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Cancer Immunotherapy Treatments

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CANCER IMMUNOTHERAPY TREATMENTS: CURRENT RESEARCH ON THE USAGE OF COINHIBITION BLOCKADE AND COMBINATORIAL APPROACHES TO TREAT CANCER

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Abstract

Cancer is the second leading cause of death in American, with over half a million deaths from cancer reported in 2009. Cancer chemotherapy treatments were developed in the nineteen hundreds and remain the backbone of current treatments; however, they have some limitations. New immunotherapy cancer treatments, where biologic agents are given to patients to influence the body's natural immune response, are being researched. Among these immunotherapy treatments are co-inhibition blockade of T cells, and combination blockade treatments together with chemotherapy treatment. This review will discuss T cell activation and the role of T cell coinhibitors such as CTLA-4 and PD-1 in immune system function. It will go through some immune system dysfunctions seen in breast cancer patients. The review will focus on the usage of anti-CTLA-4 and anti-PD-1 antibodies in coinhibition blockade treatments, as well as combination immunotherapy approaches in clinical trials. The mechanism involved in the blockade of T cell coinhibition is important for understanding why this form of immunotherapy is successful. Anti-CTLA-4 and PD-1 antibodies have resulted in objective responses in a good percentage of cancer patients. New combination immunotherapy approaches, as well as immunotherapy treatments in addition to chemotherapy, has been shown to be more effective. Also, the blockade of multiple T cell receptors combined with vaccination in mice has yielded a high survival rate. Most of the material for this paper was located from journals, and extracted via The Touro College Library search engine—primarily through Pubmed.

Introduction

Cancer is the second leading cause of death in America. The Centers for Disease Control and Prevention reported 567,628 deaths from cancer in 2009. One in six people in the U.S. and Europe will die of cancer (Paul, 1991). Cancer treatment evolution began with ancient physicians using surgery. Little progress was made until the early nineteen hundreds, when radiation therapy and chemotherapy were developed. Until the late 1990s nearly all drugs used in cancer treatment worked by killing cells that were in the process of mitosis. These chemotherapy drugs also killed some normal cells but had a greater effect on cancer cells. Better understanding of the biology of cancer cells has led to the development of a new type of cancer treatment called immunotherapy, where biologic agents like interferons, interleukins, and other cytokines are given to patients to imitate or influence the natural immune response. They function either by directly altering the cancer cell growth, or by acting indirectly to help healthy cells control the cancer (The American Cancer Society, 2012).

In adoptive immunotherapy, or cell-transfer therapy, cells involved in immune defense are removed from a cancer patient and "educated" to react against the cancer, or else to enhance the patient's native ability to kill cancer cells. The cells are then returned to the bloodstream. Molecules that are important in the immune response are administered in combination with the transfer of

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immune-system cells, or alone. Attempts to stimulate anticancer activity directly in the body's immune system cells are made with these molecules. Immunotherapy is a particularly appealing addition to all of the existing treatments because it can be delivered systemically to combat metastases (like chemotherapy). However, since the immune system is selective—it attacks only diseased cells ignoring the healthy ones—immunotherapies might be devised that are more cancer-specific than chemotherapies (Paul, 1991).

Cancer chemotherapies remain the backbone of current treatment but they are limited by a narrow therapeutic index, significant toxicities and frequently acquired resistance. A lot of hematologic malignancies and metastatic solid tumors cannot be wiped out by the available anti-cancer therapeutic agents. Improved understanding of cancer pathogenesis, such as how immunosuppresion regulates anti-tumor immune responses, has given rise to a new treatment option—cancer immunotherapy. New approaches for cancer immunotherapy are being developed and tested by researchers around the world. One developing approach focuses on activating lymphocytes by cytokines in order to maximize their therapeutic potential. Another approach being investigated is the method of sensitizing patient lymphocytes through vaccinations (Weiss, et. al., 2003). New immunotherapy treatments alone, or used in combination with chemotherapy, may be able to improve the prognosis of cancer patients as well as the long-term outcomes of cancer survivors. In this review we will discuss T cell co-inhibition, co-inhibition blockade of T cells as an immunotherapy treatment, and combination immunotherapy approaches; we will also determine if combination immunotherapy, as well as combining immunotherapy with chemotherapy proves to be more effective in cancer treatments as compared to monotherapy treatment.

Methods

The author utilized Google and Google Scholar to find general information as well as peer reviewed articles on the topic. The majority of the peer reviewed articles were found via the Touro College library search engine, specifically through Pubmed, Medline and Ebsco, to retrieve journal articles. Background knowledge on this topic came from Touro College's on-campus library, from books including, "Immunology Recognition and Response."

Discussion

The immune system plays an active role in finding and eliminating newly developing cancer cells. Since immunotherapy influences the natural immune response, it is important to have a general understanding of the immune system and tumor immunology. The immune system has two broader branches: the innate immune system and the adaptive immune system. The innate immune system is the first line of defense, and it responds generally to threats. The adaptive immune system is responsible for precise, antigen-specific, targeted immune attacks. The principle effector cells of the adaptive immune system are lymphocytes that have antigen-specific receptors on their cell surface. The three major types of lymphocytes include T cells, B cells and Natural Killer cells. T cells and their coreceptors, such as T helper cells (CD4+) and cytotoxic T cells (CD8+), play a key role in immunity

and tumor immunology. The mere presence of T cells has no effect; they need to be activated in order to contribute to the body's immune response.

