

Conference Report

4th Summer School in Immuno-Oncology, July 1st–3rd, 2021, Athens, Greece

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1. Abstract

The 4th Summer School in Immuno-Oncology was held from July 1st–July 3rd as a web meeting. Many eminent researchers and leading oncologists from Europe and the USA working on basic, translational and clinical cancer research participated, presented, and discussed the most recent advances in cancer immunology and immunotherapy. Besides sharing the newest information in the field of cancer immunology and immunotherapy, the meeting also focused on the actual translation of new knowledge acquired in the lab to the clinical setting; particular emphasis was given to the mode of action of novel therapeutic modalities and to biomarkers helpful for treatment decision-making, as well as to means that may improve

cancer immunotherapeutic protocols used for the treatment of a variety of malignancies. The main topics presented by the speakers included: (1) mechanisms of tumor immune evasion and resistance; (2) host-tumor interactions and means to regulate antitumor immunity; (3) exploitation of new biomarkers and tumor or immune signatures able to potentially guide therapeutic interventions; (4) emerging therapeutic modalities for cancer treatment and specific immunotherapeutics for thoracic, genito-urinary, gastrointestinal, skin and breast cancers; and (5) innovative treatment options and alternatives to minimize the toxic adverse events of cancer immunotherapy.

2. Tumor immune evasion

In his opening lecture, **Jörg Wischhusen (University of Würzburg School of Medicine, Department of Gynecology and Obstetrics, Germany)** provided an immunologist's view on tumor development and discussed the molecular basis of immunosurveillance, immunoediting and immune escape. By incorporating data on immunosubversive properties of pre-existing and induced tumor-initiating cells, and by referring to further physiological programs for immune tolerance, he expanded the established concept of tumor cell elimination, equilibrium and escape towards a more complete framework that helps to explain the potential and limitations of cancer immunotherapy. As there is no evolutionary benefit associated with tumor immune escape in the case of adult cancers, molecular mechanisms most likely developed to protect a semi-allogeneic fetus from the maternal immune system, to preserve tissue-resident stem cells from immune-mediated destruction, or to enable regeneration and wound healing. When these mechanisms are co-opted by cancer cells, onco-fetal proteins [like programmed death-ligand 1 (PD-L1), indoleamine 2, 3-dioxygenase 1 (IDO), human leukocyte antigen (HLA)-G or growth differentiation factor 15 (GDF-15)] emerge as most attractive targets for cancer immunotherapy.

Natalia Aptsiauri (Department of Biochemistry, Molecular Biology III and Immunology, University of Granada Medical School, Spain) presented the basic immune escape mechanisms used by cancer cells, with an emphasis mostly on alterations in HLA class I expression. Total or partial loss of HLA class I molecules on cancer cells is a frequent phenomenon, leading to reduced immunogenicity and consequently tumor immune escape. Nevertheless, in specific types of cancer (e.g., cervical, colorectal), HLA class I alterations can often be reversed by interferon (IFN)- γ treatment. Additional escape mechanisms include mutations in β 2-microglobulin, (over)expression of PD-L1, and mutations in various signaling pathways, which cannot be corrected by IFN treatment. She stressed that during tumor evolution, positive selection of HLA loss of heterozygosity and β 2-microglobulin variants may occur, as such cells avoid recognition and killing by tumor-reactive cytotoxic effectors. Finally, studying tumor-derived exosomes that bear the same alteration as the parental tumor represents a promising diagnostic tool for the future.

Panayotis Verginis (School of Medicine, University of Crete, Heraklion, Greece) discussed immune suppression in cancer emphasizing regulatory T cells (Tregs) contributing to immune escape. The mechanisms of Treg-mediated suppression, Treg stability and function in the tumor microenvironment were addressed, based on the role of the alarmin interleukin (IL)-33, which, when depleted in preclinical models, abrogates the suppressive function of Tregs, leading to reduced tumor growth and an

increase in immunotherapy efficacy. Moreover, by analyzing transcriptomic signatures in cancer patients who developed immune related adverse events, Dr. Verginis showed that Tregs in these patients had significantly upregulated inflammatory signatures, with some genes implicated also in autoimmunity, suggesting that excess manipulation of Treg tolerance in cancer patients is detrimental as it may lead to autoimmunity.

3. Tumor immune resistance

Efstratios Stratikos (Laboratory of Biochemistry, Department of Chemistry, National and Kapodistrian University of Athens, Greece) began this session by outlining intracellular tumor antigen processing and immune checkpoint blockade. Recent clinical successes of cancer immunotherapy using immune checkpoint inhibitors (ICIs) have changed the landscape of cancer treatment. Unfortunately, the therapeutic benefit of ICIs is often limited to a subset of patients and tumor types. Recent research has revealed that the potency of ICI therapies depends on the presentation of tumor-specific antigens by cancer cells and professional antigen presenting cells. Dr. Stratikos reviewed the biochemical pathways of antigen processing and presentation by major histocompatibility complex (MHC) class I molecules, HLA in humans, focusing on how this can affect cancer immune evasion, particularly in the context of ICI therapies. He also discussed ongoing efforts to leverage the pharmacological manipulation of intracellular antigen processing as a complementary approach to enhance tumor immunogenicity and the effectiveness of ICI immunotherapy.

