ACT).[1] He further underlined the need for biomarker profiling to guide immunotherapeuties against cancer which can be achieved by various approaches such as immunochips, imaging, multi-parametic immunohistochemistry, TCR repertoire profiling, multi-channel FACS and multiplex cytokine assays. Ira Mellman also suggested bringing oncolytic interventions (BRAF, MEK, VEGF inhibitors) into combination with immunotherapy as yet another important strategy for the design of more efficient therapies against cancer.

Tumor Vaccination

High-dose IL-2 immunotherapy has been a promising treatment option for metastatic cancers but is often accompanied by severe side-effects such as pulmonary edema. In this respect, it was reported by Boyman et al. already in 2006 that the injection of IL-2-neutralizing monoclonal antibodies paradoxically enhances the biological activity of preexisting IL-2 compared with that of IL-2 alone through the formation of immune complexes.[2,3] The increased potency of these immune complexes may represent a way to reduce therapeutic doses and toxicities associated with conventional IL-2 therapy, as is supported by data from Krieg et al. where prevention of tumor lung metastases was demonstrated in mice strikingly in the absence of IL-2-caused pulmonary edema.[4] Hy Levitsky (Roche Pharma Research and Early Development, Schlieren, Switzerland) presented the development of a novel generation of tumor-targeted IgG-IL2 immunocytokines addressing the key limitations of IL-2 therapy. These immunocytokines comprise of an IgG with high-affinity, bivalent binding to CEA or FAP, an Fc part with abolished FcγR binding, and a monomeric IL-2 variant (IL2v) binding only to IL2-Rβγ (abrogation of CD25 binding). In their studies they found that abolished CD25 binding of IgG-IL2v led to a lack of preferential regulatory T cell (Treg) activation while maintaining activation of NK, $CD4^+$ and $CD8^+$ T cells when compared with IL-2 alone or IgG-IL2wt. The lack of the CD25 sink and improved tumor targeting resulted in increased systemic exposure of IgG-IL2v compared with IgG-IL2wt in immunocompetent C57BL/6 mice. Both FAP- and CEA-IL2v skewed the T cell ratio toward $CD8^+$ T cells and expanded NK cells in a fashion similar to IL-2 antibody complexes,[2] and were able to superiorly target intrarenal RENCA tumors and subcutaneous CEA-expressing tumors, respectively. In addition, CEA-IL2v treatment revealed single agent efficacy as it increased median and overall survival in a syngeneic MC38-huCEA colorectal cancer model in immunocompetent hCEA/hCD16 transgenic C57BL/6 mice whereas untargeted IgG-IL2v is toxic at the same dose. Hy Levitsky concluded that this type of targeted IL-2 immunotherapy might have a great impact on the treatment of metastatic cancers, whether applied as a single agent or in combination.

Cancer immunotherapies using dendritic cell (DC) vaccination preferably apply mature DCs (mDCs) loaded with tumor-associated antigens to activate and differentiate $CD8^+$ CTLs, polarize cytokine secretion of T cells and activate NK cells. The conventional protocol for ex vivo preparation of autologous mDCs from PBMCs used frequently in early-phase clinical trials produces immature DCs with GM-CSF and IL-4 within six

*Correspondence to: Mustafa Diken; Email: Mustafa.Diken@TrOn-Mainz.de
Submitted: 06/29/13; Accepted: 07/16/13
http://dx.doi.org/10.4161/hv.25768

days, followed by maturation for 24 h with TNFα, IL-1β, IL-6, and PGE_2.[5] Although displaying surface markers characteristic for mDCs, these cells failed to produce IL-12p70 which is critical for T_h1 induction and NK cell activation.

Dolores Schendel (Helmholtz Center, Munich, Germany) described the development of a maturation cocktail containing TLR7/8 agonist CL097 or R848 in addition to TNFα, IL-1β, IFNγ, PGE_2, and TLR3 ligand poly I:C that allows the rapid production of autologous mDCs within three days.[6-8] These cells stably express high levels of CD80 rather than PD-L1 and secrete large amounts of IL-12p70 but low levels of IL-10. NK cells activated by these "fast & polarized" mDCs produced superior levels of IFNγ and were able to lyse K562 target cells more efficiently. These mDCs also polarized allogeneic $CD4^+$ and $CD8^+$ T cells toward a T_h1/T_c1 phenotype, and MART-1/Melan-A pulsed, induced higher percentages of antigen-specific CTLs with greater tumor-specific cytotoxic activity. Using NOD/scid $IL2R\gamma^{null}$ (NSG) mice reconstituted with human PBMCs and vaccinating them with 3-d mDCs electroporated with MART-1/Melan-1 in vitro transcribed (*ivt*) RNA, her group demonstrated that 3-d fast and polarized mDCs were superior in their capacity to induce human antigen-specific cytotoxic T cells in vivo.[9] Dolores Schendel proposed that easy introduction of tumor antigens to these rapidly generated mDCs via RNA electroporation could open the door for feasible, antigen-tailored individualized DCs for many different cancer types.

Benefiting from this concept, the Schendel group has recently been focusing on adoptive transfer of TCR-engineered high-affinity T cells with antitumor specificity selected on allogeneic HLA molecules. Utilizing the non-negatively selected T cell repertoire of any donor negative for the patient HLA allele, high affinity donor TCRs selected with fast and polarized mDCs electroporated to present self-peptides and the patient's HLA allotype can be retrovirally introduced into patient PBLs ex vivo and adoptively transferred.[10,11] Comparing 12 HLA-A2-restricted tyrosinase peptide-specific CTL clones, they demonstrated that enhanced peptide sensitivity correlated with polyfunctional T_c1 cytokine secretion (IFNγ, TNFα, IL-2) as well as increased tumor cell recognition and lysis in vitro. Most importantly, these characteristics of the original CTL were maintained in recipient PBLs after TCR gene transfer and thus represent functional biomarkers for early CTL clone selection to be used in transgenic TCR T cell therapy.

