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REVIEW ARTICLE

Cancer Immunotherapy: A Review

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Abstract

BACKGROUND: The goals of treating patients with cancer are to cure the disease, prolong survival, and improve quality of life. Immune cells in the tumor microenvironment have an important role in regulating tumor progression. Therefore, stimulating immune reactions to tumors can be an attractive therapeutic and prevention strategy.

CONTENT: During immune surveillance, the host provides defense against foreign antigens. By targeting surface antigens expressed on tumor cells, monoclonal antibodies have demonstrated efficacy as cancer therapeutics. Recent successful antibody-based strategies have focused on enhancing antitumor immune responses by targeting immune cells, irrespective of tumor antigens. The use of antibodies to block pathways inhibiting the endogenous immune response to cancer, known as checkpoint blockade therapy, has stirred up a great deal of excitement among scientists, physicians, and patients alike. Clinical trials evaluating the safety and efficacy of antibodies that block the T cell inhibitory molecules cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) have reported success in treating subsets of patients. Adoptive cell transfer (ACT) is a highly personalized cancer therapy that involve administration to the cancer-bearing host of immune cells with direct anticancer activity. In addition, the ability to genetically engineer lymphocytes to express conventional T cell receptors or chimeric antigen receptors has further extended the successful application of ACT for cancer treatment.

SUMMARY: The underlying basis of cancer immunotherapy is to activate a patient's own T cells so that they can kill their tumors. Reports of amazing recoveries abound, where patients remain cancer-free many years after receiving the therapy. The idea of harnessing immune cells to fight cancer is not new, but only recently have scientists amassed enough clinical data to demonstrate what a game-changer cancer immunotherapy can be. This field is no stranger to obstacles, so the future looks very promising indeed.

KEYWORDS: immune checkpoint, adoptive cell transfer, neoantigen, monoclonal antibody

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Introduction

The past few decades have seen a grounds well of research on the immune system yielding a deeper understanding of how cancer progresses and offering new ways to stop it.(1) In 1891, William Coley injected cancer patients with bacteria to ignite an immune response, a strategy is experiencing a revival. Now immunologists are finding ways to harness the immune system, including training immune cells to recognize a patient's particular cancer.(2,3) The finding that tumors can actively suppress immunity has led to the development of checkpoint blockades that prevent this suppression.(4)

Last year's Lasker DeBakey Clinical Research Award was awarded to James Allison for discovering that antibody blockade of the T cell molecule cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) unleashes the body's immune response against malignant tumors. This lead to development of multiple immune checkpoint therapies that are prolonging and saving the lives of many cancer patients.(5) The successful treatment of multiple mouse tumors with anti-CTLA-4 impressed many immunologists, but the perceived failure of many earlier immune-based therapies created a very high bar for advancing immune checkpoint therapy to the clinic. The turning point came with 3 trial comparing Ipilimumab to a melanoma peptide vaccine in metastatic melanoma patients who had the human leukocyte antigens (HLA) A0201 allele.(6) Key to the success of the study was the decision to evaluate overall survival rather than response rate, and in the large study, Ipilimumab monotherapy resulted in more than 20% long-term survival. The success of this trial led the Food and Drug Administration (FDA) to finally approve Ipilimumab for the treatment of metastatic melanoma in 2011.

At around the time when Ipilimumab was being considered for FDA approval, renewed excitement about immune checkpoint therapy came from clinical studies targeting a second immune inhibitory molecule, programmed cell death 1 (PD-1). PD-1 was discovered by Tasuku Honjo in 1992 in a screen for genes expressed during programmed cell death of a T cell hybridoma.(7) Unlike CTLA-4, which functions mainly during primary immune responses, PD-1 signaling results in exhaustion of activated T cells, an anergic-like state that is thought to be due to a shift in the utilization of metabolic substrates.(8) Antibody blockade of PD-1 was shown to enhance anti-tumor and anti-viral responses in animal models, suggesting that this could be another immune checkpoint target for cancer.

Schering Plough acquired an anti-PD1 antibody developed by Organon, and this was introduced into the clinic as Pembrolizumab, after the company was acquired by Merck, and was approved by the FDA for treating advanced melanoma in 2014. The Bristol-Myers Squibb (BMS) drug, Nivolumab, was approved very shortly thereafter. The change in attitude of clinical oncologists and immunologists toward the place of immune modulation in combating cancer guarantees that there will be many exciting advances in immune-based therapies in the years ahead.(5)

Cancer Immunology

Although originally considered as monoclonal, tumor cells show heterogeneous morphology and behavior.(9,10) This heterogeneity has traditionally been explained by the clonal evolution of tumor cells resulting from the progressive accumulation of multiple genetic (11) or epigenetic changes (12). Alterations in tumor stroma microenvironments may also promotes the development of tumor cell heterogeneity through extrinsic activation of certain tumor cell signaling pathways.(13) Moreover, recent studies have suggested that heterogeneity is a result of the hierarchical organization of tumor cells by a subset of cells with stem or progenitor cell features known as cancer stem cells (CSC).(14)

The concept of cancer as an abnormal stem cell disease was proposed based on the similar abilities of cancer cells and normal stem cells to self-renew, produce heterogeneous progeny and also divide in an unlimited fashion.(15,16) However, the CSC hypothesis has only recently been experimentally validated by the identification of a subset of certain self-renewing stem cell marker-positive cells with a hierarchical organization.(17,18) The self-renewal capacity is confirmed by serial in vitro clonogenic growth and in vivo tumorigenicity. CSC are also known as tumor-initiating cells or tumor-propagating cells. CSC are highly tumorigenic, metastatic, chemotherapy and radiation resistant, responsible for tumor relapse after therapy, and able to divide symmetrically and asymmetrically to orchestrate the tumor mass.(19) Therefore, CSC are a pivotal target for the eradication of many cancers including liver cancer.(20)

Some cancer cells may be disseminated and leave tumor relentlessly, only to perish en masse, find a good time to reinitiate a full-fledged tumor and settle there arising metastasis in distant tissues.(21,22) Yet when metastasis occurs, it creates complications that account for the vast majority of deaths from cancer. Cancer cells that succeed in doing this task possess not only the attributes of tumor-initiating cells, but also the ability to exert this capacity under harshly adverse conditions. Metastasis therefore is driven by CSC at their best, or at their worst, depending on your perspective.(23)

As tumors are heterogeneous and show distinctive genetic and epigenetic profiles, there may not be a single biomarker that will prove sufficient information for predicting treatment response and patient outcome. Examples of informative tumor biomarkers are molecular features of neoplastic cells, including epidermal growth factor receptor (EGFR) mutations in lung cancer (24,25); microsatellite instability (MSI) in colorectal cancer (26-28); estrogen receptor 1 (ESR1), progesterone receptor (PGR) and erb-b2 receptor tyrosine kinase 2 (ERBB2/HER2) expression in breast cancer (29,30); transmembrane protease, serine 2 and ETS-related gene fusion (TMPRSS2-ERG) translocation in prostate cancer (31); and CpG island methylation, and kirsten rat sarcoma viral oncogene (KRAS), B-raf proto-oncogene (BRAF), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) and tumor protein(TP)-53 mutations in multiple cancer types (32-34). In addition to tumor markers, host factors which include the immune response to the tumor might determine tumor behavior or serve as informative biomarkers.(35)

During carcinogenesis, tumor cells interact with a complex microenvironment which is composed of extracellular matrix and non-neoplastic host cells, including mesenchymal cells, vascular endothelial cells and inflammatory or immune cells. Inflammatory and immune cells are present to varying degrees (from absent to intense) in the tumor microenvironment, which can be observed routinely in pathology practice. The tumor microenvironment provides nutrients, oxygen, growth factors, cytokines, and other chemical mediators that support tumor proliferation, survival, invasion, and metastasis for the cancer cells.(36) The immune system can respond to cancer cells in two ways, which are by reacting against tumor-specific antigens (molecules that are unique to cancer cells) or by reacting against tumor-associated antigens (molecules that are expressed differently by cancer cells and normal cells).(37) Immunity to carcinogen-induced tumors in mice is directed against the products of unique mutations of normal cellular genes. These mutant proteins are tumor-specific antigens.(38)

The immunosurveillance hypothesis posits that the immune system recognizes malignant cells as foreign agents and eliminates them. This idea was contentious until the understanding of tumor immunity improved and better techniques and animal models became available to test it rigorously. Mouse models in which immune effector mechanisms such as the type 1 interferon (IFN) were eliminated by gene deletion showed a clear reduction in the incidence of tumors by the immune system.(39-43) In animal models, the encounter between the immune system and a nascent tumor initiates a process termed ‘immunoediting’ that can bring about three outcomes, those are elimination of the cancer; cancer equilibrium, in which there is immune selection of less immunogenic tumors during an antitumor immune response; and tumor escape, the growth of tumor variants that resist immune destruction.(44,45)

