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Conference Report

The 21st International Conference on Progress in Vaccination against Cancer (PIVAC-22), September 26–28, 2022, Turin, Italy

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Abstract

The 21st international congress on Progress in Vaccination against Cancer (PIVAC-22, https://www.pivac22.it/) was held from September 26 to September 28, 2022, at the Molecular Biotechnology Center "Guido Tarone" in Turin, Italy. The meeting covered the most recent advances in the field of cancer immunotherapy, with a focus on the tumor microenvironment, and addressed how alterations to the microenvironment's molecular and metabolic features and the microbiota impact upon the response to immunotherapy.

Keywords: PIVAC-22; cancer vaccination; immunotherapy; tumor microenvironment; immune resistance; microbiota

1. Introduction

The main objectives of the meeting were to gather, share and exchange experiences and ideas, promote interactions between speakers and participants, encourage stimulating discussions, provide networking opportunities and stimulate new collaborations. The scientific program was divided into five plenary sections that dealt with: (1) the tumor microenvironment and mechanisms of immune resistance and immune modulation by cancer cells; (2) the influence of metabolism and the microbiota on tumor immunology and immunotherapy; (3) progress in vaccination against cancer - from the bench to the bedside; (4) strategies to identify novel immunotherapeutic targets and monitor the immune response; and (5) novel immunotherapeutic and combined strategies for cancer treatment. Each plenary section consisted of presentations from invited speakers, and young scientists whose submitted abstracts were chosen by the scientific committee. Two afternoon poster sessions were also organized during the meeting to allow 29 young investigators to discuss their recent findings. In addition to the excellence of the scientific activities, the attendees were able to experience the beauty of Baroque Turin and Piedmontese regional cuisine in a spirit of friendship.

2. Session 1. The Tumor Microenvironment, and Mechanisms of Immune Resistance and Immune Modulation by Cancer Cells

Session 1 was co-chaired by Graham Pawelec (University of Tübingen, Tübingen, Germany) and Rienk Offringa (German Cancer Research Center, Heidelberg, Germany), and was focused on the influence that the tumor microenvironment (TME) can have on therapeutic efficacy and clinical outcomes. Cancer cells can co-opt, via various mechanisms, immune-suppressive cells, such as tumor-associated macrophages (TAMs), regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) to protect themselves from immune attacks and promote tumor growth. Deciphering the mechanisms underlying this crosstalk can lead to the identification of new therapeutic targets and the potential development of new drugs for cancer treatment. In this very context, *Suzanne Ostrand-Rosenberg* (University of Utah, Salt Lake City, Utah) demonstrated that the Receptor for Advanced Glycation Endproducts (RAGE) plays a key role in driving differentiation, accumulation in tumors and MDSC function. Indeed, signaling through RAGE by the alarmins/damage-associated proteins S100A8/A9 and HMGB1 activates many of the pro-inflammatory mediators that are established activators of MDSCs. Tumor progression and lung metastatization are delayed in RAGE-deficient mice, suggesting that RAGE, HMGB1, and S100A8/A9 may represent interesting therapeutic targets.

Per thor Straten (University of Copenhagen, Copenhagen, Denmark) centered his presentation on the impact of receptor-ligand interactions on T-cell function in the TME. With his colleagues, he characterized a novel co-stimulatory pathway in $CD8^+$ T cells, which consists in the interaction between MerTK, a type I Receptor Tyrosine Kinase, and its soluble ligand Protein S (Pros1). This co-stimulatory pathway increases proliferation, cytokine release and the cancer-cell-killing ability of $CD8^+$ T cells. Unfortunately, the MerTK-Pros1 co-stimulatory signal can be converted into an oncogenic signal as cancer cells and immunosuppressive innate immune cells compete with T cells for Pros1 binding, as they express MerTK. *thor Straten* also presented preclinical data on the impact of exercise in

decreasing tumor incidence and growth and in increasing epinephrine-dependent natural killer (NK) cell mobilization and accumulation in tumors of different histotypes. These data laid the foundation for the design of an ongoing clinical trial in which exercise is combined with immunotherapy (NCT04263467).

The central role of tumor/stroma interactions in impacting lung-cancer patient clinical outcomes and response to immune checkpoint inhibitors (ICIs) was discussed by *Paola Nisticò* (IRCCS Regina Elena National Cancer Institute, Rome, Italy). She focused on the role of human protein hMENA, which is a regulator of actin dynamics, integrin-mediated signaling, adhesion and cell motility. Two tissue-specific isoforms of hMENA exist in cancer; hMENA11a and hMENAΔv6. They have opposite functions in regulating extracellular matrix (ECM) composition and are associated with the clinical outcomes of node-negative non-small-cell lung-cancer (NSCLC) patients. Notably, high hMENA11a and low stromal fibronectin expression in these patients correlate with a low probability of recurrence when treated with ICIs. *Nisticò* also summarized her recent data on hMENA overexpression by a subset of cancer-associated fibroblasts (CAFs) that have an immunosuppressive phenotype. In these CAFs, hMENA regulates the GAS6-AXL axis, fostering their pro-tumor function. These data further support the potential use of hMENA isoforms as promising biomarkers of prognosis and response to ICIs.

