Advances in cancer immunology and cancer immunotherapeutics

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ABSTRACT

The immune system plays an important role in protection against tumor growth. The theory of immune surveillance has evolved today leading to recent advancement in field of cancer immunotherapy. Here, we have revisited the basic structure of immune system with emphasis on its interaction with tumor cells. Also discussed briefly, is the landscape of current cancer immunotherapy and the future it holds.

Key words: Immunotherapy, Immune system, Cancer immunosurveillence, Checkpoint inhibitors

Introduction

It was been now known for at least a century that the immune system plays an important role in protection against tumor growth. Dr. William Coley, back in 1891, reported successful treatment of incurable bone sarcoma by injection of streptococcal bacteria.[1] He hypothesized that somehow the bacteria generated an immune response that was active against the tumor cells. Due to the limited knowledge of immune system at that time, the exact mechanism of this response was not understood. Although he reported successful treatment of many bone and soft tissue cancers, with what was then known as Coley's toxin, the treatment fell out of favor with the development of chemo- and radio-therapy. In past two decades, researchers have explored our immune system and much has been understood about its interaction with tumor cells. The theory of immune surveillance has now evolved into a more complex "immunoediting" concept.[2-4] With the recent advancement in the field of cancer immunotherapy, especially discovery of checkpoint inhibitors (anti-cytotoxic T-lymphocyte-associated protein-4 [CTLA4] and programmed death-1 [PD1]), more and more cancer types are showing a response to these novel treatment modalities. In this article, we revisit the basic structure of the immune system with emphasis on its interaction with tumor cells, also discussed briefly, are the landscape of current cancer immunotherapy and the future it holds.

The Immune System

Immunity traditionally refers to the defense mechanism against the infectious pathogen. The immune system consists of complex interaction between various cells, organs and molecules to help the body fight against foreign pathogens. It has been long known that immune system plays an important role not only in the prevention of tumorgenesis but also in remodeling the immunogenicity of the tumor cells.[3] The immune system can be broadly divided into two types: (1) Innate and (2) adaptive immune system.

Innate Immunity

This is a non-specific defense mechanism. The receptor that recognizes the pathogens is germline encoded. The main components of innate immunity are epithelial barriers, cells (neutrophils, macrophages, dendritic cells [DCs], and natural killer cells [NK cells]) and several plasma proteins like complements. Instead of recognizing a specific antigen, innate immunity recognizes pathogen-associated molecular patterns, which makes it effective against wide range of pathogen. Together this immunity act as the first line of defense but generates no memory of prior exposure to same antigens.[5,6] Cells of the innate immune system have an important role in the tumor microenvironment (TME).

Neutrophils

These are the most important phagocytic cells of the innate immunity. These cells circulate in the bloodstream and are recruited to the site of the infection by various cytokines. They recognize the pathogens by pattern recognition receptors leading to their phagocytosis and destruction.[7] The fate of these neutrophils in the TME is determined by the dominant cytokines. TME associate neutrophils can take up either tumor preventing (N1) or promoting (N2) phenotype. Cytokines like transforming growth factor (TGF)-β promotes its differentiation into N2 phenotype that increases tumor cell survival and proliferation by production various molecules (ELA2 and oncostatin). Lack of TGF-β promotes differentiation to N2

phenotype, which exerts antitumor response by the release of certain cytokines (interleukin [IL]-12 and tumor necrosis factor [TNF]-α) and recruitment of effector T cells.[8]

DCs

These are the most important antigen-presenting cells (APCs). In normal circumstances, DCs present self-antigens to the T cells to maintain immune tolerance. This promotes the formation of regulatory T cells (Tregs) that has immunosuppressive effects and prevents autoimmunity.[9] DCs also act as a bridge between innate and adaptive immunity. On encounter with a pathogen, the antigens are processed and presented on major histocompatibility complex (MHC) molecules to the naïve T cell in the lymphoid tissues. They also express costimulatory molecules such as B7 (CD80 and CD86) and release certain cytokines that lead to complete T cell activation for effector functions. Similar to the microbial antigens, tumor-associated antigens (TAAs) are presented to T cells to mount an antitumor response.[10,11]

