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Cancer immunotherapy

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ABSTRACT

The era has begun where oncology meets immunology. The recent advancement in the field of molecular biology has led to the discovery of various pathways through which cancer establishes, proliferates, grows, and disseminates. These pathways provided major insight for targeting specific molecules with the targeted therapies that show predicted responses. This targeted therapy is usually referred to as immunotherapy. These immunotherapies possess and display a unique set of toxicities mainly immunologic in nature and different from chemotherapies. This article focuses on mechanisms of immune activity of the body and various therapies available to boost these mechanisms.

Key words: Immune system, Immunotherapy, Targeted therapies

Introduction

"The beginning of the end of the hopeless era."

The field of oncology is one of the recently popularized branches of medical sciences. Robotic surgery and video-assisted surgeries have emerged as the face of a change of surgical oncology. Radiation oncology too has witnessed the introduction and application of novel technologies such as intensity-modulated radiotherapy, image-guided radiotherapy, and stereotactic body radiotherapy. Not left behind, the field of medical oncology has entered the era which has resurrected new hopes for the patients of advanced malignancies. The era has begun where oncology meets immunology. The recent advancement in the field of molecular biology has led to the discovery of various pathways through which cancer establishes, proliferates, grows, and disseminates. These pathways provided major insight for targeting specific molecules with the targeted therapies that show predicted responses. This targeted therapy is usually referred to as immunotherapy. These immunotherapies possess and display a unique set of toxicities mainly immunologic in nature and different from chemotherapies.

This article focuses on mechanisms of immune activity of the body and various therapies available to boost these mechanisms.

Cancer cells or cancer bed is cunning enough to fool the immune system of impending health crisis. Unchecked cancer cells proliferate indefinitely and uncontrollably and metastasize to different body parts. Hanahan and Weinberg have described the eight hallmarks of cancer[1] [Table 1]. In 1950s Burnet and Thomas established the concept of "cancer immunosurveillance." It practically means protective role of immune system in cancer establishment. This concept has been modified to the present concept of "cancer immune-editing" which means the immune system can both protective and promoting roles in cancer growth.[2] With the evolving understanding of cancer development is now regarded as a multistep process with heterogeneous cell populations.[3]

Body's immune systems, innate and adaptive immunity plays a crucial role in immune surveillance and body's defense mechanism. The use of the immune system has demonstrated great potential for cancer treatment, especially for the diseases refractory to conventional treatments such as radiotherapy, surgery, and chemotherapy.[4] Effectiveness of cancer immunotherapy largely relies on the identification and detection of cancer-specific antigen, so far 403 cancer-specific antigens have been identified and reported in the database.[5] Activation of cancer-specific T-cell is the fundamental step to recognize cancer-specific antigen and kill the cancer cells. Two signals are required to achieve optimal intrinsic T-cell activation; first, recognition of antigen-presenting cells (APC) processed antigenic peptides with human leukocyte antigen molecules by T-cell receptor (TCR) and second, binding of surface molecules B7 and CD28 on APCs and T-cells, respectively.[6] Other than stimulating signals, there are inhibitory molecules such as CTLA-4 and programmed death receptor-1 (PD-1), which prevent activation of T-cells. Balance between stimulating and inhibitory signals determines the degree of T-cell activation. Recent evidences from clinical trials have shown that blockade of PD-1 and CTLA-4 signaling enhances T-cell-mediated anticancer responses with significant improvement in the survival of patients.[7] Besides, intrinsic T-cell regulation, external factors such as cytokines (e.g., interleukin [IL]-2) can also regulate T-cell activation. Presence

of myeloid-derived suppressor cells (MDSCs) and regulatory T-cell (T-reg) cells inhibit the function of cancer-specific T-cell resulting in immunosuppression. To overcome MDSC-Treg cell associated immunosuppression, anti-CD25 antibodies and cyclophosphamide have been used.[8]

Immunological methods have been the recent addition in anticancer therapeutics. Increasing anticancer immunity or eliminating the immunosuppression is of special interest. Immunotherapy can be passive (i.e., providing monoclonal antibodies) or active (i.e., stimulating antitumor activity through vaccines). Immunotherapies work better for some types of cancer and show interpatient variability.

Anticancer Immunity: A Multistep Process

Generating immunity against non-self-particle is not a simple process. It is a multi-step challenge that involves initiation, promotion, and progression for establishing anticancer immunity. Following are the steps that generate anticancer immunity.[4]

Processing antigens

Antigens can be processed and presented to T-cells through two pathways.

