The Use of the Immune System to Treat Cancer
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IMMUNE SYSTEM AND CANCER

One of the main roles of our immune system is to defend the body against infecting and other foreign agents by distinguishing our body’s own cells (self) from foreign elements (non-self). The principal immunologic cells are called leukocytes¹ or white blood cells, and some examples include macrophages and lymphocytes (natural killer cells, T-cells and B-cells).

But the immune system not only provides a line of defense against foreign agents, it can also protect us against tumor cells; indeed, most incipient cancers are eliminated by a process called immune surveillance or immunoediting².

How does it work? Tumor gene mutations can generate proteins with aberrant amino-acid sequences, called neoantigens. Antigen-presenting cells (APCs), particularly dendritic cells, internalize these neoantigens, which are either captured from dead/dying tumor cells or delivered exogenously³. The dendritic cells process the tumor fragments, display them on major histocompatibility complex (MHC) molecules and migrate then to the lymph nodes (Figure 1). There, dendritic cells interact with naïve T-lymphocytes, leading to the activation and proliferation of tumor-specific CD4+ (helper) and CD8+ (cytotoxic) T-cells. The activation of the CD4+ and CD8+ T-cells occurs through the interaction of their T-cell receptor (TCR) with the dendritic cell-presented neoantigen on MHC class II and class I, respectively. While CD4+ cells help to activate CD8+ lymphocytes, cytotoxic T-cells kill tumor cells, recognized as foreign due to the neoantigens presented through their MHC class I molecules.

Unfortunately, cancer cells evolve in order to avoid immune-mediated elimination and are selected based on antigenicity and/or immunogenicity loss (Figure 2). Loss of antigenicity occurs through emergence of tumor cells that mutate or lack immunogenic antigens, or through defect acquisition in the processing or presentation of antigens⁴, such as the loss of MHC class I proteins⁵. Moreover, tumors keeping a certain degree of antigenicity can escape immune-mediated elimination by a decrease in their immunogenicity, for example through the expression of the immunoinhibitory molecule PD-L1 or the secretion of immunosuppressive cytokines such as IL10 and TGFβ⁴. In some cancers, in addition, subsets of tumor-infiltrating leukocytes might coordinate a site or microenvironment of "immune privilege" that suppresses antitumor immunity.

Figure 1. Generation of anti-tumor immunity. MHC, major histocompatibility complex. Modified from Mellman, Coukos & Dranoff.

Figure 2. Generation of anti-tumor immunity. MHC, major histocompatibility complex. Modified from Mellman, Coukos & Dranoff.
ACT is a personalized cancer immunotherapy that exploits the antitumor features of lymphocytes. The ACT using autologous tumor-infiltrating lymphocytes (TILs) is the most effective in achieving a complete durable regression for metastatic melanoma patients. The general approach starts with the digestion of the resected melanoma into a single-cell suspension or its division into several tumor fragments that are grown in interleukin-2 (T-cell growth factor). The lymphocytes present overgrow, destroying the cancer cells in some weeks, and giving rise to pure cultures of lymphocytes. These cultures can be tested for reactivity against tumors, and selected cultures are then expanded individually; approximately 5 or 6 weeks after tumor resection, enough lymphocytes are obtained for infusion into patients (Figure 3). Moreover, the inclusion of lymphodepleting chemotherapy in patients prior to TILs infusion has resulted in a substantial increase in cell persistence and duration of clinical responses.

CANCER IMMUNOTHERAPY

The field of cancer immunotherapy, intended as the use of the immune system to treat tumors, tries to overcome cancer immune escape by acting at several stages of the immune response. Some very well-known treatments include:

1) Adoptive cell therapy (ACT),
2) Oncolytic virus immunotherapy,
3) Monoclonal antibodies and
4) Immune checkpoint regulation inhibitors.

