INTRODUCTION

Tumors have developed multiple mechanisms to avoid destruction by the immune system. There are, in fact, various inhibitory pathways in the immune system that allow this complex system to tolerate cells and antigens physiologically present in the body, and not to mount an excessive immune response. These inhibitory pathways are essential because they allow the immune T-cells to block the growth of cancer cells; however, tumors use mechanisms to escape the control of the immune system, preventing the T-cells from using their cytotoxic activity against the tumor.

Both the innate and adaptive immune responses can act against tumors. A deficiency in cytotoxic T lymphocytes and natural killer cells can, in fact, increase tumor incidence. One of the mechanisms by which tumors can escape the immune system is the expression of ligands that inhibit T-cell expression, such as CTLA-4 (cytotoxic T-lymphocyte antigen 4), PD-L1 (programmed death-ligand 1) and PD-1 (programmed death-1).

Malignant Pleural Mesothelioma (MPM) is considered an “inflammatory” tumor because it is often characterized by a prominent infiltration of lymphocytes, macrophages and T-cells. The continuous, chronic inflammation of the mesothelial cells helps at first to promote, progress and transform these healthy cells into cancer cells. Similarly, the escape mechanisms from the immune system allow the tumor to evade the immune response from the host. The role of the immune system in the biogenesis of MPM is also complex and multifaceted and appears to involve both the innate and adaptive immune responses. This is why research is being conducted in immune mechanisms that allow tumors to grow by escaping the body’s control, with the goal of identifying suitable targets for an effective immunotherapy for MPM.

PD-L1 expression in MPM appears to be associated with a greater extent of disease at the time of presentation and with a greater incidence of sarcomatoid histology. This could be a plausible explanation for the poor prognosis seen in these cases.

Immunotherapy represents a new frontier in the treatment of cancer. The significant progress made in our understanding of the immune system has led to the development of new molecules that can increase the immune response of patients. Regardless of their genetic or metabolic disorders, many cancer patients may benefit from treatment because it is the immune response itself of the patient that is targeted and not the cancer cell.
The term **immune-checkpoints** refers to a series of inhibitory pathways in the immune system that are crucial for maintaining self-tolerance and preventing the excessive, prolonged and potentially harmful activity of T-cells in peripheral tissues.

It is now clear that tumors can use these immune-checkpoints to evade the antitumor immune response, such as through the loss of expression of tumor-associated antigens (TAA) and/or major histocompatibility complex (MHC) antigens, or through the production of cytokines and the expression of new, inhibitory membrane molecules.

This continuous molecular remodeling phenomenon is known as “**cancer immunoediting**”, and consists of three main, consecutive phases:

- Elimination (complete destruction of the tumor cells by the host’s immune system)
- Equilibrium (tumor cells selected by the T-cells become resistant to the immune system)
- Escape (the tumor cells give rise to clinically detectable lesions)

The immune-checkpoints currently known to be involved in the development of lung cancer are the cytotoxic T-lymphocyte antigen-4 receptor (CTLA-4) and the programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1).

**Cytotoxic T lymphocyte antigen-4**

Because immune-checkpoints are activated in most cases by a ligand-receptor interaction, they can be inhibited by antagonist antibodies or recombinant forms of ligands/receptors. The CTLA-4 receptor, known also as CD152, is a member of the immunoglobulin (Ig) superfamily that is expressed on cytotoxic T lymphocytes. After binding with one of its ligands, B7-1 or B7-2 expressed on the antigen-presenting cells (APC), it transmits an inhibitory signal to the lymphocyte, thus helping to homeostatically regulate the immune response.

CTLA-4 plays a vital role in the maintenance of immune tolerance to the tumor. Specifically, the CTLA-4 receptor acts on the CD80 and CD86 costimulatory immune signals activated by the antigen-presenting cells, so they increase the activation threshold of the T lymphocytes. The systemic administration of anti-CTLA-4 inhibitors as monotherapy or in combination with other therapeutic cancer vaccines has been shown to induce a regression of melanoma and colon cancer in murine models.

In a Phase II study evaluating the activity of an anti-CTLA-4 antibody (tremelimumab) that enrolled 29 patients with chemotherapy-resistant MPM (28 pleural and 1 peritoneal), objective clinical responses were observed only in 29 patients, however, stable disease was
seen in 9 patients, or approximately 31% of patients with epithelial histology. The median overall survival rate at one year was 48% and 37% at two years.

**Programmed death-1 receptor**

PD-1 is a cell surface receptor belonging to the Ig superfamily that is expressed on T-cells and pro-B cells and binds two ligands, PD-L1 and PD-L2. PD-L1 is a transmembrane protein that binds to its PD-1 and B7.1 receptors on the surface of T-cells, deactivating them. The PD-1 receptor stimulates the cells into inactivation by allowing the tumor cell to escape the surveillance of the immune system. This receptor is activated by binding with its ligand: programmed death-ligand 1 (PD-L1), which is usually found in the tumor microenvironment on the surface of cancer cells.