Macrophages

These are large, mononuclear cells primarily involved in phagocytosis; secretion of pro-inflammatory cytokines and also function as APCs. Like the neutrophils, they play a dual role in TME.[12] Depending on the cytokine milieu of the TME, macrophages may assume a tumor preventing (M1) or tumor-promoting (M2) phenotype. Cytokines such as macrophage-colony stimulating factor (M-CSF), TGF-β, and IL-10 polarize differentiation of macrophages to M2 phenotype.[13,14] M2 macrophages promote tumor growth and metastasis by inducing angiogenesis, recruiting Tregs and secreting immunosuppressive cytokines IL-4, IL-6, IL-10, TGF-β, and indolamine 2,3-dioxygenase (IDO). M1 phenotype causes tumor cell or microbial death by activating an adaptive immune response, secretion of pro-inflammatory cytokines - IL-12, IL-23, TNF, and reactive oxygen metabolites.[13,14] Promoting M1 phenotype in TME may likely provide a therapeutic advantage in cancer treatment.

NK cells

NK cells are a unique subset of lymphocytes and part of the innate immune system. They lack specific surface receptors such as T-cell receptor (TCR) or immunoglobulin (Ig). They are identified by expression of surface molecule CD56 and CD16. The balance between activation of various surface inhibitory and stimulatory receptors regulates the NK cells activity. NK cells identify normal cell with self-MHC I molecules by interaction with its inhibitory killer-cell Ig like receptors.[15,16] The best-characterized NK cell stimulatory receptor is NKG2D. The expression of MHC I molecules is decreased and NKG2D ligand is increased in infected and tumor cells which lead to NK cell-mediated cytotoxicity and/or promote chemokine production.[17] NK cells induce cytotoxicity by releasing perforins and granzymes or by expression of Fas ligand or TBF-a related apoptosis-inducing ligand (TRAIL) that binds to receptors on the transformed cell to induce apoptosis.[18] Tumors cells evolve to evade NK cells mediated cytotoxicity commonly by NKG2D ligand proteolysis.[19]

Other cells

The role of basophils, eosinophils, and mast cells is less understood in tumor genesis. Due to their interaction with other cells and secretion of cytokines within the TME, basophils may have a tumor-promoting role while eosinophils may have a tumor-preventing role.[20,21] Mast cells, on the other hand, have been associated with both good and poor prognosis in different cancer types.[22-25]

Adaptive Immunity

This is an antigen-specific immune response. It mainly consists of lymphocytes (B and T cells) and their products like antibodies (Ig)/cytokines. The adaptive response is slow on the initial exposure to the pathogen or TAAs, but due to the formation of memory cells, any subsequent exposure to the same antigen generates a faster and robust response.[12] The cornerstone of lymphocyte activation is antigenic peptide presentation in the conjugation of MHC molecules. The MHC I molecule is expressed on all nucleated cell in the body while the MHC II molecule is only expressed on APCs. The MHC I and II molecule interacts with CD8+ and CD4+ T-cells, respectively. T-cells have the ability to mount an effective anticancer immune response and have been the focus of current cancer immunotherapy research.

T lymphocytes (T-cells)

T-cells are derived lymphoid progenitor cells that migrate from bone marrow to the thymus (central lymphoid organ). In the cortex of the thymus, they undergo a complex differentiation into either CD4+ or CD8+ T cells based on their affinity of their TCR to MHC II or I molecule, respectively. The T cells that do not interact with MHC molecules undergo apoptosis (positive selection). In the medulla, T cells are presented with a wide variety of self-antigens by the thymic medullary epithelial cells. Most of the T cells with high affinity to self-antigens undergo apoptosis (negative selection). The process of negative selection is a very important step to develop self-tolerance and prevent autoimmunity. The naïve T cells, thus, formed may be activated in the thymus or secondary lymphoid organs by the APCs (DCs, macrophages).[26,27] To counterbalance, the immune system has certain cells, T-regulatory cells (Tregs) that keep the immune system in check against overactivation. Tregs can either be naturally occurring positively selected high-affinity T cells in thymus (characterized by expression of forkhead box P3 – FOXP3) or differentiate in periphery from Naïve CD4+ T cells and express immunosuppressive cytokines, e.g., IL-10 or TGF-β.[28]