1) Adoptive cell transfer

ACT is a personalized cancer immunotherapy that exploits the antitumor features of lymphocytes. The ACT using autologous tumor-infiltrating lymphocytes (TILs) is the most effective in achieving a complete durable regression for metastatic melanoma patients. The general approach starts with the digestion of the resected melanoma into a single-cell suspension or its division into several tumor fragments that are grown in interleukin-2 (T-cell growth factor). The lymphocytes present overgrow, destroying the cancer cells in some weeks, and giving rise to pure cultures of lymphocytes. These cultures can be tested for reactivity against tumors, and selected cultures are then expanded individually; approximately 5 or 6 weeks after tumor resection, enough lymphocytes are obtained for infusion into patients (Figure 3). Moreover, the inclusion of lymphodepleting chemotherapy in patients prior to TILs infusion has resulted in a substantial increase in cell persistence and duration of clinical responses.
Ongoing efforts aim to broaden the reach of ACT to other cancer types. For this purpose, different techniques are being developed to introduce antitumor receptors into T-cells. One strategy involves the redirection of T-cell specificity by the integration of genes encoding chimeric antigen receptors (CARs, Figure 4). They are artificial receptors that can recognize antigens on cancer cell surface although they are not MHC-presented, circumventing in this way problems derived from the antigenicity loss.

CARs have been successful in the treatment of hematologic malignancies and will probably join the mainstream of cancer treatment in a near future; however, treatment of common epithelial solid cancers with CAR therapy is limited because suitable antigens exclusive to tumors are missing. Of note, there are many clinical trials to test safety and efficacy of these genetic engineered T-cells (check clinicaltrials.gov).

2) Oncolytic virus immunotherapy

The FDA approved in 2015 an injectable form of the virus Talimogene laherparepvec (T-VEC) for the treatment of melanoma lesions that cannot be removed completely by surgery. T-VEC is an attenuated oncolytic herpes simplex virus, type 1, engineered for expressing human granulocyte-macrophage colony-stimulating factor that favors APC recruitment to the tumor microenvironment. Moreover, it has been specifically adapted for selective tumor cell replication (Figure 5).
While the importance of T-cells in the antitumor response is well established, the potential contribution of B-lymphocytes is less well investigated. However, monoclonal antibody-based treatment of cancer, which involves the production by B-cells of antibodies against specific tumor antigens that are later given to patients (Figure 6), is another example of immunotherapy. The antibodies attach to the target antigen on cancer cells, and cell destruction happens through different mechanisms, which include antibody-dependent-cell-mediated-cytotoxicity (binding of antibodies to antigens on tumor cell surface promotes cell lysis by natural killer cells) and direct tumor cell death by inhibition of survival signalling.

Another approach to trigger the natural antitumor immune response are the immune checkpoint regulation inhibitors, which block the immune-inhibitory pathways. As mentioned above, APCs activate T-cells through an interaction between antigen-MHC complex on the APCs and TCR on naïve T-cells. Of note, a second interaction between B7 costimulatory molecules on APCs and CD28 on T-cells is needed for T-cell activation. Once activated, CTLA-4 is expressed on the surface of T-cells, binding to B7 with high affinity and thus providing an inhibitory signal to limit further T-cell activation (Figure 7).

The anti-CTLA-4 antibody ipilimumab was approved by the FDA in 2011 as first-line therapy for metastatic melanoma patients. The rational for using monoclonal antibodies against CTLA-4 was to enhance and prolong the activation and proliferation of tumor-specific T-cells, permitting therefore an effective tumor immune response. However, several limitations exist, such as the relatively small fraction of patients who obtain clinical benefit, or immune-related adverse events observed in a certain proportion of patients.

3) Monoclonal antibodies

Rituximab was one of the first anticancer antibodies used. Its antigenic target is the B-cell marker CD20, and it has significantly improved the clinical outcomes in non-Hodgkin’s lymphoma. Other cancer-directed monoclonal antibodies are the anti-epidermal growth factor receptor antibodies cetuximab and panitumumab, used for the treatment of metastatic colorectal cancer, and trastuzumab and pertuzumab, approved therapies targeting HER2 in breast cancer.