Researchers have shown that PD-L1 is present on rat murine cells in vivo. PD-L1 expression increases in response to an increase in the concentration of interferon γ (IFN)-γ and the draining of T-cells into the tumor-draining lymph nodes, supporting the hypothesis that this is an important local immunosuppressive pathway. The inhibitory action of PD-L1 on the different subpopulations of T-cells produces opposite effects on tumor progression and suggests that the immune suppression of the tumor is mediated by a specific subclass of T-cells. Researchers have demonstrated that PD-L1 was expressed in approximately 40% of 106 samples of mesothelioma analyzed, all of which were tumors with sarcomatoid histology with a poor prognosis (5.0 months versus 14.5).

**TREATMENTS**

**Targeted immunotherapies**

The first immunopotentiation study dates back to 1975, investigating the intrapleural administration of a vaccine consisting of irradiated and sterilized BCG, which led to a reduction in tumor growth, secondary to the activation of the immune system. Other studies followed the same line of research using Mycobacterium vaccae in combination with chemotherapy, for example. Another attempt at potentiating the immune response is the use of cytokines, such as interferon. Viral vectors have also been used to increase the efficacy of therapy. Interleukin-2 has also been tested in this disease to activate the immune system against the tumor. Another cytokine used in immunopotentiation studies is GM-CSF (granulocyte/macrophage colony-stimulating factor)
Several studies have evaluated the possibility of increasing the expression of major histocompatibility molecules (MHCs) to increase T-cell clones with antitumor effect.

Other studies have evaluated coadjuvant therapies such as CpGm oligodeoxynucleotide, CpG ODNs, Toll-like receptor 3 agonist, Toll-like receptor-7 agonist.

**Immunomodulation** involves modifying the lack of cell immunity within tumor sites. The first immunomodulation studies were conducted in the 1990s, evaluating therapies such as autologous and allogeneic LAK cells to reduce pleural effusions. Other studies demonstrated the cytotoxic action of CTLs against MPM cells. More recently, research has been conducted on the activity of dendritic cells and their ability to protect antitumor immunity. Other studies have investigated CD40-activated follicular B-cells and autologous T-cells expressing a chimeric antigen receptor (CAR).

**Immunodepletion**, often known as lymphodepletion, is another area of immunotherapy that involves eliminating the cells that have infiltrated the tumor foci. Promising studies have investigated the depletion of tumor-associated macrophages (TAMs) from the tumor mass and the possibility of reversing these cells from TAMs to M1 status. Similarly, other studies have demonstrated the utility of activating the TAMs infiltrating MPM tissue, promoting the release of cytokines and chemokines such as TNF-a, IFN-inducible protein 10 and IL6.

**Vaccines**

The discovery of mesothelin in the 1990s was very important because researchers hoped it could be used as a specific marker for MPM. Indeed, since this protein is also expressed in other tumor types, promising vaccines against these cancers were developed. Notwithstanding the controversy surrounding the possible role of the SV40 virus in the biogenesis of MPM, SV40 antigens have been tested as potential immunological targets for the disease. Wilms’ tumor 1 protein (WT1) has also been investigated as a potential diagnostic marker specifically for MPM.

One of the treatment strategies for this disease is developing cellular vaccines and a number of studies have evaluated the effects of cancer cells transfected with IL-4, IL-2, GM-CSF, B7-1, as
vaccine cells. Dendritic-cell based vaccines have also been investigated to activate both Th1 cells and CTLs by activating phagocytosis and apoptosis. Researchers have also reported that MPM cells in apoptosis could be used as potent inducers of anticancer CTLs.

**Monoclonal Antibodies**

Monoclonal antibodies have long been used in immunohistochemical techniques to obtain a differential diagnosis between MPM and other tumors. The first approach in this area was to test a monoclonal antibody and in combination with a toxin as a potential immunotherapy. Other studies evaluated monoclonal antibody reagents in combination with the 7D3 transferrin receptor, ricin A or doxorubicin. The monoclonal antibody K1 targeting mesothelin has been investigated as a potential immunotherapy for MPM. Various biomedical engineering methods have been used to design monoclonal antibodies combined with toxins to construct chimeric agents. Fusion proteins that can target cancer cells when subjected to radiotherapy have also been developed, and whose results suggest greater cytotoxic effects with fewer side effects. Researchers have developed an anti-MPM immunotoxin by combining Pseudomonas exotoxin with a fragment of the anti-mesothelin antibody. Human mesothelin has also been combined with an antibody targeting a dendritic cell receptor, increasing the potential for vaccination with mesothelin. Wingless-type (Wnt) protein, which is involved in various cancers, is another important antigen against which monoclonal antibodies have been developed. Antibodies against the surface antigen CD26, which is involved in tumor growth, have also been developed. Other examples of monoclonal antibodies described above include CTLA-4 inhibitors (ipilimumab and tremelimumab), and PD-L1 (pembrolizumab) and PD-1 receptor (nivolumab) inhibitors. Tremelimumab was investigated in a single-arm Phase II study of pretreated patients but the primary endpoint of objective response rate was not achieved. The disease control rate of
patients treated with tremelimumab was around 31%, progression-free survival (PFS) was 6.2 months (95% CI 1.3–11.1), and the mean survival time (mST) was 10.7 months (0.0–21.9).