The role and heterogeneity of $\gamma\delta$ T cells in cancer are the subject of a growing and dynamic research field, as discussed by *Kilian Wistuba-Hamprecht* (University of Tübingen, Tübingen, Germany). He showed that high frequencies of the Vδ1 subset of $\gamma\delta$ T cells in the peripheral blood are associated with poor overall survival under checkpoint blockade in patients suffering from metastatic melanoma. This is due to an accumulation of a certain differentiation phenotype whose defective function cannot be rescued by checkpoint blockade. The group of *Wistuba-Hamprecht* is currently performing an extensive characterization of $\gamma\delta$ T-cell subsets that will certainly contribute to a better understanding of the complex biology of these cells in cancer.

The first session involved talks by four young scientists whose abstracts were selected by the scientific committee because they are interesting contributions to our understanding of the interactions between tumor cells and their microenvironment that may lead to the development of new strategies for fighting cancer progression. *Alessio Menga* (University of Turin, Turin, Italy) described the existence of metabolic crosstalk between glutaminolytic ovarian cancer cells and TAMs, and discussed the importance of exploring the metabolite N-acetylaspartate (NAA) as a predictive biomarker for disease progression in ovarian cancer. He showed that, following glutamine-synthetase knockdown, glutamine-independent moderately aggressive ovarian cancer cells undergo phenotypic and metabolic reprogramming, leading to glutamine addiction and the production and release of Interleukin (IL)-10 and NAA. NAA deactivates the N-methyl-D-aspartate receptor (NMDAR) on macrophages and synergizes with IL-10 in inhibiting their M1 polarization. One particularly interesting point discussed by *Menga* was the possibility of re-educating macrophages towards an anti-tumor phenotype via the agonistic stimulation of NMDAR.

The role of macrophages as key drivers in the pathogenesis of glioblastoma was discussed by *Alessandra Maielli* (Humanitas Clinical and Research Center, IRCCS, Rozzano, Italy). She demonstrated that glioblastoma cells release factors that polarize macrophages toward a pro-tumoral M2-like phenotype, leading to increased CD206 expression, immunosuppression and resistance to immunotherapy. *Maielli* and colleagues also established that mitogen-activated protein kinase kinase (MEK) inhibitors (Pimasertib and Trametinib), currently approved for glioma treatment, impact upon macrophage and glioma stem-cell vitality. However, they are less effective in inducing glioma cell death when macrophages are present. Further studies will be conducted using nanovector-encapsulated drugs administered to glioma cells co-cultured with macrophages.

Alessandro Scagliotti (University of Turin, Turin, Italy) discussed the role of paired immunoglobulin-like receptor B (PIRB), the murine ortholog of human leukocyte immunoglobulin-like receptor B (LILRB), in pancreatic cancer progression. PIRB is expressed on hematopoietic cells and acts as an immune checkpoint by binding with high-affinity major histocompatibility complex (MHC) class I molecules. *Pirb* gene ablation delayed cancer growth, with an increase in the frequency of tumor-infiltrating T and B lymphocytes, in genetically modified mice that spontaneously develop pancreatic cancer. Moreover, the immunization of PIRB-deficient mice with the ovalbumin (OVA) antigen resulted in a significantly improved cellular and humoral anti-tumor response against OVA-expressing tumors, indicating that PIRB may be a novel target with which to improve immunotherapy strategies for the treatment of pancreatic cancer.

In the last presentation in this section, *Paolo E. Porporato* (University of Turin, Turin, Italy) focused on the metabolic crosstalk between skeletal muscle and cancer cells. He showed that the muscles of tumor-bearing mice contain low levels of bioavailable iron and heme, directly causing muscle wasting. In turn, muscle wasting affects tumor evolution. Indeed, despite functional iron deficiency, atrophic muscle upregulates the transcription of iron and heme export machinery. To elucidate the impact of such export in the crosstalk between skeletal muscle and cancer, *Porporato* and coworkers generated specific muscle-specific knockout mice and subcutaneously challenged them with Lewis lung carcinoma cells. While no alteration in primary-tumor growth and tumor-induced cachexia were observed, these mice displayed a significant reduction in lung-metastasis formation and muscle inflam-

mation. Further experiments are required to elucidate the model, but skeletal muscle cannot be considered an innocent bystander in cancer.

3. Session 2. Influence of Metabolism and Microbiota on Tumor Immunology and Immunotherapy

Session 2, co-chaired by Per thor Straten (University of Copenhagen, Copenhagen, Denmark) and Pierre Coulie (UCLouvain, Brussels, Belgium), addressed the role played by metabolism and the microbiota in anti-cancer immune response and tumor progression. *Massimiliano Mazzone* (Center for Cancer Biology, Leuven, Belgium) applied a systems biology approach in analyzing data derived from metabolomics and the bulk transcriptomic and single-cell sequencing of human and mouse tumors treated with ICIs in order to identify metabolic pathways associated with resistance to immunotherapy. He then developed a CRISPR/Cas9 platform to select, *in vivo*, the most relevant cancer-cell intrinsic and extrinsic mediators of resistance. These analyses led to the identification of cytidine deaminase (CDA), an enzyme belonging to the pyrimidine salvage pathway, as being overexpressed in ICI-resistant pancreatic cancer cells. The deletion of CDA increases cytotoxic T-cell response, while decreasing TAMs, thus reinstating ICI-sensitivity in preclinical models of pancreatic cancer. Since CDA levels correlate with T-cell infiltration and prognosis in pancreatic cancer patients, CDA inhibitors may represent promising drugs for the ICI-combinatory treatment of pancreatic cancer patients.

