Second Ascoli Piceno conference on gene vaccination in cancer (GVC), Ascoli Piceno, Italy, October 9–11, 2013

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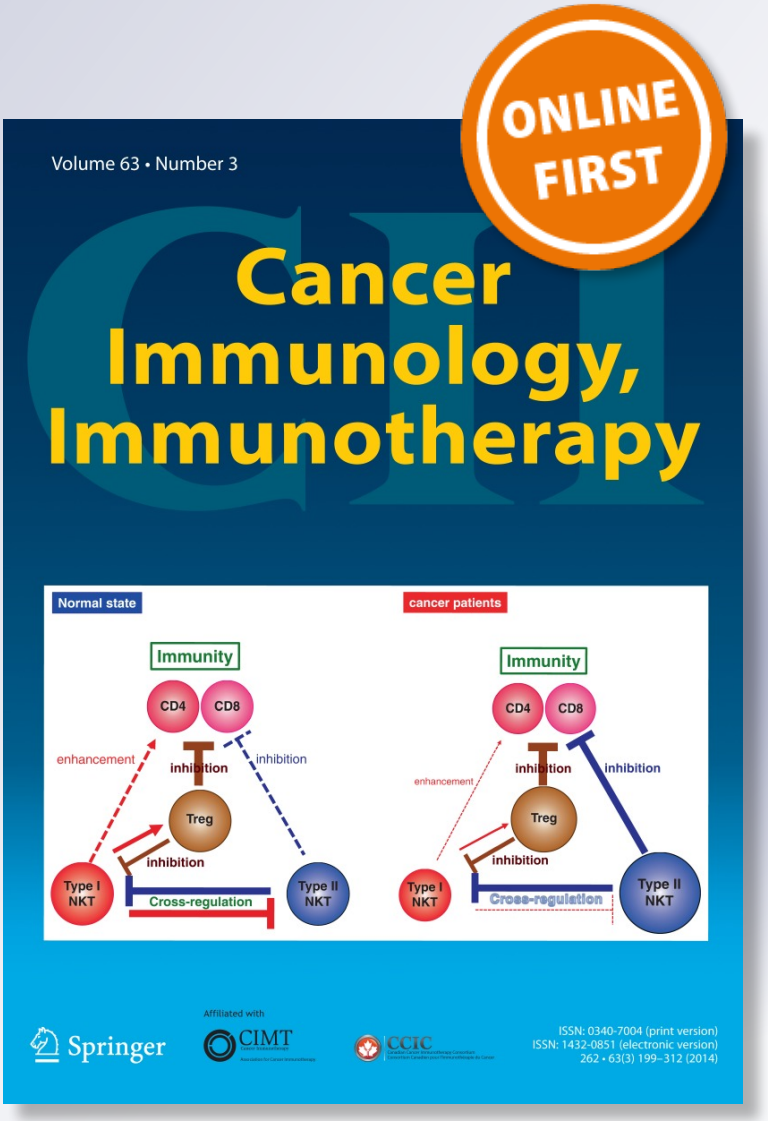

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MEETING REPORT

Second Ascoli Piceno conference on gene vaccination in cancer (GVC), Ascoli Piceno, Italy, October 9–11, 2013

Elisabetta Bolli · Laura Conti · Federica Riccardo · Cristina Marchini · Augusto Amici · Federica Cavallo · Elena Quaglino

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Abbreviations

ALK	Anaplastic lymphoma kinase
CMM	Canine malignant melanoma
CSPG4	Chondroitin sulfate proteoglycan-4
CSC	Cancer stem cells
DAA	Disease-associated antigens
ECT	Electrochemotherapy
EP	Electro-permeabilization
GVC	Gene vaccination in cancer
HPV	Human papillomavirus
MDSC	Myeloid-derived suppressor cells
MUC1	Mucin1
NHL	Non-Hodgkin lymphoma
NSCLC	Non-small-cell lung cancer
PBMC	Peripheral blood mononuclear cells
PD1	Protectin D1
PDA	Pancreatic ductal adenocarcinoma
TAA	Tumor-associated antigens
TERT	Telomerase reverse transcriptase