Daniel E. Speiser (Department of Oncology, University of Lausanne, Switzerland) pointed out that immunotherapy outcome depends on cancer genes and tumor immunity genes. Conventional views are that the immune system consists essentially of immune cells and their soluble mediators, lymphoid organs, and the structures of the lympho-haematopoietic system. While research on these topics has led to tremendous insights, there is still only limited knowledge on immune mechanisms in non-immune cells, although they may also strongly regulate immune functions. Most cancers, such as all carcinomas and sarcomas, are derived from non-immune cells. Oncogenes and tumor suppressor genes have been studied extensively, leading to great mechanistic understanding of how cancer cells deregulate cell and tissue growth. In contrast, the reasons why cancer cells are also very powerful in deregulating immune responses are still poorly understood. Interestingly, a variety of immune alterations within cancer cells were found to be associated with immune failures, typically modifications in IFN response pathways, altering many cancer cell functions important not only for adaptive immunity (particularly antigen presentation) but also innate immune mechanisms. Thus, besides oncogenes and tu-

mor suppressor genes, attention should be given to what he called “tumor immunity genes”. Immune fitness against tumors may be improved if we understood and consequently targeted those mechanisms of cancer cells that misguide immune pathways.

In his lecture, **Ferdinandos Skoulidis (Department of Thoracic and Head and Neck Medical Oncology, The University of Texas, MD Anderson Cancer Center, USA)** focused on tumor intrinsic mechanisms of primary resistance, mechanisms of acquired resistance, and mainly on the genomic determinants of primary or *de novo* resistance to ICIs in non-small cell lung cancer (NSCLC). He showed that somatic mutations in the genes *STK11* and *KEAP1* mediate a “cold” tumor microenvironment (TME) with reduced T cell and increased suppressor cell infiltration, and that these genes are major drivers of primary resistance to ICI or chemo-immunotherapy in NSCLC. Moreover, *STK11* and *KEAP1* mutations correlate with a poor clinical outcome in NSCLC patients, being more pronounced in tumors with a high mutational burden (TMB) and *KRAS* mutations. Alterations in *STK11* and/or *KEAP1* are detected in 25% of NSCLC cases, defining a subgroup of patients that needs to be treated with alternative therapeutic strategies. The major mechanisms of acquired resistance to ICIs include defects in antigen processing machinery (APM) components, the IFN- γ pathway, neoantigen loss and several tumor cell intrinsic pathways that contribute to the generation of an immunosuppressive TME. Therefore, identifying more mechanisms that lead to tumor escape is crucial for developing tailored strategies to re-establish effective immunity to tumors.

4. Host-tumor interactions

Ioannis Pateras (Department of Histology and Embryology, Medical School, National and Kapodistrian University of Athens, Greece) analyzed the features of the TME in solid tumors, highlighting its complexity. He focused on the role of inflammatory cells, the crosstalk between M2-type macrophages and tumor-derived exosomes, the oncogenic function of mutant *p53* in reprogramming tumor-associated macrophages (TAMs) to a tumor supportive phenotype, the features of CD8+ T cells bearing an exhausted phenotype [e.g., expressing programmed death-1 (PD-1) and T cell immunoglobulin and mucin domain 3 (TIM-3)] and showed that understanding the TME will be a key issue for the effective treatment of cancer. Examples from pancreatic ductal adenocarcinoma, breast and NSCLC suggest that dysfunctional T cells are present in the tumor stroma and may be used for patient stratification for targeted therapies, as with ICIs.

Teresa Frisan (Department Molecular Biology and Umeå Centre for Microbial Research, Umeå University, Umeå, Sweden) addressed the topic of host-microbiome crosstalk and its impact on tumorigenesis.

Changes in the gut microbiota composition, known as dysbiosis, and asymptomatic infections/colonization with gastrointestinal bacterial pathogens are associated with an increased risk of cancer development. The gut represents a complex microenvironment densely populated with microbes that produce a huge variety of metabolites and interact with each other and with the host mucosa. The host interface needs to fine tune the immune response to maintain tissue homeostasis, while activating the appropriate defense against invading pathogens. In this delicate context, many microbial and host factors can contribute to the induction of the enabling characteristic of cancer (genomic instability and tumor-promoting inflammation) triggering other key hallmarks of cancer (such as cell proliferation, resistance to cell death, replicative immortality, invasion, and metastasis). This ranges from: (i) chronic activation of pathogen recognition receptors, leading to the long-term activation of the NF- κ B and IL-6-STAT3 axes; (ii) chronic production of inflammatory-associated DNA damaging agents (e.g., reactive oxygen species); (iii) activation of a non-sterilizing immunity that allows the establishment of a persistent infection; (iv) direct induction of genomic instability by microbial production of DNA damaging toxins; and (v) specific predisposing conditions of the host (e.g., genetic mutations or susceptibility to inflammatory disease). Thus, at the extreme end of this view, in the context of bacterial infections/colonization cancer is a form of collateral damage that occurs as a result of concurrent events. Understanding the chain of events leading to this unfavorable and unwanted outcome can lead to improved prevention and development of targeted therapeutic approaches.

