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The future of checkpoint blockade to treat cancer patients

Abstract

The presence of several inhibitory pathways that block T cell responses - immune checkpoints, offers particular strategies for mobilizing the immune system to attack cancer cells. The best characterized of these immune checkpoints are CTLA-4 (Cytotoxic T lymphocyte associated protein 4) and PD-1 (Programmed cell death protein 1). CTLA-4 is expressed exclusively on CD4+ and CD8+ lymphocytes which restrains T cell proliferation by interfering with the interaction of CD28 with its ligands CD80 (B7-1) and CD86 (B7-2) on the surface of antigen presenting cells (APC's). PD-1 belongs to CD28 family and it is expressed on T cells, B cells, monocytes, Natural Killer (NK) and many tumor infiltrating lymphocytes (TIL's). Furthermore, PD-1 recruits a phosphatase and seems to inhibit with T cell antigen receptor mediated signaling. It has 2 ligands that have been described, PD-L1 and PD-L2, which are both expressed on dendritic cells and many tumors cells. Immunotherapeutic approaches to treat cancer patients have been evaluated during the last decades and today; immune checkpoints are the new paradigm for cancer treatment. The Food and Drug Administration (FDA) approved the antibody against CTLA-4 (Ipilimumab) in 2011 for the treatment of metastatic melanoma. To date, it is undergoing clinical trials for the treatment of non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder and metastatic hormone refractory prostate cancer. Antibodies against PD-1 (Pembrolizumab and Nivolumab) were approved in 2014 by FDA for the treatment of melanoma patients that did not respond to prior treatment. This type of therapy symbolizes an innovative concept in cancer therapy due two ways: first, these drugs totally ignore the tumor cells - they reliant on the immune system and second, they are not used to activate the immune system against a particular cancer; they remove inhibitory molecules that block a successful antitumor T cell response. Antibodies to CTLA-4, PD-1 and PD-L1 have shown objective response against several cancer types in clinical trials with response rates of about 25%. This effect represents a special challenge for immunotherapy - since certain types of cancer have presented lower burden of mutation and higher immune regulatory molecules such as VISTA, TIM-3 and LAG-3. Here, I have raised recent advances in the understanding of the cancer immunotherapy mainly the role of blockade of immune checkpoints.

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Introduction

Have you ever thought how complex our immune system is to recognize cancer cells? Researchers have tried to develop cancer vaccines for decades but unfortunately this achievement does not translate into success in clinical trials. This complexity is due to the compromise nature of how the evolution selects our immune system to respond not only against strange particles or different cells but normal cells as well. The immune system's capacity to detect and most of the time destroy abnormal cells may prevent the development of many cancers. Cancer is not only a disease but also rather a collection of several diseases - it is not only characterized by uncontrolled growth cells but a complex mechanism as proposed by Douglas Hanahan and Robert A. Weinberg as an organizing principle that provides a logical framework for understanding the remarkable diversity of cancer.[1] According to them there are six biological, distinctive and complementary capabilities that enable tumor development and progression as the follow: Sustaining proliferative signaling, resisting cell death, inducing angiogenesis, enabling replicative immortality, evading growth suppressors and activating invasion and metastasis. In this context cancer cells are able to growth, escape and avoid detection and destruction by the immune system. Cancer cells come from a normal cell driven by mutations that lift the brakes on cell proliferation. In this case cancer can be considered as a genetic disease. In the other hand most of cancer patients suffer since their immune system is weak and inefficient. How the immune system is impaired in cancer patients and how they can contribute to the tumor growth and development? One of the key issues refers to the reduction of the expression of tumor antigens on their surface making it harder for the immune system to recognize them as well as expressing protein on their surfaces that induce immune cell inactivation and releasing substances that suppress immune responses and promote tumor cell proliferation, growth and survival.[2] In this case cancer can be considered as an immunological disorder - cause or consequence?

