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Review Article

Challenges in Immunotherapies for Solid Tumors - Focus on Lung Cancer, A Review

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Abstract

This review explores the advances and potentialities of Immune Oncology in the fight against cancer and the challenges facing researchers to overcome evasive strategies developed by tumors, especially the solid ones, with a focus on lung cancer.

Keywords: *Immunoediting; TREGs; Immune Evasion; Epigenetic Silencing; CAR T Cells; Monoclonal Antibodies; Immune Checkpoints; ICI Inhibitors; NSCLC; SCLC*

Background

The immune response is present in all phases of carcinogenesis, from inflammation-induced cell damage to cell transformation, tumor formation, and tumor progression. Approximately 20% of all types of cancer are caused by chronic inflammation and solid tumors display inflammatory infiltrates. Inflammatory immune cells influence tumor onset, growth, and progression, due to the cytokines they secrete in response to antigen detection. The cytokines released are genotoxic and potentially oncogenic because they not only eventually damage the DNA but also induce the synthesis of growth factors which promote cell proliferation. Moreover, some cytokines promote tumor vascularization that facilitates the access of malignant cells to blood circulation and the onset of metastases in distant organs [1].

When properly activated, the immune system works against tumors and circulating malignant cells. However, the same natural system of checks-and-balances that prevents autoimmunity may be subverted in favor of tumors. As tumors grow, hypoxia takes place in its inner layers which, along with the inflammatory process, triggers the recruiting of macrophages by the tumor, which become tumor-associate macrophages (e.g. tumor collaborators) [2]. Associate macrophages recruit nuclear factors, and vascular and epidermal growth factors, among other molecules, to promote vascularization, tumor survival, and progression. Tumor–induced TGF-Beta (transforming growth factor beta) and/or costimulatory factors CD80, CD86, CD70 promote differentiation of naïve CD4+ and CD25+ T-cells into T Regulatory cells (TREGs) which inhibit the immune anti-tumor activity.

TREGs are nevertheless crucial for inflammation control and its deficiency leads to chronic inflammatory processes and autoimmune diseases [3]. TREGs also induce immune tolerance to tumor antigens**.** As TREG cells accumulate in tumor tissues they allow cancer evasion from the immune surveillance. In normal tissues TREGs promote self-tolerance and prevent autoimmunity, representing between

5% and 10% of the peripheral T CD4+ and T CD25+. However, when TREGs are under-expressed or when mutational defects occur, they result in hereditary syndromes such as IPEX (Immune dysfunction, polyendocrinopathy, enteropathy, X-linked) or may lead to acquired autoimmune diseases [4,5].

Antigens and Epitopes: the fundamentals for prophylactic and therapeutic vaccines

Antigens are protein fragments or peptide complexes which are presented by a class of antigen-presenting cells (APC) to the circulating T-lymphocytes (T cells) which constantly search for alien proteins, toxins, or pathogens. Even in a same protein some peptides may be more immunogenic than others, thus causing a stronger immune response. The first task of an immuno-oncology research team is to identify the best epitopes in the tumor before starting the development of a new therapeutic vaccine. Epitope is the peptide of a given protein (e.g. antigen) that triggers the strongest immunogenic response on the surveilling T lymphocytes. Therefore, the best candidates to vaccines and immunomodulation therapies are those epitopes with the strongest affinity to antibodies [6].

Immunotherapeutic approaches to cancer and tumor evasive tactics

Surgery and Radiotherapy were the first therapeutic resources in Oncology, followed by Systemic Chemotherapy and Hormone Therapy, which were developed and improved mainly from the 70s on in the 20th Century. The following decade saw the emergence of the first anti-tumor immune response modulators (polyclonal and monoclonal antibodies) and advances in Molecular Genetics led to new therapeutic modalities, from the 1990s onwards. The last two decades have also seen a resurgence of interest in the potential of the Immune System as therapy and prophylaxis in Oncology.

Immune-Oncology is now approaching the challenges through two different ways, active or passive immunization, and searching the possible benefits of combining one or more therapeutic modality, such as monoclonal antibodies plus chemotherapy or radiotherapy, or the combination of autologous vaccines with chemotherapy or radiation, for instance.

