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Aung Naing
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This book is dedicated to our parents,
Khin Maung Oo, Khin Htwe, M Issam Hajjar,
and Nada Abo Shala, who inspired us to spread
accurate and updated knowledge to humanity.

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Overview of Basic Immunology and Clinical Application

1

Bettzy Stephen and Joud Hajjar

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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2 Advances in Diagnostic Procedures and Their Applications in the Era of Cancer Immunotherapy

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Abstract

Diagnostic procedures play critical roles in cancer immunotherapy. In this chapter, we briefly discuss three major diagnostic procedures widely used in immunotherapy: immunohistochemistry, next-generation sequencing, and flow cytometry. We also describe the uses of other diagnostic procedures and preclinical animal models in cancer immunotherapy translational research.

Keywords

Immunotherapy · Diagnostic procedure · Biomarker · Immunohistochemistry · Next-generation sequencing · Genetic diagnostics · T-cell receptor sequencing · Flow cytometry · Polymerase chain reaction · Microarray · Southern blot · Western blot · Proteomic profiling · Preclinical animal models

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Introduction

Cancer immunotherapy (also called immuno-oncology) is a cancer treatment designed to stimulate and utilize the body's own immune system, or to block immune escape or immune inhibitory pathways, to fight cancer. In the past few decades, with the advancement of understanding of immunity in cancer biology and the tumor immune microenvironment, immunotherapy has demonstrated tremendous clinical progress in various cancer types [1, 2]. However, only a subset of patients have responded to and benefited from immunotherapy. Moreover, immunotherapeutic drugs have been associated with immune-related adverse events, some of them severe and even life threatening. For the diagnosis and identification of patients whose disease is likely to respond to immunotherapy without severe toxicity, diagnostic procedures must be accurate, sensitive, robust, and versatile as well as applicable in various tumor types to guide the selection of the most suitable treatment regimens. In this chapter, we briefly discuss several diagnostic techniques, preclinical animal models, and their relevance in immunotherapy.

Immunohistochemistry

Immunohistochemistry (IHC) is a simple diagnostic procedure that is well established and widely used to detect and visualize antigen (that

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is, protein) expression levels and cellular or subcellular patterns using highly specific antigen–antibody reactions in tissue sections [3]. The term "immunohistochemistry" comprises three parts, "immune", "histo", and "chemistry": (1) "immune" indicates antibody–antigen recognition; (2) "histo" indicates tissue morphology preservation; and (3) "chemistry" indicates the antibody–antigen reaction, resulting in staining. IHC assays can be performed on formalin-fixed and paraffin-embedded (FFPE) or fresh frozen (FF) tissue sections. IHC has been widely used not only for diagnostic pathology classification, but also anti-tumor drug development in cancer immunotherapy [4]. IHC provides cancer diagnostic, prognostic, and predictive guidance for immunotherapy [5–9].

IHC Applications

Cancer Pathology Diagnostics

Both adjuvant to and independent of conventional hematoxylin and eosin staining, IHC staining of cells and tissue provides comprehensive histologic and morphologic information using highly specific antibody markers. In cancer pathology diagnostics, these markers include tumor cell proliferating antigens, growth factors, tumor-specific signaling pathway factors, and tumor-infiltrating lymphocytes. IHC assays have contributed to the pathologic classification and diagnosis of a variety of cancer types, including breast, lung, and prostate cancers [4, 5, 10–13], using well-established, specific tumor markers [14].

Predictive Biomarker Tests

In addition to cancer diagnostics, IHC assays have been increasingly used for predictive biomarker tests for targeted immunotherapy, especially immune checkpoint inhibitors [15]. For instance, PD-L1 assessed by IHC has served as a predictive biomarker for identifying patients more likely to benefit from anti–PD-1/PD-L1 immunotherapy. The PD-L1 IHC 28-8 and PD-L1 IHC 22C3 pharmDx assays (Agilent) have been approved by the U.S. Food and Drug Administration as diagnostic or companion tests for anti–PD-1 therapies [16–19]. However, patients with PD-L1− status can still benefit from anti–PD-1 or anti–PD-L1 treatment.

In addition, IHC-assessed tumor-infiltrating lymphocytes (TILs) play an important role in predicting immunotherapy outcomes. Several clinical studies have shown that increased TIL density is associated with improved survival rate in patients receiving immunotherapy for melanoma, colorectal cancer, lung cancer, breast cancer, and other tumor types [15, 20–24]. TIL density can be estimated using routine hematoxylin and eosin staining without distinguishing lymphocyte types and populations [25]. With IHC, immune-related markers can be used to assess specific immune cell types and subsets of TILs, including CD3+, CD4+, CD8+, CD20+, CD45RO+, and FOXP3+ lymphocytes, as well as ratios between these subsets etc., thereby providing more comprehensive information about the tumor microenvironment. For instance, higher CD8+ TIL T-cell density and CD8+/FOXP3+ ratio were associated with clinical outcomes [26].

Another application of IHC assays in immunotherapy diagnostics involves microsatellites, which are special repeat sequences in the DNA. When DNA repair genes are not functional, microsatellite sequences can acquire or lose nucleotides, which is known as microsatellite instability (MSI). High neoantigen load or tumor mutational burden caused by deficiency in the DNA mismatch repair protein function, also interpreted as MSI-High (MSI-H), is a well-known indicator of genomic instability. Patients with high MSI or DNA mismatch repair protein deficiency (dMMR) usually carry a high number of genetic mutations in tumors. MSI-H/ or dMMR has been associated with increased T-cell activation and immune cell infiltration and therefore better response, especially in patients with colorectal tumors or metastatic colorectal cancer treated with immunotherapy [27–30].

MSI test can be assessed by detecting microsatellite DNA loci in the genome using polymerase chain reaction (PCR) or by examining loss of expression of mismatch repair proteins MLH1, MSH2, MSH6, and PMS2 using

IHC. The PCR test provides direct evidence for MSI status, whereas IHC assesses the expression levels of mismatch repair proteins, an indirect indicator of MSI. Nonetheless, comparison studies suggested high concordance between IHC testing and DNA-based MSI PCR testing (>90% coincidence rate) [28, 31–34]. Moreover, IHC assays are more feasible and economical for the clinical setting. IHC analysis requires only 4 × 3 μm formalin-fixed, paraffin-embedded sections, whereas PCR requires more biopsy material for DNA extraction. Therefore, IHC has been used as a primary detection method: if no deficiency of any repair protein is detected, indicating microsatellite stability, then no further PCR is required. On the other hand, if any repair protein is found to be deficient on IHC, PCR can be used as a complementary, secondary detection assay to further determine MSI status. Although a few studies have shown discordance between IHC and PCR tests for MSI in ovarian cancer [35], most studies have suggested that IHC is a reliable and economical method for MSI diagnostics.

Single-Marker Versus Multiplexed IHC

While conventional chromogenic IHC can detect only one targeted antigen per experimental run using chromogens such as 3,3′-diaminobenzidine tetrahydrochloride, multiplexed IHC enables the detection of multiple targeted antigens simultaneously on a single tissue section or assembled tissue microarray section. Current multiplexed IHC platforms use either fluorescence-labeled antigens (up to eight) detected by fluorescence microscopy [9] or, in "next-generation" IHC, metal-conjugated antigens (up to 60) detected by mass spectrometry [36] to maximize antigen detection capacity and the quality and resolution of image acquisition.

Compared with traditional, single-marker IHC, multiplexed IHC offers several advantages. First, by labeling multiple antibodies on a single section and obtaining maximal data sets from one sample, multiplexed IHC saves precious samples, including clinical samples with limited availability. Second, traditional IHC stains use one antibody per section, so spatial and co-localization data from multiple-antibody staining are obtained by staining serially cut sections individually and aligning the serial images. The more sections stained, the less accurate the spatial information. However, for multiplexed IHC, multiple-antibody staining is performed simultaneously or sequentially on one section, providing accurate spatial information that is easily assessable. Finally, multiplexed IHC enables the introduction of housekeeping protein markers as references for normalization, eliminating potential errors between batches and producing more accurate IHC data. Nonetheless, despite its comprehensive insights into tissue context and microenvironment, multiplexed IHC has some technical limitations and complications. Highly specific, high-quality IHC antibodies are required for a multiplexed IHC platform to produce accurate results; antibody cross-reactivity leads to unreproducible, unreliable results. Additionally, interpreting multiplexed IHC data can be challenging and more time consuming.

IHC has been an essential diagnostic procedure in cancer diagnosis and therapy for many years. With technical advancements, automation, and standardization of IHC techniques, as well as the application of multiplexed IHC platforms, IHC will play an expanding role in cancer diagnostics, predictive biomarker testing in the era of immunotherapy.

Next-Generation Sequencing

Next-generation sequencing (NGS) techniques have enabled fast, affordable analysis of the genome and transcriptome in immunotherapy. There are three major sequencing platforms: 454 sequencing, SOLiD, and Solexa. 454 sequencing, among the first NGS platforms, has the longest read length and is very fast but is expensive and has a high error rate. The SOLiD platform is the most accurate but has shorter reads. Solexa has the lowest price and highest throughput and has a low error rate but uses short reads [37–39]. Recently, third-generation sequencing has

emerged, which can generate very long reads (1–100 kb). Two such techniques are PacBio and Oxford Nanopore. Long reads at this scale are essential when a reference genome is lacking or for identification of a novel gene or isoform. However, these platforms also have high error rates [40].

Most genetic diseases are not caused by a single mutation in a single region. Instead, complex diseases are the result of variations in many different genomic regions. For years, researchers tried to connect genomic variations to complex diseases using genome-wide association studies [41]. Because individual genetic variations with a large effect size are very rare and hard to detect in these studies, studying gene interactions with this method is challenging, and epigenetic causes are often overlooked [42]. Identifying these rare variants became more achievable with NGS technologies. This approach can powerfully contribute to personalized medicine, as each cancer patient has a distinct mutational signature, and tailoring treatment to each patient can improve clinical outcomes.

High-throughput sequencing has enabled fast, unbiased genetic comparisons of patients and healthy controls. However, although NGS technologies have substantially decreased the costs of sequencing, these technologies did not render large-scale sequencing of the entire genome affordable. Thus, targeted enrichment techniques were developed to limit sequencing to areas of interest, reducing cost and time spent. Targeted enrichment techniques can be PCR- or hybridization-based. If the genomic region of interest is known, researchers can use flanking PCR primers to amplify specific regions before library preparation for sequencing. As longer PCR products have more errors, PCR enrichment requires many parallel reactions with shorter products, which increases cost. Despite its limitations, PCR enrichment can be very useful in the clinic, as sequencing of enriched regions leads to higher accuracy, which is essential in the clinical setting. In hybridization-based methods, the target regions are captured after the sequencing library is prepared, using complementary oligonucleotides. These oligonucleotides can be attached to an array or can be in solution. Generating microarrays can be costly and requires large amounts of input DNA, while hybridization to labeled oligonucleotides in solution is more affordable and can be performed using a small amount of input DNA [42].

A widely used method of targeted enrichment is whole-exome sequencing (WES). The exome comprises the protein-coding regions of the genome, and sequencing only the exome can still give essential insights about genetic diseases, many of which are caused by mutations in these regions, while sequencing only approximately 2% of the sequences required for whole-genome sequencing (WGS) [43]. So far, many studies have identified mutations that cause genetic diseases and cancers using whole-exome sequencing [42, 44].

The causes of many diseases lie not only at the DNA level but also at the RNA level. For this reason, RNA sequencing (RNA-Seq), which uses NGS technologies to analyze RNA transcripts (the transcriptome), is essential for understanding the changes in tissues and cells under different conditions. RNA sequencing quantifies the abundance levels of both mRNAs and non-coding RNAs. RNA seq is very advantageous while studying complex diseases, as it can efficiently detect gene fusions, allele-specific expressions, and non-coding RNAs, which can have regulatory functions [42, 45]. Recently, platforms such as 10x Genomics started to sequence RNAs from single cells instead of bulk samples. Single-cell RNA sequencing has the potential to identify and analyze rare cell populations that might be missed in pooled analysis [46, 47].

Many diseases involve epigenetic abnormalities [48]. Epigenetic means utilization of the genomic information to establish specific gene expression patterns. The cellular states in development and disease rely on a particular gene expression program that is facilitated by transcribing the genetic code on the DNA and making RNA [49]. This particular RNA program can then be translated to a particular protein program, which executes cellular functions and phenotypic features. It is important to understand how epigenetic mechanisms control diverse cel-

lular fates through establishing unique gene expression programs [50]. Chromatin in eukaryotic cells consists of DNA that is wrapped around highly conserved histone proteins [51]. The amino-termini tails (N-terminal tails) of histones undergo posttranslational modifications that alter the nucleosome structure [52]. Proteins that recognize these modifications or the changes in nucleosome structure play an important role in regulating gene expression [53, 54] and are frequently mutated in cancer [49].

A common technique for studying the epigenome is chromatin immunoprecipitation followed by sequencing (ChIP-seq) [55–57]. In this technique, samples are fixed to maintain DNA–protein interactions; DNA that is not bound to any protein is digested and removed; and protein–DNA complexes are precipitated using an antibody specific to the protein of interest. Then, the cross-links are reversed, and DNA sequences that bind to the protein are sequenced using NGS technologies. ChIP-seq reveals histone modifications and binding patterns of DNA-binding proteins such as transcription factors [58]. Thus, ChIP-seq can be used to determine the molecular causes of complex diseases [59].

Another technique for studying chromatin state is called Assay for Transposase-Accessible Chromatin followed by sequencing (ATAC-seq) [60]. In this method, open chromatin is mapped using a special type of transposase that can insert certain sequences into chromatin regions that are open or accessible. The inserted sequences are then used for PCR amplification followed by NGS. As DNA accessibility affects gene expression, ATAC-seq from clinical samples can identify many clinically relevant epigenetic changes [61].

NanoString gene expression panels (also known as nCounter panels) offer a distinct way of analyzing gene expression. This method does not involve any enzyme: no reverse transcription or amplification. Instead, individual mRNAs are labeled with DNA barcodes. Each barcode has a sequence of six fluorescent spots that can be one of four colors, as well as complementary sequences for the gene of interest. mRNA transcripts are hybridized to these barcodes and then imaged on a slide. Instead of quantifying the overall fluorescence intensity, the assay counts individual barcodes, which is equivalent to counting individual mRNA transcripts. This direct, single-molecule counting method is precise and reproducible and works well with formalin-fixed, paraffin-embedded tissue samples. NanoString offers many different gene panels targeted for oncology, immunology, and neuroscience. Each panel contains up to 800 targets and can be customized to an extent [62].

Most human T cells have T-cell receptors (TCRs) that comprise alpha and beta chains. TCR chains are highly diverse as a result of recombination and can detect millions of antigens. Complementarity-determining region 3 (CDR3) is a site of antigen contact in the variable region of TCRs and thus is often studied to determine T-cell repertoire diversity [63]. Most commonly used for characterizing the T-cell repertoire are NGS-based assays that involve sequencing the CDR3 region of the TCR beta chain. These assays can be used to analyze T-cell clones in formalin-fixed, paraffin-embedded tumor samples. More recently, advances in single-cell genomics have enabled single-cell TCR sequencing to identify the T-cell repertoire in patients [64]. There is growing interest in identifying T-cell clones in cancer that can be expanded to respond to specific tumor antigens after immunotherapy. Importantly, when combined with tumor cell sequencing and T-cell phenotyping, TCR sequencing can provide comprehensive information for monitoring and predicting immunotherapy response [65].

Response to immunotherapy varies widely between individuals, so there is a dire need to identify predictive biomarkers for immunotherapy response. A potential predictor of this response is a high tumor mutational burden, or the proportion of nonsynonymous mutations in the tumor genome [66]. Tumor mutational burden can be determined by whole-exome sequencing or gene-targeted sequencing. Initially, whole-exome sequencing, by comparing tumor data with matched non-tumor tissue, was preferred; however, gene-targeted sequencing can be more advantageous owing to lower costs and higher

sensitivity. Tumor mutational burden has the potential to play a key role in the immuno-oncology field [67].

NGS can also be used to predict immunotherapy response via microsatellites. MSI is observed in many cancer types and is a potential predictive marker for immunotherapy response. While MSI testing often uses PCR amplification of known repeat regions, MSI status can also be determined using NGS methods such as targeted sequencing using gene panels [68].

Tumors that lack functional DNA repair genes acquire many mutations and are thus more immunogenic and sensitive to immunotherapy. However, not all tumors that lack DNA repair genes respond to immunotherapy. It was recently shown that this variable immunotherapy response can be explained by the extent of MSI. This study suggests the potential of analyzing MSI intensity using NGS techniques as a means to predicting immunotherapy response [69].