Relation between immune system and cancer is complex and dynamic. Whereas there are a series of scenarios in which the immune system exerts an antineoplastic surveillance, there are also other situations in which immune processes contribute to the transformation and progression of malignant tumors.(46) This paradigm of tumor immunology is known as cancer immunoediting, and is composed of three stages, which are elimination, equilibrium and escape (Figure 1).(46-48) The elimination phase is difficult to study, and clear evidence of its existence is scarce in humans, as elimination is believed to take place in a preclinical stage and often times represents the resolution of the disease process. Elimination was thought to be carried by the innate and adaptive immune systems, and natural killer (NK), natural killer T (NKT), cluster of differentiation $(CD)8^+$ cytotoxic and $CD4^+$ lymphocytes, macrophages as well as dendritic cells (DC) that participating in the presentation, recognition and lysis of cells displaying tumor antigens.(49) Elimination is often limited, and some tumor cells are left alive, either due to their antigenic or immune-related gene expression profile allows these cells to survive the initial immune surveillance entering an equilibrium phase. In this phase, there is a dynamic balance between anti-tumoral immunity and tumor cells.

Cancer development was showed in patients transplanted with donor-derived tumors, even when there was no cancer clinical manifestation found. This suggest the existence of human equilibrium stage.(49,50) The equilibrium is thought to be maintained by adaptive immunity.(46) At last, escape is the stage in which those tumor cells that are not detectable or have developed mechanisms to avoid immune recognition and lysis, get selected and then grow into a symptomatic lesion. An illustrating example of this phenomenon is the use of a vaccine targeting the EGFR variant III (EGFRvIII) in glioblastoma. Clinical studies have shown some effectiveness in patients with glioblastoma that originally expressed EGFRvIII, while upon recurrence expression was lost in the tumors.(51) In addition to the induction of an immunosuppressive state, another paradigm in tumor-immunology is the tendency for tumors to minimize the display of their antigens, which is resulting in another mechanism for evasion of anti-tumor immunity. The relative lack of a tumor-specific antigenic repertoire and the impairment of antigenic presentation by major histocompatibility complex (MHC) class I by tumors can diminish tumor recognition by cytotoxic T-lymphocytes.(52)

Tumors can suppress immunity both systemically and in the microenvironment of the tumor.(53) In addition to producing immunosuppressive molecules such as transforming growth factor β (TGF-β) (54) and soluble Fas ligand (55), many human tumors produce the immunosuppressive enzyme indoleamine-2,3-dioxygenase (IDO) (56,57). This enzyme was previously known for its

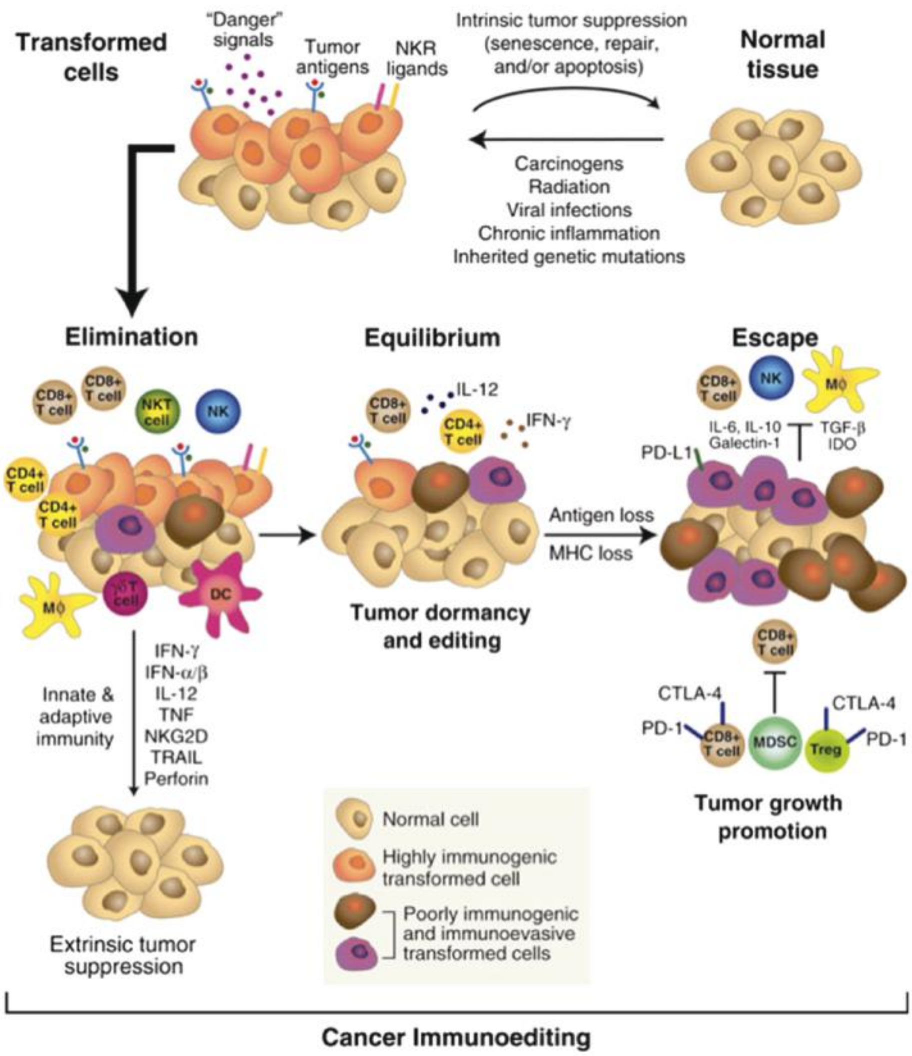

Figure 1. Cancer immunoedit-ing paradigm.(46) IDO: indoleamine-2,3-dioxygenase, TGF-β: transforming growth factor β, IL: interleukin, TNF: tumor necrosis factor, NKG2D: natural-killer group 2 member D, TRAIL: TNF-related apoptosis inducing ligand, MØ: macrophage. (Adapted with permission from Springer).

role in maternal tolerance to antigens from the fetus (58) and, more recently, as a regulator of autoimmunity that mediates inhibition of T-cell activation (59). Stereoisomers of 1-methyl-tryptophan inhibit IDO (60), and when administered to tumor-bearing mice, they restore immunity and thereby allow immune rejection of the tumor (61). Such stereoisomers might have a role in the treatment of patients with cancer.(62)

Tumor microenvironment can be dominated by regulatory T cells that suppress antitumor effector T cells by producing the immunosuppressive cytokines TGF-β and interleukin (IL)-10.(35) High numbers of these cells can be detected in non-small-cell lung cancer and ovarian cancer.(63) Murine tumors that produce TGF-β can convert antitumor effector T cells into regulatory T cells, thereby escaping their own destruction by immune cells.(64,65) The immunosuppressive effects of a tumor can also be systemic. An increase in regulatory T cells has been found in the peripheral blood of patients with head and neck cancer (66,67) or melanoma (68). Patients with colorectal cancer or pancreatic tumors have increased numbers of activated granulocytes (69) and myeloid-derived suppressor cells (70), both of which suppress tumor-specific T cells in mice (71,72). Up to now, the field of tumor immunology is providing an initial understanding of how these tumors might be avoiding immune recognition.(46)

Cancer Immunotherapy

For more than half a century, scientists have been trying to turn the body's immune system against cancer. But decades of failures have revealed that tumors have the ability to evade, tamp down and overwhelm the normal immune response. Most modern immune therapies try to get the immune system to recognize and attack tumor cells.(4) The administration of monoclonal antibodies (mAb) against tumor antigens in HER2-positive breast cancer (Trastuzumab) (73), B-cell lymphomas (Rituximab) (74), and head and neck, lung, and colorectal cancers that express the EGFR (Cetuximab) (75-77) is clinically effective (Table 1).(62) Efforts are ongoing to produce antibodies with new effector functions against known targets or to identify new targets for therapeutic antibodies. These targets could be tumor antigens or molecules produced by tumors to promote their own survival, such as vascular endothelial growth factor (VEGF) (78) and TGF-β (64). Antibodies can also target immune cells at the tumor site to aid the activation of effector cells and promote more effective antitumor immunity.(79)