E. Bolli · L. Conti · F. Riccardo · F. Cavallo (✉) · E. Quaglino
Department of Molecular Biotechnologies and Health Sciences, Molecular Biotechnology Center, University of Torino, Via Nizza, 52, 10126 Turin, Italy
e-mail: federica.cavallo@unito.it

C. Marchini · A. Amici
Department of Bioscience and Biotechnology, University of Camerino, Camerino, Macerata, Italy

Introduction

The gene vaccination in cancer (GVC) meeting offered the opportunity to continue and strengthen the existing national and international collaborations between basic scientists, clinical researchers and scientists in industry to overcome obstacles in gene vaccination against cancer. The 2013 GVC meeting covered the following topics: (1) new technologies for gene vaccination; (2) emerging targets for cancer vaccines; (3) therapeutic vaccination against human papillomavirus (HPV)-dependent diseases; (4) preclinical challenges; (5) veterinary applications of gene vaccination in cancer; (6) in vivo gene-electro-transfer: from technology development to clinical applications.

To provide the broad background for the topics covered by the meeting, two important lectures, one on the discovery of tumor-associated antigens (TAA) and the other on the importance of tumor-induced immunoregulatory networks, were given by Olivera J. Finn and Vincenzo Bronte, respectively. Olivera J. Finn (University of Pittsburgh School of Medicine, Pittsburgh, PA, USA) emphasized the critical role of the disease-associated antigens (DAA) that are TAA expressed in premalignant lesions or during infections. She pointed to the fact that the efficacy of immunotherapy may be greatly improved by vaccinating patients with very early lesions, boosting the immunological memory developed against DAA during previous infections. It is thus necessary to focus on DAA in order to boost preexisting responses generated earlier in life. Mucin1 (MUC1) is a typical DAA overexpressed in an abnormal hypoglycosylated form in preneoplastic and cancerous lesions of various histotypes. A recent clinical study in patients with advanced adenoma of the colon showed that anti-MUC1 vaccination was able to elicit anti-MUC1 IgG and long-term memory in about 50 % of the vaccinated patients. Peripheral blood mononuclear

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cells (PBMC) of non-responders contained increased levels of myeloid-derived suppressor cells (MDSC). The importance of modulating MDSC differentiation and activity in the tumor microenvironment was further discussed by Vincenzo Bronte (University Hospital and Department of Pathology, Verona, Italy). He identified miR-142-3p as a master regulator of the expression of the signaling elements of the IL-6 receptor, showing that a decrease in this miRNA expression is essential in allowing myeloid cells to acquire the suppressive phenotype. Moreover, he demonstrated that miR-142-3p can be considered a new target for cancer therapy since mice constitutively expressing miR-142-3p in the bone marrow have altered tumor-induced macrophage differentiation. These mice display a marked increase in survival following the adoptive transfer of tumor-specific T cells.

New technologies for gene vaccination

In this session, attention was given to the efforts by companies in the design of carriers, adjuvants, viral vectors, self-amplifying mRNA vaccines as well as prime and boost strategies to be used for anticancer immunotherapy. **Ennio De Gregorio** (Novartis, Siena, Italy) reported the development of new synthetic compounds optimized to stimulate specific immune cells with minimal side effects. A significant increase in vaccine potency, as well as reduced local and systemic reactogenicity, was shown by a synthetic compound specific for Toll-like Receptor 7. New mRNA-based vaccines have also been developed. Of particular note is a non-viral delivery system for self-amplifying RNA from Novartis that has been successfully tested in preclinical models. There are also the RNActive® vaccines reported by **Karl-Josef Kallen** (CureVac, Tübingen, Germany). RNActive® vaccines are two component vaccines engineered in a way that maximizes the antigen encoding properties of mRNA and bestows self-adjuvanticity by activation of TLR7, causing a balanced activation of the adaptive and innate immune system. Initial phase I/IIa studies in patients with prostate and non-small-cell lung cancer (NSCLC) showed that RNActive® vaccines are safe and effective in inducing a long lasting, humoral and cellular immune response. Currently, a multicenter controlled phase IIb study is performed in castrate-resistant prostate cancer in 8 European countries to investigate the clinical efficacy of RNActive® vaccines. A complementary phase Ib study tests the combination of radiotherapy and RNActive® vaccines in metastatic NSCLC.

