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MEETING REPORT

Fifteenth Meeting of the *Network Italiano per la Bioterapia dei Tumori (NIBIT)* on Cancer Bio-Immunotherapy, Siena, Italy, October 5–7, 2017

Michele Maio[1,2] · Maria Fortunata Lofiego[2] · Carolina Fazio[2] · Sara Cannito[2,3] · Carla Chiarucci[2] · Gianluca Giacobini[2] · Monica Valente[2] · Patrizia Tunici[2] · Alessia Covre[2] · Vincenzo Russo[1,4]

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Abbreviations

ACT	Adoptive cell therapy
APM	Antigen-presenting machinery
BM	Bone marrow
CAR	Chimeric antigen receptors
CDA	Cytidine deaminase
CSC	Cancer stem cells
DC	Dendritic cells
DCR	Disease control rate
DITC	Dacarbazine
GIC	Italian society of cytometry
GITR	Glucocorticoid-induced tumor necrosis factor receptor
HF	Heart failure
HMA	Hypomethylating agent
ICI	Immune checkpoint inhibitors
IDO1	Indoleamine 2,3-dioxygenase-1
iNKT	Invariant natural killer T cells
MDP	Mutations and drugs portal
MDSC	Myeloid-derived suppressor cells
MØ	Macrophage orchestration
MSA	Methylseleninic acid
MV	Microvesicles
NSCLC	Non-small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PCa	Prostate cancer
PDAC	Pancreatic ductal adenocarcinoma
pDC	Plasmacytoid dendritic cells
ROS	Reactive oxygen species
STS	Short-term starvation
TAM	Tumor-associated macrophages
TCF-1	Transgenic T-cell factor 1
TCR	T-cell receptors
TENM4	Teneurin-4
TIL	Tumor-infiltrating lymphocytes
TLS	Tertiary lymphoid structures
TME	Tumor microenvironment
TNBC	Triple-negative breast cancer
TNFR	Tumor necrosis factor receptor
Treg	Regulatory T cells
T-VEC	Talimogene laherparepvec

This meeting report is a summary of presentations from the Fifteenth Meeting of the *Network Italiano per la Bioterapia dei Tumori (NIBIT)* on Cancer Bio-Immunotherapy, published together with a series of Focussed Research Reviews based on lectures given at the conference.

✉ Michele Maio
mmaiocro@gmail.com

[1] Italian Network for Tumor Bio-immunotherapy (NIBIT), Siena, Italy

[2] Center for Immuno-Oncology, Medical Oncology and Immunotherapy, University Hospital of Siena, Istituto Toscano Tumori, V.le Bracci, 16, Siena, Italy

[3] University of Siena, Siena, Italy

[4] Immuno-biotherapy of Melanoma and Solid Tumors Unit, Division of Experimental Oncology, San Raffaele Hospital Scientific Institute, Milan, Italy

Introduction

The XV annual meeting of the Italian Network for Tumor Biotherapy (NIBIT) took place in Siena, Tuscany, on October 5–7, 2017. International leading scientists from academic institutions and pharmaceutical companies presented updates on new research achievements in cancer bio-immunotherapy. The meeting aimed to build a close connection between clinical and pre-clinical research. Many topics were

discussed during the meeting, such as (a) tumor microenvironment; (b) epigenetics, immune cells and cancer; (c) commensal microorganisms, food, immune cells and cancer; (d) immune agonists and antagonists; (e) active and adoptive immunotherapy; (f) prospective NIBIT and NIBIT Foundation collaborations. There follows a brief summary of the topics discussed in the meeting.

Session 1: tumor microenvironment 1

Growing experimental evidence points to a significant role of the host immune system and its interactions with cancer cells inside the tumor microenvironment (TME) in modulating cancer development and metastasis. TME, with its highly complex tumor-promoting features, is the third emerging component in this scenario, playing a huge role in enhancing or decreasing anti-cancer immune responses.

