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The fourteenth International Conference on Progress in Vaccination Against Cancer (PIVAC-14), September 24-26, 2014, Rome, Italy: rethinking anti-tumor vaccines in a new era of cancer immunotherapy.

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Précis

The PIVAC-14 conference brought together translational and clinical oncologists and immunologists who provided an up-to-date overview of the most recent advances in active cancer vaccination and adoptive cell therapy.

Abbreviations:

ACT adoptive cell transfer

CDR complementarity determining regions

CMM canine malignant melanoma

CSC cancer stem cells

DC dendritic cells

DTH delayed-type hypersensitivity

ECM extracellular matrix

ECT electrochemotherapy

EP electropulsation

HCC hepatocellular carcinoma

HCV hepatitis C virus

HDAC histone deacetylases

HPV human papilloma virus

IFN interferon

Ii MHC class II-associated invariant chain protein

IL interleukin

M2 alternatively activated macrophages

MDSC myeloid-derived suppressor cells

PBMC peripheral blood mononuclear cells

PSA prostate specific antigen

PTX3 pentraxin 3

ROS reactive oxygen species

SPARC secreted protein acidic and rich in cysteine

TAM tumor-associated macrophages

TCR T cell receptor

TGF tumor growth factor

TIL tumor infiltrating lymphocytes

TNF tumor necrosis factor

TRAIL-R TNF-related apoptosis-inducing ligand receptor

Treg T regulatory cells

VEGF vascular endothelial growth factor

PIVAC-14, the fourteenth annual conference on Progress in Vaccination Against Cancer (http://www.tati-group.de/pivac/) has been held September 24-26, 2014, at the Donna Camilla Savelli Hotel in Rome. More than 110 attendees presented and discussed a plethora of new and promising results in a relaxed and informal atmosphere.

The main topics covered by PIVAC-14 were: cancer immunoprevention; the role of the tumor microenvironment in determining immunotherapy outcomes; the use of comparative oncology in predicting immunotherapy effectiveness; tumor escape mechanisms and the latest experimental and clinical advances in various vaccination strategies, including peptide-, dendritic cell- and T cell-based vaccines. Special attention was paid to DNA vaccination and electrotransfer, which benefited from a dedicated section co-organized by COST Action TD1104 - EP4Bio2Med, a European network of researchers working on the development of electroporation-based technologies and treatment in the field of medicine, biotechnology and environment preservation (see: http://www.electroporation.net).

The promise of preventive cancer vaccines

Cancer immunoprevention has demonstrated its efficacy against infection-associated tumors like those induced by the human papilloma virus (HPV), as overviewed by **Aldo Venuti** (Regina Elena National Cancer Institute, Rome, Italy) and **Marij Welters** (Leiden University Medical Center, Clinical Oncology, Leiden, Netherlands) in their lectures. Recently the possibility to apply cancer-vaccines in a preventive setting against non-infection-related cancers has gained consensus and is now considered the next frontier in the battle against cancer. This was the main topic of **Olivera J. Finn** (University of Pittsburgh School of Medicine, Pittsburgh, PA, USA) opening lecture. Most therapeutic cancer vaccine trials have so far only furnished marginal results. So-called push-pull strategies, where vaccination is combined with the administration of adjuvants, cytokines, immune checkpoint inhibitors or other strategies that may counteract tumor-induced immunosuppression, hold the potential to increase the efficacy of therapeutic cancer vaccines. These treatments are expensive and some are also very toxic. A practical and feasible alternative is to vaccinate patients in the pre-malignant setting, targeting antigens expressed by premalignant lesions. The hypothesis is that the immune system will be less suppressed in the premalignant state. Finn and coworkers have chosen advanced adenomas of the colon as premalignant precursors of colon cancer and MUC1 antigen, whose expression during colon cancer development increases from small adenomas to metastasis. Patients were vaccinated with a MUC1 100mer peptide, admixed with the TLR3 agonist Hiltonol®, after removal of the polyps; the vaccine elicited

anti-MUC1 IgG responses and long-term memory in 50% of the patients. No correlation between response and HLA expression, age or gender was found. However, the peripheral blood mononuclear cells (PBMC) of non-responders contained increased levels of myeloid-derived suppressor cells (MDSC), but not of T regulatory cells (Treg) prior to vaccination, which eventually impaired the response to the vaccine. In order to verify the presence of other response predictive factors, responder and non-responder PBMC gene expression profiles were analyzed in pre-vaccination PBMC. Close to 350 genes involved in interferon (IFN) signaling, innate immunity and T cell pathways, showed completely different expression in responders and non-responders.

