Overview of Basic Immunology and Clinical Application

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Abstract

Tumor exists as a complex network of structures with an ability to evolve and evade the host immune surveillance mechanism. The immune milieu which includes macrophages, dendritic cells, natural killer cells, neutrophils, mast cells, B cells, and T cells are found in the core, the invasive margin, or the adjacent stromal or lymphoid component of the tumor. The immune infiltrate is heterogeneous and varies within a patient and between patients of the same tumor histology. The location, density, functionality, and cross-talk between the immune cells in the tumor microenvironment influence the nature of immune response, prognosis, and treatment outcomes in cancer patients. Therefore, an understanding of the characteristics of the immune cells and their role in tumor immune surveillance is of paramount importance to identify immune targets and to develop novel immune therapeutics in the war against cancer. In this chapter, we provide an overview of the individual components of the human immune system and the translational relevance of predictive biomarkers.

Keywords

Adaptive · Biomarkers · Checkpoint inhibitors · Immune cells · Immune checkpoints · Immunology · Immunotherapy · Innate · Resistance · Response · T cells · Translational

The human immune system is an elaborate and dynamic network of cells that work together to defend the human body against attacks by foreign agents including malignant cells. There are two levels of immunity, the innate immunity and the adaptive immunity. The innate immunity constitutes the first line of defense against pathogens, which includes the anatomic and physiologic barriers, phagocytic leukocytes, dendritic cells (DC), natural killer (NK) cells, and the circulating plasma proteins [1]. Elie Metchnikoff, a pathologist and Father of natural immunity, was the first to describe the concept of leukocyte recruitment and phagocytosis of microorganisms [2]. The adaptive immune system is a more versatile mechanism of defense provided by the B lymphocytes and the T lymphocytes, which has been attributed to Paul Ehrlich, the physicist who described the side-chain theory of antibody

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formation [3]. The innate and adaptive immune systems are distinct but interactive components of the human immune system that collectively contribute to the defense operations against foreign proteins [4]. In this chapter, we discuss the fundamental components of the immune system and their development, how innate immunity interfaces with adaptive immune responses to eliminate tumor cells, and the development of immunotherapeutic strategies to combat cancer.

Innate Immune System

An association between inflammation and tumorigenesis has long been described, but it has been established with turn of the century [5]. The human body is constantly exposed to a highly diverse world of foreign proteins every day, which are rapidly eliminated in a normal healthy individual by the components of the innate immune system. Speed is the essence of innate immune response; however, they are nonspecific in nature, of limited duration, and lack immunologic memory [6]. Traditionally, the cellular components of the innate immune system, which includes the macrophages, neutrophils, eosinophils, basophils, mast cells, NK cells, and DCs, are associated with elimination of microbial agents and activation of the more efficient, antigen-specific adaptive immune response in the event of failure [4, 6]. In addition, the humoral elements of the innate immune system that includes the complement proteins and C-reactive protein are considered as a regulator of inflammatory process [4]. However, accumulating evidence suggests that the innate and adaptive immune system, triggered by the tumor antigens, play a significant role in the recognition and elimination of malignant cells as well [7]. In the process, several noxious reactive chemicals, cytokines, and chemokines are released, which damages the surrounding healthy tissue [8]. The inflammatory microenvironment also induces genomic instability and enhances rate of molecular alterations [9]. The resultant process of repeated cell renewal and proliferation sets the stage for chronic inflammation that produces a microenvironment conducive for malignant transformation of cells [10]. For this reason, tumors are sometimes described as "wounds that do not heal." [11]

Cellular Components of the Innate Immune System

All the cells of the immune system originate from the pluripotent hematopoietic stem cells (HSCs) in the bone marrow. The HSCs divide to produce the common lymphoid progenitor (CLP) and the common myeloid progenitor (CMP) cells. The CLP cells give rise to the T and B lymphocytes that are responsible for adaptive immunity and the NK cells, while the CMP cells give rise to the cells of the innate immune system, leukocytes (neutrophils, monocytes, basophils, and eosinophils), mast cells, DCs, erythrocytes, and megakaryocytes.

Leukocytes

The primary function of the leukocytes is to protect the body against invading microorganisms. However, microenvironmental factors at the site of inflammation produce substantial changes in the phenotype and functional status of individual cells that favor initiation and progression of tumor [12, 13].

Neutrophils

They account for 50–70% of circulating leukocytes [14] and form the indispensable first line of defense against pathogenic microorganisms. They originate from the CMP cells in the bone marrow in response to several cytokines including granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) [14, 15]. They circulate in the blood as dormant cells and are recruited to sites of infection by specific chemokines, cytokines, and cell adhesion molecules [16]. The microbes are then taken up by the process of phagocytosis and destroyed by high concentrations of microbicidal granules or by respiratory burst associated with production of highly toxic reactive oxygen species in the

pathogen-containing vacuole [14]. In addition, the activated neutrophils upregulate the production of cytokines [including tumor necrosis factor-α, interleukin (IL)-1β, IL-1Rα, IL-12, and vascular endothelial growth factor (VEGF)] and chemokines (including IL-8) critical for chemotaxis and recruitment of additional neutrophils, macrophages, and T cells [17, 18].

Beyond the classical role of professional phagocytes, neutrophils play a significant role in tumor biology [1, 19]. Neutrophils are recruited to the tumor microenvironment (TME) through local production of chemokines, such as IL-8, macrophage inflammatory protein-1α (MIP-1α/CCL3), and human granulocyte chemotactic protein-2 (huGCP-2/CXCL6) [20]. Tumor-associated neutrophils (TANs) are markedly different from naive neutrophils. TANs exhibit dual conflicting roles at the molecular level [20]. They take up either an antitumorigenic (N1) or a pro-tumorigenic (N2) phenotype [14, 21]. In untreated tumors, the regulatory cytokine transforming growth factor-beta (TGF-β) in the tumor cells drives the differentiation of TANs toward N2 phenotype [13]. These neutrophils locally produce neutrophil elastase (ELA2) [22], oncostatin M [23], and alarmins S100A8/9 [24] that promote proliferation, survival, metastasis, and resistance of tumor cells to chemotherapy. In addition, N2 TANs promote immunosuppression and tumor progression by releasing growth-stimulating signals, angiogenic factors, and matrix-degrading enzymes [13, 20, 25]. Furthermore, neutrophils with a pro-tumor N2-like phenotype have been found to form clusters around circulating tumor cells in the peripheral blood of breast cancer patients [26]. These neutrophil-circulating tumor cell clusters favor the development of blood-borne metastasis in an accelerated manner, resulting in shorter overall survival. Neutrophils, thus, assume multiple roles in the development and progression of tumor cells [27]. However, under certain conditions such as TGF-β blockade, TANs assume a N1 phenotype, which are more cytotoxic due to enhanced expression of immune-activating cytokines and chemokines and lower levels of arginase [13]. N1 TANs also communicate with DCs to trigger an adaptive immune response [28]. In addition, they facilitate intratumoral CD8+ T-cell infiltration and activation through the production of chemokines (like CCL3, CXCL9, and CXCL10) and pro-inflammatory cytokines (i.e., IL-12, TNF-α, GM-CSF, and VEGF) [29]. This phenotype has the potential to inhibit progression of the tumor, indicating the possibility of immune stimulation through TGF-β blockade [13].

Monocytes and Macrophages

Monocytes are derived from the CMP cells. They are large, mononuclear cells that account for 5–7% of circulating leukocytes. These monocytes migrate into the tissues, where they differentiate rapidly and mature into distinct macrophages depending on tissue of activation, the Langerhans cells in the epidermis, Kupffer cells in the liver, and microglial cells in the central nervous system [30]. Macrophages perform many functions. Primarily, they engulf and destroy the invading microorganisms. They also release cytokines and chemokines to recruit other cells of the immune system to the site of inflammation. Macrophages also induce expression of co-stimulatory molecules on the antigen-presenting cells (APCs) to initiate adaptive immune response and help in the disposal of pathogens destroyed by adaptive immune response [2].

Similar to TANs, monocytes are attracted to the TME by tumor-derived chemokines, such as CCL2, CCL5, CCL7, and CCL8, or cytokines, such as VEGF, platelet-derived growth factor (PDGF), TGF-β, GM-CSF, and M-CSF [31–34], where they differentiate into tissue-resident macrophages [35]. The tumor-associated macrophages (TAMs) assume either antitumorigenic M1 phenotype (classically activated) or pro-tumorigenic M2 phenotype (alternatively activated) reflecting the functional plastic nature of these cells [36]. The cytokine profile of the TME plays a central role in the phenotype orientation of the differentiating macrophages [37]. In general, M-CSF, TGF-β, and IL-10, the principal cytokines present in the TME, strongly inhibit IL-12 production and NF-κB activation in TAMs

[38]. This skews the differentiation of monocytes to macrophages M2 phenotype, characterized by IL-12^{low} IL-10^{high} [31, 39]. These macrophages migrate to hypoxic areas within the tumor and promote tumor progression by inducing angiogenesis through expression of factors such as VEGF, angiopoietins, pro-angiogenic cytokines, and IL-1; by remodeling of stromal matrix by producing a variety of matrix metalloproteinases (MMP) such as MMP1 and MMP9; and by suppressing adaptive immunity through production of prostaglandins, IL-4, IL-6, IL-10, TGF-β, and indoleamine 2,3-dioxygenase (IDO) metabolites, and induction of T regulatory (Treg) cells [34, 39]. This enables the tumor cells to escape into surrounding stroma and ultimately metastasize to distant sites. However, classical macrophage activation occurs under certain conditions, for example, in the presence of GM-CSF, microbial products, lipopolysaccharides (LPS), or interferon (IFN)-γ, where TAMs are educated to assume the more cytotoxic, antigen presenting, IL-12^{high} IL-10^{low} M1 phenotype [34]. They kill microbes and tumor cells by producing copious amounts of proinflammatory cytokines such as IL-12 and IL-23, toxic intermediates-nitric oxide, reactive oxygen intermediates (ROI), and TNF [31, 34]. The cytokines also initiate T-helper 1 (Th1) adaptive immunity. Although high macrophage content is often correlated with poor patient prognosis in breast [40, 41], bladder [42], endometrial [43], and cervical cancers [44], TAMs in tumor tissue confer survival advantage to patients with prostate cancer [45] and colon cancer [46]. Pharmacological skewing of macrophage polarization from M2 to M1 phenotype is likely to provide therapeutic benefit to cancer patients. Melittin, a major polypeptide of bee venom, is reported to have antitumor properties by virtue of their ability to selectively reduce M2-like TAMS [47]. This action increases the M1/M2 ratio. Further, when fused with mitochondrial membrane-disrupting peptide dKLA, melittin selectively induces apoptosis of M2-like macrophages in orthotopic lung cancer models. These findings suggest a novel therapeutic approach to target TAMs in the TME [48].