In this framework, as discussed by Wolf H. Fridman (Cordeliers Research Centre, University Paris Descartes, Paris, France), the occurrence of high density of CD8+ T cells, and tertiary lymphoid structure (TLS)-containing dendritic cells (DC), in the TME of clear cell renal cell carcinoma, was associated with good clinical response to immunotherapy. Conversely, tumors with low levels of T cells, lack of TLS and the presence of immature DC, high levels of regulatory T cells (Treg) and exhausted CD4+ and CD8+ T cells were correlated with poor prognosis. These results strongly suggest that specific immune TME signatures could better predict overall survival (OS) and facilitate investigation of mechanisms to improve the efficacy of cancer immunotherapy. Soldano Ferrone (Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA) discussed the beneficial effects of neoadjuvant polychemotherapy with Oxaliplatin, 5-Fluoruracil and Irinotecan, the Folfirinox combination, in patients with pancreatic ductal adenocarcinoma (PDAC). The treatment effects, characterized by reduced frequency of HLA-A defects, increase of tumor CD8+ T-cell infiltration and reduction of tumor Treg infiltration, in PDAC patients are mediated in part by induction of changes in immune balance, with an increase of tumor cell recognition by the immune system. To implement immunotherapy approaches in cancer patients and to better understand mechanisms of resistance, Zlatko Trajanoski (Biocenter, Division for Bioinformatics Medical University of Innsbruck, Innsbruck, Austria) developed *in silico* and *in vitro* models to identify molecular factors that could be responsible for the resistance to combination immunotherapy. Moreover, immune-oncology 3D models represent progress for better understanding melanoma TME. Immunosuppression is the major obstacle to cancer immunotherapy and these models provide support for investigating cell survival, migration and function of all cell types, allow the real-time study of immune cells within a complex tissue architecture, exploring the interaction between cancer and non-cancer cells and immune system in melanoma TME, as reported by Dr. Marcella Tazzari (Department of Tumor Immunology, RIMLS, Radboud UMC, Netherlands). Other components actively involved in the immunosuppressive TME, allowing cancer growth and resistance to treatments, are genetic alterations. In this scenario, the Genomics and Drugs integrated Analysis (GDA) developed by Dr. Silvio Bicciato's group (Department of Life Sciences, University of Modena and Reggio Emilia, Italy), interconnecting pharmacological information from the NCI-60 Discovery & Development Services with genomic data of the Cancer Cell Line Encyclopedia, could be a very useful tool to develop novel and more effective targeted therapies.

Session 2: tumor microenvironment 2

Cancer should be considered as a highly heterogeneous functional system, connecting and depending on the TME. In this context, tumor cells, changing the mechanical properties of the microenvironment, create favorable conditions for their proliferation via different immune evasion mechanisms. Strategies overcoming the ability of cancer to escape the immune system are of increasing interest to the scientific community to develop new and more efficacious therapies. One of these includes the targeting of the IFN-γ-inducible enzyme indoleamine 2,3-dioxygenase-1 (IDO1), as discussed by Alexander J. Muller (Lankenau Institute for Medical Research, Pennsylvania, USA). IDO1 catalyzes the degradation of the essential amino acid tryptophan, which is the first and rate-limiting step in the kynurenine pathway, and affects immunological regulatory pathways. In addition to enabling suppressive FoxP3+ Tregs to inhibit effector T-cell activity, pre-clinical evidence suggests that high IDO1 activity has an important role in the neovascularization process and represents a tumor-promoting inflammatory stimulus. Moreover, IDO2 has recently been shown to have similar activity to IDO1 and can be considered a predictive marker of patient responsiveness to neoadjuvant radiotherapy. This evidence raised the possibility that IDO enzymes might have potential roles as targets for future therapeutic approaches. Supporting this hypothesis, in the pre-clinical results shown by Maura Camozzi (Medical Manager Oncology, Incyte, Milan, Italy), IDO1 alone or in combination with arginase contributes to an immunosuppressive TME through the activation of Treg cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs). Epacadostat (an IDO1 inhibitor) blocks IDO1-mediated immunosuppression and shifts the TME toward a state of supporting immune surveillance. Pre-clinical models have shown that epacadostat combined with CTLA-4 and