The hypothesis presented was that responders to the vaccine had previous immune memory against abnormal MUC-1, which may have been generated during viral infections and other nonmalignant acute inflammatory events, and the vaccine simply acted as a boost. People that had not previously had acute inflammation due to viral infections had low levels of specific T cells and high MDSC and did not respond to the vaccine. A new multi-center, randomized, placebo-controlled phase II efficacy trial of the MUC1 prophylactic vaccine started in May 2014; results are expected in 3-5 years.

Inflammation and extracellular matrix shape the tumor microenvironment

While acute inflammation may have a positive role in predisposing the host to react against tumors and respond to cancer vaccines, chronic inflammation instead is known to promote tumor progression. As discussed by **Alberto Mantovani** (Istituto Clinico Humanitas IRCCS, University of Milan, Rozzano, Italy) in his opening lecture, there are two pathways that connect cancer and chronic inflammation: the intrinsic pathway, in which the genetic events that cause cell transformation force the transformed cell to produce inflammatory mediators and promote the generation of an inflamed tumor microenvironment; and the extrinsic pathway, in which a preexisting inflammatory microenvironment increases the risk of neoplastic transformation. Both pathways induce the production of inflammatory mediators that recruit and activate various leukocytes, mainly myeloid cells, which in turn contribute in maintaining inflammatory conditions. A key role in the maintenance of the inflammatory environment is played by tumor-associated macrophages (TAM) that display the alternatively activated (M2) phenotype. Patients that have high TAM levels generally show poor prognosis. Targeting TAM may therefore be an effective anti-tumor strategy. The anti-tumor agent Trabectedin, a natural product derived from a marine tunicate that binds the minor groove of DNA, may be particularly effective in this context. It is selectively cytotoxic for monocytes as it

activates an extrinsic apoptosis pathway that is dependent on tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor (TRAIL-R). Besides depleting macrophages *in vivo*, Trabectedin also significantly reduces the expression of vascular endothelial growth factor (VEGF) and of the chemokine CCL2 in tumor vessels, thereby limiting tumor angiogenesis as well as monocyte recruitment and differentiation into tumor-promoting TAM. Another key component of cancer-related inflammation is the complement system. Mantovani and co-workers have recently shown that a molecule from the interleukin (IL)1-R family, the humoral pattern recognition molecule Pentraxin 3 (PTX3), protects against cancer development by regulating complement-dependent tumor-promoting inflammation. PTX3 expression in human tumors is frequently inhibited by gene methylation. PTX3 deficient (PTX $3^{-/-}$) mice have increased susceptibility to chemically induced carcinogenesis (earlier tumor appearance, increased growth and angiogenesis, early increase in TAM, increased p53 mutations and gene instability in tumor cells). PTX3 deficiency is also associated with increased complement activation at the tumor site and increased cancer-related inflammation. Moreover, C3 deficiency, C5aR inhibition and macrophage recruitment inhibition in tumors all significantly reduce susceptibility to 3-methylcolanthrene-induced carcinogenesis in $PTX3^{-/-}$ mice. These data bring to light a new connection between inflammation and cancer by showing that PTX3, an effector molecule in innate immunity, may act as an extrinsic oncosuppressor.