Endogenous pathway

Antigens (such as tumor and viral proteins) synthesized in the cytosol are degraded in proteosomes into peptides; which are then transported through peptide transporter, known as TAP complex, into endoplasmic reticulum of APC (mainly dendritic cells [DC]) where they bind to freshly synthesized major histocompatibility complex (MHC)-I class proteins.

Exogenous pathway

Exogenous antigens (like bacteria) are phagocytosed by APC, and their peptides are processed and packed into endocytic vesicles. MHC-II class proteins are synthesized in the endoplasmic reticulum of these cells and transported to the endocytic vesicle. Antigen load onto MHC-II and presented on the surface of by APC.

DC and macrophages are "professional APC."

Tumor antigens may be products of non-mutated genes but preferentially expressed by cancer cells (like cancer-testis antigen), differentiation antigens to which central or peripheral tolerance has not been developed (like melanosome-associated proteins in melanoma), and mutated proteins (like ectopic presentation of cytosol proteins on plasma membrane, e.g., calreticulin) or delivered exogenously as a part of therapeutic vaccine.[9]

Generating T-cell response

Tumor antigen-loaded DC reach lymphoid organs where T cell responses are initiated. These T cell responses depend on the maturation stimulus of DC and costimulatory molecules. Interaction of CD28 (T-cell) with CD80/86 (DC) will promote protective T cell responses while the interaction of CTLA4 (T cell) with CD80/86 (DC) or PD-1 (T cell) with PD-L1/L2 (DC) will suppress T cell responses and possibly promote Treg formation. MHC-I and tumor antigen complex initiates cytotoxic T cell response by stimulating CD-8$^+$ cells. DC may also trigger antibody, natural killer (NK) or NK T (NKT) cell responses. Anticancer T cells must enter the tumor bed to perform their function. Tumor microenvironment is gruesome for effector T cell functions.[8]

Following is the account of how tumor metabolism and microenvironment evade the anticancer immune mechanisms.

Tumor Metabolism and Microenvironment

Tumor metabolism and microenvironment have been argued as one of the sources of immunosuppression that compromises the effectiveness of cancer immunotherapy. Lymphocytes, on stimuli recognition, undergo activation and differentiation. This is the result of metabolic reprogramming. Similar type of reprogramming for utilizing nutrients and engaging metabolic regulation is reported in pre-cancerous or cancerous cells. This leads to "metabolic competition" between lymphocytes and cancer cells and the winning side establishes the fate of tissue.[10]

Role of immunosuppressive tumor microenvironment

Baitsch elaborated on three major hurdles that weaken the antitumor immunity and fails cancer immunotherapy[11] [Table 2].

The issue of low antigenic cells may be solved with cancer vaccines, adoptive cell therapy and chimeric-antigen receptor (CAR) T cells but even these fail miserably in the immunosuppressive microenvironment.[12] The immunosuppressive effect of tumor microenvironment is so horrendous that T cells forget their effector function and

Table 1: Eight hallmarks of cancer

Sustaining proliferative signal
Evading growth suppressors
Resisting cell death
Enabling replicative immortality
Angiogenesis
Activating invasion and metastasis
Reprogramming of energy metabolism
Evading immune destruction

Table 2: Three stumbling blocks for anticancer T cells

Low number of tumor antigen-specific T cells due to clonal deletion
Poor activation of innate immune cells and accumulation of tolerogenic APC in the tumor microenvironment
Formation of immunosuppressive tumor microenvironment

APC: Antigen-presenting cells

get hypnotized to express coinhibitory receptors compared to circulating T cells. Two major mechanisms have been elucidated that explain the immunosuppressive effect of the tumor microenvironment.

1. Accumulation of cells that counter antitumor activity of T cells either through direct contact or through cytokine milieu. These cells are M2-type macrophages, immature DC, T-regs, and MDSC.[4,13]
2. Expression of PD-1 receptor ligands (PD-L1/PD-L2) and reduced expression of tumor antigens and MHC in the tumor.[4]

Metabolic reprogramming guides T cell activation, differentiation, and effector function

Stimuli recognition leads to changes in the metabolic activity of T cells that consequently differentiate into cytotoxic, helper. or Tregs.[14] Naive T cells, T regs. and memory cells utilize oxidative phosphorylation for meeting energy demands, survival and differentiation whereas activated T cells rely on aerobic glycolysis.[15] Eliminating glucose uptake or impairing glycolysis suppresses mammalian target of rapamycin (mTOR) but increases AMP-activated protein kinase (AMPK) activity. This leads to suppression of effector T cell differentiation, cytokine production, and promotes Treg.[16] Thus, mTOR and AMPK are antagonistic in action.