Eosinophils

Eosinophils are derived from the CMP cells, and they constitute less than 5% of circulating leukocytes [2, 49]. Traditionally, eosinophils are associated with host defense against large, multicellular parasitic helminths and fungi with allergic conditions [50]. Eosinophils express a number of receptors such as chemokine receptors, cytokine receptors, immunoglobulin (Ig) receptors, Toll-like pattern recognition receptors, and histamine receptors [51]. Engagement of these receptors causes the release of highly cytotoxic proteins, such as major basic protein, eosinophil-derived neurotoxin or eosinophil peroxidase (EPO), proinflammatory cytokines and growth factors (IL-2, -3, -4, -5, -6, -10, -12, and -13, IFN-γ, TNF-α, GM-CSF, TGF-α/β), chemokines, including RANTES(CCL5), eotaxin-1 (CCL11), CXCL5, and lipid mediators (platelet-activating factor and leukotriene C4) from the large, highly cytotoxic, secretory cytoplasmic granules at the sites of allergic inflammation [51, 52].

In addition, eosinophils are found in the tumor-infiltrating area [1]. Tumor-associated tissue eosinophilia has been associated with improved patient outcomes in a variety of solid tumors including colorectal cancer [53], oral squamous cell carcinoma (SCC) [54] laryngeal, and bladder carcinoma [55]. Although an understanding of the function of eosinophils in cancer has remained elusive, it has become apparent that eosinophils express major histocompatibility complex (MHC) class II and co-stimulatory molecules [CD40, CD28/86, cytotoxic T lymphocyte-associated protein 4 (CTLA-4)] [56, 57], whereby they function as APCs and initiate antigen-specific immune responses by the T cells [58]. Kinetic studies have demonstrated that chemotactic factors such as eotaxins and damage-associated molecular patterns (DAMPs), high mobility group box 1 (HMGB1) released by necrotic tumor cells, preferentially induce eosinophilic migration to tumors [59, 60] prior to infiltration by CD8+ T cells [61]. Tumor-associated tissue eosinophils in its active form release chemokines such as CCL5, CXCL9, and CXCL10 that attracts CD8+ T cells to the tumor [62]. Tumor-associated tissue eosinophilia in the

presence of tumor-specific CD8+ T cells produces significant changes in the TME such as polarization of TAM to M1 phenotype and vascular normalization of the tumor, resulting in increased T-cell infiltration, enhanced tumor rejection, and improved patient survival [61]. Eosinophils also exhibit antitumor immune response in a T-cell-independent manner [63]. Tumor-derived alarmin IL-33 mediates intratumoral migration and activation of eosinophils. Subsequent degranulation of eosinophils releases cytotoxic granules that has a direct action on the tumor cells resulting in reduced tumor growth [64]. Although this dual mechanism of tumor-associated tissue eosinophilia mediates antitumor activity in several solid tumors, tumor-associated blood eosinophilia is associated with worse prognosis in breast cancer, hematological malignancies, and myelodysplastic syndromes [65].

Basophils

They originate from the CMP cell in the bone marrow and are released into circulation as mature cells [2]. They account for less than 1% of circulating leucocytes and were, therefore, considered redundant to mast cells functionally till about 15 years ago [66]. Basophils travel to the sites of allergic inflammation and microbial assault in response to cytokines and chemokines released locally [66]. IgE-mediated activation of basophils induces proliferation and rapid release of several inflammatory mediators, such as histamine, leukotriene C4, prostaglandins, and significant amount of IL-4 and IL-13 [67]. IL-4 and IL-13, released within an hour of stimulation, serve as chemo attractants for other immune cells and direct the differentiation of naive T cells toward Th2 phenotype, resulting in Th2-(allergic)-type immune responses in an IgE-dependent and IgE-independent manner [68, 69]. Further, basophils express CD40 ligand, which on binding with CD40 on B cell induces transformation of B cells to plasma cells and promotes production of IgE antibodies [69].

Although the role of basophils in tumorigenesis has not been clearly understood, it is believed that basophils promote neoplastic angiogenesis [70]. Basophils express angiopoietin-1 and angiopoietin-2 messenger RNAs in the cytoplasmic vacuoles and VEGFR-2 and Tie1 receptors on the cell surface. In addition, activation of basophils releases pro-angiogenic factors VEGF-A and VEGF-B through a cross talk between the basophils and the mast cells, contributing to neoplastic angiogenesis. Further, the correlation between basophils in the tumor draining lymph node with Th2 inflammation in patients with pancreatic ductal adenocarcinomas and the emergence of basophils as an independent prognostic factor of poor survival after surgery suggests a role for basophils in tumor development and disease recurrence [71].

Mast Cells

Mast cells are tissue-based inflammatory cells of hematopoietic origin [72]. The origin of mast cell has long been debated. Recently, Qi et al. identified prebasophil and mast cell progenitors (pre-BMP), a population of granulocyte-macrophage progenitors (GMPs) with a capacity to differentiate into basophils and mast cells while retaining a limited capacity to differentiate into myeloid cells [73]. The pre-BMPs circulate in the blood and reach the peripheral tissue, where they are differentiated into basophils and mast cells in the presence of mutually exclusive transcription factors, C/EBPα and MITF, respectively [73]. Basophils and mast cells share many characteristics such as expression of IgE receptors, presence of same granules, and secretion of similar mediators of immune response and cytokines when stimulated. Both offer protection against parasites and are key players in the Th2-(allergic)-type immune responses [74, 75]. However, mast cells show marked differences in their histochemical, biochemical, and functional characteristics based on their phenotype and the cytokine milieu, a phenomenon called "mast cell heterogeneity." [76] Mast cells express several surface receptors including KIT IgG receptor and Toll-like receptors (TLRs) [76]. The characteristic feature of mast cells is the presence of dense metachromatic granules in the cytoplasm-containing histamine and heparin, which are explosively released on contact with allergens

[77]. Tissue mast cells besides being the largest storehouse of histamine, with the exception of gastrointestinal tract and central nervous system, also contain several preformed mediators such as heparin, serotonin, tryptases, and chymases; lipid mediators; cytokines such as TNF-α/β, IFN-α/β, IL-1α/β, IL-5, −6, −13, −16, and − 18; chemokines such as IL-8 (CXCL8), I-309 (CCL1), MCP-1 (CCL2), MIP-1αS (CCL3), MIP1β (CCL4), MCP-3 (CCL7), RANTES (CCL5), eotaxin (CCL11), and MCAF (MCP-1); and growth factors such as SCF, M-CSF, GM-CSF, bFGF, VEGF, NGF, and PDGF [77], which are synthesized and rapidly released on activation by IgE- or IgG-dependent mechanisms. Strategic location of the mast cells at the interface between mucosal and environmental surfaces, for example, near blood vessels, nerves, glands, and beneath epithelial surfaces [74, 76], and their ability to store TNF-α in a preformed state allows mast cells to orchestrate the first response to invading pathogens [72]. Different stimuli activate different pathways resulting in different cocktail of molecules released by mast cells, which significantly influences T-cell differentiation and the subsequent adaptive immune response [72].

Increased numbers of mast cells found in many tumors may have a double-edged function in tumor development. Infiltration of tumor by mast cells has been associated with poor prognosis in some cancers, such as prostate cancer [78], lip cancer [79], and diffuse large B-cell lymphoma [80]. This may be because intratumoral mast cells, which are a rich source of pro-angiogenic and tumor growth stimulatory mediators, stimulate or modulate angiogenesis; and peritumoral mast cells, which are rich sources of tryptase and chymase, promote extracellular matrix degradation and tumor invasion, resulting in tumor progression [79, 81, 82]. On the contrary, mast cell infiltration has been associated with good prognosis in breast [83], ovarian [84], lung [85], and colorectal cancers [86]. This is due to release of several antitumoral factors by stromal mast cells including cytotoxic endogenous peroxidase, cytokines like IL-1, IL-4, IL-6, and TNF-α that induce apoptosis of endothelial cells, chymase, which inhibits angiogenesis, and tryptase leading to tumor fibrosis [84, 87, 88]. It is, therefore, evident that the density and location of mast cells within the tumor samples and the crosstalk between mast cells and stromal cells are predictors of patient survival as they modulate the immune response [1].

Dendritic Cells

DCs are professional APCs that are resident in most tissues of the body and concentrated in the secondary lymphoid tissues [89]. In the steady state, they originate from the monocyte and dendritic cell progenitor (MDP) derived from the CMP cells in the bone marrow [90]. The MDPs gives rise to monocytes and common DC progenitors (CDPs) in the bone marrow [91]. The CDPs give rise to pre-DCs, which migrate from the bone marrow through the blood to lymphoid and nonlymphoid tissues, where they differentiate to produce conventional DCs (cDCs). The pre-DCs lack the form and function of DCs, but with microbial or inflammatory stimuli, they develop into DCs [92]. Plasmacytoid DCs are an example of pre-DCs found in blood, thymus, bone marrow, and secondary lymphoid tissue, which produce type I IFN-α in response to viral exposure. The cDCs are broadly classified into migratory DCs and lymphoid tissue-resident DCs. The migratory DCs (Langerhans cells and dermal DCs) are immature DCs present in the peripheral tissue, which are very effective in capturing antigens. They sample the environment using several receptors including the TLRs and NOD-like receptors (NLRs). On encountering a pathogen, endocytosis is upregulated transiently to facilitate the accumulation of large quantities of antigens by the immature DCs that are phagocytic and macropinocytic in the peripheral tissue [3]. Immature DCs are relatively inefficient in presenting the peptide-MHC complexes at the surface due to reduced formation of antigenic peptides [3], ubiquitination of MHC class II molecules in the lysosomes, and poor expression of co-stimulatory ligands (CD80, CD86) [3, 93]. Shortly thereafter, functional maturation of DCs ensues triggering the antigen-presenting machinery, which is the critical link between innate and adaptive immunity [94]. Endocytosis by the DCs decreases and expression of MHC-I, MHC-II,

and co-stimulatory molecules increases at the surface possibly due to cessation of ubiquitination of MHC class II molecules [93]. As a result, the mature DCs degrade the pathogen and present the antigenic peptides on MHC class I or II molecules on the cell surface to naive T cells, express co-stimulatory ligands (CD80, CD86) simultaneously, and migrate to the T-cell zones of the lymphoid tissue [3]. Binding of the ligands to the co-stimulatory molecules on T cells leads to activation of T cells [93]. Based on the type of pathogen and other maturation signals received, the activated T cells are educated to proliferate and differentiate to become potent effector cytotoxic T cells or helper T cells [3]. DCs can also directly present the intact antigen to and activate the antigen-specific B cells [3]. The lymphoid tissue-resident DCs (CD8+ and CD8-splenic cDCs and thymic cDCs) are immature DCs uniquely located in regions where naive T cells are activated [93]. They present the antigens in the lymphoid organ to the T cells [92]. They are likely responsible for maintaining peripheral tolerance in the steady state. Under inflammatory conditions, some DCs may arise from the CLP cells and from the monocytes [2]. An example of inflammatory DC is the tumor-necrosis factor and inducible nitric-oxide synthase-producing DCs (Tip DCs) [92].