Targeting host immunity is an attractive strategy for cancer therapy and prevention because therapy resistance is less likely to develop when host cells are targeted instead of altered molecules within tumor cells.(37,38) The latter approach frequently results in resistance to the initial targeted therapy owing to, for example, an acquired mutation in a domain of the therapeutic target that interacts with the drug. Host immunity can be targeted by the use of activated autologous peripheral-blood mononuclear cells (sipuleucel-T) (80,81), or the use of specific immunoregulatory molecules, such as recombinant vaccinia vector (targeting prostate-specific antigen) (82). One such treatment, the vaccine sipuleucel-T (marketed as Provenge by Dendreon Corporation in Seattle), was approved by the US FDA in 2010 for use in prostate cancer, which is a move that generated a lot of excitement. But the drug has proven disappointing, with benefits limited to a small percentage of patients, Dendreon is now reported to be for sale. The problem, researchers have slowly been realizing, is that stepping on the immune system's gas pedal isn't enough. It is also necessary to release its brakes, and that is where immune checkpoint blockades come in.(4)

In 2011, the US FDA approved the anti-CTLA4 drug Ipilimumab (developed by BMS and marketed as Yervoy), which was based on Allison's research and eventually saved the lives of some of Ribas's patients. CTLA-4 is not the only checkpoint being targeted by researchers and drug developers. Early trials suggest that drugs that block a different checkpoint, which is PD-1, are even more effective and have fewer side effects than Ipilimumab.(83) In recent studies, checkpoint blockades produced improvements in between 20% and 65% of patients, depending on the

Table 1. Immunologic reagents approved by FDA for cancer therapy.(62) (Adapted with permission from Massachusets Medical Society).

Table 1. Immunologic Reagents Approved by the Food and Drug Administration for Cancer Therapy.

Reagent	Reagent Target	Indications
Antibodies		
Trastuzumab (Herceptin)	HER2 receptor	HER2-positive breast cancer
Bevacizumab (Avastin)	Vascular endothelial growth factor	Non–small-cell lung cancer, colorectal cancer, and breast cancer
Cetuximab (Erbitux)	Epidermal growth factor receptor	Colorectal cancer and head and neck cancer
Panitumumab (ABX-EGF)	Epidermal growth factor receptor	Colorectal cancer
Ibritumomab tiuxetan (Zevalin)	CD20 B-cell surface antigen (nonradioactive and radiolabeled)	Non-Hodgkin's B-cell lymphoma
Alemtuzumab (Campath)	CD52 lymphocyte surface antigen	Chronic lymphocytic leukemia and T-cell lymphoma
Gemtuzumab ozogamicin (Mylotarg)	CD33 leukemic-cell surface antigen	Acute myeloid leukemia
Rituximab (Rituxan)	CD20 B-cell surface antigen	Non-Hodgkin's lymphoma
Tositumomab (Bexxar)	CD20 B-cell surface antigen (nonradioactive and radiolabeled)	Non-Hodgkin's B-cell lymphoma
Other	**Reagent Type**	
Denileukin diftitox (Ontak)	Recombinant interleukin-2 and fragments of diphtheria toxin (binds CD25 receptor on T cells)	Cutaneous T-cell lymphoma
Aldesleukin (Proleukin)	Interleukin-2	Melanoma and renal-cell carcinoma
Interferon alfa-2b (Intron A) and interferon alfa-2a (Roferon-A)	Recombinant interferon	Hairy-cell leukemia, chronic lymphocytic leukemia, Kaposi's sarcoma, melanoma, non-Hodgkin's lymphoma, multiple myeloma, and renal cancer
Imiquimod (Aldara)	Toll-like receptor 7 agonist	Basal-cell carcinoma

drug, dosage and type of cancer. In one long-term study of Ipilimumab in patients with advanced melanoma, 22% of the 1,861 patients survived for three years, and 17% for seven years or longer (with median survival nearly a year).(84) Historically, average survival was six to nine months.(84) Early research suggests that Ipilimumab may be even more effective when combined with other drugs. In further evidence for the value of drug combinations, Ipilimumab and Nivolumab appear to complement each other.(4) A few years ago, when Michel Sadelain spoke about adoptive cell transfer (ACT) therapy at cancer meetings, his colleagues were dubious about what seemed a drastic and unconventional approach: harvesting and genetically altering his patient's immune cells to train them to attack her cancer.(3)

There are three strategies for ACT therapies, the most developed of which is the simplest. The tissue surrounding a tumor is likely to contain immune cells with antitumor activity, so doctors take a sample of this tissue and select those T cells that have been primed to attack the cancer. They culture these cells in the lab until they have enough, and re-infuse the cells back to patients along with the T-cell growth factor IL-2, which promotes the proliferation of antigen-specific T cells.(3) This approach, called tumor-infiltrating lymphocyte (TIL) therapy, has been used successfully to treat only one type of cancer, that is metastatic melanoma. T cells that have been primed to attack a specific cancer are difficult to collect in a blood sample, but in melanoma these lymphocytes enter the tumor and are easy to biopsy. Currently, the success of TIL in melanoma is not transferable to other cancers, because it is harder to collect tumor-specific T cells. For those cancers, researchers are working hard in order to genetically modify T cells to hone their cancer killing skills.(3)

To do this, researchers are taking a couple of approaches. One option, called T cell receptor (TCR) therapy, involves giving the cells new receptors that allow them to recognize specific cancer antigens; the receptors can even be modified to improve their ability to find and bind to their targets. A more flexible tactic, called chimeric antigen receptor (CAR) therapy, avoids this constraint. It uses a gene that encodes artificial, antibody-like proteins that bind the antigens studding the tumor cell's surface without needing to match the patient's immune type. So part of the optimization process includes giving both TCR- and CAR-based T-cell therapies the optimal mix of enhancing molecules and targets to achieve the best response.

As ACT therapies move closer to the mainstream, the next big step will be investigating whether and how to integrate them with other cancer immunotherapies. Despite lingering questions, scientists and clinicians are enthusiastic about the potential of ACT. It represents a flexible platform for cancer treatment that can be tweaked and adapted as further discoveries are made.

Immunity results from a complex interplay between the adaptive immune system (which is antigen-specific) and the innate immune system (which is not). B cells and T cells of the adaptive immune system use receptors that recognize antigens, or their derived peptides, in a highly specific manner. DC provide an essential link between the innate and adaptive immune responses. The generation of anticancer immunity depends on DC presenting cancer antigens to T cells. But cancers can create an environment that inhibits T cells. The aim of DC vaccination is to boost cancer-specific effector T cells that can not only fight existing cancer but also induce immunological memory to control the recurrence of cancer.(2)

Topalian notes that patients treated with immune therapies could potentially gain a lifetime of protection, similar to the buffer against certain diseases offered by childhood vaccines. “We hope that the same thing is happening in cancer,” she says. “We hope that we are re-educating the immune system and that, even if it doesn't completely destroy every last cancer cell, it can keep it in check for a very long time.”(85) It is tempting to ask whether immunotherapy is evolving to become standard care for cancer patients, beyond those with advanced disease. Is there a place for therapeutic regimens that combine checkpoint blockade with other strategies? While we are nowhere near having all the answers, these studies provide a wealth of data supporting the idea that somatic mutations in cancer cells are an important target of endogenous anti-tumor responses. Checkpoint blockade is effective at rescuing the anti-tumor effect and it is plausible that understanding the dynamics of the response to this therapy will also help the development of alternative and personalized approaches to treat cancer.(86)

mAb Targetting Cancer-associated Proteins

Immune system is regulated by a reptilian complex balance of signals transmitted by stimulatory and inhibitory receptors. More than any other discovery, mAb have enabled us to identify and manipulate these molecules, provide an important new class of immunostimulatory therapeutics that can complement small-molecule therapeutics under active development.(87,88) Specific recognition by mAb has permitted the identification of cytokines and cell-surface molecules involved in humoral antibody-mediated and cellular immune responses.(79)

Antibodies may target tumor cells by engaging surface antigen differentially expressed in cancers. For example, Rituximab target CD20 in non-Hodgkin B cell lymphoma, Trastuzumab targets HER2 in breast cancer, and Cetuximab targets EGFR in colorectal cancer. Blocking the ligand-receptor growth can evoke the tumor cell death and survival pathways. Innate immune effector mechanisms engaging the Fc portion of antibodies via Fc receptors including complement-mediated cytotoxicity (CMC) and antibody-dependent cellular cytotoxicity (ADCC) are emerging as equally important.(89,90)

