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MEETING REPORT

4th Symposium on Advances in Cancer Immunology and Immunotherapy, November 29–December 1, 2018, Athens, Greece

Sotirios P. Fortis[1] · Athanasios Kotsakis[2] · Christophe Le Tourneau[3] · Ourania E. Tsitsilonis[4] · Vassilis Georgoulias[5] · Constantin N. Baxevanis[1]

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Abbreviations

ALK	Anaplastic lymphoma kinase
ATF	Activating transcription factor
BCSC	Breast cancer stem cells
CHOP	CCAAT-enhancer-binding protein homologous protein
CPI	Checkpoint inhibitor
EMA	European medicines agency
EML4	Microtubule-associated protein-like 4
EV	Extracellular vesicles
FLIP	FLICE-inhibitory protein
GO	Gemtuzumab ozogamicin
GPCRs	Protein-coupled receptors
HNSCC	Head and neck squamous cell carcinoma
ICIs	Immune checkpoint inhibitors
ICIR	Immune checkpoint inhibitory receptor
irAEs	Immune-related adverse events
iTreg	Induced regulatory T cell
miTRAP	miRNA trapping by RNA in vitro affinity purification
MM	Multiple myeloma
NSCLC	Non-small cell lung cancer
NTRK	Neurotrophic tropomyosin receptor kinase
PARP	Poly (ADP-ribose)
pTreg	Peripheral Treg
TEX	Tumor-derived exosomes
TIGIT	T-cell immunoglobulin and ITIM domain
TIM-3	T-cell immunoglobulin, mucin domain-3
TLS	Tertiary lympoid structure
TME	Tumor microenvironment
TP53	Tumor protein 53
Treg	T regulatory cells
UPR	Unfolded protein response
VLP	Virus-like particles

✉ Sotirios P. Fortis
fortis@ciic.gr

[1] Cancer Immunology and Immunotherapy Center, Saint Savas Cancer Hospital, 171 Alexandras avenue, 11522 Athens, Greece

[2] Department of Medical Oncology, University Hospital of Larissa, Larissa, Thessaly, Greece

[3] INSERM U900 Research Unit, Department of Drug Development and Innovation (D3i), Institut Curie, Paris-Saclay University, Paris, France

[4] Department of Biology, National and Kapodistrian University of Athens, Athens, Greece

[5] 1st Department of Medical Oncology, Metropolitan General, Athens, Greece

Introduction

The 4th symposium on advances in cancer immunology and immunotherapy was held from November 29 to December 1, 2018, in Athens, Greece. The 4th symposium aimed to bring together many of the leading researchers and clinicians from Europe and the US to report on most recent advances in cancer immunology and immunotherapy and to promote interactions between speakers and participants, thus encouraging stimulating discussions and providing network opportunities and new collaborations. The main objectives were to gather, share and exchange current knowledge from the field of tumor biology and cancer immunology and immunotherapy, and to translate and extend this knowledge to the clinic. The main topics included: (1) immune suppression; (2) innate immunity in cancer; (3) monitoring of immunomodulatory mechanisms; (4) combinational treatment; (5) biomarkers for immunotherapy; and (6) clinical trials.

Overcoming immune suppression

The tumor microenvironment (TME) contains a suppressor network which promotes tumor growth, invasion and