Yi-Ru Yu (University of Lausanne, Lausanne, Switzerland) investigated the mechanisms responsible for T-cell exhaustion, as this phenomenon influences patient responsivity to ICIs. Indeed, ICI treatment is effective on partially exhausted T cells, while terminally exhausted T lymphocytes do not respond to treatment. The passage to this fully exhausted state is probably caused by epigenetic alterations, including changes in chromatin architecture and DNA-methylation pattern, which prevent their proliferation and cytokine production. In his search for the underlying mechanisms that drive this epigenetic reprogramming, *Yu* identified a population of tumor-infiltrating lymphocytes (TILs) that harbor damaged mitochondria and that correspond to the terminally exhausted T-cell subset. However, IL-10 can rescue these T cells by reprogramming their metabolism. Based on this observation, *Yu* developed an IL-10/anti-PD-L1 antibody fusion protein, whose administration reduced tumor progression in preclinical models of liver cancer.

The influence exerted by cancer-cell metabolism on the tumor immune microenvironment was the focus of the talk given by *Cristina Muñoz-Pinedo* (Bellvitge Biomedical Research Institute, Barcelona, Spain), who studied the effects exerted by nutrient restriction on the cancer secretome. She demonstrated that acute starvation and glucose deprivation triggered the secretion of the neutrophil chemoattractant IL-8 and the inflammatory mediator IL-6. These two cytokines, whose presence in the TME correlates with poor prognosis in patients, induced the recruitment of neutrophils and macrophages in the tumor, while decreasing infiltration by T lymphocytes and NK cells. Moreover, glucose starvation stimulated lung-cancer cells to secrete the leukemia inhibitory factor (LIF), which is an IL-6 family cytokine that promotes angiogenesis and tumor growth. Tumor hypoglycemic conditions thus promote paracrine responses in the immune system and endothelial cells, which together induce persistent inflammation, immunosuppression, angiogenesis and cancer progression.

Emerging evidence points to the presence of a specific microbial composition in, and adjacent to, tumors that differ from the normal tissue counterpart. These microorganisms actively participate in the complex tumor ecosystem by recruiting immunosuppressive and inflammatory populations, thus playing a role in subverting the local immune microenvironment. *Maria Rescigno* (Humanitas University, Milan, Italy), in her research on metastatic colorectal cancer (CRC), demonstrated that the gut microbiota may induce modifications in the gut vascular barrier (GVB) that promote the hematogenous dissemination of metastatic tumor cells. *Rescigno* and her team demonstrated that some bacteria can disrupt the GVB and disseminate into the liver, where bacteria boost the formation of a pre-metastatic niche. It is worth noting that the endothelial cell marker plasmalemmal vesicle associated protein-1, which increases following GVB disruption, is a new promising prognostic marker for distant recurrences in CRC. Overall, vascular impairment, bacteria and tumor metastatization are linked processes, and this opens new perspectives for CRC therapy.

Besides influencing cancer growth and metastases, bacteria can be exploited to develop new cancer therapies. In this context, *Guido Grandi* (University of Trento, Trento, Italy) and his team are investigating the use of outer membrane vesicles (OMVs) as anti-cancer compounds. OMVs are spheroid particles that are released from all Gram-negative bacteria via a "budding out" of the outer membrane. *Grandi* showed that OMVs can be engineered to express cancer neoepitopes and effectively used for either systemic or *in-situ* vaccination. Moreover, in studying the mechanisms through which the intestinal microbiome affects cancer development and immunotherapy, he demonstrated that the presence of microbial species that express proteins with amino-acid sequences homologous to cancer neoepitopes can inhibit tumor development in experimental mouse models. This anti-tumor activity appears to correlate with the presence of neoepitope-specific T cells that accumulate in the lamina propria and in the TME. The existence of T cells with identical T cell receptors (TCRs) at both sites suggests that such T cells originate at the mucosal level and subsequently reach the tumor. Inter-

estingly, if homologous epitopes are expressed in Gram-negative intestinal species, the anti-tumor activity can be, at least partially, mediated by the release of OMVs that carry such homologous sequences. By artificially administering neoepitope-decorated OMVs to mice, epitope-specific T cells are elicited and animals are protected from tumor challenge.

