Overview of advances in cancer immunotherapy

Cancers are tumors and tumors are by definition multicellular three-dimensional structures, measured at least at the millimeter level. A single cell with inappropriate features, even if it can be recognized under a microscope, will never be called a "tumor". Tumors are tissues and as such, they are composed out of different types of cells with altered properties in a permissive environment. Potentially malignant cells in terms of genetic alterations, as we currently conceive them, may be produced upon programmed cell divisions but these are either non-viable or they are timely recognized by our immune system as "alien" or "non-self", and destroyed. The fact that such alien cells eventually form measurable structures and spread locally and distantly has always a dual aspect: the so called "tumor" cells have acquired features that allow them to grow, and the host immune system has failed in stopping them from growing. The involvement of the "lymphoreticular system" in tumors was already described by Rudolf Virchow in his series on the cellular organization of tumors under the microscope, in the second half of the nineteenth century (1). In the late 1800s, reports from both sides of the Atlantic highlighted that hospitalized patients with certain cancer types who were accidentally infected with erysipelas actually improved from cancer, and Coley's toxins are considered as the first anti-cancer vaccine ever documented (2). The immediate connection between cancer and the immune system was apparent in these early days but progress in using this knowledge delayed for over a century due to lack of a scientific basis for understanding this connection. Pathologist reports on tumor immune cell characteristics could not be systematized in order to be used in the clinic and the sporadic success in treating certain tumor types with immunological interventions was counteracted by painful failures [reviewed in (3)]. Progress was regained in the 1980s–1990s, the mechanisms involved in preventing the immune system from recognizing and destroying the alien cancer cells progressively started to be elucidated, drugs to temper the immune system were developed and successfully applied in the first decade of the present century, the relationship between Oncology and Immunology was progressively established in the last decade and, as confirmed from all points of view, immuno-oncology is here to stay [reviewed in (4)]. Main hallmarks that have strengthened this relationship are: progress in elucidating the pathophysiology of cancer—host immune system interactions and the biology of cancer immuno-editing at the molecular level; affordable large-scale sequencing; development, validation and approval for clinical use of further classes of immune checkpoint inhibitors; understanding the need for modified consensus in the design of clinical trials and, correspondingly, in patient management. The comprehensive review of all above topics, critical discussion on their impact in current clinical practice and on the potential expansion of cancer immunotherapy implications constitute the canvas of the present issue.

The pathophysiology of cancer—host immune system interactions may well be described in the context of inflammatory response (1). The current knowledge on the multiple types and functional aspects of participating cells of the "self" in tumor inflammatory infiltrates, the pro- and anti-tumor immune system activation, and particularly the frequently overlooked dual role of the ubiquitously present complement in the complement-dependent cytotoxicity that may interfere with, and impede antibody-dependent cellular cytotoxicity (ADCC) are reviewed by Rafail and Kourtzelis (5). In the context of histopathology, Kourea and Kotoula (6) review the characterization of the various types of tumor immune cell infiltrates with immunohistochemistry (IHC) and discuss the potential prognostic utility of immune cell markers at the tissue level. Emphasis is given on the morphologically assessed stromal tumor infiltrating lymphocyte (TILs) density in tumor tissues. High TILs density has consistently been reported as a favorable prognostic marker in many tumor types, especially in breast carcinomas. Further, single IHC markers, e.g., CD8, may also be of prognostic value in ovarian cancer, while the ratio of CD3+:CD8+ positive cells, which has been coined as "immunoscore" is of prognostic value in colon carcinomas and is particularly discussed in this context by Nearchou and Pentheroudakis (7).

The "self" immune cells are attracted to the tumor area because they respond to messages by the local antigen presenting machinery on the existence of "non-self" cells. Cancerous cells are recognized as "non-self" because they produce neoantigens, i.e., short peptides containing mutated epitopes that are cleaved from mutated proteins and presented to helper and cytotoxic T cells through the Human Leukocyte Antigen (HLA) system on the cell surface so that immune response is generated. As outlined by Liontos *et al*. (8), DNA damage leads to the accumulation of mutations, specific types of damage to mutational signatures and these signatures are related to the production of neoantigens. Bobisse *et al*. (9) discuss biological aspects of mutational load and neoantigens and their implications in cancer immunotherapy. With increasing applications

of next-generation sequencing it has been possible to map and characterize the entire mutational spectrum of thousands of tumors, which is called the "mutagenome"; with bioinformatics and continuously developing computational tools it is possible to predict the antigenicity of the entire spectrum of neoantigens, which is collectively called the "immunopeptidome". The number of neoantigens and the mutational load of a tumor are strongly associated; both characteristics are increasingly considered in cancer immunotherapeutic strategies.

Similarly to the common practices in infectious diseases, the therapeutic strategies addressing the immune system in cancer make use of the passive and active immunity concepts. Papaioannou *et al*. (10) comprehensively review the mechanisms and general effectiveness of the different types of passive immunotherapy including ADCC that is triggered upon tumor specific monoclonal antibodies, cytokine administration, *ex vivo* expanded autologous cells and other variations of adoptive immunotherapy; and, of active immunotherapy, including the various types of immune checkpoint inhibitors and of anti-cancer vaccines. As with infectious diseases, mobilizing the intrinsic defense in the context of active immunotherapy would be expected to yield more prolonged responses and so it has been achieved.