Hans-Georg Rammensee (University of Tübingen, Tübingen, Germany) described a strategy to identify cancer-associated peptides via HLA ligandome analysis for individualized immunotherapy. He presented the potential and limitation of target identification by ligandome analysis, based on the findings from 10 paired samples of renal cell carcinoma (RCC) tumor and adjacent benign tissue. He further highlighted the clinical and immunological activity of IMA901 in RCC. IMA901 consists of 10 tumor–associated peptides (TUMAPs) confirmed to be naturally presented on tumor cells in patients with RCC. He mentioned that the number of peptide-specific immune responses is associated with increased survival in phase I and phase II clinical trials.[12] Rammensee presented an additional clinical study of IMA910 consisting of 13 TUMAPs in colorectal carcinoma and concluded that the effect was most pronounced in patients responding to multiple class I and class II TUMAPs. In yet another study, his group investigated the benefit of multi-peptide immunotherapy in prostate cancer which was well tolerated and induced T cell responses in most patients. The study also showed clinical benefit in a fraction of patients and the best benefit was obtained in combination with TLR7 ligands as adjuvants.

He also used both next generation sequencing and mass spectrometry for identification and validation of mutated peptides in tumors. For this purpose, tumor tissue from an RCC patient was sequenced and mutated HLA-ligands were predicted by MHC binding algorithms. Many unique mutated peptide sequences fitting to the patients HLA-haplotype were identified. However, two selected mutated peptides could not confirmed by mass spectrometry underscoring the difficulty to validate the predicted peptides by mass spectrometry due to the diffuse growth pattern in locally advanced RCC.

Two young investigators provided insights to the importance of dynamics as well as the route of administration for vaccine efficacy. In his talk, Yared Hailemichael (MD Anderson Cancer Center, Houston, USA) reported findings, why vaccine-activated killer T cells can eventually lose their tumor-killing function and become unable to kill cancer cells.[13] He showed that in mice anti-melanoma vaccines based on Incomplete Freund's Adjuvant (IFA), commonly used for the experimental treatment of patients with cancer, results in the persistence of the vaccine. By visualizing luciferase gene-transduced pmel-1 effector T cells transferred to vaccinated mice bearing gp100-expressing B16 melanoma, he observed, very surprisingly, that T cells travel to the vaccination site, become activated, but do not leave, never reaching the tumor. Persistent antigen presentation drove gp100-specific pmel-1 T cell accumulation at the injection site where these T cells largely became hyporesponsive and underwent apoptosis through a mechanism involving interferon-g and Fas ligand. Addition of immunostimulatory CD40-specific antibody, TLR7 agonist and interleukin-2 (IL-2) (together referred as covax), induced strong pmel-1 T cell expansion and reduced T cell death, however killer T cells continued to become trapped at the vaccination site. Replacing the non-biodegradable IFA formulation with a water-based, biodegradable formulation allowed the T cells to leave the vaccination site and traffic toward tumors, causing their regression. This short-lived vaccine also prevented T cell dysfunction and enabled long-term memory formation by these primed T cells. While water-based vaccination induced preferential T cell localization and tissue destruction at the tumor site, IFA based vaccination induced T cell sequestration, dysfunction and tissue destruction at vaccination sites.

Federico Sandoval (INSERM, Paris, France), on the other hand, presented the influence of mucosa-specific homing instructions to the immune system and how it controls mucosal tumor outgrowth.[14] Many tumors are located at mucosal sites, however most of the vaccines are tested against subcutaneous tumors. He showed that the growth of orthotropic head and neck or lung cancer was inhibited when a cancer vaccine was administered by the mucosal route but not after systemic administration.

Sandoval demonstrated that this benefit was dependent on $CD8^+$ T cells. Based on his findings, only intranasal vaccination elicited mucosa-specific $CD8^+$ T cells expressing the mucosal integrin CD49a and the blockade of CD49a attenuated intratumoral $CD8^+$ T cell infiltration.

Cellular Therapies

ACTs relying on the transfer of autologous ex-vivo expanded tumor-infiltrating lymphocytes or on the engineering of T cells with tumor-specific TCRs as well as chimeric antigen receptors (CARs) are powerful approaches to target immune responses to tumor cells. In contrast to conventional TCRs, CARs consist of a single chain and target exclusively cell surface antigens by an antibody-derived domain that is linked to one or more signaling molecules to activate engineered T cells. CARs overcome some limitations of TCRs as they are HLA independent, active in both $CD4^+$ and $CD8^+$ T cells and based on their single-chain character they have a reduced risk to generate autoimmunity.

Preclinical and clinical studies have demonstrated the curative potential of CARs, but also revealed issues, such as emergence of tumor escape variants, limited persistence of transferred cells and an immunosuppressive tumor microenviroment, that have to be addressed in order to improve CAR-based immunotherapy. Renier Brentjens (Memorial Sloan-Kettering Cancer Center, New York, USA) summarized clinical trial results using CD19-targeted T cells in low grade B cell malignancies of different trials[15-17] and reported on early-stage clinical success in acute lymphoblastic leukemia (ALL), a very aggressive disease.[18] He also pointed out that CAR T cells can be further modified to "armored" CAR T cells that co-express cytokines such as IL12 thereby overcoming inhibitory factors around the tumor.[19] He concluded that in spite of promising anti-tumor efficacy of CD19 CARs in the clinical setting, optimal CAR design, conditioning chemotherapy regimens and gene transfer technologies have to be further investigated in multicenter studies.

The latter subject was addressed by Laurence Cooper (MD Anderson Cancer Center, Houston, USA) who reported on the first in human application of the Sleeping Beauty (SB) system for engineering T cells to target $CD19^+$ tumor cells in cancer patients after hematopoietic stem-cell transplantation. This non-viral approach for gene delivery into T cells uses a clinical-grade plasmid-based transposon/transposase system as an inexpensive and flexible alternative to recombinant viruses to express a second-generation chimeric antigen receptor (CAR) that activates T cells via CD28 and CD3-zeta. He reported on the manufacturing process to generate the clinical products for three active clinical trials based on the combination of the SB system with an artificial APC (aAPC) platform[20] to propagate CAR-transgenic T cells to sufficient numbers. He showed initial clinical trial data demonstrating feasibility, safety, and persistence of transferred SB-modified and aAPC-propagated CAR^+ T cells.