The two selected young-investigator talks in Section 2 were by *Giancarla Bernardo* (University of Milan, Milan, Italy) and *Francesco De Sanctis* (University of Verona, Verona, Italy). *Bernardo* demonstrated that the mammary microbiota can directly promote breast-cancer growth. Indeed, she demonstrated that treatment with oral absorbable antibiotics reduced tumor growth in syngeneic models of mammary cancer. This was accompanied by a reshaping of the tumor immune microenvironment to one with anti-tumor character, with increased T-cell and M1-macrophage infiltration and decreased mast-cell levels. Gene Set Enrichment Analysis reported reduced toll like receptor (TLR)2 and TLR7 expression and a low deposition of complement C3 fragments in ampicillin-treated tumors. Furthermore, 16S rRNA sequencing revealed a drop in Staphylococcaceae levels, including in *Staphylococcus epidermidis*, which induces potent proinflammatory activity via complement activation and cytokine release. Conversely, bacteria species expanded by oral ampicillin administration reduced immunosuppressive populations, and limited tumor growth when transferred *in vivo*. The combination of paclitaxel and oral ampicillin strongly improved the efficacy of chemotherapeutic agents, suggesting that antibiotics can complement chemotherapy regimens in breast cancer patients.

Francesco De Sanctis explained that pancreatic ductal adenocarcinoma (PDAC) progression is marked by the stepwise infiltration of myeloid cells, which enforce the highly immunosuppressive microenvironment that is induced by the activities of arginase 1 and inducible nitric oxide synthase. These enzymes mediate the production of large amounts of reactive oxygen and nitrogen species, which impair tumor infiltration by T lymphocytes by altering the chemokine milieu. Pharmacological treatment with AT38 ([3-(aminocarbonyl)furoxan-4-yl]methyl salicylate), which is a drug that downregulates arginase 1 and inducible nitric oxide synthase levels and thus decreases cell nitrosative stress, re-educates the myeloid-cell compartments to support antitumor immunity. This reinstates T-cell recruitment and activation. The combination of AT38 and the adoptive cell transfer of tumor-antigen-specific T cells thus potentiates the efficacy of immunotherapy in PDAC models, indicating that this drug may be an interesting approach to overcoming tumor immunosuppression.

4. Section 3. Progress in Vaccination against Cancer: From the Bench to the Bedside

Rolf Kiessling (Karolinska Institutet, Stockholm, Sweden) and Guido Grandi (University of Trento, Trento, Italy) co-chaired this section on the possibility of using immunotherapy as a pillar of cancer care. Although the clinical translation of cancer vaccines into efficacious therapies has been challenging for decades, promising results are now emerging from several clinical trials. *Cécile Gouttefangeas* (University of Tübingen, Tübingen, Germany) highlighted the importance of developing vaccines that not only activate $CD8^+$ T cells, but also $CD4^+$ T cells. $CD4^+$ T cells are essential for the development and expansion of antitumor $CD8^+$ T cells, the recruitment of further immune cells, and, in addition, a percentage of them are endowed with cytotoxic activity. *Gouttefangeas* and team worked on developing a vaccine based on a 20mer peptide from Ras homolog family member C (RhoC), a small GTPase associated with tumor progression, metastasis and poor prognosis in several human cancers. They tested the vaccine, emulsified in Montanide ISA-51, in a phase I trial for prostate cancer patients. Most vaccinated patients developed a robust and long-lasting T-cell response that was dominated by multifunctional effector/memory $CD4^+$ T cells. However, a phase II clinical study failed to demonstrate clinical efficacy, suggesting that there is a need to refine the vaccine formulation.

Besides ameliorating vaccine formulation, another strategy to improve clinical responses consists of combining multiple immunotherapies, as discussed by *Stina Wickström* (Karolinska Institutet, Stockholm, Sweden) who combined the adoptive cell transfer (ACT) of patient-derived TILs with the administration of a dendritic cell (DC)-based vaccine, with promising results in a phase I clinical trial. Since not all patients are suitable for TIL purification, an alternative approach involves the *in-vitro* stimulation of patient peripheral blood T lymphocytes with tumor-neoantigen-pulsed autologous DCs. Moreover, as the transferred T cells find high levels of reactive oxygen species (ROS) in the TME, which impairs their activity and survival, *Wickström* pre-treated them with a low dose of auranofin, the FDA-approved nuclear factor erythroid 2–related factor 2 (Nrf-2)-activating drug that decreases intracellular ROS levels and thus preserves T-cell antitumoral activity, further improving ACT effectiveness.

The characterization of vaccine-induced T-cell responses may provide us with fresh information on immunotherapy efficacy and function. In this context, *Pierre Coulie* (UCLouvain, Brussels, Belgium) analyzed the role of $CD8^+$ T cells in patients affected by bladder carcinomas and treated with Bacillus Calmette-Guérin (BCG), a widely used immunotherapy whose mechanism of action is still unknown. RNAseq analyses of T lymphocytes in the tumor, blood and urine identified two kinds of clonotypes: (1) those that are present in the tumor before BCG instil-

lation and remain detectable after therapy; and (2) those that appear during therapy, which suggests that BCG induces new tumor-specific T cells and boosts pre-existing ones. TCR repertoire analyses showed that these T cells are polyclonal and recognize different antigens. Furthermore, a spontaneous tumor-specific T-cell response directed against neoantigens is observed in some patients.