Activation of T cells

At least two interactions are required between the naïve T cells and APCs for activation of T-cells. The cytokines secreted helps in proliferation and survival of the activated T-cells:

1. Interaction between TCR complex and antigen presented in conjugation with MHC molecules. In 95% of T cells, TCR is a heterodimer of alpha and beta chains, which forms a complex with CD3 and zeta proteins for signal transduction.[12]

2. Coregulatory stimulus: There are various stimulatory and inhibitory receptors that are involved in T-cell activation process. While the costimulatory stimulus is required for complete activation of T-cell, the inhibitory stimulus keeps the immune system in check to prevent overactivation. The major stimulatory receptors are CD28 (classical) that interacts with B7 molecules, ICOS (inducible costimulator) and CD27 (TNF family receptor).[29] The major inhibitory receptors are CTLA-4, PD-1, and T cell immunoglobulin mucin-3 (TIM-3). Inadequate stimulus may lead to T-cell anergy and apoptosis. The balance between the stimulatory and inhibitory signals determines the regulation of T-cells. There are various other coregulatory signals under investigation.[30]

A third signal is known to determine the ultimate fate of activated T-cells. DCs secreted various CD4+ T-cells polarizing molecules have been identified. Molecules such as IL-12, IL-23, IL-27, type I interferons (IFNs), and ICAM1 polarize naïve CD 4+ T-cells to TH-1 while MCP1, OX40L polarize them to TH-2 cells.[31,32] Polarization to TH-2 response has been associated with poor prognosis in many cancer types. It is also known that depending on the conditions, in which DCs are activated, they may secrete factors such as IL-10 and TGF-β that transforms CD4+ T-cells to regulatory T-cells.[28] Activated effector T-cells migrate to the site of antigen origination and perform various functions. CD8+ T-cells (CTLs) may be activated by APCs in conjugation with MHC I molecule or by TH-1 cells. These CD8+ T-cells are directly cytotoxic. TH-2 cells promote humoral immunity by activation of B-cells. Effector T-cells secrete various cytokines (IL-2), vital for their proliferation and survival. Memory cells are also formed that generates a robust immune response on a subsequent encounter with the same antigen.[12]

B-lymphocytes

B-lymphocytes are derived from lymphoid progenitor cells and mature in the bone marrow. They recognize antigen by B-cell receptor complex (BCR). BCR consist of membrane-bound antibodies of IgM and IgD isotypes in conjugation with heterodimer of Ig alpha and Ig beta proteins (similar to CD3 and zeta proteins of TCR). Activation of B-cells also requires costimulatory signals from interaction of complement receptor-2 (CR2 or CD21) that binds to complement proteins and CD40 that binds to CD40L on TH-2 cells. Once activated, B-cells transforms to plasma cells for immunoglobulin (Ig) production and memory cells.[12,33] Igs are heterodimer of identical two light (L) and two heavy chains (H).[34] Each chain has a constant and a variable region. The variable region of H and L chains determines the specificity of the Igs while the constant region of H chains determines the isotypes (IgM, IgD, IgG, IgE, and IgA).[34] Several monoclonal

antibodies, currently used in cancer immunotherapy, target specific molecule/receptor to augment immune response - Ipilimumab (anti CTLA-4), nivolumab and pembrolizumab (anti-PD1) are few of many examples.

Cancer Immunology

Recently, research has been focused to understand the mechanics of the TME. The TME consists of genetically altered tumor cells normal cells of immune system, stromal cells, and cytokines. The interaction of these cells and cytokines with the tumor cells may be both inhibitory and stimulatory. In the attempt to evade the immune system, tumor cells promote the recruitment of cells and cytokines that support tumorigenesis. This tumor sustaining TME helps the tumor cells to attain the hallmark traits that lead to a clinically apparent neoplastic disease. Hanahan and Weinberg enumerated these hallmark traits - Sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, and evading immune destruction. This knowledge of TME has enabled the researchers to develop innovative drugs and techniques to target these traits for favorable cancer immunotherapy.[35]