Under normal conditions, DCs are responsible for maintaining immune tolerance to host cells [3]. DCs are generally phenotypically and functionally immature in the steady state. Immature state is characterized by ubiquitination and intracellular accumulation of MHC class II molecules and low levels of co-stimulatory molecules [89]. Therefore, in the absence of infections, though DCs continuously present self-antigens and non-pathogenic environmental antigens to T cells, this induces the production of Tregs instead of effector T cells. In the development of cancer, where the tumor cells are more similar to normal cells, DCs are, therefore, more likely to induce peripheral tolerance in the absence of inflammation. Further, other mechanisms of immune suppression such as expression of PD-L1 and PD-L2, TGFβ, and IDO inhibit DC and T-cell function and facilitate escape of tumor cells from immune recognition. This may explain why vaccines did not succeed as an effective treatment modality in cancer patients [3]. DCs are aptly called the gatekeepers of the immune system because of their ability to inspect the microenvironment, interpret the cues in the environment, and instruct the immune cells to respond quickly and appropriately between tolerogenic and immunogenic function [89]. However, recruitment of DCs in the TME is influenced by tumor cell intrinsic factors [95]. For example, activation of the WNT/β--catenin signaling pathway prevents DC recruitment and inhibits T-cell activation resulting in immune exclusion [96]. On the contrary, tumor-infiltrating NK cells recruit and promote survival of DCs in the TME [97]. Hence, initiation of antitumor response by DCs is largely dependent on the immune milieu in the TME.

Natural Killer Cells

NK cells are the most powerful lymphocytes of the innate immune system with robust cytotoxic activity. They originate from the CLP cells in the bone marrow and account for 15% of all the circulating lymphocytes [1]. Besides, they are located in many peripheral tissues. Although NK cells do not express antigen-specific surface receptors such as the classical membrane-bound Igs of B cells or the T-cell receptor (TCR) of the T cell, they express a wide range of activating and inhibitory cell surface receptors. As the primary function of NK cells is to identify and eliminate cells that fail to produce self-MHC class I molecules, NK cells during the process of maturation are educated to identify "missing self" through the expression of several cell surface inhibitory receptors such as killer cell inhibitory receptor–L (KIR-L), which specifically binds with MHC class I ligands [98]. Engagement of these receptors by cognate MHC class I ligands constitutively expressed in normal cells in steady-state conditions ensures self-tolerance by transducing inhibitory signals [99]. It is the absence of these MHC class I ligands on tumor cells and cells in distress as in viral infection that marks them for destruction by NK cells [98].

The effector function of NK cells is triggered by the engagement of cell surface-activating receptors including the potent NKG2D receptor,

killer-cell Ig-like receptors (KIR-S), TLR, and NLR that identifies non-self-infected cells and self-cells under stress by recognizing pathogen-associated molecular patterns (PAMPs) [100]. However, activation of the NK cells is dependent on cellular crosstalk with accessory cells such as DCs, neutrophils, macrophages, and mast cells, and/or a cytokine microenvironment that includes IL-2, IFN-α/β, IL-12, IL-15, IL-18, or IL-21 [101, 102]. The DCs, which are key partners to NK cells, lie in close proximity to the NK cells and prime the NK cells either directly by contact or by secretion of the cytokines, IFN-α, IL-2, IL-12, IL-15, or IL-18 [103]. Activated NK cells induce cytotoxicity and/or promote cytokine production [103]. NK cells kill tumor cells by releasing cytoplasmic granules containing perforin and granzymes or by expressing Fas ligand (CD95) or TNF-α–related apoptosis-inducing ligand (TRAIL) that binds with death receptors on the tumor cells triggering apoptosis [104]. Tumor cells, however, evolve and evade destruction by NK cells [104]. A common escape mechanism used by tumor cells is the proteolytic shedding of NKG2D ligands [105]. Further, chronic stimulation of NKG2D pathway by tumor-associated expression of TGF-β and NKG2D ligands (including MHC class I homologues MICA and MICB) on the surface of tumor cells can functionally impair NKG2D pathway by inducing endocytosis and destruction of the potent-activating NKG2D receptors on NK cells [106, 107]. This results in markedly reduced expression of NKG2D on NK cells, which promotes T-cell silencing and evasion of immune surveillance by tumor cells. Nevertheless, NK cells prosecute tumor cells through other mechanisms such as antibody-dependent cell cytotoxicity [108]. NK cells express other activating receptors such as CD16, Fc-γ receptor IIIa (FCGR3A), which bind to the Fc region of Ig [109]. This enables the NK cells to identify antibody-coated tumor cells and destroy them by releasing perforins.

At least two functional subsets of NK cells have been described based on the expression of CD56 and CD16 [110]. The $CD56^{dim}$ $CD16^{+}$ NK cells account for 90% of circulatory NK cells. These cells are attracted to peripheral tissues by several chemokines. They express perforin, natural cytotoxicity receptors (NCR), and KIRs. On activation, the $CD56^{dim}$ $CD16^{+}$ NK cells are more cytotoxic and secrete low levels of cytokines. On the other hand, $CD56^{bright}$ $CD16^{-}$ NK cells are primarily located in the secondary lymphoid tissue and account for less than 10% of circulatory NK cells. They lack perforin, NCR, and KIRs. On activation by IL-2, the $CD56^{bright}$ $CD16^{-}$ NK cells produce cytokines, mainly IFN-γ, GM-CSF, and TNF-α. However, on prolonged stimulation by IL-2, they express perforin, NCR, and KIRs and acquire cytotoxic function.

Although NK cells are traditionally characterized as cells of innate immunity, they also exhibit T-cell characteristics and are capable of mounting rapid and robust immune response on secondary exposure [111]. The immune memory function of NK cells lasts for several months after the initial exposure, is antigen-specific, and is transferable to naive animals [111]. Although NK cells are potent killers with immune memory, only modest success has been achieved in clinical setting as their effectiveness has been hampered by their limited ability to infiltrate tumor cells [112]. In recent years, NK cells have been engineered to express TCRs (TCR-NK-92) that are functional and capable of cytotoxic activity [113]. Based on the demonstrated antitumor activity in preclinical studies and their ability to expand indefinitely, this TCR-redirected cell line provides proof-of-principle for use of engineered NK cells in adoptive cell-based cancer therapy.

Adaptive Immune System

The hallmark of adaptive immunity, mediated by the T lymphocytes (T cells) and B lymphocytes (B cells), is the specificity of the immune response to antigenic stimuli. Another unique feature of adaptive immunity is its ability to confer lasting immunological memory that results in more rapid and robust immune response with subsequent exposure to the same antigen [2]. Contrary to innate immune response, which is immediate in onset due to the presence of germ line-encoded cell surface receptors, the adaptive

immune response is a slower processes, as the lymphocytes on activation undergo clonal expansion to attain sufficient numbers before the effector cells mount an immune response [30]. There are two classes of adaptive immune response, the humoral and cell mediated. The humoral immune response is mediated by the B lymphocytes against antigens present outside the cells, in the blood and body fluids. On the other hand, the cell-mediated immune response is mediated by the T lymphocytes against intracellular pathogens presented as small antigenic determinants on MHC molecules.

Cellular Components of the Adaptive Immune System

The T and B lymphocytes originate from the CLP, a specialized type of stem cell originating from the pluripotent HSCs [2].

T Lymphocytes

The lymphoid progenitor cells migrate from the bone marrow to the thymus, where they undergo four stages of differentiation and proliferation, including developmental check points to ensure that cells which fail to recognize antigen-MHC complexes or distinguish self-antigens do not mature [114]. As the lymphoid progenitor cells migrate through the cortex, they undergo an education program based on the constant interaction with the thymic epithelial cells [115]. The lymphoid progenitor cells that enter the thymus at the corticomedullary junction do not express CD4 or CD8 co-receptors and are therefore called CD4/CD8 double-negative (DN) lymphocytes (DN1) [116]. As they move through the cortex from the corticomedullary junction to the capsule, the lymphoid progenitor cells lose their ability to form B cells or NK cells and become committed T-cell precursors (DN2) [117]. Following T lineage commitment and expression of recombination-activating gene 1 (RAG1), the TCRβ chain is rearranged and paired with the pre-Tα chain, resulting in the expression of pre-TCRs (DN3) [114]. Subsequently, intense proliferation results in the generation of multiple thymocytes (DN4). With appropriate cytokine stimulation, they express CD8 co-receptors first and then CD4 co-receptors to become double-positive (DP) thymocytes. This is accompanied by rearrangements in the TCRα chain, which results in the generation of complete αβ TCRs. Then, DP thymocytes interact with TECs, and further development into naive T cells is dependent on their ability to bind with MHC class I or class II molecules associated with self-peptides (positive selection) [114, 118]. Approximately 90% of DP thymocytes express TCRs that fail to bind with MHC molecules, resulting in delayed apoptosis of these cells (death by neglect). Based on their interaction with MHC molecules, the DP thymocytes differentiate into single-positive T cell by silencing of the transcription of one co-receptor locus [115, 119].