metastasis. Detailed investigation of the immunosuppressor circuits within the TME will improve our understanding of tumor biology and evolution and at the same time will enable the design of novel therapeutic modalities against cancer. In this session, **Vincenzo Bronte** (School of Medicine and Surgery, University of Verona, Verona, Italy) provided an overview of the role of myeloid-derived suppressor cells (MDSC) in tumor immune evasion. Low doses of different chemotherapeutic agents selectively eliminated the monocytic-MDSC (M-MDSC) and enhanced the therapeutic efficacy of adoptively transferred, tumor-specific CD8+ T cells. His team found that chemotherapeutics affecting M-MDSC all shared the ability to modulate the anti-apoptotic protein FLICE-inhibitory protein (FLIP) both in vitro and in vivo, and that enforced FLIP expression which partially protected the cells from chemotherapy-induced death. Unexpectedly, FLIP induction in monocytes regulated a complex transcriptional program, which included several MDSC-related genes such as CD274, CD273, IL-10 and IDO1, promoting their transition towards fully immune suppressive monocytic-MDSC (M-MDSC). To prove the immunoregulatory power of this molecular axis, they showed that injection of c-FLIP-expressing monocytes was able to control GvHD progression in a setting of xenogeneic transplantation. Patients with pancreatic ductal adenocarcinoma presented an increased frequency of cellular FLICE (FADD-like IL-1β-converting enzyme) inhibitory protein (c-FLIP) + PD-L1 + CD14 + cells in the blood and the higher circulating levels of these cell subsets together with increased concentrations of plasma IL-6 correlated with shorter overall survival. Collectively, these data suggest a critical role of c-FLIP in cancer promotion by regulating the immunosuppressive properties of mature myeloid cells. **Viktor Umansky** (German Cancer Research Center (DKFZ), Heidelberg, Germany) reported on the immunosuppressive network in the melanoma microenvironment, where MDSC induced by chronic inflammation play a major role. The accumulation and activation of MDSC could be mediated not only by soluble inflammatory factors but also by tumor-derived extracellular vesicles (EV). Recent studies from his laboratory showed that EV isolated from Ret mouse melanoma cells induced an upregulation of PD-L1 on immature myeloid cells, leading them to acquire the ability to suppress T-cell activation. PD-L1 expression and the immunosuppressive ability of EV-generated MDSC were dependent on the interaction between TLR4 on myeloid cells and HSP86 on EV followed by NF-κB activation. EV from human melanoma cells or plasma of melanoma patients induced PD-L1 upregulation on normal monocytes leading to their conversion into immunosuppressive cells. Thus, these findings highlight novel mechanisms of MDSC generation from normal myeloid cells by melanoma EV, suggesting the importance of EV targeting for tumor therapy. A different mechanism of immunosuppression was described by **Theresa L. Whiteside** (University of Pittsburgh Cancer Institute, Pittsburgh, USA) on the functional role of regulatory T cells (Treg) in cancer. Although Treg were discovered more than 20 years ago and have been a subject of intense interest and investigation ever since, many questions about their origin, development, functions and clinical significance in cancer remain elusive. No definitive marker for human Treg has been identified; the most frequently used markers HELIOS, neuropilin-1 (NRP-1) and CD15s are not specific for all Treg. Sakaguchi's classification of Treg based on expression of CD45RA and FOXP3 identifies fraction II Treg (FOXP3 + CD45RA^{low}) as the true suppressor cells that accumulate in tumors. Strikingly, there exists a lack of clarity in interpretation of Treg responses to checkpoint blockade. Treg that accumulate at tumor sites and in the circulation of patients with cancer are referred to as induced Treg (iTreg). They differ phenotypically and functionally from peripheral Treg (pTreg) and mediate vigorous suppression of immune cells utilizing a variety of mechanisms. They may or may not be FOXP3+ but co-express numerous immune checkpoint inhibitory receptors (ICIRs), e.g., cytotoxic CTLA-4, PD-1, T-cell Ig and mucin domain-containing protein-3 (TIM3), lymphocyte activation gene 3 (LAG3) and T-cell Ig and ITIM domains (TIGIT). It was, therefore, expected that the suppressor activity of Treg expressing ICIRs would be silenced following delivery of checkpoint inhibitory Abs that block negative signaling. Unexpectedly, responses of Treg to immune checkpoint blockade differed from those of effector T cells: while the blockade released all other immune cell types from suppression and rejuvenated their anti-tumor activities, Treg-mediated suppression, phenotype, frequency and stability were not reduced. It appears that Treg are resistant to ICIR blockade with Abs, and it has been suggested that this resistance may underlie unresponsiveness of cancer patients to immune checkpoint inhibitors (ICIs). A number of questions and controversies about Treg remain, including their "transcriptional signature," the nature of the primary target of Treg, the role of APC in Treg functions, the redundancy of Treg suppressive mechanisms and the requirement for overexpression of multiple ICIRs on Treg. Recent data suggest that Treg are functionally highly diverse regulatory cells that are involved in a broad spectrum of immune and non-immune activities in various tissues and organs. In the tumor microenvironment, Treg are subverted by the tumor, and their immunosuppressive activities represent a major barrier to successful cancer immune therapies.

The role of innate immunity in cancer

Chemokines and their specific receptors constitute a complex network within the TME which depending on the context may promote or suppress tumor growth.

Toll-like receptors (TLR) and their agonists play a significant role in inducing and/or optimizing anti-tumor immune pathways. Nathan Karin (Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel) summarized the role of regulatory chemokines in cancer progression. Chemokines are small (~ 8–14 kDa) secreted proteins, structurally similar to cytokines. They regulate cell trafficking through interactions with a subset of seven-transmembrane G protein-coupled receptors. In oncogenesis, chemokines and their receptors are involved in supporting tumor development and metastatic spread by four major complementary mechanisms: (1) by attracting cancer cells to sites of metastatic spread; (2) by supporting tumor growth through an autocrine loop; (3) through mobilization of bone marrow-derived leukocytes from the bone marrow to the blood, followed by supporting their colonization at the tumor site and shaping their biological function there; and (4) through chemokines and chemokine receptors as immune checkpoints in the regulation of anticancer immunity. Focusing on the last two features. Karin's group, in collaboration with Viktor Umansky from the DKFZ, have recently shown that the chemokine receptor CCR5 is essential for supporting the mobilization of polymorphonuclear MDSC (PMN-MDSC) from the bone marrow to the blood and later their accumulation at the tumor site to support tumor development and suppress anti-tumor immunity. They also identified a chemokine that enhances anti-tumor immunity, CXCL10, and developed a stabilized form of this chemokine (CXCL10-Ig) for cancer therapy, alone, or in combination with ICIs, and CCR5 blockers. **Tina Bagratuni** (School of Medicine, National and Kapodistrian University of Athens, Athens, Greece) provided a general overview on TLR. The systematic study of TLRs role and function in tumor cells can contribute substantially to the development of new anti-tumor pharmaceuticals with TLR-dependent mechanisms of action. To this end, Dr. Bagratuni focused on a recent study from her group investigating the role of TLR4 in multiple myeloma (MM) cell biology. Their data support the importance of TLR4-mediated effects in MM and suggest that TLR4 activation may protect MM cells from apoptosis by suppressing the CCAAT-enhancer-binding protein homologous protein (CHOP)-activating transcription factor (ATF) unfolded protein response (UPR) (CHOP-ATF4 UPR) branch. The data from this study deepen our understanding of the function and diversity of TLR4 in MM and the association between innate immune signaling and MM cell proliferation. It also offers new insights into potential new strategies for MM therapy, as it can be assumed that TLR4 targeting could be used as a potential therapeutic strategy that could reduce tumor burden and provide an adjuvant therapy to standard treatments.