The key role of the extracellular matrix (ECM) in shaping the tumor microenvironment was discussed by **Mario Paolo Colombo** (Istituto Nazionale Tumori, Milan, Italy). He showed that four breast cancer prognostic groups can be identified according to differing ECM gene expression enrichment which leads to different ECM signatures. The most prominent gene that was overexpressed in the signature and which identifies high-grade tumors with poor response to therapy encodes the Secreted Protein Acidic and Rich in Cysteine (SPARC), an ECM regulator that is expressed *in vivo* by both tumor and stromal cells. SPARC overexpression triggers the epithelial-to-mesenchymal transition, tissue invasion and regulates the recruitment and effector function of myeloid infiltrating cells. Indeed, SPARC regulates collagen assembly that in turn delivers inhibitory signals through its receptor, LAIR-1, which is expressed by myeloid cells, particularly neutrophils. This is in line with the role SPARC plays in lymphoid tissue homeostasis. Stromal cells, which are unable to secrete SPARC, induce an alteration in the hematopoietic niche that favors myeloid precursor expansion in the bone marrow. SPARC deficiency alters mesenchymal follicular dendritic cell networking in secondary lymphoid organs and impairs humoral immunity while also promoting lymphomagenesis in persistent lymphoid tissue homeostasis perturbation. Faulty

ECM organization may lead to the emergence of otherwise silent genetic defects that in turn lead to the development of hematologic and solid malignancies which act on several aspects of the tumor immune microenvironment and thus influence almost all the characteristics that tumor cells need to acquire if they are to become fully tumorigenic. SPARC and the other matricellular proteins are key players in these events and act as the main regulators of ECM remodeling during tumorigenesis and endow proper immune regulatory functions.

Lessons from phase I/II clinical trials

Active immunotherapy is emerging as a potential therapeutic approach for prostate cancer. Constantin **N. Baxevanis** (Cancer Immunology and Immunotherapy Center, St Savas Cancer Hospital, Athens, Greece) summarized the results of the clinical trial in prostate cancer that he has performed using the AE37 cancer vaccine; a 15mer peptide (776-790, GVGSPYVSRLLGICL) from the intracellular domain of the HER2 protein that is chemically liked to a tetramer (LRMK) from the MHC class II-associated invariant chain (Ii) protein. The AE37 vaccine was safe and could induce HER-2/neu–specific cellular immune responses and clinical efficacy in patients. Retrospective analyses showed that pre-vaccine levels of tumor growth factor (TGF)-β and IFN-γ displayed an inverse correlation with immunological responses to the AE37 vaccine, and clinical efficacy. Low pre-existing TGF-β and/or high IFN-γ levels are prognostic factors for overall survival. Vaccine-induced delayed-type hypersensitivity (DTH) has also a prominent role as a prognostic factor for overall survival. Baxevanis and co-workers also found that patients that express the HLA-A24 and HLA-DR11 alleles showed better overall survival, low TGF-β levels and high DTH and IFN-γ responses. On the other hand, HLA-A2 positive patients trended towards shorter overall survival. When AE37/DR11 tetramers were used to evaluate the frequencies, phenotype and function of vaccine-induced $CD4^+$ T cells in patients that express the HLA-DR11 allele, it was found that AE37 vaccination induced peptide-specific central and effector memory cells as well as low numbers of Treg and exhausted ($PD1^+$ and $CTLA4^+$) cells. Finally, they showed pre-vaccine increased frequencies of CD8+ T cells against the HLA-A24-restricted peptide (152-161) of the prostate specific antigen (PSA) which were boosted during vaccination. Such an increased T cell immunity against PSA was also positively correlated with prolonged overall survival reinvigorating the essential role of endogenous antitumor immunity for the therapeutic management of prostate cancer.

Per thor Straten (Center for Cancer Immune Therapy, Copenhagen University Hospital Herlev, Denmark) reported that adoptive cell transfer (ACT), using tumor infiltrating lymphocytes (TIL), has provided impressive clinical results in phase I/II clinical trials for the treatment of melanoma, with around 20% giving complete lasting responses. However, some patients do not respond at all or only temporarily. Therefore, Per thor Straten and co-workers have comprehensively monitored the patients included in a phase I/II study which is ongoing at their institution. With this aim in mind, they looked for all known melanoma associated immunogenic peptides, restricted by HLA-A1, -A2, -A3, -A11 and -B7, and found 174 peptides that were used to study recognition by TILs using tetramers. The testing of *in vitro* expanded TIL with these tetramers, showed that TIL cultures had surprisingly low frequencies of cancer specific T cell prior to administration. When TIL were tested for reactivity against autologous or HLA-A-matched allogeneic melanoma cell lines, an association was found between clinical response, higher absolute numbers of tumor-reactive $CD8^+$ T cells infused and their *in vivo* persistence. The next steps in the study of TIL based ACT will be the initiation of a multicenter phase III clinical trials. Moreover, various combination treatments will be tested, e.g. combining ACT with the administration of IFN-α, anti- PD1 and genetic engineering of TIL to improve T cell homing to tumor sites and sustained functionality in a hostile tumor microenvironment. Finally, the identification of other cellular sources, e.g. γδ T cells, may imply that ACT can be used for the treatment of other solid tumors, for which TIL cannot be expanded properly.