PD-L1 immune checkpoint inhibitors improves anti-tumor efficacy vs single treatment through a direct reactivation of T cells in situ. In addition, arginase inhibition has been shown to enhance anti-tumor immunity and inhibit tumor growth. Thus, blocking IDO1 and/or arginase may lead to a novel strategy in combination with immune checkpoint inhibitors (ICI) to relieve immunosuppression and to enhance anti-tumor activity. Evidence of enhanced T-cell responsiveness and anti-tumor immunity was also demonstrated combining OX40 (CD134) or glucocorticoid-induced tumor necrosis factor receptor (GITR) agonist antibodies, members of the tumor necrosis factor receptor (TNFR) superfamily, with ICI. As underlined by Aurelia Rughetti (Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy), tumor growth depends strictly on the interaction/balance of the highly complex network of molecular and metabolic cross-talk signals among cancer cells, immune cells and the TME. She described how tumor microvesicles (MVs), that promote tumor progression encouraging immunosuppression, as well as anti-tumor responses, play a contradictory role in this complex scenario. They can promote tumor growth by enhancing immunosuppression or induce efficacious anti-tumor immune responses by mediating antigen transfer to DC. This mechanism is important mainly for the cross-presentation of molecules that are blocked by HLA class II when internalized as soluble molecules. Among them, the transfer of MUC1, a tumor-associated glycoproteins, mediated by MVs to DC was demonstrated to stimulate IFN-ɣ responses. These results have important implications for the use of glycoprotein-based immunogens in anti-cancer therapy. Paolo Dellabona (Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Hospital Scientific Institute, Milan, Italy) showed that invariant natural killer T cells (iNKT cells) represent an active component that plays a major role in the regulation of the cellular adaptive immune response via their ability to mature DCs and improve CD4 and CD8 T cells, as demonstrated in prostate cancer (PCa). Modulation of TMEs and pro-inflammatory tumor-associated macrophages (TAMs) through CD40L/CD40 binding, supported anti-tumor macrophage type 1 (M1) survival and FasL/Fas to selectively kill pro-tumor type 2 (M2) macrophages. Inverse correlation between iNKT cells and M2-like TAMs has clinical relevance in human PCa and this subset of cells may be harnessed as a platform to therapeutically reprogram the TME. Andrea Alimonti (Department of Medicine, Venetian Institute of Molecular Medicine, University of Padua, Padua, Italy) showed that MDSCs and TAMs are the major immune cell subsets infiltrating PCa at different disease stages. MDSCs can promote the development of metastatic castration-resistant PCa and CXCR2 antagonists can reprogram TAMs to an M1 phenotype. Conversely, tumor immunotherapy can occasionally induce adverse events also in normal tissues, especially cardiotoxicity, as discussed by Marinos Kallikourdis (Adaptive Immunity Laboratory, Humanitas Clinical and Research Center, Milan, Italy). Indeed, he described in pre-clinical mouse models and in human heart failure (HF) patient biopsies a correlation between T-cell infiltration and cardiovascular disease. Thus, in this scenario, he explained how drugs that interfere with T-cell function can be used to treat experimentally-induced HF. This treatment resulted in a block of HF progression that was more efficient than the standard pharmacological treatment of cardiac diseases. So, a deeper investigation of the mechanism(s) of cardiotoxicity occasionally induced by cancer immunotherapy should be considered, to develop more efficacious immunotherapy protocols.

Session 3: epigenetics, immune cells and cancer

Epigenetic events are emerging as important novel hallmarks contributing to cancer development and progression through their regulation of different mechanisms, including host immune recognition of cancer cells. Epigenetic programs regulating normal differentiation of innate and adaptive lymphocytes have already been described, but epigenetic processes of T-cell differentiation and dysfunction in tumors remain unknown. The exciting findings described by Andrea Schietinger (Immunology Program, Memorial Sloan Kettering Cancer Center, New York, USA) define the chromatin state dynamics underlying tumor-specific T-cell dysfunction in the tumorigenesis process. Her group identified novel membrane proteins (CD38, CD101, CD30L, CD5), which can demarcate a plastic (therapeutic reprogrammable) *vs* a fixed (not epigenetic therapeutic reprogrammable) chromatin state of tumor-specific $PD1^{hi}$ T cells in mouse models. Hypothesizing that $PD1^{hi}$ TIL in patients resistant to immune checkpoint blockade may be in a fixed dysfunctional state, in contrast to those of responders that are in a plastic state, amenable to reprogramming, they pointed towards discovering new targets and strategies to transform TIL into potent anti-tumor agents. In addition to increasing the functionality of TIL, the possibility of up-regulating MHC class I cell surface expression is a well-known critical step in the induction of tumor rejection. Alterations of HLA and Antigen-Presenting Machinery (APM) components are found in a large proportion of malignant cells, and among the different mechanisms proposed to revert this phenomenon, epigenetic remodelling plays a fundamental role. The group of Barbara Seliger (Institute for Medical Immunology, Martin Luther University Halle-Wittenberg, Halle, Germany) demonstrated that both DNA hypomethylating agents and histone deacetylase inhibitors could up-regulate