The natural properties of antibodies which enable specific antigen engagement can be leveraged and improved upon by engineering approaches that increase antitumor activity. One example is the creation of bispecific antibodies (bsAb) with dual affinities for a tumor antigen and either another tumor antigen or a target in the tumor microenvironment. As the Fc domain of mAbs does not directly activate T cells, CD3, the activating receptor for T cells, is a common target of bsAb. Catumaxomab is a bsAb that binds the tumor antigen epithelial cell adhesion molecule (EpCAM), CD3, and innate effector cells through an intact Fc portion.(91) This bsAb, termed a Triomab, effectively kills tumor cells in vitro and in vivo and induces protective immunity, most likely through the induction of memory T cells. Catumaxomab's success in a phase II/III clinical trial led to its approval by the European Commission in 2009 for the treatment of malignant ascites. This success spurred the development of other Triomabs targeted against the tumor antigens HER2/neu (Ertumaxomab), CD20 (Bi20/FBTA05; NCT01138579), GD2 and GD3 (Ektomun).(90)

Some efforts that made earlier to enhance the antitumor effects of mAbs focused on boosting their direct cytotoxic effects on the targeted cells. Conjugation of radionuclides (radioimmunotherapies (RIT)), drugs (antibody-drug conjugates (ADC)), toxins (immunotoxins), and enzymes (antibody-directed enzyme prodrug therapy (ADEPT)) yielded a multitude of antibodies, or antibody-like molecules, with varying clinical efficacy. Three conjugated antibodies have translated into FDA-approved therapies for hematological malignancies. Two are RIT agents targeting CD20 and are indicated for treatment of relapsed and/or Rituximab-refractory follicular or low-grade lymphomas: 90Y-ibritumomab tiuxetan and 131I-tositumomab. At the minimum of a dozen other RIT agents are in active development, including ten that target solid tumors.(92) Brentuxima vedotin, the third approvedimmunoconjugate, is a CD30 targets ADC, carries the antimitotic drug monomethyl auristatin E. It has recently approved as a treatment of anaplastic large cell lymphoma (NCT00866047) and Hodgkin lymphoma (NCT00848926).

Nine mAbs targeting six cancer-associated proteins (HER2/neu, EGFR, VEGF, CD20, CD52 and CD33) are approved for the treatment of solid and hematotogical malignancies. In addition to antagonizing oncogenic pathways, these biotherapeutics may act by opsonizing tumor cells and triggering their death or removal by ADCC or phagocytosis.(93) Ongoing investigations in murine models and patients increase the possibility that they may also stimulate adaptive immune responses in some settings.(94) Recently, the successful conjugation of toxins to antibodies has been achieved, and these have induced a clinical response in patients who are refractory to the naked antibody.(95) The concurrent administration of immunostimulatory cytokines such as IL-2 and granulocyte-macrophage colony-stimulating factor may also enhance the efficacy of antibody therapy.(96)

Immune Checkpoint Therapy

The myriad of genetic and epigenetic alterations which are characteristic of all cancers provide a diverse set of antigens that the immune system can use to distinguish tumor cells from their normal counterparts. In the case of T cells, the ultimate amplitude and quality of the response, which is initiated through antigen recognition by the TCR, is regulated by a balance between co-stimulatory and inhibitory signals, that is immune checkpoints.(97,98) Under normal physiological conditions, immune checkpoints are crucial for the maintenance of self-tolerance (the prevention of autoimmunity) and also to protect tissues from damage when the immune system is responding to pathogenic infection. The expression of immune-checkpoint proteins can be dysregulated by tumor as an important immune resistance mechanism.

A novel strategy of immunotherapy called checkpoint inhibition is hovered to dramatically revamp the treatment of a broad spectrum of malignancies. Checkpoint inhibitors function by modulating the immune systems' endogenous mechanisms of T cell regulation Ipilimumab (YervoyTM, BMS, New York, NY) has become standard treatment for metastatic melanoma.(6,99) Ipilimumab binds and blocks inhibitor signaling mediated by the T cell surface coinhibitory molecule cytotoxic T lymphocyte antigen 4 (CTLA-4). Because the mechanism of action is not specific to one tumor type, and because a wealth of preclinical data support the role of tumor immune surveillance acros multiple malignancies (100,101), Ipilimumab I being investigated as a treatment for patient with prostate, lung, renal, and breast cancer among other tumor types.(102) The field of immune checkpoint therapy has joined the ranks of surgery, radiation, chemotherapy, and targeted therapy as a pillar of cancer therapy. Therefore, in contrast to most currently approved antibodies for cancer therapy, antibodies that block immune checkpoints do not target tumor cells directly, instead they target lymphocyte receptors or their ligands in order to enhance endogenous anti tumor activity.(103)

Three new immune checkpoint agents now have been approved by the US FDA for the treatment of melanoma, arising new hopes more approved agents for treating lung cancer, kidney cancer, bladder cancer, prostate cancer, lymphoma, and many other tumor types. Ipilimumab an antibody against CTLA-4 was approved in 2011, and two antibodies against PD-1 (Pembrolizumab and Nivolumab) were approved in 2014.

Another category of immune-inhibitory molecules includes certain metabolic enzymes, such as IDO, which is expressed by both tumor cells and infiltrating myeloid cells, and arginase, which is produced by myeloid-derived suppressor cells.(60,104-109) These enzymes inhibit immune responses through local depletion of amino acids that are essential for anabolic functions in lymphocytes (particularly T cells) or through the synthesis of specific natural ligands for cytosolic receptors that can alter lymphocyte functions. These enzymes can be inhibited to enhance intratumoral inflammation by molecular analogues of their substrates which act as competitive inhibitors or suicide substrates.(110-112) These drugs represent a radical and disruptive change in cancer therapy in two ways. First, they target molecules involved in T cell regulation as the soldiers of the immune system, rather than the tumor cell. Second, perhaps in a more radical shift, the therapy is not designated to activate the immune system to attack particular targets on tumor cells, but to remove inhibitory pathways that block effective antitumor T cell responses.

Understanding of immune checkpoint therapy has led to new weapons against cancer which is elicit durable clinical responses and showed long-term remission for patiets, and provide an important advance in clinical advances about regulatory pathways in T cells and enhancing antitumor immune responses. Tumor cells express tumor-specific antigens in the form of complexes of tumor-derived peptides bound to MHC molecules on the cell, this will be the target of T cells in this therapy. Tumor antigens can be derived from oncogenic viruses, differentiation antigens, epigenetically regulated molecules such as cancer testis antigens, or neoantigens derived from mutations associated with the process of carcinogenesis.(113)

Recognition of antigen-MHC complexes by the T cell antigen receptor is not sufficient for activation of naïve T cells, but additional co-stimulatory signals are required that are provided by the engagement of CD28 on the T cell surface with B7 molecules (CD80 and CD86) on the antigen-presenting cell (APC).(97,115) Expression of B7 molecules is limited to subsets of hematopoietic cells, especially DC, which have specialized the processes for efficient antigen presentation.(114)

Further insights into the fundamental mechanisms which regulate early aspects of T cell activation may provide one from many possible explanations for the limited effectiveness of these early vaccine trials. By the mid-1990s, it became clear that T cell activation was even more complex, and in addition to initiating proliferation and functional differentiation T cell activation also induced an inhibitor pathway that could eventually attenuate an terminate T cell responses. Expression of CTLA-4 a gene with very high homology to CD28, is initiate by T cell activation, and, like CD28, CTLA-4 binds B7 molecules, albeit with much higher affinity. Although CTLA-4 was first thought to be another co-stimulatory molecule (116), two laboratories independently showed that it oppose CD28 co-stimulation and down-regulated T cell responses (117,118). Thus, activation of T cells result in induction of expression of CTLA-4, which accumulates in the T cell at the T cell-APC interface reaching a level where it eventually block co-stimulation and abrogates an activated T cell response (Figure 2).(114)

The preclinical successes of anti-CTLA-4 I achieving tumor rejection in animal models and the ultimate clinical success which opened a new field of immune checkpoint therapy.(103,119) It is now known that there are many additional immune checkpoints. PD-1 was shown in 2000 to be another immune checkpoint that limits the responses of activated cells.(120) PD-1, like CTLA-4, has two ligands, PD-L1 and PD-L2, which are expressed on man cell types. The function of PD-1 is completely distinct from CTLA-4 in that PD-1 does not interfere with co-stimulation, but interferes with signaling mediated by the T cell antigen receptor.(97) Also, one of its ligands, PD-L1 (B7-H1), can be expressed on many cell types, including T cells, epithelial cells, endothelial cells, and tumor cells after exposure to the cytokine IFN-γ, produced by activated T cells.(121) This leads to the notion that rather than functioning early in T cell activation, PD-1/PD-L1 pathway acts to protect cells from T cell attack.