In the medulla, T cells are screened for reactivity against wide range of tissue-specific proteins including self-peptides expressed by the thymic medullary epithelial cells [30]. The T cells that express TCRs with high affinity for self-peptides undergo rapid apoptosis and are later cleared by thymic macrophages (negative selection). T cells that express intermediate level of TCR signaling enter into a maturation phase by the process of positive selection. The T cells that express TCRs that bind with MHC class I molecule mature into a single-positive CD8 mature T cell (CD8+ T cell), while those that express TCRs that bind with MHC class II molecule mature into a single-positive CD4 mature T cell (CD4+ T cell). These naive T cells then sample the environment in the medulla for antigen-presenting DCs. On exposure to antigenic determinants presented by the APCs, the T cells are activated in the presence of co-stimulation of CD28 by B7 molecules (CD80 and CD86) on the APCs to form effector T cells that either destroy the pathogenic agent or attract other immune cells to the site. In the absence of antigenic stimuli in the medulla, the naive T cells enter the blood stream, travel to the peripheral lymphoid tissue, and enter the paracortical region of the LN. In the tumor draining LNs, naive T cells are activated on encountering tumor antigen in the context of MHC molecule and co-stimulation of

the constitutively expressed CD28 on the surface of T cells by B7 proteins (CD80 or CD86) expressed on the same APC [120]. This results in clonal expansion and differentiation of naive T cells in the lymph nodes into effector T cells (CD4+ helper T cells or CD8+ cytotoxic T cells). Depending on the cytokine milieu and the transcription factors in the TME, the CD4+ helper T cells differentiate into several subtypes that include Th1 [121], T-helper 2 (Th2) [122], T-helper 17 (Th17) [123], induced Tregs (iTregs) [124], follicular helper T cell (Tfh) [125], and T-helper 9 (Th9) [126]. These helper T cells secrete cytokines and chemokines that regulate the immune response. Th1 cells favor cell-mediated immunity by activation of CD8 T cells to mount an immune response against intracellular pathogens, while Th2 cells favor humoral immunity by activation of B cells against extracellular parasites. On the other hand, CD8+ effector T cells activated by antigen presentation on the MHC class I molecule or through CD4 helper T cells are directly cytotoxic. Hence, they migrate to the tumor and destroy the tumor cells. In addition, some of the activated T cells and B cells differentiate into memory cells that are responsible for the long-lasting immunological memory [127]. Subsequent exposure to the same antigen results in more rapid and robust immune response.

Regulation of T-cell response is a delicate balance between co-stimulatory and inhibitory signals that serve as immune checkpoints. Under normal physiologic conditions, these T-cell receptors serve to maintain immune homeostasis and prevent autoimmunity. Co-stimulatory receptors include CD28, inducible T-cell co-stimulator (ICOS), 4-1BB (CD-137), OX40 (CD-134), CD40, and glucocorticoid-induced TNFR-related protein (GITR), while CTLA-4, programmed cell death 1 (PD-1), lymphocyte activation gene-3 (Lag-3), T-cell immunoglobulin-3 (Tim-3), and T-cell immunoglobulin and ITIM domain (TIGIT) are coinhibitory [128]. CD28 is the primary co-stimulatory molecule constitutively expressed on the surface of naive T cells. On ligand binding with B7–1 and B7–2 on APCs, they provide the essential co-stimulatory signal for T-cell activation and downstream signaling [129]. ICOS is another member of the CD28 family [130]. Although structurally similar to CD28 and CTLA-4, it is not constitutively expressed, but it is induced on activated CD4+ and CD8+ T cells. On ligand binding with B7-H2 expressed on activated DCs, ICOS enhances T-cell proliferation, but unlike CD28 which upregulates IL-2, ICOS stimulation upregulates IL-10 expression. Further, ICOS induces co-stimulation of T cells, causes upregulation of CD40 ligand, and promotes synthesis of immunoglobulins by B cells.

Besides CD28 and ICOS, there are other co-signaling receptors that belong to the TNF receptor superfamily such as 4-1BB [131], OX40 [132], CD40 [133], and GITR [134]. These receptors synergize with TCR signaling to promote cytokine production and T-cell survival. 4-1BB, OX40, and GITR are transiently upregulated on activated CD4+ and CD8+ T cells and their ligands on activated APCs [135]. On ligand binding, co-stimulatory signaling augments T-cell expansion and cytotoxic effector functions. However, its effect on the Tregs is dependent on the cytokine milieu in the TME. In general, engagement of T-cell-activating receptors impairs conversion of naive T cells into FoxP3+ Tregs, depletes tumor-infiltrating Tregs, and, thus, blocks the immune suppressive function of Tregs [136]. However, in the absence of IFNγ or IL-4, stimulation of activating receptors enhances Treg proliferation and accumulation. Thus, activation of co-stimulatory receptors has a dual effect on Tregs. CD40 differs from other members of the TNF receptor superfamily in that it is predominantly expressed on APCs and macrophages, and its ligand, CD40L, is expressed transiently on activated T cells [135]. Activation of CD40 induces tumor regression indirectly by licensing of DCs and by promoting macrophage-dependent tumoricidal action [137]. Stimulation of CD40 also exhibits direct cytotoxic effects by mediating antibody-dependent cellular cytotoxicity, complement-mediated cytotoxicity, and programmed cell death. The stimulatory effect of T cells is counterbalanced by a suppressive mechanism in order to maintain immune homeostasis. Activated T cells simultaneously express CTLA-4

and PD-1 on their surface as immune checkpoints [138–140]. CTLA-4, a CD28 homologue with a higher affinity to bind with B7 molecules, is an early co-inhibitory signal that regulates T-cell activity during the priming phase. On engagement with B7, CTLA-4 blocks CD28 co-stimulation and abrogates T-cell activity and cytokine production. On the other hand, PD-1, a CD28 family member, is a late co-inhibitory signal that regulates T-cell activity during the effector phase in the peripheral tissue. PD-1 interacts with two ligands, PD-L1 and PD-L2. PD-L1 is expressed on many cells including the tumor cells and activated B and T cells in response to IFN-γ produced by the activated T cells, while PD-L2 is expressed exclusively on macrophages and DCs [141]. Unlike CTLA-4, the PD-1 to PD-L1 ligand binding does not interfere with co-stimulation, but it downregulates B- and T-cell proliferation and cytokine production by interfering with signaling pathways downstream of TCRs and BCRs [142]. Besides CTLA-4 and PD-1, there are other next-generation co-inhibitory receptors, such as Lag-3, Tim-3, and TIGIT, which are expressed on distinct lymphocyte subsets that are responsible for differential suppression of immune response [143]. For example, Tim-3 pathway may regulate immune responses in the gut, while TIGIT may regulate in the lungs and Lag-3 in the pancreas. Similarly, they exhibit functional specification in that TIGIT may selectively suppress pro-inflammatory response of Th1 and Th17 cells, while promoting Th2 cell response [144]. Besides immune checkpoints, a chief contributor to this immunosuppressive effect is the regulatory T cells (Tregs), which are specialized T cells that suppress the cytotoxic function of other T cells [145]. They are classified as thymus-derived natural Tregs (nTregs) and peripherally derived Inducible Tregs (iTregs). nTregs characterized by surface expression of the CD4 and CD25 antigens and by the nuclear expression of forkhead box P3 (FOXP3) are positively selected thymocytes with relatively high affinity for self-antigens presented on MHC class II molecules. On the contrary, iTregs differentiate from naive CD4 T cells in the periphery in the presence of TGF-β. They exert their immunosuppressive action by the expression of immunosuppressive cytokines such as IL10 and TGF-β [124]. Decreasing the activity of Treg cells enhances both innate and adaptive immune responses, which can be utilized to treat cancer [146]. Thus, under normal conditions, coordinated regulation of immune activation and suppressive pathways play an important role in the maintenance of peripheral tolerance and regulation of the amplitude and duration of T-cell responses [147].

B Lymphocytes

The B cells develop from the HSCs in the liver during fetal life and continue in the bone marrow in adult life [2]. The four subsets of B-cell precursors that develop from the lymphoid progenitor cells, pre-pro-B cells, early pro-B cells, late pro-B cells, and pre-B cells, are devoid of surface Ig [148]. In the presence of RAG 1 and 2, these cells constantly interact with the bone marrow stromal cells that provide critical growth factors, chemokines, and cytokines for B-cell development. The B-cell precursors undergo sequential rearrangement of the genes encoding for the heavy chain (H) [149]. The DJ rearrangement occurs in the early pro-B cells followed by VDJ rearrangements in the late pro-B cells, resulting in the formation of a large pre-B cell with a complete Ig μ heavy chain in the cytoplasm [2]. The μ heavy chain combines with the surrogate light chain (L) and two invariant accessory chains Igα and Igβ to form the pre-B-cell receptor (BCR), which is transiently expressed on the surface of pre-B cells, positively selecting these cells for further development. This initiates a negative feedback loop by which it shuts down RAG expression, halts the H gene rearrangement in the pre-B cell, prevents the rearrangement of the second H (allelic exclusion), and signals the proliferation of pre-B cells. The RAG genes are re-expressed, which induces rearrangement of the genes encoding the L in positively selected pre-B cells that leads to formation of an immature B cell with the expression of a complete IgM BCR on the surface of the cell. This triggers the cessation of L gene rearrangement. As a vast repertoire of BCRs capable of recognizing a huge

diversity of antigens including self-antigens are developed, the immature B cells are tested for reactivity to autoantigens before leaving the bone marrow. When immature B cells express a nonautoreactive BCR with optimal downstream signaling, RAG expression is downregulated, which allows for positive selection of these cells to enter the spleen as transitional B cells. However, immature B cells that express a nonautoreactive BCR with low basal BCR signaling insufficient to downregulate RAG expression and immature B cells that are strongly self-reactive are negatively selected for elimination by apoptosis (clonal deletion). Alternatively, these cells may be inactivated (anergy) or may undergo receptor editing, a process by which secondary rearrangement of L leads to formation of new BCRs that are not self-reactive, which allows for subsequent positive selection of these cells for further development [150].

The immature B cells enter the spleen as transitional cells. Very few cells progress from T1 to T2 stage as most of the T1 cells undergo clonal deletion or anergy due to strong reactivity to self-antigens that are expressed only in the peripheral tissue [151]. In addition, the transition from T1 to T2 cell is dependent on basal tonic BCR signaling. The T2 cells receive pro-survival signals through B-cell-activating factor (BAFF)-R and differentiate into naive B cell expressing both IgM and IgG surface receptors. Guided by the strength of BCR signal, naive B cell differentiates into either follicular (FO) B cells with intermediate BCR signals and expression of Bruton tyrosine kinase (BTK) or marginal zone (MZ) B cell with weak BCR signal and expression of NOTCH2 [151, 152]. The MZ B cells located within the splenic white pulp are resting mature B cells that do not circulate. They have limited antigen specificity and are activated by nonprotein antigens such as common blood-borne pathogens independent of T cells. On activation, they rapidly develop into short-lived plasma cells secreting low-affinity IgM antibodies and do not produce memory cells. The FO B cells that circulate between the blood and the spleen are located adjacent to T-cell-rich areas in secondary lymphoid organs and are activated by foreign proteins in a T-cell-dependent manner [153]. The antigens bound to membrane bound Ig are internalized by FO B cells and presented on MHC class II molecules to the CD4 helper T cells. The activated T cells express CD40L, a co-stimulatory molecule, and other cytokines required for B-cell activation [2]. The activated B cells undergo clonal expansion to differentiate into plasma cells that produce large amounts of high affinity secreted antibody. Some of the activated B cells migrate into the lymphoid follicle to form a germinal center, where they undergo extensive proliferation, Ig class switching, and somatic hypermutation to generate long-lived plasma cells or memory B cells. These plasma cells leave the germinal center and migrate to the bone marrow, where they continue to produce antibodies even after elimination of the antigens. On reinfection, these circulating antibodies provide immediate protection and activate the memory cells located in the peripheral lymphoid tissue.