Burnet and Thomas introduced the Immune surveillance hypothesis in late 1950s. They proposed that adaptive immune system prevents the growth of tumor cells in immunocompetent hosts.[2,4] The research in past few decades has evolved our understanding of cancer immunology leading to abandonment of immune surveillance hypothesis and adoption of a more evolved concept of immunoediting. This concept explains the dual interaction of tumor cells with an immune system that can be both tumor promoting and preventing roles. Of note, the data behind this concept is largely based on animal studies. Immunoediting has three phases: Elimination, equilibrium and escape, which occur in the TME.[3,36] In the elimination phase, the transformed cells are destroyed by both innate and adaptive immune response. Although the exact mechanism of this phase is not clearly understood, different experimental models have been suggested. The tumor antigens are presented to T-cells by various APCs, mainly DCs. T effector and NK cells identify the tumor antigens presented in conjugation with MHC-I and/or NKG2D ligand, respectively, and induce tumor cell death. Effector T cells also interact with Fas and TRAIL receptors on tumor cells and secrete IFN-γ which inhibits tumor growth. Tumor protective phenotype of neutrophils (N1) and macrophages (M1) also contribute to tumor cell death by increased production of TNF-α, IL-1, IL-12, and ROS. [3,36-38] Overall, during this phase the scale is tipped toward the cells and cytokines that prevent tumor growth. If the tumor is not completely eliminated, it enters into the equilibrium phase. Unlike the elimination phase, this phase is mostly governed by the adaptive immune system. During this phase, the tumor remains dormant due to a relative balance between the tumor-promoting (IL-10 and IL-23) and antitumor (IL-12

and IFN-γ) cytokines within the TME.[39,40] This phase may be very prolonged, and tumor may never become clinically apparent. Throughout the equilibrium phase, tumor cells are under constant immunologic pressure, which may cause genetic modifications, allowing the tumor to enter the escape phase. In this phase, the tumor cell variant develops the ability to evade antitumor immune response and manifests as a clinically apparent tumor. The tumor escape can occur through various intrinsic (loss of tumor antigen or resistance to cytotoxic effects by induction of antiapoptotic pathways, e.g., STAT3 or BCL- 2) and extrinsic mechanisms (increased immunosuppressive molecules, e.g., Vascular endothelial growth factor (VEGF), TGF-β, IDO, PD1- programmed death ligand 1 (PDL-1), CTLA-4, and cells, e.g., regulator T-cells, and myeloid-derived suppressor cells), which makes the TME conducive for tumor growth.[3,36,41] The tumor cells exploit various signal transduction pathways to evade the immune response. One of such pathway is JAK/STAT. Tumor activates this pathway for overexpression of STAT3 which promotes tumor growth by increasing concentration of various molecules in TME, e.g., TGF-β, IL-6, IL-10, and VEGF.[42] Examples of other pathways modulated by tumor cells are NF-KB, PI3K/AKT and BRAF-MAPK.[43-47] The alteration in surface MHC expression on tumor cells, by downregulation of proteins such as TAP1/2 or LMP2/7 or mutation in HLA genes, has also been associated with their ability for tumor evasion.[48]

Besides recruiting Tregs, M2 macrophages and N2 neutrophils, TME also promotes the high concentration of immature myeloid cells called myeloid-derived suppressor cells (MDSCs). These cells are a heterogeneous population of immature myeloid cells. Under normal circumstances, these immature cells differentiate into granulocytes, macrophages, or DCs. The expansion of the immature cells occurs in the presence of tumor or inflammation. MDSCs can be found abundantly in the TME and suppresses the immune system, esp. T-cells, by upregulating expression of arginase-1 (ARG-1) and inducible nitric oxide (NO) synthase. There is increased production of reactive oxygen species (ROS) and NO by MDSCs. ROS, e.g., peroxynitrite negatively affects TCRs while NO decreases MHC II expression and induces T-cell apoptosis.[49] MDSCs have been identified as major immune suppressive cells in the TME and various methods to eliminate the effects of these cells are being investigated. Some of the suggested approaches are to promote differentiation of the MDSCs into mature myeloid cells with no suppressive effects (Vitamin D3 and all-trans retinoic acids), inhibit MDSCs expansion (KIT-specific Ab, VEGF-trap) or inhibit MDSCs functions (cyclooxygenase-2 inhibitors).[50-54] Polarization to TH2 response has been associated with poor prognosis and promoted in TME. Recently, matrix metalloproteinase-2 (MMP-2) produced by the tumor cells has been linked to promoting TH2 response by acting directly as an antigen, increased OX40L expression and DCs receptor alteration.[55] Tumor cells also develop perforin and granzyme inhibitors to resist CD8+ T-cell and NK cell-induced cytotoxicity.[56] Various other molecules are also involved (discussed elsewhere in this article) which may ultimately lead to immune evasion by tumor cells.