Flow Cytometry

Clinicians have recently started to use flow cytometry techniques as flow cytometers have become smaller and more affordable, allowing the rapid analysis of many characteristics of a wide variety of samples, including blood and bone marrow [70]. Flow cytometers take cells in suspension, focus the cells into a stream using a fluidics system, and create liquid droplets that each contain a single cell. Thus, each cell can be analyzed individually. To analyze the cells, flow cytometers use lasers to record single cells' optical and fluorescence properties. While light scattering patterns can indicate the size and internal complexity of cells, fluorescence can be used to analyze many different properties that the researchers are interested in through the use of fluorescence-labeled antibodies, which can stain cell-surface proteins and internal proteins. Samples can be stained with several antibodies at once, so many different properties can be obtained simultaneously. After optical and fluorescent signals are detected, amplification and conversion steps enable data analysis on computers. These data are often visualized using two-dimensional dot plots and histograms.

Flow cytometry can assess the DNA content of cells using dyes that stain DNA. The signal from these DNA-intercalating dyes is directly proportional to the amount of DNA, allowing ploidy and cell cycle kinetics of tumor cells to be determined. DNA analysis can also have prognostic value in several types of cancer [71]. A frequently used application of flow cytometry in the clinic is immunophenotyping, which characterizes cell populations according to the antigens they express either on their surface or intracellularly. Immunophenotyping is used to diagnose and classify lymphoma and leukemia, diagnose immune deficiency disorders, quantify stem cells in the blood, monitor HIV+ patients, and so forth [71].

Immune cells have many different subtypes that express various cell-surface markers. Recent advances allow analysis of many different antigens simultaneously using antibodies that are tagged with different fluorescent colors. This multicolor analysis allows precise gating of cell populations. For instance, one can quantify the proportions of B cells and different subtypes of T cells using sequential gating based on the markers these cells are known to express. In the clinic, CD4+ T cells can be quantified by flow cytometry to monitor the infection stage of HIV+ individuals [71]. However, not all antigens are on the cell surface. Other recent advances in flow cytometry allow staining of intracellular antigens as well, by permeabilization of the samples before staining. This intracellular staining of lymphoid and myeloid differentiation markers can be very useful in leukemia diagnosis [72].

Flow cytometry can also be used to assess the functionality of immune cells. Cell proliferation can be measured using fluorescently labeled antibodies that recognize the thymidine analog 5-bromo-2′-deoxyuridine (BrdU), as proliferating cells incorporate BrdU into their DNA. The cytotoxicity of natural killer cells can be measured using fluorescently labeled target cells; as the target cells are killed by natural killer cells, the amount of fluorescence decreases. Moreover, neutrophil function can be measured by analyzing phagocytosis, which is done by incubating

neutrophils with fluorescently labeled bacteria and then quantifying the neutrophils' fluorescence levels.

Tumor-specific T-cell responses are often studied in immunotherapy patients, as antigen-reactive T cells are crucial for a successful anti-tumor response. Antigen-specific T cells can be detected either directly through their TCRs or by functional assays measuring cytokine secretion, proliferation, cytotoxicity, and so forth. Flow cytometry can be used for direct detection of antigen-specific T cells using fluorescently labeled major histocompatibility complex–peptide complexes, although this direct detection does not give information about cell function. Flow cytometry can also analyze various functionality parameters using in vitro stimulation of cells with peptides or protein lysates. One way to assess T-cell activation is to quantify cytokine secretion. By inhibition of cytokine secretion using chemicals such as brefeldin A, intracellular cytokines can be quantified by flow cytometry as discussed above. Another method of measuring T-cell activation is to quantify cell-surface molecules that are known to be upregulated upon T-cell activation, such as CD69 and CD25. T-cell function can also be studied by measuring their proliferation and cytotoxicity with flow cytometry [73].

Fluorescence-activated cell sorting separates cells according to their characteristics. The analysis of particles by this method is the same as that used for flow cytometry with some additional steps. After the properties of each droplet are determined by the computer, each droplet is charged and deflected in a specific direction based on its properties. For instance, cells that express green fluorescence can be directed into one tube, and cells that express red fluorescence can be directed into another tube. Using multiple colors allows more precise separation of cells based on the markers they express. Although sorting is not yet a common clinical procedure, it has significant clinical potential, as it can allow high-purity isolation of very specific cell types, which can then be cultured and expanded in vitro and reinfused into patients in cell-based therapies. For instance, while chemotherapy can be highly toxic to the hematopoietic compartment, autologous transplant of hematopoietic stem cells sorted by fluorescence-activated cell sorting can increase the survival of cancer patients [74].

Mass cytometry, also known as cytometry by time-of-flight (or CyTOF), is a fusion of flow cytometry and mass spectrometry that allows the simultaneous characterization of over 40 properties of single cells. In mass cytometry experiments, cells are labeled with antibodies of interest. Unlike in flow cytometry, these antibodies are not labeled with fluorescence but with heavy metals. Samples that are labeled with antibodies are charged and deflected in a magnetic field. Their time of flight in the magnetic field is then recorded. Lighter ions deflect more than heavier ions, and the specific heavy-metal probes can be identified using their mass-to-charge ratio. These signals are recorded for each cell, and the quantity of probes in each cell corresponds to the expression levels of the antigen that was labeled with the specific antibody–heavy metal complex. As the signal overlap with different heavy metals is minimal, many parameters can be quantified simultaneously with mass cytometry. In contrast, emission spectra of fluorophores can overlap easily, limiting the number of antigens that can be characterized in a flow cytometry experiment [75].

Furthermore, mass cytometry can identify molecular changes that cause diseases and thus has potential in the clinic for observing disease progression and predicting therapy response [76]. For instance, Yao et al. analyzed inflammatory cells in the airway from patients with cystic fibrosis and asthma patients using mass cytometry and found differences in the frequencies and functions of different immune cell subtypes [77]. In another study, Corneau et al. investigated CD4+ T cells from healthy and HIV+ individuals for activation, differentiation, exhaustion, and cell cycle markers. The researchers concluded that many "resting" cells express cell cycle markers or co-inhibitory receptors, which challenge the current definition of resting T cells in the HIV context [78]. Mass cytometry is often used to characterize immune cells but can also be applied to other cell types from any tissue [79].

Another modified version of flow cytometry is imaging flow cytometry, which captures fluorescence, bright-field, and dark-field images of each cell as it flows through the cytometer. Imaging flow cytometry includes many magnifying objectives, two cameras, and up to 10 fluorescence channels, allowing the measurement of thousands of parameters of a single cell. This method can be used to diagnose leukemia from even unstained blood samples, which would not only make sample preparation in the clinic easier but also allow analysis of samples that are close to their native state. Moreover, imaging flow cytometry can be used to study rare cell types in liquid biopsy and can efficiently identify circulating tumor cells [80].

Other Preclinical and Clinical Diagnostics Techniques in Immunotherapy Research

Besides the major diagnostic procedures we discussed above, here, we briefly present additional preclinical methods and clinical diagnostic techniques and concepts used in immunotherapy research.

PCR is a frequently used, fundamental molecular biological technique that amplifies a DNA region of interest. While NGS uses massive, simultaneous deep sequencing to generate comprehensive genomic information with low cost and fast turnaround time in many clinical genetic diagnostic applications, routine PCR (including reverse transcriptase PCR (RT-PCR)) is still a very sensitive molecular genetic test for cancer diagnosis and has a wide application in cancer clinics. On the other hand, before the explosion of NGS techniques, hybridization-based gene expression microarray (also known as chip assay) technologies, including RNA and DNA microarrays, have been extensively used in cancer diagnosis to evaluate alterations in the expression of large numbers of cancer-related pathway gene sets, in many types of cancer [81–84].

Another well-established molecular technique to examine gene expression patterns is Southern blot (also called Southern blot hybridization), named after Edwin Southern, who developed this technique in the mid-1970 s [85]. In brief, Southern blot detects and locates specific gene sequences using designated labeled DNA probes that hybridize with denatured DNA fragments that have been pre-transferred and immobilized on a supporting membrane from an electrophoresis separation gel.

Like other blotting techniques, Western immunoblotting emerged from the Southern blot and is a semi-quantitative biological technique for detecting protein–protein interaction via a highly specific antibody–antigen binding blot. Western blot has been widely used in biology research since its development in the late 1970s [86, 87]. In addition, Western blot has been used for clinical diagnosis, including the detection of infectious diseases such as HIV, bovine spongiform encephalopathy, feline immunodeficiency, hepatitis B, and hepatitis C as well as autoimmune diseases such as paraneoplastic disease and myositis conditions. Western blot has also been used to identify malignant lymphoma and stomach cancer antigens [88]. Nonetheless, to date, Western blot has had limited clinical diagnostic use in cancer immunotherapy. The labeled probes, detection targets, and applications of five blotting techniques using similar principles are shown in Table 2.1.

Like Western blotting, *enzyme-linked immunosorbent assay* (ELISA) is an antibody-based bioassay with extensive uses, from basic research to clinical diagnostics. However, unlike other antibody-based assays, ELISA is a plate-based, cell-based quantitative bioassay that detects not only proteins but also other protein-binding ligands, including hormones, drugs, small-molecule compounds, and cytokines. As a fast, sensitive quantitative immunoassay, ELISA has been widely used in preclinical and clinical cancer immunotherapy research [89–91].

mRNA-based arrays and sequencing assays are usually carried out to monitor mRNA or gene expression profiles at the transcriptional level and infer protein expression levels. However, in some circumstances, RNA levels are not consistent with protein levels. Thus, direct detection of protein level and activity is desirable. Nowadays,

Table 2.1 Summary of five blotting techniques

Blotting	Labeled probe	Detection targets	Applications
Southern	DNA oligonucleotides complementary to target DNA sequence	DNA	Detection or identification of DNA or gene of interest
Northern	DNA or RNA oligonucleotides complementary to target RNA sequence	RNA	Detection of gene expression pattern or profile
Western	Protein, antibody, or peptide	Protein	Detection of protein expression level and pattern
Eastern	Protein, antibody, or peptide	Protein post-translational modifications	Detection of post-translational modifications such as phosphorylation and glycosylation
Southwestern	DNA oligonucleotides	DNA-binding protein	Detection of DNA–protein interactions

with the advancement of quantitative mass spectrometry techniques, proteomic arrays (also called proteomic profiling) provide more direct protein measurement for discovery of tumor-specific and tumor-associated antigens as predictive diagnostics biomarkers [92–94]. Proteomic arrays also have specific antibody–antigen recognition-based clinical diagnostic applications. As with other array assays, the complex data sets from proteomic arrays are usually recorded and visualized as comprehensive heat maps [95].

Western blotting, IHC, flow cytometry, ELISA, and proteomic arrays are all based on antibody–antigen interaction. In IHC, formalin fixation preserves tissue section morphology and architecture, but antigen retrieval is required to break the cross-link introduced by fixation and unmask antigen sites and therefore may limit antibody usage. IHC assays are multiplexible, but standardizing IHC assays is a challenge. On the other hand, Western blot detects target proteins from cells or tissue extraction, so cell morphology and tissue architecture information are lost, and Western blot is not multiplexible, although target protein data can be semi-quantified or quantified. With recent advances in mass spectrometry, proteomic arrays offer more an effective, global, and direct way to measure, monitor, and identify immune-related proteins in the tumor microenvironment. Proteomic arrays thus play an increasingly important role in the discovery of tumor-specific and tumor-associated antigens and potential drug targets in immunotherapy [96–99].

Preclinical Tumor Models in Immunotherapy Research

In the research and development of new immunotherapeutic drugs, in vivo preclinical data from animal tumor models are critical for evaluation of drug activity, understanding drug action mechanisms, and optimizing drug administration plans before drugs enter clinical trials. Because only a subset of patients respond to immunotherapy, it is critical to develop and establish animal models with functional immune systems and tumors that resemble human cancer as closely as possible for the testing of novel immunotherapeutic treatments. Common techniques for generating animal models used in cancer immunotherapy research and cancer biology include spontaneous tumors, genetic engineering, graft transplantation, and carcinogenesis induced chemically, physically, virally, or by radiation [100–103]. Below, we describe preclinical animal model types and related concepts in immunotherapy.

Immunodeficient and Immunocompetent Mouse Models, Nude Mouse

In general, preclinical animal models can be divided into two categories: immunodeficient and immunocompetent. Immunodeficient models include nude mice, which have a T-cell production deficiency, and severe combined immunodeficiency (SCID) mice, which have defects in both T-cell and B-cell function, but normal natural

killer cell and macrophage function [104, 105]. An even more severely immunodeficient mouse strain, developed by the Jackson Laboratory, is nonobese diabetic/SCID mice. In addition to T-cell and B-cell deficiency, nonobese diabetic/SCID mice also have reduced natural killer cells and reduced mature macrophage populations [106, 107].

A nude mouse, or athymic nude mouse, is a laboratory mouse bearing a spontaneous deletion in the *FOXN1* gene. Phenotypically, nude mice lack body hair (hence their name) and have no functional thymus gland, leading to a defective immune system for production of mature T cells [108–110]. In cancer immunotherapy research, since nude mice are immunodeficient and incapable of rejecting tumor cells or transplants from humans or other species, these mice are often used to grow grafted tissue to test novel therapies.

In immunocompetent models, however, the immune system is preserved or reconstituted. With the success of cancer immunotherapy agents, the development of immunocompetent models is urgently needed to test novel immunotherapeutic agents. There are three major immunocompetent mouse model types, as follows.

Syngeneic tumor models are generated by inoculating allografts (also called homografts) of mouse cancer cell lines into host mice from the same inbred strain to induce and establish a tumor-bearing system. Through the use of syngeneic allografts, immune rejection of transplants can be avoided. Syngeneic tumor models are fully immunocompetent.

In genetically engineered mouse models, the tumor-bearing system is introduced by genetic manipulation techniques, such as transgenic methods, knock-in, or knock-out to develop endogenously arising tumors, genetically mimicking human disease that is caused by gene mutation, deletion, insertion, or other alteration. For instance, the introduction of double deletion of the *Trp53* and *Pten* genes in mice leads to invasive bladder cancer [111].

Carcinogen-induced tumor models develop tumors after carcinogenic induction by chemicals, virus, radiation, physical stress, etc. For instance, Fantini et al. developed a muscle-invasive bladder cancer mouse model induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine, bearing histologic resemblance to human tumor as well as a competent immune system [112].

Xenograft Tumor Models

Xenograft tumor models are generated by inoculating xenograft tumors from a different species into a host animal to establish a tumor-bearing system, including patient-derived xenografts and cell line–derived xenografts.

A translational cancer patient-derived xenograft model is a humanized tumor model, in which human tumor grafts or primary human cancer cells are transplanted to a host animal. Xenograft mouse models of human cancer can be generated heterotopically (usually subcutaneously) or orthotopically; however, orthotopic tumor models, in which the specific tissue site of the tumor remains the same, are preferred.

Immunocompetent humanized xenograft models are of particular value for immuno-oncology research, allowing human tumors to be assessed in a functional immune system. Since graft transplantation requires an immunodeficient recipient as host, the immunocompetency of humanized xenograft models can be achieved by reconstitution of the host immune system via co-engraftment. The transplant types used to generate these preclinical animal models are summarized in Table 2.2.

Common Translational Research Techniques and their Biospecimen Requirements

Biospecimen types used in various translational tests are summarized in Table 2.3 [113].

Table 2.2 Transplant types for generating preclinical animal models

Transplant type	Description
Autograft	The transplantation of cells, tissues, or organs from one part of the body to another in the same individual
Allograft/ Homograft	The transplantation of cells, tissues, or organs, to a recipient from a genetically non-identical donor of the same species
Xenograft	The transplantation of cells, tissues, or organs, to a recipient from a different species

Table 2.3 Summary of biospecimens used in common translational research techniques

Common translational tests		Biospecimen
Nucleotide-based assays	DNA-sequencing, RNA sequencing	DNA/RNA extracted from any type of tissue, liquid biopsy
	Cell-free DNA sequencing	Total cell-free DNA isolated from plasma, cell-free samples
	TCR sequencing	DNA/RNA extracted from any type of tissue, liquid biopsy
	Southern blot	DNA extracted from any type of tissue, liquid biopsy
	Northern blot	RNA extracted from any type of tissue, liquid biopsy
	Microarray	DNA/RNA extracted from any type of tissue, liquid biopsy
Antibody-based assays	Western blot	Homogenized tissue, liquid biopsy
	Proteomic array	Homogenized tissue, liquid biopsy
	IHC	Tissue specimen (FFPE, FF, FNA, etc. section)
	Flow cytometry	Any tissue specimen, liquid biopsy

NGS next-generation sequencing, *TCR* T-cell receptor, *IHC* immunohistochemistry, *FFPE* formalin-fixed paraffin-embedded, *FF* fresh-frozen; fine-needle aspirated

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Immunotherapy for Melanoma

3

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Abstract

While melanoma is less common than some other skin cancers, it is responsible for nearly 10,000 deaths in the USA each year alone. For many decades, very limited treatment options were available for patients with metastatic melanoma. However, recent breakthroughs have brought new hopes for patients and providers. While targeted therapy with BRAF and MEK inhibitors represents an important cornerstone in the treatment of metastatic melanoma, this chapter carefully reviews the past and current therapy options available, with a significant focus on immunotherapy-based approaches. In addition, we provide an overview of the results of recent advances in the adjuvant setting for patients with resected stage III and stage IV melanoma, as well as in patients with melanoma brain metastases. Finally, we provide a quick overview over the current research efforts in the field of immuno-oncology and melanoma.