Immunoglobulins

Immunoglobulins are Y-shaped heterodimers composed of two identical L chains and two identical H chains [154]. The two H chains are attached to each other by multiple disulfide bonds, and each L chain is attached to an H chain by a disulfide bond. Each L and H chain is divided into a variable and constant region. The variable region in each L and H chain has three complementarity determining regions (CDRs). The three CDRs in one L chain pair with the three CDRs in the H chain in each arm of the Y to form a paratope, the antigen-binding site. Each paratope is specific for an epitope of the antigen, which determines the specificity of the Ig. The constant region of the H chain is identical for all the Igs of the same class, but different between classes. So also, all the Igs in a class have either λ or κ L chains. Proteolytic digestion with papain divides the Ig into three functional units, two antigen binding fragments (Fab) and the crystallizable fragment (Fc). Each Fab fragment contains a complete L chain and one variable and one constant domain of H chain, which includes the antigen-binding site. The Fc fragment contains two constant domains of the H chain. This is the

effector domain of the Ig which activates the NK cells, classical complement pathway, and phagocytosis [155].

Based on the amino acid sequences in the constant region of the H chains, human antibodies are classified as IgM, IgD, IgG, IgE, and IgA [154]. Accordingly, they have diverse biologic functions. IgM is the earliest antibody expressed on the surface during B-cell development, and it is the major class of Ig that is secreted on first exposure to the antigen. IgG is the major antibody in the blood that is produced in large quantities during secondary immune response and is responsible for clearance of opsonized pathogens and neutralization of toxins and viruses. IgA is the principal antibody in body secretions and contributes to nearly 50% of protein content in colostrum and protects mucosal surfaces from toxins, virus, and bacteria. Membrane-bound IgD is expressed in small amounts when the immature B cells leave the bone marrow, and it regulates the cell's activation. IgE is found in trace amounts in the blood, but it is a very potent Ig expressed during hypersensitivity or allergic reactions and parasitic infestations.

Each B cell in the body produces only one kind of antibody [155]. When a naive B cell is activated, it proliferates and differentiates into a clone of plasma cells, which produces large amount of secreted antibodies that have the same antigen-binding site as the BCR that was activated and is specific for a single epitope. Hence, they are called monoclonal antibodies (mAb). Polyclonal antibodies are secreted by different B-cell clones that bind with different epitopes on the same antigen.

Monoclonal antibodies have revolutionized the use of Igs as a therapeutic agent. However, engineering mAb is not without challenge. The first mAb engineered for human use was a murine antibody [156]. They were highly immunogenic with limited biological efficacy and very short half-life. This limitation was overcome by genetically engineering human protein formats of mAb. Chimeric mAbs that are 70% human are created by fusing murine variable region with human constant region [157]. Later, humanized mAbs that are 85–90% human, where only the CDRs are murine, were developed [158]. Currently, fully human mAbs produced by phage display are available [159]. The process of humanization has made the mAbs less immunogenic than murine mAbs. As a result, several mAbs that target growth factor receptor [such as epidermal growth factor (cetuximab), human epidermal growth factor receptor 2 (trastuzumab)], TME, and tumor antigens have been approved for treatment of colorectal, breast, and lung cancer [160]. The humanness of mAbs is indicated by the nomenclature. For example, −xi- indicates chimeric mAbs (rituximab), −zu- indicates humanized (bevacizumab), and -u- indicates fully human mAb (ipilimumab).

Besides antibody production, B cells play a role in the regulation of cell-mediated immune response [161]. Ligand binding of CD40 expressed on B cells promotes germinal center formation, Ig isotype switching, somatic hypermutation of the Ig to enhance affinity for antigen, and formation of plasma cells and memory B cells [162]. In addition, CD40/CD40L ligation on resting B cells induces surface expression of MHC and co-stimulatory molecules and produces pro-inflammatory cytokines, thus contributing to APC licensing of B cells. Thus, B cells serve as professional APCs. Although preclinical studies provide a strong rational for the clinical application of CD40B cells as a cellular cancer vaccine, B cells are being investigated for their potential use a cancer immunotherapeutic agent in a limited number of clinical trials [161].

The Immune System in Action!

Summary of the Immune Responses against Tumor Cells

In the fight against cancer, greater understanding of the immunoregulatory processes of TME is critical for development of immunotherapy. The TME is composed of a variety of cells, such as macrophages, DCs, NK cells, mast cells, naive lymphocytes, B cells, cytotoxic T cells, helper T cells, memory cells, Tregs, myeloid-derived suppressor cells (MDSCs), and stromal cells [163].

Despite the dynamic interaction between these elements in the TME and the tumor, the cancer cells develop cellular processes to subvert the immune attack and become resilient. Thus, a comprehensive understanding of the interactions between the tumor and the elements in the TME will help to identify novel targets and therapeutic strategies to combat resistance to therapy.

The human immune system exhibits a dual role in cancer. Although the primary function of the immune system is to eliminate tumor cells, they also shape immunogenicity and promote tumor progression through a dynamic process called cancer immunoediting [164]. This process includes three distinct phases: elimination, equilibrium, and escape. During the elimination phase (cancer immunosurveillance), the challenge lies in the ability of the immune system to recognize the subtle differences between self and transformed self of the malignant cells [165]. The tumor cells express several danger signals, such as NKG2D ligands and surface calreticulin, and produce minor disruptions in the surrounding tissue, resulting in the release of inflammatory signals such as IFNγ, IFN α/β, TNF, and IL-12, which recruit NK cells, DCs, and macrophages to the tumor site. This results in apoptosis and death of tumor cells. The liberated tumor antigens are then presented by the APCs on MHC molecules to T cells. This initiates tumor-specific adaptive immune response. The cytotoxic T cells interact with the Fas and TRAIL receptors on tumor cells or secrete granzymes and perforins to induce tumor cell apoptosis. Thus, innate and adaptive immune cells have the capacity to completely eliminate the tumor cells and halt the immunoediting process.

During the equilibrium phase, there is continuous interaction between the immune cells and tumor cells that have escaped elimination phase. The tumor and the immune cells exist in a state of equilibrium that prevents expansion of the tumor cells. However, this continuous immune pressure selects or promotes the formation of new variants of tumor cells with reduced immunogenicity that escapes recognition by immune system [165]. This is the longest phase in the immunoediting process, when the tumor cell variants reside in a latent form before escaping eventually [166].

During the escape phase, tumor cells adopt several mechanisms to evade immunosurveillance [167]. Tumor cells downregulate expression of tumor antigens or MHC class I molecules to reduce immune recognition and antigen presentation to tumor-specific T cells, preventing activation of T cells. Tumor cells may also upregulate expression of pro-survival growth factors such as EGFR and HER2. In addition, the tumor cells frequently develop a host of immunosuppressive defense mechanisms to escape immune surveillance through a process called immune tolerance [7]. For example, tumor cells may express suppressive surface ligands, PD-L1 or PD-L2, that engage with PD-1 receptors on activated T cells resulting in T-cell exhaustion or release immunosuppressive molecules such as IDO [168]. Under hypoxic conditions, the TME may release VEGF, which suppresses T-cell adhesion to tumor endothelium and impedes T-cell infiltration of the tumor. Similarly, TAMs in the presence of IL-4, IL-10, and TGF-β may polarize to assume M2 phenotype and express high levels of IL-10 and low levels of IL-12. These macrophages suppress T-cell activity and promote angiogenesis and tumor growth [169]. In addition, MDSCs, which are immature innate immune cells in the TME, utilize various mechanisms such as expression of IL-10, TGF-β, and Tregs to produce immune suppression, resulting in tumor progression [170, 171]. As a result, immunologically sculpted tumor cells with increased resistance emerge, resulting in uncontrolled growth of the tumor with overt clinical disease. It is, therefore, critical to overcome these barriers to elicit clinical response to therapeutic agents.

Cancer Immunotherapy

Immunotherapy has revolutionized cancer treatment due to its ability to produce durable responses in patients with certain types of advanced cancer. In the early days, several cytokines were investigated, which ultimately led to

the US Food and Drug Administration (FDA) approval of IFN-α for hairy cell leukemia and high-dose IL-2 for the treatment of renal cell carcinoma and metastatic melanoma [172]. However, their use in anticancer treatment was limited due to systemic toxicities, induction of immune checkpoints, and activation of Tregs and MDSCs. Recently, NKTR-214, an IL-2 pathway agonist, was found to selectively favor activation and expansion of CD8+ T cells and NK cells over Tregs in the TME and increase in cell surface expression of PD-1 [173]. Based on this finding, NKTR-214 in combination with Nivolumab, a PD-1 inhibitor, is being investigated in immunotherapy-naive patients with melanoma, renal cell carcinoma, NSCLC, and urothelial cancer (phase II PIVOT-02 study). In the melanoma cohort, an objective response rate (ORR) of 53% and disease control rate of 76% were reported in 38 efficacy evaluable patients [174]. The cytokine-related adverse events (AEs) were low grade and easily manageable compared to those reported with high-dose IL-2.

Generally, IL-10 is perceived as an immune-inhibitory anti-inflammatory molecule. However, higher concentrations of IL-10 achieved with the use of PEGylated IL-10 (Pegilodecakin) enhanced intratumoral infiltration and cytotoxic activity of CD8+ T cells [175]. In addition, IL-10-induced IFNγ secretion in CD8+ tumor-infiltrating lymphocytes (TILs) produced upregulation of MHC molecules in the TME, leading to rejection of well-established tumors in mice models. On investigating the clinical activity of pegilodecakin in a patient population with refractory cancers, remarkable antitumor activity was observed in renal cell carcinoma and uveal melanoma [176]. The clinical activity of pegilodecakin was extended to non-small-cell lung cancer when used in combination with a PD-1 inhibitor [177] and to pancreatic cancer when used in combination with FOLFOX [178]. Translational studies revealed that while pegilodecakin induced sustained elevation of Th1 and Th2 cytokines in the serum, it led to a reduction of the immune suppressive cytokine TGFβ and Th17-related cytokines, which mediate tumor-associated inflammation [179]. Notably, these changes were sustained throughout the treatment and were consistent across tumor types. Further, pegilodecakin leads to clonal expansion of CD8+ T cells not present at baseline to become a sizable fraction of the T-cell repertoire. This novel mechanism of action together with induction of long-lasting immunologic memory was responsible for the durable objective tumor response. Further, with the notable absence of immune-related adverse events [176] usually associated with the use of immunotherapeutic agents, pegilodecakin is emerging as a potential anticancer therapeutic agent worthy of further exploration.