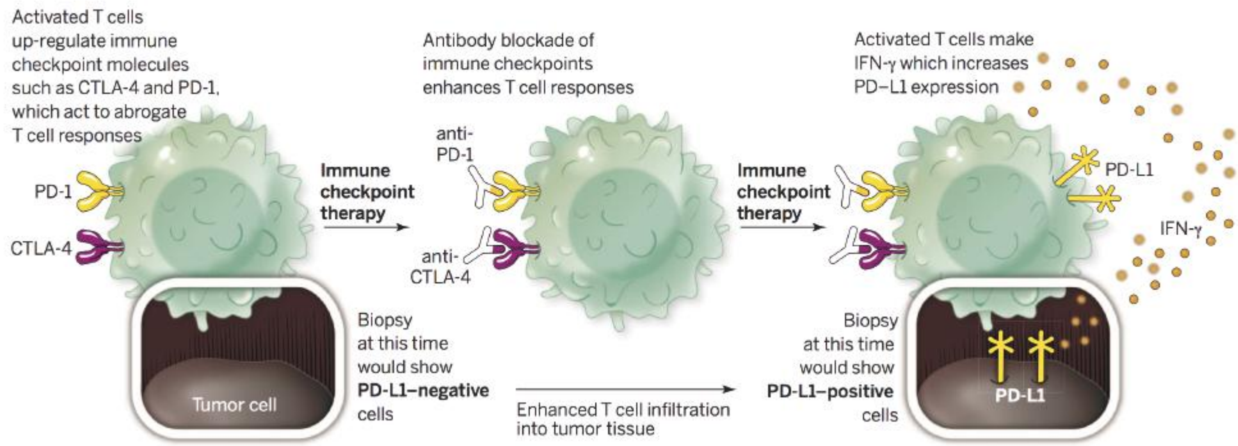

Figure 2. Blockade of immune checkpoints to enhance T cell responses. After T cell activation, T cells express immune checkpoints such as CTLA-4 and PD-1.(114) (Adapted with permission from American Association for the Advancement of Science).

Two anti-PD-L1 inhibitory antibodies, MPDL3280A (Genentech, South San Francisco, CA) and BMS-936559 (BMS, New York, NY), have undergone clinical investigation. Like Nivolumab an MK-3475, these antibodies are thought to function principally by blocking PD-1/PD-L signaling. Unlike PD-1 antibodies, PD-L antibodies spare potential interactions between PD-L2 and PD-1, but additionally block interaction between PD-L1 and CD80.(122) In addition to CTLA-4 and PD-1/PD-L1, plenty other immunomodulatory targets have been identified preclinically, many with corresponding therapeutic antibodies that are being investigated in clinical trials (Figure 3).(102) Majority of these targets are T cell surface receptors, but targets in other immunologic cell populations are currently being investigated. For example, NK cells express killer immunoglobulin-like receptors (KIR), which bind HLA class I molecules on target cells, so that delivering an inhibitory signal preventing NK cell-mediated cytotoxicity.(123) Anti-KIR antibodies may release these inhibitory KIR-mediated signals, thereby enabling tumor cytotoxicity and immune clearance.

Efficacy in checkpoint modulation is associated with certain immunologic changes, raising the hope that biomarkers for response may be identified. Only a minority of patients experience long-term survival with Ipilimumab; therefore, considerable efforts are ongoing to discover predictors of response.(102) Immune and tumor response to therapy has been monitored by utilizing a variety of laboratory techniques (Figure 4) (102), and numerous correlates of response have been retrospectively identified.

During the past 2-3 years, outcomes of clinical trials with Ipilimumab and the PD-1 or PD-L1 inhibitors, alone or in combination, have dominated the news coming out of clinical oncology meetings. Combination therapy blocking both checkpoint pathways has been particularly effective, with response rates in advanced melanoma is over 80%. These new issues has embraced the biopharmaceutical enthusiastically, acquisitions and licensing deals for new approaches are almost every week in the news, and investigations about new checkpoint targets, negative regulators of both adaptive and innate immune cells, combination therapy such as with cytokines, co-stimulatory

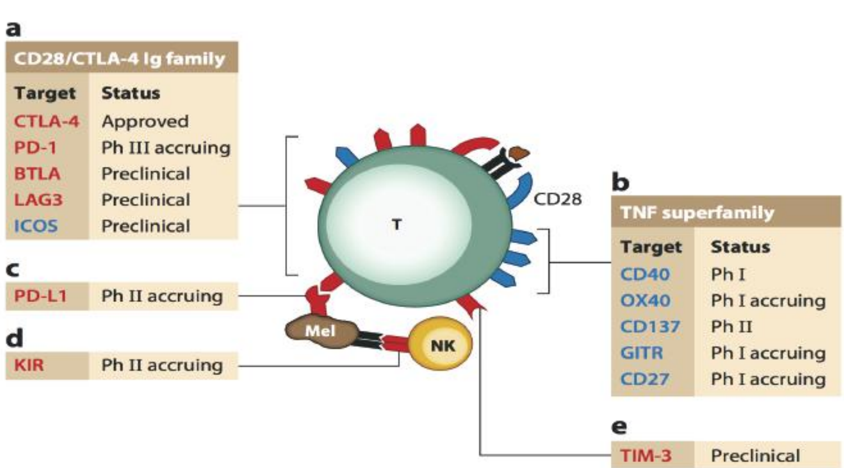

Figure 3. Targets of antibody immune modulators.(102) BTLA: B- and T-lymphocyte attenuator, LAG3: Lymphocyte-activation gene 3, ICOS: inducible T-cell costimulator, GITR: glucocorticoid-induced TNFR family related, TIM-3: mucin-domain containing-3. (Adapted with permission from Annual Reviews).

molecules, antigen vaccines, and small-molecule modulators of signaling pathways and enzymes are being actively done.(46) The ability of an activated immune response to generate a diverse T cell repertoire that adapts to heterogeneous and genetically unstable tumors and persistence of memory T cells with specificity for tumor antigens, which provide efficient recall responses against recurrent disease, make it absolutely essential to expand our efforts to find the rational combinations to unleash antitumor immune responses for the benefit of cancer patients. If it is properly done, seems likely that cures for many types of cancer will soon become reality.(114)

Adoptive Immunotherapy

T cells move through tissues, scanning for MHC-peptide complexes that specifically activate their TCR. T cells are also capable of sensing a variety of signals that can alert them to potentially threatening pathogens and to cancer. Tumor-specific T cells are probably activated through encounters with tumor-associated antigens that are presented by specialized APC, including DC. However, activated T cells are capable of directly recognizing antigens which were presented on the surfaces of tumor cells. Based on intravital imaging, there is a growing body of evidence showing that the migration of tumor-specific T cells is rapidly arrested when they encounter their cognate antigens.(124,125)

Sufficient number of active in vivo antitumor T cells was necessary to mediate cancer regression in many forms of cancer immunotherapy. ACT has advantages, because for use in ACT, the antitumor lympochyte can be readily grown in vitro up to 10^{11}, then selection for the high-avidity recognition tumor can be performed before applied effectively, and the inhibitory factors exist in vivo could be released. Perhaps most importantly, ACT enables manipulation of the host before cell transfer to