The first part of T-cell activation is the T cell maturation/selection process. In the maturation process a T cell is committed to one of two lines: the CD4+ T helper cell line or the CD8+ cytotoxic T cell line. Once they have matured in the thymus, CD4 T cells and CD8 T cells are released into the bloodstream in search of an antigen. These newly matured T cells are naïve in the sense that they have never encountered the antigen that their T cell receptors were built to recognize. There are two determinants that decide what a naïve T cell will mature into: the type of coreceptor it expresses (CD4 or CD8) and the nature of its first contact with its antigen (Inman, et al., 2007). The relationship between the T cell and its antigen presenting cell (APC) is critical to understanding how cancers can trick the immune system into a state of unresponsiveness. In other words, it helps explain how cancers can suppress the body's immune system, so that the body cannot eliminate the cancer. Also, the role of the antigen presenting cell is important in many immunotherapies. If antigens from tumors can be injected into naïve T cells, for example in the form of a vaccine, they have the potential to help the body become immune to that tumor's antigen. Of course, the cell needs to undergo the proper activation signal in order to promote cancer immunity.

For naïve CD4 T cells, activation requires the presence of a mature dendritic cell with antigen loaded with Major Histocompatibility Complex (MHC) II. The activation of a CD8 T cells requires a target cell, and antigen presenting cell (APC) with antigen loaded MHC I and an antigen-specific effector CD4 T cell for cytokine support. Activation of any T cell requires a team effort. The many steps involved show that T cell activation is not a chance occurrence. For the T cell to be activated by an MHC bound target antigen, several things must happen. First, cell adhesion molecules must be present on both the APC and the T cell. The cell adhesion molecules serve two main purposes. One is to adhere the T cell to the APC and help these two cells remain in contact long enough to allow as many T cell receptors on the T cell as possible to become activated. The adhesion molecules also help the formation of an immunologic synapse, which is necessary for T cell activation. After binding the right MHC antigen combination, the T cell receptor is phosphorylated and a suitable signal is directed into the T cell for processing. If enough T cell receptors bind to the MHC antigen complexes, the T cell has the opportunity to test itself at signal II (Inman, et al., 2007).

After processing the first signal, the T cell must receive a second confirmatory signal in order to avoid apoptosis, or programmed cell death. The molecules that give this second signal are called costimulatory molecules. Certain molecules give the T cell the activation signal (costimulation) it is programmed for, while others do the opposite (coinhibition). Some costimulatory molecules can be either stimulatory or inhibitory such as B7-1, B7-2 and B7-H1 (Subudhi, et al., 2005). The basic costimulatory molecule is CD28, a receptor that is expressed on the surface of nearly all CD4 T cells, and most CD8 T cells. When the T cell receptor and CD28 bind to their ligands at the same time, their respective intracellular signals act together to stimulate the cell's replicative mechanism and secretory apparatus. A coinhibitor receptor called CTLA-4 travels to the T cell plasma membrane upon activation by the second signal. CTLA-4 expression is rapidly upregulated following T cell activation. A number of autoimmune diseases including insulin-dependent diabetes mellitus, rheumatoid arthritis and multiple

sclerosis, have shown genetic linkage to the CTLA-4 locus (Greenwald, et al., 2005). This means that the gene encoding for CTLA-4 may be involved in causing different immune diseases. CTLA-4 is significant in current research on T cell immunotherapy and will be discussed in detail as an example of coinhibition blockade.

Ultimately, there are a lot ways a T cell can mature, which will lead to different T cell functions. Some of these will be helpful in fighting the cancer and some hold back the immune system from ridding the body of the cancer. If a tumor-antigen is presented to a T cell, and it receives the proper activation signal, the T cell will cause anti-cancer responses in the body. However, if the T cell does not receive the right stimulus it can result in the body tolerating the cancerous cells. If a T cell receives a signal from coinhibitory receptors like CTLA-4 this can suppress the T cell response. Interaction of a T cell with CTLA-4 might promote T regulatory formation. T regulatory cells are important because they suppress immune responses in the body which can help prevent autoimmune diseases. Unfortunately, they are not helpful when they prevent the body's immune system from destroying cancerous cells.

Several new costimulatory molecules have been discovered more recently like the PD-1 (programmed death 1) receptor, which is found on T cells and numerous other cell types. This is another coinhibitor receptor currently being researched. Evidence shows that PD-1 signals inhibit T cell activation and proliferation (Dong, et al., 1999). PD-1 has two known ligands, PD-L1 and PD-L2. PD-L2 appears to have a stronger attraction for the PD-1 receptor than PD-L1 and it is expressed on dendritic cells and macrophages. Contrarily, PD-L1 is expressed on T cells, B cells and macrophages in response to inflammatory cytokines. When PD-1 interacts with PD-L1/PD-L2 it can suppress T cell responses. It might also promote T regulatory formation. If the PD-1/PD-L1 pathway can be blockaded, T cell activation activity may be enhanced. This explains why the blockade of the PD-1/ PD-L1 pathway is being promoted as a potential cancer treatment.