IL-6 is another cytokine overexpressed in several cancers and is associated with aggressive growth and poor prognosis [180]. In addition, IL-6 through activation of downstream JAK/STAT3 signaling pathway exerts a profound negative effect on tumor-infiltrating immune cells, producing an immunosuppressive TME [181]. Further, upregulation of IL-6 by chemotherapeutic agents results in therapeutic resistance to anticancer treatment. Thus, targeting IL-6 may offer a potential therapeutic approach to treat cancer. Siltuximab (IL-6 inhibitor), Tocilizumab (IL-6 receptor inhibitor), and Ruxolitinib (JAK1/JAK2 inhibitor) have been FDA approved for treatment of multicentric Castleman disease, chimeric antigen receptor (CAR) T-cell-induced cytokine-release syndrome, and myelofibrosis/polycythemia vera, respectively. Drugs targeting IL-6/JAK/STAT3 signaling pathway are currently under clinical investigation for the treatment of solid tumors.

Several mAbs have also been used in the treatment of cancer [182] based on their ability to inhibit ligand binding and downstream signaling (cetuximab), target the tumor microenvironment (bevacizumab), and target immunosuppressive cytokines (GC-1008, an anti-TGFβ antibody) [183]. But it is the discovery of immune checkpoints and a deeper understanding of the immune regulatory pathways that led to a major breakthrough in cancer immunotherapy [184]. With the discovery that CTLA-4 expressed on activated T cells on binding with B7 molecules expressed on the APC blocks co-stimulation of T cells and produces immune suppression,

a series of experiments were performed to unleash the immune harnessing power of T cells to combat cancer. This led to the development of the concept of immune checkpoint blockade and breakthrough discovery of ipilimumab, a CTLA-4 inhibitor, which was FDA approved for the treatment of patients with metastatic melanoma in 2011 due to the durable responses observed in about 20% of patients and considerable improvement in the median OS of patients [185]. The dramatic response with ipilimumab laid the foundation for exploration of other T-cell inhibitory pathways. Based on strong preclinical evidence, several clinical trials were conducted to evaluate the efficacy of PD-1/PD-L1 pathway blockade by mAbs [186–190]. As a result of durable responses and survival benefits produced in several tumor types, FDA granted accelerated approval of several immune checkpoint inhibitors (ICPis) as listed in Table 1.1 [191]. This offers proof of concept that checkpoint inhibition provides durable and meaningful response in a subset of patients with responsive tumors.

Besides CTLA-4 and PD-1/PD-L1 signaling pathways, other immune regulatory pathways are being investigated as potential therapeutic targets. IDO is one such immunosuppressive pathway exploited by tumor cells to evade immune surveillance [192]. Several IDO inhibitors, such as INCB024360 [193, 194], indoximod [195],

Table 1.1 FDA-approved immune checkpoint inhibitors and indications[a]

Drug	Immune checkpoint(s)	FDA-approved tumor type[b]
Ipilimumab	CTLA-4	Melanoma
Nivolumab	PD-1	Melanoma
		Non-small-cell lung cancer
		Small-cell lung cancer
		Renal cell carcinoma
		Classical Hodgkin lymphoma
		Squamous cell carcinoma of the head and neck
		Urothelial carcinoma
		Hepatocellular carcinoma
		Mismatch repair-deficient and microsatellite instability-high metastatic colorectal cancer
Pembrolizumab	PD-1	Melanoma
		Non-small-cell lung cancer
		Esophageal squamous cell cancer
		Small-cell lung cancer
		Squamous cell carcinoma of the head and neck
		Classical Hodgkin lymphoma
		Urothelial carcinoma
		Gastric or gastroesophageal junction
		Microsatellite instability-high or mismatch repair-deficient solid tumors
		Cervical cancer
		Merkel cell carcinoma
		Hepatocellular carcinoma
Atezolizumab	PD-L1	Urothelial carcinoma
		Non-small-cell lung cancer
		PD-L1-positive triple-negative breast cancer
Durvalumab	PD-L1	Urothelial carcinoma
		Non-small-cell lung cancer
Avelumab	PD-L1	Merkel cell carcinoma
		Urothelial carcinoma

(continued)

Table 1.1 (continued)

Drug	Immune checkpoint(s)	FDA-approved tumor type[b]
Nivolumab with Ipilimumab	PD-1 and CTLA-4	Melanoma
		Renal cell carcinoma
		Microsatellite instability-high or mismatch repair-deficient colorectal cancer
Pembrolizumab with carboplatin and either paclitaxel or nab-paclitaxel	PD-1	Squamous non-small-cell lung cancer
Pembrolizumab with axitinib	PD-1	Renal cell carcinoma
Pembrolizumab with lenvatinib	PD-1	Endometrial carcinoma that is not microsatellite instability-high or mismatch repair deficient
Atezolizumab with bevacizumab, paclitaxel, and carboplatin	PD-L1	Nonsquamous, non-small-cell lung cancer
Atezolizumab with carboplatin and etoposide	PD-L1	Small-cell lung cancer
Avelumab with axitinib	PD-L1	Renal cell carcinoma

[a]List of FDA-approved immune checkpoint inhibitors as of October 9, 2019, adapted from: https://www.fda.gov/drugs/resources-information-approved-drugs/hematologyoncology-cancer-approvals-safety-notifications
[b]Tumor type must meet the criteria listed in the above-mentioned website

IDO peptide vaccine [196], BMS-986205 [197], and NLG919 [198], were investigated as single agents and in combination with PD-1 inhibitors and chemotherapy. Despite promising results in early-phase clinical trials, the combination of epacadostat with pembrolizumab failed to recapitulate the response in a phase III trial in melanoma patients [199].

A robust therapeutic immune response is produced not only by releasing the "brakes" on T cells but also by stepping on the "gas." T-cell costimulation through receptors, like OX40 or 4-1BB, provides a potent "go" signal that actively promotes the optimal "killer" CD8 T-cell responses [200]. Several ongoing clinical trials are investigating immune checkpoint agonist therapies as single-agent or in combination with other immunotherapies, chemotherapy, targeted therapy, or radiotherapy. Treatment with T-cell agonist is generally well tolerated. The most common side effects with these agents are fatigue and infusion-related reaction. However, two hepatotoxicity-related deaths were reported in a phase II study of a 4-1BB agonist at a dose range of 1 and 5 mg/kg every 3 weeks, respectively, resulting in termination of the study in 2009 [201]. The study was restarted in 2012 at lower dose levels (0.1 mg/kg every 3 weeks and 0.3 mg/kg every 3 weeks) and was found to be safe.

Despite the success with ICPis (CTLA-4, PD-1/PD-L1 blockade) in various tumor types, many patients are primarily resistant or develop resistance to treatment after an initial period of response [202]. Among several therapeutic strategies being investigated in the clinic to overcome primary and secondary resistance to the ICPis, there is growing evidence that combination therapies are far more effective than monotherapies to combat resistance mechanisms as tumors use multiple pathways to evade immune elimination [203]. Further, as these co-inhibitory receptors have nonredundant signaling pathways, a combined blockade of these mechanistically different pathways may be synergistic in restoring T-cell-mediated immune response [143]. Recently, FDA approved nivolumab in combination with ipilimumab for the treatment of patients with BRAF V600 wild-type, unresectable, or metastatic melanoma and advanced renal cell carcinoma [191]. There is intense research to identify optimal combinations that would increase the response rate and the duration of response. Targeted therapies are known to produce rapid onset of tumor regression [204]. However, the response is short lived. On the contrary, immunotherapies take

longer to initiate tumor regression, but produce responses that are more durable. Due to their complimentary outcomes, combinations of targeted and immunotherapy are being investigated in several clinical trials and emerging data suggest that such combinations may potentially be synergistic [205]. Similarly, radiation-induced immunomodulatory changes provide local control and prolong survival, but it is insufficient to shift the balance of the immunosuppressive TME to achieve tumor rejection [206]. To overcome this limitation, clinical studies evaluating the combination of radiotherapy and ICPis are currently underway [207, 208].

As immunotherapy-based combinations are being increasingly investigated, identifying optimal combination strategies remains a challenge as timing and sequencing of the drugs may affect treatment outcomes. For example, majority of patients with breast cancer do not respond to PD-1 inhibitor monotherapy. As TILs in breast cancer are known to express OX40, combination of anti-PD-1 and OX40 agonist was investigated in a PD-1 refractory murine mammary cancer model [209]. The antitumor response was weak and short lived on concurrent administration of these two agents, whereas the response was not only durable on sequential administration of these agents but also complete in more than 30% of the mice. Furthermore, timing of immunotherapy is very critical for improved treatment outcomes. For example, effects of radiation in combination with immunotherapy were investigated in a colorectal cancer tumor-bearing mice [210]. Response was optimal when OX40 agonist antibody was delivered immediately after radiation therapy during the postradiation window of increased antigen presentation [210], whereas anti-CTLA-4 was most effective when given prior to radiation. Thus, it is important to pay attention to sequence and timing of immunotherapeutic agents when used in combination.