Lindy Durrant (Scancell and University of Nottingham, Nottingham, UK) presented the results of a phase I/II clinical trial of the SCIB1 ImmunoBody® vaccine in stage III/IV melanoma patients. ImmunoBody® vaccines are DNA vectors that encode engineered human IgG1 antibody molecules that target the high affinity FcγR1 (CD64) receptor on activated dendritic cells (DC). T cell epitopes are inserted into the complementarity determining regions (CDR) of the antibody framework to make a genetic antigen/antibody complex. SCIB1 ImmunoBody® has 3 epitopes from gp100 and one from TRP-2 antigens grafted into its CDR. A vaccine dose of 0.4, 2, 4 or 8 mg was injected intramuscularly in patients with metastatic disease (Part 1), a dose of 4 mg was injected intramuscularly in patients with resected disease (Part 2) and electroporation was administered. Trial results demonstrate that SCIB1 is safe and immunogenic. 24/28 patients developed melanoma-specific immune responses and a dose response effect was evident; 5/11 (45%) Part 1 patients who received a 2-8 mg dose of SCIB1 displayed evidence of a clinical response (reduction in tumor size or complete tumor

destruction, progression free survival, stable disease); all 14 Part 2 patients are still alive, show a median survival time of 21 months since study entry and only 3 have progressed.

Anne Merete Tryggestad (Department of Cellular Therapy, Oslo University Hospital, Oslo, Norway) described a phase I/II trial of adjuvant therapeutic vaccination in resected prostate cancer patients using autologous DC loaded with mRNA from primary prostate cancer tissues in combination with hTERT and survivin mRNA. Two different DC maturation protocols were used: a 2 day Jonuleit-maturation cocktail and a 3 day TLR7/8-maturation cocktail. Only 2 of the 18 patients enrolled have so far experienced PSA relapses, 3 and 9 months after vaccination started. Extensive immune monitoring is ongoing, making use of the autologous tumor cell lines established from each patient using an in-house cell culture method.

Increasing the effectiveness of immunotherapy

Several studies have demonstrated that vaccination induced T cells have an effector phenotype. However, these cells are not often able to kill tumors that express PD1 on their surface. This fact has driven **Suzanne Ostrand-Rosenberg** (University of Maryland Baltimore Country, Baltimore, MD, USA) to investigate novel strategies for neutralizing PD1 mediated immune suppression, as she reported in the sixth session, chaired by Constantin N. Baxevanis. She hypothesized that CD80 on T cells may bind to PD-L1 on tumor cells and sterically inhibit its binding to PD1 thus preventing PD-L1 from causing apoptosis, anergy and tolerance in activated $PD1^+$ T cells. At the same time, CD80 binding to CD28 could provide costimulation. Indeed, a soluble form of CD80 binds to tumor cell-expressed PD-L1, prevents PD1 binding and maintains $PD1^+$ T cell activation. It appears to be more effective than PD1, PD-L1 or PD1-Fc specific antibodies. This enhanced efficacy of soluble CD80 is probably due to its ability to simultaneously neutralize PD-L1-PD1 suppression and to costimulate T cells, whereas antibody therapy only neutralizes suppression. Soluble CD80 thus provides all the effects of a "combination therapy" in a single reagent.