Keywords

Melanoma · Immunotherapy · Ipilimumab · Pembrolizumab · Nivolumab · CTLA-4 · PD-1 · PD-L1 · Adjuvant therapy · Brain metastasis

Introduction

Melanoma represents the malignant transformation and proliferation of melanocytes, which are primarily found in the skin, but can also be identified in the uvea, gastrointestinal mucosa, genitourinary mucosa, as well as meninges/CNS [1]. While it only comprises about 1% of all skin cancer cases, it is accountable for the majority of all deaths in this group [2]. Furthermore, the annual incidence has been increasing worldwide [3]. While some of the rise may be caused by increased skin cancer awareness and earlier detection, sun-related behaviors such as indoor tanning have been contributing to the incidence [4]. Based on data from the American Cancer Society, 96,480 new cases of melanoma were diagnosed in 2019 in the United States alone, with 7230 people expected to die of the disease [5]. Melanoma can affect anyone; but risk factors like fair skin, exposure to ultraviolet radiation

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(sun exposure, tanning beds), history of blistering sunburns in early age, dysplastic or atypical nevi, 50 or more of small nevi, and familial dysplastic nevus syndrome increase the likelihood of melanoma [3, 6]. It is important to note that although melanoma can be associated with preexisting nevi, about 70% of cases can develop de novo (i.e., not from a preexisting pigmented lesion) [4]. Prognosis is related to many factors; and late stage, depth (thicker than 4 mm), advanced age, male sex, location (chest and back), and ulceration are associated with poorer prognosis [7, 8]. The survival rate depends primarily on the stage, with 98% 5-year survival for stages I and II, 64% for stage III, and it decreases to 23% for stage lV [2, 3].

Treatment for early stage melanoma is surgery, and is highly curable. Based on thickness of the primary melanoma and presence of ulceration, initial surgery might include sentinel node biopsy for staging. For patients with advanced and nonresectable disease, systemic therapy most often represents the backbone of therapy. Encouraging, we have seen a significant change in the treatment landscape for metastatic melanoma since 2011, changing the outcomes in a substantial number of patients.

While this chapter focuses primarily on immunotherapy, with a concise summary of its past, present, and anticipated future use, it should be mentioned that we have also seen tremendous results with the use of targeted therapies in melanoma. RAS/RAF/MAPK signaling pathway is known to be involved in melanoma transformation [9, 10]. BRAF mutations are observed in up to 50% of cutaneous melanoma and in 10–20% of mucosal melanomas [11]. Combinations of BRAF and MEK inhibitors (inhibiting the RAS/RAF/MAPK pathway) are very effective in *BRAF* mutated melanoma (Table 3.1).

As the use of both targeted and immunotherapies has extended, it only makes sense that combinations of BRAF/MEK inhibitors and immunotherapy are currently in clinical testing. (e.g., NCT01754376, NCT02902029, NCT03235245, NCT02631447, NCT02968303, NCT02910700, NCT03554083, NCT02908672, NCT02224781, NCT02967692, NCT01683188, NCT02967692, NCT02027961, NCT02130466, and NCT03178851).

Short Overview of the History of Melanoma Treatment Options up to 2011

High-Dose Interleukin-2

Interleukin-2 (IL-2) is a T cell growth factor, which stimulates T cell proliferation and cytotoxic activity [15]. It was the first immunotherapy to receive regulatory approval in 1998 for the treatment of metastatic melanoma, based on durable objective responses observed in these patients.

In a pooled analysis of 270 melanoma patients treated with high-dose IL-2 (HD IL-2) between 1985 and 1993, the overall objective response rate (ORR) was 16% [with complete response (CR) 6%, and partial response (PR) 10%] [16].

Table 3.1 Selection of pivotal phase III trails that assessed BRAF and MEK inhibitors combinations in BRAF mutated melanoma

Reference	Phase	#Patients enrolled	Studied combination	Control	ORR (combi vs. single)	Median PFS in months	Median OS in months
Long et al. [12]	III (COMBI-d trial)	423	Dabrafenib + Trametinib	Dabrafenib + placebo	69% vs. 53%	11.0 vs. 8.8	25.1 vs. 18.7
Larkin et al. [13]	III (coBRIM trial)	495	Vemurafenib + Cobimetinib	Vemurafenib	68% vs. 45%	9.9 vs. 6.2	22.3 vs. 17.4
Dummer et al. [14]	III (COLUMBUS trial)	577	Encorafenib + Binimetinib	Vemurafenib	63% vs. 40%	14.9 vs. 7.3	33.6 vs. 16.9
			Encorafenib + Binimetinib	Encorafenib	63% vs. 51%	14.9 vs. 9.6	NR

Difference was not significant. HR 0.75 (85% CI 0.56–1.00); two-sided $p = 0.051$.
NR not reported

Importantly, in patients with an ongoing response at 30-month mark no progression was noted, supporting the proof of concept that immunotherapy can lead to long-term responses.

A retrospective chart review of 45 renal cell and 245 melanoma patients treated with HD IL-2 showed median overall survival (OS) of 16.8 months [17]. For patients who experienced a favorable response to treatment, median OS had not been reached, and for patients with stable disease (SD), the median OS was 38.2 months, compared to patients with progressive disease (PD) who had a median survival of 7.9 months. In patients who achieved a PR or CR, the 3-year OS was 78%, confirming the durability of responses.

However, the significant toxicities observed with HD IL-2 require intensive monitoring and limit its use to specialized centers [18]. The majority of the major side effects, such as hypotension, renal impairment, shortness of breath, pulmonary and generalized edema, as well as neuropsychiatric alterations are thought to be caused by capillary leak syndrome and lymphoid infiltration, but toxicities typically resolve after discontinuation of treatment.

Nowadays, while its use has significantly decreased, HD IL-2 is still being used in numerous adoptive cell protocols (see next sections).

Chemotherapy

While chemotherapy rarely ever led to durable responses, it was the only option available for numerous patients until 2011. Various agents have been tested in melanoma in phase II and phase III trials, with an overview of an extract of the clinical data provided in Table 3.2.

Biochemotherapy (BCT) consists of the chemotherapy triplet CVD, as well as HD IL-2 and interferon. The efficacy of this regimen compared to CVD was evaluated in a phase III trial [33]. Response rates were only numerically higher for BCT (CVD, n = 195; BCT, n = 200; 19.5% vs. 13.8%, p = 0.140) and median progression-free survival (PFS) was significantly longer for BCT than for CVD (4.8 vs. 2.9 months; $P = 0.015$), but it should be mentioned that the improved PFS did not translate into longer OS (9.0 vs. 8.7 months). In addition, grade 3 and 4 toxicities were more commonly observed with BCT regimen (95% vs. 73%; $p = 0.001$).

While chemotherapy is nowadays rarely used in front line, multiple trials are ongoing to explore the efficacy of chemotherapy agents in combination with immunotherapy (Table 3.3).

Finally, melphalan has been used for decades as part of isolated limb infusion (ILI) protocol for patients with localized in-transit metastases [34]. While its use has significantly diminished in the era of new effective targeted and immunotherapy, it should be pointed out that melphalan-based ILP (M-ILP) had high ORR of 75% and CR of 45% [35].

Adoptive Cell Therapy (ACT)

Adoptive cell therapy represents a patient-tailored therapeutic approach, using autologous derived T cells, which typically are derived from the tumor (TILs, tumor-infiltrating lymphocytes), or via pheresis. While this approach has been used for decades, its use has been limited by the need for specialized laboratories as well as the need for hospital units able to manage the toxicities from HD IL-2, which is most commonly administered in conjunction with the T cell product [36]. In addition, most patients are undergoing lymphodepletion (fludarabine and cyclophosphamide) prior to ACT, with its use dating back to 1994 [37]. The ORR was 34% for all patients (N = 86), and side effects stemmed mainly from the HD IL-2. Another clinical trial reported an ORR of 51% (9% CR) in 35 patients with metastatic melanoma. Mean duration of response was 11.5 ± 2.2 months [38]. Since then, different approaches have been developed and tested to improve efficacy and toxicity profile of adoptive cell therapy, including CAR T cell therapy (NCT03893019) as well as modified/transduced T cells (NCT01955460, NCT03060356) [39, 40].

Table 3.2 Selected Phase II and III chemotherapy clinical trials in melanoma

Reference	Patients (*n*)	Agent	Comparator	Phase	Line of therapy	ORR	Median PFS	Median OS	AEs
Hill et al. and Serrone et al. [19, 20]	62	Dacarbazine (DTIC)	No treatment	III	First line	8–20%	4–6 months	NR	Myelosuppression, mild nausea, vomiting, minimal alopecia, and fatigue [21, 22]
Middleton et al. [23]	305	Temozolomide (TMZ), an oral analog of DTIC	DTIC	III	First line	13.5% vs. 12.1%	1.9 vs. 1.5 months; HR: 1.37; $P = 0.012$	7.7 vs. 6.4 months; HR: 1.18; $P = 0.20$	No major difference in drug safety was observed [24, 25]
Bedikian et al. [26]	393	DHA-paclitaxel (DHA-P)	DTIC	III	First line	5.2% vs. 5.5%	TTP 48 days for both groups	267 vs. 226 days	In DHA-P, 73.6% had grade ≥3 adverse events including neutropenia compared to 34.9% in the DTIC arm.
Hersh et al. [27]	529	Nab-paclitaxel	DTIC	III	First line	15% vs. 11% (NS)	4.8 vs. 2.5 months; HR: 0.792; $P = 0.044$	12.6 vs. 10.5 months; HR: 0.897; $P = 0.271$	Grade ≥3: neuropathy (25%) and neutropenia (20%).
Hodi et al. and Rao et al. [28, 29]	17	Paclitaxel plus carboplatin	N/A	II	Treated once but no previous platinum or taxane	20%	NR	9 months	Grade ≥3: granulocytopenia (17%), thrombocytopenia (9%).
Kottschade et al. [30]	76	Nab-paclitaxel and carboplatin	N/A	II	Treatment-naïve (CN) and previously treated (PT)	25.6% (CN) 8.8% (PT)	4.5 months (CN), 4.1 months (PT)	11.1 months	Grade ≥3: neutropenia (28–41%), thrombocytopenia, neurosensory problems, fatigue, nausea, and vomiting (<10%).
Keith et al. [31]	823	Carboplatin plus paclitaxel with sorafenib (CPS)	Carboplatin plus paclitaxel (CP)	III	First line	20% vs. 18% (NS)	4.9 vs. 4.2 months	11.3 vs. 11.1 months	Grade ≥3: 84% vs. 78%; $P = 0.027$. Increased rash, hand-foot syndrome, and thrombocytopenia accounted for most of the difference.
Legha et al. [32]	52	Cisplatin, vinblastine, and dacarbazine (CVD)	N/A	II		40%		9 months	Nausea, vomiting, diarrhea, partial hair loss, neutropenia, and significant anemia required blood transfusions in a majority of the patients after three to four courses of chemotherapy. The dose-limiting toxicity was peripheral neuropathy.

AEs adverse events, *NS* difference was not statistically significant, *NR* not reported, *N/A* not applicable

Table 3.3 Examples of recent trials combining checkpoint inhibitors with chemotherapy. Patients must have either unresectable stage III or stage IV metastatic melanoma

Trial	Phase	Primary outcome	Estimated enrollment	Chemotherapy	Immunotherapy	Line of therapy
NCT02617849	II	ORR	44	Carboplatin/ paclitaxel	Pembrolizumab	First-line
NCT01827111	II	PFS	21	Abraxane	Ipilimumab	First-line
NCT01676649	II	Safety	30	Carboplatin and paclitaxel	Ipilimumab	First-line

Immune Checkpoint Inhibitors

The development of checkpoint inhibitors (CPIs) has revolutionized the treatment in metastatic melanoma, and these agents are now successfully used in various other cancer types. However, research to understand the mechanisms of T cell signal transduction and regulation was initiated decades ago [41]. The cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) was first described in 1987 and competes with CD28 to bind to CD80 (B7-1) and CD86 (B7-2) [42]. By binding, CTLA-4 downregulates pathways of T cell activation by competitively binding to B7 proteins (required for stimulation of T cells). Recently, it also has been shown that anti-CTLA-4 induces the expansion of an $ICOS^+$ Th1-like CD4 effector population, which means it engages a different cellular pathway than the Programmed cell death protein 1 (PD-1) antibody. Th1-like CD4 expansion leads to the expansion of specific tumor-infiltrating exhausted-like CD8 T cell subsets [43]. Similar to CTLA-4, PD-1 negatively regulates the antitumor response. Based on their different mode of action compared to chemotherapy or targeted therapy, CPIs also can cause a different set of side effects, commonly referred to as immune-related adverse events (irAEs). Early recognition and management are essential to expedite resolution of symptoms, and irAEs may affect any organ at any time [44]. CPIs can also lead to delayed toxicities occurring weeks or months after discontinuation of therapy [45]. Additionally, the combination of two CPIs results typically in greater risk of and also earlier onset for clinically significant irAEs [46].

Anti-CTLA-4: Ipilimumab

Ipilimumab is a fully human, monoclonal IgG1 antibody that inhibits CTLA-4. Ipilimumab was initially approved in 2011 by the FDA for the treatment of unresectable metastatic melanoma. In a randomized, double-blind, phase III study, 676 patients were treated with either ipilimumab plus gp100 peptide vaccine, gp100 alone, or ipilimumab alone [47]. The OS in the combination arm and single ipilmumab was significantly longer (10.0 months). Ipilimumab as single agent resulted in a relative risk (RR) of 10.9%, with a disease control rate of 28.5%.

In another phase III trial, 502 untreated metastatic melanoma patients were randomly assigned to either ipilimumab (10 mg/kg) plus DTIC (850 mg/m^2) versus DTIC plus placebo ($n = 252$) [48]. The response rate (CR + PR) was 15.2% in patients who received ipilimumab/DTIC combination versus 10.3% in the DTIC/placebo group ($p = 0.09$). Addition of Ipilimumab led to a significantly longer median OS, as survival was 11.2 months and 9.1 months for the DTIC group (HR for death with ipilimumab/DTIC 0.72; $p < 0.001$). The combination therapy resulted in more grade III and IV toxicities (56.3% vs. 27.5%), with the most common grade 4 toxicity being elevation in liver enzymes.

Furthermore, ipilimumab is currently being tested in various combinations, including chemotherapy, radiotherapy, vaccines, cytokines, and other CPIs (NCT02644967, NCT02259231, NCT02307149, NCT02203604, NCT02073123, NCT01940809, NCT03297463) [49].

Anti-PD-1

Programmed cell death protein 1 or PD-1 is a negative regulator of T cell activity and is expressed by T cells with excessive exposure to antigens. Its primary ligand, PD-L1, is frequently expressed throughout cancerous cells and TILs [50]. The other ligand, PD-L2, is expressed mainly by antigen-presenting cells (APCs). Both ligands are members of B7 protein family. An association between overexpression of PD-1 and PD-L1 on tumor cells and TILs and disease outcomes has been observed in some tumor types [51].

Nivolumab

Nivolumab is a fully human immunoglobulin IgG4 monoclonal antibody directed against PD-1 and was granted regulatory approval in 2014 for the treatment of metastatic melanoma. In CheckMate-066, a phase III randomized double-blind study, 418 previously untreated patients with metastatic melanoma without a BRAF mutation were randomly assigned to receive either nivolumab (3 mg/kg) and DTIC-matched placebo or DTIC (1000 mg/m^2) with nivolumab-matched placebo [52]. The ORR was 40% (95% CI, 33.3–47.0) in anti-PD-1-treated patients, with over 7% achieving a CR versus 13.9% overall response (95% CI, 9.5–19.4) and only 1% CR in the DTIC group. Very encouraging was also the 1-year OS for the nivolumab group which was 72.9% as compared to 42.1% in the DTIC group. Nivolumab also compared favorably to dacarbazine in regard to Grade 3 and 4 adverse events.