Cancer Immunotherapy

The interaction between cancer cells and immune system, especially T-cells, is aptly explained by Chen *et al.*'s cancer-immunity cycle. These series of steps have various checkpoints and modulators, which may augment or dampen the immune response. First, the DC captures the TAAs which are released by the cancer cells (Step 2). The DCs present the TAAs to the T cells in conjugation with MHC molecules in the lymphoid tissue. The DC may capture the TAAs in the tumor bed or the lymphoid tissue (Step 3). The naïve T-cells in the lymphoid organs are activated and differentiate into either effector or regulator T-cells (Step 4). The effector T-cells then migrate and infiltrate the tumor bed (Step 5). Due to the acquired specificity, T-cells recognize the tumor cells and (Step 6) kills the tumor cell which again releases the TAAs to be captured by DCs. In an ideal situation an effective anticancer response should be generated but more often than not this does not happen. Due to the constant immune pressure, the tumor cells undergo several genetic and epigenetic changes, and may ultimately evade the immune system.

Most of the recent immunotherapeutic agents target one of the steps in the cancer immunity cycle to attempt to amplify the immune response against the tumor cells.[57] Table 1 summarizes the list of FDA approved immunotherapeutic drugs.

Checkpoint Inhibitors

CTLA-4 inhibitors

CTLA-4, a transmembrane glycoprotein expressed on T cell surface, may interact with B7 molecules but unlike CD28 it inhibits the T-cell response and maintains immune tolerance.[12] This interaction occurs early in cancer immunity cycle in the lymphoid organs. Till date, two monoclonal antibodies against CTLA-4 have been developed - Ipilimumab (Human IgG1 mAb) and tremelimumab (Human IgG2 mAb). Ipilimumab is the most studied CTLA-4 inhibitor. FDA approved it in 2011 for treatment of unresectable or metastatic melanoma. The approval was based on 2 phase III RCTs that showed improvement in overall survival in melanoma patients. In one of the RCTs, 676 patients with unresectable Stage III or IV melanoma with the progression of disease on standard therapy were assigned to receive either ipilimumab plus gp100 peptide vaccine, ipilimumab alone or gp 100 alone. The median overall survival was significantly higher with ipilimumab with or without gp100 vaccine (10 months for ipilimumab plus gp100, 10.1 months for ipilimumab alone compared to 6.4 months for gp100 alone).[58] In the other study, 502 patients were randomly assigned to ipilimumab plus dacarbazine or dacarbazine plus placebo in previously untreated metastatic melanoma. The overall survival (OS) was significantly higher in ipilimumab

group (11.2 vs. 9.1 months).[59] Tremelimumab, IgG2 mAb CTLA4 inhibitor, failed to demonstrate a statistically significant response in a phase III RCT comparing it to the standard of care chemotherapy in patients with metastatic melanoma.[60] It has shown some activity against malignant mesothelioma in a small study.[61] The hypothesis that chemotherapy can cause the release of tumor antigens, which augments T-cell activation, has lead to studies with combination cytotoxic chemotherapy and CTLA4 inhibitors.[62] CTLA4 inhibitors have also shown positive results renal cell carcinoma (RCC), metastatic castration-resistant prostate cancer (CRPC), and urothelial cancer.[63-65]

Programmed Death-1/Ligand Inhibitor (PD-1/L Inhibitors)