Active Immunization refers to antigen-specific vaccines which activate and direct the immune cells to their targets (e.g. cancer cells), usually providing prolonged protection. An example of active immunization in cancer is that obtained with autologous vaccines, which are developed using both the immune T cells and tumor samples of a patient in cell cultures. Once obtained a population of immune cells with a strong anti-tumor response, such population is expanded and then reinjected in the patient.

Passive Immunization includes the administration of monoclonal antibodies or of pre-activated T cells of a patient through genetic engineered T cells against his tumor epitope, such as CAR-T cells (Chimeric-Antigen Receptor T cells). Examples of monoclonal antibodies are those industrially developed against molecular targets that are relatively common in tumor subtypes such as HER2-neu in breast cancer tumors. Another example are the monoclonal antibodies developed to inhibit or block the ligand or the immune checkpoint receptors present on T cells surface, thus restoring the anti-tumor activity of T cells and macrophages. However, differently from the active immunization, passive immunotherapies or vaccines do not promote long lasting immune memory and therefore have only a temporary effect [6,7].

Passive immunotherapies have shown more benefits so far - and even some spectacular ones - in non-solid tumors such the hematologic cancers (leukemia, lymphoma) and, in a certain measure, also in melanoma (a solid tumor). Solid tumors pose a greater challenge to immunotherapies due to their complex structure rich in stromal tissue, fibers, vascularization, and better ability to evade immune surveillance. Briefly, the main evading tumoral strategies to escape the immune surveillance are condensed in a single word: immunoediting. The currently known immunoediting tactics are as follows [8,9]:

- Recruitment of tumor-associated macrophages and promotion of an immunosuppressive microenvironment via increased expression of immunosuppressive B cells, regulatory T cells (TREGs), and myeloid-derived suppressor cells (MDSCs);
- Desensitization to epitopes via epigenetic and/or post-transcriptional silencing of the involved histocompatibility complex (MHC or HLA);
- Reduced levels of immunoglobulins and increased tolerogenic enzymes (arginase-1, indoleamine 2,3-dioxygenase);
- Elimination of the more reactive tumor antigens (epitopes);
- Increased expression of immune checkpoints receptors, or of their ligands, thus co-inhibiting the antigenic response.

One of the pathways to escape immunity is the epigenetic silencing of genes that regulate the expression of MHC complex or antigen-presenting cells (APCs). Clinical studies combining inhibitors of aberrant methylation and deacetylation with Immune Checkpoint Inhibitors (ICIs) or other immunotherapies plus chemotherapy are underway, and some have already published results, targeting solid tumors such as NSCLC (non-small cell lung cancer), renal, high-grade glioma, medulloblastoma, colorectal, ovaries, among others [10].

In many cancer patients, immunosuppression is mediated by two immune checkpoints which act complementary: cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), and the programmed death-1 receptor (PD-1). As mentioned before, these two immunomodulatory receptors are expressed on T cells surface and through the transmembrane portion. Monoclonal-antibodies were developed for targeting CTLA-4 and/or PD-1 (or its ligand PDL-1) and promote the checkpoint blockade, being generally termed Immune Checkpoint Inhibitors (ICI). These therapies have yielded significant clinical benefits with durable responses to patients with different types of cancer, and several drugs were approved mainly for solid tumors, such as colorectal cancer, non-small cell lung cancer, small cell lung cancer, squamous cell carcinoma, cervical cancer, melanoma, gastric cancer, triple negative breast cancer, etc. However, tumors eventually acquire or use innate mechanisms by which resistance is developed against these therapies [11].

Several next-generation immune checkpoint inhibitors are currently being developed or undergoing clinical trials, such as Relatimab, Ieramilimab, Miptenalimab, Sabatolimab, Etigilimab, BMS-93=86207, Enoblituzumab, among others. They target other immune checkpoints such as LAG-3 (Lymphocyte activation gene-3 (LAG-3 or CD223), TIM-3 (T cell immunoglobulin-3), TIGIT (T cell immunoglobulin and ITIM domain), VISTA (V-domain Ig suppressor of T cell activation), B7-H3 (B7 homolog 3). Such renewed effort is justified by the prior successes with anti PDL-1 and anti CTLA-4 in several tumor types, especially in melanoma patients, rising from 12,2% to 22% the in-3-years survival rate of these patients. Whereas promising response rates in patients with Hodgkin's lymphoma or with melanomas may be between 40% and 70%, in most other tumors they are much lower (10% - 25%) [11].