Linda Nocchi (Nouscom Srl, Rome, Italy) described a novel immunomodulatory approach aimed at inducing TME reprograming and overcoming suppressive mechanisms via *in-situ* treatment with a modified Vaccinia Ankara virus that encodes IL-12. She demonstrated that this "vector-aided microenvironment programing" system was effective in impairing the growth of tumors in preclinical models, and in the context of checkpoint-inhibitor-resistant tumors.

Another hurdle to vaccine efficacy in solid tumors can be found in the need for vaccine-induced T cells to migrate into the tumor, and this is still a major gap in knowledge. *Craig L. Slingluff* (University of Virginia, Charlottesville, Virginia) discussed the possible barriers to T-cell infiltration in melanoma, underlying the lack of homing receptor ligands on tumor blood vessels and the low production of chemokines. These two barriers can be overcome by combining anti-cancer peptide vaccines with the right immunostimulatory molecules, as demonstrated by *Slingluff* in two clinical trials that used IFNγ intratumoral administration and a topical TLR7 agonist (imiquimod).

Vaccines for melanoma were also the topic of the talk given by *Gustav Gaudernack* (Ultimovacs ASA, Oslo, Norway), who presented data from three completed phase I/IIa clinical trials of vaccination against the tumor antigen human telomerase reverse transcriptase (hTERT) performed in melanoma- (in combination with Ipilimumab), NSCLC- and prostate-cancer patients. The vaccine consisted of three peptides derived from the active site of hTERT that had been identified as immunogenic in previous hTERT vaccination clinical trials. *Gaudernack* presented the results obtained from the longitudinal immunomonitoring of patients up to 8 years after vaccination, and these data clearly demonstrate that the activation of specific immune responses is induced in the vast majority of patients, independently of their human leucocytes antigen (HLA) alleles, suggesting that multi-epitope recognition occurred as a result of the peptides' promiscuous HLA binding features.

The last invited speaker in Session 3, *Jolanda De Vries* (Radboud University Medical Center, Nijmegen, The Netherlands), presented an overview of techniques that can be used to characterize the immune-cell types present in the TME, with a focus on the application of multiplex immunohistochemistry to study melanoma, in which the presence of cytotoxic T cells, DC and NK cells correlates with favorable clinical outcomes. Based on these datasets she set up a DC vaccine against frameshift-derived neo-peptides and tested it in Lynch-syndrome carriers, demonstrating its safety and its ability to give rise to specific immune responses.

The efficacy of immunotherapy is also influenced by the type of cancer-cell death induced. While necroptosis and immunogenic apoptosis are currently known to induce immune-system activation, the immunogenicity of cells that die via different mechanisms has not yet been fully deciphered. *Elena Catanzaro* (Ghent University, Ghent, Belgium), the first selected speaker for Section 3, discussed data showing that ferroptosis is immunogenic. During its early phases, the cells release the danger-associated molecular patterns adenosine triphosphate (ATP) and High Mobility Group Box 1 (HMGB1), which induce DC maturation. The injection of early ferroptotic cells into immune-competent mice therefore induces an effective anti-cancer immune response, suggesting that they can act as a vaccine.

Junbiao Wang (University of Camerino, Camerino, Italy) applied phage-display technology to develop cancer vaccines that can break immune tolerance to self-antigens and trigger a protective immune response. Vaccines that are based on M13 bacteriophages and are specific for either Erb-B2 receptor tyrosine kinase 2 (HER2), or its Δ16HER2 splice variant, were effective in reducing tumor growth in preclinical mouse models of mammary cancer when administered in preventive and therapeutic settings. This antitumor protection was associated with significant anti-HER2 antibody production and antibody-dependent cellular cytotoxicity (ADCC). Similarly, *Francesca Ruzzi* (University of Bologna, Bologna, Italy) investigated the use of a virus-like particle HER2 vaccine (based on the Acinetobacter phage AP205) in human HER2 transgenic mouse models. She demonstrated that this vaccine prevents tumor growth and lung metastasis and induces strong and persistent antibody responses that inhibit human HER2$^+$ breast cancer cells *in vitro*, suggesting that it is a promising candidate vaccine for human HER2$^+$ cancers.

5. Session 4. Strategies to Identify Novel Immunotherapeutic Targets and Monitor Immune Response

This section was co-chaired by Suzanne Ostrand-Rosenberg (University of Utah, Salt Lake City, Utah) and Michael Nishimura (Loyola University Chicago, Chicago, Illinois) and dealt with the need to identify immune biomarkers for use as tools for tumor monitoring and the identification of patients that will most likely respond to therapy. In this context, *Sotirios Fortis* (Cancer Immunology and Immunotherapy Center, Cancer Research Center, Saint Savas Cancer Hospital, Athens, Greece) and his collaborators identified radiotherapy-, radiochemotherapy- and ICI-induced alterations in the peripheral blood TCRVβ repertoire of prostate-, head-and-neck-, and NSCLC patients. The detection of tumor(neo)epitope-specific TCR clones that develop after therapy has potential clinical relevance as they can act as biomarkers for the timely identification of patients that will respond to therapy. Be-