Immunotherapy records a pivotal moment in cancer as long sought attempt to promote the immune system against tumors. The standard treatments for patients with several cancer types are in most cases, surgery, radiation and chemotherapy.[3] Surgery offers a huge chance for a cure for many types of cancer, principally those that have not metastasis and diagnosed from the beginning. Radiotherapy is involved in many therapeutic treatments of cancer; however, severe side effects can occur months to years after treatment. Additionally, some cancer cells are strong enough to tolerate and retrieve from the damage to their DNA caused by radiation therapy.[4] Although chemotherapy remain an effective treatment for many types of cancer often causes side effects such fatigue, pain, diarrhea, nausea and vomiting, blood disorders, nervous system, among others. Thus, there is an urgent need to develop new therapies for cancer treatment. Some strategies including cytokines, signal transduction inhibitors, oncolytic

viruses, cancer vaccines, T cell adoptive transfer and angiogenesis inhibitors have been tried, generally with low percentages of positive response.[5] Here, I review recent advances in the understanding of the cancer immunotherapy mainly the role of blockade of immune checkpoints. The concept of using activation of the immune system and an inflammatory response to investigate an anti tumor immune response was studied in the 1960's. In 2015, James Allison, PhD, who received the Lasker-DeBakey Clinical Medical Research Award for his pioneering work in enabling T cells to attack cancer cell by removing the brakes of T cells called checkpoints, has paved the way for a new perspective on cancer therapy.

Immune checkpoints

One of the most notorious questions in immunology for over a century has been whether an effective immune response could be generated against cancer. The answers to this particular question, can the immune system identify and destroy cancer cells? - has been widely dependent on fundamental immunological theories. In 1893, William B. Coley created a purified mixture of bacterial lysate (*Streptococcus pyogenes* and *Bacillus prodigiosus*) to treat a 21years old patient who was facing an inoperable sarcoma. After the treatment with Coley's toxin the patient had a complete remission.[6] Answer number 1, yes - the immune system can be activated to recognize and destroy cancer cells. In 1909 Paul Erlich suggested that immune system could control cancer development.[7] Unfortunately the arguments rose by Coley and Erlich were not enough in order to sustain that the immune system is indispensable to recognize and kill cancer cells. The role of immune system in tumor recognition faced a gloomy period for a while. Due the difficulty of reproducing tumor regression in different types of cancer using Coley's toxin,[8] treatment extremely toxic,[9] rejection of transplantable tumors[10] (alloreactivitty) by the fact that tumor cells are self[11,12] and thymic selection removes all auto reactive T cells,[13] the answer at this time is No, the immune system is not able to recognize and destroy cancer cells. After 1980's a plenty of experiment were done in order to attest the immune system could be the effective against cancer development. Auto-reactive T cells can escape from thymic deletion[12–15] several TAA (tumor associated antigens) were identified,[16,17] dendritic cells can present tumor antigens to T cells[18,19] and immunodeficient mices to STAT1-/-, perforin-/-, IFN-gamma-/-, RAG-/- have much higher frequency of cancer than wild type mices.[20]

Based on current immunological developments there is no doubt that the immune system can recognize and eliminate transformed cancer cells. Several studies have investigated the immune system of cancer patients, and they suffer from large immunosuppression mainly due to decrease lymphocyte proliferation and cytotoxic activity. This means that the immune system, responsible for immunosurveillance now becomes weak, inactive and inefficient.

Cancer immunotherapy is one of the best therapies compared to traditional therapies that may cause potential toxicities such as chemotherapy and radiation. The potential use of immunotherapy is to restore the immune system of patients in attempt to stimulate it to reject and destroy cancer cells.[21] The immune system can recognize and destroy tumor cells in a process called cancer immunosurveillance. After a century of scientific controversy, the notion that the immune system contributes to cancer development is experiencing a new resurgence-cancer might be seen as a failure of immune surveillance. Recent evidences suggest that the mechanism of tolerance that commonly exist to avoid autoimmune disease may also preclude the development of an proper anti tumor response and tumors themselves have the capability to antagonize the development of effective immune response against their antigens.[22] Thus, the major challenge has been to develop strategies to breaking this tolerance. Advances in our discerning of antigen presentation and tolerance have conduct to some promising strategies. Tumor cells are not just a provincial mass of proliferating abnormal cells, but they are defined as a heterogeneous and structurally complex tissue. These cells can recruit diversity of cell types, including endothelial cells, fibroblasts and immune cells, and, through production and secretion of stimulatory growth factors. This collection of cells and molecules together compose the tumor microenvironment.[23] We know the microenvironment plays a major role during the initiation and development of tumor progression. During tumor development monocytes and macrophages are actively recruited into tumors where they change the tumor microenvironment to accelerate tumor progression. Several researchers had been showed that distinct microenvironments where tumor-associated macrophages (TAM) promote cancer cell motility, angiogenesis and metastasis. In addition, there is strong evidence that regulatory T cell populations (Treg) can migrate into tumors and suppress adequate anti-tumor responses in the tumor microenvironment, thus contributing to the prosperity and growth of human tumors.[24]