Emerging data suggest that activation of innate immune system could disrupt the immunosuppressive dynamics of TME to evoke an effective antitumor immune response. Importantly, this process leads to initiation of adaptive immune response by enhancement of the T-cell priming process. Toll-like receptors (TLRs), the most important receptors in innate immunity, exhibit dual role in cancer [211]. While some TLRs on cancer cells favor tumor progression [212, 213] and promote resistance to chemotherapy, most TLRs on immune cells serve as sensors [211]. Activation of these TLRs by foreign antigens triggers a cascade of pro-inflammatory reactions that ultimately initiates an adaptive immune response. Thus, TLRs have been identified as potential targets, and several TLR agonists (TLR3, TLR4, TLR5, and TLR7 agonists) are being investigated for clinical application [214, 215]. Similarly, an endoplasmic reticulum membrane protein STING (Stimulator of Interferon Genes) that is highly expressed in the APCs mediates potent antitumor activity by induction of innate immunity and initiation of adaptive immunity [215]. Typically, self-DNA is located in the nucleus or mitochondrion, while microbial/tumor-derived DNA is located in the cytoplasm. By virtue of their location, the tumor-derived DNA is identified by several cytosolic DNA sensors triggering activation of STING signaling in the APCs [216]. The resultant downstream signaling through STING pathway results in phosphorylation of interferon regulatory factor 3 (IRF3) and nuclear factor-κB and subsequent induction of pro-inflammatory molecules, IFN β, and cytokines, such as TNF, IL-1β, and IL-6. In the process, IFNs also promote cross-priming of T cells by the DCs resulting in initiation of adaptive immune response [217]. As activation of STING pathway promotes T-cell priming and induction of adaptive immune mechanism, several STING agonists as vaccine adjuvants and in combination with other immunomodulators are being investigated [218–220]. Macrophages are cells of the innate immune system that serve as a double-edged sword in response to cytokines in the TME [221]. Typically, in the presence of IFN-γ, TAMs acquire M1 phenotype and are tumoricidal. However, in the hypoxic TME, TAMs acquire a pro-tumoral M2 phenotype and engage in proliferation and migration of tumor cells. Thus, TAMs are potential therapeutic targets. Several strategies to reduce recruitment of TAMs or deplete TAMs using CSF1R inhibitors [222, 223] and reprogramming

TAMs to acquire an antitumor M1-like phenotype using bioconjugated manganese dioxide nanoparticles [224] or ferumoxytol nanoparticles [225] or concurrent CSF-1R blockade and CD40 agonism [226] are now under investigation. Thus, strategies that bridge the innate and adaptive immune response may have therapeutic utility.

Besides targeting the cellular components of the innate and adaptive immune system, manipulation of metabolic pathways is a promising strategy to induce immune response in the management of cancer. In general, L-arginine is metabolized by nitric oxide synthases in M1 macrophages to produce nitric oxide, which is cytotoxic in function [227]. However, in the TME, increased MDSCs express arginase I that metabolizes L-arginine to L-ornithine and urea [228]. This depletion of L-arginine induces T-cell anergy and profoundly suppresses T-cell immune response. Modulation of L-arginine metabolic pathway by direct inhibition of arginase I using arginase inhibitors and by supplementation of L-arginine has been promising [229].

Translational Relevance

Immunotherapeutic agents have revolutionized the treatment paradigm of patients with advanced cancer. However, significant survival benefit has been observed only in a subset of patients. Biomarker-driven drug development is, therefore, critical, as it may help physicians to preselect patients who are most likely to derive benefit and more, importantly, allow patients who are less likely to benefit to look for alternate therapies and spare them from avoidable immune-related toxicities and cost of treatment [230]. Some of the important biomarkers of response are provided in the following.

PD-L1 Expression

Early-phase I trials suggest that cell surface expression of PD-L1 on tumor cells in pretreatment tissue samples could serve as biomarker of response to treatment with anti-PD-1/PD-L1 therapies. In a phase I study of MDX-1106, an anti-PD-1 inhibitor, in 39 patients with advanced cancers, tumor biopsies from nine patients were analyzed for PD-L1 expression by immunohistochemistry (IHC) [186]. Objective response was observed in three of four patients (75%) with PD-L1-positive tumors, while none of the five patients with PD-L1-negative tumors had a response. Similar results were observed in another phase I study of BMS-936558 (nivolumab), an anti-PD1 therapy, in which pretreatment tumor tissue from 42 patients with advanced cancer was analyzed for PD-L1 expression by IHC [231]. Nine of 25 patients (36%) with PD-L1-positive tumors had objective response, while none of the 17 patients with PD-L1-negative tumors had a response, indicating the possibility of an association between PD-L1 expression on pretreatment samples and objective response. Recently, FDA approved expression of PD-L1 by IHC using 22C3 pharmDx as a diagnostic test for selecting NSCLC patients for treatment with pembrolizumab [232]. However, PD-L1 expression in pretreatment tumor tissue as an absolute biomarker to predict response to PD-1/PD-L1 pathway inhibitors has been questioned for various reasons. In a phase I study conducted to evaluate the safety and efficacy of MPDL3280A, an anti-PD-L1 inhibitor, ORR of 46% was reported in patients with high PD-L1 expression on pretreatment immune cells, 17% in patients with moderate PD-L1 expression, 21% in patients with minimal PD-L1 expression, and 13% in patients with absent PD-L1-expression in tumor immune cells [233]. Surprisingly, response to treatment was observed even in patients with PD-L1-negative disease. In addition, the association between response to therapy and PD-L1 status was discordant depending on PD-L1 expression on tumor cells or tumor immune cells. PD-L1 expression on tumor-infiltrating immune cells was significantly associated with response to MPDL3280A ($P = 0.007$), whereas PD-L1 expression on tumor cells was not significantly associated with response ($P = 0.079$). In addition, in a phase III study, survival benefits were seen in NSCLC patients treated with Atezolizumab compared to docetaxel

regardless of PD-L1 expression in the tumor or immune cells [234]. There is also marked heterogeneity in PD-L1 expression between samples from the primary and metastatic sites in the same individual [235]. Further, the predictive potential of PD-L1 expression is challenged due to technical issues, such as lack of standardized PD-L1 diagnostic assay, use of different PD-L1 antibody clones by multiple immune assays, different staining procedures for IHC staining, and different cutoff values and scoring patterns [236]. As a result, there is lack of defined criteria to determine PD-L1 status of the patient. The above findings suggest that although PD-L1 expression in tumor tissue may indicate an increased likelihood of response to treatment with PD-1/PD-L1 inhibitors, it may not be a definitive biomarker to exclude PD-L1-negative patients from therapy [233, 237].

Tumor-Infiltrating Lymphocytes

There is a broad literature of evidence that infiltration of tumor tissue by T cells, specifically CD8+ T-cell density at the invasive tumor edge, is associated with improved survival in patients with melanoma, breast, ovarian, lung, esophageal, gastric, renal cell, colorectal, and bladder carcinoma among other solid tumors [238–240]. On the contrary, infiltration of the tumor tissue by Tregs is associated with poor survival in ovarian cancer, breast cancer, and hepatocellular carcinoma [241–243]. Interestingly, strong intratumoral infiltration by CD8+ T cells and Th1 cells did not favor immune elimination of tumors in patients with mismatch repair-deficient colorectal cancer [244]. Despite a hostile TME, the tumors survived due to strong co-expression of several immune checkpoints, such as PD-1, PD-L1, CTLA-4, Lag-3, and IDO, in the invasive margin, stroma, and TILs. This finding suggests that the tumors may be responsive to checkpoint blockade. As a result, mismatch repair status may be predictive of response to checkpoint inhibition.

Further, the type, density, and location of immune cells within the tumor (collectively known as immune contexture) have prognostic value. Multiple immune markers including total T lymphocytes (CD3), T-cell effectors (CD8), their associated cytotoxic molecule (GZMB), and memory T cells (CD45RO) in the center of tumor (CT) and the invasive margin (IM) were quantified using IHC in tumors from 415 colorectal cancer patients [245]. The immune cell densities in each tumor region were higher in patients without recurrence than in patients with recurrence and were predictive of disease-free survival (DFS) and OS. These results were independent of the staging of the tumor, indicating the role of adaptive immune response in preventing tumor recurrence. In addition, the presence of markers for Th1 polarization and cytotoxic and memory cells was predictive of low recurrence rate.

Baseline expression of TILs may not always suggest response to immune checkpoint blockade. For example, CD8+ T cells at the IM were positively associated to response with pembrolizumab in patients with metastatic melanoma [246], but not in patients with unresectable stage III/IV melanoma treated with ipilimumab [247]. However, increase in the levels of tumor-infiltrating T cells at the CT and IM in on-treatment biopsies were predictive of response to treatment with ICPi in several studies [246–248]. The antitumor activity was largely dependent on preexisting adaptive immune mechanism as evidenced by the presence of higher numbers of CD8-, PD-1-, and PD-L1-expressing cells in the baseline samples [246].

Immunoscore

Immunoscore is a methodology by which in situ immune infiltrate is quantified. This supersedes the TNM classification of tumors used for the estimation of the degree of progression of the tumor to make informed treatment decisions [245]. Marked variations in clinical outcomes among patients with the same stage of disease were observed with TNM classification, partly due to failure to include the immune cells in the TME in TNM classification of tumors. As the interaction between the tumor cells and the immune cells play an important role in immune escape and progression of the tumor, immune

contexture discussed above is a better prognostic indicator than TNM classification [249]. Therefore, a new scoring system was derived from immune contexture called the immunoscore, which is a ratio of the densities of two lymphocyte populations, CD3/CD45RO, CD3/CD8, or CD8/CD45RO, in the CT and IM. Due to difficulty in staining methods, a combination of two markers (CD3+ and CD8+) in CT and IM has been used by the worldwide immunoscore consortium in the development and validation of immunoscore as prognostic markers in different patient populations. The score ranges from immunoscore 0 (I0), when the densities of both the lymphocyte populations are low in both the regions, to immunoscore 4 (I4), when the densities of both the lymphocyte populations are high in both the regions. This score is the strongest prognostic indicator of DFS and OS in patients with local and metastatic disease [250]. Recently, the consensus immunoscore was validated in a study conducted by an international consortium of centers in 13 countries [251]. In the analysis that included tissue samples from 2681 colorectal cancer patients, patients with a high immunoscore had the lowest risk of recurrence in 5 years and prolonged DFS and OS, a finding that has been confirmed in both the internal and external validation set. This scoring system will help to stratify patients based on the risk of recurrence. However, the universal application of immunoscore across tumor types has to be determined.

T-Cell Receptor Diversity

As T cells play an important role in recognition and eradication of cancer cells, a diverse TCR repertoire will allow for detection of wide range of foreign antigens. On activation, TCR undergo clonal expansion. Thus, characterization and estimation of TCR repertoire diversity by next-generation sequencing of complementarity determining region 3 (CDR3) region may provide insight into antitumor activity of ICPis. In a melanoma patient with metastatic lesion to the brain that progressed on ipilimumab, a durable complete clinical response was achieved with sequential whole-brain radiation therapy and pembrolizumab [252]. A high-throughput CDR3 sequencing of the intratumoral T cells in the brain metastasis obtained before treatment and the circulating peripheral T cells obtained sequentially during treatment showed that the dominant CD8+ T-cell clone in the brain metastasis (pretreatment) had clonally expanded on treatment with pembrolizumab and was detected as the most frequently occurring clone in the blood. This indicates the presence of preexisting but inadequate adaptive immune response that was bolstered by treatment with pembrolizumab. Similar on-treatment clonal expansion of a CD8+ T-cell clone present in the metastatic site prior to treatment was seen in a NSCLC patient who experienced pathological complete response with nivolumab [253]. In 10 patients with metastatic melanoma treated with nivolumab [254], oligoclonal expansion of certain TCR-β clonotypes was observed in posttreatment tumor tissues of responders. Similar results were also observed in 25 patients with metastatic melanoma treated with pembrolizumab [246]. TCR sequencing of pre- and posttreatment samples showed the number of clones that had expanded was 10 times more in the responders than in nonresponders. Further, clinical response was associated with a more restricted TCR beta chain usage in predosing samples. Thus, a diverse TCR repertoire at baseline and on-treatment tumor antigen-specific clonal expansion may be predictive of response to treatment with ICPis.