Emerging targets for cancer vaccines

Besides the development of more effective gene vaccination technologies, one of the main issues in anticancer vaccines is still the identification of effective tumor antigen targets. A number of different strategies have been used to identify new targets, including protein kinases, metabolic enzymes, chaperone proteins, transporters and angiogenesis-related receptors.

Anaplastic lymphoma kinase (ALK) is known to be a good therapeutic target for the treatment of anaplastic large cell lymphoma. **Claudia Voena** (University of Torino, Torino, Italy) and coworkers had previously demonstrated that anti-ALK DNA vaccination through electro-permeabilization (EP) is a good strategy for the treatment of anaplastic large cell lymphoma. They reported similar results in the treatment of NSCLC in two independent transgenic mouse models of ALK-driven lung cancers that express two different fusions of ALK (EML4-ALK and TFG-ALK). It is worth noting that anti-ALK DNA vaccination was also effective against tumors harboring ALK mutations that abrogate the efficacy of the ALK inhibitor crizotinib. Voena and coworkers also tested the combined administration of anti-ALK DNA EP and crizotinib, showing that ALK vaccine could prevent the growth of crizotinib-resistant tumors in combined therapy.

The glycolitic enzyme α-enolase is a promising target for the immunotherapy of patients affected by pancreatic cancer. It is involved in both growth and metastasis of ductal pancreatic adenocarcinoma (PDA). **Francesco Novelli** (University of Torino, Torino, Italy) and coworkers used serological proteomic analysis to discover that patients with PDA produce antibodies against two isoforms of α-enolase, which are also recognized by T lymphocytes. A good correlation between these immune responses and prognosis has also been demonstrated. For this reason, a DNA vaccine targeting α-enolase has been developed. The EP-mediated α-enolase vaccination was tested in two genetically engineered mouse strains ($Kras^{G12D}$/Cre mice and $Kras^{G12D}$/Tr $p53^{R172H}$/Cre mice) that develop autochthonous lethal PDA with different kinetics. A specific anti-α-enolase antibody and cellular immune responses were both elicited in the vaccinated mice. The induction of this immunity was correlated with increased survival times.

Serenella Pupa (Istituto Nazionale dei Tumori, Milan, Italy) discussed an approach for identifying new targets for B cell non-Hodgkin lymphoma (NHL) immunotherapy. Using sera from autologous tumor-loaded dendritic cells from vaccinated NHL responder patients, Pupa and coworkers identified the chaperon protein HSP105 as a candidate target for NHL immunotherapy. While in vitro administration of a commercial polyclonal anti-HSP105 antibody failed to provide any direct anti-tumor effect, it significantly reduced tumor burden through antibody-dependent cellular cytotoxicity in vivo. Therefore, HSP105 can be added to the list of non-oncogenes that can be exploited as a new target for B cell lymphoma therapy.

A growing body of evidence points to cancer tissues being hierarchical systems, where cellular heterogeneity is the result of multi-lineage differentiation processes, and tumor progression, metastasis, recurrence and resistance to therapy being sustained by a subset of cells with stem cell properties, named cancer stem cells (CSC). The identification of molecules involved in CSC self-renewal is a necessary step toward the development of effective therapies. To this aim, **Ronald Rooke** (Transgene S.A., Illkirch Graffenstaden, France) and **Stefania Lanzardo** (University of Torino, Torino, Italy) presented two different approaches to identifying specific CSC targets in glioblastoma and breast cancer, respectively. Rooke used proteomic analysis to identify markers overexpressed in neurospheres generated from human samples, while using transcription profiling of RNA from mammospheres generated from murine breast cancer cells, Lanzardo identified the overexpression of cystine/glutamate exchange transporter (xCT) in breast CSC. EP-mediated delivery of a plasmid encoding xCT prevents the formation of lung metastasis, decreasing cancer growth in a preclinical model of $HER2^+$ breast adenocarcinoma.