Reasons for limited immune response against tumor cells include immune regulation mediated by cancer cells and immune cells profile on microenvironment. Indeed, cancer cells are able to shape the innate immune response to obtain growth factors, pro-angiogenic factors, and other elements that stimulate tumor growth, development, invasion, and metastasis. Additionally, this tumor-promoting activity is able to control the principal type of immune response that is able to kill tumor cells-mediated by cytotoxic CD8+ T lymphocytes, M1 macrophages and Natural Killer (NK) cells among others.[25]

With the recent approval of the monoclonal antibodies against CTLA-4 and PD-1 for the treatment of melanoma, renal cancer and non-small cell lung besides the success in several clinical trials with chimeric antigen receptor (CAR-T cells) have attracted wide interest for strategies that enhance T-cell-mediated response against cancer.[26–27] A complex network of biological pathways governs interactions between the immune system and cancer cells. The balance of signaling via co-inhibitory or co-stimulatory molecules expressed on T cells has demonstrated to be a powerful approach to intensify antitumor immune responses. This approach has been used effectively for the generation of a new class of anticancer therapies called checkpoint-blocking antibodies, represented by the FDA-approved agent, Ipilimumab, an antibody that blocks the co-inhibitory receptor CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4). Exploiting on the success of CTLA-4 blockade, agents that target a second co-inhibitory receptor, PD-1 (Programmed cell death protein 1), or its ligand, PD-L1, are in clinical development.[28] Inhibitory molecules like CTLA-4 and PD-1 such as PD-L1, LAG-3, TIM-3, VISTA and BTLA besides co-stimulatories molecules such as ICOS, OX40 and 4-1BB are potent agents for combination therapy in order to improve antitumor responses. Until now, according to Clinical Trials.gov there are more 900 clinical trials ongoing in cancer immunotherapy based on checkpoints inhibitor. Among them we can cite: 208 studies involving CTLA-4, 375 studies involving PD-1, 340 studies involving PD-L1, 25 studies involving OX40, 14 studies involving 4-1BB, 7 studies involving GITR, 9 studies involving TIM-3, 15 studies involving LAG-3 and 256 studies involving ICOS molecules.[29]

The treatment with Ipilimumab was the first agent to show enhanced survival in a randomized phase III trial that enrolled patients with metastatic melanoma. Currently, it is well know that treated patients has an increase in the frequency of T cells expressing

the inducible costimulator (ICOS) molecule, a T-cell-specific molecule that belongs to the CD28/CTLA-4/B7 immunoglobulin superfamily. ICOS and its ligand (ICOSL) have been shown to play diverse roles in T-cell responses such as mediating autoimmunity as well as enhancing the development/activity of regulatory T cells.[30] Furthermore, the treatment with Ipilimumab resulted in higher CD4+ ICOS+ T frequency and IFN-y levels in malignant prostate tissue.[31] Engagement of the ICOS pathway markedly enhances efficacy of CTLA-4 blockade in cancer immunotherapy.[32]