Metabolic pathways govern macrophage polarization

Macrophages are highly secretory and phagocytic cells of the immune system. Depending on stimuli these may differentiate into M1 or M2 like macrophages. Lipopolysaccharides lead to M1 polarization whereas IL-4 favors M2 polarization. M1 macrophage possesses pro-inflammatory properties whereas M2 possess anti-inflammatory properties.[17] Accumulation of M2 macrophages disarm antitumor immunity through preventing Type 1 immune responses, the formation of abnormal vasculature and facilitating dissipation of metastatic cancer cells.[10,13]

Nutrient specialized tumor microenvironment

Cancer cells use Warburg glycolysis to consume glucose and increase lactic acid production. This lactic acid suppresses T cell effector and cytotoxic function and favors the polarization of macrophages toward the M2 phenotype. Cancer cells with higher glycolytic activity have a strong capacity to evade immunosurveillance and glucose depleted microenvironment contributes to diminished anti-T cell tumor response.[18] More invasive cancers have a high rate of *de novo* fatty acid synthesis.[19] Lipid-enriched tumor microenvironment can contribute to metastasis[20] and favors the generation of Tregs and M2-like macrophages. Various studies have also demonstrated that reduced availability of amino acids in tumors may lead to defective T cell immunity.[10]

Hypoxia in tumor microenvironment

Hypoxia in tumor microenvironment may inhibit effector T cell function by promoting the generation of adenosine or CCL28 which attracts immigration of T-regs.[21]

To define which metabolic pathways link to effective antitumor responses [Table 3], it will be critical to determine the metabolic signature and profile in tumor infiltrating lymphocytes (TILs) and tumor-associated macrophages, from progressed and regressed tumors.[10]

Immunotherapeutic Strategies

Immunotherapy drugs are now used to treat many different types of cancer. Many new types of immunotherapy are now being studied for use against cancer.

The generation of immunity to cancer is a cyclic process that can be self-propagating, leading to an accumulation of immune-stimulatory factors that in principle should amplify and broaden T cell responses. The cycle is also characterized by inhibitory factors that lead to immune regulatory feedback mechanisms, which can halt the development or limit the immunity. This cycle can be divided into seven major steps[22] [Figure 1], starting with the release of antigens from the cancer cell and ending with the killing of cancer cells.

The goal of cancer immunotherapy is to initiate or reinitiate a self-sustaining cycle of cancer immunity,[22] enabling it to amplify and propagate, but not so much as to generate unrestrained autoimmune inflammatory responses. Cancer immunotherapies must, therefore, be carefully configured to overcome the negative feedback mechanisms. Although checkpoints and inhibitors are built into each step that opposes continued amplification and can dampen or arrest the antitumor immune response, the most effective approaches will involve selectively targeting the rate-limiting step in any given patient. Amplifying the entire cycle may provide anticancer activity but at the potential cost of unwanted damage to normal cells and tissues. The most promising results have been achieved with T-cell-based therapies. These include adoptive cell transfer (ACT) of TILs, genetically engineered T cells, and immune checkpoint inhibitor antibodies.

Table 3: Cancer and microenvironment[10]

Tumor-promoting factors
Pro-tumorigenic cells
Tregs
M2-like macrophages
Immature DC
MDSC
Immunosuppressive metabolic factors
In tumor
Increase lactic acid production
Higher glycolytic activity
High rate of *de novo* fatty acid synthesis
Reduced availability of amino acids
Lipid-enriched tumor microenvironment
Hypoxia
Expression of PD-1 receptor ligands (PD-L1/PD-L2)
Reduced expression of tumor antigens and MHC in tumor

Tregs: Regulatory T-cell, MDSC: Myeloid-derived suppressor cells, DC: Dendritic cells, PD-1: Programmed death receptor-1, MHC: Major histocompatibility complex