APM components and HLA class I surface expression in melanoma cells and that deficient IFN signalling (i.e., STAT1 and JAK2) in these cells was associated with low APM component expression. They also proposed methylseleninic acid (MSA) as a new drug for reversion of MHC defects, showing a dose-dependent induction of MHC class I surface antigens in murine melanoma cells by MSA treatment. Epigenetic events are linked to the TME and in particular to the orchestration of tumor macrophage (MØ) polarization in response to local environmental signals. Renato Ostuni (Genomics of the Innate Immune System Unit, San Raffaele-Telethon Institute for Gene Therapy (SR-Tiget), Milan, Italy) explained how the biology of MØ activation in cancer is not based on individual stimuli that dictate specific functional states. He demonstrated how concomitant exposure of MØ to IFN-γ (polarizing MØ with immunostimulatory functions) and IL-4 (polarizing MØ with immunosuppressive functions) results in a complex cross-antagonistic behavior. Indeed, many IFN-γ-induced genes were completely shut down upon co-administration of IL-4, and their regulatory elements lacked activating histone marks, leading to the hypothesis that the antagonistic effect of IL-4 occurs at the epigenomic level. In the complex scenario of cancer growth and progression, in which epigenetic modeling has a demonstrably crucial role, the possibility of utilizing drugs reverting epigenetic dysfunctionality is a new strategy of great clinical interest. Roberta Ferraldeschi (Astex Pharmaceuticals Inc., Cambridge, UK) provided an overview of the immunomodulatory activity of the next-generation hypomethylating agent (HMA), guadecitabine, a dinucleotide of deoxyguanosine resistant to deamination by cytidine deaminase (CDA), designed to prolong exposure to decitabine, its active metabolite. Guadecitabine improves the immunogenicity and immune recognition of neoplastic cells in vitro. Moreover, it resulted in improved anti-tumor activity when it was followed by the administration of anti-CTLA4 mAb in syngeneic mouse models. Based on this rationale, several clinical trials using guadecitabine in combination with different immunotherapeutic agents are ongoing. This session was closed by Matteo Bellone (Cellular Immunology Unit, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy) who discussed clinical progression in lymph node-positive patients with PCa. His group conducted a retrospective study in which a cohort of 51 patients with lymph node-positive PCa were treated with radical prostatectomy and extended pelvic lymph node dissection. Patients with at least 1% of PD-L1$^+$ tumor cells had shorter metastasis-free survival than those with PD-L1$^-$ tumors. In addition, PD-L1 expression was significantly associated with CD8$^+$ T-cell density, suggesting that PD-L1 and CD8 can be used as biomarkers for lymph node-positive PCa patients with high risk of progression. Additionally, patients with PD-L1$^+$ and CD8$^+$ tumors might be the ideal candidates for immune checkpoint blockade therapies.

Session 4: commensal microorganisms, food, immune cells and cancer

The relationships among cancer, the immune system, commensal microorganisms, and diet is becoming an interesting topic for the scientific community. The commensal microbial community, known as the microbiota, has been found to be crucial for immunological, hormonal and metabolic homeostasis of the host. The human microbiota is an aggregate of microorganisms (e.g., bacteria, fungi, archaea and viruses) that resides on or within a number of human biofluids and tissues, including the gastrointestinal tract. In this organ, the colonization and growth of commensal microorganisms are directly influenced by the presence of mucus. Changes in mucus composition have been described by Chiara Pozzi (Department of Experimental Oncology, European Institute of Oncology-IEO, Milan, Italy) in ApcMin/+mice, a mouse model of spontaneous intestinal tumorigenesis. In these mice, the analysis of the 16S rDNA sequencing data identified the lack of an anaerobic bacterium belonging to the *Erysipelotrichaceae* family at 8 weeks of age, coinciding with the beginning of tumor development. Interestingly, they also found that the same family was under-represented in advanced adenoma patients. The intestinal microbes seem also to influence the progression of extra-mucosal tumors, as explained by Arianna Brevi (Division of Immunology, Transplantation and Infectious Diseases, Cellular Immunology Unit, San Raffaele Scientific Institute, Milan, Italy). She described how the microbiota can guide the differentiation of Th17 cells, that colonize Peyer's patches and migrate to the bone marrow (BM) of transgenic Vk*MYC mice, harboring oncogene-driven plasma cell proliferative disorders, and lead to the progression of multiple myeloma. Indeed in Vk*MYC mice, an altered microbiome, as well as the lack of IL-17, imply a reduction in the levels of this cytokine in BM and a delayed MM insurgence. Likewise, patients with high levels of IL-17 in the BM had faster disease progression. The imbalance in the microbiota can have a strong impact on immunity and inflammation as described by Romina S. Goldszmid (Inflammatory Cell Dynamics Section, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA). Considering that inflammatory processes are implicated in all stages of cancer, Goldszmid's group investigated how, influencing inflammation, the microbiota contribute to cancer insurgence and its response to therapy. They focused their attention on a specific cell subset, the infiltrating mononuclear phagocytes (MPs), the composition and function of