The treatment with anti-CTLA-4 enhances the production of IFN-γ, which is a critical cytokine for tumor immune responses. The loss of the IFN-γ signaling pathway is highly associated with primary resistance to Ipilimumab. Gao et al.,[33] analyzed patients identified as non-responders to Ipilimumab and they found that these patients had tumors with genomics defects in IFN-γ pathways. Experimentally, to endorse these findings the authors used knockdown mices to IFN-γ receptor (IFNGR1) bearing B16BL6 (syngeneic melanoma). As result, the mices had impaired tumor rejection upon Ipilimumab treatment[33] This was not the first time that IFN-γ showed to be important against murine melanoma. The hypothesis of interferon gamma (IFN-gamma) accumulation and consequent cytotoxicity to implanted tumor cells was confirmed in vitro and ex vivo by Rodrigues et al.[34] Recent studies showed that LAG-3 and PD-1 are co-expressed on tolerized tumor infiltrating lymphocytes (TILs) implying that these molecules may contribute to tumoral immune escape[35] Preclinical models using antibodies to block LAG-3 demonstrate a boosted activation of antigen-specific CD8+ T cells at the tumor site[36] Currently, there are a plenty of articles showing within tumor microenvironment a exhausted T cell population expressing high levels of inhibitory receptors, including PD-1, LAG-3, TIM-3, CTLA-4, BTLA and TIGIT.[37–42]

Immune monitoring as a tool to predict immune response

There are some strategies to modulate the microenvironment - targeting regulatory cells, blocking differentiation or recruitment, blocking immunosuppressive enzymes, regulatory cell depletion, re-programming immunosuppressive cells, modifying the chemokine and cytokine profile are some examples. The attractively of new strategies for immunotherapy is driven by immune response and microenvironment discovery. Usually, scientists have relied on conventional laboratory research tools to identify, for example, altered genes and changes in mRNA and protein expression. To put these cancer biomarkers in the context the researchers can use several strategies to find a good parameter to take care of patient and drug development. Since Ipilimumab arrived on the scene, a number of other molecules, such 4-1BB, TIM-3, LAG-3, OX40, VISTA, GITR and PD-1 have gathered researcher's attention. Most famous is an antibody that targets a molecule on immune cells called PD-1. Data collected from analysis of tumor tissue can then guide rational searches for important markers in the blood. For example, the initial phase I trial with anti-PD-1 (Nivolumab) therapy reported that PD-L1 expression on tumor cells may serve as a prognostic marker to suggest which patients would benefit from treatment suggesting a correlation between pre treatment tumor PD-L1 expression and clinical response.[43] Tumor samples with PD-L1 positive had an objective response rate of 36% (9 of 25 patients) whereas tumors with PD-L1 negative didn't show any objective clinical response (0 of 17 patients).[44] From now on, it is going to be more often the presence of specialized laboratories (Facilities) doing translational research - studies of cellular immunity including assays of cell populations and response in clinical trials. They will be dedicated to support immune monitoring during novel cancer immunotherapy, being essential for characterizing the immune status in patients receiving immune-modulating therapies such as levels of serum cytokine, cancer biomarkers on tumor samples, microenvironment, status of T cell activation, Natural Killer cells (NK), presence of immunosuppressive profile - T regs and MDSC (Myeloid-derived suppressor cell) and some molecules like IDO (indoleamine 2,3 dioxygenase), Galectin among others. A harmonized struggle to assess the value nongenetic biomarkers that address different aspects of the cancer-immunity cycle in T cell checkpoint blockade will allow us to integrate information on individual aspects of tumor-immune interaction. The Figure 1 shows the balance between co-stimulatory and inhibitory signals and some possible candidates to checkpoints combinations, blocking or inducing.