The effectiveness of adoptive immunotherapies might be increased by T cell transduction with miR-155, as suggested by **Luca Gattinoni** (National Institutes of Health, Bethesda, MD, USA). In effect, he said, tumor-specific CD8+ T cells that constitutively express miR-155 display sustained proliferation and cytokine production, which results in enhanced anti-tumor responses even in the absence of lymphodepletion. In particular, he found that miR-155 promoted the activity of Akt and Stat5, two pivotal pathways that are downstream of homeostatic cytokine signaling, by restraining the expression of the Akt inhibitor Ship1 and multiple negative regulators of Stat5, such as Socs1 and Ptpn2. Enforced miR-155 expression

is therefore a cell intrinsic means to enhance T cell-based immunotherapy without the need for life-threatening, lymphodepleting maneuvers.

Cancer, a disease of the elderly, is and will primarily remain a problem for public health in our ageing societies and likely will become an epidemic. In this context, the identification of suitable vaccine delivery systems for older individuals is important for increasing cancer vaccine efficacy. **Claudia Gravekamp** (Albert Einstein College of Medicine, Bronx, New York, USA) discussed the crucial points of curing cancer via immunotherapy in older individuals and proposed the use of *Listeria monocytogenes* as a cancer vaccine delivery system. Listeria acts on multiple levels, by directly infecting tumor cells and killing tumor cells with reactive oxygen species (ROS), and Listeria infects MDSC resulting in the production of IL-12 and activation of T cells at young and old age. New immunization protocols in which low concentrations of Listeria at short time intervals is administered, induce strong innate immune responses that assists adaptive immune responses to the tumor cells. This resulted in a dramatic reduction in the number of metastases (90% compared to untreated mice), in both young and old mice with metastatic breast cancer (4T1 syngeneic model). Very interesting results were also presented on Radioactive Listeria, which may become a new alternative strategy for pancreatic cancer treatment in both young and old, since it is able to eliminate pancreatic cancer in 80% of treated mice and to almost completely remove liver metastases in a Panc-2 mouse model.

Vaccines and other immune interventions against infection-associated cancer diseases

The two currently available HPV vaccines are expensive and not suitable for use in developing countries. Moreover, they are mainly based on the generation of antibodies to the virus that are able to eliminate the virus itself and therefore to prevent the development of cervical cancer; nevertheless they are unable to eliminate pre-existing cancer cells. Therefore there is still a need for therapeutic vaccines against HPV and for more informative preclinical models. Potentially therapeutic vaccines have been designed to generate T cells to recognize the HPV16 antigens (E6 and E7) expressed by the cancer cells. **Aldo Venuti** and coworkers have developed two new orthotopic mouse models of HPV-associated, ano-genital and oropharyngeal cancers. Both models are based on the use of transplantable cell lines that express luciferase. The bioluminescent epithelial TC-1 line, which expresses HPV16 E6 and E7 proteins, is injected into C57BL/6 mice intravaginally and gives rise to genital tumors, while the bioluminescent, HPV16 E7 protein expressing AT-84 cell line is injected into the floor of the mouth in C3H mice via an extra-oral route. These models were used to test the efficacy of

gene based and plant derived vaccines against HPV16 E7. New models for other high risk HPVs, which will study the cross activity of the different vaccines, are under development.

Marij Welters described the results of the clinical testing of an HPV16 synthetic long peptide (HPV16-SLP) based therapeutic vaccine. The vaccine proved to be clinical effective and induced a HPV16-specific effector T-cell response in patients with HPV16-induced premalignant vulvar lesions, a disease which shows a spontaneous complete regression in <2% of the patients. As expected from their immunosuppressed immune profile, the vaccine induced a weaker T-cell response in late stage HPV16-induced cervical cancer patients and did not improve clinical outcome. The observations that late stage patients display transient immune profile normalization upon standard carboplatin-paclitaxel chemotherapy and that the HPV16-SLP vaccination and chemotherapy combination increases the cure rate of mice bearing established $HPV16^+$ tumors spurred Welters and co-workers to start a clinical trial in which patients receive the combined treatment.