CTLA-4 and PD-1 are similar in that they are coinhibitor receptors on T cells that block T cell activation and proliferation. From the other end, the antigen presenting cells (APC) which are necessary in T cell activation have ligands that play a role in coinhibition as well. The role of a ligand called B7-H3 is not completely understood; conflicting studies found evidence suggesting that B7-H3 is a positive costimulator while others found the exact opposite. In mice, the B7-H3 molecule is found to have the same effect as CTLA-4 and PD-1; it blocks T cell activation and proliferation (Prasad, et al., 2004).

In summary, the immune system is an important defense mechanism against cancer and is therefore a worthy target for cancer therapy. The adaptive branch of the immune system is responsible for antigen-specific immune attacks. The principle effector cell of the adaptive branch, the T cell, plays a major role in cancer immunotherapy. During T cell activation the T cell matures and expresses CD4 or CD8 coreceptors. In another step to T cell activation the T cell receives a signal from costimulatory or coinhibitory molecules. Some of these coinhibitory molecules, such as CTLA-4 and PD-1, inhibit T cell activation and proliferation. The role of these coinhibitor T cells is explored as a new immunotherapy approach in cancer treatment, specifically in coinhibition blockade. If coinhibitory receptors can be blockaded, there is a chance that immune function may be enhanced instead of suppressed.

As previously noted, costimulation and coinhibition through the B7 and CD28 family plays a role in regulating T lymphocyte activation. This can result either in cancer tolerance, or anti-cancer reactions by the immune system. Up-regulation of coinhibitory B7/ CD28 and PD-L1 members on tumor cells can create tumor evasion pathways. Chemotherapy can affect the expression of these molecules, for example, by increasing expression of PD-L1 on tumor cells (Janakiram, et al., 2012). This dampens the immune response against cancer. This is just one of many ways a tumor cell can suppress the immune system. While immunotherapy approaches offer the hope of activating a patient's immune system (even if the cancer is already far-reaching), this hope is not reflected by the current reality. The slow pace of progress is likely due to incomplete understanding of immune regulation in the context of cancer, and the fact that the tumor microenvironment is inherently immunosuppressive. However as researchers expand their understanding in this regard, new ideas are advancing and being translated into improved anti-tumor immunotherapy. Immunotherapy targeting T cell coinhibition as monotherapy or combined with standard therapies, such as chemotherapy, are in early stages of clinical development but hold great potential for treatment of human cancer.

Studies show that breast tumors, among other tumors, have the ability to suppress the immune system, specifically by suppressing the T cell response through T cell coinhibition. As previously discussed, activation of a T cell requires two signals: the presentation of a specific antigen on the MHC of an antigen presenting cell, together with a costimulatory signal delivered by the antigen presenting cell to the T cell. CTLA-4 is a coinhibitory member of B7/CD28 group and it is expressed in breast tumors. It negatively regulates the proliferation and the effector functions of T cells. In breast cancer patients, CTLA-4 is up-regulated in activated T cells and binds to B7-1 or B7-2. CTLA-4 reduces T cell activation in two ways. The first is through direct inhibitory signals, and the second is through opposition of CD28 binding (Krummel, Allison, 1995). CTLA-4 is strongly expressed in breast cancer patients, in contrast to normal breast tissue where it is weakly expressed. This abnormal expression helps explain the evasion of anti-tumor immune responses in breast cancer patients. Some costimulators are limited in where they might be expressed in the body. Costimulators B7-1 and B7-2 are limited to lymphoid organs. PD-L1 and B7-H3 are not limited; they can be expressed in non-lymphoid organs and on tumor cells in cancers. Since abnormal expression of costimulators partially explains tumor evasion pathways, the balance of costimulation and coinhibition can be used as an important checkpoint in T cell function. CTLA-4 in particular is important in the development of breast cancer.

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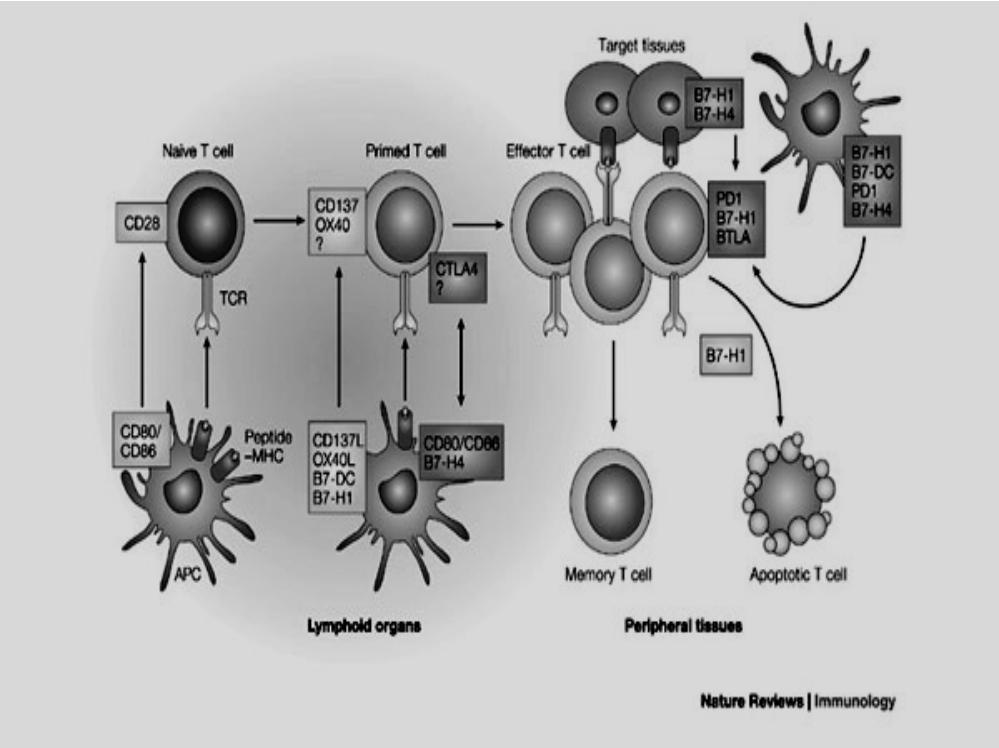
Figure 1: The Co-Signal Network Model (Chen, 2004)