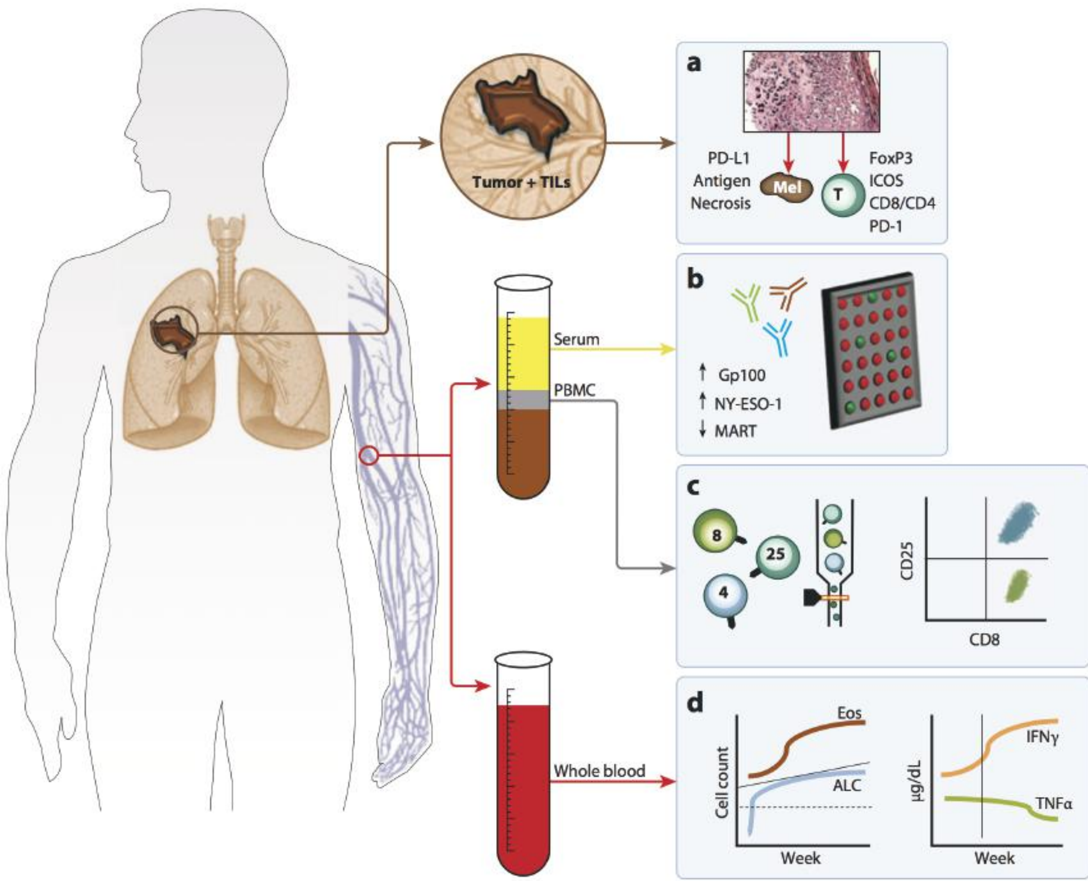

Figure 4. Strategies for immune monitoring in patients receiving checkpoint agents. (a) Surgical specimens may be analyzed using immunohistochemical or immunofluorescence techniques to evaluate tumor antigen expression, T cell infiltrate, tumor necrosis, or expression of surface markers such as PD-L1. (b) Using enzyme-linked immunoabsorbent assays or protein arrays, treatment-related production of tumor-specific antibodies can be detected in the serum. (c) Flow cytometric analysis of TILs and peripheral blood mononuclear cells (PBMC) can quantitate the effect of therapy on immune subsets such as $CD25^+$ T regs, activated $CD8^+$ T cells, or myeloid-derived suppressor cells. (d) Whole blood can be used to evaluate changes in cell count with therapy or changes in cytokine levels.(102) FoxP3: forkhead box P3, Eos: eosinophil, ALC: absolute lymphocyte count. (Adapted with permission from Annual Reviews).

provide a favorable microenvironment that better supports antitumor immunity. ACT is a living treatment because the administered cells can proliferate in vivo and maintain their antitumor effector functions.(126)

ACT has used either natural host cells that exhibit antitumor reactivity or host cells that have been genetically engineered with antitumor TCRs or CAR. With the use of these approaches, ACT has mediated impressive regressions in a variety of cancer histologies, including melanoma, cervical cancer, lymphoma, leukemia, bile duct cancer, and neuroblastoma.(126)

TILs are primarily Cytotoxic T Lymphocytes (CTL) which recognize proteolytically cleaved intracellular tumor antigen fragments which have become associated with specific MHC class I antigens on the cell surface.(127) Expanded TILs have cytolytic activity against the original tumor and in contrast to lymphokine activated killer (LAK) cells the killing is MHC class I restricted. They are selectively broaden from either tumor or draining lymph node cells via IL-2, and then re-stimulated with irradiated or killed tumor cells to maintain T cell specificity.(128)

Three forms of ACT using T cells have been practiced. The first one is TIL therapy, using lymphocytes expanded from a tumor biopsy sample (129); the second one is antigen-specific T cell therapy, using endogenous T cells sourced from peripheral blood (130-132); and the last one is the more recently, the use of gene modified T cells engineered to express the desired TCR or CAR with occasional remarkable results (133). Immunotherapy based on the adoptive transfer of naturally occurring or gene-engineered T cells can mediate tumor regression in patients with metastatic cancer.(125)

The capacity to use ACT was facilitated by the description of T cell growth factor IL-2 in 1976, which provided a means to grow T lymphocytes *ex vivo*, often without loss of effector functions.(134) The direct administration of high doses of IL-2 could inhibit tumor growth in mice (135), and studies in 1982 demonstrated that the intravenous injection of immune lymphocytes expanded in IL-2 could effectively treat bulky subcutaneous virus-induced lymphoma cells (FBL3) (136). Moreover, administration of IL-2 after cell transfer could enhance the therapeutic potential of these adoptively transferred lymphocytes.(137)

From many tumor histologies culture grown, only melanoma appeared to reproducibly gave rise to TIL cultures for specific antitumor recognition. Studies of genetic engineering led to lymphocytes capability to express antitumor receptors. Following mouse models (138), it was shown for the first time in humans in 2006 that administration of normal circulating lymphocytes transduced with a retrovirus encoding a TCR that recognized the melanoma antigen recognized by T-cells 1 (MART-1) could mediate tumor regression (139). Administration of lymphocytes genetically engineered to express a CAR against the B cell antigen CD19 was shown in 2010 to mediate regression of an advanced B cell lymphoma.(140) These findings of the use of either naturally occurring or genetically engineered antitumor T cells set the stage for extended development of ACT for the treatment of human cancer (Figure 5).(126)

In an attempt to broaden the reach of ACT to other cancers, techniques were developed to introduce antitumor receptors into normal T cells that could be used for therapy (Figure 6).(126) The specificity of T cells can be redirected by integrating genes encoding either conventional alpha-beta TCRs or CARs. CARs were pioneered by Gross and colleagues in the late 1980s and can be constructed by linking the variable regions of the antibody heavy and light chains to intracellular signaling chains such as CD3-zeta (141), often including co-stimulatory domains encoding CD28 (142) or CD137 to fully activate T cells (143,144). CARs can provide non-MHC-restricted recognition of cell surface components and can be introduced into T cells with high efficiency using viral vectors.

For more patients-specific product approach, a paradigm shift required from conventional medicine such as pills, vaccines, small molecule inhibitor molecules, and antibodies to autologous engineered cell therapies. Some have dismissed adoptive T cell immunotherapy as a fringe or boutique therapy that would be impossible to commercialize. (145). Indeed, several challenges must be overcome before this disruptive therapy can become widely applicable and widely available. Currently, the barriers that we perceive fall into two areas. First, robust and reproducible cell culture system. T cell engineering process needs complex logistics, and some variables standardization in order to scale this out for widespread use include developing a leukapheresis network, standardizing and scaling up the manufacturing of lentiviral vectors, and developing validated cell-shipping and chain-of-custody procedures.(146)

Second, personalized cell therapies cannot become broadly available if the cell culture process requires extensive manipulation by highly skilled scientists and technicians.(147) Hence, automated culture systems need to be developed. Previous case in automotive industry, cars were initially manufactured in assembly lines, but manually. Today's automobiles are assembled largely by robots and other forms of automation.(148) As engineered T cell processing becomes more automated, cell products will be produced for greater number of patients more

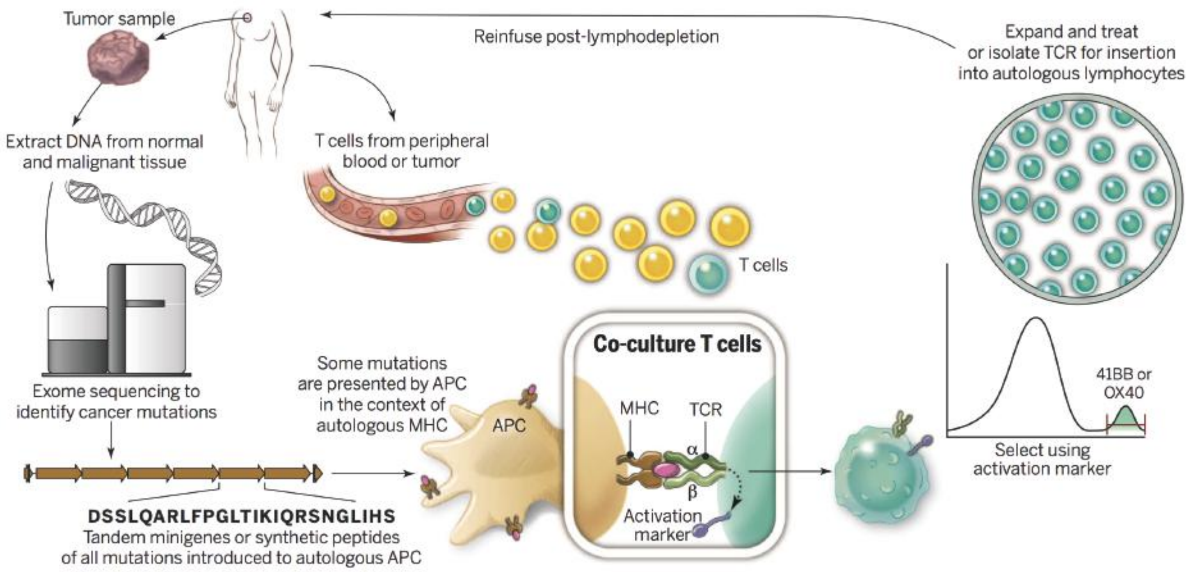

Figure 5. A blueprint for the treatment of patients with T cells recognizing tumor-specific mutations.(126) (Adapted with permission from American Association for the Advancement of Science).