sides changes in the TCR repertoire, prostate-cancer patients displayed alterations in the expression levels of 6 immune-related genes (IRG) in their blood cells after therapy. By interrogating the PRAD (PRostate ADenocarcinomas) dataset, *Fortis* and co-workers found that lower IRG expression levels after therapy are associated with a significantly lower risk of recurrence, suggesting that this signature has possible uses as a prognostic tool. The single-cell sequencing of the TILs of 9 pancreatic tumor samples allowed *Rienk Offringa* (German Cancer Research Center, Heidelberg, Germany) and coworkers to observe that the fraction of tumor-specific TCRs was larger in samples from genetically unstable than in those from stable tumors. Moreover, they also found a specific gene signature that distinguishes tumor-specific from bystander T cells, and, interestingly, demonstrated that this signature, developed from pancreatic tumors, can be successfully applied in multiple tumor types to predict T-cell reactivity.

The novel role of phosphoinositide-3-kinase (PI3Kγ) and IL17 in restraining the anti-tumor immunity induced by tumor-associated antigen (TAA)-based immunotherapy in PDAC was the focus of the presentation by *Francesco Novelli* (University of Turin, Turin, Italy). In the past, he and his group had demonstrated the presence of an increased immune response against the TAA α enolase 1 (ENO1) in chemotherapy-treated PDAC patients, and that DNA vaccination against ENO1 halts tumor growth in genetically engineered mice that spontaneously develop PDAC. Recently, they have shown that the genetic and/or pharmacological inhibition of PI3Kγ in these mice, especially in combination with chemotherapy, induces a stronger anti-ENO1 immune response, which is responsible for prolonged mouse survival. Moreover, by exploiting a transplantable preclinical PDAC model, they also found that IL-17 inhibition, through the administration of monoclonal antibodies, plays a critical role in increasing tumor $CD8^+$ T-cell infiltration and the cytotoxic ability of T cells to kill the PDAC target cells in ENO1-vaccinated mice. Taken together, these data point to PI3Kγ and IL-17 depletion being effective as a strategy to strengthen the anti-tumor response elicited by the ENO1 DNA vaccine.

The design of immunotherapeutic strategies against cancer was also the focus of the talk by *Else Marit Inderberg* (Oslo University Hospital-The Norwegian Radium Hospital, Oslo, Norway). Together with her collaborators, she designed a clinical trial to test the efficacy of a DC-based vaccine that targets cancer stem cells (CSC) in glioblastoma (GBM). mRNA from GBM CSC, in addition to mRNA that encodes telomerase and survivin, was transferred into autologous DCs that were then injected intradermally. A specific T-cell response was induced in the majority of vaccinated patients, three of whom were long-term survivors. Specific survivin and telomerase T-cell clones were detected post-vaccination in both the periphery and tumor-specific T cell clones were isolated from tumor biopsies. Interestingly, TILs taken from a progressing tumor displayed phenotypic heterogeneity, suggesting reduced tumor specificity. A randomized study that compares the efficacy of the DC-based vaccine to standard treatment in GBM patients has been started thanks to the results of this trial.

The presentation by *Federico Garrido* (University of Granada, Granada, Spain) provided evidence to show that several altered HLA class I phenotypes are present in primary tumors and metastases, regardless of histological origin, and that these can range from a total loss of HLA class I expression to partial alterations, including HLA-haplotype loss or the absence of a single locus or single HLA allele. Some of these alterations are reversible, or "soft", leading to T-cell-mediated tumor regression, while others are irreversible, or "hard", leading to cancer progression. Therefore, HLA analyses of primary tumor tissues and, if present, of metastatic lesions must be included within the context of cancer immunotherapy in order to incorporate HLA class I up-regulation into treatments, at least where lesions are "soft".

Two young investigators, *Nandita Noronha* (University of Montreal, Montreal, Canada) and *Sofie Deschoemaeker* (Vrije Universiteit Brussel, VIB, Brussels, Belgium), whose abstracts were selected by the scientific committee, closed out this section. *Noronha*'s work focuses on the identification of the mechanisms responsible for the well-known 5-azacytidine (AZA)-induced immune response in patients under treatment for acute myeloid leukemia (AML). By inhibiting DNA methyltransferases (DNMT), AZA induces the re-expression of normally silenced genes, including cancer-testis antigens (CTAs) and endogenous retroelements, leading to a change in the MHC I-associated peptide repertoire (immunopeptidome). Using transcriptomic and mass spectrometry analyses on moderately non-cytotoxic AZA-treated AML cell lines, *Noronha* demonstrated that the changes in the immunopeptidome only arose from CTA-derived peptides and not from EREs, which only act at the RNA level by forming double-stranded RNA that stimulates the immune system via viral mimicry. In addition, *Noronha* found a higher amount of unfolded protein in AZA-treated cells, in parallel with increased autophagy. The combination of AZA with autophagy inhibitors resulted in an increase in AML-cell death and in a decrease in AML-cell proliferation, indicating that this combinatorial treatment may be a novel and successful strategy for counteracting AML. The presentation by *Sofie Deschoemaeker* focused on the evaluation of the treatment of established E07771 triple-negative breast cancer (TNBC) with a nanobody-Fc fusion antibody with ADCC capacity targeting C-C Motif Chemokine Receptor 8 (CCR8), whose effectiveness had already been tested in lung and colorectal cancer models. By targeting CCR8, which is expressed by the most immunosuppressive tumor-infiltrating Treg, the drug induced the complete rejection of the E07771 tumor in 100% of mice. These