Adoptive transfer of autologous genetically modified T cells for the treatment of hepatitis C virus (HCV) infections was the focus of **Timothy Spear**'s presentation (Loyola University Chicago, Maywood, IL, USA). HCV infects approximately 3% of the world population. The rapidly mutating HCV genome may lead to immune escape variants, chronic infections and eventually hepatocellular carcinoma (HCC) in some patients. Conversely, a variety of T cell receptors (TCR) with the capacity to recognize many viral protein epitopes may exist in patients with a resolved HCV infection. Indeed, Spear and co-workers isolated an HLA-A2 restricted TCR specific for the HCV peptide NS3:1406-1415, from an HCV^+ $HLA\text{-}A2^-$ patient who received an $HLA\text{-}A2^+$ liver allograft. Normal PBL-derived T cells engineered to express this high-affinity TCR recognize the wild-type peptide and naturally occurring mutant peptides expressed by HCC cells in a CD8-independent manner. Adoptive transfer of these engineered T cells can mediate human HCC tumor regression in a mouse xenograft model. Therefore, HCV-reactive TCR gene modified T cells may be a promising form of clinical therapy for treating HCV infection and associated HCC.

Comparative oncology in translational medicine

In 2003 the National Cancer Institute's Center for Cancer Research of the United States launched the Comparative Oncology Program to help researchers better understand the biology of cancer and improve the assessment of novel treatments for humans by treating pet animals - primarily cats and dogs - with naturally occurring cancer. **Philip J. Bergman** (Katonah-Bedford Veterinary Center, Bedford Hills, NY, USA) espoused the translational

power of cancer vaccine trials in client-owned pets by summarizing the data he obtained from dogs with canine malignant melanoma (CMM) using ONCEPT, USDA approved DNA vaccine against tyrosinase commercialized by Merial. Various clinical trials in CMM patients demonstrate that ONCEPT is safe, induces specific anti-tyrosinase humoral and cell-mediated immune responses and is therapeutic in stage II/III local-regional controlled disease. CMM are spontaneous cancers that develop in outbred immuno-competent hosts that share living environments and cancer-bearing ages with us. Both CMM and advanced human melanoma are initially treated with aggressive local therapies including surgery and/or radiotherapy; however, the development of systemic metastatic disease is very common. Based on these similarities, CMM appears to be a clinically faithful model for human malignant melanoma. The results obtained with ONCEPT are thus of high translational value.

Guillermo Marshall (Departamento de Computacion, Universidad de Buenos Aires, Argentina) briefly discussed results of electrochemotherapy (ECT – a combination of membrane permeabilisation by high voltage electric pulses and injection of cancer drugs that have limited access to the cytosol) performed in pet animals, affected by different solid tumors. He pointed-out that ECT not only allows efficient treatment of tumors with non-permeant cytotoxic drugs, it also induces per se a low level humoral immune response. One dog affected by a grade 2 desmoplastic melanoma, another one affected by a grade 3 giant cell sarcoma, as well as a cat with a squamous cell carcinoma, have been treated with bleomycin administered into the tumors by ECT. Due to the promising results revealed in the two-three months follow-up, Marshall and co-workers are planning to couple ECT with gene electrotransfer protocols based on immunotherapy with plasmid vectors expressing IL-2 and IL-12.

DNA vaccination and gene electrotransfer

Lectures in the section co-organized by COST TD1104 Action (www.electroporation.net) were organized in such a way as to provide a complete range of views on electrotransfer, from its basic concepts (lab-bench) to the most efficient patient protocols (bench-bedside). **Guillermo Marshall** described the associations between electric fields, pH fronts and tissue damage in gene electrotransfer protocols. He presented examples of *in vitro*, *in silico* and *in vivo* pH front modeling and showed evidence of pH fronts generated by local electric field application, suggesting they are principal causes of the tissue damage. **Justin Teissié** (CNRS-Institute of Pharmacology and Structural Biology, Toulouse, France) briefly summarized 35 years of studies on membrane permeabilisation by high voltage electric pulse mechanisms with

respect to cell membrane structural changes and the molecular transport of differently sized nucleic acids. He discussed how the high voltage electric pulses (EP) gives rise to cellular membrane structural changes and to molecular transport, pointing out EP is a reversible but stressful event for the cells, which undergo stretching and swelling phenomena. The delivery of small siRNA and larger pDNA with respect to the delay between the membrane permeabilization and transport into the cytoplasm and then into the nucleus of the cells was also reported. He demonstrated that only properly selected electric pulse physical parameters can provide efficient nucleic acid transfer and good cell survival.