5. Regulation of antitumor immunity

Barbara Seliger (Institute of Medical Immunology, Martin-Luther-University Halle-Wittenberg, Halle, Germany) talked about micro RNAs (miRNAs) and immune escape. Oncogenic and tumor suppressive miRNA overexpression inhibit HLA class I APM components [e.g., transporter associated with antigen processing-1 (TAP1), tapasin], whereas inhibition of other miRNAs enhances HLA-G and PD-L1 expression. miRNAs also play a critical role in tumor-TME interactions, some of which are associated with promotion of tumor growth. All these miRNAs, now termed immune modulatory miRNAs (im-miRNAs), can be used (including inflammatory miRNAs) as diagnostic and maybe prognostic tools [as shown in myelodysplastic syndromes and acute myeloid leukemia (AML)]. The goal is to develop approaches that can alter these miRNAs, like miRNAs mimics or anti-miRNAs, as therapeutic targets.

Gosse Adema (Radiotherapy and Onco-immunology laboratory, Department of Radiation Oncology Radboud UMC, Nijmegen, The Netherlands) presented means of immune modulation for

cancer treatment, focusing on sialic acids. Sialic acids are overexpressed on the tumor cell surface, rendering tumor cells resistant to apoptosis and therapy, facilitating their migration, metastasis, and immune evasion, and are associated with patients' poor prognosis. Developing sialic acid inhibitors results in tumor cell desialylation and this blockade was shown to suppress tumor growth and prevent metastasis formation in the therapeutic setting in preclinical models. Moreover, sialic acid blockade shapes the TME, making cold tumors hot, and in combination with toll-like receptor (TLR) ligands, it induces dendritic cell (DC) activation and enhances T cell responses *in vivo*. Thus, sialic acid blockade via glycomimetics seems to affect multiple pathways, targeting tumor cells, activating immune cells (like DCs) and inhibiting immune suppression [like Tregs and myeloid-derived suppressor cells (MDSCs)].

Graham Pawelec (Department of Immunology, University of Tübingen, Germany; Health Sciences North Research Institute, Sudbury, ON, Canada) discussed anti-cancer immunity and ageing. Age is a major risk factor for solid cancers. This is often viewed in the context of the cell biology and accumulations of driver mutations, but cancers are immunogenic, and the immune system can protect against tumorigenesis ("immunosurveillance"). Vaccination or other immunomodulation is therapeutically beneficial. However, if immunity decreases with age ("immunosenescence"), this may contribute to increased disease in the elderly. It was pointed out that it is commonly stated in the literature that immunosenescence does in fact lead to more cancers in the elderly, but one needs to question the actual evidence for this. A critical evaluation of the available data on vaccination against infectious disease reveals that the responses of older adults to neoantigens can be excellent, provided that the vaccine is appropriately formulated, as dramatically illustrated by protection against COVID-19 by vaccines against SARS-CoV-2 spike antigens. One of the most successful SARS-CoV-2 mRNA vaccines, so successful at protecting against COVID-19 was in fact pioneered by BioNtech in Mainz, Germany, for use in melanoma vaccines several years earlier. Similarly, memory responses against Varicella zoster virus (VZV) can be re-activated by herpes zoster subunit vaccines to protect older adults against shingles. Thus, both naïve and memory immune responses can be essentially intact in older adults. Although there are as yet no data on mRNA vaccine efficacy in elderly cancer patients, experience with immune checkpoint blockade "*in situ* vaccination" suggests that not only do older people do as well as younger patients on anti-PD-1 therapy, they may actually do *better*. Thus, we should not assume that "immunosenescence" really does contribute to increased solid cancer in older adults.

6. Therapeutic modalities for cancer

George Vassilopoulos (Hematology Clinic of the University Hospital of Larisa, University of Thessaly; Genetics Laboratory & Gene Therapy, BRFAA, Athens, Greece) presented an overview of the promising approach exploiting chimeric antigen receptor (CAR)-T cells, although the high efficacy of CAR-T cell treatment in hematological malignancies is still limited by its high cost. He extensively discussed the means used to construct universal CAR-T cells, to minimize dose and accordingly the severe adverse events post-CAR-T cell infusion, to optimize lymphodepletion prior to infusion and how to handle chronic B cell depletion in the clinic, and the marginal need to incorporate ON/OFF switches to silence CAR-T cells. His suggestion for increasing the efficacy of CAR-T cells included the use of this type of therapy at earlier stages of the disease when tumor burden is still low. Finally, he provided evidence that CAR-T cells are clinically far more effective in comparison to the bi-specific T cell engager (BiTE) technology.

Alejandro Madrigal (University College London Cancer Institute, Royal Free NHS Trust, UK) talked about natural killer (NK) cells for cancer immunotherapy and specifically a novel technology for developing universal cord blood induced pluripotent stem cells (iPSC)-derived CAR-NK cells as an "off-the-shelf" immunotherapy for cancer. In his laboratory, they have designed and constructed a multi-loading docking platform for mammalian cells and tested it by loading iPSCs with genes for CAR19, IL-15, inhibitory or activating killer receptors, and suicide genes. The advantages of this technology are its versatile genetic modification, high capacity, and high potential for commercial use. Moreover, it allows for a universal type of therapy with readily available cell products at a lower cost and, most importantly, reduced toxicity compared to CAR-T cell therapies.