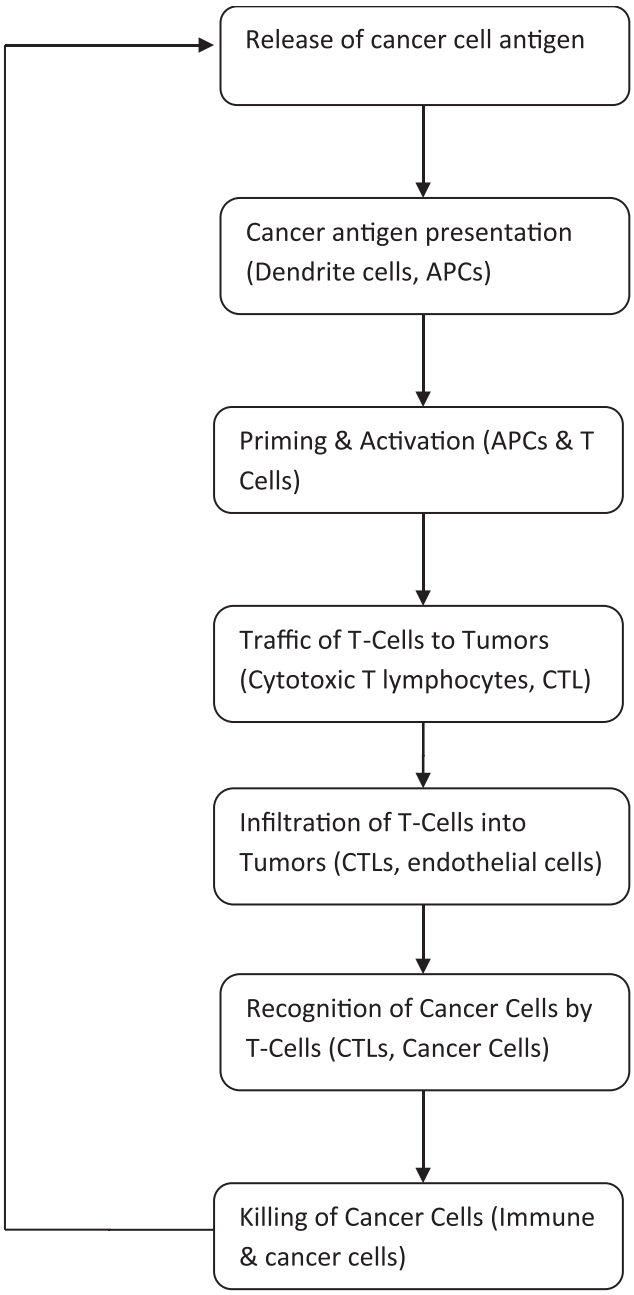

Figure 1: Cancer immunity cycle

Three main immunotherapeutic approaches to target cancer cells are non-specific stimulation of immune reactions (stimulating the effector function or inhibiting the regulatory function), active immunization to enhance antitumor reactions (cancer vaccine), and passively transfer activated immune cells with antitumor activity (adoptive immunotherapy). The classification is based on if the approach is cell-based or non-cell based [Table 4].

Non-specific immunotherapies

These therapies do not target cancer cells specifically. They stimulate the immune system in a more general way. Following are the types of non-specific anticancer immunotherapies.

Cytokines

These are the chemical messengers that are secreted by and act on immune system cells. The popular therapeutic cytokines are IL-2 and interferon (IFN).

IL-2 or IL-2 therapy

This therapy opened up the new window for targeting cancer in terms of immunological manipulation. High dose IL-2 resulted in objective tumor regression in metastatic melanoma and renal cell cancer.

IFN or IFN

These have three types (alpha, beta, and gamma). Only IFN-alpha is used to treat cancers. It may act directly on cancer cells or through anti-angiogenesis. It uses include hairy cell leukemia, chronic myeloid leukemia, follicular non-Hodgkin lymphoma, cutaneous T cell lymphoma, renal cancer, melanoma, and Kaposi sarcoma.[23] Their use, in general, is limited by serious adverse drug reactions.

Immune checkpoint inhibitors

Immune system attack foreign cells (or rather non-self-antigens) while spare the normal cells. To do this, it uses "checkpoints"-molecules on certain immune cells that need to be activated (or inactivated) to start an immune response.