Like the CTLA-4 pathway, PD-1/PDL-1 axis is another inhibitory signal exploited by the tumor cells to evade the immune response. This interaction occurs mostly in the TME. PD1 is an inhibitory receptor expressed on the activated T-cell (also on B-cell and NK cells) that interacts with two ligands, PDL-1 and PDL-2, on the tumor cell. In physiologic conditions, this interaction prevents T-cell from generating an autoimmune response. The tumor cells have increased expression of PD-1 ligands, mostly PDL-1, on its surface to prevent T-cell mediated tumor cell lysis.[12] In recent studies, inhibition of this axis has shown a promising antitumor response in different tumor types. Theoretically, PD1 inhibitors, which block its interaction with both PDL-1 and -2, should have greater response compared to inhibitors of either of the ligands alone. Two PD-1 inhibitors, nivolumab and pembrolizumab, have gained popularity in the past few years. In 2 phase III trials, nivolumab was associated with higher OS and better response rate in patients with advanced melanoma.[66,67] In a recent trial of nivolumab versus everolimus in previously treated advanced RCC, nivolumab was statistically significant better overall survival (median OS 25 months vs. 19.6 months) and objective response rate (25% vs. 5%).[68] In 2 phase III trials, nivolumab has shown prolonged OS, and better objective response rate (ORR) compared to docetaxel in previously treated advanced non-small-cell lung cancer (NSCLC).[69,70] Similarly, pembrolizumab has shown improved RR and prolonged survival in different advanced tumor types - melanoma, NSCLC, head and neck squamous cell carcinoma, and Hodgkin lymphoma.[71] Atezolizumab is a PDL-1 inhibitor approved by FDA for locally advanced or metastatic urothelial cancer and metastatic NSCLC.[72] Another PDL-1 inhibitor, durvalumab, has been granted breakthrough therapy designation for urothelial carcinoma.[73]

Table 1: FDA approved drug list

Drug class	Drugs	Mechanism	Indication*
Checkpoint inhibitors	Ipilimumab	Anti-CTLA4	Melanoma
Virotherapy	Nivolumab	Anti-PD1	Melanoma
Vaccine	Pembrolizumab	Anit-PD1	NSCLC
BiTE	Atezolizumab	Anti-PDL1	RCC
Cytokines	Ipilimumab + Nivolumab	Anti-CTAL4 + Anti PD-1	Hodgkin lymphoma
	Talimogene laherparepvec (T-VEC)	Oncolytic virus	HNSCC
	Sipuleucel-T	DCs based vaccine	Urothelial cancer
	Blinatumomab	Anti CD3/CD19 mAb	Melanoma
	Aldesleukin	Recombinant IL-2	NSCLC
	IFN-α	IFN-α	HNSCC
			Hodgkin lymphoma
			Urothelial cancer
			NSCLC
			Melanoma
			Melanoma
			Prostate cancer
			B-cell ALL
			RCC
			Melanoma
			HLL
			CLL
			AIDS related Kaposi
			Melanoma
			Follicular NHL

*Refer to dailymed.com for detailed indications. BiTE: Bi-specific T-cell engager, IFN: Interferon, CTLA4: Cytotoxic T-lymphocyte-associated protein-4, PD1: Programmed death-1, IL: Interleukin, NSCLC: Non-small-cell lung cancer, RCC: Renal cell carcinoma, HNSCC: Head and neck squamous cell carcinoma, NHL: Non-Hodgkin lymphoma, CLL: Chronic lymphocytic leukemia

Adoptive T-Cell Therapies

Tumor infiltrating lymphocytes (TILs)

TILs are the activated T-cells within the TME. This therapy essentially has 2 phases - removal the TILs from immunosuppressive tumor environment, *ex vivo* clonal expansion with re-establishment of antitumor activity and infusion of these T-cells back into the host. Most commonly used a source of TILs has been the excised tumor itself, which may not be available in many cancer types. These TILs are then grown in lymphocyte favorable medium mainly containing IL-2. The TILs with tumor reactivity, as shown by increased IFN-γ production, then undergo clonal expansion and infused back into the patient. Although the concept has been around for few decades, clinical experience with TILs has been limited mostly to melanomas. In the study by Rosenberg *et al.*, patients with metastatic melanomas received TILs plus IL-2 after a lymphodepleting regimen. The authors reported RRs ranging 49–7%. The factors, which were significantly associated with favorable response, were longer telomeres of infused cells, number of CD8+CD27+ cells infused and persistence of infused cells in circulation after 1 month.[74]