Monitoring of immunomodulatory mechanisms

Immunomodulatory mechanisms may act in various ways to establish conditions relevant for the clinical benefit of immunotherapies but also other types of cancer therapies. These may include the generation of (1) an immune stimulatory TME, (2) robust tumor infiltration with lymphocytes, (3) immunogenic phenotype of cancer cells and (4) functionally active anti-tumor effector cells. **Theodora Katsila** (Department of Pharmacy, University of Patras, Patras, Greece) introduced proteomics and proteogenomics as a toolbox toward monitoring immunomodulatory mechanisms. Emphasis was put on exosomes (and their cargos) as means to interpret cell-to-cell communication, also in the context of non-classical secretion. The secretome and membrane proteome may add an extra information layer to shed light on molecular mechanisms and drug resistance. Coupling proteomics or proteogenomics to other omics approaches empower data reliability and map inter-individual variability, attempting to define the so-called 'actionable genome'. Colorectal cancer and glioblastoma multiforme served as paradigms. **Gosse J. Adema** (Department of Radiation Oncology, RIMLS, Radboud University Medical Center, Nijmegen, The Netherlands) considered "in situ cancer vaccines" following tumor ablation. Tumors can serve as their own antigenic vaccine "in situ" provided that appropriate tumor ablation approaches are combined with immune activating compounds such as adjuvants or checkpoint mAbs. Dr. Adema presented data to support the role of saponins as highly effective adjuvants able to synergize with tumor ablation. Subsequent studies revealed that these adjuvants induce an unprecedented level of DC cross-presentation in vitro and in vivo. The presence of saponin adjuvants increased cytosolic translocation of antigen, resulting in proteasome-dependent cross-presentation in monocytic $CD11b^+$ DC. Strikingly, specifically in this monocytic $CD11b^+$ DC subset, saponins enhanced DC cross-presentation by lipid body induction. Both pharmaceutical and genetic interference with lipid body formation inhibited saponin-based adjuvants (SBA)-induced cross-presentation in these DCs in vitro and in vivo. How lipid bodies affect DC cross-presentation, type I IFN production and other immune processes are just beginning to be studied in detail. **Panayiotis Vergis** (Biomedical Research Foundation of the Academy of Athens, Athens, Greece) presented data on the role of Treg in immune-related adverse events (irAEs) during immunotherapy. The advent of ICIs has revolutionized cancer immunotherapy. However, despite the enormous success, a significant proportion of patients do not respond, while responses are frequently accompanied by life-threatening (auto)irAEs.

Therefore, predictive biomarkers of clinical responses are urgently needed. Moreover, deciphering new therapeutic strategies to harness anti-tumor immunity while keeping autoimmunity in check remains a daunting task. Achieving these goals has been hampered by the immunosuppressive nature of the TME. Treg comprise an ideal candidate, since they are physiologically engaged in maintenance of self-tolerance, are the dominant suppressive population in TME, promote tumor growth, associate with poor prognosis and importantly represent a fundamental impediment of cancer immunotherapy success. Towards this, Dr. Verginis and his group have analyzed the transcriptomic signatures of CD4 + CD25hiCD127lo Treg from melanoma patients that developed or not irAEs after immunotherapy. The findings of this approach demonstrate significant alterations in the expression of inflammatory genes expressed by Treg in patients with irAEs. Decoding Treg signatures in cancer will empower the discovery of personalized predictive biomarkers and novel targeted therapy. **Dimitris Kletsas** (NCSR "Demokritos", Athens, Greece) introduced the concept of cellular senescence. He summarized the mechanisms leading to replicative or stress-induced premature senescence, focusing on the activation of the DNA damage response (i.e., the activation of the ATM-Chk2-p53-$p21^{WAF1}$-Rb axis), as a common motif in both types of senescence. At the functional level, he presented data indicating the putative anticancer role of senescence. The pro-inflammatory and catabolic nature of these cells and their role in tissue homeostasis and the development of age-related diseases leads to the suggestion that the short-term presence of these cells may be beneficial in some cases (e.g., in tissue repair), while their chronic presence may be detrimental. In particular, his group has shown that stromal fibroblasts can become senescent as a result of conventional anticancer treatments (e.g., exposure to ionizing radiation) both in vitro and in vivo. These cells can enhance the growth of cancer cells through increased secretion of soluble factors (e.g., matrix metalloproteases) or insoluble extracellular matrix components (e.g., the proteoglycan syndecan 1), which create a permissive environment for tumor growth. The mechanisms leading to these alterations and especially the participation of TGF-β were considered. Finally, he presented new means for controlling the presence and role of senescent cells in vivo by the use of a new class of compounds which can lead to preferential killing of these cells (senolytics) or to inhibition of their inflammatory phenotype (senomorphics), and discussed the limitations in their use. **Barbara Seliger** (Institute for Medical Immunology, Martin Luther-University Halle-Wittenberg, Halle/Saale, Germany) overviewed other tumor immune escape mechanisms. Immune escape strategies developed by the tumors are linked with the biology of the tumor itself or occur within the TME. With respect to the former, various types of tumors downregulate their antigen-presenting machinery (APM) components leading to loss or reduced expression of HLA class I molecules. As a result, tumor cells are not properly recognized by CTL either endogenously induced (e.g., via vaccination or immune checkpoint blockade) or exogenously transferred in the context of adoptive T-cell therapies. Another mechanism which leads to downregulation of HLA class I molecules is based on the activity of small leucine-rich proteoglycans (SLRP). When overexpressed, the SLRP biglycan (Bgn) leads to upregulation of MHC class I surface expression, while downregulation of Bgn causes reduced MHC class I expression. Interestingly, there is an inverse correlation between Bgn and TGF-β expression, since overexpression of Bgn leads to downregulation of TGF-β receptors as well as their ligands. Experimental in vivo data showed a significant delay in tumor growth by Bgn overexpression, which was apparently caused by immune infiltrates in those tumors. The cancer genome atlas (TCGA) data analyses also confirmed the role of Bgn in the prognosis of various types of cancer. Dr. Seliger also addressed the important role of microRNAs (miRNAs) as modulators of the expression of MHC class I APM components as well as of HLA-G, which is known to suppress T-cell and NK cell responses and to be overexpressed in various types of tumors. The miRNAs identified by combining miTRAP (miRNA trapping by RNA in vitro affinity purification) with RNA sequencing were either downregulating or enhancing HLA class I antigens or HLA-G and thus represent possible therapeutic targets. SLRPs and miRNAs emerge as new tools, which can be used by tumor cells to evade immune surveillance, but can be also therapeutically targeted.