Andrea Facciabene (University of Pennsylvania, Philadelphia, PA, USA) reported on the efficacy of targeting tumor vasculature as a promising strategy for the treatment of cancer. He showed that the EP of a DNA vaccine targeting TEM1 (endosialin, CD284), one of the most abundantly expressed tumor endothelial or stromal antigens in humans, is safe and that it does not affect physiological angiogenesis. Moreover, it results in the induction of TEM1-specific T cell-mediated immunity, in the disruption of the tumor vasculature and in the induction of tumor necrosis/apoptosis and cross-presentation of TAA. Combination of TEM1 vaccination with the administration of regulatory T cell inhibitors resulted in increased vaccine efficiency.

Therapeutic vaccination against HPV-dependent diseases

Two HPV vaccines, which are effective in the prevention but not in the treatment of established tumors, are currently on the market. Furthermore, they are expensive and not suitable for use in the developing countries. Therefore, the development of new vaccines and more informative preclinical models are needed. **Aldo Venuti** (Regina Elena National Cancer Institute, Rome, Italy) reported on the new orthotopic mouse models of HPV-associated cancers that can be used for testing therapeutic vaccines. By using the combination of a DNA vaccine and a fusion protein vaccine produced in plants, he obtained complete protection against HPV^+ head and neck orthotopic tumors in vaccinated mice.

Mark Bagarazzi (Inovio Pharmaceuticals, Blue Bell, PA, USA) reported on the efficacy of VGX-3100, a new therapeutic DNA vaccine against HPV-related diseases. This EP administered DNA vaccine led to the induction of antigen-specific antibodies and T cells in the majority of vaccinated patients. In order to significantly increase T cell responses, Bagarazzi's group proposed to combine DNA vaccination against HPV with IL-12 administration or with vaccination against telomerase (hTERT), an antigen that is known to be immunogenic, as demonstrated by the presence of existing naturally occurring T cell responses in cancer patients.

Preclinical challenges

The improvement of preclinical models of cancer is one of the issues that need to be dealt with for the further development of immunotherapy, since many of the currently used models do not closely enough recapitulate human disease. In the context of breast cancer, emerging evidence suggests that the real transforming form of HER2 is its splice variant, the Δ16HER2. **Cristina Marchini** (University of Camerino, Camerino, Italy) described a new preclinical breast cancer model, the Δ16HER2 transgenic mice that develop mammary carcinomas with 100 % penetrance. Vaccination of these mice with a plasmid encoding a chimeric human/rat HER2 protein resulted in an effective antitumor response that was greater than the protection induced by vaccination with a plasmid coding for a non-chimeric human HER2 protein.

Further evidence of the great tumor immunotherapy potential that lies in DNA EP came from data presented by **Maarten Ligtenberg** (Stockholm, Sweden), showing Cripto-1 as a prime target for DNA vaccination. Cripto-1 is involved in cell fate regulation during embryogenesis, in cell proliferation and in epithelial-mesenchymal transition in several cancers. Immunization with mouse and human Cripto-1 generates in vivo protective immune responses against Cripto-1^+ murine tumors. In order to improve the efficacy of anti-Cripto-1 vaccination, Ligtenberg pointed out the possibility of modulating the signaling pathways involved in T cell activation through the use of shRNA targeting of the NF-kB signaling pathway.

Preclinical evaluation of the safety of therapeutic vaccines to cancer is an important issue. **Isabella Andreini** (RTC S.p.A., Pomezia, Italy) emphasized the need for critically interpreting preclinical data in order to obtain correct and useful information for the design of clinical studies and where necessary to find a suitable compromise between regulatory requirements and scientific logic. Indeed, preclinical models have several limitations: few animal species are available, and animals are young, healthy and inbred, while patients are older, sick and genetically heterogeneous. Moreover, the required number of animals for

statistically significant data is impossible to achieve when using primates, not to mention the inter-species differences that may indeed affect the results.