Pembrolizumab

Pembrolizumab is also a fully humanized IgG4 antibody directed against PD-1 receptor that has regulatory approval since 2014. In KEYNOTE-002, a multicenter phase II study, 540 previously treated patients were randomly assigned (in a ratio of 1:1:1) to receive pembrolizumab 2 mg/kg ($n = 180$), pembrolizumab 10 mg/kg ($n = 181$) given IV every 3 weeks, or investigator-choice chemotherapy ($n = 179$) [53]. Progression-free survival was improved in patients assigned to pembrolizumab 2 mg/kg and those assigned to pembrolizumab 10 mg/kg compared with those assigned to chemotherapy.

In KEYNOTE-006, a phase III study, 834 metastatic melanoma patients were randomized (1:1:1 ratio) to receive either pembrolizumab (10 mg/kg every 2 weeks or every 3 weeks) or four doses of ipilimumab (3 mg/kg every 3 weeks) [54]. The majority of the patients were treatment-naïve. Both pembrolizumab arms yielded higher response rate (33.7% for every 2 weeks, 32.9% for every 3 weeks) ($P < 0.001$ vs. ipilimumab) and 11.9% for ipilimumab. Six-month PFS was nearly 47% for pembrolizumab in both groups versus 26.5% for ipilimumab. In addition to improving PFS, 12-month OS was 74.1% for pembrolizumab every 2 weeks, 68.4% for pemrolizumab every 3 weeks as compared to 58.2% for ipilimumab. Endocrine events related to thyroid were more frequently observed in the pembrolizumab groups, whereas colitis and hypophysitis were more frequent in the ipilimumab group. In general, pembrolizumab has a similar toxicity profile as nivolumab, with both anti-PD1 agents exhibiting a favorable toxicity profile with fewer high-grade AEs than ipilimumab.

Ipilimumab and Nivolumab in Combination

Based on the outcomes of melanoma patients treated with either CTLA-4 or PD-1 CPI monotherapy and a better understanding of the mechanism involved in the activation of T cells, the combination of ipilimumab and nivolumab was evaluated. CheckMate-069 was a double-blinded phase ll study, randomly assigned (in a 2:1 ratio) to 142 previously untreated patients with metastatic melanoma to receive ipilimumab 3 mg/kg combined with either nivolumab 1 mg/kg or placebo, once every 3 weeks for four doses, followed by nivolumab 3 mg/kg or placebo every 2 weeks [55]. The ORR for the combination therapy was 56%, with 22% of patients achieving a CR. Similar to prior reports, the RR for patients with ipilimumab was only 11% ($p < 0{\cdot}0001$ compared to nivolumab) and no patient had a CR. At median follow-up of 24.5 months, median PFS had not been reached for the ipilimumab/nivolumab group and was 3.0 months (95% CI 2·7–5·1) in the CTLA-4 only group (HR 0.36,

95% CI 0.22–0.56; $p < 0.0001$). In the combination group, 49% of patients discontinued study drug due to toxicities, compared to 22% in the ipilimumab group, and grade 3 or 4 adverse events were reported in 54% of the patients who received the ipilimumab/nivolumab versus 24% of the patients who received ipilimumab monotherapy respectively.

A larger, randomized, double-blind, phase III study (CheckMate-067), compared nivolumab (3 mg/kg) alone or nivolumab (1 mg/kg) every 3 weeks plus ipilimumab (3 mg/kg) for a maximum of three doses, followed by 3 mg/kg of nivolumab every 2 weeks with ipilimumab (3 mg/kg) alone in patients with metastatic melanoma [56]. A total of 945 previously untreated patients were assigned to the treatment arms in a 1:1:1 ratio. Overall response rates ranged from 19% (2.2% CR) in the ipilimumab group to 43.7% (8.9% CR) in the nivolumab group to 57.6% (11.5%) in the nivolumab/ipilimumab combination group. PFS was significantly longer in the combination group (11.5 months) compared to the ipilimumab group (2.9 months) and the nivolumab group (6.9 months). Subgroup analysis showed that patient with high baseline lactate dehydrogenase, low baseline tumor PD-L1 expression, or a BRAF mutation might benefit from the combination over monotherapy. As expected, more treatment-related grade 3 and 4 adverse events were observed in the combination group (55.0%) compared to either single agent group [nivolumab group (16.3%) or ipilimumab group (27.3%)].

Given the demonstrated efficacy but higher incidence of treatment-related AEs, a different dosing schedule of nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (NIVO3 + IPI1) was recently studied. This phase IIIb/IV (CheckMate-511) study met its primary end point, demonstrating a significantly lower incidence of treatment-related grade ≥3 adverse events of 34% with NIVO3 + IPI1 versus 48% with NIVO1 + IPI3 ($P = 0.006$). In descriptive analyses, objective response rate was 45.6% in the NIVO3 + IPI1 group and 50.6% in the NIVO1 + IPI3 group. Median PFS was 9.9 months in the NIVO3 + IPI1 group and 8.9 months in the NIVO1 + IPI3 group. Median OS was not reached in either group. It should be emphasized that this study was not designed to formally demonstrate noninferiority of NIVO3 + IPI1 to NIVO1 + IPI3 for efficacy end points [44].

Ipilimumab and Pembrolizumab in Combination

Pembrolizumab was also tested in combination with dose reduced ipilimumab (1 mg/kg) in the KEYNOTE-029, a phase 1b trial [57]. Prior targeted therapy or chemotherapy was allowed, but 87% of patients were treatment-naïve. Patients ($n = 153$) were treated with the combination of IV regular dose pembrolizumab (2 mg/kg) and ipilimumab, followed by pembrolizumab (2 mg/kg) maintenance therapy. ORR was 61%, with 15% CR, and with estimated 1-year PFS of 69%, and estimated 1-year OS of 89%, grade 3 and 4 adverse events occurred in 45% of patients.

Anti-PD-L1

Antibodies directed at PD-L1, and therefore blocking PD-L1 from binding its receptors PD-1 and B7-1, have also been tested in metastatic melanoma patients [58]. While these agents have shown efficacy in the treatment of metastatic melanoma, none of the currently three available PD-L1 agents (atezolizumab [59], avelumab [60], and durvalumab [61]) have been approved for the treatment of metastatic melanoma, with multiple combination trials with PD-L1 inhibitors still ongoing (NCT02535078, NCT02639026, NCT03273153, NCT03178851).

Vaccination and Intratumoral Approaches

Multiple vaccination and intratumoral approaches have been tested for the treatment for advanced melanoma. The vaccines aim to elicit immune response against antigens expressed by melanoma tumor cells, such as tumor-associated antigens (TAAs) or mutation-derived antigens (neoantigens). Various TAAs have been identi-

fied such as melanoma antigen A1 (MAGE-A1), gp100, or melanoma antigen recognized by T cells (MART-1/Melan-A) [62]. However, as single agents the results have been underwhelming, and combinatorial approaches might be more promising. For example, gp100, a synthetic polypeptide found to carry immunogenic epitopes that can be recognized by T cell lymphocytes to induce antitumor activity, was tested in combination with HD IL-2 [63]. In this phase III trial, a total of 185 metastatic melanoma patients (prior chemotherapy, interferon, and low-dose IL-2 were allowed) were randomized to receive either HD IL-2 alone or HD IL-2 with GP100. The response rate was 10% among patients who received HD IL-2 alone and 20% among patients receiving the combination ($P = 0.05$). The median OS was 11.1 months among patients receiving HD IL-2 alone and 17.8 months among patients receiving combination therapy ($P = 0.06$). The toxicities were similar in both treatment groups; however, arrhythmias, metabolic changes, and neurologic events were more likely among patients in the vaccine/HD IL-2 group than among patients in the HD IL-2 only group.

PV-10 (Rose Bengal)

Rose Bengal (RB) is a water-soluble injectable iodinated fluorescein derivative. After intralesional injection, PV-10 accumulates in tumor lysosomes resulting in rapid lysis of tumor cells and is able to produce cytotoxic reactive oxygen species when exposed to ionizing radiation [64]. PV-10 may also stimulate an antitumor immune response against distant lesions. In a phase II study, 80 patients with refractory stage III and IV melanoma received intralesional PV-10, which resulted in the best ORR of 51% (CR in 26%) and 8% of patients still had no evidence of recurrence after 52 weeks [65]. Importantly, noninjected lesions also showed regression. Toxicity profile was favorable, with no treatment-related grade 4 adverse event. The most recently published prospective phase II trial reported an ORR of 87% (42% CR) in the 45 treated patients [66]. Complete responses were associated with having less than 15 metastases at time of PV-10 injection. PV-10 is currently not FDA approved for the treatment of metastatic melanoma, and clinical trials are ongoing to evaluate its safety and efficacy in combination with CPIs (NCT02557321).

T-VEC

Talimogene Laherparepvec (T-VEC), a genetically modified herpes simplex virus (HSV) type 1, is currently the only intralesional oncolytic virotherapy with regulatory approval in 2015 for melanoma. It exerts its effect on regional and systemic antitumor immunity by selective intratumoral replication and expression of GM-CSF (granulocyte macrophage colony-stimulating factor) within the infected melanoma cells [67]. The approval was based on a randomized phase III trial in 436 patients with unresectable stage III or IV melanoma [68]. Patients were randomly assigned at a two-to-one ratio to intratumoral T-VEC or subcutaneous GM-CSF. The ORR for T-VEC were higher (26.4% vs. 5.7%) and more durable responses were observed with T-VEC compared with GM-CSF (16.3% vs. 2.1%) ($p < 0.001$). Median OS was numerically longer with T-VEC than with GM-CSF (23.3 months vs. 18.9 months), but failed to reach statistical significance ($P = 0.051$). T-VEC injections were well tolerated, and other grade 1 and 2 toxicities included were fatigue, chills, pyrexia, nausea, flu-like illness, reaction at injection site, and vomiting. Incidence of grade 3 and 4 adverse effects was considerably low (11% vs. 5% for GM-CSF).

T-VEC has also shown efficacy in combination with CPIs. In a phase Ib trial of T-VEC in combination with ipilimumab in 19 previously untreated melanoma patients (prior adjuvant therapy ≥6 months from last therapy was allowed) [69]. The ORR was 50%; durable responses were seen in 44% of patients lasting ≥6 months. With a median follow-up time of 20 months (1.0–25.4 months), PFS was 50% and OS 67% at 18 months. No unexpected toxicities were observed. In MASTERKEY-265, a phase Ib study, 21 advanced melanoma patients with no prior systemic treatment received T-VEC (in day

1, day 22 then every 2 weeks), and pembrolizumab (200 mg) on day 36 and then every 2 weeks [70]. Confirmed RR was 62% with a CR rate of 33%, and responses were seen in 43% of noninjected nonvisceral, and 33% of noninjected lesions. At time of the report, median PFS and OS had not been reached. No unexpected adverse events were noted. Multiple clinical trials are currently ongoing, and investigating the efficacy of T-VEC in combination with other CPIs, targeted therapy as well as radiation (NCT02263508, NCT03088176, NCT02819843, NCT02965716).

Brain Metastases and Immunotherapy

Clinical and autopsy data show that a significant number of patients with metastatic melanoma will develop brain metastases (MBM) during their course of disease [71]. However, recent advances in immunotherapy and targeted therapy are improving the outcomes for these patients [72]. A phase II study using pembrolizumab (10 mg/kg) in patients with melanoma with one or more asymptomatic, untreated 5- to 20-mm brain metastases not requiring corticosteroids was recently published [73]. Of the 23 patients enrolled, six patients (26%) had an objective response (two PRs and four CRs), one patient had SD, eight patients (35%) had PD, and eight patients (35%) were unevaluable due to progression or need for radiation. The median PFS time was 2 months (95% CI, 2 months to not reached), with a median OS time of 17 months (95% CI, 10 months to not reached). Importantly, all responses were durable and all six intracranial responses (100%) were ongoing at 24 months [74].

Importantly, two recent studies have shown that in patients with untreated MBM, the combination of ipilimumab and nivolumab can yield intracranial response rates similar to extracranial response rates as observed in CheckMate-067 [56]. CheckMate-204 enrolled 94 MBM patients, using standard dosing of up to four doses of ipilimumab (3 mg/kg) plus nivolumab (1 mg/kg) followed by nivolumab (3 mg/kg) every 2 weeks until progression or unacceptable toxicities [75]. At median follow-up of 14.0 months, the intracranial clinical benefit rate was 57% (CR 26% and PR 30%), with a similar extracranial clinical benefit rate of 56% [(95% CI, 46–67)]. Treatment-related grade 3 or 4 adverse events were reported in 55% of patients, with the overall safety profile similar to CheckMate-067 [76]. Importantly, central nervous system-specific grade 3 or grade 4 adverse events were seen in only 7%. The second phase II trial led by the Australian group (ABC trial) randomized 79 patients with MBM to receive either combination therapy with ipilimumab (3 mg/kg) plus nivolumab (1 mg/kg) for four doses then nivolumab 3 mg/kg every 2 weeks (cohort A, $n = 36$), or to receive single agent nivolumab (3 mg/kg) (cohort B, $n = 27$). Patients who were symptomatic or had leptomeningeal disease (LMD) were treated in nonrandomized fashion with single agent nivolumab (3 mg/kg) (cohort C, $n = 16$). Compared to CheckMate-204, these patients had a higher number of brain metastases and allowed patients with LMD on trial. With a median follow-up of 17 months, intracranial responses were achieved by 16 (46%) of 35 patients in cohort A, five (20%) of 25 in cohort B, and one (6%) of 16 in cohort C. Complete responses occurred in six (17%) patients in cohort A, three (12%) in cohort B, but none in cohort C. Grade 3 or 4 treatment-related adverse events occurred in 19 (54%) patients in cohort A, four (16%) in cohort B, and two (13%) in cohort C.

As patients with MBM still have an unmet need, multiple clinical trials are currently ongoing, including for symptomatic patients requiring corticosteroids. Examples of ongoing combination studies include bevacizumab with CPIs (NCT03175432, NCT02681549), chemotherapy with ipilimumab (NCT02460068), radiotherapy + CPI (NCT02716948), and targeted therapy + CPI (NCT02910700). Furthermore, patients with involvement of the leptomeninges have the worst prognosis of all patients with melanoma, and multiple approaches using CPI either intrathecally or intravenously, as well as with or without the addition of radiation are currently under investigation (NCT02939300, NCT03719768, NCT03025256).

Adjuvant Therapies

The goal of systemic adjuvant therapy is to decrease the risk for high-risk melanomas to recur after surgery. Traditionally, this approach has focused mainly on patients with stage III disease, which is defined as the presence of lymph-node and/or in-transit metastasis. Furthermore, higher number of involved lymph nodes, deeper invasion of the primary tumor, higher mitotic rate as well as the presence of ulceration in the primary tumor are all associated with worse outcomes [77]. Stage III disease is associated with heterogeneous outcomes. Therefore, it has been redefined into four subgroups in the eighth edition of the American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma. The 5-year melanoma-specific survival rates range from 93% for stage IIIA disease to 32% for stage IIID disease (thickness >0.4 mm with ulceration and >4 involved lymph nodes) [78]. Adjuvant therapy remains an important area of research as immediate complete lymph-node dissection is now frequently omitted due to lack of improved melanoma-specific survival [79]. Furthermore, anti-PD-1 agents are now being tested in the adjuvant setting for patients with high-risk stage II disease (NCT03553836).

Adjuvant Therapy with Interferon

Interferon is now rarely used in the adjuvant setting for patients with either stage II or stage III melanoma. Initial trials showed and improved recurrence-free and overall survival benefit for treatment with high-dose interferon alpha-2 (HD INF-α) compared to observation [80–82]. However, at a median follow-up of 12.1 years, the OS benefit was no longer observed [80]. Pooled analysis of two ECOG trials (E1684 and E1690) showed indeed a benefit in relapse-free survival (RFS), but no OS survival benefit from HD INF-α [83]. Another pooled analysis showed that increased benefit was observed in patients with ulcerated primary melanomas [84]. Worth mentioning, 50% and 47% of patients had grade 3/4 toxicity in the induction and consolidation phase, respectively [85]. Pegylated interferon (longer half-life, less injections per week) has slightly more favorable side-effect profile compared to HD INF-α. However, while showing improvement in RFS similar to HD INF-α, there was no improvement in OS, and the positive impact on RFS appeared to decrease over time [86]. As its use completely stalled, peginterferon alfa-2b (Sylatron) was discontinued in December 2019 [87].