Combinational treatments

The hostile tumor microenvironment downregulating antitumor immunity and thereby minimizing the clinical efficacy of immunotherapies can now be reversed via application of different immunomodulatory approaches. **Ed Lavelle** (Adjuvant Research, School of Biochemistry and Immunology Trinity College, Dublin, Ireland) gave a talk on targeting strategies to enhance the effectiveness of immunotherapies. He emphasized that while there is great interest in the development of cancer vaccines, this is impeded by the lack of safe and effective adjuvants that strongly promote cellular immunity. To this end, he addressed a number of promising strategies including polymeric nanoparticles. Although the role of particle size in adjuvanticity is contested, there is now ample information to show that particle size was critically important in the ability of particulate adjuvants to promote antigen-specific CD8+ T cells and Th1 responses. In contrast, a broad range of particle sizes could promote antigen-specific antibody responses. These results indicate than nanoparticles may have significant advantages as adjuvants

for cancer vaccines. He then described a second adjuvant system based on chitin-derived polymers with highly deacetylated polymers being most effective by promoting acyclic GMP-AMP synthase stimulator of interferon genes (cGAS-STING) and nucleotide-binding domain leucine-rich containing (NLR) pyrin domain-containing 3 (NLRP3) inflammasome-dependent Th1 response. The activation of this DNA sensing pathway was proposed to be mediated through induction of mitochondrial reactive oxygen species and release of mitochondrial DNA, which is sensed by cGAS. These adjuvants have unique cellular immunity promoting properties that are not shared with alum, the most widely used adjuvant, which supports their further investigation as candidates for inclusion in cancer vaccines. **Michael I. Koukourakis** (Radiobiology and Radiopathology Unit, Democritus University of Thrace, Alexandroupolis, Greece) presented data on the interplay of radiation and immunotherapy. The role of host immunity in the efficacy of radiotherapy against experimental tumors has been recognized for many decades. Heavily irradiated autologous cancer cells have been applied as vaccines for the treatment of cancer since 1920. Indeed, irradiated cancer cells function as a vaccine (1) by switching on the IFN type I pathway; (2) by inducing secretion of cytokines and chemokines, or even (3) by restoring HLA class I molecule expression by cancer cells. DCs are activated and, in turn, activate cytotoxic T cells in lymph nodes. T cells have a direct cytolytic effect on the irradiated local tumor and, also, against metastatic 'out-of-portal' deposits (abscopal effects). Handling immune checkpoint pathways with novel immunotherapies, in combination with radiotherapy-induced tumor vaccination, is expected to become a potent therapeutic tool against locally advanced and metastatic cancer. **Christoph Schultes** (Global Program Lead Oncology, Biopharma | Global R&D, Merck, Frankfurt, Germany) gave a general overview on combinations and bifunctional molecules as the next generation of immuno-oncology therapies. He pointed to the fact that in attempting to address the broad range of possible immuno-oncology combinations and ascertaining which are likely to benefit patients, the industry has adopted a number of approaches to ensure that the most promising molecules are combined in a manner maximizing the benefit/risk ratio for cancer patients. On one hand, this comprises scientific and clinical alliances and partnerships, in which assets from different pipelines can be combined and trials run collaboratively. The selection criteria for molecules in such collaborative ventures may vary significantly, though there are general trends becoming established in the field. Fundamentally, these have to do (1) with the scientific rationale of specific combinations (2) whether a synergy with anti-PD-1/PD-L1 is expected, and (3) with safety aspects of the molecules to be combined. Given recent clinical trial setbacks, the importance of defining surrogates for clinical monotherapy efficacy also needs to be taken into consideration. This can be demonstrated using examples of how combinations of anti-PD-1/PD-L1 therapies with certain classes of agents (chemotherapy, VEGFR-targeted molecules) have led to practice-changing progress in specific indications. Another approach will be to examine targeting two promising biological targets with a single, bifunctional (bispecific) antibody. Two clinical-stage examples (anti-PD-L1/TGF-β TRAP, anti-PD-L1/LAG-3) demonstrate how such bifunctional antibodies are anticipated to provide improved clinical results with less toxicity compared to combinations of individual molecules. Enhanced benefit in preclinical models has been shown with respect to specificity and the physical proximity of the target ligands, providing novel insights on how synergies may be achieved compared to combinations of separate agents. **Rhoda Molife** (European Clinical Development, Merck Sharp and Dohme (MSD), London, UK) gave a talk on the novel ICIs and their combinations in genitourinary malignancies with a focus on urothelial carcinoma (bladder cancer). Bladder cancer is the ninth most common cancer in the world with nearly half a million new cases recorded per year. Clinically, there are three main disease states: non-muscle invasive disease (70%), muscle invasive disease (20%) and metastatic disease (10%; de novo presentation). In 2017, the European Medicines Agency (EMA) approved three checkpoint inhibitors (CPI) as monotherapy for both untreated and pretreated advanced/metastatic disease, thus providing alternate and effective treatments for a significant proportion of patients that are not suitable for first-line platinum-based chemotherapy, and a more effective therapy for relapsed patients. As such, these agents are suitable partners for combination approaches that may provide even more effective treatment options for these patients in the future. Several of these approaches are being tested in all disease states (as well as in earlier disease) in phase I to III trials, some of these combine CPI with: (1) chemotherapy; (2) other CPI; (3) anti-angiogenic agents; (4) poly (ADP-ribose) polymerase (PARP) inhibitors and (5) BCG. The results of some of these trials should be reported soon and are eagerly anticipated. **Manuel Fernández Bruno** (Institute of Oncology Rosell (IOR), University Hospital Sagrat Cor, Quiron Salud Group, Barcelona, Spain) addressed the important topic of combinatorial treatments consisting of ICI and targeted therapies. The identification of tumor oncogenic drivers and the subsequent development of targeted therapy represent a recent milestone in the treatment of lung cancer. However, ICI that are widely used for the treatment of non-small cell lung cancer (NSCLC) have yielded disappointing results for patients with oncogenic drivers like EGFR mutations. Despite the induction of PD-L1 expression due to constitutive oncogenic signaling that has been reported in NSCLC models harboring microtubule-associated protein-like 4 (EML4), anaplastic lymphoma kinase