Konstantin Kosmatopoulos (Vaxon Biotech, Paris, France) explained the advantages of vaccinating cancer patients with cryptic tumor peptides. Cryptic self-peptides that have lower affinity for HLA molecules and are expressed at lower levels, escape immune self-tolerance, can be amplified *in vivo*, and therefore when administered as vaccines, can induce a strong anti-tumor immune response. Results from preclinical models showed that optimized peptides (of increased immunogenicity via optimizing their HLA-affinity through anchor amino-acid substitutions) lead to notable tumor regression. The designed Vx-001 vaccine [composed of a cryptic telomerase (TERT) nonapeptide and its optimized variant] induced a strong TERT-specific immune response in the majority of vaccinated NSCLC patients, which was also long lasting (>1 year). Most importantly, it was efficient in patients with non-immunogenic (cold) tumors refractory to ICIs. Phase 3 trials will be needed to confirm the clinical efficacy of optimized cryptic peptide-based anticancer vaccines.

Dimitris Mastellos (Molecular Immunology Group, Division of Biodiagnostic Sciences and Technologies, I/NRASTES, National Center for Scientific Research Demokritos, Athens, Greece) presented recent data on the therapeutic targeting of the complement system in cancer. This system is a vital component of innate immunity comprising a network of soluble and membrane-bound proteins and pattern recognition receptors that mediate tissue homeostasis and pathogen immunosurveillance. For many years, complement activation was considered a bystander mechanism that augments the cytolytic activity of tumor-targeting antibodies. However, a growing list of discoveries in syngeneic mouse models of cancer have reshaped our understanding of its role in tumor-associated inflammation and cancer progression. These studies have also provided proof-of-concept for the therapeutic targeting of complement in the context of cancer immunotherapy. Recently, a monoclonal antibody targeting the proinflammatory C5a-C5aR1 axis has entered clinical development in cancer patients, in combination with PD-L1 blockade. Complement activation, however, is not uniformly associated with cancer progression and its role in the TME appears to be context-dependent, modulating tumor inflammation, antigen presentation and cytotoxic T cell responses associated with radio- or chemotherapy. Several complement biomarkers are being validated as prognostic markers in human cancers, facilitating the development of tailored therapeutic interventions. More than 20 complement-targeting candidate drugs are currently in clinical development for various indications. Combinatorial immunotherapy employing anti-complement agents alongside PD-1 or cytotoxic T lymphocyte-associated protein 4 (CTLA-4) blockade, holds promise as a new therapeutic modality for cancer patients refractory to standard treatment. Future studies will have to discern the precise spatiotemporal regulation of complement activation in the TME and validate more complement biomarkers for disease staging and prognosis. Cancer patient stratification based on a prognostic score of complement gene expression/protein activation could ultimately guide tailored complement intervention in cancer.

7. Biomarkers and mutational landscape within the tumor

Alexandra Giatromanolaki (Department of Pathology, Medical School, Democritus University of Thrace, Greece) presented a series of tumor biomarkers used in routine practice. The discovery of biomarkers that could guide immunotherapy is imperative. An important group of biomarkers that can be used to characterize anti-tumor immunity refers to the quantification of the tumor infiltrating lymphocytes (TILs) and TAMs. These markers (e.g., CD4, CD8, FOXP3, CD68 and others) have an established role in defining the postoperative prognosis, but their role in guiding immunotherapy is still under evalua-

tion. Another group of biomarkers relates to the expression of immune checkpoint molecules (ICMs). PD-L1 expression by cancer cells, TILs and TAMs has an established role in predicting response to anti-PD-1/PD-L1 immunotherapy. Other molecules, like CD80/86, CTLA-4/CD28, are under evaluation. Microsatellite instability (MSI), mismatch repair deficiency and the TMB have been also approved as biomarkers that guide the administration of ICIs. There are many other ICMs with an eventual biomarker value to be assessed in the future when relevant immunotherapies enter clinical practice.

Valsamo Anagnostou (Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, USA) discussed the genomic landscape of response and resistance to immune checkpoint blockade. In frame of revealing determinants of response to ICIs, TMB simulation analysis led to corrected TMB scores that more accurately predicted outcome. In NSCLC, genomic features associated with a molecular smoking signature and activating mutations in kinase genes were enriched in non-responders to immune checkpoint blockade. HLA class I and class II genetic diversity, intratumoral T cell density, IFN- γ -induced gene expression, T cell clonal dynamics, neoantigen depletion, differential B cell infiltration and immunoglobulin (Ig) rearrangements were also linked to therapeutic benefit, suggesting that integration of multi-omic data and meta-analyses may provide an optimized predictive model. Circulating tumor DNA (ctDNA) dynamics during ICI therapy are also of clinical importance and reduction of ctDNA has been associated with improved survival.