efficiently. Seeing the recent entry of the pharmaceutical industry to this field, we are optimistic that the resources and expertise of the pharmaceutical industry will create the infrastructure required for the widespread availability of this disruptive technology. Further clinical development of engineered T cell therapies in large numbers of patients will be challenging but is justified given the magnitude of therapeutic effects recently observed (Figure 7).(149)

The need to develop highly personalized treatments for each patient does not fit into the paradigm of major pharmaceutical companies that depend on off-the-shelf reagents that can be widely distributed. However, curative immunotherapies for patients with common epithelial cancers will probably dictate the need for more personalized approaches.(126) Widespread of ACT can not depend only on multiple commercial models, but more to the development of centralized facilities for tumor-reactive TILs production and genetically modified lymphocytes, that later can be delivered to the institution who do the treatment. New effective approaches for cancer immunotherapy will need to trump the convenience of applicable administration in treatment.

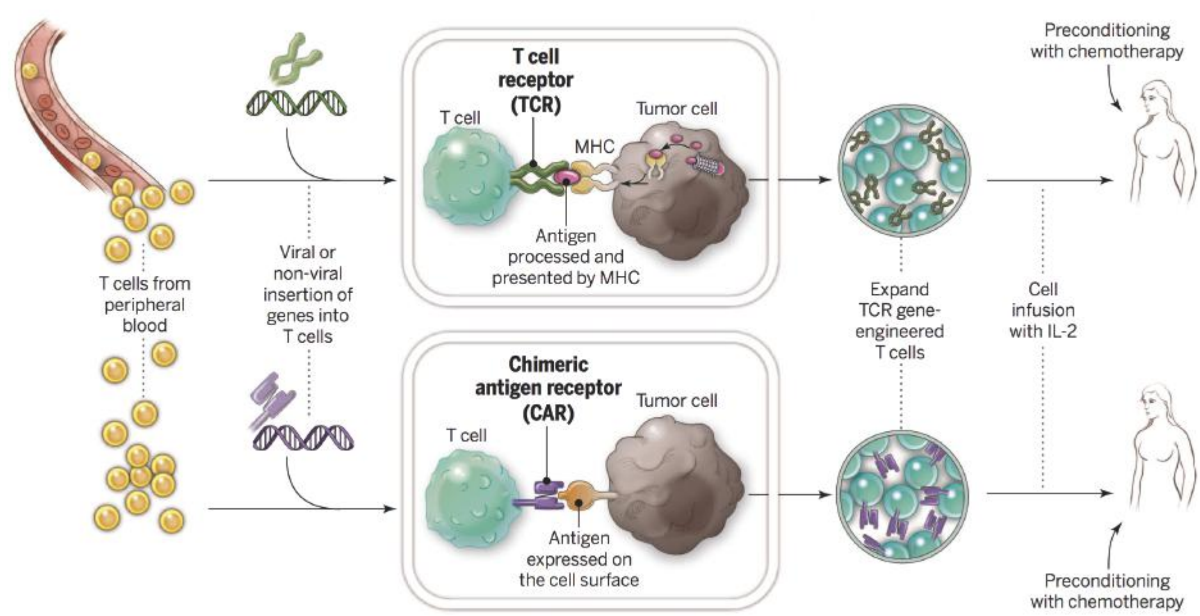

Figure 6. Gene-modification of peripheral blood lymphocytes.(126) (Adapted with permission from American Association for the Advancement of Science).

γδ T cells are a subset of T cells that express alternative, clonally distributed TCRs and function as innate effectors and therefore are restricted in TCR diversity. Compared to αβ T cells, they do not target specific peptide antigens and are not constrained by the selectivity and restriction of the MHC. Although γδ T cells absolute counts are decreased and their proliferative capacity is decreased in the setting of glioblastoma, they can be expanded and activated ex vivo and have shown the ability to recognize and kill glioma cells in vitro while sparing cultured normal astrocytes.(150) Expanded and activated γδ T cells can mediate killing of glioblastoma and reduce tumor progression in mouse models.(151)

Cancer immunotherapy trials with autologous cd T cells have been investigated in parallel by Japanese, Australian and French groups. The French company, Innate Pharma, has conducted a phase I study in 10 patients with metastatic renal cell carcinoma to determine the maximum-tolerated dose of autologous TCRVγ9Vγ2^{+} γδ T cells and the safety of these cells as a therapeutic product.(152) In parallel with these studies, Kobayashi's group at Tokyo Women's Hospital investigated seven patients with advanced renal cell carcinoma engrafted with autologous γδ T cells.(153) Therefore, the authors concluded that γδ cell-based immunotherapy is a clinically beneficial and safe therapeutic option for patients with advanced renal cell carcinoma, whose rates of circulating γδ cells might constitute a favorable prognostic indicator.(154) To be successful, cancer immunotherapies involving γδ cells will require updated protocols which limit anergy and the use of drugs able to overcome immunoescape. Even though the former contingency is currently an open issue, the second one is already well underway.(155)

Neoantigens in Cancer Immunotherapy

Immunotherapies which are boosting the ability of endogenous T cells to destroy cancer cells have showed therapeutic efficacy in a variety of human malignancies. Until now, evidence that the endogenous T cell compartment could help control tumor growth was in big part restricted to preclinical mouse tumor models and to human melanoma.(113) With respect to human studies, effects of the T cell cytokine IL-2 in a small subset of melanoma patients provided early clinical evidence of the potential of immunotherapy in the said disease. A randomized clinical trial was performed in 2010 showed that Ipilinumab, an antibody targets T cell checkpoint protein CTLA-4 could improve patient survival, even with metastatic melanoma.(6) As a direct test of the tumoricidal potential of the endogenous T cell compartment, a study by Rosenberg and colleagues exhibited that infusion of autologous *ex vivo* expanded TIL can induce objective clinical responses in metastatic melanoma (156), and at least part of this clinical activity is due to cytotoxic T cells (157). Basically, recent studies show that T cell-based immunotherapies are also effective in a range of other human malignancies.

With recent technology, we can check the uplevel of neoantigens as a tumor-specific mutation consequences, and emerging data suggest that this neoantigen play a role in the activity of clinical immunotherapies, thus this load may be used as cancer immunotherapy biomarker and an incentive development of novel therapeutic approaches can be provided.(113) In cancer, so-called neoepitope peptides are derived from proteins encoded by mutated genes. Recent

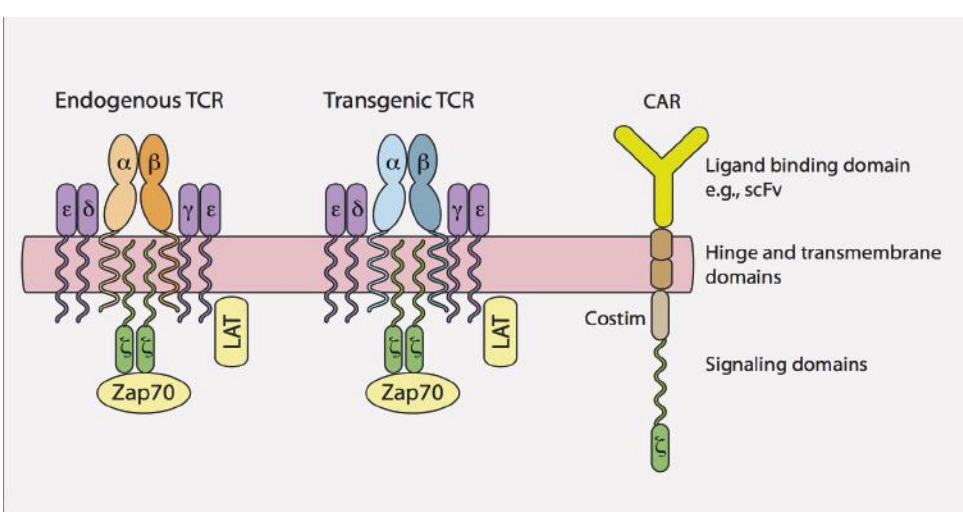

Figure 7. Engineered T Cells that have retargeted specificity.(149) Zap70: Zeta-chain-associated protein kinase 70, LAT: linker for activation of T cells, scFv: single-chain variable fragment. (Adapted with permission from Elsevier).

advances in next-generation DNA and RNA sequencing now enable rapid mapping of all expressed mutated genes in an individual tumor, and it is possible to predict epitopes that are efficiently presented on the surface of cancer cells. Thus, it has been demonstrated that $CD8^+$ T cells in human melanomas are able to recognize one or more neoepitopes from mutant proteins unique to that specific melanoma. However, efficient methods for studying $CD4^+$ T cells that recognize neoepitopes arising from somatic mutations in cancer have been lacking.(158) By using this approach, the authors identified neoepitope-specific $CD4^+$ T cells in two out of three melanoma patients.(159) The patients' T cells only recognized neoepitopes from the host's tumor, and they preferentially or exclusively noticed the neoepitope over the corresponding native, non-mutated peptide, demonstrating the exquisite specificity of the $CD4^+$ T cells.