This figure shows the co-inhibitory molecules of the B7-CD28 family in the control of T cell immunity (Chen, 2004). Costimulators and coinhibitors function in controlling the many stages of T cell activation. These may include priming, differentiation, maturation and memory responses. Naïve T cells in lymphoid organs constitutively express CD28. After ligation by the T cell ligands (CD80 or CD86) from an antigen presenting cell, the priming stage occurs. Then a series of costimulator and coinhibitor interactions occur that lead to the differentiation and maturation of primed T cells into effector T cells. Interactions between costimulatory molecules like CD137 and OX40 with their respective ligand receptors promote T cell maturation. Interactions
between coinhibitors such as CD80/CD86 with the CTLA-4 receptor can *negatively affect the maturation of a primed T cell into an effector T cell. It is important that strongly self-reactive T cells are destroyed in the lymphoid organs (like the thymus)* during the maturation process. If they were not inhibited they might cause damage to
the body. After maturation the effector T cells travel into peripheral tissues, where they are regulated by co-signals from their target cells. At this stage, the negative regulation of coinhibitors again is needed to prevent potential damage of tissues. One coinhibitory pathway is B7-H1-:D1 (a.k.a. programmed cell death 1) with receptor B7-H1-B7-H4.

In breast cancer, the costimulatory signal balance is skewed towards coinhibition due to dysregulation of the expression of some B7 and CD28 family members. Studies indicate at least three types of immune dysregulation in breast cancer patients. First, there is some dysregulation in breast cancer patients involving CTLA-4, not only in the tumor microenvironment but also possibly extending

to the systemic immune system (Zhang, et al., 2011). Another dysregulation recorded in breast cancer patients is that PD-L1 is highly expressed in breast cancer tissue samples, while PD-L1 is not expressed in normal breast tissue. In fact, PD-L1 expression is higher specifically in tumors that have a higher proliferation index (Ghebeh, et al., 2007). This suggests that the PD-L1/ PD-1 pathway is more important in certain, more serious and dangerous, breast cancer subtypes; this includes breast cancer subtypes that have a higher proliferation rate, and high lymphocytic response. A third dysregulation prominent in breast cancer is that B7-H3 is expressed in breast cancer tissue samples but not in normal breast tissue (Arigami, et al., 2010). Expression of B7-H3 is associated with an increase in tumor size and overall lymph node metastasis, which also indicates a more dangerous subtype of breast cancer. All of these dysregulations show that cancer affects the balance of costimulation and coinhibition through the expression of T cells. Researchers wish to reverse these dysregulations, and that is why it is imperative that they understand the mechanism behind the co-signal network, and T cell expression. Developing T cell-based immunotherapy strategies focus on coinhibition blockade of T cells as a cancer treatment, to rectify the skewed balance of coinhibition in cancer patients.

After decades of minimal results, researchers have finally managed to achieve some therapeutic success with the blockade of T cell coinhibitory molecules in the past few years. The development of the antibodies of coinhibitory molecules in the form of drugs may be the most important development in cancer immunotherapy history yet. Clinical studies in metastatic melanoma have shown tumor regression and an improvement in overall survival of patients after treatment with the anti-CTLA-4 antibody. Both CTLA-4 and PD-1 antibodies have produced cases of long-lasting remission in patients treated with these antibodies. However, these drugs can also result in adverse autoimmune side effects including pneumonitis, colitis, hepatitis and diabetes mellitus. The most common drug-related negative effects include fatigue, rash, nausea, pruritis and headache. Majority of the drug-related adverse events in trials were low grade, and were handled with interruption or discontinuation of treatment. The side effects in general do not seem to be as harsh as those caused by cancer chemotherapy treatments. Many chemotherapy side effects occur because the treatment affects non-cancerous parts of the body in addition to destroying cancer cells. Since this does not happen in immunotherapy treatments, there is a smaller risk of painful side effects. The mechanism behind this treatment is coinhibition blockade, in order to reverse the dysregulation of T cell coinhibitory molecule expression. Therefore, the development of antibody based immunotherapy not only gives cancer patients the hope of survival, but also the possibility of a gentler treatment option. The overall results of coinhibition blockade therapy prove that stimulating the immune system in this way can effectively induce long standing anti-tumor immunity even in advanced cancers.