Chimeric antigen receptor (CAR) T-cells

This immunotherapy is mostly investigated in hematological malignancies. The CARs are genetically engineered mAbs on the surface of T-cells. The CARs have an antigen binding and a T-cell activating domain. More recent generations of CAR T-cells have various costimulatory domains such as CD28, OX40L, or 4-1BB that leads enhanced T-cell proliferation and survival.[75] Another approach that has been under investigation is genetic modification of CAR cells to enhance cytokine production that favorably alters the TME on activation. After successful transduction of mAb on the T-cell surface, they are clonally expanded and infused back into the patients. Expression of mAb can impart specificity to an antigen of interest. This technique essentially circumvents the need for tumor antigen presentation to the T-cell with subsequent priming and activation.[76] The most investigated CAR cell therapy is against CD19 for B cell lineage malignancies with overwhelming response across multiple clinical trials.[77-81] Unfortunately, the data are not very encouraging in solid tumors.[82] Obvious on target-off tumor side effects should be expected as the target antigen might be expressed on the normal tissue. Another safety concern is "cytokine storm" associated with excessive T-cell activation.[81,83] A plethora of target antigens specific to tumor cells is currently under investigation to minimize the normal tissue destruction by the CAR T cells.[75]

TCR therapy

In this therapy, the alpha and beta chain of the TCR is genetically modified to confer TAA specificity to the T cells. Like CAR-T cells, these engineered T-cells are expanded and infused back into the patient. This therapy theoretically should be feasible in any tumor type that expresses the specific antigen in conjugation with a MHC molecule. Destruction of normal tissue expressing the same antigen is always been a concern for this therapy.[84] Efforts to identify highly specific tumor antigen is required to minimize the on target-off tumor effects.[85] At present, TCR therapy against NY-ESO antigen for advanced synovial sarcoma in LA-A*201, HLA-A*205, or HLA-A*206 allele-positive patients has been granted breakthrough therapy designation by the FDA.[86,87]

Vaccines

The major hurdle for the development of a successful cancer vaccine has been identification of tumor-specific antigen and adequate delivery mechanism for activation of T-cells.[84] FDA approved Sipuleucel-T, an adoptive DCs therapy, in 2010, for metastatic CRPC.[88] It consists of *ex vivo* activated DCs and recombinant expression of fusion protein PA2014 (prostatic acid phosphatase plus GM-CSF). In a Phase III RCT, Sipuleucel-T was associated with improved median OS (25.8 vs. 21.7 months, $P = 0.032$).[89] PROSTVAC, a recombinant poxvirus, genetically modified to express three costimulatory signals (LFA-3, ICAM-1, and B7.1) is being studied in a Phase III RCT after a successful Phase II trial.[90,91] Even with recent developments, cancer vaccines have a long way to go before they are considered standard cancer treatment.

Oncolytic Viruses

Although the idea of oncolytic viral therapy has been there for a while, it has recently been recognized as a viable option for cancer treatment.[92] The anticancer response of oncolytic viral therapy is based on three complementary mechanisms - preferential infection of tumor cells and direct lysis, release of tumor antigens, and systemic immune response against tumor cells. Preferential tumor cell infection is naturally achievable since cancer cells frequently have impaired antiviral immune response.[93] Martuza *et al.* introduced the concept of genetically engineered oncolytic virus in 1991. They demonstrated that HSV-1 with thymidine kinase gene mutation was selective for cancer cells.[94] Till date, only 1 genetically modified oncolytic viruses have been approved for cancer treatment in the USA. T-vec (Talimogene laherparepvec) is an HSV-1 with deletions in y34.5 and a47 gene with the insertion of GM-CSF gene at former loci. FDA approved T-vec in 2015 for treatment of unresectable Stage IIIB-IV melanoma.[95] The approval was based on phase III clinical trial that compared T-vec to GM-CSF alone that showed superior OS and durable response rate.[96] Several other oncolytic viruses (G47Δ for Glioblastoma, JX-594 for HCC, CG0070, or bladder cancer) are currently under clinical investigation.[97] In the near future, oncolytic viral therapy has the potential to be a part of standard cancer treatment.