(ALK) rearrangements and EGFR mutations, the combination of targeted therapies and ICI does not seem to improve outcomes, but still have detrimental side effects. Different combinations of tumor protein 53 (TP53), EGFR, and serin/threonine kinase 11 mutations, together with PD-L1 expression by tumor cells, may represent robust parameters to identify best responders to PD-1 blockade. Elucidating the factors responsible for the lack of sensitivity to CPI is a crucial issue, and novel combination approaches are urgently needed in this patient population. Other approaches include adoptive cell therapy in solid tumors, as summarized by **Troels Holz Borch** (Center for Cancer Immune Therapy-Herlev Hospital, Herlev, Denmark). He emphasized that across multiple phase II trials, adoptive cell therapy using TIL has consistently shown response rates of about 40–50% with durable complete responses in about 15% of treated metastatic melanoma patients. It has been suggested that tumors with a pre-existing immune cell infiltrate respond well to immunotherapy with ICI, whereas those without do not respond. This would implicate that the tumors progressing on CPI should be immunologically ignorant. However, in a recently reported clinical trial, it was possible to grow tumor-reactive TIL from 83% of CPI-resistant patients, which is comparable with previously published findings. Nevertheless, of 11 evaluable patients, only 2 achieved partial responses (18%) of which only 1 was durable. More data are needed to elucidate whether the poor clinical outcome of this study was due to the low number of patients recruited or due to shared resistance mechanisms with CPI treatment. For other cancer types, the clinical results of TIL therapy has only been reported in small cohorts or case reports. However, it has been shown that growing TIL to a clinically relevant number is possible from a wide range of tumors and many trials testing their clinical efficacy are ongoing. **Christophe Le Tourneau** (Department of Drug Development and Innovation (D3i), Institut Curie, Paris-Saclay University, INSERM U900 Research Unit, Paris, France) talked about precision medicine in cancer, based on recent large clinical studies. Precision medicine has been a reality for a long time in oncology. Now, the term "precision medicine" has effectively emerged with this advent of high-throughput technologies, especially sequencing. The use of geneexpression signatures has been shown to be able to help prognosis, especially in early breast cancer. Beyond refining prognosis, sequencing might have a predictive value and therefore help guiding molecularly targeted therapy and immunotherapy. Several types of studies have tried to bring the proof of principle that sequencing cancer patients might improve their outcome, including retrospective studies and prospective clinical trials. While results from retrospective and non-randomized clinical trials were encouraging, no robust conclusions could be drawn because of methodological issues. SHIVA01 has been the only randomized trial to date and it failed to demonstrate a survival benefit. Now, for the first time in the history of oncology, drugs were approved by the FDA in 2017 in a histology agnostic way, including larotrectinib in neurotrophic tropomyosin receptor kinase (NTRK)-translocated cancer patients and pembrolizumab in microsatellite instability-high cancer patients. They encourage the use of sequencing in patients with recurrent cancer independent of tumor type, provided they would have access to these latter drugs or to clinical trials with drugs matching molecular alterations that might be identified.

Biomarkers for immunotherapy

Cancer alters various features over the course of its development, generating heterogeneity which contributes to the development of resistance to therapies. Therefore, it is of great interest to discover biomarkers to appropriately and accurately appreciate the risk of relapse at the time of diagnosis but also to select for patients most likely to respond to therapies. **Federica Cavallo** (Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Turin, Italy) presented data on the specific targeting of cancer stem cells in breast cancer as a novel therapeutic modality. Breast cancer is still the leading cause of cancer death in women, with about 30% of the affected patients dying because of metastasis and disease recurrence. As the normal mammary gland epithelium, breast cancer is hierarchically organized, with a sub-population of cancer cells with stem cell properties (breast cancer stem cells, BCSC) at the top of the hierarchy. BCSC are the reservoir for the relapse, metastatic evolution and progression of the disease. Moreover, the transient decrease in the extent of cancer heterogeneity induced by conventional anti-tumor treatments results in the enrichment of BCSC. Therefore, targeting BCSC is necessary to obtain tumor eradication. Her team has recently shown that BCSC overexpress xCT, a transmembrane protein that imports extracellular cystine in exchange for intracellular glutamate, affording protection against harmful reactive oxygen species generated by altered metabolic states and by chemotherapeutic drugs. Accordingly, the immune targeting of xCT with DNA-, virus-like particles (VLP)-, and bovine herpex virus (BoHV) 4-based vaccines resulted in reduced tumor growth and metastasis formation in mice and sensitization to chemotherapy. Therefore, xCT represents a relevant target for breast cancer treatments. In her second presentation, **Theresa L. Whiteside** (University of Pittsburgh Cancer Institute, Pittsburgh, USA) reviewed the role of exosomes as biomarkers of tumor progression and response to therapy. EV are produced by all cells and are present in all body fluids. They represent a heterogeneous mix of membrane-bound nanovesicles of various sizes and different cellular origins. Among them, EV produced by tumor cells, referred