Michalis Liontos (Department of Clinical Therapeutics, Alexandra Hospital, Athens, Greece) reviewed how homologous recombination deficiency (HRD) impacts on anti-cancer immune response and how drug combinations affect the DNA damage response in the context of immunotherapy. He focused on BRCA1/2 mutations in various types of cancer, presenting results of preclinical studies and clinical trials, and concluded that gene mutations implicated in HRD do not seem to correlate with the presence of neoantigens or the overall immunogenicity of tumors; however, drugs used in HRD tumors affect immune responses via various pathways (overexpression of PD-L1, IFN- γ -mediated immune reactions), whereas poly (ADP-ribose) polymerase (PARP) inhibition and immunotherapy may provide a new combination treatment option for patients refractory to previous therapies.

8. Immunotherapy for different types of cancers

Dionysis Papadatos-Pastos (University College London Hospitals and The Princess Alexandra Hospital, London, UK) discussed the current status of immunotherapy in NSCLC. Almost 60% of NSCLC patients

present with metastatic disease and until 2013, their outcome was very poor, as the 5-year overall survival (OS) was less than 5% and median OS only 10 months. Since 2015, immunotherapy, particularly with nivolumab and pembrolizumab for stage IV patients and durvalumab for stage III non-operable NSCLC patients, greatly increased response rates and have become a standard-of-care treatment for NSCLC patients who do not progress on receiving concurrent chemotherapy and radiotherapy. Recent trial results also suggest that immunotherapy with atezolizumab in PD-L1-positive tumors is very effective in the adjuvant setting.

Konstantinos Leventakos (Chest oncology and clinical studies phase 1, Mayo Clinic, Rochester, USA) presented data on the clinical use of immunotherapy in small cell lung cancer (SCLC) and mesothelioma. The results of several clinical trials showed impressive progress and immunotherapy has been incorporated in the first-line setting in combination with chemotherapy for both diseases. The issues to be addressed refer to the question of who benefits from such treatments, how non-responders can respond to immunotherapy and how to predict and minimize serious side effects.

Filippos Koinis (Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece) discussed the immunotherapy landscape in **renal cell cancer (RCC)**. He presented clinical trials, showing that until recently there were no phase III data supporting the use of adjuvant systemic treatment in RCC, the major question being whether ICIs may play a role in the adjuvant setting. An important issue is RCC classification and molecular data have shown that RCC patients are better classified into subgroups depending on their tumors' signatures, i.e., angiogenic, immunogenic or proliferative. It is likely that patients with an immunogenic signature will benefit from immunotherapy. Indeed, although until 2006 anti-angiogenic factors were the only treatment option, anti-PD-1 therapy, tested since 2015, has shown promising results at 12-month follow up. Currently, a combination approach that incorporates immunotherapy early in treatment of metastatic RCC (first-line) is preferred, either with a tyrosine kinase inhibitor (TKI) (for favorable risk disease) or with a different immunotherapeutic or TKI (for intermediate/poor risk patients) depending on comorbidities. Second-line treatment options generally include TKIs, but results from ongoing trials will confirm the use of immunotherapy beyond progression.

Petros Grivas (Department of Medicine, Division of Medical Oncology, Genitourinary Cancers Program, University of Washington, USA) presented the state-of-the-art immunotherapy position in urothelial cancer. Traditionally, urothelial cancer was treated with immunotherapy comprising Bacillus Calmette-Guerin (BCG) intravesical installations, resulting in modulating the TME and generating an anti-cancer immune response. Follow-

ing a series of clinical trials, BCG-unresponsive cancer, besides radical cystectomy or intravesical chemotherapy, can be treated with anti-PD-1 or anti-PD-L1. Indeed, pembrolizumab has FDA approval for BCG-unresponsive high-risk carcinoma *in situ* for patients not subjected to radical cystectomy. In an ongoing trial, adjuvant nivolumab prolonged disease-free survival, whereas atezolizumab or pembrolizumab have been tested in a phase II trial in first-line cisplatin-ineligible patients; anti-CTLA-4 remains experimental; lastly, patients should be educated about toxicity, and biomarkers need to be validated for optimal selection of patients with urothelial malignancies.

George Papaxoinis (Second Department of Internal Medicine, Saint Savvas Anticancer Hospital, Athens, Greece) focused on the current status of immunotherapy in gastrointestinal (GI) tumors. Although ICIs have revolutionized the systemic treatment of many malignant tumors, GI cancers are generally resistant to ICIs. A possible explanation is that GI cancers usually are not characterized by high expression of PD-L1 and/or a high TMB. However, MSI and polymerase E (POLE) mutations are associated with high TMB, and tumor sensitivity to ICIs; notably, KEYNOTE-177 compared pembrolizumab to standard first-line chemotherapy in patients with metastatic colorectal cancer and MSI, and demonstrated significant improvement in all outcomes. However, these genetic aberrations are observed in only a minority of GI cancers. Recently, clinical trials testing ICIs in upper GI and liver cancers yielded encouraging results. KEYNOTE-590 and CheckMate-648 showed that pembrolizumab and nivolumab, respectively, added to first-line chemotherapy significantly prolong progression-free (PFS) and OS in patients with advanced esophageal cancer. Also, CheckMate-649 showed that nivolumab, when added to first-line chemotherapy, was associated with improved PFS and OS in patients with advanced gastric cancer. Finally, IMbrave-150 showed that the combination of atezolizumab with bevacizumab was superior to sorafenib as first-line treatment in patients with hepatocellular carcinoma.