Veterinary applications of gene vaccination in cancer

The use of naturally occurring cancer in pet animals as models of human cancer has gained an important role in translational medicine over the last decade, and several companion species, such as cats, dogs and horses, have successfully contributed to this effort. This is due to the striking similarity in the histological appearance, tumor genetics, environmental influence, biological behavior and response to therapy between the spontaneous tumors diagnosed in pet animals and the human tumors. **Wei-Zen Wei** (Karmanos Cancer Institute, Detroit, MI, USA) reported on numerous studies in which DNA EP against HER2 was used successfully in murine models but did not lead to effective results in human clinical practice. She displayed a comparative study on feline mammary tumors showing the expression of HER2 in 40–85 %, but the absence of gene amplification, in these tumors. The feline HER2 is very similar in its amino acid sequence to the human ortholog and is recognized by antibodies against human HER2. A vigorous anti-HER2 immune response after DNA EP with a plasmid encoding a mutated form of feline HER2 sequence can be obtained.

Luigi Aurisicchio (Takis s.r.l., Rome, Italy) reported a double-arm cancer vaccine study based on targeting of the canine telomerase (TERT) in dogs affected by $TERT^+$ B cell lymphosarcoma. The combination of standard-of-care chemotherapy and heterologous prime/boost, i.e., adenovirus/DNA EP-based vaccination, gave a good clinical efficacy in terms of survival of B cell lymphosarcoma in canine patients. These data support the suitability of this approach to the evaluation of other novel immune therapies for cancer. **Joe Impellizeri** (Veterinary Oncology Services, Vassar College, New York, NY, USA) reported additional data showing increased survival of dogs affected by B cell malignant lymphosarcoma when the standard-of-care chemotherapy is combined with a genetic vaccine against canine TERT developed by Aurisicchio and coworkers. He also reported HER2 immunotherapy trials ongoing with feline mammary cancers and canine osteosarcoma, using the same immunotherapy platform (adenovirus followed with DNA plasmid via EP).

Soldano Ferrone (Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA) described the use of Chondroitin Sulfate Proteoglycan-4 (CSPG4) as a target of antibody-based immunotherapy for melanoma patients. Several advanced melanoma patients who were immunized with a CSPG4 mimic and those who developed CSPG4-specific humoral immunity had a statistically significant longer survival. Human CSPG4 displays more than 80 % homology with its canine counterpart. This has provided the rationale to use human CSPG4 as an immunogen to implement active specific immunotherapy in dogs with melanoma, as discussed by **Federica Riccardo** (University of Torino, Torino, Italy). She showed that CSPG4 is expressed by most canine malignant melanoma (MM) and can thus be considered a new diagnostic marker and a target for cancer immunotherapy. In dogs with surgically resected stage II–III $CSPG4^+$ oral MM, EP-mediated vaccination with a plasmid encoding human CSPG4 resulted in the induction of anti-human and canine CSPG4 antibodies and a significantly longer overall and disease-free survival. Therefore, xenogeneic vaccination against CSPG4 is able to overcome host unresponsiveness to the self-antigen and appears to be effective for the treatment of canine MM.

Philip Bergman (Katonah-Bedford Veterinary Center, Bedford Hills, NY, USA) emphasized that the evaluation of gene-based cancer vaccines, and the assessment of the delivery technologies in pet dogs, could provide a predictive model for human clinical trials and to serve as a tool for the development of novel therapeutic strategies in veterinary oncology. He described the promising results obtained by vaccinating dogs following the surgical resection of stage II–III CMM with the FDA-approved ONCEPT (Merial) vaccine, a plasmid that codes for human tyrosinase, administered intramuscularly with a bio-injector.

In vivo gene-electro-transfer: from technology development to clinical applications

Several preclinical and clinical trials have demonstrated the safety and tolerability of EP-mediated transfer of both genes and other therapeutic agents. **Mattia Ronchetti** (IGEA S.p.A., Carpi, Italy) reported the efficacy of electrochemotherapy (ECT) in patients with cutaneous cancer manifestations and the ongoing clinical validation of a new medical device by IGEA, the Cliniporator™ VITAE, for the treatment of deep seated tumor nodules such as bone, liver and soft tissue metastases. He highlighted the importance of using a single EP technology from preclinical experiments to clinical trials, in order to reduce the regulatory requirements needed to be satisfied for translation to clinical practice.