Drugs that inhibit CTLA-4

Ipilimumab is an anti-CTLA-4 monoclonal antibody that has shown improvement in median survival in metastatic melanoma patients.[24] The most common side effects include diarrhea, endocrinopathies, enterocolitis, hepatitis, and dermatitis. Grade 3 or 4 immune-related events occurred in 10–15% of patients. Tremelimumab is another CTLA-4 antibody (IgG2) that has shown antitumor activity in terms of prolonged response duration [Figure 2].

Drugs that target PD-1 or PD-L1

Discovered in 1992, PD-1 is a member of the B7-CD28 superfamily. It is expressed on activated T-cells (Cd8+ and CD-4+), B-cells, monocytes, NKT cells, and APCs. It keeps T-cells in toned down activity and prevents the destruction of other cells. It attaches to PD-L1/2 (PD ligand) on normal cell or cancer cells [Figure 3]. This ultimately leads to decreased cytokine production and antibody formation, thereby inhibiting autoimmunity and antitumor and anti-infectious immunity.[25]

The first drug approved for inhibition of PD-1 is pembrolizumab. Its approved for treatment of metastatic melanoma, metastatic non-small cell lung cancer, and head and neck cancers.[26]

The other approved drug for PD-1 inhibition is nivolumab (humanized IgG4 anti PD-1 monoclonal antibody) for metastatic non-small cell lung cancer, melanoma, renal cell cancer, and Hodgkin's lymphoma.

The approved drug that inhibits PD-L1 is Atezolizumab (humanized IgG1 anti PD-L1 monoclonal antibody) and is

Table 4: Immunotherapeutic approaches[22]

A. Cell-based
1.DC vaccination
2.CIK cells
3.NK cells or genetically modified T-cells
B.Non-cell-based
1.Cytokines, e.g., interferon-a
2.Oncolytic viruses
3.Peptide vaccines
4.Checkpoint inhibitor antibodies

DC: Dendritic cells, CIK: Cytokine-induced killer, NK: Natural killer

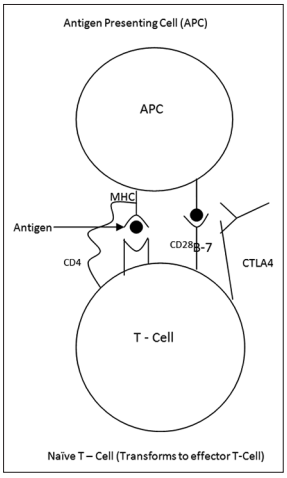

Figure 2: Early immune response; priming phase; first signal T-cell recognizes antigen presented by major histocompatibility complex of antigen-presenting cells. Costimulation-7 through engagement of CD-28 and B-7. If CTLA-4 interacts with B-7, the effector function of T-cell decreases. Hence, blocking antibody against CTLA-4 enhances effector T-cell function. (Lymph node response)

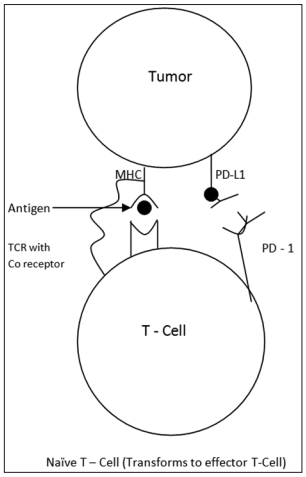

Figure 3: Effector phase: T-cell interacts with tumor. PD-1 and PD-L1 interaction silences T-cell. Hence, antibodies against both may be used to enhance T-cell effector function. (Peripheral tissue)

approved for metastatic bladder cancer.[27] Adverse events of these immune drugs are generally immune-related and managed with the use of corticosteroids or dosing interruptions. The common ones are fatigue, diarrhea, hyperthyroidism, hypothyroidism, rash, and pruritis.[27]

When patients are initially treated with these immune checkpoint inhibitors, they may show signs of initial worsening which later stabilizes or regresses. This is called pseudoprogression. Based on these observations, alternate response criteria, termed the immune-related response criteria were proposed and under investigation.[28]

Targeted agents

These agents may boost DC priming and activities of T cells. Monoclonal antibodies coat tumor cells and boost phagocytosis. Numerous therapies increase the expression of tumor antigens on tumor cell surfaces, increasing TCR signaling and activation. Enforcement of exogenous costimulatory signals potentiates the production of inflammatory cytokines. Targeted agents may antagonize immunosuppression in tumor microenvironment. Examples of these targeted agents include monoclonal antibodies such as transtuzumab, cetuximab, and bevacizumab; TKIs such as imatinib and sunitinib; JAK2 inhibitors; mTOR inhibitor-temsirolimus; proteasome inhibitor-bortezomib and immunomodulatory drugs such as thalidomide and lenalidomide.[29]