Studies of anti-PD-L1 and PD-1 monoclonal antibodies are also currently underway.

Recent clinical trials
Below is a short list of recent clinical studies that have been completed or currently ongoing. Please see www.clinicaltrials.gov for further details about these studies.

Completed studies:
- Dendritic Cell-based Immunotherapy in Mesothelioma (tumor lysate-loaded autologous dendritic cells)
- Dendritic Cell-based Immunotherapy Combined With Low-dose Cyclophosphamide in Patients With Malignant Mesothelioma (DC+CTX)
- Pilot Study of Allogeneic Tumor Cell Vaccine With Metronomic Oral Cyclophosphamide and Celecoxib in Patients Undergoing Resection of Lung and Esophageal Cancers, Thymic Neoplasms, and Malignant Pleural Mesotheliomas (Allogeneic Tumor Cell Vaccine (K562); Drug: Celecoxib; Drug: cyclophosphamide).
- Cyclophosphamide Plus Vaccine Therapy in Treating Patients With Advanced Cancer (allogeneic tumor cell vaccine; Biological:autologous tumor cell vaccine; Biological: recombinant interferon alfa; Biological: recombinant interferon gamma; Biological:sargramostim; Drug:cyclophosphamide)
- Study of Safety and Tolerability of Intravenous CRS-207 in Adults With Selected Advanced Solid Tumors Who Have Failed or Who Are Not Candidates for Standard Treatment (Biological: CRS-207, Live-attenuated Listeria monocytogenes expressing human Mesothelin)
- Safety and Immune Response to a Multi-component Immune Based Therapy (MKC1106-PP) for Patients With Advanced Cancer (PSMA/PRAME).

Ongoing studies:
- Safety and Efficacy of Listeria in Combination With Chemotherapy as Front-line Treatment for Malignant Pleural Mesothelioma (Immunotherapy plus chemotherapy; Biological: Immunotherapy with cyclophosphamide plus chemotherapy)
- Dendritic Cells Loaded With Allogeneous Cell Lysate in Mesothelioma Patients (MesoCancerVac)
- CAR T Cell Receptor Immunotherapy Targeting Mesothelin for Patients With Metastatic Cancer (Fludarabine; Biological:Anti-mesothelin CAR; Drug:Cyclophosphamide; Drug:Aldesleukin)
- Genetically Modified T Cells in Treating Patients With Stage III-IV Non-small Cell Lung Cancer or Mesothelioma (Aldesleukin; Biological: Autologous WT1-TCRc4 Gene-transduced CD8-positive Tcm/TnLymphocytes; Drug:Cyclophosphamide; Other: Laboratory Biomarker Analysis; Procedure: Therapeutic Conventional Surgery)
- The Anti-CTLA-4 Monoclonal Antibody Tremelimumab in Malignant Mesothelioma (Tremelimumab)
- Adjuvant Tumor Lysate Vaccine and Iscomatrix With or Without Metronomic Oral Cyclophosphamide and Celecoxib in Patients With Malignancies Involving Lungs, Esophagus, Pleura, or Mediastinum
RESPONSE TO IMMUNOTHERAPY

Immunotherapy has changed the way in which we measure objective responses in both clinical studies as well as clinical practice. Melanoma immunotherapy studies have shown that the antitumor response is not seen until weeks or months after the start of treatment, with a survival gain seen after several months. This is because immunotherapy drugs activate the immune system, which in turn elicits a cell-mediated response. The response to treatment is measured by using RECIST or WHO criteria. During immunotherapy, these conventional criteria are not capable of adequately measuring the presence of peritumoral inflammatory infiltrate which may mimic a pseudoprogression, a phenomenon typically encountered during this type of treatment. To avoid this problem, immune-related response criteria (irRC) have been developed, in which an initial radiographic progression, in other words the appearance of new lesions and/or an increase in the size of existing lesions, in the absence of clinical progression, must be confirmed by another evaluation. The correct use of irRC can identify long-term survivors, including patients who would be considered by conventional criteria to be progressing and so may not continue to benefit from targeted treatment.

Another aspect evidenced by immunotherapy is the need to understand whether this treatment is suitable for everyone or only certain patients who are most likely to benefit from immunotherapy.

CONCLUSIONS

The association between the immune system and MPM is complex and multifaceted. Immunity certainly plays a key role in inducing damage to the DNA of mesothelial cells, which is strongly and pathogenically linked to exposure to asbestos.

Dividing the immune system into innate response and adaptive response also helps balance
the inhibition and activation of the cells involved in this complex mechanism.

Although the results from immunotherapy studies have not yet been earth-shattering, they are nevertheless extremely promising and provide a new view of this disease in anticipation that it will lead to new therapeutic approaches.

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