The creation of CTLA-4 antibody based therapy in breast cancer initially began with clinical trials from mouse models. In the trials, the mice were treated with a vaccine followed by anti-CTLA-4 antibody. The result of this treatment was that the mice rejected submucosa1 cell line-induced mammary carcinoma. In addition, they were immune to re-challenge of the same cancerous cell line afterward. Following this success in mice, a human anti-CTLA-4 antibody called Tremelimumab was developed. Tremelimumab worked by blocking the binding of CTLA-4 to B7-1 and B7-2 on the antigen presenting cell. It was tested in combination with Exemestane, an inhibitor of the production of estrogen

(Vonderheid et al., 2012). In a phase 1 study, 26 patients with advanced hormone receptor positive breast cancer were treated with Tremelimumab every 28 days or every 90 days, along with Exemestane orally daily. Due to an increase in diarrhea, the 28 day dosing schedule was discontinued later in the trial. The discontinuation of treatments that cause overly harsh side effects shows the caution that researchers are displaying in their methods. In order to ensure the safety of the patients involved in the trial the maximum tolerated dose is not reached. The overall response rate was rewardingly positive—stable disease in 11 out of 26 patients (42 percent). The treatment was associated with an increase in the ratio of CD4 and CD8 T cells in comparison with T regulatory cells. This suggests enhanced cellular immune function due to this treatment because it led to proliferation of effector T cells. As previously mentioned, while T regulatory cells are important in immune regulation, they are not so helpful in eliminating cancer cells. Since treatment with the anti-CTLA-4 antibody increased effector T cell activation, and reduced Treg cells comparatively, it resulted in enhanced cellular immune function overall. The results of this trial were extremely encouraging, motivating the creation of Phase II and III trials in a similar vein, many of which are currently in progress.

Based on the results of anti-CTLA-4 therapy in mouse tumor models, two human anti-CTLA-4 antibodies, Ipilimumab and Ticilimumab, were developed and have entered into multiple clinical trials. In addition to prostate, ovarian, breast, and colon carcinoma, these antibodies have been tested in metastatic melanoma and in renal cell cancer. As previously mentioned, there are a lot metastatic tumors that cannot be wiped out by the available anti-cancer therapies, so any progress that can be made in this area (i.e. metastatic melanoma) is vital. Encouraging observations can be drawn from these phase I/II clinical trials. Anti-CTLA-4 monotherapy is capable of inducing objective tumor responses (meaning partial or even complete response) in patients with melanoma, renal cell, and non-Hodgkin's lymphoma. Objective tumor responses were observed to be as high as 20 percent in heavily pretreated melanoma patients. This shows that anti-CTLA-4 can enhance the immune system and be used as a successful treatment even after many other treatments have failed to cure the patient. Another positive result was that CTLA-4 blockade does not appear to inhibit the function of T regulatory cells, which are necessary to prevent autoimmune diseases. The most common adverse reactions to these trials involved the skin (rash and pruritus) and the gastrointestinal tract (diarrhea and colitis). In the event of severe reactions the therapy was either discontinued, or the reaction was reversed with the treatment of steroids (Zang, Allison, 2007).

The most successful anti-CTLA-4 drug, Ipilimumab, has already entered phase III trials in late-stage metastatic melanoma (Hodi, et al., 2010). This immunotherapy treatment can be used either as an initial treatment or after relapse. What sets Ipilimumab apart from previous cancer treatments is its ability to increase the chance of survival in cancer patients with no other therapeutic options. In March 2011 the FDA granted broad approval for its usage in cancer patients suffering from metastatic melanoma. One of the initial goals for developing cancer immunotherapy approaches was the hope of being able to activate a patient's immune system, even in the event of late-stage, advanced cancer. Impilimumab may have the ability to finally bring this goal to fruition.

Many human cancers have been reported to express the ligand PD-L1, including melanoma and cancers of the lung, ovary and breast. There as inverse correlation between PD-L1 expression in tumor

cells and poor prognosis of patients (Konishi, et al., 2004). This means that an increase in PD-L1 expression is linked to poor prognoses of cancer patients. Patients with high expression levels of PD-L1 displayed aggressive tumors and were at a noticeably increased risk of death from renal cell carcinoma. The trend appears to be that the more PD-L1 expressed by the patient the worse off they are. Studies also showed that intratumor expression of PD-L1 had a significant correlation with clinical outcome in breast cancer, ovarian cancer and pancreatic cancer. For example, 34 percent of breast cancer patients had intratumor expression of PD-L1. This is a decidedly significant percentage, and worthy of noting. Due to the correlation between PD-L1 expression and poor prognoses in cancer patients, researchers are looking to reverse this effect through immunotherapy strategies. One possibility of an immunotherapy approach may be to blockade the PD-1/PD-L1 pathway.