The limited persistence of transferred cells is a major obstacle for successful T cell-based immunotherapies. Retrospective analysis of ACT trials demonstrated that the differentiation state of transferred T cells critically determines the effectiveness of antitumor T cell-based immunotherapies.[21-23] Luca Gattinoni (NIH, Bethesda, USA) pointed out that specific modulation of the T cell metabolism can be employed to enhance long-term T cell survival and antitumor function. He presented data demonstrating that T cell differentiation is associated with metabolic reprogramming. Naïve T cells dramatically shift to a glucose metabolism following antigen stimulation and effector differentiation leading to an increase in glycolytic metabolites. Moreover, he showed that constitutive activation of glycolysis limited memory formation in vivo. He demonstrated vice versa that inhibition of glycolysis by 2-deoxyglucose represents a strategy to enhance T cells survival and antitumor function upon adoptive transfer. His data indicate that inhibition of glycolysis promotes the transcriptional program of memory T cells, enhances memory T cell formation and the functional quality of these cells.

Improving Immunity

Although several studies showed induction of T cell responses against cancer, there are only few examples of objective clinical responses upon cancer immunotherapy highlighting the need for improving the immunity, novel means of which were presented in this session. Jill Slanksy (University of Colorado, Denver, USA) presented a collection of data on the strategy of mimotope based vaccination for the improvement of immune responses. Her group hypothesized that mimotopes, mimics of peptide epitopes, can be designed to improve anti-tumoral immunity by stimulating the antigen-specific T cells with a higher affinity signal compared with wild-type epitope through the TCR. For this purpose, they employed the syngenic transplantable CT26 colon carcinoma cells as a model system and generated a range mimotopes of the immunodominant $gp70_{423-431}$ (AH1) antigen against which tolerance is evident.[24] These mimotopes had similar relative binding affinity to the respective MHC molecule however formed higher affinity interactions with CT-TCR which recognize the wild-type peptide. Vaccination with mimotopes elicited a range of anti-tumoral immunity and it was revealed that tumor-protective mimotopes led to more AH1-specific T cells[24] which express a shared CDR3beta motif.[25] Interestingly, the use of an suboptimal mimotope for primary vaccination and boosting with the wild-type epitope led to higher amount of AH1-specific T cells which have increased affinity and decreased off-rate for AH1.[26] Moreover, this strategy enhanced the tumor protection elicited by the sub-optimal mimotope. Slansky also underlined the importance of considering TCRs from tumor-specific T cells present in the naturally responding repertoire versus rare TCRs with high affinity for the tumor antigen for the design of mimotope vaccines.

ACTs against various cancer types but especially against melanoma have been proved to be effective yet experimental investigation bringing tumor biology and tumor immunology together is required for explaining the development of resistance by tumors to T cell immunotherapy. Thomas Tüting (University Hospital, Bonn, Germany) shed light on this resistance through investigating the plasticity of tumor and immune cells. After

generating an in vivo mouse model (Hgf-Cdk4[R24C]) that mimics the regression, remission and relapse of human melanoma,[27] he could induce the regression of macroscopically visible melanoma lesions using an ACT protocol comprising of cyclophosphamide, adoptive transfer of gp100-specific CD8$^+$ T cells activated in vivo by an gp100 expressing adenovirus vector vaccine combined with immunostimulatory TLR ligands (CpG and poly I:C).[28] However, two months after remission, tumors frequently reappeared and progressed implying for a resistance to the therapy. Interestingly, he observed appearance of hypomelatonic or amelatonic areas in relapsing tumors and these showed partially reduced expression of the target antigen gp100 together with increased frequency of tumor infiltrating immune cells.[29] Interestingly, the relapse melanomas showed an increased frequency of spindle-shaped tumor cells strongly expressing the nerve growth factor receptor, NGFR (CD271), a marker also expressed by human melanoma initiating cells.[30] When cell lines generated from melanoma tumors were administered to mice, their relapse melanoma tumors also exhibited a dedifferentiated phenoype and were poorly recognized by antigen-specific T cells both of which were reversible in vivo upon retransplantation. Tüting could show that inflammatory mediators like TNFa can promote this reversible dedifferentiation in vitro, however upregulation of PD-L1 and MHC class I molecules on melanoma cells was largely mediated by IFNg instead. He provided evidence that both mouse and human melanoma cells exist in a dynamic equilibrium of two states of differentiation that rapidly adapts to the inflammatory signals in the environment and concluded that this inflammation induced phenotypic plasticity of tumor and immune cells can be regarded as a new mechanism of resistance to adoptive T cell therapies.[31]

John Bell (Ottawa Hospital Research Institute, Ottawa, Canada) described yet another means of enhancing anti-tumoral immunity via oncolytic viruses. These viruses can be regarded as miniature biological battleships which can spread between tumor cells by self-amplification and kill tumor cells in multiple ways.[32] After presenting examples of activity of oncolytic viruses upon locoregional or systemic therapy in various in preclinical and clinical settings,[33,34] he introduced oncolytic vaccines in which the virus is used as both an oncolytic and a tumor vaccine which enable benefiting the viral oncolysis as well as the potent induction of anti-tumor immune responses.[35] These designer immune responses against tumors were mediated by oncolytic rhabdovirus such as vesicular stomatitis virus (VSV) that encode and express a relevant tumor antigen which in turn result in more efficient induction of antigen-specific T cells, augmented tumor infiltrating lymphocyte frequencies and more importantly antigen spreading. The safety of another genetically engineered Maraba rhabdovirus based MAGE3 expressing oncolytic vaccine was shown in non-human primates and clinical trials with this vaccine is underway. Improvement of anti-tumoral immunity via rhabdoviral oncolytic vaccines holds promise as the infection and lysis of the tumor not only reverses the immunosupressive tumor microenvironment but also infection of immune cells in lymphoid organs like spleen potentiates the boosting of anti-tumor immune responses.