4) Immune checkpoint regulation inhibitors

Another approach to trigger the natural antitumor immune response are the immune checkpoint regulation inhibitors, which block the immune-inhibitory pathways. As mentioned above, APCs activate T-cells through an interaction between antigen-MHC complex on the APCs and TCR on naïve T-cells. Of note, a second interaction between B7 costimulatory molecules on APCs and CD28 on T-cells is needed for T-cell activation. Once activated, CTLA-4 is expressed on the surface of T-cells, binding to B7 with high affinity and thus providing an inhibitory signal to limit further T-cell activation (Figure 7).
A second immune checkpoint inhibitor is programmed cell death ligand-1 (PD-L1). Upon neoantigen recognition on the surface of tumor cells, T-cells produce cytokine interferon gamma. This results in the expression of interferon-stimulated genes in cancer cells through Janus kinases JAK1 and JAK2 (among others)\(^\text{16}\). Most of these expressed genes have antitumor effects, for example an increase in antigen presentation; however, interferon gamma also causes adaptive expression of PD-L1, which reacts with programmed cell death protein-1 (PD-1), inhibiting T-cells (Figure 8). Like anti-CTLA-4, antibodies targeting PD-1 or PD-L1 reactivate T-cell activity, and they have reached the clinic. Nivolumab (anti-PD-\(1\)) has been FDA approved for treatment of several tumors, including renal cell carcinoma, metastatic melanoma and non-small cell lung cancer\(^\text{18}\). In addition, pembrolizumab (anti-PD-\(1\)) was approved by the FDA in May 2017 for a subset of microsatellite instability-high or mismatch repair deficient solid tumors (adult and pediatric patients)\(^\text{19}\).

Regarding antibodies targeting PD-L1, two examples are atezolizumab, which has been FDA approved for a subset of non-small cell lung cancer patients\(^\text{20}\), and avelumab, approved by the FDA for the treatment of patients with metastatic Merkel cell carcinoma (12 years and older)\(^\text{21}\).

Interestingly, targeting the PD-1-PD-L1 axis shows a reduced toxicity compared to ipilimumab\(^\text{22}\). Moreover, there are other immune checkpoints pathways that could be target of novel treatments; some newly discovered molecules are being studied in clinical trials\(^\text{15}\) (check clinicaltrials.gov).

There are several predictive biomarkers of immune checkpoint therapy. PD-L1 protein expression (measured by immunohistochemistry (IHC)) in pre-treatment tumor specimens predicts a higher probability of response to anti-PD-1/anti-PD-L1 therapies. However, the use of these IHC assays as biomarker tests has some limitations\(^\text{23}\). An emerging biomarker for response to immunotherapy is tumor mutational burden, a measurement of the total number of mutations in a cancer genome. It is hypothesized that highly mutated tumors have an increased number of neoantigens on the cell surface capable of eliciting an immune response. In fact, higher numbers of mutations were shown to correlate with clinical
responses to pembrolizumab in several tumor types\textsuperscript{24,25}. In addition, colorectal cancers harboring microsatellite instability respond to anti-PD-1/anti-PD-L1 antibodies, whereas tumors harboring microsatellite stability are much less responsive\textsuperscript{26}. Based on these findings, two clinical trials are ongoing, where anti-PD-1 agents are tested in patients selected based on microsatellite instability\textsuperscript{27}.

There are two additional factors that can affect tumor response to PD-1 blockade. One is the presence of certain gene alterations: JAK1/2 mutations leading to interferon gamma signaling loss can cause a lack of response to PD-1 blockade therapy\textsuperscript{16}. Moreover, mutational inactivation of STK11/LKB1 in KRAS-mutant lung adenocarcinomas predicts for de novo resistance to immune checkpoint blockade\textsuperscript{28}, and some patients with cancers harboring MDM2 gene family amplification had poor clinical outcome and remarkably increased tumor growth rate after anti-PD1/PD-L1 treatment\textsuperscript{29}. The second one is the presence of pre-existing CD8\textsuperscript{+} T-cells negatively regulated by PD-1/PD-L1-mediated adaptive immune resistance, needed for tumor regression (metastatic melanoma) after therapeutic PD-1 blockade\textsuperscript{30}.

**CONCLUSION**

Immunotherapy has emerged as a significant addition to conventional cancer therapies; particularly, immune checkpoint regulation inhibitors have been one of the most dramatic advancements made in tumor treatment in recent years. Undoubtedly, these inhibitors and their combination with other (immune)therapies are the leading path to achieve an increased success in the fight against cancer. That’s why, at OncoDNA we create for each cancer patient who comes to us a specific immunogram that predicts the clinical response to immune checkpoint regulation inhibitors (Figure 9).

**PERSONALIZED IMMUNOGRAM**

Figure 9. Proprietary graph model that predicts patient response to immunotherapy in a personalized way. A axis, PD-L1 protein expression; B axis, tumor mutational burden; C axis, CD8\textsuperscript{+} T-cells infiltration; D axis, microsatellite instability; E axis, gene alterations leading to resistance or sensitivity.
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