Adjuvant Biochemotherapy

In an effort to increase the efficacy of adjuvant therapy, a shorter course of biochemotherapy (up to three cycles) was compared to standard HD-INF-α monotherapy [88]. Southwest Oncology Group (SWOG) S0008 was a phase III study that enrolled 402 patients who had undergone complete lymph-node resection for stage III melanoma. Patients were randomly assigned to either biochemotherapy (CVD as previously described, IL-2 at 9 MU/m^2 administered as a 96-hour continuous IV infusion on days 1 through 4, and INF at 5 MU/m^2 administered on days 1 through 5; treatment was repeated every 21 days for a total of three cycles), or to HD INF-α (20 MU/m^2 IV per day for 5 days for 4 weeks, followed by 10 MU/m^2 subcutaneously three times per week for 48 weeks) monotherapy [88]. In the HD-INF-α group, 43% of patients were able to complete therapy as planned, whereas in the biochemotherapy group, 80% of patients were able to receive all three treatment cycles ($p < 0.001$). With a median follow-up of 7.2 years, the median PFS was 4.0 years versus 1.9 years for biochemotherapy and HD-INF-α, respectively ($p = 0.029$). The 5-year RFS was 48% versus 39%, respectively. No statistically significant difference was found between the two groups but a trend toward favoring biochemical group was reported. As expected, both treatment groups experienced different toxicities, however, none unexpected.

CPIs in the Adjuvant Setting

The improvement of overall survival and durable responses that were observed with CPI in unresectable advanced melanoma patients led to study its efficacy in the adjuvant therapy.

EORTC 18071 was a phase III double-blind randomized study comparing high-dose ipilimumab (10 mg/kg every 3 weeks for four doses, then every 3 months for up to 3 years) to placebo in patients with fully resected stage III melanoma who had not received any other prior systemic therapy. At a median follow-up of 2.74 years, median RFS in the ipilimumab group was higher (26.1 months) than in the placebo group (17.1 months, $p = 0.0013$) [89]. As expected, toxicities in the treatment group were significant, grade ≥3 gastrointestinal 16%, hepatic 11%, and endocrine 8%. It should be noted that five (1%) participants died due to irAEs. A recent update at a median follow-up of 5.3 years, the 5-year OS was 65.4% in the ipilimumab group, as compared with 54.4% in the placebo group [90].

In a randomized double-blind phase III trial (CheckMate-238), 906 patients with complete resection of stage IIIB, IIIC, or IV melanoma were randomized to receive either ipilimumab (10 mg/kg) or nivolumab (3 mg/kg), with the primary end point of RFS [91]. The 12-month RFS was remarkably higher in the nivolumab group (70.5%) versus (60.8%) in the ipilimumab group ($P < 0.001$). An updated 24-month analysis showed RFS of 63% in the nivolumab group compared to 50% in the ipilumumab group [92]. In a prespecified subgroup analysis, benefit for nivolumab was observed, regardless of PD-L1 and BRAF mutation status. However, having >5% PD-L1 expression showed increased 24-month RFS benefit (76% for nivolumab vs. 58% for ipilumumab). Similar to previous reports, nivolumab had a favorable toxicity profile, as only 14.4% of patients experienced grade ≥3 compared to 45.9% patients in the ipilimumab group.

The KEYNOTE-054 phase III enrolled 1019 patients with completely resected stage III melanoma, randomly assigned to receive 200 mg of pembrolizumab ($n = 514$) or placebo ($n = 505$) every 3 weeks for a total of 18 doses or until disease recurrence or unacceptable toxic effects occurred. The 1-year rate of RFS in pembrolizumab group was 75.4% versus 61.0% in placebo. Grade 3/4 toxicities were reported in 14.7% of the patients in the pembrolizumab group and in 3.4% of patients in the placebo group. Of note, KEYNOTE-054 included patients with stage IIIA disease, who were excluded from the CheckMate-238 trial [93].

Given the promising results of the nivolumab/ipilimumab combination in the metastatic setting, studies have looked into testing it in the adjuvant setting. A small trial (NCT01176474) carried out at the Moffitt Cancer Center, FL, is assessing two treatment schedules of NIVO1 + IPI3 (cohort 1) versus NIVO3 + IPI1 (cohort 2) for resected stage IIIC/IV melanoma. At median follow-up of 21.3 months and 11 months, respectively, for the two cohorts, the median PFS and OS have not been reached. CheckMate-915 (NCT03068455) is a phase III trial comparing adjuvant ipilimumab and nivolumab versus ipilimumab or nivolumab.

Furthermore, KEYNOTE-716 (NCT03553836) is a phase III placebo-controlled trial investigating pembrolizumab in resected high-risk stage II melanoma.

The Future of Melanoma Treatment

As our understanding of the tumor microenvironment and T cell homeostasis deepens, numerous new targets have been identified and are being currently tested in clinical trials. We will highlight some of these developments in the section below.

Indoleamine Dioxygenase (IDO) Inhibitors

IDO Inhibitors block enzymes involved in catalyzing tryptophan. T cells need tryptophan for function, and tumors can increase IDO levels, thereby suppressing the function of T cells [94].

Epacadostat, a selective inhibitor of the IDO1 enzyme, moved into phase III trial based on the results of a phase I/II study (ECHO-202/KEYNOTE-037, NCT02178722) [95, 96]. However, reported results from phase III ECHO-301/KEYNOTE-252 (NCT02752074) did not show a clinical benefit of the combination over pembrolizumab alone. PFS was 4.7 versus

4.9 months, and the OS rate at 12 months was 74% in both groups [97]. Possible explanations for the discrepancy of results between phase II and III trials include different treatment populations, relatively low dosing of epacadostat, and incomplete suppression of intratumoral kynurenine [98]. Other trials studying IDO inhibitors are ongoing (NCT02327078, NCT02658890).

Lymphocyte-Activation Gene 3 (LAG-3)

LAG-3 is an immune checkpoint receptor (CD223) found on the surface of activated CD4 and CD8 T cells, NK cells, B cells, and plasmacytoid dendritic cells [99]. LAG-3's main ligand is MHC class II. LAG-3 has various biologic effects on T cell function, including the negative regulation of T cell proliferation, activation, and homeostasis, and LAG-3 is upregulated during T cell exhaustion. Recently, its role in the maturation and activation of dendritic cells has also been described [100]. The development of LAG-3 blockade has now moved into clinical testing. In a phase I/IIa clinical trial, 43 melanoma patients who progressed on prior PD1/PD-L1 exposure were treated with relatlimab (previously known as BMS-986016) in combination with nivolumab [101]. Disease control rate was 45%, and ORR was 16% in the 31 efficacy-evaluable patients. Benefit was even observed in patients refractory to prior anti-PD-1 therapy. Importantly, relatlimab did not appear to add toxicity, as grade 3 or 4 toxicities were only observed in 9% of the treated patients. Multiple clinical trials are currently evaluating the efficacy of anti-LAG-3 in combination with other immunotherapies, in the neoadjuvant setting (NCT02519322) and in other tumor types (NCT02676869, NCT01968109, NCT03250832, and NCT03219268) [102].

T-Cell Immunoglobulin-3 (Tim-3)

TIM-3 is a co-inhibitory receptor, which is expressed on specific subtypes of INF-γ-producing CD4+ and CD8+ as well as dendritic cells, NK, and monocytes [103]. It was shown that a subset of T cells in patients with advanced melanoma upregulate Tim-3 expression and that cells positive for this marker appear to be dysfunctional [104]. It was also shown that concurrent blockade with anti-PD1 acted synergistically in reversing tumor-induced T cell exhaustion and dysfunction. Currently, a few Tim-3 antagonists are in early-phase clinical development, either as single agent or in combination with anti-PD-1 or PD-L1 (NCT03099109, NCT03489343, NCT02817633, NCT02608268). While most of these trials focus on safety, the results are eagerly awaited.

OX40

OX40 (or CD134) is a member of tumor necrosis factor (TNF) receptor superfamily (TNFRSF), and in vitro studies have shown that stimulation of its ligand can lead to proliferation, improved effector function, and prolonged survival of T cells, and treatment with OX40 agonists can increase antitumor immunity [105]. In an initial phase I trial using an OX40 agonistic murine monoclonal antibody, regression of metastatic lesions was noted in 12 out of 30 patients (7 patients with metastatic melanoma). Grade 3 and 4 lymphopenia was noted in seven patients, and other grade 1 and 2 toxicities included fatigue, nausea, vomiting, rash, and flu-like symptoms. Multiple clinical trials are currently ongoing, including in combination with atezolizumab (NCT02410512), durvalumab (NCT02705482), or tremelimumab (anti-CTLA-4; NCT02705482). In preclinical models, MEDI6383, a human OX40 ligand fusion protein, can initiate an intracellular signaling pathway to enhance T cell survival and activity, and proliferation, and is being evaluated in combination with durvalumab (NCT02221960) [106].

4-1BB

4-1BB (CD137) is another member of TNFRSF, and is an inducible costimulatory receptor expressed on T cells and other immune cells, and can restore effector function [107]. 4-1BB and 4-1BBL interaction results in cytokine secretion

and increased survival of CD8+ T cells. Urelumab (BMS-663513) is a fully humanized 4-1BB agonist mAb that has been tested in a phase I dose-escalation study. Only 3 out of 54 melanoma patients had a response to the monotherapy [108]. However, because of the synergistic activity of urelumab with nivolumab in preclinical data, this combination is currently being evaluated in a phase I dose-escalation clinical trial. In addition, PF-05082566, another 4-1BB agonist mAb, has also been evaluated in combination of pembrolizumab in patients with solid tumors (NCT02253992, NCT02179918). PF-05082566 (4-1BB agonist) is also being studied in combination with avelumab in advanced melanoma patients (NCT02554812).

Toll-Like Receptors (TLRs)

Toll-like receptors are members of immune recognition receptor family and were initially discovered through their role within the innate as well as adaptive immune response [109]. Furthermore, it was discovered that many tumor types express functional TLRs, leading to tumor proliferation, formation of metastases, and resistance to apoptosis. Numerous studies are now underway to see if TLR-based therapeutic approaches (especially intratumoral) can increase the efficacy of anticancer immunotherapies (NCT02644967, NCT03052205, NCT00960752, NCT03445533). Intratumoral TLR9 agonist, CMP-001, plus pembrolizumab demonstrated an ORR of 22% in a phase Ib trial for anti-PD-1 refractory disease [110]. Furthermore, another TLR9 agonist (SD-101) plus pembrolizumab demonstrated an ORR of 78% in treatment-naïve patients [111].

Bempegaldesleukin (NKTR-214): A CD-122-Biased IL-2 Receptor

NKTR-214 preferentially activates IL2 receptor beta over IL2 receptor alpha, due to the location of PEG molecules. Compared to aldesleukin, NKTR-214 induced higher ratio of tumor-killing CD8+ T cells to Foxp3+ regulatory T cells [112]. The phase I study with NKTR-214 enrolled 28 patients (melanoma $N = 7$) and demonstrated a favorable tolerance profile. Only 21.4% of patients experienced grade ≥3 treatment-related AEs [113]. PROPEL (NCT03138889) will evaluate NKTR-214 combined with pembrolizumab or atezolizumab. PIVOT-02 (NCT02983045) is phase II trial assessing NKTR-214 plus CPIs.

Melanoma Immunotherapy and the Gut Microbiome

Analysis of fecal microbiome samples from anti-PD-1-treated melanoma patients ($n = 43$, 30 responders, 13 nonresponders) showed significantly higher diversity and relative abundance of bacteria of the Ruminococcaceae family in responding patients [114]. Additionally, fecal transplants from responding patient given to germ-free mice led to enhanced antitumor immunity [114]. Creating more diversity in the patient's gut microorganisms by means of fecal transplant may improve the response to immunotherapy. Multiple studies are now assessing the role of gut microbiome alteration and response or toxicity to CPI therapy (NCT03817125, NCT03772899, NCT03819296).

Conclusion

The numerous breakthrough discoveries that have been made with regard to the treatment of melanoma over the last decade have translated into successful therapeutic approaches for other tumor types. While there is reason for optimism, much still remains unknown, and the results of ongoing trials are eagerly awaited and hopefully will guide the treating physician to be able to choose the best combination therapy for each individual patient.

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Immunotherapy in Lung Cancer: From a Minor God to the Olympus

4

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Abstract

Over the last decade, we have witnessed a paradigm shift in cancer treatment, with the advent of novel therapeutic approaches that target or manipulate the immune system, also known as immunotherapy. Blocking immune checkpoints has emerged as an effective strategy with unprecedented results in several solid tumors, including lung cancer. Since 2012 when PD(L)-1 inhibitors showed first clinical signals of activity in lung cancer, immune checkpoint blockade (ICB) has emerged as a novel effective therapeutic strategy in different settings, determining a dramatic change in the therapeutic landscape of both non-small cell lung cancer (NSCLC) and, more recently, small cell lung cancer (SCLC). Although the benefit from this novel therapeutic approach is undeniable, several open questions still remain unanswered. Herein, we summarize the major breakthroughs in the immunotherapy journey in lung cancer and how it is changing our clinical practice.

Keywords

Non-small cell lung cancer · Small cell lung cancer · Immunotherapy · Programmed death 1 · Programmed death-ligand 1 · Cytotoxic T-lymphocyte antigen-4 · Nivolumab · Pembrolizumab · Atezolizumab · Durvalumab · Tumor mutation burden

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Introduction

The past decade has witnessed a paradigm shift in cancer treatment, with the advent of novel therapeutic approaches that target or manipulate the immune system (immunotherapy) [1] demonstrating unprecedented results in several solid tumors, including lung cancer. The cancer-immunity cycle refers to the delicate balance between the recognition of self while minimizing toxicities related to autoimmunity [2]. The exploitation of the immune system with agents that

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stimulate it to react against tumor cells has been extensively studied in oncology and traditionally this strategy has not been effective in lung tumors, with multiple vaccination or immunostimulating strategies failing to prove any significant benefit. Recently, a renewed interest on immunotherapy emerged with the identification of immune checkpoints. Each step of the cancer-immunity cycle requires the coordination of numerous factors that have stimulatory and inhibitory actions [2] and among these, recently, two immune checkpoints have emerged as promising therapeutic targets, CTLA-4 (cytotoxic T-lymphocyte antigen-4) and PD-1 (programmed death 1) (Fig. 4.1).

CTLA4 was the first immune checkpoint receptor to be clinically targeted. It is expressed exclusively on T cells and inhibits the development of an active immune response. CTLA-4 acts at the level of T-cell development and proliferation by counteracting the activity of the T-cell costimulatory receptor CD28 through competing for the binding of the same ligands (CD80 also known as B7.1 and CD86 also known as B7.2) [2, 3]. In contrast to CTLA-4 that is involved in early steps of the cancer-immunity cycle, PD-1 and its ligands have a crucial role in the killing of cancer cells. Physiologically, PD-1/PD-L1 have the task of limiting the activity of T cells in peripheral tissues at the time of an inflammatory response to infection thereby limiting autoimmunity [2, 3]. Similar to CTLA-4, PD-1 is expressed on activated T cells and inhibits T-cell responses by interfering with T-cell receptor signaling. PD-1 has two ligands, PD-L1 (B7-H1) that is expressed on antigen-presenting cells (APCs), macrophages, fibroblasts, and T cells and PD-L2 (B7-DC) that is predominantly expressed on antigen-presenting cells (APCs). PD-L1 is also overexpressed in several solid tumors, while PD-L2 is expressed relatively rarely [4, 5]. The role of CTLA-4 and PD-1/PD-L1 in immune suppression and their expression in solid tumors provided the rationale for their therapeutic exploitation. Moreover, CTLA-4 and PD-1 exert their effects through separate pathways and therefore simultaneous targeting of both pathways has also been evaluated to restore antitumor immunity [6].

Since the first demonstration of activity of PD(L)-1 agents in lung cancer in early clinical trials in 2012 [7, 8], immune checkpoint blockade (ICB) has emerged as a novel effective therapeutic strategy in different clinical settings and determined a dramatic shift in the therapeutic landscape of both NSCLC and SCLC (Fig. 4.2). Several biological prognostic and predictive factors in blood and tissue samples have been identified, but unfortunately no single biomarker can perfectly discriminate between responders and non-responders and PD-L1 still remains the only applicable marker in clinical practice to date [9].

Herein, we summarize the major breakthroughs in the immunotherapy journey in lung cancer and how it is changing our clinical practice.