Julie Gehl (Copenhagen University Hospital, Herlev, Denmark) discussed the need for optimization of EP conditions for each therapy and for each tissue. A list of the protocols for ECT in muscle and skin, and for EP-mediated gene delivery for the treatment of several cancers, is developed by the Center for Experimental Drug and Gene Electrotransfer, in the Copenhagen University Hospital. This

list is available at http://www.herlevhospital.dk. Interestingly, a clinical brain electrode for ECT has recently been developed. This electrode, which is approved for trial use, is now in a clinical study in patients suffering from brain metastases.

The second part of this session was focused on the discussion of new strategies that may enhance DNA vaccination efficacy. **Emanuela Signori** (Institute of Translational Pharmacology, Rome, Italy) reported that muscular fiber transfection and consequent antigen expression is significantly enhanced by the combination of hyaluronidase administration and DNA EP. These effects rest on the ability of the coupled treatment to induce in the vaccinated muscle an early release of inflammatory cytokines favoring the regeneration of muscle fibers and allowing lower levels of electric fields to be applied. Another important strategy for enhancing vaccination efficacy is the use of DNA fusion vaccines. **Christian Ottensmeier** (University of Southampton, Southampton, United Kingdom) reported that DNA vaccines containing tumor antigen sequences fused to microbial genes are safe and close to becoming licensed for clinical use. Cancer patients vaccinated with anti-CEA EP showed responses that included preliminary indications of reduced tumor growth and evidence of clinically manageable concomitant autoimmunity. A direct correlation between the density of tumor-infiltrating effector T lymphocytes and a better outcome was found in these patients. This suggests that T cells induced by vaccination have an effector phenotype. However, often these cells are not able to kill the tumor because they express protectin D1 (PD1) on their surface. For this reason, the use of anticancer vaccines in combination with immuno-stimulant monoclonal antibodies is needed. A new promising way to increase antigen immunogenicity is to combine peptides of interest with viral particles. These chimeric products can be produced in plants, further increasing their immunogenicity. Preclinical testing of these new vaccine products resulted in the induction of an effective T cell response and will soon be brought into clinical testing.

Special report: the three best abstracts awardees

A prize was given to three young scientists who contributed to the GVC meeting with a relevant poster presentation. The first prize was given to **Moitza Principe** (University of Torino, Torino, Italy), who demonstrated the involvement of the glycolitic enzyme α-enolase in PDA metastasis. The second prize went to **Giuseppina Barutello** (University of Torino, Torino, Italy), who demonstrated that maternal immunization against a TAA could confer effective protection to the offsprings that were genetically predestined to developing carcinomas. The third prize was awarded to **Caterina Bartolacci** (University of Camerino, Camerino, Italy), who showed the ability of anti-HER2 vaccines to inhibit tumor growth in Δ16HER2 transgenic mice.

Conclusions

The closing lecture delivered by **Gennaro Ciliberto** (Istituto Nazionale Tumori, Fondazione G. Pascale, Naples, Italy) provided an overview of possible future trends in the development of an easily scalable vaccination platform technology. Several important points have to be taken into account when starting a new cancer vaccine clinical trial. Firstly, the identification of reliable preclinical animal models to test vaccine efficacy is crucial, and to this extent, the use of pet animals as models of human cancer promises to be a successful strategy in translational medicine. Secondly, since it is difficult to eradicate a tumor, especially if metastatic, the technology used to treat cancer patients should be compatible with prolonged treatment. Thirdly, it is important to combine vaccines with other drugs, and it is mandatory to find new biomarkers. Ultimately, the best vaccination strategy to use depends on the kind of immune response that is needed: to elicit an antibody response, it is better to use a xenogeneic antigen, while to activate T lymphocytes, it is better to use a poly-epitope vaccine that is different from the whole antigen. To get both responses, a heterologous prime-boost strategy might be needed.

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Conflict of interest The authors declare that they have no conflict of interest.