Intravesical BCG (for early-stage bladder cancer) and imiquimod (for very early-stage skin cancer or pre-cancer) are the other drugs that boost the immune system in a non-specific way.[23]

Cancer vaccines

Vaccines try to mount an attack on cancer cells by active immunization. These may be either prophylactic (such as against viruses hepatitis B or Human papillomavirus where the etiological agent is known) or therapeutic (to treat the already existing disease like cancer).[30] These vaccines are expected to augment or induce the functions of effector cells. Some cancer treatment vaccines are made up of cancer cells, parts of cancer cells, or pure antigens. Patients own immune system may be used to create vaccines in the laboratory and then injecting it back into the body.[23] Vaccines may be combined with other substances called adjuvants to boost the efficacy. Sipuleucel-T is the only vaccine approved so far to treat cancer. It is an autologous dendritic-cell-based vaccine supplemented with an incompletely characterized complex mixture of peripheral blood mononuclear cells (PBMC), cytokine and tumor-derived differentiation antigen[4] [Figure 4]. This vaccine did not cure prostate cancer but showed improvement in median survival.

Many different types of vaccines are now being studied to treat a variety of cancers.[23] These are:-

a. Tumor cell vaccines: Made from actual cancer cells.
b. Antigen vaccines: Made from one or few antigens rather than the whole cell.
c. DC vaccine: Like sipuleucel-T.
d. Vector-based vaccine: Use special delivery systems to make them more effective.

e. Cancer immunotherapeutic RNA vaccines: Nucleic acid vaccines, "naked" RNA cancer vaccines, RNA encapsulated vaccines, targeting RNA vaccines, and RNA-loaded nanoparticles are under investigational role.

Vaccines are being studied in a variety of cancers including lung, kidney, melanoma, pancreas, prostate, colorectal, cervical, breast, brain, and lymphoma.[23]

Adoptive immunotherapy

This is passive immunization against cancer. It is also called ACT.[31] Following is the general mechanism of action of the ACT [Figure 5].

The various types of ACT are as follows:-

Lymphokine-activated killer (LAK) cell therapy

Development of ACT started with the generation of LAK cells. LAKs cells are a heterogeneous population of cells consisting primarily of NK, NKT, and T cells. PBMC either from cancer patient or healthy donor are cultured *in vitro* with IL-2. This generates LAK cells. NK cells are the predominant effector cells within LAKs and are mechanistically equivalent to peripheral NK cells, but more cytotoxic again tumor cells. These cells can kill cancer cells of colon, pancreatic, adrenal, esophageal, renal tumors, and sarcomas.[32] However, the efficacy of LAK cells appeared to be low and showed increased toxicity associated with IL-2 (coadministered to maintain LAK activation *in vivo*).

Cytokine-induced killer (CIK) cell therapy

In general, CIK cells are a heterogeneous cell population generated from human PBMC after *in vitro* stimulation by multiple factors including IL-2, IL-1, IFN-gamma, and anti-CD3 monoclonal antibody. The various clinical studies concluded the efficacy of CIK cells to be unsatisfactory.[31,33]

DC CIK cell therapy

Due to the failure of CIK cells, these cells were further cultured in the presence of DC, and the resultant DC-CIK cells showed enhanced lytic action *in vitro* for cancer cells.[34] The investigational role involves lung cancer, myeloma, CML, osteosarcoma, and leukemia.[31]

TIL therapy

Some immune cells, especially lymphocytes, are embedded in neoplastic lesions of tumor tissue that is called TIL. Only a fraction of TILs express TCRs unique for tumor-associated antigens and exerts a cytotoxic effect on malignant cells. TCRs expressing TILs are isolated from resected tumors, scrutinized, and expanded *ex vivo*. In metastatic melanomas, TILs were unable to persist in the presence of T-cell growth factor, IL-2. However, administration of lymphodepleting preparative regimen (cyclophosphamide and fludarabine) significantly improved TILs function. Although this approach has shown a durable response in patients with better overall response rates and complete response rates, there are several limitations too. Feasibility is the main concern where the tumors need to be get resected for the extraction and expansion of TILs *in vitro* which may take several weeks and becomes expensive too. Another concern is the suitability and tolerability of lymphodepleting preparative regimen consist of cyclophosphamide and fludarabine to the patients.[35] Till date, TILs can be isolated from many types of cancers, but so far only TILs isolated from melanomas have shown specific cytolysis activity. The investigational role involves lung cancer, melanoma, renal cancer, and squamous cell cancer.[31]