Helen Gogas (1st Department of Medicine, Laiko Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece) presented the state-of-the-art of immunotherapy for malignant melanoma. Melanoma dominated the field in the development of immunotherapy, as there were no effective treatments available until 2011; during the last decade, the approval of new drugs, targeted therapy, immunotherapy, and combinations thereof, revolutionized the outcome of patients with metastatic disease. As of now, 3 combinations of BRAF and MEK inhibitors, anti-CTLA-4, anti-PD-1, and their combination, and most recently, a combination of targeted therapy and immunotherapy (anti-PD-L1, BRAF and MEK inhibitors) are approved for treating metastatic melanoma. The combination of a BRAF and a MEK inhibitor, and anti-PD-1 have been approved

in the adjuvant setting. The results from most recent and ongoing clinical trials reaffirmed the approved combinations for metastatic or unresectable disease and nivolumab as an adjuvant standard-of-care. New ICIs, e.g., the anti-lymphocyte activation gene 3 (LAG-3), and cellular therapies, e.g., with autologous tumor infiltrating lymphocytes and IL-2, are under investigation.

Dimitrios Tryfonopoulos (Saint Savvas Anti-cancer Hospital, Athens, Greece) reviewed advances in the immunotherapy of **breast cancer (BCa)**. Although traditionally BCa is not considered as an immunogenic cancer and is highly heterogeneous, advances in tumor biology and genetics have shown interactions between immune system components and disease progression. Triple-negative BCa (TNBC), HER-2+ tumors and luminal-B subtypes are immunogenic and aberrantly express tumor antigens, mainly products of mutated genes, supporting the therapeutic use of immunotherapy. The subclassification of TNBC by molecular gene signatures that appear to be immunomodulatory, facilitates selection of TNBCs prone to ICIs. Several clinical trials, many of which are still ongoing, show that combination treatments, i.e., immunotherapy with chemo-, radio-, anti-angiogenic and targeted therapies may be beneficial, although better predictive biomarkers are needed to select BCa patients who will benefit from such treatments.

Ioannis Samaras (University Hospital of Larissa, Greece) talked on the role of cemiplimab in the management of advanced cutaneous squamous cell carcinoma (CSCC). Cemiplimab is a PD-1 inhibitor approved in 2018 as monotherapy for the treatment of adult patients with metastatic or locally advanced CSCC who are not candidates for curative surgery or curative radiation. A series of clinical trials was presented, concluding that cemiplimab is safe, well-tolerated and effective in advanced CSCC, with an overall response rate of 45.5%, whereas no treatment-related deaths were reported. Follow up data will be very useful in confirming the efficacy of cemiplimab.

9. New immunotherapy agents and toxicity

Roman Kischel (BiTE Technology, AMGEN Research (Munich) GmbH, Germany) presented means to target cancer with BiTE constructs, a technology recently moved to the clinical setting. The mode of action of BiTEs is in principle to redirect the cytotoxicity of T cells to tumor cells driving them to apoptosis. Because BiTEs ligate CD3, they require neither engagement of T cell clones with a specific T cell receptor, nor MHC class I and peptide antigens for recognition; thus, they can cross-link any T cell with a tumor-associated antigen present on the tumor surface. Moreover, BiTEs stimulate the proliferation of T cells in a strictly target-cell-dependent manner, they are highly potent in redirecting lysis of target cells, and were shown to selectively expand CD8+ effector memory T cells *in vivo*.

Importantly, no active internalization of target antigens or CD3 was noticed. New generation BiTEs bear an Fc-based moiety which extends their half-life from 2 to >100 h. In the first-in-human studies half-life extended (HLE) BiTEs are administered once weekly or every second/third week. BiTE constructs in clinical development are being tested for ALL (CD19-specific), AML (CD33- and FLT3-specific), multiple myeloma (BCMA-specific), prostate and NSCLC (PSMA-specific), SCLC (DLL3-specific), and GI cancer (MUC17- and CLDN18.2-specific).

Maria Karasarides (Early Assets and Biomarkers, Worldwide Medical Oncology at Bristol Myers Squibb) talked about emerging immunotherapy targets. At present, FDA-approved immunotherapies include seven ICIs (targeting CTLA-4, PD-1 and PD-L1) and four CAR-T cell therapies for hematological malignancies. Since 2014, the number of patients treated with ICIs has increased from 2500 to over 200,000 in 2018. Although 5-year OS rate estimates for nivolumab are ~35%, still a significant percentage of cancer patients does not respond. A lot of effort is now focused on immunotherapy combinations; in 2019 there were over 2200 active combination trials using 295 targets, exploiting 9 different mechanisms of action in late-stage and 62 in early-stage disease. We need to follow biology to restore anti-tumor immune responses, focusing on improving tumor cell recognition, preventing immunosuppression, and enhancing effector cell functions. Available datasets from patients treated with immunotherapy (genomics, exomes, transcriptomes) should be used to identify drug targets, understand the molecular profiles and resistance mechanisms of the disease, and identify patient subpopulations with different immune status that would benefit from different therapeutic regimens. To further increase responses to ICIs, we should explore the dynamics of early vs. metastatic disease (e.g., by tracking the evolution of immunity from the primary through to metastasis), define and look for more precise resistance patterns (e.g., evaluate host immune capacity) and develop next generation innovation (e.g., utilize synthetic biology to design antigen-targets for new agents and use systems biology tools to understand tumor heterogeneity).