mice did not develop tumors when re-challenged with the same cells, which indicates the induction of a long-term memory anti-tumor response. A detailed analysis of the tumor -microenvironment using scRNAseq indicated a shift from $CD8^+$ T-cells expressing more exhausted cell markers in the control mice to $CD8^+$ T-cells showing an expression pattern of naïve and effector T-cells upon anti-CCR8 treatment. Consequent CD8 depletion experiments confirmed that $CD8^+$ T-cells are required for the strong anti-tumor response observed upon anti-CCR8 treatment of E0771 tumor-bearing mice. Although further studies will be needed, these results support the clinical development of anti-CCR8 treatment as a therapy for TNBC patients.

6. Session 5. Novel Immunotherapeutic and Combined Strategies for Cancer Treatment

The last session of PIVAC22 was chaired by Else Marit Inderberg (Oslo University Hospital-The Norwegian Radium Hospital, Oslo, Norway) and Gustav Gaudernack (Ultimovacs ASA, Oslo, Norway), and aimed to provide an update on the new strategies that can be used to treat cancer, with a particular focus on combined therapies. *Gosse J. Adema* (Radboud University Medical Center, Nijmegen, The Netherlands) contributed to the search for tumor-promoting mechanisms that can be targeted to improve cancer therapy by demonstrating that the aberrant levels of sialoglycans that are induced by the upregulation of sialyltransferases in cancer cells provide protection against apoptosis and therapy, and thus favor their metastatic spread. The administration of a sialic-acid mimetic that can impair sialic-acid production in melanoma cells therefore decreases tumor progression in preclinical models. It is interesting to note that this mechanism not only mediated a direct effect on cancer cells, but, in addition, sialic-acid blockade also improves DC maturation and their T-cell-activation capacity, inducing immune-mediated cancer-cell killing.

While anti-cancer immune responses can also be stimulated by therapies that induce immunogenic cell death, and consequently promote the activation of tumor-antigen-specific lymphocytes, cancer cells have unfortunately evolved immunosuppressive mechanisms to bypass this phenomenon. In fact, *Michael A. Curran* (University of Texas, MD Anderson Cancer Center, Houston, Texas) showed that radiotherapy rarely induces an abscopal effect in CRC patients, and this is due to the upregulation of the "don't-eat-me signals" CD47 and programmed death-ligand 1 (PD-L1) on CRC cells, which thus escape from phagocytosis by antigen-presenting cells and limit tumor antigen presentation to T cells, preventing immune activation. Consequently, a combination of ICIs that target these two phagocytosis checkpoint pathways potentiates the effectiveness of radiotherapy by restoring tumor antigen cross-presentation.

The route of immunotherapy administration is another important point that can determine efficacy, especially in cancers affecting the brain, whose accessibility by systemically administered ICIs is dampened by the blood-brain barrier. *Johannes vom Berg* (University of Zurich, Zurich, Switzerland) discussed the possibility of using IL-12 to rewire the immunosuppressive TME characteristics of glioblastoma and thus potentiate immunotherapy efficacy. However, IL-12 systemic administration has demonstrated low efficacy and highly significant side effects, while the low molecular weight of IL-12 impairs its retention in the brain even when locally administered. To overcome these problems, *vom Berg* developed a hybrid protein by combining IL-12 with human IgG4 Fc. Moreover, because the neonatal Fc receptor (FcRn) is expressed in brain tissue and contributes to the brain export of IgG, he substituted some key amino acids in Fc to prevent its binding to FcRn. The resulting compartment-locked fusion protein showed promising results in preclinical glioblastoma models, improving the tolerability and efficacy profile of IL-12 for local delivery. Interestingly, this approach could be used to improve the tumor targeting of other immunomodulators.

Michael I. Nishimura (Loyola University, Chicago, Illinois) gave a brief overview of the history of the strategies developed for the adoptive transfer of TCR- and (chimeric antigen receptor) CAR-transduced T cells that target tumor antigens. Most of these engineered T cells have been developed to target TAAs that are also expressed in healthy tissue, consistently limiting the efficacy and amplifying the side effects of these therapies in clinical trials. The development of T cells that are specific for neoantigens, which are tumor-specific, may be a means of overcoming this issue. This strategy, however, is cumbersome because neoantigens are usually patient-specific, which increases costs and regulatory requirements. The ideal antigen would thus be a tumor-specific antigen that is shared by several tumors. *Nishimura* found such an antigen in defective endogenous retrovirus group E (HERV-E), which is expressed in many tumors, and he tested T cells that express a HERV-E-specific TCR in patients with clear cell renal cell carcinoma. These clinical trials showed objective clinical responses and no serious toxicity, paving the way for new trials targeting such antigens that are selectively expressed in cancer cells.