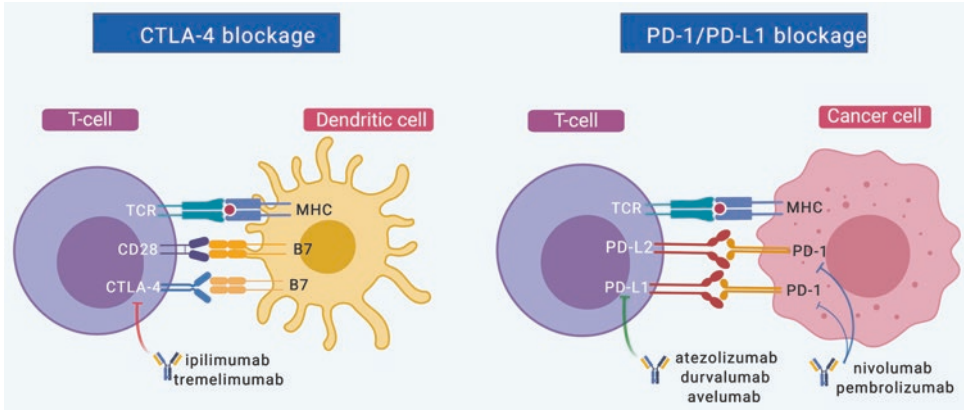

Fig. 4.1 Mechanism of action of CTLA-4 and PD-1/PD-L1 inhibitors. (*Credit: created with BioRender)*

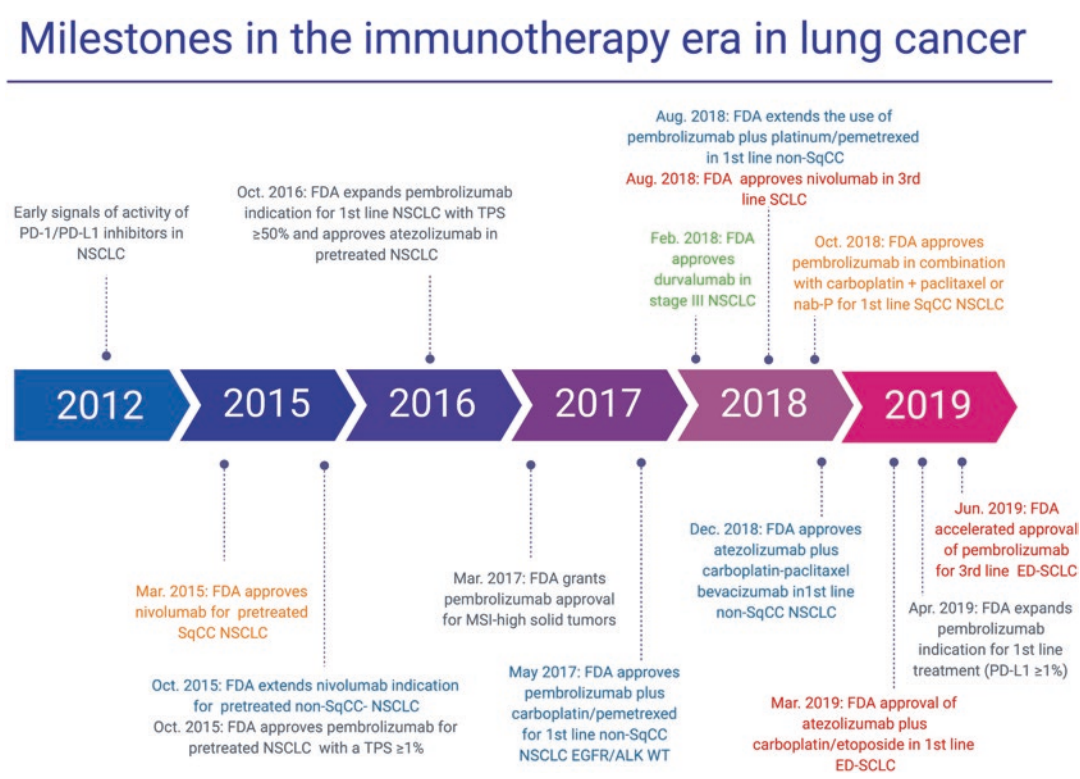

Fig. 4.2 Timeline of major breakthroughs in the immunotherapy era in lung cancer. In orange and in blue FDA approvals in squamous and non-squamous in metastatic NSCLC, respectively; in black data and FDA approvals in metastatic NSCLC independently of histology; in green FDA approval in locally advanced NSCLC; in red FDA approvals in extensive disease SCLC. (*Credit: created with BioRender)*

Early-Stage NSCLC and Locally Advanced NSCLC

Medical treatment of early stage and locally advanced NSCLC has changed little over the last two decades with platinum-based chemotherapy as the cornerstone of treatment either as adjuvant/neo-adjuvant therapy or in association with radiotherapy in inoperable patients. Meta-analyses of randomized phase III trials conducted in 1990s and early 2000s reported an absolute survival benefit at 5 years of 5% from adjuvant/neo-adjuvant approaches in stage IB-IIIA NSCLC compared with surgery alone [10, 11] and 4.5% with concurrent versus sequential chemoradiation in inoperable stage III NSCLC [12]. However, major breakthroughs in molecular biology translated little in early stage NSCLC and no targeted therapies have been approved to date in both early stage and locally advanced NSCLC.

Recently, immune checkpoint blockade has emerged as a new effective therapeutic modality in advanced NSCLC either alone or in combination with platinum-based chemotherapy. The activity and relatively favorable safety profile prompted the evaluation of immune checkpoint inhibitors (ICIs) in earlier lines of treatment, including neo-adjuvant and inoperable stage III NSCLC, leading to the approval of durvalumab as the first in class PD-L1 inhibitor approved as maintenance therapy after concurrent chemoradiation. The role of ICIs as neo-adjuvant therapy has been evaluated in small non-randomized studies with promising results (Table 4.1).

Collectively, single agent PD-1/PD-L1 inhibitors in the palliative setting have been associated with a 7–22% ORR per RECIST. In the neo-adjuvant setting, two to three cycles have resulted

Table 4.1 Clinical studies with immune checkpoint inhibitors in the neo-adjuvant setting

Study name	Resected patients (*n*)	Stage	Drug(s)	Cycles	MPR[a] (%)	ORR (%)
Forde et al. [13]	20	IB-IIIA	Nivolumab	2	45	10
LCM3 [14]	84	IB-IIIB	Atezolizumab	2	18	7
NEOSTAR [15]	23 (arm A) 21 (arm B)	IA-IIIA	Nivolumab Nivolumab + ipilimumab	3 3	17 33	22 19
NADIM [18]	30	IIIA	Nivolumab + carboplatin/paclitaxel	3	80	70
Shu et al. [19]	11	IB-IIIA	Atezolizumab + carboplatin/ nab-paclitaxel	2	64	73

[a]MPR (major pathologic response) defined as <10% residual viable tumor (RVT) in post-therapy specimen

in a major pathological response rate (MPR) of 17–45% in stage I-IIIA NSCLC [13–15]. MPR has been defined as 10% or less residual viable tumor after neo-adjuvant chemotherapy and has been proposed as a surrogate endpoint in neo-adjuvant studies in NSCLC [16]. Recently, immune-related pathologic response criteria (irPRC) have been proposed to better characterize the response of neo-adjuvant ICIs [17]. In contrast, chemo-immunotherapy combos have been associated with higher ORR (70–73%) and MPR (64–80%) [18, 19] and seem to be a more effective strategy in this setting. These data compare favorably with historical controls reporting a MPR of 19–27% [20, 21] and an ORR of approximately 35–50% with platinum-based chemotherapy [22, 23]. Several phase III studies are currently being conducted to evaluate the role of different chemo-immunotherapy combos for three to four courses as neo-adjuvant therapy compared with chemotherapy alone, including CheckMate 816, KEYNOTE-617, IMpower030, and AEGEAN. The results of these trials will provide definitive conclusions on the potential role of ICIs in this therapeutic setting.

Another potential neo-adjuvant approach is the concurrent use of ICIs and radiotherapy. This strategy is under evaluation in a pilot phase II study (NCT03237377).

The role of ICIs in the adjuvant setting is unclear and is currently under evaluation in multiple phase III clinical trials (NCT02273375, PEARLS, ANVIL, and IMpower010). Moreover, the phase II study CheckMate 9TN is currently evaluating the role of nivolumab in patients with residual disease after surgery.

The role of ICIs in inoperable stage III NSCLC is much more defined and durvalumab has been FDA and EMA approved as maintenance therapy in non-progressing patients after concomitant chemoradiation. The goal of using ICIs concomitantly with radiation therapy or immediately after is to augment the antitumor responses typically observed with either modality alone, exploiting the synergistic effect observed with both modalities through multiple mechanisms that include the release of signals and chemokines that recruit inflammatory cells into the tumor microenvironment, including antigen-presenting cells that activate cytotoxic T-cell function, release of neoantigens that can evoke the antitumor response, and upregulation of PD-L1 expression on tumor cells [24, 25]. After a decade of failures with alternative strategies to concurrent chemoradiation with platinum-based chemotherapy by adding a targeted agent [26] or replacing the non-platinum agent with a less toxic compound [27], increasing radiation dose [26], or using a tumor-derived vaccine [28], the PACIFIC trial changed the standard of care, adding durvalumab in the therapeutic armamentarium of inoperable locally advanced NSCLC. This randomized phase III trial evaluated durvalumab at the dosage of 10 mg/m^2 I.V. every 2 weeks versus placebo (2:1 randomization) as consolidative therapy in patients with inoperable stage III NSCLC who did not have disease progression after two or more cycles of platinum-based chemoradiation [29]. The trial met its two co-primary endpoints, demonstrating a statistically significant improvement in both PFS (17.2 months in the durvalumab group vs. 5.6 months in the placebo group; HR

0.51, 95% CI, 0.41–0.63) and OS (not reached vs. 28.7 months; HR 0.68, 99.73% CI, 0.47–0.997; $p = 0.0025$). Moreover, durvalumab treatment was associated with a higher ORR (28.4% vs. 16.0%; $p < 0.001$) and a longer time to death or distant metastasis (28.3 months vs. 16.2 months in the placebo group; HR 0.53, 95% CI, 0.41–0.68) [29, 30]. Treatment with durvalumab was well tolerated with an incidence of grade 3/4 adverse events of 30.5% in the durvalumab group versus 26.1% in the placebo group. An unplanned post hoc analysis requested by a health authority evaluated the role of pre-treatment PD-L1 status (unknown in 37% of patients) and showed no benefit in terms of OS in PD-L1 <1% patients (HR 1.36) [30]. However, these data should be considered only exploratory and no firm conclusions can be made due to the sample size (only 60 patients). Based on this analysis, it has been concluded that EMA restricted durvalumab use in PD-L1 ≥1% patients only.

The role of nivolumab and pembrolizumab as consolidative therapy after chemoradiation is under evaluation in phase II/III studies (RTOG 3505, MP-LALC, and HCRN LUN14–179).

PACIFIC evaluated durvalumab after concomitant chemoradiation. However, sequential chemoradiation is a valid alternative in patients who are not candidates for concurrent treatment and therefore the role of consolidative immunotherapy in this setting is not known. The phase II study PACIFIC-6 will address this issue.

Furthermore, several studies (PACIFIC-2, RATIONALE001, NICOLAS, DETERRED, and KEYNOTE-799) are evaluating the addition of PD-1/PD-L1 inhibition during concurrent chemoradiation followed by consolidation with immunotherapy.

First Line Metastatic NSCLC

The success of ICI use in pre-treated NSCLC patients prompted the evaluation of these agents in the upfront setting either alone or in combination with platinum-based chemotherapy or other immunotherapeutic agents. The positive results of the KEYNOTE-024, demonstrating the superiority of pembrolizumab compared with platinum-based chemotherapy in strong PD-L1 expressors (TPS ≥50%) of the EGFR/ALK wild type [31, 32], represented a major improvement in non-oncogene-addicted NSCLCs, which were minimally influenced by major therapeutic innovations in the last two decades [33]. The trial reported an impressive median OS of 30 months in the experimental arm with a statistically significant advantage over chemotherapy despite extensive crossover (64.2%) [32] and represented a major shift in the therapeutic landscape of NSCLC, adding a new molecularly defined subgroup of patients with improved outcome after a chemotherapy-free regimen. Subsequent studies tried to extend the benefit of ICB to a higher patient population with different therapeutic strategies, including evaluation of ICIs in PD-L1 ≥1% patients, chemo-immunotherapy combinations in PD-L1 all comers, and dual blockade with PD-1/PD-L1 inhibitors in combination with anti-CTLA4 agents. The results of these trials are summarized in Table 4.2 and contributed to the expanded use of ICIs in chemotherapy-naïve patients.

The KEYNOTE-042 trial aimed to evaluate the role of pembrolizumab in patients with weak and strong PD-L1 expression (TPS ≥1%) compared with standard-of-care platinum-based chemotherapy. The trial met its primary endpoints, with a statistically significant advantage in terms of OS in patients with a TPS of 50% or greater (HR 0.69, 95% CI 0.56–0.85; $p = 0.0003$), 20% or greater (HR 0.77, 95% CI 0.64–0.92; $p = 0.0020$), and 1% or greater (HR 0.81, 95% CI 0.71–0.93; $p = 0.0018$) [34]. However, when restricting the analysis to the subgroup of patients with a TPS 1–49% no differences in OS were observed (HR 0.92, 95% CI 0.77–1.11), suggesting that strong PD-L1 expressors mostly drove the benefit observed in the study population. These data lead to the extension of the FDA approval of pembrolizumab in chemotherapy-naïve EGFR/ALK wild-type NSCLC patients with a TPS ≥1%. The relatively favorable safety profile and activity seen in this trial make the regimen particularly useful in patients who are not candidates or refuse platinum-based chemotherapy.

Table 4.2 Phase III studies with PD-1/PD-L1 inhibitors in first-line NSCLC

Study	*n*	Arms	Population	PD-L1	Treatment BPD	IO duration	Crossover rate	Median FU (mos)	ORR	PFS (mos)	PFS (HR)	OS (mos)	OS (HR)
KEYNOTE-024 [31, 32]	154 vs. 151	Pembrolizumab vs. Platinum-CHT	NSCLC EGFR/ALK WT	≥50%	Allowed	Up to 35 cyles	64.2%	25.2	44.8% vs. 27.8%	10.3 vs. 6.0	0.50	30.0 vs. 14.2	0.63
KEYNOTE-042 [34]	637 vs. 637	Pembrolizumab vs. CP or carbo-pem	NSCLC EGFR/ALK WT	≥1%	Allowed	Up to 35 cyles	N.A.	14.0	27.2% vs. 26.5%	5.4 vs. 6.6	1.05	16.4 vs. 12.1	0.82)
CheckMate 026	271 vs. 270	Nivolumab vs. Platinum-CHT	NSCLC EGFR/ALK WT	≥1%	Allowed	Until PD	60%	13.5	26% vs. 33%	4.2 vs. 5.9[c]	1.15[c]	14.4 vs. 13.2[c]	1.02[c]
KEYNOTE-407 [35]	278 vs. 281	CP or carbo/ nab-P + pembro vs. CP or carbo/nab-P	SqCC	All comers	Allowed	Up to 35 cyles	31.7%	7.8	57.9% vs. 38.4%	6.4 vs. 4.8	0.56	15.9 vs. 11.3	0.64
KEYNOTE-189 [36, 37]	410 vs. 2016	Ccis/carbo-pem + Pembro vs. cis/carbo-pem	Non-SqCC EGFR/ALK WT	All comers	Allowed	Up to 35 cyles	53.9%	18.7	48.0% vs. 19.4%	9.0 vs. 4.9	0.48	22.0 vs. 10.7	0.56
IMpower150 (ARM B vs. C) [38, 39]	400 vs. 400	ABCP vs. BCP	Non-SqCC (EGFR/ALK allowed)	All comers	Allowed	Until PD	N.A.	19.6 19.7	63.5%[a] vs. 48%[a]	8.3[a] vs. 6.8[a]	0.59[b]	19.2[a] vs. 14.7[a]	0.78[a]
IMpower150 (ARM A vs. C) [38, 39]	402 vs. 400	ACP vs. BCP	Non-SqCC (EGFR/ALK allowed)	All comers	Allowed	Until PD	N.A.	19.6 19.7	40.6% vs. 40.2%	N.A.	0.91	19.4[a] vs. 14.7[a]	0.88[a]
IMpower 130 [40]	451 vs. 228	Atezo + carbo/ nab-P vs. Carbo/ nab-P	Non-SqCC (EGFR/ALK allowed)	All comers	Allowed	Until PD	19.3%	18.5 19.2	49.2%[a] vs. 31.9%[a]	7.0[a] vs. 5.5[a]	0.64[a]	18.6[a] vs. 13.9[a]	0.79[a]
IMpower 131 (ARM B vs. C) [41, 42]	343 vs. 340	Atezo + carbo/ nab-P vs. Carbo/ nab-P	SqCC	All comers	Allowed	Until PD	43%	17.1	49% vs. 41%	6.5 vs. 5.6	0.74	14.6 vs. 14.3	0.92
IMpower132 [43]	292 vs. 286	Atezo + cis/carbo + pem vs. Cis/ carbo+ pem	Non-SqCC EGFR/ALK WT	All comers	Allowed	Until PD	37.1%	14.8	47% vs. 32%	7.6 vs. 5.2	0.60	18.1 vs. 13.6	0.81