Active anti-cancer immunotherapy is increasingly based on the use of neoantigens for vaccines and the use of immune checkpoint inhibitors. The concept of neoantigens for the generation of patient-specific vaccines is theoretically ideal; in practice however, a major burden would be the validation of the effectiveness of such vaccines, the number of which would approximately equal the number of patients. In addition, dominant immunogenic peptides are the ones related to self-tolerance. As explained by Menez-Jamet *et al*. (11) an alternative way to overcome this burden would be to use widely expressed non-immunogenic peptides, the so called "cryptic" peptides, render them immunogenic, and then vaccinate them for evoking immunogenic response in patients. Such vaccines are currently under trial but until they prove effective, and also until neoantigens are tamed for the production of personalized vaccines, immune chek-point inhibitors rule the field of active cancer immunotherapy (10).

These drugs are monoclonal antibodies targeting the inhibitory molecules of immune checkpoints, i.e., molecules that inhibit T cell activation. First generation checkpoint inhibitors that have been introduced in the clinic for specific types of cancer target two classes of T cell inhibitory molecules, cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) targeted by ipilimumab; programmed cell death protein-1 (PD1) targeted by nivolumab and pembrolizumab; and, PD1 ligand, PD-L1, targeted by atezolizumab. Diamantopoulos and Gogas (12) review ipilimumab and nivolumab that have become the new standard of care in advanced melanoma; Mountzios *et al*. (13) discuss the beneficiary effects of pembrolizumab that replaces chemotherapy in advanced pre-treated non-small cell lung cancer (NSCLC), and of nivolumab with the same indications and with particular efficacy in squamous lung cancer; Tsiatas and Grivas (14) report on atezolizumab that was recently FDA approved for bladder cancer, while promising results have been presented for renal cell cancer as well. In addition, pembrolizumab seems to be efficient in MMR-deficient hypermutated colorectal cancer (7), while nivolumab shows promise in platinum-failed squamous head and neck cancer (15).

A still unmet need in the clinical application of checkpoint inhibitors concerns predictive markers for these drugs despite that three companion diagnostic PD-L1 assays have recently been FDA approved for an equal number of PD1 or PD-L1 blocking drugs (6). The clinical value of these assays remains to be evaluated in practice but it is already known that patients are concordantly identified with all assays in cases with true over-expression of PD-L1 (>50% positive cancer cells); in the absence of underlying up-regulating genetic alterations, PD-L1 is transiently expressed, and staining of tumor tissues is heterogeneous. One of the effects of cancer neoantigens (and corresponding mutational load) seems to be that they up-regulate inhibitory checkpoints of cytotoxic T cell activation (9,10). Not surprisingly, checkpoint inhibitors have as yet proven efficient in tumors with expected high neoantigen/high mutational load which is established because of specific inherited or acquired DNA repair defects and environmental carcinogen contribution. Surrogate predictive markers of response to PD1/PD-L1 inhibitors may include simple IHC for mismatch repair (MMR) proteins in cases with suspected MMR deficiency or tumor mutational load. The latter may be evaluated in association with specific mutation characteristics (i.e., clonal mutations, specific gene mutations) or with stromal TILs density (6). The possibility to use such markers for predicting response to currently used immunotherapeutic drugs and/or to conventional chemotherapeutics is progressively investigated.

Plausibly, because checkpoint inhibitors interfere with immune system balance outside cancer in the patient, this type of immunotherapy generates dysimmune toxicities that are called immune-related adverse events (IRAEs) (16). These are more pronounced and frequent for CTLA-4 blockade, but less frequent and severe for blockers of the PD1/PD-L1 axis, especially

for the latter. Skin, gastrointestinal and hepatic toxicity, pneumonitis, and dysfunction of basically all systems may be noticed, including late onset endocrinopathies, prompting for alertness by patients and caregivers for the early recognition and reporting of such events, as suggested by Linardou and Gogas (17).

Except for checkpoint inhibitors, different immunotherapy modalities are in use in certain types of cancer, such as the sipuleucel-T vaccine (14); HPV16 E6 and E7 peptide vaccines are tested in HPV positive oropharyngeal cancer with many more options of immunotherapy drug combinations with each other or with standard treatments (15); multiple vaccines are tested for NSCLC (13); passive immunotherapy with tumor-specific monoclonal antibodies is established in colorectal cancer where also adoptive immunotherapy, active immunotherapy with vaccines and oncolytic viruses, and combinations thereof are already under trial or proposed for future testing (7).

At present, only a few active immunotherapeutic agents have been FDA and EMA approved for cancer as of mid 2016, meaning that hundreds of other new and promising cancer immunotherapy treatments are only available to patients in clinical trials. The design of such trials and the endpoints to evaluate response and patient benefit pose statistical challenges (4,18), including new consensus in the criteria for response evaluation, delayed treatment effect, and long survival. These challenges and also the need to motivate patients to enter such trials are discussed by Menis *et al*. (19). By participating in an immunotherapy clinical trial, patients have the opportunity not only to access a potentially lifesaving treatment, but also to help advance this new approach and bring immunotherapy to more patients in the future.

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