Genetically engineered T cell therapy

Considering the TIL therapy limitation of identifying antigen-specific T cells in other cancer types, it is impossible to obtain tumor-reactive TILs from all types of cancers. Hence, there is a need to modify T cells to increase antitumor activity. This

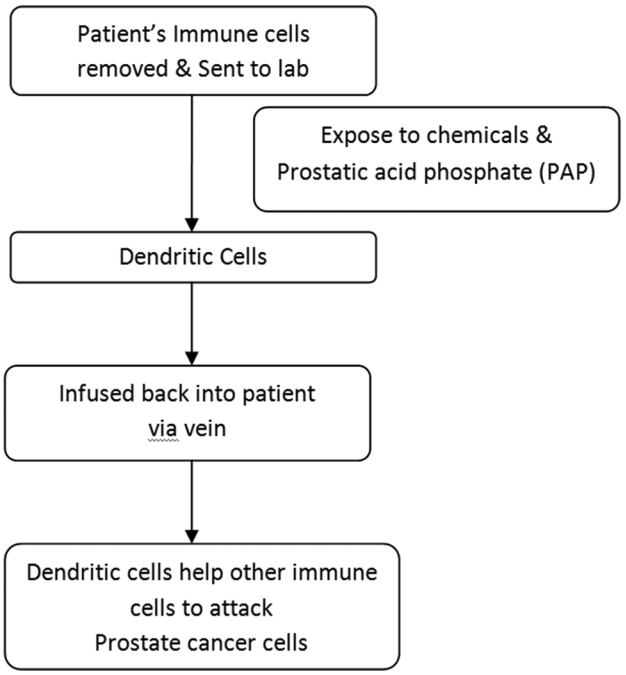

Figure 4: Mechanism of sipuleucel-T vaccine

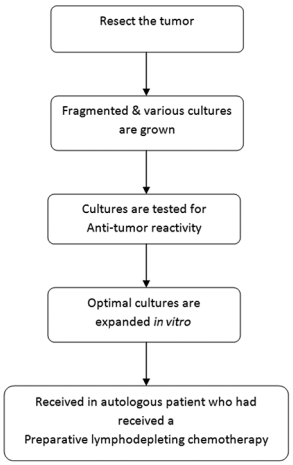

Figure 5: Mechanism of adoptive cell therapy

modification is genetic engineering and gives T cells with high avidity and tumor reactivity. Viral vectors carrying genes coding TCR specific to tumor antigens or CARs are genetically introduced into PBMC or TILs and transferred into patients treated with preconditioning lymphodepletion. [Figure 6] At present, there are two types of genetically engineered T cells available for ACT for cancer, TCR gene, and CAR gene-modified T cells. This is a promising approach for hard-to-treat leukemias and lymphomas and has an investigational role in melanomas.[31]

TCR T cells

These T cells are cloned with TCR specific to tumor antigens either from a patient or humanized mice immunized with specific tumor antigen and recognize tumor-specific antigens in the context of MHC. Many clinical trials tested TCRs against tumor antigens with observed clinical responses such as for MART1 for metastatic melanoma, gp100 for melanoma, carcinoembryonic antigen in colorectal cancer, and MAGE-3, and NY-ESO-1 in synovial sarcoma and melanoma.[36] Unlike TILs, TCR T cells can be produced by peripheral blood T cells and unlike CARs, can detect intracellular antigens. Expressions of tumor-specific antigen on healthy tissues are also recognizing by TCRs which lead to on-target toxicity, for example, gp100 and MART-1 are expressed by normal melanocytes present in the skin and retina.[37] Some other clinical trials have also reported issues of neurological or cardiac toxicity.