to as tumor-derived exosomes (TEX) have recently emerged as potential noninvasive biomarkers of cancer. TEX are of special interest as liquid biopsies, because their molecular and genetic profiles resemble those of parent tumor cells and TEX can be viewed as clinically relevant surrogates of cancer cells. In the tumor microenvironment, TEX carry and deliver unique messages from the tumor to normal cells. This TEX-mediated information transfer results in reprogramming of recipient normal cells to tumor-promoting cells which produce their own exosomes and play an active role in tumor progression. In body fluids of cancer patients, TEX represent a fraction of total exosomes, and the ratio of TEX/non-TEX varies among patients. To study the molecular profiles of TEX, it is necessary to isolate them from body fluids. Isolation and subsetting of TEX are accomplished by immunoaffinity capture with antibodies specific for antigens carried by TEX. Immunocapture offers an opportunity for identification of proteins, lipids, glycans, nucleic acids and other molecular components that TEX carry and use to promote tumor progression. At the same time, non-TEX can also be captured, so that the same liquid biopsy can serve as a source of TEX as well as, e.g., a source of CD3+ exosomes produced by T cells. Once isolated by immunoaffinity capture, molecular profiles of TEX and non-TEX can be characterized by Western blot or by on-beads flow cytometry. The same liquid biopsy can inform about a state of the tumor and simultaneously evaluate the competency of immune cells for mediating anti-tumor activities. Preliminary data correlating protein or miRNA profiles of TEX and CD3+ exosomes from cancer patients' plasma with disease activity and responses to immune therapies suggest that these exosome subsets serve as sensitive surrogates of clinical endpoints. **Anastasios Boutis** (Theagenio Cancer Hospital, Thessaloniki, Greece) reviewed recent advances in biomarkers for immunotherapy beyond simple PD-L1 expression. He started his talk with the comment on the landscape of NSCLC management which has evolved rapidly during the last 3 years with the incorporation of ICIs in everyday clinical practice. However, currently, PD-L1 is the only predictive marker in use for immunotherapy, and its use is limited due to intrinsic biological complexity as well as technical and logistical problems. He also mentioned that tumor mutational burden is the most promising evolving biomarker for immunotherapy having been tested in several clinical trials and consistently shown to correlate with clinical outcome. However, it still lacks validation and until this has been done it cannot be routinely implemented in clinical practice. **Cécile Gouttefangeas** (Department of Immunology, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany) presented a new method for monitoring antigen-specific T cells which is based on the specific detection of activated integrins using fluorescent multimers of ICAM1 molecules. During T-cell stimulation, inside-out signaling leads to rapid integrin activation through affinity increase and clustering of integrins on the cell membrane. The assay described by Dr. Gouttefangeas is simple (no sophisticated reagents are needed), is combinable in multiparametric flow cytometry, and preserves cell viability. She presented data to show that the assay is applicable for assessing a broad range of virus-, tumor- and vaccine-specific CD8+ T cells. Staining of activated β2-integrins presents the unique advantage of requiring activation times of only several minutes, and could be rapidly implemented in the clinical setting, including for monitoring T-cell-based immunotherapy. **Aristides Eliopoulos** (School of Medicine, National and Kapodistrian University of Athens, Athens, Greece) presented an overview on tumor-associated and nontumor-associated microbiota. The microbial cosmos that colonizes mammals has major physiological roles in the development and function of the immune system, in host defense against pathogens and in a plethora of metabolic processes. Imbalances in microbiota, termed dysbiosis, have been linked to various inflammatory pathologies such as *Clostridium difficile*-associated diarrhea, Crohn's disease and ulcerative colitis, where transplantation therapies with healthy fecal microbiota have demonstrated significant efficacy. Dysbiosis is also linked to various types of cancer and experimental evidence supports a role for certain microbial species or general in tumor growth and metastasis. A fascinating aspect of host–microbial interactions is the influence of distinct microbiota clusters on the efficacy of immunotherapy. Specific clusters of 'tolerogenic' bacteria correlate with poor response to both anti-CTLA-4 and anti-PD1/PD-L1 immunotherapy in melanoma patients, whereas 'immunogenic' bacteria appear to act in concert with ICIs to boost T-cell responses and therapeutic efficacy. Modulation of intestinal microbial composition through specific types of diet (including the Mediterranean diet), fecal microbiota transplantation or administration of synthetic stools may serve as adjuvant to immunotherapy. Future progress in synthetic biology and application of multi-omic diagnostic approaches is anticipated to assist in the identification and correction of microbiota defects that compromise therapeutic efficacy. **Wolf H. Fridman** (Laboratory Cancer, Immune Control and Escape, Cordeliers Research Centre, Paris, France- University Paris Descartes, Paris, France) presented a series of previous and most recent data from his laboratory on the immune contexture as a prognostic biomarker. In addition to the well-known association between high tumoral infiltration of CD8+ T cells and favorable prognosis, he also highlighted the impact of tertiary lymphoid structures (TLS) as a prognosticator. TLS are ectopic lymphoid organs found in tumors and their microenvironment. They are composed of a T-cell zone containing mature dendritic cells which surrounds a B-cell-rich germinal center intricated with follicular dendritic cells. T follicular helper cells are found at the interface of the T-cell