Anna Koumarianou (4th Department of Internal Medicine, Attikon University Hospital, Athens, Greece) gave an overview of therapeutic approaches to deal with immune adverse events. She noted that immunotherapy has increased the survival of cancer patients and presented data of many clinical trials assessing the efficacy of immunotherapeutic approaches, mostly of ICIs, either as standalone or combination treatments. However, immune checkpoint blockade has been associated with inflammation-mediated (auto)immune-related adverse events, due to excess activation of immune system components. Most commonly, these affect the skin, the musculoskeletal, GI and endocrine systems, the liver, and to a lesser extent, the cardiovascular, pulmonary, hematologic

and central nervous systems. Most adverse events are reversible and responsive to corticosteroids, except for endocrine system failures that require lifelong hormone replacement therapy. At present, deaths due to immune-related adverse events (severe myocarditis, pneumonitis, colitis) are very rare; however, oncologists need to be aware and provide the necessary supportive coverage to cancer patients treated with immunotherapy. Moreover, pseudoprogression may occur, so careful imaging and detailed clinical assessment are required. Finally, TMB and PD-L1 scoring may be useful in predicting response to immunotherapy but are not directly associated with the rate of immune-related adverse events.

10. Conclusions

The presenters in the 4th Summer School in Immuno-Oncology addressed various aspects of antitumor immunity and resistance, analyzed immunoregulatory pathways and showed how genes (oncogenes, tumor-suppressor genes), immune components (Tregs, MDCs, HLA molecules, immune checkpoints, complement) and other molecules (miRNAs, sialic acids, ctDNA) shape the TME and host immunity. The design of improved immunotherapeutic modalities, likely combinatorial, that lead to higher PFS and OS rates, necessitates a deeper understanding of these pathways, to ultimately attain tumor cell elimination avoiding tumor resistance and escape. The ternary aims need to be concurrently met in improved immunotherapy protocols, namely, specific recognition of tumor cells, minimization of immunosuppression and maximization of tumor-reactive effector cell functions. Advances in cancer immunotherapy via clinical assessment have been remarkable and novel therapeutic options emerge constantly for patients with otherwise untreatable tumors. We are moving towards a new era of cancer immunotherapy, where advanced technologies and targeted therapies, along with prognostic and predictive biomarkers, will eventually bring more personalized therapeutic alternatives for patients with cancer.

11. Awards

The three Best Presentation Awards were given to: (1) **Niki Prekete** (Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London, EC1M6BQ, UK), (2) **Ioannis M. Koukourakis** (Department of Radiotherapy/Oncology, Medical School, Demokritos University of Thrace, Greece) and (3) equally to **Sotirios Fortis** (Cancer Immunology and Immunotherapy Center, Saint Savas Cancer Hospital, Athens, Greece) and **Pantelis Rousakis** (Department of Biology, School of Sciences, National and Kapodistrian University of Athens, Athens, Greece).

12. Author contributions

IVK, NOS, NA and OET wrote the manuscript. ISP, AK, VG, CNB and GP edited the manuscript.

13. Ethics approval and consent to participate

Not applicable.

14. Acknowledgment

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16. Conflict of interest

The authors declare no conflict of interest.

17. Appendix

1. Locally far-advanced Squamous Cell Carcinoma of the skin: curable disease with anti-PD-1 'cemiplimab' monotherapy?

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Introduction: Cancer cells have the ability to express inhibitory immune checkpoint molecules (ICMs), suppressing the immune system, allowing cancer cell evasion from immune surveillance. An important inhibitory ICM is the PD-L1 (programmed death ligand 1) that binds to the PD-1 receptor of cytotoxic T cells leading to their inactivation and blockage of their anti-tumor activity. Cemiplimab, a therapeutic moAb that blocks PD-1, has been approved for the treatment of SqCC of the skin.

Materials: We present two patients with locally far advanced inoperable SqCC, from an ongoing study trying to investigate the role of cemiplimab and the time-sequence of radiotherapy vs. immunotherapy in the treatment of this aggressive disease. The dose of cemiplimab was 350 mg, diluted in 250 mL N/S and given as an 1 h infusion every 3-weeks.

Results: *Case 1:* a male patient 88 years old with SqCC of the left cheek that recurred after local radical radiotherapy. The patient had been treated with 11 cycles of

chemo (5fu and cetixumab), with local relapse after a short period of remission of the disease. A large rapidly growing mass appeared, and we decided to proceed to immunotherapy with cemiplimab. Immediately after the first cycle we noticed a substantial improvement. The disease was gradually regressing and complete response was documented at the 10th cycle. *Case 2:* a male 82 years old with locally far advanced skin SqCC covering the right upper frontal thoracic wall/shoulder area. The patient was treated with cemiplimab immunotherapy. Complete response was achieved at the 5th cycle.