Immunoguiding

Innovative technologies that are able to monitor multiple parameters at the same time, some also at single cell level will show their full potential in the ongoing studies. Holden Maecker (Stanford University, Boston, USA) gave an exciting overview of the current state of play in immunomonitoring to look at immunocompetence and measure antigen specificity. With new technologies now available, intracellular cytokine secretion assays using a flow cytometer have been relegated to being a "simple" technology. Up to 17 markers, though typically fewer, can be stained at one time using a state of the art flow cytometer. The technical progress allows now a simultaneous analysis of multiple parameters like produced cytokines and differentiation status. Such panels allow a more detailed comparison of cancer patients with healthy donors, to identify cancer specific biomarkers. Important is that more than a cytokine secretion profile can be seen using flow cytometry, the phenoytpe of the cytokine secreting cells can be even more important than simply quantifying the activated cell numbers.

Besides flow cytometry, quantitative microengraving is a tool used to look at individual cells cytokine secretion profiles. It allows for cells to be seen in a live image, rather than at a static time point as is the case with flow cytometry. Using quantitative microengraving, it can be shown that "polyfunctional" cells sequentially secrete cytokines, rather than all at once as had previously been thought.

Finally the Cy-ToF is a mass spectrometer that allows for the staining of over 40 markers simultaneously using heavy metal labeled monoclonal antibodies.[36] The output is very similar to "classical" flow cytometry, but due to the extreme complexity, new analysis software is required. SPADE is such a software and is able to run multiparametric analysis, showing the cell populations in a dendrogram, allowing each population to be identified based upon its exact expression of each marker in the panel.

Philipp Beckhove (German Cancer Research Center, Heidelberg, Germany) showed the importance of Tregs in cancer immunotherapy. As also discussed by other speakers he mentioned the increasing complexity of vaccination regimen and therapies against cancer. The results of several approaches do not always lead to the same conclusions, as the mode of action and the individuality of each patient is not known in enough detail. Vaccination might be able to induce a tumor-specific cytotoxic immune response, but also boost inhibitory cells like Tregs. He explained that though spontaneous anti-tumor responses are often found ex vivo, they do not usually offer a survival advantage to the patient. Depleting Tregs is often an effective treatment and leads to an augmented immune response, however is also associated with side effects. However, the positive effect of an increased immune response can also be observed by a specific depletion of tumor antigen-specific Tregs. Monitoring tumor antigen-specific Tregs via multimers combined with CD25 and FoxP3 staining might be a prognostic marker to differ between potential responders—without pre-existing tumor antigen-specific Tregs—and non-responders—with pre-existing tumor antigen-specific Tregs.

Suzanne Ostrand-Rosenberg (University of Maryland, Baltimore, USA) discussed the role of myeloid-derived suppressor cells (MDSC) in cancer patients. MDSC numbers are elevated in virtually all cancer patients. MDSCs along with Tregs and M2 macrophage make the tumor microenvironment very tolerogenic. She showed that HMGB1 is an important differentiation factor for MDSCs. It regulates inflammation and binds to TLR-4 and the pattern recognition receptor RAGE, both of which are expressed on MDSCs. HMGB1 has both pro-inflammatory and anti-inflammatory domains and its action depends upon its secondary structure. RAGE deficiency in mice delays tumor progression, and a reduction of HMGB1 delays primary tumor progression along with reducing MDSC quantity and hydrogen peroxide content, restores L-selectin levels to naive T cells, and shifts macrophages toward an M1 phenotype. It was thus suggested that HMGB1 could be the master regulator of MDSC function in mice.

Combination Therapies

After several years of disappointing results from clinical trials, the approval of Ipilimumab in 2010 by the FDA set the stage for a new era in cancer immunotherapy. The study from Hodi et al. had a tremendous value for the field of cancer immunotherapy since it demonstrates that Immunotherapy on its own is able to achieve prolonged survival in patients with metastatic melanoma.[37] Accumulating evidence now suggests that combination therapies could be the next wave of innovations in oncology. The idea to combine cancer immunotherapy with other cancer therapies is driven by three main scientific hypotheses which were summarized by Rami Ibrahim (BMS, Gaithersburg, USA) during his talk at CIMT. One rational is to achieve complementary response kinetics. In most cases immune mediated effects in cancer show a delayed but sustained effect for a small proportion of patients, whereas small molecules like the BRAF signaling inhibitors show a rapid but transient improvement for many patients. Ideally such a combination could unlock the full potential of Immunotherapies and lead to a faster and sustained response in more patients than administration of one drug alone. The second is to combine with vaccine treatment which can steer the non-specific immune activation towards the tumor. Vaccine alone is usually not sufficient to induce complete tumor regression and overcome the suppressive tumor microenvironment therefore one way to boost immunotherapies is to utilize the direct and indirect effects on the immune system of conventional anticancer drugs. Intensive experimental efforts devoted to elucidate the mechanisms beyond their anti-proliferating potential revealed that cytotoxic agents induce different cell death modalities, show immunomodulatory potential and can selectively target immunosuppressive cell populations.[38] Combination of Ipilimumab with chemotherapy in NSCLC patients revealed that the concurrent Ipilimumab regime did not significantly improve immune-related progression-free survival (irPFS) compared with the control regime, whereas the phased ipilimumab regime, administered after chemotherapy, significantly improved the irPFS, which could be potentially contributed to the augmentation of T-cell activation by Ipilimumab after chemotherapy.[39] Cytotoxic agents which have direct effects on the tumor cells can also be used for combination. Ramy Ibrahim also presented preclinical data on sorafinib, an approved angiogenesis inhibitor, the use of which was also associated with reduction of MDSCs indicating that even anti-angiogenic agents could have an immune effect. It is also described that a chemotherapeutic agent, 5-Flurouracil (5-FU) can selectively kill MDSCs[40] however it also leads to secretion of IL1-β, which curtails anticancer efficacy.[41] The combination of 5-FU with an IL-1 receptor antagonist was shown to enhance the antitumor efficiency.[41]