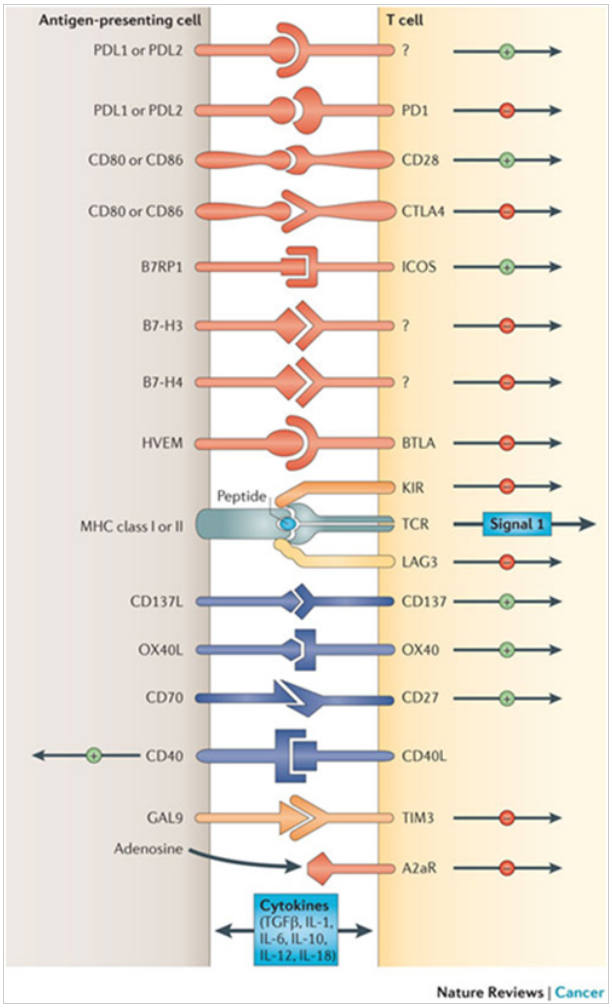

Figure 1 Multiple co-stimulatory and inhibitory interactions regulating T cells. Drew Pardoll.The blockade of immune checkpoints in cancer immunotherapy.[26]

Discussion

Have you ever thought how complex our immune system is to recognize cancer cells? Researchers have tried to develop cancer vaccines for decades but unfortunately this achievement does not translate into success in clinical trials. This complexity is due to the compromise nature of how the evolution selects our immune system to respond not only against strange particles or different cells but normal cells as well. The immune system's capacity to detect and most of the

time destroy abnormal cells may prevent the development of many cancers. Cancer is not only a disease but also rather a collection of several diseases - it is not only characterized by uncontrolled growth cells but a complex mechanism as proposed by Douglas Hanahan and Robert A. Weinberg as an organizing principle that provides a logical framework for understanding the remarkable diversity of cancer. According to them there are six biological, distinctive and complementary capabilities that enable tumor development and progression as the follow: Sustaining proliferative signaling, resisting cell death, inducing angiogenesis, enabling replicative immortality, evading growth suppressors and activating invasion and metastasis. In this context cancer cells are able to growth, escape and avoid detection and destruction by the immune system.

Besides this cancer cells can be extremely adaptable and responsive. Cancer cells can resist chemotherapies and other treatments through a variety of mechanisms that can sometimes seem perplexing. The fundamental mechanism, by which several cancers develop resistance to therapy, is a major feature in the failure of many forms of treatment, including chemotherapy and radiotherapy. While most cancers initially can respond to the given treatment unfortunately some cancers will relapse following treatment. The resistance can be caused by alteration to drug metabolism such drug uptake and efflux. Another important feature of drug resistance is that development of resistance to one drug can lead to resistance to other drugs. The loss of a drug transporter (responsible of putting the chemotherapeutic agent to inside the cell) can lead to resistance to structurally diverse compounds that resulting from one therapy will affect the efficacy of many other compounds. Additionally, some cancer cells are strong enough to tolerate and retrieve from the damage to their DNA caused by radiation therapy. Although chemotherapy remain an effective treatment for many types of cancer often causes side effects such fatigue, pain, diarrhea, nausea and vomiting, blood disorders, nervous system, among others. Thus, there is an urgent need to develop new therapies for cancer treatment. Some strategies including cytokines, signal transduction inhibitors, oncolytic viruses, cancer vaccines, T cell adoptive transfer and angiogenesis inhibitors have been tried, generally with low percentages of positive response. Immunotherapy records a pivotal moment in cancer as long sought attempt to promote the immune system against tumors. The immunotherapy agent (Nivolumab, Pembrolizumab and Ipilimumab) is being used in conjunction with chemotherapy on patients with advanced sarcoma, breast, lung, ovarian, head and neck, colorectal, and pancreatic cancers. Early results indicate that this combination with several types of chemotherapy appears to be safe and effective in treating advanced cancer patients.

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Conflicts of interest

Author declares there are no conflicts of interest.

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