Response rates to immunotherapy with molecular-targeted monoclonal antibodies vary not only due to the histology of disease but also among tumors of same histology. Tumors of same histology may have different levels of mutational burden, varying therefore among them in their degree of immunogenicity [12]. For instance, tumors with high expression of certain specific mutations (or high expression of PDL-1 receptors) are more responsive to monoclonal antibody therapies designed to target those mutations (or the receptor of interest), whereas others with low expression will not respond satisfactorily or not respond at all [13].

Current therapeutic strategies in immuno-oncology

For the sake of providing a general view of the current and near-future potentials of immunotherapies against cancer, here follows a brief description of the immune strategies currently available for cancer patients.

Immune checkpoint inhibitors: Drugs that prevent the interruption of the immune response against malignant cells. They do not target the tumor directly, but rather interfere with the ability of malignant cells to escape the attack by the immune system.

Adoptive therapies with TILs and TCR

TILs (Tumor infiltrating lymphocytes) methodology: Cells are removed from the tumor and the most active ones are grown in large batches in the laboratory for 2 to 8 weeks. After radiation of the patient to reduce levels of other immune cells, autologous cultured T cells are injected through an intravenous access.

TCR (T cell receptor): Adoptive Therapy with T cells CAR (T cells Chimeric Antigen Receptor) = T Cells with Synthetic Chimeric Receptors, designed to bind the tumor associate antigen (TAA).

As mentioned, Monoclonal Antibodies are laboratory-synthesized immune proteins designed to adhere to specific molecular targets in malignant cells. They can be designed to make cancer cells more antigenic (increased immune attack; or directly inhibit tumor proliferation; or induce apoptosis; or to transport cytotoxins to cancer cells.

Therapeutic vaccines do increase the immune response against cancer. The different approaches to therapeutic vaccines are as shown below:

- **Cytokines:** The two main types of cytokine lymphocytes in oncology are interferons and interleukins.
- **BCG (Bacille Calmette-Guérin):** is used to treat bladder cancer. It is inserted directly into the bladder with a catheter. BCG triggers an immune response against cancer cells. It is also being studied in other types of cancer.

Adoptive therapy with T cells (TACT) comprise two modalities - activated TILs (tumor infiltrating lymphocytes) and CAR (chimeric antigen receptor) T cell:

- **TILs:** Autologous vaccine with T cells activated endogenously by tumor antigens presented by MHC I, which can kill tumor cells. TILS requires *in vitro* expanded population using growth factors, lymph ablation with whole-body radiotherapy (elimination of TREGs and other co-inhibiting factors); and the re-administration of the expanded autologous T cell population.

The advantages of this method are a) polyclonal population with antigenic specificity for several tumor antigens makes it difficult tumor immunoediting and prolongs response duration; b) in melanomas, metastatic expanded TILs eliminated multiple lesions bearing common antigens.

The disadvantages are a) difficulty in collecting T lymphocytes and expanding their population; b) low affinity of TCRs for TAAs (tumor associated antigens), due to their presence in healthy tissues as well; c) restricted to the MHC Type I system [14,15].

What is new in the realm of CAR-T cells biotechnology?

CAR-T cell immunotherapies are being developed and tested in several clinical studies, including trials for solid tumors wherein resides the greatest challenge [16-20].

The chimeric antigen receptor (CAR) is a transmembrane receptor comprised of fragments or domains of synthetic antibodies. It contains an extracellular antigen-recognition domain that binds to the tumor-associated antigen, and an intracellular signaling domain or series of domains which activate T cells. The domains that are used can affect the ability of the receptor to recognize or bind to the antigen on the tumor cell. Antigen receptors depend on intracellular stimulation signals to be activated. Thus, each T CAR cell has signaling and co-stimulating domains, within the cell, which signal to the surface receptor. The different domains used can affect the overall function of the T cell [20,21].

New advances in intracellular T cell biotechnology have improved the ability of T cells with chimeric antigen receptors to produce more CAR T cells after infusion in the patient, thus being able to expand its population and prolonging CAR-T cells survival in the circulation. Advances have also been made in production time and reduction of it to seven days or less. However, many strategies are followed by different clinical trials using many different CAR formats, application routes, and extra features introduced into the T cells [22]. Schaft [22] suggested that this could be an indication that the ideal methodology for treating solid tumors with CAR-T cells has not been found yet. Moreover, the clinical outcomes seem to confirm that assumption because the trials that reported on this found that only 52 of 375 patients had clinical response. Further development of CAR T cell technology and clinical testing is needed to meet the medical need for more effective treatments of solid cancers.