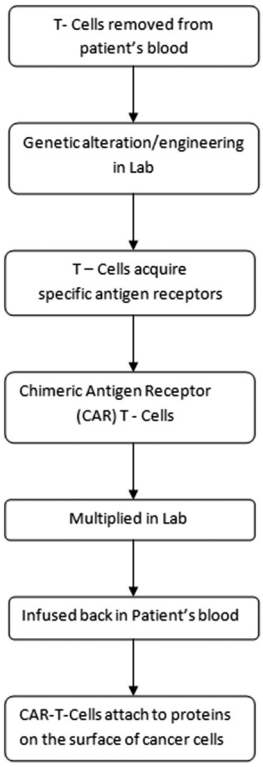

Figure 6: Genetic engineering of T-cells create chimeric antigen receptors

Table 5: Receptors on T cells[4]

Activating receptors	Inhibiting receptors
CD28	CTLA-4
OX40	PD-1
GITR	TIM-3
CD137	BTLA
CD27	VISTA
HVEM	LAG-3

PD-1: Programmed death receptor-1

CAR T cells

The limitation with TCR T cell therapy is that it is only suitable for MHC compatible patients, and often MHC is downregulated because of reduced antigen expression on tumors. Variable domains of a monoclonal antibody (Fv) is fused with T-cell signaling domains to construct CAR T cells, which exhibit high-affinity MHC independent recognition of, any surface antigen, including carbohydrates and phospholipids.[35] Encouraging results have been obtain in B-cell malignancy with use of CAR T cells targeting CD19 antigen, by far the most common antigen targeted in hematological B-cell cancers.[38] Other investigational trials are underway to target other antigens such as CD20, CD22, CD30, CD33, CD123, CD133, CD138, ROR1, κ light chain, and B-cell maturation antigen.[39] Clinical reports have also demonstrated a realistic clinical response in patients with partial remission follicular lymphoma treated with second-generation CD19 CAR with a CD28 costimulation domain and in leukemia with CD19 CAR with a 4-1BB costimulation domain.[40] Importantly, such study also demonstrated the capability of the CAR T cells to expand *in vivo* correlated with clinical responses, where two patients with the complete response shown prolonged persistence and functional CAR T cells beyond 4 years without relapse. Various clinical trials reported use of developed CAR T targeting GD2 in neuroblastoma, epidermal growth factor receptor (EGFR) members in breast, ovarian, bladder, salivary gland, endometrial, pancreatic, and non-small cell lung cancer, folate receptor-α for ovarian cancer, vascular endothelial growth factor receptor 2 overexpressed in tumor vasculature, and carbonic anhydrase IX (CAIX) for renal cell carcinoma. With many advantages of CART technology, extensive use of CAR therapy may be limited because of On/Off target toxicity.[39] For example, use of CART against CAIX in RCC patients experienced liver toxicity and no tumor regression because of CAIX expression in normal tissues including, small intestine, and gastric mucosa.[41] Same was the case with anti-human EGFR 2 (HER2) (EGFR family member) CAR T therapy in a subset of tumors overexpressing HER2 and B-cell aplasia resulting from CD19 CAR T cells.[42] Another issue with CART therapy is CAR-mediated cytokine release syndrome, which can sometimes life-threatening complication.

It is very important to address these limitations, and several approaches are under investigation to avert it, for instance, careful choice of target antigen to avoid on target and off-target toxicity. Selection of target should be specific to the tumor and should not be present on vital organ or normal tissue.

Oncolytic viruses

Largely investigational

Altered viruses may kill cancer cells directly or alert the immune system against them. An example is talimogene laherparepvec that may be used to treat inoperable melanoma limited to skin and lymph nodes.[23]

Prospects in Cancer Immunotherapy

Due to the multi-step nature of cancer development, numerous genetic clones of cancer cells, and tumor antigen heterogeneities, and effective therapies for cancer patients may require highly personalized treatment. Cancer research is being actively pursued worldwide.

Merkel cell polyoma oncoprotein associated vaccine, hepatitis protein derived vaccine for hepatocellular cancer, JC virus derived vaccine for colorectal cancer, and HTLV-1 protein derived vaccine for adult T cell leukemia are under investigational role.[31]

T cell activation may be stimulated through activating receptors on surface and inhibition may be augmented by stimulating inhibitory receptors. Following Table 5 shows various such receptors.

Agonistic antibodies for activating receptors and blocking antibodies for inhibitory receptors may be the next generation T cell modulators that enhance T cell stimulation to promote tumor destruction.

Prospects of combining immunotherapies, targeted therapies and conventional chemotherapies in various permutations and combinations are under consideration and in future may expand the armamentarium against cancer.

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