zone and the germinal center. High endothelial venules allow the entry of T and B lymphocytes into TLS. High density of TLS in the tumor core or at its close vicinity was reported to be associated with longer patient survival in many cancers, as illustrated in NSCLC, hepatocellular carcinoma and soft-tissue sarcoma. In these cases, the presence of TLS is associated with a strong infiltration of memory Th1, CD8+ T cells and B cells likely with anti-tumor activity. Their clinical impact is more significant than that of CD8+ T cells. In contrast, high density of TLS in inflammatory regions, at distance of the tumoral nodules bears no positive clinical efficacy, such as in hepatocellular carcinoma. These observations strengthen the fact that not only T cells, but also B lymphocytes, mature DCs and the overall organization of the tumor microenvironment are major parameters in the control of tumor growth and spreading, and have to be considered in designing immunotherapeutic approaches. **Rienk Offringa** (University Hospital Heidelberg and German Cancer Research Center, Heidelberg, Germany) emphasized the importance of intratumoral heterogeneity detected via next-generationsequencing of TCRs. In spite of the fact that pancreatic cancer has long been portrayed as a 'cold' tumor that cannot be targeted by means of immunotherapy, they found that most primary tumors contain significant numbers of infiltrating T cells and that their TCR repertoire is highly enriched for certain TCR-Vα/β pairs. Moreover, ex vivo expanded T cells isolated from these tumors display HLA-restricted activity against autologous tumor cells. They are currently investigating the antigen specificity of tumor-dominant TCRs with a focus on neo-epitopes encoded by the tumor mutanome. While the outcome of these experiments is still awaited, they have demonstrated in an orthotopic pancreatic cancer mouse model that the dominant TCRs target neo-epitopes. In parallel, they are isolating TCRs from human TCR-locus-transgenic HLA-transgenic mice immunized with candidate neo-epitopes, some of which were shown to selectively react against the mutated neo-epitope and not against its wild-type counterpart. Taken together, they aim at the development of adjuvant immunotherapeutic strategies for countering the devastating recurrence rate in patients with primary resectable pancreatic cancer.

Immunotherapy and clinical trials

A deeper understanding of the complex interactions between tumor cells and elements of the immune system has paved the way for novel immunotherapy modalities. The field has been revolutionized by therapies based on anti-PD1, anti-PD-L1 and/or anti-CTLA-4 antibodies for several types of cancer, with impressive clinical outcomes in melanoma and NSCLC. **Dominique Heymann** (University of Nantes, Nantes, France and the University of Sheffield, Sheffield, UK) presented an overview on the therapeutic advances of immunotherapy in the field of mesenchymal tumors. Mesenchymal tumors comprise numerous tumor entities (> 50 histological subtypes) including benign and malignant subtypes. Malignant mesenchymal tumors include soft-tissue and bone sarcomas, they represent 1% of adult and 20% of pediatric cancers and no substantial improvement in overall survival has been achieved in the last 4 decades. Immune infiltrates consisting of lymphocytes and macrophages are detectable in all of these tumors and the first immunotherapy in sarcoma was assessed by William Coley in 1891 by injection of microorganisms into the tumor mass. Soft-tissue and bone sarcomas are considered as an immune desert compared to a large number of immune privileged cancers and this immune desert is mainly explained by a poorly characterized tumor microenvironment. Among the promising therapies, targeting immune checkpoints using specific antibodies would be an alternate, however, the data available on the expression of such negative regulators are contradictory in sarcomas. Cytokine-induced killer cells, oncolytic viruses and autologous T cells expressing cancer testis antigens are potential new therapeutic options as revealed by recent results of clinical trials. Even if sarcomas remain an immune desert, some oasis can be now identified. **Nikolaos Pistamaltzian** (Oncology Department "MITERA" Hospital in Athens, Athens, Greece) talked about immunotherapy in bladder (urothelial) cancer. Urothelial cancer is considered among the most immunogenic cancers, carrying a high mutational load and having a prevalence of PD-L1 positivity between 35 and 65% in tumor samples. Apart from intravesical administration of BCG, no other form of immunotherapy had made any clinically meaningful impact until now. During the last 2 years, emerging data from large phase II and randomized phase III trials suggest that immunotherapy with PD-1 inhibitors like nivolumab and pembrolizumab, and PD-L1 inhibitors like atezolizumab, have superseded chemotherapy in terms of response rates and survival. This benefit has been observed in both patient groups: those ineligible for cisplatin administration and those who have been exposed to cisplatin, and has been associated with durable remissions. All immunotherapy drugs have also exhibited a more favorable safety profile compared to chemotherapy. Selecting patients based on PD-L1 expression has produced conflicting results and there is an effort to develop more biomarkers based on immune gene signatures, tumor mutation burden and tumor infiltrating lymphocytes that will eventually be used complimentary to each other. There are also many ongoing trials studying the combination of immunotherapy with chemotherapy in the front-line setting, and second-line trials studying anti PD1/PD-L1 agents in combination with targeted therapies and other immunotherapies. **Nikolaos Giannakoulas** (Internal Medicine and Hematology Dept, Faculty of Medicine, University of Thessaly, Larissa, Greece) presented current data on the immunotherapy