Cytokine Therapy

Like most of the other components of immune system, cytokines play a dual role in cancer immunology. Major hurdles for cytokine therapy have been lack of specific target cell population and overlapping functions. Till date, FDA has approved only IL-2 and IFN-α for cancer treatment. Current indications for recombinant IL-2 are metastatic RCC and melanoma. In a pooled analysis of 270 patients with metastatic melanoma, high dose IL-2 had an ORR of 16% (CR 6% and PR 10%)[98,99] Similarly for mRCC, ORR of 20% (CR 9% and PR 11%).[100] IFN-α showed improved survival rates for Hairy cell leukemia and chronic myeloid leukemia.[101,102] The utilization of IFN-α has significantly decreased in CML due to development of imatinib (BCR-ABL inhibition). Patients with Stage IIB or III melanoma also have better overall survival with high dose IFN-α.[103,104]

Other Therapies

Bi-specific T-cell engager (BiTE)

These mAbs enable T-cell interaction with tumor cells without the need of MHC molecules. The BiTE mAb binds to CD3 domain of T-cells and tumor-specific peptides on tumor cell surface. This interaction is analogous to the physiologic T-cell activation process that leads cytotoxic effects. FDA has approved blinatumomab, a CD3-CD19 BiTE mAb, for treatment of Philadelphia chromosome negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia. BiTE mAbs targeting various other tumor surface molecules (CEA, EpCAM, MCSP, EGFR, and Her2/neu) are under investigation.[105,106]

Future Directions

Combination therapies

Several trials are registered on Clinicaltrials.gov to study the advantage of combination immunotherapeutic modalities. The rationale behind these studies is to give maximum boost to the immune system to overcome the various rate-limiting steps in its activation and generate an effective antitumor immune response. Obvious concerns with overactivation of immune system and damage to normal tissue are associated with this approach. At present, FDA has approved a combination of ipilimumab and nivolumab, for treatment of advanced or metastatic melanoma. Although higher toxicity was reported with the combination treatment compared to either of the drug alone.[107] The combination of immunotherapy and conventional chemo- or radio-therapy is also under investigation. Conventional therapy is thought to increase the release of TAAs that can further augment the response to the immune modulatory therapies.[108,109]

Other Immunomodulatory Molecules

Checkpoint inhibitors

Besides CTLA-4/PD-1, there are other checkpoints that are being exploited for effective cancer immunotherapy.

Lymphocyte-activation gene 3 is expressed of T-cells, B-cells, and DCs. It keeps the immune system in check by transmitting inhibitory signals when it interacts with MHC molecule on APCs. It also enhances suppressive actions of Tregs.[110] Similarly, inhibitory receptors such as T-cell membrane protein 3, V-domain Ig suppressor of T cell activation, and B- and T-lymphocyte attenuator (BTLA) can be a potential target of checkpoint inhibitors.[111-113]

Costimulatory molecules: Several agonists of costimulatory molecules expressed on the surface of T-cells or NK cells are under investigation. 4-1BB (CD137) agonist, urelumab, prevents T-cell apoptosis and promotes NK-mediated antibody-dependent cell cytotoxicity.[114] Agonists for stimulatory receptors such as OX40, CD40, and glucocorticoid-induced TNFR-related protein are also being studied.[115-117]

IDO, a heme-containing enzyme, is being studied in various clinical trials. IDO is overexpressed by the tumor cells in TME and converts tryptophan to kyneurin that promotes T-cell energy, and differentiation to Tregs.[118]

Biomarkers

Although cancer immunotherapeutic agents have shown promising results across multiple trials, still only a small subset of patients actually benefit from these agents. Several predictive biomarkers have been suggested to screen patients who might benefit from the cancer immunotherapy and avoid unnecessary adverse effects in others. Biomarkers such as high mutational load in the tumor, increased lymphocytes infiltrates, overexpression of PDL-1 on tumor surface, and various genetic profiling have shown to be predictive of better response to immunotherapy in some studies.[119-121] However, this positive association has not been observed across different tumor types.[122,123] Thus, there exists a paucity of good predictive tool to assess the potential candidates for immunotherapy. With our increasing knowledge of cancer immunology, especially the immune milieu of TME and their immune evasion mechanism, an improved biomarker may be expected in the near future.

Conclusions

The field of cancer immunotherapy is rapidly expanding with increasing FDA approved indications. Recent positive response with immunotherapy in multiple trials of different cancer types has rekindled the interests of clinical investigators. With improvement understanding of the immune system, new receptors/molecules are being identified with a potential to be the target of future immunotherapeutic drugs. Past few decades have seen great advancement in immunotherapy, but the peak is yet to be achieved.

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