Conclusions: Locally far-advanced skin SqCC is an inoperable disease, difficult to treat even with Radiotherapy, due to the large field dimensions and the extensive ulcerations. Anti-PD-1 immunotherapy with cemiplimab proved highly effective. The position of Radiotherapy in cases with incomplete response or relapse after immunotherapy is under investigation.

2. SIRP α expression by tumor associated macrophages (TAMs) is associated with poor prognosis in non-small cell lung cancer

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Introduction: CD47/SIRP α is an important immune-checkpoint pathway. Normal cells escape the phagocytic activity of macrophages by expressing the CD47 (integrin associated protein, IAP) glycoprotein that binds to the SIRP α ligand (signal regulatory protein alpha) expressed by macrophages. Cancer cells can exploit the same pathway by overexpressing CD47 to mask their alien nature against macrophages. The clinicopathological and prognostic value of SIRP α expression by macrophages (CD68+ cells) is unknown.

Materials and methods: We studied ninety-eight (98) patients with operable non-small cell lung cancer (NSCLC). The stage of the disease ranged from stage I to III and all patients were treated with surgery alone. Formalin-fixed paraffin-embedded tissue sections were studied with immunohistochemistry for the detection of SIRP α and CD68 (pan-macrophage marker). The number of macrophages stained for SIRP α and CD68 per 400 \times optical field was recorded and the mean value was used to score each case (SIRP α -score and CD68-score). In addition, the SIRP α /CD68-ratio was calculated, which reflects the percentage of tumor-associated macrophages (TAMs) expressing SIRP α .

Results: The SIRP α /CD68-ratio ranged from 0 to 1, and this was not statistically associated with the SIRP α

score, histology type, stage of disease or MIB-1 proliferation index. Moreover, Kaplan-Meier analysis of disease-specific survival did not reveal any significant association with SIRP α -score ($p > 0.95$). A strong association of high CD68-score with better overall survival was noted ($p = 0.005$). Finally, a direct association of high SIRP α /CD68-ratio with poor prognosis was observed ($p = 0.02$). This shows that the quality of macrophages in the tumor stroma is what actually defines patients' prognosis.

Conclusions: The SIRP α /CD47 pathway emerges as a critical immune checkpoint target for the development of adjuvant immunotherapy policies for NSCLC, while prevalence of SIRP α expression among TAMs arises as a reliable marker of activation of this pathway. SIRP α /CD68-ratio may prove of importance as a biomarker to guide the administration of anti-SIRP α /CD47 immunotherapy.

3. HLA-class-I expression in breast cancer cell lines under simulated micro-environmental conditions

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Introduction: Cytotoxic T-cells recognize foreign-antigens expressed by cancer cells through MHC/HLA-class-I molecules. These present foreign peptides that are recognized by specific T-cell membrane receptors (TCRs). Cytotoxic T-cells bind to this antigen presenting complex and secrete perforin and granzymes that induce apoptosis of the target cell. Loss of HLA-class-I expression is an important mechanism used by cancer cells to evade immune surveillance.

Materials: The estrogen receptor (ER) dependent/HER2 negative (MCF7 and T47D), the HER2 positive (BT474 and SKBR3), and the triple negative (MDA-MB231 and HCC180) cell lines were treated as followed: (i) co-cultured with fibroblasts for 72 h, (ii) exposed to hypoxia (1% O₂) for 48 h, and (iii) exposed to acidosis (pH 6.2) for 24 h.

Results

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APM, antigen processing machinery; BCa, breast cancer; BCG, Bacillus Calmette-Guerin; BiTE, bi-specific T cell engager; CAR, chimeric antigen receptor; CSCC, cutaneous squamous cell carcinoma; ctDNA, circulating tumor DNA; CTLA-4, cytotoxic T lymphocyte associated protein 4; DC, dendritic cell; GDF-15, growth differentiation factor 15; GI, gastrointestinal; HLA, human leukocyte antigen; HLE BiTEs, half-life extended BiTEs; HRD, homologous recombination deficiency; ICI, immune checkpoint inhibitor; ICMs, immune

checkpoint molecules; IDO, indoleamine 2, 3-dioxygenase 1; IFN, interferon; Ig, immunoglobulin; IL, interleukin; iPSC, induced pluripotent stem cell; LAG-3, lymphocyte activation gene 3; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; miRNAs, micro RNAs; MSI, microsatellite instability; NK, natural killer; NSCLC, non-small cell lung cancer; OS, overall survival; PARP, poly(ADP-ribose) polymerase; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PFS, progression free survival; POLE, polymerase E; RCC, renal cell cancer; SCLC, small cell lung cancer; TAM, tumor-associated macrophage; TAP, transporter associated with antigen processing; TERT, telomerase reverse transcriptase; TILs, tumor infiltrating lymphocytes; TIM-3, T cell immunoglobulin and mucin domain 3; TKI, tyrosine kinase inhibitor; TLR, toll-like receptor; TMB, tumor mutational burden; TME, tumor microenvironment; TNBC, triple negative breast cancer; Tregs, regulatory T cells; VZV, varicella zoster virus.

Keywords: Cancer biomarkers; Cancer immunology; Cancer immunotherapy; Immune checkpoint inhibitors; Immune monitoring; Immunomodulatory mechanisms

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