In addition to the aforementioned combinatorial approaches, Zaima Mazorra Herrera (Center for Molecular Immunology, Havana, Cuba) presented the combination of two vaccines targeting EGF and NeuGcGM3 ganglioside in stage IIIB or IV NSCLC. CIMAvax administration alone leads to a deprivation of circulating EGF resulting in an inhibition of proliferation signals. Preliminary data from a conducted Phase III study showed a median overall survival (OS) of 11.2 mo in the vaccine group in comparison to 7.7 mo in the control group. Furthermore the study revealed that the anti-EGF antibody titer could be used as an immunological surrogate of clinical benefit. Good antibody responders (Ab titer > 1:4000) showed an OS of 18.6 mo against 11,2 mo in the poor antibody responder group (Ab titer < 1:4000). Racotumumab targets N-glycosylated variant of GM3 known to impair DC and $CD4^+$ cell function.[42] Additionally it was recently demonstrated that NSCLC patients with high NeuGc-containing gangliosides had a low OS and a significant lower survival rate.[43] As well as CIMAvax administration, Racotumumab treatment leads to an improved OS in stage IIIB/IV NSCLC patients. The preliminary data of the combination regime look promising: Improved antibody response against EGF and Racotumomab were achieved and 30% of treated patients were alive at 18 mo after the inclusion. These findings suggest a benefit for first line chemotherapy progressors in NSCLC. In order to confirm these results a new randomized clinical trial in advanced NSCLC patients unfit for chemotherapy will be performed.

Alex Hoos (GSK, Collegeville, USA) summarized that progress has to be made on two fronts to foster the development of combination therapies. On the scientific side, there is a need to improve our understanding of the tumor environment and its suppressive signals, gain more insights in the basic immune biology and take the opportunity to find new biomarkers. In addition, going back and rediscovering the influence of conventional therapies on the immune system to build a valid basis for a science driven approach for further investigations is necessary. Beside these scientific improvements, there is also a need to proceed tailoring the response criteria and clinical endpoints to address the unique features of immunotherapeutic agents.[44,45] Moreover, challenges like abundance of targets, increasing number of drug actions and tumor heterogeneity have to be confronted. Combination therapy, therefore, requires operational discipline and innovate study designs, accustomed regulatory concepts and intensive collaboration. But hard work pays off—combination therapy can deliver greater patient benefit, innovation and an improved understanding of cancer immunotherapies.

Tumor Microenvironment

Various immunotherapeutic strategies against cancer aim to reduce tumor growth and push immune system to eradicate tumors. However, all these approaches should show effectiveness in the tumor environment where tumor, endothelia, stroma cells as well as immune cells share their space for survival. Speakers in tumor microenvironment session, described some of the currently used strategies to prevent tumor growth by locally attacking tumor cells and tumor microenvironment with classical anti-tumor chemotherapies, novel inhibitors and emerging molecular tools (miRNAs).

George Prendergast (Lankenau Institute for Medical Research, Wynnewood, USA), who opened the session, gave a charming overview through the history of the relationship among immunological and genetic approaches to eradicate cancer.[46] The presentation was then focused on indoleamine 2,3-dioxygenase (IDO), as a good example of how these elective affinities merged. IDO is overexpressed in many types of tumor cells where its presence has been associated with poor prognosis. Preclinical data showed that IDO-deficient mice are resistant to inflammatory skin carcinogenesis[47] and Ido1 gene disruption in mouse models of pulmonary metastasis resulted in reduced lung tumor burden and improved survival.[48] He described that IDO may act at multiple sites to blunt anti-tumor immunity and is involved in immune escape, invasion, tolerance, angiogenesis and metastasis. IDO is not per se critical for cancer development, however, it rather programs a cancerous inflammation. IDO inhibitors are recently promoted for treatment of cancer and one such inhibitor 1-methyl-D-tryptophan (D-1MT) was shown to relieve the IDO mediated tryphophan related inhibition of mammalian target of Rapamycin (mTOR) suggesting its broader clinical use against cancer.[49]

Antonio Mantovani (Instituto Clinico Humanitas, Milan, Italy) presented data on the characterization of tumor environment during cancer progression. Among the complex chemokine systems involved in tumor development and immunesuppression, chemokine decoy receptor D6, one of the atypical chemokine receptors (ACKRs), which are required for the generation of chemokine gradients in tissues, showed a remarkable effect in tumor regression.[50] Up to now, ACKRs were considered "silent receptors" because no G protein–dependent signaling activity has been described to be involved after their engagement by cognate ligands. However, it was published recently that β-arrestin1 is the adaptor molecule engaged by D6 upon its ligation.[51] This signaling pathway is required for the increased abundance of D6 protein at the cell surface and for its chemokine-scavenging activity as regulator of chemokine-mediated responses in inflammation and immunity. Interestingly, D6 transfected Karposi's sarcoma cell line (KS-IMM) has a slower tumor growth in mice due to reduced expression of inflammatory chemokines, reduced angiogenesis, lower infiltration of macrophages and neutrophils in comparison to control tumors. These insights into the signaling properties of D6 may lead to identification of new therapeutic approaches aimed at regulating inflammatory responses.

In addition, his group investigated the changes in tumor microenvironment during anti-cancer therapy using trabectedin, a natural product derived from marine tunicate, currently approved by EMA for treatment of soft tissue sarcomas and ovarian cancer. However, the mode of action for this clinically-approved chemotherapeutic agent has been not described so far. Mantovani showed that trabectedin selectively decreased circulating monocytes, depleted TAM, reduced tumor angiogenesis as well as expression of some inflammatory cytokines (such as IL6 and CCL2). The elegant work was performed first in several mouse tumor models and later in human on soft tissue sarcoma patients receiving trabectedin as a single dose. They showed that trabectedin acted via an intrinsic pathway mediated by TRAIL-R and caspase 8 followed by caspase 9 expressed selectively on monocytes and macrophages inducing cell death. In conclusion, among the different hallmarks for anti-tumor therapies, he favored targeting immunosuppressive tumor associated macrophages (TAM). Interestingly, in addition to elimination of these (via trabectidin or anti-CCL2 antibody) as a powerful anti-cancer strategy, their re-education (via anti CD40 agonist mAb or IFN-g) can also be an attractive way to modulate tumor environment.