CheckMate 227 (TMB ≥10) [44]	139 vs. 160	Nivolumab-ipilumumab vs. Platinum-CHT	NSCLC EGFR/ALK WT	All comers	Allowed	Until PD	N.A.	11.2[d]	45.3% vs. 26.9%	7.2 vs. 5.5	0.58	23.03 vs. 16.72	0.77
MYSTIC (ARM A vs. C) [45, 46]	374 vs. 372	Durvalumab vs. Platinum-CHT	NSCLC EGFR/ALK WT	All comers	Allowed	Until PD	39.5%	30.2	35.6%[b] vs. 37.7%[b]	4.7[b] vs. 5.4[b]	0.87[b]	16.3[b] vs. 12.9[b]	0.76[b]
MYSTIC (ARM B vs. C) [45, 46]	372 vs. 372	urvalumab-tremelimumab vs. Platinum-CHT	NSCLC EGFR/ALK WT	All comers	Allowed	Until PD	39.5%	30.2	34.4%[b] vs. 37.7%[b]	3.9[b] vs. 5.4[b]	1.05[b]	11.9[b] vs. 12.9[b]	0.85[b]

[a]*EGFR/ALK WT intention-to-treat (ITT) population;*[b]*ITT population (PD-L1 ≥ 25%);*[c]*ITT population (PD-L1 ≥ 5%);*[d]*minimum follow-up*

N.A. Not Available, *ABCP* atezolizumab + bevacizumab/carboplatin/paclitaxel, *ACP* atezolizumab + carboplatin/paclitaxel, *BCP* bevacizumab/carboplatin/paclitaxel, *Cis* cisplatin, *CP* carboplatin/paclitaxel, *nab-P* nab-paclitaxel, *Platinum-CHT* platinum-based chemotherapy

In contrast, the CheckMate 026, evaluating nivolumab in chemotherapy-naïve NSCLC EGFR/ALK WT with a PD-L1 expression ≥1%, failed to meet its primary endpoint, showing no statistically significant difference between ICB and chemotherapy in terms of PFS in the intention-to-treat (ITT) population (PD-L1 ≥5%) (HR 1.15, 95% CI 0.91–1.45, $p = 0.25$ for PFS). Furthermore, nivolumab was not associated with any differences in terms of OS (HR 1.02, 95% CI 0.80–1.30) and ORR compared with platinum-based chemotherapy (26% vs. 33%, odds ratio 0.70, 95% CI 0.46–1.06) [47]. Moreover, an exploratory subgroup analysis involving patients with a PD-L1 expression level ≥50% showed no differences between the two treatment arms in both PFS (HR 1.07, 95% CI 0.77–1.49) and OS (HR 0.90, 95% CI 0.63–1.29) [47]. Differences in the study design and population included might have contributed to the differences seen with trials evaluating pembrolizumab monotherapy. Similarly, durvalumab monotherapy failed to prolong both PFS (HR 0.87, 95% CI 0.593–1.285; $p = 0.324$) and OS (HR 0.76, 95% CI 0.564–1.019; $p = 0.036$) in the ITT population (PD-L1 ≥25% with SP263 IHC assay) compared with chemotherapy in the phase III MYSTIC trial (arm A vs. B) [45]. However, subgroup analyses of both studies evaluated the predictive role of tumor mutation burden (TMB) with ICIs. In the CheckMate-026 trial, TMB was evaluated in the tissue using a whole exome assay, dividing patients in three tertiles (<100, 100–242, or ≥243 total missense mutations) [47]. Nivolumab in TMB high (≥243 total missense mutations) patients was associated with improved ORR (47% vs. 28%) and PFS (HR 0.62, 95% CI 0.38–1.00) versus chemotherapy, but not OS (HR 1.10), likely secondary to extensive crossover in the control arm (68%). Interestingly, there was no association between TMB and PD-L1 expression, albeit patients with both PD-L1 ≥50% and high TMB seemed to derive the greatest benefit [47]. Whole exome sequencing is impractical in clinical practice and, therefore, smaller targeted-gene next-generation sequencing (NGS) panels have been used to evaluate this potential biomarker with comparable results [48]. However, the impact of the mutational study of different genes on TMB calculation using different NGS platforms (MSK-IMPACT, Foundation Medicine, etc.) has not been analyzed yet [49]. In the MYSTIC trial, a TMB analysis was conducted in both tissue (Foundation Medicine 315-gene panel) and plasma (GuardantOMNI 500-gene panel). Unfortunately, tissue availability for TMB analysis was limited to only 41% of the ITT population. However, despite these limitations high TMB (≥10 mutations/Mb) predicted a better OS with durvalumab compared with chemotherapy (HR 0.70, 95% CI 0.47–1.06). There was a good correlation between tissue and plasma results for TMB in patients with matched specimens (Spearman's rho = 0.6; Pearson's $r = 0.7$) and blood. TMB ≥20 mutations/Mb were associated with improved OS (HR 0.72, 95% CI 0.50–1.05) and PFS (HR 0.77, 95% CI 0.52–1.13) with durvalumab [46]. As reported previously, TMB and PD-L1 were independent predictive factors, suggesting that these biomarkers can be used as complementary tools when selecting patients for immunotherapy treatment. However, standardization of methods used and robust analytical/clinical validation are needed before extensive clinical implementation of this biomarker is implemented [49].

Avelumab is also under clinical development in first-line versus chemotherapy in PD-L1 positive patients in the ongoing randomized phase III study JAVELIN Lung 100.

The addition of chemotherapy to ICIs is based on the rationale that chemotherapy may expose the immune system to high levels of tumor cell antigens through tumor cell killing, induce secretion of cytokines that ultimately enhance T-cell responses, eliminate immunosuppressive cells (i.e., MDSCs and Tregs), and induce tumor PD-L1 overexpression [33]. Several studies have evaluated the safety and efficacy of multiple chemo-immunotherapy regimens. Most of these trials excluded EGFR-mutated and ALK rearranged NSCLCs, due to the lower activity seen in previous studies in pre-treated patients with PD(L)-1 inhibitors in these molecular subgroups [50–53] and included PD-L1 all comers patients.

KEYNOTE-021 was a multi-cohort phase 1/2 study evaluating different chemotherapy regimens in addition to pembrolizumab. One of the most promising chemotherapy combinations was pembrolizumab plus carboplatin-pemetrexed that was further evaluated in the phase II part of the study in a randomized cohort (cohort G). Preliminary efficacy data showed a significant increase in both ORR (55% vs. 29%, $p = 0.0016$) and PFS (13.0 vs. 8.9 months, HR 0.53), but there were no differences in OS (HR 0.90, at a median follow-up of 10.6 months), likely to the extensive use of PD-1/PD-L1 inhibitors as salvage therapy in the chemotherapy arm (74%) [54]. Based on these preliminary results, FDA approved this regimen for first-line treatment of non-squamous NSCLC EGFR/ALK wild-type lung cancer. Final results of the study after a median follow-up of 23.9 months further confirmed the advantage in terms of ORR (56.7% vs. 30.2%, $p = 0.0016$) and PFS (24.0 vs. 9.3 months; HR 0.53, 95% CI 0.33–0.86; $p = 0.0049$). A statistically significant advantage in terms of OS was also reported in the experimental arm (median OS not reached in the chemo-immunotherapy arm vs. 21.1 months; HR 0.56, $p = 0.0151$), despite an extensive crossover (73.3%), with a relatively favorable safety profile (AEs G3–5 41% vs. 27%) [55]. The subsequent phase III randomized trial KEYNOTE-189 evaluated pembrolizumab in association with platinum-pemetrexed chemotherapy in non-squamous NSCLC EGFR/ALK wild type, PD-L1 all comers. At the first interim analysis (median follow-up of 10.5 months), the addition of pembrolizumab was associated with a statistically significant advantage in both of the two co-primary endpoints of the study, OS (N.R. vs. 11.3 months, HR 0.49; $p < 0.001$) and PFS (8.8 vs. 4.9 months, HR 0.52, $p < 0.001$), independent of PD-L1 IHC expression. Higher ORR (47.6% vs. 18.9%, $p < 0.001$) was reported in the chemo-immunotherapy arm, with higher response rates among PD-L1 strongly positive patients (61.4% vs. 22.9% in PD-L1 ≥50%) [36]. The updated survival data of the trial at a median follow-up of 18.7 months continued to show a statistically significant advantage in both OS (22.0 vs. 10.7 months; HR 0.56, 95% CI 0.45–0.70, $p < 0.00001$) and PFS (9.0 vs. 4.9 months; HR 0.48, 95% CI 0.40–0.58; $p < 0.00001$) across all PD-L1 TPS groups. Furthermore, chemo-immunotherapy was also associated with a significant prolongation of PFS2 (17.0 vs. 9.0 months; HR 0.49, 95% CI 0.40–0.59; $p < 0.00001$) [37], suggesting that the combinatorial approach is superior to the sequential use of chemotherapy and ICB (crossover rate of 53.9%). In August 2018, the FDA approved an expanded label for pembrolizumab in combination with pemetrexed and platinum-based chemotherapy for the first-line treatment of patients with metastatic non-squamous NSCLC with no EGFR or ALK aberrations.

Three phase III trials evaluated atezolizumab in non-squamous NSCLC in association with different platinum-based chemotherapy regimens: carboplatin/paclitaxel with or without bevacizumab (IMpower150), carboplatin/nab-paclitaxel (IMpower130), and cisplatin or carboplatin/pemetrexed (IMpower132).

IMpower150 was a large randomized phase III trial evaluating atezolizumab in association with carboplatin-paclitaxel (ACP – arm A) versus atezolizumab plus bevacizumab/carboplatin/paclitaxel (ABCP – arm B) versus bevacizumab/carboplatin/paclitaxel (BCP – arm C) in all comer chemotherapy-naïve non-squamous NSCLCs. The trial also enrolled EGFR-mutated and ALK rearranged tumors that had previously been treated with appropriate tyrosine kinase inhibitor (TKI) therapy. The two primary endpoints of the study were PFS both among patients in the ITT population (EGFR/ALK wild-type patients) and among patients in the wild-type (WT) population who had high expression of an effector T-cell (Teff) gene signature in the tumor (Teff-high WT population), and overall survival in the WT population. Efficacy and safety results of arm B and C were presented. ABCP was associated with longer PFS than BCP in the entire study population (8.3 vs. 6.8 months; HR 0.62; 95% CI 0.52-–0.74; $p < 0.001$), in the ITT population (WT) (8.3 vs. 6.8 months; HR 0.61; 95% CI 0.52–0.72; $p < 0.001$), and in the Teff-high WT population (11.3 vs. 6.8 months; HR 0.51, 95% CI 0.38–0.68; $p < 0.001$) [38]. At first interim analysis

(median duration of follow-up approximately 20 months), OS was significantly longer in the WT population with ABCP than with BCP (19.2 vs. 14.7 months; HR 0.78, 95% CI 0.64–0.96; $p = 0.02$) [38]. Interestingly, improved OS with ABCP versus BCP was observed in patients with sensitizing EGFR mutations (Not estimable vs. 17.5 months; HR 0.31, 95% CI 0.11–0.83) and in patients with baseline liver metastases (13.3 vs. 9.4 months; HR 0.52, 95% CI 0.33–0.82). The benefit was independent of PD-L1 expression. A synergistic effect between bevacizumab and atezolizumab can be hypothesized, since no OS benefit was observed with the addition of atezolizumab to carboplatin/paclitaxel in both EGFR-positive patients (21.4 vs. 18·7 months; HR 0.93, 95% CI 0.51–1.68) and in patients with liver metastases (8.9 vs. 9.4 months; HR 0.87, 95% CI 0.57–1.32) [39]. These data suggest that ABCP can be a novel treatment option in first-line non-squamous NSCLC. The use in EGFR-mutated patients progressing after an EGFR TKI is promising, but these data should be confirmed prospectively in a larger cohort of patients. In December 2018, the FDA granted approval for ABCP combination as first-line therapy in EGFR/ALK wild-type NSCLC patients.

IMpower130 was a phase III randomized trial evaluating the addition of atezolizumab to carboplatin/nab-paclitaxel in chemotherapy-naïve non-squamous NSCLC patients. Pemetrexed maintenance was permitted after —four to six chemotherapy cycles in the control arm. Co-primary endpoints of the study were PFS and OS in the ITT EGFR/ALK wild-type population. The trial met its co-primary endpoints, showing a statistically significant improvement in both OS (18.6 vs. 13.9 months; HR 0.79, 95% CI 0.64–0.98; $p = 0{\cdot}033$) and PFS (7.0 vs. 5.5 months; HR 0.64, 95% CI 0.54–0.77; $p < 0{\cdot}0001$) in the ITT WT population. The benefit was observed across all PD-L1 subgroups, but no benefit was observed in the EGFR/ALK positive cohort (HR 0.98 for OS and 0.75 for PFS) [40].

KEYNOTE-407 and IMpower131 evaluated the addition of a PD(L)-1 agent to platinum-based chemotherapy (carboplatin/nab-paclitaxel or paclitaxel) in patients with squamous cell carcinoma of the lung. The addition of pembrolizumab to carboplatin/nab-paclitaxel or paclitaxel compared to chemotherapy alone was associated with a statistically significant improvement of both PFS (6.4 vs. 4.8 months; HR 0.56, 95% CI 0.45–0.70; $p < 0.001$) and OS (15.9 vs. 11.3 months; HR 0.64, 95% CI 0.49–0.85; $p < 0.001$), primary endpoints of the KEYNOTE-407 study, independent of PD-L1 status and taxane used [35]. Based on these results, in October 2018 FDA extended first-line pembrolizumab approval in combination with carboplatin/nab-paclitaxel or paclitaxel in chemotherapy-naïve NSCLC with squamous histology. This represented a major improvement in the upfront treatment of squamous NSCLC that had little changed in the last two decades with marginal incremental benefits with the addition of anti-EGFR mAb [56] or the use of novel chemotherapy agents [57]. The IMpower131 trial evaluated the addition of atezolizumab to either carboplatin/paclitaxel (arm A) or carboplatin/nab-paclitaxel (arm B) versus carboplatin/nab-paclitaxel alone (arm C). Preliminary data of arm B versus C were presented at the 2018 ASCO annual meeting. At a median follow-up of 17.1 months, addition of atezolizumab to first-line carboplatin/nab-paclitaxel was associated with a statistically significant improvement in PFS compared with carboplatin/nab-paclitaxel alone (6.3 vs. 5.6 months; HR 0.71, 95% CI 0.60–0.85; $p = 0.0001$), but failed to meet the other co-primary endpoint, with no statistically significant differences in terms of OS (14.0 vs. 13.9 months; HR 0.96, 95% CI 0.78–1.18; $p = 0.6931$) [58]. The definitive results of this trial, including those of arm A, are awaited and could clarify the role of atezolizumab in first-line treatment of squamous NSCLC.

Finally, IMpower132 evaluated atezolizumab in combination with platinum-pemetrexed in chemotherapy-naïve non-squamous NSCLC without EGFR or ALK genetic alterations. The study met one of its two co-primary endpoints with a significant advantage in terms of PFS (7.6 vs. 5.2; HR 0.60, 95% CI 0.49–0.72; $p < 0.0001$), but did not show any statistically significant advantage in terms of OS (18.1 vs. 13.6 months; HR

0.81; 95% CI 0.64–1.03; $p = 0.0797$) at the first interim analysis (median follow-up of 14.8 months), despite a 4.5-month survival gain [43]. A longer follow-up can provide definitive conclusions on the efficacy of this combination.