Damijan Miklavcic (University of Ljubljana, Faculty of Electrical Engineering, Slovenia) presented data on plasmid DNA transport across the membrane, the effects of pulse polarity in DNA transfer and the importance of electrode selection for successful *in vitro* and *in vivo* gene transfer protocols. He showed that electric field distribution depends on electrode geometry, placement and tissue anatomy, and proposed predictive mathematical models for determination of electric field distribution, useful for the success of electrotransfer treatments. The importance of the tissue geometry and its passive electrical properties that influence the effects of the applied pulse amplitude has been also discussed. Thanks to a better understanding of the mechanisms governing the electrotransfer, an accurate choice of electrical parameters to be applied with appropriate electrodes chosen among a large variety, is now possible.

Gregor Sersa (Institute of Oncology, Ljubljana, Slovenia) presented the results from therapeutic electrogene therapy protocols for the delivery of IL-12 in cancer treatment. Data on local intratumoral and intramuscular plasmid DNA delivery, peritumoral or intratumoral gene therapy combined with tumor irradiation, human clinical trials and veterinary applications were discussed. He demonstrated the effectiveness of IL-12 electrogene therapy on subcutaneous solid tumors and in induced lung metastasis. Data on the successful combination of electrochemotherapy and electrogene IL-12-based therapy treatment in mastocytoma-affected dogs were also presented. The importance of electrode design, the proper choice of electric pulse parameters to obtaining favorable therapeutic outcomes and the role of the comparative oncology was stressed.

The HLA ligandome analysis to identify new vaccination targets

The loss of MHC class I expression is a widespread mechanism in tumor escape from $CD8^+$ T cell responses and one of the main reasons for tumor resistance to immunotherapy. Nevertheless, in many tumors MHC class I defects are “soft”, reversible, and MHC class I cell

surface expression recovery can be achieved via different methods, e.g. cytokines, radiotherapy and chemotherapy, as reviewed by **Federico Garrido** (Department of Immunology, Universidad de Granada, Spain) in his lecture. In these cases, immunotherapy may result in a T cell mediated tumor rejection. Therefore, peptides naturally presented as HLA ligands on tumor cells may represent ideal vaccine antigens to induce a T cell response. To this end, **Janet Peper** (Department of Immunology, University of Tübingen., Germany) conducted an HLA ligandome analysis on 6 ovarian cancer samples and tested the ability of the identified peptides to prime $CD8^+$ T cells from healthy HLA-matched blood donors. Immunogenic HLA ligands derived from histone deacetylases (HDAC) 1 and 2 were represented in all analyzed tumor samples. HDAC molecules are overexpressed in many different cancer types, including ovarian cancer; they increase cell proliferation, cell migration, angiogenesis and invasion by reducing tumor suppressor gene transcription. HDAC inhibitors are currently being tested in several clinical tumor therapy trials and are giving promising results. Hence, HDAC are suitable targets for T-cell mediated immunotherapy. Peper and co-workers are planning to use immunogenic HDAC1/2 derived peptides in an upcoming phase I/II vaccination trial in ovarian cancer patients.

Monitoring of cancer-specific B cell responses

The Satellite Symposium sponsored by Cellular Technology Limited – CTL gave **Paul V. Lehmann** (President and CEO of Cellular Technology Limited – CTL, Shaker Heights, OH, USA) the opportunity to highlight the potential importance of monitoring cancer-specific B cell responses in early diagnosis and the prediction of cancer vaccine responses. Serum antibodies are not fully reflective of B cell immunity as their production results from random memory B cell activation, they can be absorbed by a persisting antigen and because they can be locally produced if the antigen persists. For these reasons it is important to directly monitor antigen specific B cells. While antigen specific T cells can be stained by tetramers and similar 'mers', B cell staining, using labeled antigens, has not so far been successful. Therefore, ELISPOT is still the only way of identifying antigen specific B cells and CTL has developed protocols and tools for this purpose. This interesting approach has been extensively used in monitoring autoimmune diseases and now is available for cancer studies.