which in the TME is strictly influenced by the microbioma imbalance. Indeed, reduced activity and lower cytokine production of MPs were evidenced in antibiotic-treated or germ-free mice under cancer therapy. These data suggest the importance of an intact commensal microbiota for a better response to cancer therapy through the modulation of myeloid-derived cell functions. Thus, novel immunotherapeutic strategies targeting MPs could represent a powerful approach to increase immune responses in cancer patients. Improvement in the response to cancer therapy seemed to be influenced also by short-term starvation (STS) as explained by Valter Longo (Longevity Institute and Davis School of Gerontology, University of Southern California, Los Angeles, CA, USA). STS increases resistance to chemotherapy in normal but not cancer cells by reducing proto-oncogene product activity. Specifically, a reduction in insulin-like growth factor-1 signaling, which is associated with an increased stress activity, has been observed in mammalian cells under STS. Another interesting aspect mentioned by Dr. Longo is the link between fasting mimicking diets and the reduction of the Heme Oxygenase-1, which seem to promote T cell-mediated tumor cytotoxicity. Finally, Christoph Huber (Department of Hematology and Oncology, Mainz University Medical Center, Mainz, Germany) presented the European Network for Cancer Immunotherapy (ENCI), a new initiative on behalf of the Association for Cancer Immunotherapy (CIMT) aiming at expanding collaborations between different European institution working on the immunotherapy of cancer.

Session 5: immune agonists and antagonists

Another topic in the field of immunotherapy that has recently attracted much interest in the scientific community is new co-stimulatory and inhibitory molecules. Agonist and antagonist antibodies are, respectively, able to boost or block immune signaling pathways, to potentiate immunotherapeutic strategies. The session was opened by Mario P. Colombo (Molecular Immunology Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy), who presented results on the activity of OX86 antibody (agonist of the activating receptor OX40) in reducing the secretion of IL-10 by Tregs, in murine cancer models. OX86 might encourage tumor rejection by stimulating DC maturation/migration (via CD40-CD40L axis) and inducing a CD8+ anti-tumor response. Several ongoing clinical trials funded by pharmaceutical companies are focused on agonistic and antagonistic antibodies. Jean Viallet (Brystol-Myer Squibb, Princeton Pike, New Jersey, USA) presented updates on the BMS pipeline. Through the use of agonist antibodies (e.g. anti-CD137, -CD27, -CD40, -OX40), they essentially aimed to stimulate effector T-cell activation and proliferation. Antagonist molecules developed by BMS (i.e. anti-CCR2/5 and anti-LAG-3) are aimed at reducing recruitment of immunosuppressive cells in the TME and potentiating effector T-cell activity. LAG-3 was also discussed by Chiara Camisaschi (Unit of Immunotherapy of Human Tumors, Department of Experimental Oncology and Molecular Medicine, IRCCS Istituto Nazionale Tumori, Milan, Italy). Her group investigated whether this inhibitory receptor could have a role in alternative activation of plasmacytoid DC (pDC). She reported that LAG-3 ligation led to an enhanced immunosuppressive activity of Tregs and that semi-mature LAG-3+ pDCs released IL-6 at the tumor site, contributing to the regulation of pDC and partially to the development of a tolerogenic environment. In the framework of the development of novel molecules, Martina Canestraro (Immunocore Ltd., Abingdon, UK), gave an overview of the ImmTAC™ platform and its leading molecule IMCgp100. ImmTAC molecules are bi-specific reagents formed from an affinity-enhanced monoclonal TCR (mTCR) linked to an anti-CD3-specific antibody fragment (CD3-scFv). The mTCR recognizes intracellular antigens that have been processed and displayed on class I MHC molecules, binding as few as 5–10 epitopes on a cancer cell; the anti-CD3-scFv leads to the recruitment and activation of T cells against the tumor. IMCgp100 targets the melanoma-associated antigen gp100 presented by an HLA-A*0201 molecule. In vitro the molecule induces potent and specific killing of gp100-positive melanoma cells. This observation led to phase I/II clinical trials in cutaneous and uveal melanoma, to investigate these therapeutic options, alone and/or in combination with checkpoint inhibitors for cutaneous melanoma.