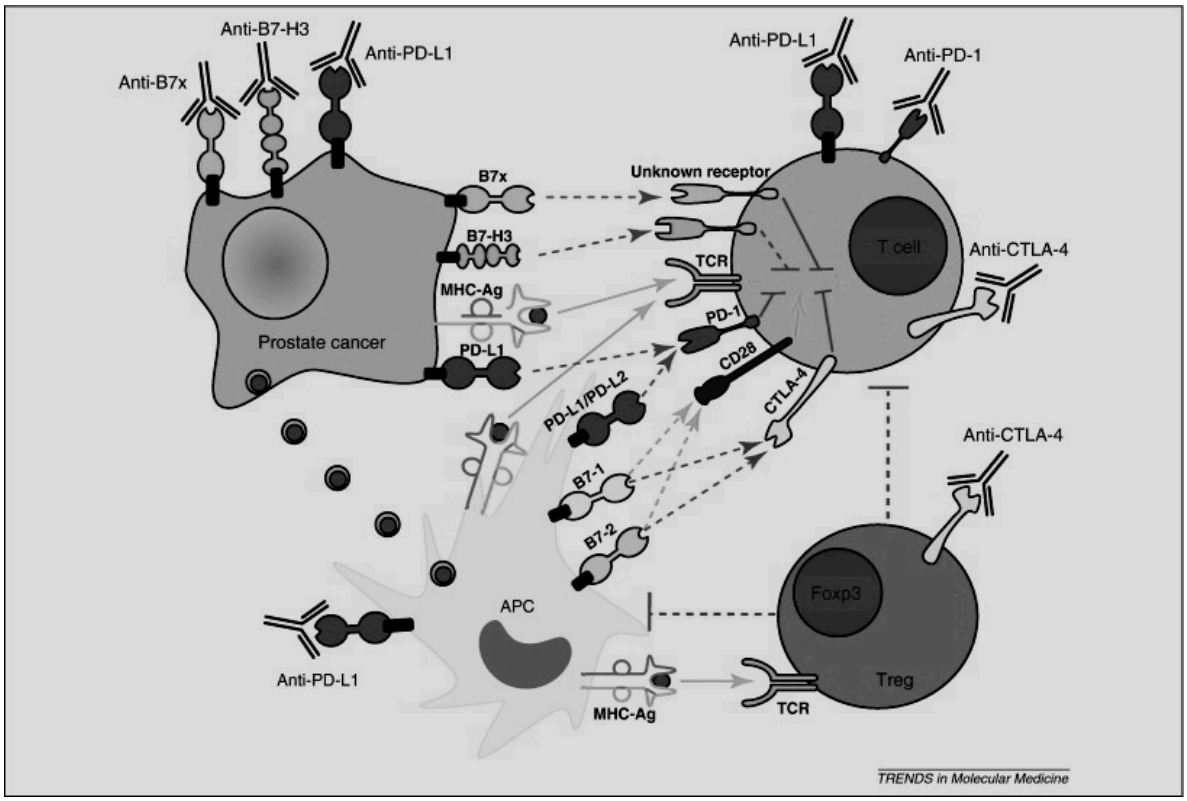
Clinical trials testing the treatment of anti PD-1 antibody in cancer are in progress. In a phase 1 study, the anti-PD-1 antibody was evaluated in 296 patients with advanced refractory solid tumors. It was studied at different doses of 1.0, 3.0 or 10.0 mg per kilogram of body weight (Topalian et al., 2012). Researchers need to experiment with different doses in order to determine the most effective one. As is the case in most immunotherapy trials, the maximum tolerated dose was not reached and the common side effects were skin rash, diarrhea and pruritus. The overall response rate was 18 percent in patients with non-small cell lung cancer, 28 percent in melanoma, and 27 percent in patients with renal cell cancer. 36 percent of patients whose tumors expressed PD-L1 demonstrated an objective response. An objective response means that the patients responded partially or even completely. The patients whose tumors did not express PD-L1 did not demonstrate a response. This is only logical because the PD-1 receptor binds to the PD-L1 ligand. If the ligand is not expressed by the tumor, it is likely that the PD-1 is not causing the adverse effect of coinhibition in these cancer patients. Therefore, if the tumor is not being assisted via PD-1 expression, then it cannot be cured through anti-PD-1 antibody treatment. Due to the effectiveness in this treatment in patients whose tumors expressed PD-L1 additional studies of different anti-PD-L1 antibodies are being evaluated. However, since the effectiveness of the anti-PD-1 antibody is limited to patients whose tumors express PD-L1, it seems a less important development than the anti-CTLA-4 antibody.

Both CTLA-4 blockade and PD-1 blockade have specific mechanisms that are used in coinhibition therapy. The basis of this therapy lies in the T cell maturation process. The cross-priming process is critical for the initiation of T cell responses to tumors (Huaung, et al. 1994). During this activation process antigen presenting cells (APCs) can pick up antigens released from tumor cells, and present them to naïve T cells in the context of B7-1 and B7-2 costimulation (see figure 2). Upon T cell activation, CTLA-4 is expressed and it starts to carry out its inhibitory function. Therefore, the mechanism behind anti-CTLA-4 antibody treatment lies in the specific blockade of CTLA-4 signals. The result of the blockade is intact T cell receptor and CD28 signals, and enhanced effector T cell function.

The molecular mechanisms regulating PD-L1 expression are not as clear as CTLA-4 expression. Two striking trends have been recorded regarding PD-L1 expression. Apparently, inflammatory mediators are responsible for up-regulation of PD-L1 expression on the surface of several tumor cell lines. Additionally, PD-L1 expression was higher in freshly isolated tumor tissue specimens than in

cultured tumor cell lines (Dong, et al., 2002). The expression of PD-L1 in newly isolated tumor tissue suggests that cytokines in the tumor microenvironment induce the expression of PD-L1 on tumor cells. It follows that these cytokines are not present in cultured tumor cell lines, which would explain the lower level of PD-L1 expression in these samples. Understanding the regulation of PD-L1 expression in cancer better will help clarify the links between oncogenesis and cancer immune evasion. This will in turn help to refine immunotherapy approaches. Although the extent to which PD-L1 protein expression directly affects tumor progression remains to be determined, it is generally accepted that expression of PD-L1 on tumor cells impairs antitumor immunity in the body. Therefore the blockade of the PD-L1/ PD-1 pathway is another possibility for tumor immunotherapy.