Cancer stem cells as strategic cancer vaccination targets

Most tumors display an intrinsic cell hierarchy, with a small population of undifferentiated tumorigenic cells - the so-called cancer stem cells (CSC) - at the apex of the hierarchy. CSC

posses all the unique biological properties necessary to maintain and spread tumors as well as displaying a plethora of cytoprotective mechanisms which make them therapy resistant. They are thus a reservoir for disease relapse and metastatic evolution. CSC are consequently a strategic target for treatments that interrupt tumor cell generation and dissemination, as pointed out by **Giorgio Parmiani** (Division of Molecular Oncology, San Raffaele Hospital, Milan, Italy) in his closing lecture. The identification of immunologically relevant markers that are expressed on CSC may thus lead to effective immunotherapies. Parmiani suggested possible targets that include both differentiation antigens (i.e. MUC1, carcino-embryonic antigen, tyrosinase, survivin, aldehyde dehydrogenase, EpCAM, etc.) and tumor specific mutated antigens. Indeed, CSC may contain many missense somatic mutations that can generate new T cell epitopes. These mutations are now detectable thanks to new deep sequencing technology, making the preparation of individual, mutated peptide-based vaccines possible. Nevertheless, CSC have various mechanisms that suppress any vaccine-induced immune response (i.e. indoleamine 2,3-deoxygenase-1, IL-4, PD-L1, IL-10, B7-H3, TGF-β, Fas-L). Counteracting these mechanisms is thus necessary for the success of anti-CSC immunotherapies.

Maarten Ligtenberg (Karolinska Institutet, Stockholm, Sweden) described the effects of DNA vaccination against a broadly expressed tumor-associated antigen, Cripto-1, whose expression is increased in breast CSC. Cripto-1 is a cell membrane tethered glycoprotein that plays a critical role during embryogenesis by regulating left-right axis development. Poorly expressed in normal tissues, it is overexpressed on melanoma, breast, colon, pancreas lung and many other human cancers where it induces cell proliferation, migration, epithelial-mesenchymal transition and angiogenesis. Plasmid DNA encoding mouse Cripto-1 generates a protective adaptive immune response in B16F10 and 4T1 models, reduces metastatic burden in Her2 transgenic (BALB-neuT) mice and specifically limits cancer stem cell growth *in vivo*. These results demonstrate that Cripto-1 is an immunologically relevant marker of CSC and a good target for the development of prophylactic and therapeutic tumor vaccines.

Awards

The European Federation of Immunological Societies (EFIS) granted two Meeting Bursaries that went to **Tania Lahera** (National Institute of Oncology, Havana, Cuba) and **Constantinos Televantos** (The University of Nottingham, UK). The two EACR Poster Prizes were awarded to **Regina Heidenreich** (CureVac, GmbH, Tübingen, Germany) and **Belinda Sanchez**

Ramirez (Center of Molecular Immunology, Havana, Cuba). The committee for poster evaluation also addressed a special mention to **Stefano Ugel** (University of Verona, Italy).

Conclusion

The PIVAC-14 conference brought together translational and clinical oncologists and immunologists who provided an up-to-date overview of many aspects of tumor immunology and of the most recent advances in active cancer vaccination and adoptive cell therapy. The data from retrospective analysis of patients enrolled in phase I/II clinical trials has highlighted the possibility to identify immune parameters that may discriminate between responders and non-responders. Most of these parameters are related to tumor induced immune suppression, further confirming the need to move towards combinatorial immunotherapeutic approaches able to counteract immune suppression and, where possible, towards cancer immune prevention. **Graham Pawelec** (Centre for Medical Research, University of Tübingen, Germany) concluded his summing up of the meeting with the announcement that the 15th PIVAC ~~edition~~ conference will take place in Tübingen, Germany, on October 6-8, 2015.

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Conflict of interest

The authors declare that they have no conflict of interest.