Mutation Load and Molecular Alterations

Tumors with high mutational load such as melanoma, NSCLC, and head and neck squamous cell carcinoma (HNSCC) are more likely to respond to treatment with ICPis as neoepitopes generated by somatic mutations function as neoantigens and elicit a brisk immune response [255]. In several clinical trials, higher clinical benefit rate and longer progression-free survival had been reported in patients with high mutation burden treated with ICPis [255–257]. It is for the same reason that improved treatment outcomes with ICPis have

been reported in patients with solid tumors, colorectal cancer patients in particular, with defects in the mismatch repair (MMR) mechanism [258, 259]. However, Snyder and colleagues described that while high mutational load correlated to sustained response to CTLA-4 blockade, not all melanoma patients with high mutational load responded to therapy [256]. However, the presence of tetrapeptide neoepitope signature in these patients with high mutation load correlated strongly with long-term clinical benefit and OS. On the contrary, tumors with low mutational loads (e.g., pancreatic and prostate cancer) were not responsive to ICPi. In addition, molecular alterations in the PI3K pathway may promote tumor immune evasion through constitutive expression of PD-L1 [260]. Assessment of PD-L1 expression in such conditions may predict response with PD-1/PD-L1 inhibitors. Similarly, increased expression of VEGF promotes angiogenesis and is associated with poor prognosis [239].

Immune Gene Signature

Differential expression of genes may help to identify phenotypes responsive to treatment with ICPis. For example, loss-of-function *BRCA2* mutations with specific mutational signatures were identified in responding melanoma tumors sampled from patients on treatment with anti-PD-1 agents [257]. Likewise, in melanoma patients treated with pembrolizumab, an IFNγ 10-gene and an expanded immune 28-gene signatures in pretreatment samples were significantly associated with ORR and PFS [261]. On further evaluation, more refined immune signatures were found to produce similar results in patients with HNSCC and gastric cancer [262]. A high pretreatment levels of IFNγ mRNA and PD-L1 protein expression were associated with increased ORR and longer OS in NSCLC patients treated with durvalumab [263]. A similar association between high expression of T-effector-associated, interferon-γ-associated, and PD-L1 genes in tumor tissue and improved OS was seen in NSCLC patients treated with atezolizumab [264]. The T-effector-associated and interferon-γ-associated gene expression was associated with PD-L1 expression on immune cells and not on tumor cells, suggesting the role of preexisting adaptive immune response. On the contrary, a group of 26 innate anti-PD-1 resistance (IPRES) signature characterized by higher expression of mesenchymal transition, angiogenesis, hypoxia, and wound-healing genes were identified in pretreatment melanoma tumors resistant to anti-PD-1 therapy [257]. The IPRES signature was also found in nonresponsive pretreatment tumor samples from patients with other solid tumors such as adenocarcinoma of the lung, colon, and pancreas and clear cell carcinoma of kidney. Thus, immune-related gene expression signatures may be associated with treatment outcomes.

Cancer Immunogram

The cancer immunogram model was developed to overcome the limitation that no single biomarker can truly reflect the dynamic interaction between the immune cells and tumor. Based on the assumption that T cells are the ultimate effectors of antitumor activity, seven parameters were included in the model to understand the interaction between the tumor and the immune cells in the TME of the patient [265]. The seven parameters and their potential biomarkers in parenthesis are as follows [1]: tumor foreignness (mutation load) [2], general immune status (lymphocyte count) [3], immune cell infiltration (intratumoral T cells) [4], absence of checkpoints (PD-L1) [5], absence of soluble inhibitors (IL-6 and C-reactive protein [CRP]) [6], absence of inhibitory tumor metabolism (lactate dehydrogenase [LDH], glucose utilization) [7], and tumor sensitivity to immune effectors (major histocompatibility complex expression, IFNγ sensitivity). The data points for each of the seven parameters are plotted in a radar plot, and the line joining the individual data points provides a personalized framework reflecting the interaction in the TME. The gaps in the radar plot indicate potential therapeutic strategies that may evoke an effective immune response in the patient.

A modified immunogram has been developed based on the seven steps in the cancer immunity cycle for use in NSCLC patients [266]. The eight axes of the immunogram score (IGS) are as follows: IGS_1, existence of T-cell immunity in the tumor; IGS_2, tumor antigenicity (existence of neoantigens and cancer germ line antigens), IGS_3, priming and activation (presence of activated DCs); IGS_4, trafficking and T-cell infiltration; IGS_5, recognition of tumor antigens; IGS_6, absence of inhibitory cells (Tregs and MDSCs); IGS_7, absence of checkpoint expression (PD-1, PD-L1, etc.); and IGS_8, absence of inhibitory molecules (IDO 1; arginase 1 etc.). High scores for $IGS_{1–5}$ indicate a favorable environment for development of T-cell immunity. On the contrary, high scores for $IGS_{6–8}$ indicate immune suppression. Based on the radar plot, three groups of patients have been identified. Patients' high $IGS_{1–5}$ and low $IGS_{6–8}$ represent T-cell-rich phenotype, where antitumor activity is dampened by an immunosuppressive TME, patients with low IGS_1, $IGS_{3–5}$ represent T-cell–poor phenotype with defects in the T-cell priming process, and patients in whom IGS_2, $IGS_{6–8}$ are maintained represent an intermediate phenotype. Thus, the immunogram helps to identify areas of therapeutic focus to elicit an effective antitumor response. Cancer immunograms are promising for personalized approach to immunotherapy.

Serum Biomarkers

Several routinely available peripheral blood parameters have been evaluated as a biomarker of response to treatment with checkpoint inhibitors [248, 267–274]. Most common among them are absolute lymphocyte count (ALC), absolute eosinophil count (AEC), LDH, and CRP. In patients with advanced refractory melanoma, ALC ≥1000/μL after two treatments with ipilimumab was significantly associated with clinical benefit and OS [270, 271]. Although ALC at baseline and after one dose of ipilimumab showed only a trend for improved treatment outcomes, they may be prognostic because a threshold ALC of 1000 cells/μL may be required for adequate activation of the immune system for patients to derive meaningful antitumor response with therapy. Similar results were seen in several clinical trials in patients with melanoma treated with ipilimumab [270–274], where an increase in ALC levels from baseline was associated with improved OS and disease control compared to patients with stable or decreasing levels. Likewise, increase in AEC levels after two courses of ipilimumab was associated with OS [270] and was an independent predictor of response in patients with melanoma [275]. On the other hand, elevated levels of LDH at baseline was an independent predictor of poor survival [270, 276]. Despite the association between these peripheral blood parameters and treatment outcomes, there is no validated biomarker available for use in the clinic.

Circulating Biomarkers

Serial assessment of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), which is a measure of tumor burden, may predict response to treatment with checkpoint inhibitors. The association between ctDNA and treatment outcomes was evaluated in three groups of patients treated with PD-1 inhibitors as single agents or in combination with ipilimumab [277]. Group A included patients with undetectable ctDNA at baseline and during treatment, Group B had patients with detectable ctDNA at baseline but undetectable early during therapy, and Group C included patients with detectable ctDNA at baseline and during therapy. Compared to baseline ctDNA, persistent on treatment levels of ctDNA was associated with decreased ORR and poor survival. On the other hand, increase in circulating levels of immune cells, Ki-67+ T cells, was associated with clinical benefit in NSCLC patients on treatment with PD-1 inhibitors [278]. If these findings are validated in large prospective cohorts, in the context of intratumoral heterogeneity, minimally invasive and easily accessible liquid biopsies may serve as a more comprehensive alternate technique for biomarker assessment.

Microbiome Assessment

Emerging data indicate that gut microbiome may be associated with response to treatment with PD-1 inhibitors. Alpha diversity of gut microbiomes in fecal samples was significantly higher in patients with metastatic melanoma responding (CR/PR/SD ≥6 months) to treatment with PD-1 inhibitors [279]. In addition, patients with higher alpha diversity had longer PFS compared to patients with low or intermediate diversity. Further, the gut microbiome was enriched for Clostridiales in responders and Bacteroidales in nonresponders. In addition, patients with abundance of Faecalibacterium genus in Clostridiales order had significantly longer PFS compared to patients with abundance of Bacteroidales. Thus, favorable gut microbiome may enhance antitumor response in patients treated with checkpoint inhibitors.

Due to the dynamic nature of immune response, development of immune oncology biomarkers is challenging. To this end, immune monitoring assays have been developed to perform genomic, proteomic, and functional studies on paired tumor and blood samples obtained before and after treatment with immunotherapeutic agents [237]. It is expected that correlation of changes in these biomarkers to treatment outcomes would provide mechanistic insight into pathways of response or resistance to immunotherapeutic agents that could guide the development of biomarker-driven, synergistic, immunotherapy-based treatment combinations. In addition, biomarkers may vary depending on the mechanism of action of the immunotherapeutic agent [186, 231]. Therefore, identification of a single immunologic biomarker may not be predictive of response [237]. This indicates a need to identify multifactorial biomarker panels that would help to determine the immunogenic nature of the tumor and predict response or resistance to treatment. For example, presence of intratumoral CD8+ T cells, expression of PD-L1 on tumor cells, and increased mutational load have been associated with greater likelihood of response to PD-1/PD-L1 checkpoint inhibition [230].

Conclusion

Seminal studies have described the different components of the innate and adaptive immune system. Although they are two distinct arms of the human immune system, they are intricately organized in time and space and are critically dependent on one another. While the blockade of immune checkpoints by mAbs to unleash the antitumor immune response by T cells has now emerged as a powerful therapeutic tool in the treatment of advanced cancer, components of the innate immune system contribute to the activation and development of adaptive immunity. Improved understanding of the interaction between the tumor cells and the immune cells in the complex TME through rigorous immune profiling will guide the future development of new immunotherapeutic strategies as well as the identification of potential biomarkers of clinical response.

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