T lymphocytes are not the only cells that can be engineered for adoptive cell therapy. *Dario Sangiolo* (University of Torino, Torino, Italy) proposed the use of cytokine-induced killer lymphocytes (CIK) that have been engineered to express CAR. CIK can be obtained by expanding *in-vivo* T-NK lymphocytes in the presence of cytokines and CD3 stimulation, which grant them HLA-independent anti-cancer activity mediated by the NKG2D receptor. Thus, CIK can overcome the HLA downregulation that is often exploited by cancer cells to evade T-cell responses, efficiently killing HLA-negative chemoresistant CSC. CIK that express a tumor-specific CAR can combine their intrinsic anti-cancer effect with CAR-specificity, and *Sangiolo*

demonstrated that these cells are able to infiltrate solid tumors and impair tumor growth in preclinical models, with this now being a promising tool for the development of adoptive cell-transfer therapies.

The importance of targeting the programmed cell death protein 1 (PD-1) pathway in treating malignancies led the scientific committee to the selection of the abstract of *Federica Pericle* (ImmunoGenesis Inc, Houston, Texas) who presented a new monoclonal antibody that can recognize and block both PD-L1 and PD-L2. Since these molecules are expressed both on cancer cells and immunosuppressive cell populations (such as TAMs and MDSCs), the antibody was engineered to induce increased effector functions (both ADCC and antibody-dependent cellular phagocytosis, ADCP) in order to eliminate PD-L$^+$ cells, thus remodeling the TME.

There is still a lack of specific target antigens that could be used for the development of vaccines and CAR T therapies for some cancers, such as metastatic osteosarcoma (OS). *Nadia Mensali* (Oslo University Hospital, Oslo, Norway) was selected to discuss her data on the identification of two antibodies endowed with high specificity to OS tissues. With her coworkers, she designed a single chain variable fragment based on the sequences of these antibodies, linked them to a second-generation CAR-signaling tail and called them OSCAR-1 and OSCAR-3. *Mensali* demonstrated that T cells that are transduced with OSCAR-1 and 3 control tumor progression and prolong survival in several human xenograft mice models, without inducing any critical cross-reactivity towards healthy tissues, paving the way for their clinical translation.

7. Conclusions

The PIVAC-22 meeting was held at the Molecular Biotechnology Center "Guido Tarone", at the University of Turin, and more than 100 attendees presented and discussed a plethora of new and promising results in a relaxed and informal atmosphere. Recent investigations into cancer immunotherapy approaches, immunotherapeutic targets and vaccines were impeccably covered in this meeting, and special attention was paid to the influence of the TME on their effectiveness. One of the main points of focus for the future is determining which immunomodulatory strategies can be combined to ameliorate patient outcomes. The meeting ended with the promise to meet again for PIVAC-23.

Abbreviations

ACT, adoptive cell transfer; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; AML, acute myeloid leukemia; AT38, [3-(aminocarbonyl)furoxan-4-yl]methyl salicylate; ATP, adenosine triphosphate; AZA, azacytidine; BCG, bacillus Calmette-Guérin; CCR8, C-C Motif Chemokine Receptor 8; CDA, cytidine deaminase; CAFs, cancer-associated fibroblasts; CAR, chimeric antigen receptor; CIK, cytokine-induced killer lymphocytes; CRC, colorectal cancer; CSC, cancer stem cells; CTA, cancer-testis antigen; DC, dendritic cell; DNMT, DNA methyltransferase; ENO1, α-enolase; FcRn, neonatal Fc receptor; GBM, glioblastoma; GS, glutamine synthetase; GVB, gut vascular barrier; HER2, Erb-B2 receptor tyrosine kinase 2; HERV-E, endogenous retrovirus group E; HLA, human leucocytes antigen; HMGB1, high mobility group box 1; hTERT, human telomerase reverse transcriptase; ICIs, immune checkpoint inhibitors; IDH, isocitrate dehydrogenases; IL, interleukin; LIF, leukemia inhibitory factor; LILRB, leukocyte immunoglobulin-like receptor B; MDSCs, myeloid-derived suppressor cells; MEK, mitogen-activated protein kinase kinase; MHC, major histocompatibility complex; NAA, N-acetylaspartate; NK, natural killer; NMDAR, N-methyl-D-aspartate receptor; Nrf-2, nuclear factor erythroid 2–related factor 2; NSCLC, non-small-cell lung cancer; OMV, outer membrane vesicles; OS, osteosarcoma; OVA, Ovalbumin; PD-1, programmed cell death protein 1; PDAC, pancreatic ductal adenocarcinoma; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; PIRB, paired immunoglobulin-like receptor B; PI3K, phosphoinositide-3-kinase; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; RhoC, Ras homolog family member C; TAA, tumor associated antigen; TAMs, tumor-associated macrophages; TCR, T cell receptor; TERT, telomerase reverse transcriptase; TILs, tumor infiltrating lymphocytes; TLR, toll like receptor; TME, tumor microenvironment; TNBC, triple-negative breast cancer; Treg, regulatory T cells.

Author Contributions

MY, wrote the manuscript. FC, LC, and EQ reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.