A deeper investigation of the therapeutic potential of ICI was discussed by Luana Calabrò (Center for Immuno-Oncology, University Hospital of Siena, Siena, Italy), who presented results of the phase II NIBIT-MESO-1 clinical study. This trial combines anti-CTLA-4 (Tremelimumab) and anti-PD-L1 (Durvalumab) mAbs, in patients with unresectable malignant mesothelioma. A median OS of 15.3 months, with an objective response rate (ORR) of 27.5% and a disease control rate (DCR) of 65%, was achieved by patients enrolled in the study, demonstrating clinical improvements compared to previous studies using Tremelimumab as a single agent. Preliminary results of the phase II MAPS-2 clinical trial combining Ipilimumab (Ipi) and Nivolumab (Nivo) versus Nivo alone, in patients with malignant pleural mesothelioma, as second-/third-line treatment were presented by Arnaud Scherpereel (Pulmonary and Thoracic Oncology, CHU de Lille, Univ. Lille, France). After 12 weeks, treatment with Nivo + Ipi resulted in a DCR of 50% as against 44.4% of the Nivo-alone arm. These results translated into an increase of the progression-free survival of 1.6 months in favor of the combination arm, whereas the OS was not yet comparable, since more than half of patients

of the Nivo + Ipi arm were still in treatment. To sum up the therapeutic alternatives with ICI in non-small cell lung cancer (NSCLC), Claudia Proto (Medical Oncology Department, Fondazione IRCCS Istituto Nazionale di Tumori, Milan, Italy) reported clinical data concerning anti-PD-1/PD-L1 mAbs. She discussed results with Pembrolizumab, the unique anti-PD-1 molecule approved as first-line treatment in patients with PD-L1-positivity higher than the 50%, in the KEYNOTE-024 study. As second-line therapy, she mentioned the possibility of using Nivo, Atezolizumab or Pembrolizumab that showed statistically significant increase in the OS, compared to standard docetaxel treatment. This session was concluded by Cornelis J.M. Melief (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands; ISA Pharmaceuticals B.V, Leiden University Medical Center, the Netherlands) who demonstrated how therapeutic vaccination with HPV16 vaccine ISA101 could increase the effects of standard chemotherapy in cervical cancer and other HPV16-positive cancers. He reported that synthetic HPV16 long peptide vaccination is more efficient in the setting of premalignant or early-stage diseases, whereas a combinatorial approach (i.e., with chemotherapy, ICI, agonist/antagonist Abs) is needed for late-stage disease to overcome the immunosuppressive microenvironment. This was the rationale for the phase II combinatorial trial of Nivo and ISA101 for HPV-16+ incurable solid tumors, which supports the idea that T-cell responses induced by vaccination could be improved by anti-PD-1 therapy. An ORR of 36% was seen in patients with HPV16+ incurable oropharyngeal cancer treated with the combination, higher than that observed with anti-PD-1 monotherapy in the CHECKMATE 141 and KEYNOTE 55 studies, with durable responses and similar side effects.