in blood cancers. Hematological malignancies are a heterogeneous group of disorders mostly of immunological origin. A significant proportion of patients with hematological malignancies remain with limited treatment options after failure of chemotherapy. Allogeneic hematopoietic stem cell transplantation and monoclonal antibodies like rituximab have been an essential part of the treatment of hematological malignancies for decades. Newer anti-CD20 antibodies (ofatumumab and obinutuzumab) for B-cell malignancies, anti-CD38 (daratumumab) and anti-SLAM (elotuzumab) for MM, antibody–drug conjugates like gemtuzumab ozogamicin (GO) for acute myelogenous leukemia, brentuximab vedotin for Hodgkin's lymphoma and CD30+ anaplastic T-cell lymphomas, bispecific antibodies like blinatumomab for B-cell acute lymphoblastic leukemia have been incorporated in the treatment of some hematological malignancies. Cellular adoptive immunotherapies utilizing T cells genetically engineered to express chimeric antigen receptors or T-cell receptors for tumor-associated antigens or neoantigens have yielded impressive clinical results mostly in hematological cancers and in certain solid tumors. Inhibiting immune checkpoints such as programmed death-1 (PD-1) and its ligand (PD-L1) have yielded remarkable results in some types of solid tumors and recently in some highly refractory lymphoma subtypes such as relapsed classical Hodgkin's lymphoma. Immunotherapy is a pillar of management for hematological malignancies and is likely to cure a subset of patients previously considered incurable. Obtaining maximal benefit with minimal toxicity from all these new immunotherapies remains a challenge. **Athanassios Argiris** (Department of Medical Oncology, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, USA) demonstrated the impact of immunotherapy on head and neck cancer. A major breakthrough in the management of head and neck squamous cell carcinoma (HNSCC) has been the introduction of ICIs. Currently, pembrolizumab and nivolumab, two agents that target PD-1, are approved in both the US and Europe for the treatment of patients with recurrent or metastatic HNSCC with disease progression on or after platinum-containing therapy (i.e., in second-line therapy and beyond). Pembrolizumab and nivolumab have been shown to confer survival advantage over standard therapies (i.e., docetaxel, methotrexate, cetuximab) in two phase III clinical trials in platinum refractory HNSCC, KEYNOTE-040 and CheckMate 141, respectively. PD-L1 inhibitors, such as durvalumab and avelumab, are being tested in clinical trials in HNSCC as well. A further step in development was the investigation of ICIs either as monotherapy or in combination regimens in the first-line treatment of recurrent or metastatic HNSCC (i.e., chemo-naive or platinum-sensitive setting). In the three-arm phase III KEYNOTE-048 trial, patients were randomized to receive either (a) the EXTREME regimen which consists of platinum (cisplatin or carboplatin), 5-FU and cetuximab, (b) platinum, 5-FU and pembrolizumab, or (c) pembrolizumab alone. KEYNOTE-048 showed survival benefit with pembrolizumab monotherapy over the EXTREME regimen in PD-L1 positive tumors. Moreover, in all patients, regardless of PD-L1 expression, pembrolizumab added to platinum and 5-FU resulted in longer OS versus the EXTREME regimen. This important study that will be changing standard treatment paradigms awaits final analysis and publication. Nivolumab has been under further investigation in combination with the CTLA-4 inhibitor ipilimumab in the first-line therapy setting. CheckMate 651 is a phase III trial in the first-line treatment of recurrent or metastatic HNSCC in which patients are randomized to receive either the standard of care EXTREME regimen or the combination of nivolumab and ipilimumab. This study recently completed accrual and results are pending. Finally, accumulating evidence suggests that the ICIs can work synergistically with radiation. Several phase III clinical trials are investigating ICIs combined with radiation therapy for the treatment of locally advanced HNSCC. Combination approaches and the study of predictive biomarkers are of major interest in future development of immunotherapy strategies in HNSCC. **Monica Neagu** ("Victor Babes" National Institute of Pathology, Bucharest, Romania) presented current data on immunotherapy for melanoma. With the advent of the new era of immune therapy, new rapidly emerging aspects are highlighted from both fundamental research and clinical applications. Actual trends in melanoma therapy were presented, highlighting the most recent ones. Therefore, one of the most important points in melanoma immunotherapy is that, due to the approval of several immune-related therapies, clinical experience is gained in terms of adverse effects types, their management, acquired resistance, modalities to overcome resistance, adjuvant therapies and, prognosis/efficacy biomarkers. Another recent trend in this domain is combining newly approved immune-related therapies, looking for the best time frame, drug(s) concentration and stratifying patients for the combination. The limitation of the currently approved drugs has triggered the design of new ones like the T-VEC oncolytic therapy that was presented. Newly discovered biomarkers or combination of biomarkers that can better predict immune-related therapy efficacy were also presented. **Anna Koumarianou** (Fourth Department of Internal Medicine, Attikon University Hospital, Haidari, Athens, Greece) gave a general overview on immunotherapy and the management of toxicities. Dr. Koumarianou underlined that immunotherapy has recently been added in the therapeutic armamentarium of a large number of cancer clinical trials. Among the tested immunotherapeutic approaches, immune checkpoint blockade has shown remarkable benefit. However, by increasing the activity of immune cells, immune checkpoint blockade has been associated with inflammatory side effects,

termed irAEs. Novel advances in the technology of monoclonal antibodies targeting immune checkpoints are expected to associate with fewer side effects. Although any organ system can beafflicted, most common irAEs involve the musculoskeletal, gastrointestinal and endocrine systems, the skin, and the liver. Other systems less often involved are the cardiovascular, pulmonary, hematologic and the central nervous system. Most of the adverse events are reversible and responsive to corticosteroid treatment, except from those affecting the endocrine system that are permanent and require lifelong hormone replacement therapy. Albeit deaths due to immune-related side effects resulting in severe myocarditis, pneumonitis, colitis are exceptionally rare, physicians have to pay particular attention and provide the supportive coverage including expert multidisciplinary team when is required.

Conclusions and perspectives

The speakers in this conference addressed many different aspects of immune regulatory pathways in anti-tumor immunity including the contribution of oncogenes, tumor-suppressor genes and immune genes. The understanding of such pathways which may lead either to tumor destruction or tumor escape from immune surveillance clearly provide novel information for the design of improved immunotherapeutic modalities. From the presentations and the discussions which followed the presentations, it also became clear that although immunotherapy has attracted interest as an effective cancer treatment, response rates are still low due to immune resistance developed by the tumor cells. Therefore, to overcome resistance and in this way achieve long-lasting clinical responses, it will be of paramount importance to better understand (1) the dynamic interactions of immune infiltrates within the tumor microenvironment; (2) the role of tumor stroma in regulating intratumoral anti-tumor reactivity; and (3) alterations in tumor phenotype and tumor biology which may generate tumor heterogeneity, and tumor-induced suppression. In-depth knowledge of these aspects will lead to the discovery of biomarkers to predict clinical outcomes which will also be valuable for immunomonitoring and for the selection of patients most likely to respond to immune- and targeted therapies, thus opening the way for the application of precision medicine in cancer immunotherapy. Furthermore, the role of exosomes, TLS, microRNAs and extracellular matrix molecules as biomarkers and/or novel targets for immunotherapeutic approaches were strongly emphasized in this conference.

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Compliance with ethical standards

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