Figure 2: Blockade of T-cell Coinhibition (Barach, et al. 2012)



This figure illustrates the blockade of T cell coinhibition as an immunotherapy approach for cancer, specifically in prostate cancer (Barach, et al., 2012). It shows the relationship between the tumor cell, antigen presenting cell, T cell and T regulatory cell. The blockade of CTLA-4 serves a dual purpose: first, it enhances antitumor immunity by leaving T cell receptors and CD28 signaling intact, and second, the blockade of CTLA-4 on T-regulatory cells reduces T-regulatory immunosuppression. The figure also shows that immune cells infiltrating prostate cancer have increased expression of PD-L1 and PD-1.

In clinical trials of PD-1 antibodies two metastatic tumor models have already proven to be sensitive to PD-1 blockade (Thompson, et al, 2004) (Konishi, et al., 2004). Administration of PD-1

blocking antibodies markedly inhibited colon carcinoma metastasis to the lung and melanoma metastasis to the liver. Both antigen presenting cells and T cells express PD-L1; therefore, enhanced antitumor immunity via blockade of the PD-L1 pathway is likely the result of inhibition of interaction between PD-L1 on tumor cells and PD-1 on T cells. The anti-PD-1 antibody trials must be monitored carefully because PD-L1 is expressed widely, not only in tumors, but also in immune cells and other tissue cells. Thus, there is a possibility of a scenario where anti-PD-1 blockades PD-L1 expression in parts of the body where there are no cancer cells. This would not be ideal because one of the aspirations of immunotherapy approaches is to target cancer cells without negatively effecting normal cells (which chemotherapy does). Potential autoimmune diseases may be induced by the blockade of PD-L1/PD-1, thus necessitating meticulous attention and caution in clinical trials.

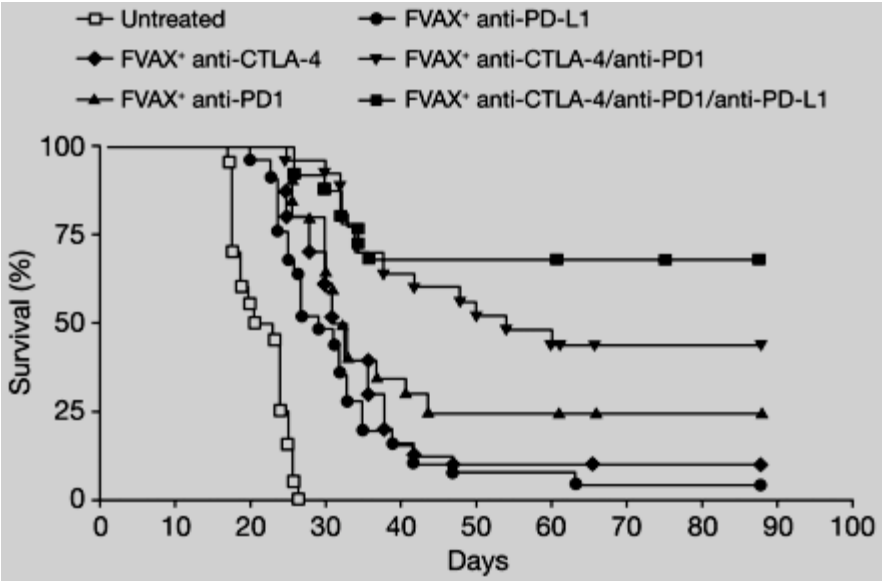
Combination immunotherapy approaches involving chemotherapy have been studied extensively in animal models, setting the stage for clinical trials. Chemotherapy may boost the immune response to cancer; however, the potential immunosuppressive effects of chemotherapy render issues of dosing and timing critical. An exciting combinatorial approach is the co-administration of multiple immunological treatments. For example, the combined blockade of PD1 and CTLA-4 has shown to enhance antitumor immune responses compared with either agent alone. Combining immunotherapies either with each other or with other modalities of cancer treatment, such as chemotherapy, could lead to enhanced effectiveness with diminished toxic effect.

Ipilimumab, a fully human monoclonal antibody against CTLA-4, has been clinically evaluated in combination with Dacarbazine, a chemotherapy agent. In a recent phase III trial, patients with metastatic melanoma receiving Ipilimumab in combination with Dacarbazine had significantly improved overall survival (11.2 months) compared with patients receiving Dacarbazine alone (9.1 months) (Thomas, et al., 2011). Additionally, an important phase II trial in patients with stage IIIb/1V non-small-cell lung cancer or extensive-disease small-cell lung cancer investigated whether it would be optimal to initiate Ipilimumab at the same time as chemotherapy, or after two cycles of treatment. The goal was to determine the timing of treatments that would yield the best prognoses for the patients. The results from this trial showed that a ‘phased regimen’ in which immunotherapy began after chemotherapy resulted in substantially improved progression-free survival compared with chemotherapy alone. The data clearly shows that the clinical effects of administering immunotherapy in combination with chemotherapy are strongly dependent on the sequencing of treatment. The study did not actively investigate dosing effects, so additional studies are necessary to gather more information in that regard. Since chemotherapy treatment was used it is likely that the typical side effects were experienced. Since the general adverse effects of immunotherapy are not very harsh, the addition of Ipilimumab as a treatment after chemotherapy appears to be a valuable option, especially considering the clear increase in survival rate it causes.