Session 6: active and adoptive immunotherapy

Therapeutic vaccines represent a relevant strategy for cancer treatment. This approach allows the generation of an active immunotherapy using the patient's immune system. The most studied types of vaccines are: (1) cell-based cancer vaccines, which use cancer cells to stimulate lymphocytes that are subsequently re-infused into the patient; (2) peptide vaccines, that uses specific tumor antigens administered to patients to elicit an immune response; (3) nucleic acid vaccines, plasmids containing antigens-encoding genes, that, once internalized by host cells, express antigens able to activate the immune system. Another therapy that showed advantages compared to classical approaches of cancer immunotherapy, is adoptive cell therapy (ACT). ACT is based on the use of genetically-modified T cells able to recognize specific antigens through the expression of chimeric antigen receptors (CARs) or engineered T-cell receptors (TCRs), so as to enhance antitumor immunity. The first speaker of the session, Federica Cavallo (Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy) reported on a new cancer stem cell (CSC) target for vaccination, the cysteine–glutamate antiporter protein xCT (SLC7A11). xCT expression is rarely present in normal cells, but highly expressed in a large percentage of solid tumors, including Her2+ and triple-negative breast cancer (TNBC). High expression of xCT seems to correlate with poor prognosis. Pre-clinical data show that vaccination of mice against xCT prior or after breast tumor development induces antibodies that impair CSC function and slows down tumor growth, impairing lung metastasis formation. Immunization against xCT, moreover, increases the chemosensitivity of CSC, and when combined with chemotherapy demonstrated a decrease of metastatic disease compared to single agent therapies. Together, these results suggest xCT as a potential target for anti-tumor vaccination. Regarding new vaccination strategies, Paola Nisticò (Tumour Immunology and Immunotherapy Unit, Regina Elena National Cancer Institute, Rome, Italy) presented the results of two trials: a phase I/II trial conducted on 10 stage III/IV melanoma patients treated with peptide vaccination (Melan-A/gp100 + IFN-α) alone or in combination with Dacarbazine (DTIC), and a phase II study with 30 stage III/IV melanoma patients treated with vaccine (Melan-A/NY-ESO-1 + IFN-α) alone or in combination with DTIC. Compared to peptide vaccination alone, long-lasting persistence of antigen-specific CD8+ T-cell responses significantly increased after chemo-immunotherapy, and seemed to reduce tumor recurrence risk. Moreover, the long-surviving patients present a high frequency of preexisting melan-A-specific clonotypes that are diversified after chemo-immunotherapy. In patients treated with a combination of DTIC plus melan-A peptide vaccination, enhanced TCR diversity of late-differentiated (CD28−), high-avid, polyfunctional and tumor-reactive antigen-specific T cells was elicited. The anti-tumor activity of melan-A-specific T-cell clones does not appear to be compromised by high PD-1 expression. Chemo-immunotherapy-driven gp100-specific CD8+ T-cell clones displayed an oligoclonal TCR repertoire of late-differentiated (CD28−), high-avidity and tumor-reactive Ag-specific T cells with low expression of PD-1. In contrast, in patients treated with peptide vaccination alone, melan-A elicited a restricted TCR repertoire of late-differentiated (CD28−), low-avidity, poorly polyfunctional and non-tumor-reactive PD-1-negative T cells. gp100 also elicited an oligoclonal TCR repertoire, of early-differentiated (CD28+), low-avidity, poorly polyfunctional and non-tumor-reactive PD-1-positive T cells. The role of this co-inhibitory molecule was confirmed by

blocking PD-1, partially restoring the polyfunctionality of gp100-specific CD8+ T cell. Moreover, PD-1 inhibitory function is significantly correlated with co-stimulatory CD28 expression in all clones evaluated, regardless of the treatment.