Vincent Bronte (University of Verona, Verona, Italy) presented two examples of adjuvant therapies to ACT. His first example involved characterization of myeloid cell subsets circulating in tumor bearing mice which were able to tolerize anti-tumor $CD8^+$ T cells in spleens. Those MDSCs were susceptible to low-dose chemotherapy (5-FU), which has been demonstrated to be exploited as an adjuvant for ACT.[52]

In the second part of his talk, Bronte presented a new approach to modulate immunity and tumor environment, altering infiltration of immune cells by miRNA overexpression.[53] In this approach, he performed an miRNA analysis of $CD11b^+$ myeloid cells in the spleen of healthy and tumor bearing mice which revealed around 80 differentially expressed. Among those, miRNA-142–3p showed an interesting function in controlling M2 macrophage generation from bone marrow cells in vitro. In particular, miRNA142–3p inhibited suppressive function of M2 macrophages in an in vitro CD8 T cell proliferation assay. The inhibition of suppressive mechanism relied at least in part on inhibition of IL6 pathway in macrophages. He further provided evidence that miRNA 142–3p was regulated by isoform ratio of Cebpβ. Although miRNA 142–3p has been recently described to be relevant for DC,[54] Bronte showed for the first time the in vivo effect of overexpression of this miRNA in bone marrow precursors during tumor growth. This overexpression sustained the decrease of monocytes and macrophages in bone marrow while in blood and spleen only macrophages were continuously low represented. Interestingly, tumor experiments using these chimeric overexpressing miR 142–3p showed a strong reduction of MCA203 tumor infiltrating macrophages, while infiltrated DC were increased. When tumor injection was followed by ACT of mTERT specific T cells in combination with miRNA 142–2p overexpression on bone marrow cells, a remarkable benefit on survival of mice in comparison to the single monotherapies was

observed highlighting novel efficient adjuvant tools for ACT against tumors.

Key Note Lecture

In his key note lecture, Tak W Mak (Ontario Cancer Institute, Toronto, Canada) provided the listeners a view on the "bigger picture" of specific therapeutic options against cancer including but not restricting himself to immunotherapy. To describe the state of development he introduced the analogy of the cart being pulled by the horses. Up to now, the oncogenes represented by the horses driving the cart where the focus of therapeutic developments (e.g., anti Her2 antibodies, Braf inhibitors) is. By his definition the cart represents the transformed state of the cells as a consequence of the actions of oncogenes and tumor suppressor genes. Tak W Mak proposed that targeting the cart could open new prospects for development of effective anti-cancer drugs. Possible areas of attack could be the altered metabolic state of the cancer cells and the possibility to reprogram the immune system in order to recognize the transformed cells.

In the following he provided different examples for the suggested concept by giving insight into studies performed in his group. Among others he discussed interleukin 7 (IL-7) and demonstrated the multifaceted effects cytokines can have enabling the immune system to overcome immunosuppression and foster anti-tumoral effects.[55,56] Starting from completely different point of origin Tak W. Mak shared the fascinating observation that Nervus vagus stimulation protects from sepsis in animal models. He then demonstrated the tight connection of immune system and nerval system by dissection of the causal mechanisms of this observation.[57,58]

Conclusion

Highlights of the advancements in the field presented in this report constitute a valid body of evidence for the efficacy of cancer immunotherapy which has reproven itself as a promising approach for the fight against cancer. Understanding the nature of combinatory approaches, identifying mediators and biomarkers together with careful selection and evaluation of target antigens can pave the way for significant improvements which will be hopefully presented at the 12th Annual CIMT Meeting (CIMT2014) which is to be held between May 6–8, 2014 in Mainz, Germany.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Authors would like to thank all speakers of CIMT2013 whose lectures formed the basis of this report.

References

1. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature 2011; 480:480-9; PMID:22193102; http://dx.doi.org/10.1038/nature10673
2. Boyman O, Kovar M, Rubinstein MP, Surh CD, Sprent J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. Science 2006; 311:1924-7; PMID:16484453; http://dx.doi.org/10.1126/science.1122927
3. Prlic M, Bevan MJ. Immunology. An antibody paradox, resolved. Science 2006; 311:1875-6; PMID:16574856; http://dx.doi.org/10.1126/science.1126030
4. Krieg C, Létourneau S, Pantaleo G, Boyman O. Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. Proc Natl Acad Sci U S A 2010; 107:11906-11; PMID:20547866; http://dx.doi.org/10.1073/pnas.1002569107
5. Jonuleit H, Kühn U, Müller G, Steinbrink K, Paragnik L, Schmitt E, et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. Eur J Immunol 1997; 27:3135-42; PMID:9464798; http://dx.doi.org/10.1002/eji.1830271209
6. Zobywalski A, Javorovic M, Frankenberger B, Pohla H, Kremmer E, Bigalke I, et al. Generation of clinical grade dendritic cells with capacity to produce biologically active IL-12p70. J Transl Med 2007; 5:18; PMID:17430585; http://dx.doi.org/10.1186/1479-5876-5-18
7. Spranger S, Javorovic M, Bürdek M, Wilde S, Mosetter B, Tippmer S, et al. Generation of Th1-polarizing dendritic cells using the TLR7/8 agonist CL075. J Immunol 2010; 185:738-47; PMID:20511554; http://dx.doi.org/10.4049/jimmunol.1000060
8. Bürdek M, Spranger S, Wilde S, Frankenberger B, Schendel DJ, Geiger C. Three-day dendritic cells for vaccine development: antigen uptake, processing and presentation. J Transl Med 2010; 8:90; PMID:20920165; http://dx.doi.org/10.1186/1479-5876-8-90
9. Spranger S, Frankenberger B, Schendel DJ. NOD/scid IL-2Rg(null) mice: a preclinical model system to evaluate human dendritic cell-based vaccine strategies in vivo. J Transl Med 2012; 10:30; PMID:22364226; http://dx.doi.org/10.1186/1479-5876-10-30
10. Wilde S, Sommermeyer D, Leisegang M, Frankenberger B, Mosetter B, Uckert W, et al. Human antitumor CD8+ T cells producing Th1 polycytokines show superior antigen sensitivity and tumor recognition. J Immunol 2012; 189:598-605; PMID:22689880; http://dx.doi.org/10.4049/jimmunol.1102165
11. Wilde S, Schendel DJ. High-quality and high-avidity T cell clones specific for tumor-associated antigens and how to find them. Oncoimmunology 2012; 1:1643-4; PMID:23264922; http://dx.doi.org/10.4161/onci.21717
12. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 2012; PMID:22842478; http://dx.doi.org/10.1038/nm.2883
13. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, et al. Persistent antigen at vaccination sites induces tumor-specific $CD8^+$ T cell sequestration, dysfunction and deletion. Nat Med 2013; 19:465-72; PMID:23455713; http://dx.doi.org/10.1038/nm.3105
14. Sandoval F, Terme M, Nizard M, Badoual C, Bureau MF, Freyburger L, et al. Mucosal imprinting of vaccine-induced $CD8^+$ T cells is crucial to inhibit the growth of mucosal tumors. Sci Transl Med 2013; 5:72ra20; PMID:23408053; http://dx.doi.org/10.1126/scitranslmed.3004888
15. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 2012; 119:2709-20; PMID:22160384; http://dx.doi.org/10.1182/blood-2011-10-384388
16. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011; 3:95ra73; PMID:21832238; http://dx.doi.org/10.1126/scitranslmed.3002842
17. Brentjens RJ, Rivière I, Park JH, Davila ML, Wang X, Stefanski J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 2011; 118:4817-28; PMID:21849486; http://dx.doi.org/10.1182/blood-2011-04-348540
18. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 2013; 5:77ra38; PMID:23515080; http://dx.doi.org/10.1126/scitranslmed.3005930
19. Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood 2012; 119:4133-41; PMID:22354001; http://dx.doi.org/10.1182/blood-2011-12-400044
20. Singh H, Figliola MJ, Dawson MJ, Olivares S, Zhang L, Yang G, et al. Manufacture of Clinical-Grade CD19-Specific T Cells Stably Expressing Chimeric Antigen Receptor Using Sleeping Beauty System and Artificial Antigen Presenting Cells. PLoS One 2013; 8:e64138; PMID:23741305; http://dx.doi.org/10.1371/journal.pone.0064138

21. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, 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
22. Gattinoni L, Klebanoff CA, Restifo NP. Paths to stemness: building the ultimate antitumour T cell. Nat Rev Cancer 2012; 12:671-84; PMID:22996603; http://dx.doi.org/10.1038/nrc3322
23. Klebanoff CA, Gattinoni L, Restifo NP. Sorting through subsets: which T-cell populations mediate highly effective adoptive immunotherapy? J Immunother 2012; 35:651-60; PMID:23090074; http://dx.doi.org/10.1097/CJI.0b013e31827806e6
24. McWilliams JA, Sullivan RT, Jordan KR, McMahan RH, Kemmler CB, McDuffie M, et al. Age-dependent tolerance to an endogenous tumor-associated antigen. Vaccine 2008; 26:1863-73; PMID:18329760; http://dx.doi.org/10.1016/j.vaccine.2008.01.052
25. Jordan KR, Buhrman JD, Sprague J, Moore BL, Gao D, Kappler JW, et al. TCR hypervariable regions expressed by T cells that respond to effective tumor vaccines. Cancer Immunol Immunother 2012; 61:1627-38; PMID:22350070; http://dx.doi.org/10.1007/s00262-012-1217-5
26. Buhrman JD, Jordan KR, U'ren L, Sprague J, Kemmler CB, Slansky JE. Augmenting antitumor T-cell responses to mimotope vaccination by boosting with native tumor antigens. Cancer Res 2013; 73:74-85; PMID:23161490; http://dx.doi.org/10.1158/0008-5472.CAN-12-1005
27. Tormo D, Ferrer A, Bosch P, Gaffal E, Basner-Tschakarjan E, Wenzel J, et al. Therapeutic efficacy of antigen-specific vaccination and toll-like receptor stimulation against established transplanted and autochthonous melanoma in mice. Cancer Res 2006; 66:5427-35; PMID:16707471; http://dx.doi.org/10.1158/0008-5472.CAN-06-0399
28. Kohlmeyer J, Cron M, Landsberg J, Bald T, Renn M, Mikus S, et al. Complete regression of advanced primary and metastatic mouse melanomas following combination chemoimmunotherapy. Cancer Res 2009; 69:6265-74; PMID:19622767; http://dx.doi.org/10.1158/0008-5472.CAN-09-0579
29. Landsberg J, Kohlmeyer J, Renn M, Bald T, Rogava M, Cron M, et al. Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. Nature 2012; 490:412-6; PMID:23051752; http://dx.doi.org/10.1038/nature11538
30. Boiko AD, Razorenova OV, van de Rijn M, Swetter SM, Johnson DL, Ly DP, et al. Human melanoma-initiating cells express neural crest nerve growth factor receptor CD271. Nature 2010; 466:133-7; PMID:20596026; http://dx.doi.org/10.1038/nature09161
31. Hölzel M, Bovier A, Tüting T. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? Nat Rev Cancer 2013; 13:365-76; PMID:23535846; http://dx.doi.org/10.1038/nrc3498
32. Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. Nat Biotechnol 2012; 30:658-70; PMID:22781695; http://dx.doi.org/10.1038/nbt.2287
33. Heo J, Reid T, Ruo L, Breitbach CJ, Rose S, Bloomston M, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. Nat Med 2013; 19:329-36; PMID:23396206; http://dx.doi.org/10.1038/nm.3089
34. Hwang TH, Moon A, Burke J, Ribas A, Stephenson J, Breitbach CJ, et al. A mechanistic proof-of-concept clinical trial with JX-594, a targeted multi-mechanistic oncolytic poxvirus, in patients with metastatic melanoma. Mol Ther 2011; 19:1913-22; PMID:21772252; http://dx.doi.org/10.1038/mt.2011.132
35. Bridle BW, Stephenson KB, Boudreau JE, Koshy S, Kazdhan N, Pullenayegum E, et al. Potentiating cancer immunotherapy using an oncolytic virus. Mol Ther 2010; 18:1430-9; PMID:20551919; http://dx.doi.org/10.1038/mt.2010.98
36. Bandura DR, Baranov VI, Ornatsky OI, Antonov A, Kinach R, Lou X, et al. Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. Anal Chem 2009; 81:6813-22; PMID:19601617; http://dx.doi.org/10.1021/ac901049w
37. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, 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
38. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov 2012; 11:215-33; PMID:22301798; http://dx.doi.org/10.1038/nrd3626
39. Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol 2012; 30:2046-54; PMID:22547592; http://dx.doi.org/10.1200/JCO.2011.38.4032
40. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010; 70:3052-61; PMID:20388795; http://dx.doi.org/10.1158/0008-5472.CAN-09-3690
41. Bruchard M, Mignot G, Derangère V, Chalmin F, Chevriaux A, Végran F, et al. Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. Nat Med 2013; 19:57-64; PMID:23202296; http://dx.doi.org/10.1038/nm.2999