Immunotherapeutic agents, with differing mechanisms of action, could be combined as a means of further enhancing immune responses against tumors. In this regard, recent data suggests that antitumor T cells may express multiple inhibitory receptors. In order to effectively blockade the antitumor T cell entirely, it is likely that more than one antibody must be employed. Single blockade of either CTLA-4 or PD-1 have been shown to enhance the infiltration of activated T cells into tumors, but

the T cells accumulated high levels of unblocked negative coreceptors that eventually limited their expansion. Hence, singular blockade with merely one antibody can have serious limitations. Blocking CTLA-4, PD1 and PD-L1 simultaneously allowed T cells to continue to survive, and resulted in enhanced infiltration, activation and cytokine production (Curran MA, 2010). This resulted in decreasing tumor-induced immune suppression, which ultimately promoted tumor rejection.

Figure 3: Combination Blockade of PD-1, CTLA-4, PD-L1 with FVAX (Drake, 2012)



Combination blockade of the PD1, CTLA-4 and PD-L1 coinhibitory molecules coupled with Fvax vaccination increased survival of mice challenged with antigen-presenting melanoma cells (Drake, 2012). Untreated mice had survival rate of 0 percent after 27 days. Mice treated with different combinations such as Fvax plus anti-CTLA-4, Fvax plus anti PD-1, and Fvax plus anti-PD-L1 showed a survival rate of up to 25 percent after 90 days. The mice that were treated with Fvax, anti-CTLA-4, anti-PD1-1 and anti-PD-L1 had the highest survival rate, a rate of up to 75 percent survival after 90 days. This study clearly shows the advantages of blockading multiple coinhibitors at once. In this case, combinatorial blockade treatment was experimented with vaccination as a treatment as well, and it resulted in an increased survival rate in those mice.

Similar results were obtained in a mouse model of metastatic colon carcinoma, evaluating the combination of IL-15 with antibodies against CTLA-4 and PD-L1. IL-15 is another promising approach in cancer immunotherapy. In this study, although IL-15 significantly prolonged survival in mice with metastatic tumors, it also increased the expression of PD1 and the secretion of the immunosuppressive cytokine, IL-10. These unexpected side effects could potentially have a negative impact on the immune system. However, the mice were also given anti-CTLA-4 and anti-PD-1 antibodies, which blockade the expression of coinhibitors. In this situation, they could be employed to reverse the effect that the IL-15 had in increasing coinhibitorial expression. Combining the immune stimulatory properties of IL-15 with the simultaneous removal of two critical immune system inhibitors significantly increased antitumor

activity compared with IL-15 alone or combined with either anti-PD-L1 or anti-CTLA-4 (Steel, et al., 2010). This data supports the idea that the synergistic blockade of multiple checkpoints can enhance immune responses. No part of the treatment combated the secretion of additional immunosuppressive IL-10, so that is an aspect that might need to be addressed. However, the results of combining IL-15 and antibodies of CTLA-4 and PD-L1 thus far are encouraging. They prove that immunotherapy approaches that work to stimulate the natural immune system are effective, in that they enhance anti-tumor activity and increase the overall chance of survival for cancer patients.

Conclusion

Immunotherapy, a relatively new form of cancer treatment, functions through influencing the immune system's natural response. The approach offers the hope of activating a patient's immune system even if the cancer is already far-reaching. However, progress has been slow in this field in the past due to incomplete understanding of immune regulation in the context of cancer. New understanding is being translated into improved anti-tumor immunotherapy. T cells in particular play an important role in many new developing clinical trials. Coinhibitor T cells such as CTLA-4 and PD-1 block T cell activation and proliferation. Their coinhibitory functions are explored in an immunotherapy approach called coinhibition blockade. Researchers have achieved some therapeutic success with the blockade of T cell coinhibitory molecules in the past few years. CTLA-4 and PD-1 antibodies have produced cases of durable remission in patients treated with these antibodies. Two human anti-CTLA-4 antibodies—Ipilimumab and Ticilimumab—have been developed and entered into clinical trials. Ipilimumab in particular appears to be a valuable new option in cancer immunotherapy. The rationale behind Ipilimumab monotherapy is that anti-tumor T cells exist in the patient before the therapy, and these cells will exert anti-tumor activity if CTLA-4 is blockaded. PD-1 antibody therapies are also an option to be explored in new cancer treatments. There is an inverse correlation between PD-L1 expression in tumor cells and poor prognosis of patients. Administration of PD-1 blocking antibodies has markedly inhibited colon carcinoma metastasis to the lung and melanoma metastasis to the liver. Even more effective than coinhibition blockade immunotherapy, are new combination immunotherapy approaches, as well as immunotherapy plus chemotherapy combination treatments. A study on Ipilimumab, an antibody against CTLA-4, combined with a chemotherapy drug called Dacarbazine showed significantly improved survival compared with treatment of Dacarbazine alone. Another study in combination immunotherapy showed that the blockade of multiple T cell receptors (CTLA-4, PD-1 and PD-L1) combined with Fvax vaccine yielded the highest survival rate in mice. Defining the optimum dose and schedule of combination therapies remains a major challenge, and clinical investigations to optimize dose and schedule in patients are required. Combining immunotherapies, particularly agents that target different T cell coinhibitors, is a promising cancer treatment approach, with the potential for an increase in overall survival.

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