A new approach for cancer vaccination, that introduces the concept of harnessing individual patient-specific antigens, was described by Matthias Miller (BioNTech AG, Mainz, Germany). The team developed the IVAC® MUTANOME vaccine, a fully individualized polytopic RNA vaccine, manufactured with personalized mutated antigens, so-called neoantigens, which is suitable for potentially all tumor indications. A multi-centric, international phase I-study (EudraCT 2013-001645-13) was conducted to evaluate the safety, tolerability and immunogenicity of intra-nodal administration of IVAC® MUTANOME vaccine with or without initial treatment with vaccines targeting NY-ESO-1 and tyrosinase in 13 patients with advanced melanoma. Results demonstrated a high activity of IVAC® MUTANOME with 60% of the selected neoantigens eliciting an immune response in post-vaccination samples. Notably, the majority of patients showed prolonged progression-free survival relative to historical controls. Another therapeutic approach was presented by Pier Francesco Ferrucci (Oncology of Melanoma Unit, European Institute of Oncology, Division of Medical Oncology of Melanoma and Sarcoma, European Institute of Oncology, Milan, Italy) who showed clinical results obtained with Talimogene Laherparepvec (T-VEC), a herpes simplex virus type-1-derived oncolytic immunotherapy designed to selectively replicate in tumors. T-VEC produces GM-CSF and enhances local and systemic antitumor immune responses. In the Phase III Study OPTiM, 436 unresectable stage IIIB–IV melanoma patients were treated with intralesional T-VEC or with subcutaneous GM-CSF. Results showed an improved Disease Response Rate and ORR of T-VEC compared to GM-CSF (16.3% vs 2.1% and 26.4% vs 5.7%, respectively). Responses were seen in both injected and un-injected lesions, including visceral lesions (underlining systemic effect) showing a tolerable safety profile. Results from a randomized (1:1), open-label phase II study of T-VEC and Ipi vs Ipi alone in 200 unresected stage IIIB–IV melanoma patients demonstrated a higher ORR of T-VEC and Ipi (39%) compared to Ipi alone (18%). Last, the phase Ib trial of T-VEC in combination with Pembrolizumab in unresectable Stage IIIB–IVM1c Melanoma (NCT02263508) demonstrated a confirmed response rate of 57.1% and a disease control rate of 71.4%, supporting the idea that T-VEC combined with an ICI may have important clinical activity in advanced melanoma. Another topic discussed during the session was CAR-T cells bearing receptors composed of an antigen-targeting moiety and a TCR-derived signaling domain. As discussed by Attilio Bondanza (Innovative Immunotherapies Unit, Division of Immunology, San Raffaele Hospital Scientific Institute, Milan, Italy), these receptors are HLA independent, show high affinity and are highly modular that can fit additional signaling domains. Regarding antitumor responses, in B-cell tumors CD19 CAR-T cells lead to a highly heterogeneous response (from 30 to 40% in chronic lymphocytic leukemia to 80–90% in acute lymphocytic leukemia). In the field of CAR-T cells, the major unmet needs are the validation of new targets, the increase of in vivo persistence, the mitigation of toxicity and an easier manufacturing process; possible strategies to overcome these limitations were presented during this session. As TNBC patients showed a quicker relapse and metastatic progression compared to other subtypes of breast cancer, the group of Elena Quaglino (Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy) hypothesized that CSC could play a central role in TBNC. So, TBNC CSC-associated molecules could be used as target for anti-cancer vaccination. RNA-Seq was used to identify differences in gene expression between tumor-spheres and their monolayer counterparts from 4T1 and HCC1806 mammary cancer cell lines. Results demonstrated overexpression of TENM4 mRNA in TNBC tumor-sphere-derived cells that correlated with a shorter relapse-free survival of patients. TENM4 silencing through RNAi significantly impaired the tumor-sphere-forming potential of 4T1 cells.

Session 7: prospective NIBIT and NIBIT Foundation collaborations

The last session of the meeting focused on possible collaborations between different research centers and NIBIT. Daniela Fenoglio (Centre of Excellence for Biomedical Research, University of Genoa, Genoa, Italy) presented the Italian Society of Cytometry (GIC), founded in 1982, that operates in the fields of biology and medicine for the development and innovation of analytical cytology methods applied to research and clinical practice. GIC collaborates with other national and international scientific societies. It promotes the training and scientific information of Flow Cytometry and Fluorescence Microscopy and on all related topics organizing conferences and scientific training events. Thereafter, Ramy Ibrahim (Parker Institute for Cancer Immunotherapy, San Francisco, CA, USA) presented the Parker Institute for Cancer Immunotherapy that involves immunologists from more than 300 countries in academic cancer centers and more than forty companies in the immunotherapy field. Finally, Aiman Shalabi (Cancer Research Institute, New York, USA) presented the Cancer Research Institute, founded in 1953. It is a no-profit organization focused exclusively on cancer immunotherapy. The

institute strongly supports critical research, mainly in the early development and new treatment strategies.

Conclusions

The NIBIT Meeting, providing a highly interactive forum in which leading experts in the field of clinical and translational cancer immunotherapy provided in-depth reviews of emerging topics in cancer bio-immunotherapy, allowed the chance to strengthen national and international scientific collaborations among pre-clinical and clinical researchers, companies, and cancer societies.

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Compliance with ethical standards

Conflict of interest Michele Maio is a consultant/advisory board member for Bristol-Meyers Squibb, Incyte, Merck Sharp & Dohme Corp. Oncology, Roche, Astex pharmaceuticals, Amgen, AstraZeneca and Merck Serono. No potential conflicts of interest were disclosed by the other authors.