42. Hayashi N, Chiba H, Kuronuma K, Go S, Hasegawa Y, Takahashi M, et al. Detection of N-glycolyated gangliosides in non-small-cell lung cancer using GMR8 monoclonal antibody. Cancer Sci 2013; 104:43-7; PMID:23004020; http://dx.doi.org/10.1111/cas.12027
43. de León J, Fernández A, Clavell M, Labrada M, Bebelagua Y, Mesa C, et al. Differential influence of the tumour-specific non-human sialic acid containing GM3 ganglioside on CD4+CD25− effector and naturally occurring CD4+CD25+ regulatory T cells function. Int Immunol 2008; 20:591-600; PMID:18310617; http://dx.doi.org/10.1093/intimm/dxn018
44. Hoos A, Ibrahim R, Korman A, Abdallah K, Berman D, Shahabi V, et al. Development of ipilimumab: contribution to a new paradigm for cancer immunotherapy. Semin Oncol 2010; 37:533-46; PMID:21074069; http://dx.doi.org/10.1053/j.seminoncol.2010.09.015
45. Hoos A, Eggermont AM, Janetzki S, Hodi FS, Ibrahim R, Anderson A, et al. Improved endpoints for cancer immunotherapy trials. J Natl Cancer Inst 2010; 102:1388-97; PMID:20826737; http://dx.doi.org/10.1093/jnci/djq310
46. Prendergast GC. Immunological thought in the mainstream of cancer research: Past divorce, recent remarriage and elective affinities of the future. Oncoimmunology 2012; 1:793-7; PMID:23162746; http://dx.doi.org/10.4161/onci.20909
47. Muller AJ, DuHadaway JB, Chang MY, Ramalingam A, Sutanto-Ward E, Boulden J, et al. Non-hematopoietic expression of IDO is integrally required for inflammatory tumor promotion. Cancer Immunol Immunother 2010; 59:1655-63; PMID:20640572; http://dx.doi.org/10.1007/s00262-010-0891-4
48. Smith C, Chang MY, Parker KH, Beury DW, DuHadaway JB, Flick HE, et al. IDO is a nodal pathogenic driver of lung cancer and metastasis development. Cancer Discov 2012; 2:722-35; PMID:22822050; http://dx.doi.org/10.1158/2159-8290.CD-12-0014
49. Metz R, Rust S, Duhadaway JB, Mautino MR, Munn DH, Vahanian NN, et al. IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: A novel IDO effector pathway targeted by D-1-methyl-tryptophan. Oncoimmunology 2012; 1:1460-8; PMID:23264892; http://dx.doi.org/10.4161/onci.21716
50. Graham GJ, Locati M, Mantovani A, Rot A, Thelen M. The biochemistry and biology of the atypical chemokine receptors. Immunol Lett 2012; 145:30-8; PMID:22698181; http://dx.doi.org/10.1016/j.imlet.2012.04.004
51. Borroni EM, Cancellieri C, Vacchini A, Benureau Y, Lagane B, Bachelerie F, et al. β-arrestin-dependent activation of the cofilin pathway is required for the scavenging activity of the atypical chemokine receptor D6. Sci Signal 2013; 6:1-11, S1-3; PMID:23633677; http://dx.doi.org/10.1126/scisignal.2003627
52. Ugel S, Peranzoni E, Desantis G, Chioda M, Walter S, Weinschenk T, et al. Immune tolerance to tumor antigens occurs in a specialized environment of the spleen. Cell Rep 2012; 2:628-39; PMID:22959433; http://dx.doi.org/10.1016/j.celrep.2012.08.006
53. Sonda N, Simonato F, Peranzoni E, Calì B, Bortoluzzi S, Bisognin A, et al. miR-142-3p Prevents Macrophage Differentiation during Cancer-Induced Myelopoiesis. Immunity 2013; 38:1236-49; PMID:23809164; http://dx.doi.org/10.1016/j.immuni.2013.06.004
54. Mildner A, Chapnik E, Manor O, Yona S, Kim KW, Aychek T, et al. Mononuclear phagocyte miRNome analysis identifies miR-142 as critical regulator of murine dendritic cell homeostasis. Blood 2013; 121:1016-27; PMID:23212522; http://dx.doi.org/10.1182/blood-2012-07-445999
55. Pellegrini M, Calzascia T, Toe JG, Preston SP, Lin AE, Elford AR, et al. IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. Cell 2011; 144:601-13; PMID:21295337; http://dx.doi.org/10.1016/j.cell.2011.01.011
56. Toe JG, Pellegrini M, Mak TW. Promoting immunity during chronic infection-The therapeutic potential of common gamma-chain cytokines. Mol Immunol 2013; 56:38-47; PMID:23685259; http://dx.doi.org/10.1016/j.molimm.2013.04.008
57. Rosas-Ballina M, Olofsson PS, Ochani M, Valdés-Ferrer SI, Levine YA, Reardon C, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 2011; 334:98-101; PMID:21921156; http://dx.doi.org/10.1126/science.1209985
58. Reardon C, Duncan GS, Brüstle A, Brenner D, Tusche MW, Olofsson PS, et al. Lymphocyte-derived ACh regulates local innate but not adaptive immunity. Proc Natl Acad Sci U S A 2013; 110:1410-5; PMID:23297238; http://dx.doi.org/10.1073/pnas.1221655110