Nevertheless, tisagenlecleucel was the first CAR T cell therapy to receive FDA approval, in 2017, for the treatment of children and young adults with acute lymphoblastic leukemia. In the same year the FDA approved the CAR T cell therapy, axicabtagene ciloleucel (Yescarta®), for the treatment of diffuse large B cell lymphoma (DLBCL). In both cases, the target cells expressed the CD19 antigen on their surface membrane. In May 2018 the FDA approved the T CAR tisagenlecleucel (Kymriah®) for a new indication, adults with non-Hodgkin lymphomas, such as high-grade B-cell lymphoma and DLBCL derived from follicular lymphoma which have relapsed or that progressed after previous treatment. Between 60% to 70% of patients with DLBCL have lasting responses to initial therapy [23].

The lung cancer challenge

More than 80% of all lung cancer cases are associate with smoking, including the most difficult-to-treat subtypes, such as neuroendocrine tumors and small cell lung cancer. Lung tumors are characterized by a strongly immunosuppressive environment. They usually contain over 200 non-synonymous mutations per tumor - with tumors from smokers and former smokers presenting 10 times as many mutations than those from never-smokers [24,25].

Tumors with high non-synonymous mutational burden are more responsive to immunotherapeutic approaches than those with low non-synonymous burden, because they are highly immunogenic. A study comparing these two subgroups of patients using the ICI inhibitor with pembrolizumab obtained 91% of partial or stable responses lasting >6 months in the higher mutation burden arm than in the lower non-synonymous arm of the study. However, some highly mutated tumors do not respond to ICI inhibitors [13].

On October 9, 2015, the FDA granted regular approval to nivolumab due to evidence of favorable benefit in patients with metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy. The study data showed that patients with PD-L1- positive tumors had longer survival when treated with nivolumab compared with docetaxel. Nivolumab was associated with a 2.8-month increase in median survival and a 27% risk reduction of death. Those who responded to nivolumab had durable responses [26]. Currently, the list of FDA-approved ICIs for NSCLC includes Pembrolizumab, Durvalumab, and Atezolizumab, in combination with other drugs in different protocols.

The small cell lung cancer challenge and promising perspectives

Small cell lung cancer (SCLC) accounts for 10% to 15% of lung cancer cases, being characterized by an aggressive tumor progression and metastatic spread. Most SCLC respond very well to first-line chemotherapy, the only treatment available for SCLC until recently. However, most tumors acquire chemo resistance and relapse. One of the reasons for some SCLC tumors to be more responsive than others that also have high mutational burden is the higher presence of neoantigens. Anti-PD-1 therapy is more effective in the presence in the tumor environment of neoantigens, which are available for presentation to T cells and can ultimately trigger an immune response [27].

Clinical trials using immune Checkpoints Inhibitors (ICIs) in SCLC patients showed no promising results, despite the high mutational burden of this lung cancer subtype. In 2018, however, the IMPOWER 133 randomized phase III study of carboplatin and etoposide with

or without atezolizumab has shown that the addition of atezolizumab to the protocol resulted in a statistically significant improvement in progression-free survival (PFS) (HR 0.77; 95% CI 0.62 - 0.96; p = 0.02) and overall survival (OS) (HR 0.70; 95% CI 0.54 - 0.91; p = 0.007) in that group of SCLC patients [28].

Across several other studies a small subset of SCLC patients appeared to substantially benefit from treatment with immune checkpoint inhibitors, with durable responses observed in patients treated with either single agent anti PDL-1 therapy or combined PD-(L)1 plus CTLA4 blockade [29].

In late 2018, another ICI, nivolumab, was granted accelerated FDA approval for third-line treatment of metastatic SCLC based primarily on response data from a subset of patients treated on the nivolumab arm of the study. Treatment with nivolumab in the third-line setting was associated with a response rate of only 12% - but notably these responses were durable for at least 6 months in 77%, and at least 12 months in 62% of cases [29].

Conclusion

In conclusion, as new findings widen the understanding of tumor immunology and new biomarkers of cancer vulnerability to immunotherapies are identified, we may hope for significant advances in Immune Oncology as an additional tool for prolonging cancer survival and perhaps, indefinite cancer remission control in the coming years.

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