$CD4^+$ T cells can antecedent cancer regression through direct killing of cancer cells, by altering the tumor-promoting function of cells in the surrounding tumor microenvironment, and by facilitating the induction, function and tumor infiltration of cancer-specific $CD8^+$ T cells.(160) These studies show that cancer-specific $CD4^+$ T cells can cause human tumor regression, adding to the importance of the findings by Linnemann, *et al*.(159) The ability to detect neoepitope specific $CD4^+$ T cells now allows validation of the hypothesis which the presence of neoepitope-specific $CD4^+$ T cells in human tumors correlates with overall clinical outcome.

The mutation in human tumor was considered to be individually different at meaningful frequencies and fractions, so the T cell reactivity against putative mutation-derived neoantigens interrogate technologies have to be developed based on individual tumor genome. With the development of deep-sequencing technologies, it has become doable to identify the mutations present within the protein-encoding part of the genome (the exome) of an individual tumor with relative ease and thereby predict potential neoantigens.(161)

Subsequent studies have demonstrated that cancer exome-based analyses can also be exploited in a clinical setting, to dissect T cell reactivity in patients who are treated by either TIL cell therapy or checkpoint blockade.(162,163) Furthermore, following this early work, the identification of neoantigens on the basis of cancer exome data has been documented in a variety of experimental model systems and human malignancies.(159,164-173) Based on data collected over the past few years, it is plausible that neoantigen-specific T cell reactivity forms a major active ingredient of successful cancer immunotherapies. In other words, the genetic damage that on the one hand leads to oncogenic outgrowth can also be targeted by the immune system to control malignancies. Based on this finding, it will be important to engineer therapeutic interventions by which neoantigen-specific T cell reactivity is selectively enhanced. As it may be, the boosting of neoantigen-specific T cell reactivity which can be achieved with such personalized immunotherapies will further increase the spectrum of human malignancies that respond to cancer immunotherapy.(113)

Cancer Vaccine Therapy

DC are designated as professional APC because of their capacity to provide T cells with all the signals required for antigen-specific T cell activation.(174) For optimal activation, T cells must receive at least three coordinated signals.(175,176) The first signal is delivered to the TCR by MHC molecules presenting antigen-derived peptides. The second signal was provided by the binding of co-stimulatory molecules with their respective ligands on T-cells. A crucial positive co-stimulatory signal is provided by the interaction of the B7 family ligands CD80 and CD86, expressed by the DC, with the CD28 receptor expressed on the T cell surface. The third signal, that called polarization signal, determines the commitment of naive CD4+ T helper (TH0) cells towards TH1,TH2 or other fates. In the setting of cancer immunotherapy, the induction of a TH1 (cellular) immune response is highly desirable as this enables the generation of CTL capable of recognizing and destroying tumor cells in an antigen-specific fashion.(177)

As well as inducing antigen-specific CTL, which are part of the adaptive immune system, DC also capable of activating NK cells, which are prime players in the innate immune system.(178) NK cells have both cytotoxic and immunoregulatory functions and require priming to accomplish their full effector potential. Many of the cytokines that play a principal role in stimulating NK cell functions can be provided by DC, *e.g.*, IL-12, IL-15, IL-18 and type I IFN.(179)

A more detailed understanding of the mechanisms leading to strong cellular immunity is necessary to enable rational approaches to the vaccine design. Two recent conceptual breakthroughs in this regard are our understanding that DC play a pivotal role in initiating the immune response to foreign antigens and the realization that adjuvants act primarily because they are DC activators.(180) Therapeutic vaccines in chronic infections (or cancer) have two objectives: one is priming, whereas the other one is the modulation or reprogramming of memory cells, i.e., to transition from one type of immunity to another (e.g., regulatory to cytotoxic).

Tumor cells themselves are poor APC, which raises the question of how such potent immunity can be generated. Mouse models demonstrate that the generation of protective anti-tumor immunity relies on the presentation of tumor antigens by DC.(181,182) These cells special features in coordinating innate and adaptive immune responses provided an idea for a DCs-involved vaccination strategies, aiming to induce tumor-specific effector T cells that can reduce the tumor mass specifically and induce the immunological memory to control tumor relapse. First step for these processes is to provide DCs with tumor-specific antigens. This can be achieved either by culturing ex vivo patients-derived DCs that have been induced with an adjuvant (induces DC maturation) and the tumor-specific antigen, and then injecting these cells back into the patient, or by inducing DCs to take up the tumor-specific antigen in vivo. To improve therapeutic use of DC vaccination strategies, it is necessary to understand the biology of DC and how they regulate the innate and the adaptive immune systems, particularly in the context of the tumor microenvironment.(183)

Most phase 1 and phase 2 trials of cancer vaccines have involved patients with an extensive cancer burden, impaired immune function, or both.(184) An alternative to infusion of preformed tumor-specific antibodies or T cells, known as passive immunotherapy, is active specific immunotherapy (*i.e.*, cancer vaccines) designed to elicit or boost similar tumor antibodies and T cells in patients. Some examples are vaccines against breast cancer (the HER2 antigen) (185-7), B-cell lymphoma (the tumor immunoglobulin idiotype) (188), lung cancer (the Mucin 1 cell surface associated (MUC1) antigen) (189), melanoma (DC loaded with tumor peptides or killed tumor cells) (190,191), pancreatic cancer (telomerase peptides) (192), and prostate cancer (DC loaded with prostatic acid phosphatase) (193). The results of these trials are promising because in each there was evidence of an immune response to the vaccine, and in a few cases there were clinical responses with minimal or no adverse effects. Regarding the limited number of completed phase 3 trials, most have failed to presentate a significant benefit with respect to predetermined end points (194), but nevertheless provided information for design of future trials, especially concerning the choice of patients and stage of disease.

The immunosuppressive microenvironment of a tumor can inhibit the effect of therapeutic vaccines, both during the immunity induction and in the response effector phase. So, the negative regulators of the activation of effector T cells need to be blocked to improve the induction phase.(195) Antibodies against one such molecule, CTLA-4 are being evaluated in clinical trials.(196,197) CTLA-4 is expressed on activated T cells, where it serves as a brake halting the activation. Blocking activity of CTLA-4 allows larger expansion of all T-cell populations, presumably including those with antitumor reactivity. In a recent pilot trial involving 14 patients with hormone-refractory prostate cancer, systemic treatment with anti-CTLA-4 antibody increased antitumor immunity, resulting in a reduction in prostate-specific antigen of more than 50% in two patients and less than 50% in eight patients.(197) The side effects were rash and pruritus, which required treatment with corticosteroids in the two patients with the best response.(62)

Approaches to eliminating immunosuppressive regulatory T cells before vaccination are also being tested. One promising reagent is Denileukin diftitox (Ontak, Seragen), a recombinant fusion protein composed of IL-2 and diphtheria toxin. It targets the high-affinity IL-2 receptor (CD25), which is displayed in abundance by regulatory T cells. When administered to patients with melanoma, protein depletes the blood of regulatory T cells. In most patients (90%), this treatment has resulted in the production of melanoma-specific CD8 T cells.(198)

Conclusion

We now have detailed knowledge of the molecular basis of cancer to allow a more personalized treatment based on genomic sequencing of an individual's cancer cells to identify specific mutations in genes. These mutations can then be targeted with compounds to blockade the downstream pathways which drive cancer development and progression. Thus, each specific mutation serves as the predictive biomarker for selecting patients for treatment with a given agent.

Clinical data generated principally over the past 5 years offer that we are at the threshold of golden era for adoptive T cell therapy, where advances in basic immunology have informed the development of a new field of synthetic immunology which may increase the potency of approaches that target cancer. Cancer immunotherapies, a no stranger to obstacles, looks as a very promising future for incurable cancers therapy, using anti-CTLA-4 and anti-PD-1 antibodies or adoptively transferring T cells for blocking inhibitory immune signaling.

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