Another potential strategy is to combine PD(L)-1 inhibitors with other immune checkpoint inhibitors in order to optimize the blockage of immune suppressive signals. One of the most promising combinatorial approaches is to combine PD(L)-1 and CTLA-4 inhibitors. The combination has shown efficacy in metastatic melanoma [59] and renal cell carcinoma [60]. The safety and efficacy of nivolumab-ipilimumab was first tested in NSCLC in the multi-cohort phase 1 CheckMate-012 study. Different schedules were tested and the results of the two arms with nivolumab 3 mg/kg every 2 weeks in combination with ipilimumab 1 mg/kg every 12 weeks or every 6 weeks of the randomized part of the study were presented. Dual blockage was associated with a promising clinical activity with ORR of 47% and 38% and median PFS of 8.1 months and 3.9 months, respectively. High PD-L1 expression (≥1%) was associated with higher ORR (57% in both treatment arms). The combination was associated with high frequency of serious adverse events with 37% and 33% of patient experiencing irAEs G3–4 in patients treated with ipilimumab 1 mg/kg every 12 weeks and every 6 weeks, respectively [61]. Moreover, evaluation of tissue TMB through whole exome sequencing showed that this biomarker strongly predicted efficacy with combination PD-1 plus CTLA-4 blockade, independent of PD-L1 expression [62]. Based on these data, nivolumab, 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks, was further evaluated in the phase II CheckMate-568 study, with ORR in PD-L1 ≥1% patients as primary endpoint. The combination was associated with increased activity among PD-L1 positive patients (ORR was 41% in PD-L1 ≥1% vs. 15% in PD-L1 <1%). Efficacy on the basis of TMB, evaluated with the FoundationOne CDx assay, was included as a secondary endpoint. TMB ≥10 mut/Mb was identified as the optimal cut-off value for efficacy and was associated with improved ORR (43.7% vs. 23.5% for TMB high and low, respectively) and PFS (7.1 vs. 2.6 months for TMB high and low, respectively), regardless of PD-L1 expression. Safety profile was in line with previous studies, with G3–4 treatment-related AEs seen in 29% of patients [63]. These results were confirmed in the randomized phase III CheckMate-227 study, which met its co-primary endpoints of PFS with the nivolumab-ipilimumab combination versus chemotherapy in first-line advanced NSCLC with high TMB (≥10 mutations/Mb), using the FoundationOne CDx assay, regardless of PD-L1 expression. Among patients with TMB ≥10%, dual blockage was associated with higher ORR (45.3% vs. 26.9%) and longer PFS (7.2 months vs. 5.5 months, HR 0.58; $p < 0.001$) compared with platinum-based chemotherapy. Responses were durable, with 43% of patients progression-free at 1 year and the advantage in PFS was independent of PD-L1 expression (≥1% vs. <1%) compared with 13% with chemotherapy. No differences were observed in terms of PFS in patients with low TBM (<10 Mb) (HR 1.07) [44]. Based on these promising efficacy data, nivolumab-ipilimumab was submitted for FDA approval in July 2018. Unfortunately, in October 2018 updated OS data, the other co-primary endpoint of the trial, for the combination showed no difference in OS between patients whose tumors had TMB ≥10 mut/Mb or <10 mut/Mb compared with chemotherapy (23.03 vs. 16.72 months; HR, 0.77; 95% CI, 0.56–1.06). In January 2019, Bristol-Myers Squibb (BMS) withdrew the application for FDA approval while awaiting the final data from part 1a of the study (nivolumab-ipilimumab vs. chemotherapy in PD-L1 ≥1% patients).

The role of TMB as predictive biomarkers for dual immune checkpoint blockage was also explored in the randomized phase III MYSTIC trial. This was a three arm randomized phase III trial comparing durvalumab (arm A) or durvalumab-tremelimumab (arm B) with chemotherapy in stage IV NSCLC EGFR/ALK wild type, irrespective of PD-L1. Primary endpoints were PFS and OS with durvalumab-tremelimumab versus chemotherapy in PD-L1 ≥25% patients. The trial failed to meet its co-primary endpoints

due to the absence of any statistically significant differences between the two treatment arms in both PFS (3.9 vs. 5.4 months; HR 1.05, 97.54% CI 0.722–1.534; $p = 0.705$) and OS (11.9 vs. 12.9; HR 0.85, 98.77% CI 0.611–1.173; $p = 0.202$) in PD-L1 ≥25% patients. However, an exploratory analysis evaluated TMB in tissue (using FoundationOne CDx assay) and in the blood (using the 500-gene GuardantOMNI panel). TMB in the tissue was evaluable only in 41% of the ITT population and high TMB (≥10 mut/Mb) predicted improved OS with durvalumab-tremelimumab compared to chemotherapy (16.6 vs. 10.9 months; HR 0.72, 95% CI 0.48–1.09). In contrast, in patients with low TMB (<10 mt/Mb), dual blockage was inferior to chemotherapy (8.4 vs. 13.8 months; HR 1.39, 95% CI 1.0–1.32). Blood TMB was assessed in 72.4% and showed a good correlation with tissue in patients with matched tumor samples. Interestingly, increasing blood TMB values correlated with increased OS HR and a TMB ≥20 mut/Mb was selected as the optimal cut-off value. Indeed, patients with high TMB in the blood experienced longer OS (21.9 vs. 10 months; HR 0.49, 95% CI 0.32–074) with durvalumab-tremelimumab compared with chemotherapy, but not in those with low TMB (≤20 mut/Mb) in the blood (median OS 8.5 vs. 11.6 months; HR 1.16, 95% CI 0.93–1.45) [46].

These results are promising and suggest that TMB can be a valid biomarker for patient selection, albeit several open questions still remain unanswered, including optimal cut-off value and standardized detection method. There is an urgent need to overcome the naïve vision of a single biomarker to identify patients who are most likely to respond to ICB therapy, moving to the integration and simultaneous evaluation of multiple clinically relevant biomarkers [64] (Fig. 4.3).

Pre-treated NSCLC

Immune checkpoint inhibitors targeting PD-1/PD-L1 dramatically changed the therapeutic landscape of pre-treated NSCLC.

In 2012, the first in human trial of nivolumab in heavily pre-treated solid tumors, including NSCLC, showed promising activity for this agent with a response rate of 18% and durable responses, exceeding results with historical controls using conventional therapeutic agents [7], proving the activity of ICB in a disease not traditionally considered to be immunogenic. Since the initial study, several PD(L)-1 compounds were tested in second-/third-line NSCLC, demonstrating superiority over the standard of care at that time (docetaxel) and now nivolumab, pembrolizumab, and atezolizumab are approved in this

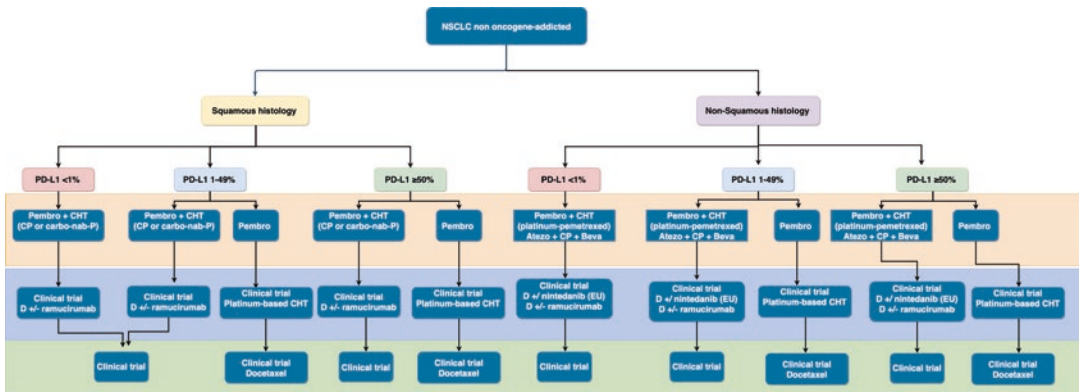

Fig. 4.3 New therapeutic algorithm in advanced/metastatic NSCLC with available therapeutic options. *Legend: Pembro, pembrolizumab; Atezo, atezolizumab; D, docetaxel; CP, carboplatin/paclitaxel; nab-P, nab-paclitaxel; Beva, bevacizumab; EU, approved only by European Medicine Agency; CHT, chemotherapy*

setting. Development of these drugs followed different pathways, since some of them were tested in unselected patient populations (nivolumab, atezolizumab, and avelumab), whereas others followed biomarker-driven development (pembrolizumab).

Nivolumab was evaluated in two large randomized phase III studies with similar designs using docetaxel as the control arm. CheckMate 017 evaluated nivolumab in second-line squamous NSCLC [52], whereas CheckMate 057 addressed second−/third3-line non-squamous NSCLC [53]. PD-L1 IHC expression was retrospectively analyzed using the 28–8 assay. Both studies met the primary endpoints, showing a statistically significant advantage in terms of OS compared with docetaxel in both squamous (9.2 vs. 6.0 months; HR 0.59, 95% CI 0.44–0.79; $p < 0.001$) and non-squamous NSCLC (12.2 vs. 9.4 months; HR 0.73; 96% CI, 0.59–0.89; $p = 0.002$) [52, 53]. Nivolumab was also superior to docetaxel in terms of ORR (19–20% vs. 9–12%) and safety profile (treatment-related AEs G3–4 in 7–10% vs. 54–55%) in both studies, as well as in PFS in squamous histology only (3.5 vs. 2.8 months; HR 0.62, 95% CI 0.47–0.81; $p < 0.001$) [52, 53]. Interestingly, PD-L1 expression as a predictive biomarker produced contrasting results between the two trials, despite similar study designs and the same assessment methods. The different mutational burden of squamous and non-squamous histology, as well as the frequency of oncogene-addicted tumors, might have contributed to this discrepancy. Moreover, a landmark analysis of the CheckMate 057 demonstrated that, excluding patients who had died in the first 3 months, nivolumab was superior to docetaxel in both PD-L1 positive and negative patients [65]. For this reason, nivolumab was approved in both squamous and non-squamous pre-treated NSCLC patients, irrespective of PD-L1 status. Recently, a pooled analysis of both studies showed an encouraging 3-year OS of 17% [66]. These results are noteworthy when compared to conventional chemotherapy. Only 8% of the patients in the docetaxel arm were alive at 3 years, and the plateau in the survival curves suggests a potential long-term benefit.

Atezolizumab was compared with docetaxel in pre-treated NSCLC in phase II (POPLAR) and phase III randomized studies (OAK), showing improved OS across all PD-L1 expression levels with incremental efficacy results at the increase of PD-L1 IHC expression in tumor cells (TC) or tumor-infiltrating immune cells (IC) using the SP142 assay [51, 67]. However, this IHC assay reported in some harmonization study lower tumor cell staining than other tests [68, 69] and is not FDA approved for lung cancer patients. An exploratory analysis was conducted in plasma samples collected in both trials to evaluate blood TMB using the FoundationOne CDx NGS assay, using POPLAR samples as training sets and validating the optimal cut-off value with the OAK samples. Blood TMB ≥16 mut/Mb (27% of the blood evaluable population of the OAK trial) was clearly predictive of improved PFS, showing a good correlation with tissue TMB values and no association with strong PD-L1 expression [70]. Based on the results of the OAK trial, in October 2018, FDA granted atezolizumab approval for pre-treated NSCLC, irrespective of PD-L1 status.

The development of pembrolizumab in NSCLC started with the phase 1 multi-cohort study KEYNOTE-001, which evaluated the safety and activity of this compound, and also validated the companion diagnostic 22C3 IHC assay for PD-L1 expression. Pembrolizumab was well tolerated with few treatment-related AEs of grade 3 or more (9.5% of the patients) and showed good clinical activity with an ORR of 19.4%, a median PFS of 3.7 months, and a median OS of 12.0 months in the overall population. No significant differences in efficacy or side-effect profile were reported with different schedules used (2 mg/kg or 10 mg/kg every 3 weeks) and a PD-L1 TPS ≥50% was associated with a higher response rate and longer PFS and OS [71]. In October 2015, the U.S. FDA granted accelerated approval for pembrolizumab for NSCLC patients whose disease had progressed after other treatments and with tumor expression of PD-L1, assessed with the companion diagnostic PD-L1 IHC 22C3 pharmDx test. The subsequent randomized phase II/III study KEYNOTE-010

compared pembrolizumab at two different dosages (2 mg/kg or 10 mg/kg every 3 weeks) to docetaxel in pre-treated NSCLC patients, PD-L1 positive (TPS ≥1%). The trial met its primary endpoint, reporting a statistically significant advantage in OS in both pembrolizumab arms (10.4 vs. 8.5 months and 12.7 vs. 8.5 months, respectively, for pembrolizumab 2 mg/kg and 10 mg/kg, with a HR of 0.71 and 0.61). Similarly to previous immunotherapy studies in pre-treated NSCLC, no differences were observed in PFS curves between the three treatment arms. Patients with strong PD-L1 expression (TPS ≥50%) derived the greatest OS benefit with both pembrolizumab 2 mg/kg (14.9 vs. 8.2 months; HR 0.54, 95% CI 0.38–0.77; p = 0·0002) and 10 mg/kg schedules (17.3 vs. 8.2 months; HR 0.50, 95% CI 0.36–0.70; $p < 0.0001$) [50].

Avelumab was evaluated in the phase III randomized study JAVELIN Lung 200, which compared this PD-L1 inhibitor with docetaxel in pre-treated NSCLC, independent of PD-L1 expression. The study failed to meet its primary endpoint, showing no statistically significant differences in terms of OS between the two treatment arms in the overall study population (10.5 vs. 9.9 months; HR 0.90, 96% CI, 0.75–1.08; p = 0.12) and in PD-L1 positive patients (≥1%) (11.4 vs. 10.3; HR 0.90, 96% CI 0.72–1.12; p = 0.16) [72]. The lack of OS benefit might be attributable to the better performance of the control arm than expected on similar randomized trials of anti–PD-1/PD-L1 agents (8.5–9.6 months) [50, 51], likely due to the subsequent use of ICIs. Exploratory subgroup analyses showed an increasing clinical activity with avelumab in patients with higher PD-L1 expression (HR 0.67 and HR 0.59 with ≥50% and ≥80% PD-L1 expression) [72], consistent with other PD(L)-1 inhibitors in NSCLC.

Durvalumab was evaluated as third-line option in the single-arm phase II study ATLANTIC. The trial included three cohorts of patients: EGFR+/ALK+ NSCLC with PD-L1 expression ≥25% (cohort 1), EGFR/ALK wild-type NSCLC with PD-L1 expression ≥25% (cohort 2), and PD-L1 ≥90% (cohort 3). The clinical activity and safety profile of durvalumab were consistent with those of other PD(L)-1 inhibitors. Responses were higher in EGFR/ALK wild-type patients and increased with higher PD-L1 expression levels (30.9% in PD-L1 ≥90% and 16.4% in PD-L1 ≥25% among EGFR/ALK wild-type patients) [73]. The 12.2% ORR reported among EGFR/ALK positive patients suggests that a subgroup of oncogene-addicted NSCLCs can derive benefit from ICB and supports further evaluation of this strategy in these patients.

Neither durvalumab nor avelumab is approved in stage IV NSCLC.

ICIs and SCLC

Treatment of extensive small cell lung cancer (ED-SCLC) has not changed over the last three decades with platinum-etoposide as the standard-of-care first-line option and topotecan or cyclophosphamide, doxorubicin, and vincristine (CAV) mostly used in subsequent treatment lines [74]. Several attempts to improve outcomes of ED-SCLC patients by incorporating novel chemotherapy agents (irinotecan, pemetrexed) or using targeted therapies (bevacizumab) failed to show any significant survival benefits [75–77]. As a consequence, survival of ED-SCLC patients enrolled in phase III trials did not improve significantly over the years [78].

The use of ICIs is attractive in SCLC due to the high number of somatic mutations seen in this tumor type that is one of the highest reported across human solid tumors [79].

The first ICI tested in ED-SCLC was the anti-CTLA4 agent ipilimumab. A phase II study evaluated the addition of ipilimumab to carboplatin/paclitaxel as first-line treatment in two alternative regimens, concurrent ipilimumab (ipilimumab + paclitaxel/carboplatin followed by placebo + paclitaxel/carboplatin) or phased ipilimumab (placebo + paclitaxel/carboplatin followed by ipilimumab + paclitaxel/carboplatin). Phased ipilimumab was associated with a non-statistically significant longer median OS compared with paclitaxel/carboplatin alone (12.9 vs. 9.9 months; HR, 0.75, 95% CI 0.46–1.23; p = 0.13) and a statistically significant improvement of immune-

related PFS (HR, 0.64, 95% CI 0.40–1.02; $p = 0.03$) [80]. Based on these promising results, a subsequent randomized phase III trial evaluated the addition of phased ipilimumab to platinum-etoposide versus platinum-etoposide alone. The trial failed to show a significant improvement in OS (11.0 vs. 10.9 months; HR 0.94; 95% CI, 0.81–1.09; $p = 0.3775$), the primary endpoint of the study, and in PFS (4.6 vs. 4.4 months; HR 0.85; 95% CI, 0.75–0.97) with the addition of phased ipilimumab [81].

The positive results of PD(L)-1 inhibitors in NSCLC prompted the evaluation of these compounds in SCLC in multiple clinical settings, including upfront treatment, maintenance therapy in non-progressing patients after standard platinum-etoposide chemotherapy, and in subsequent lines of therapy (Table 4.3).

Nivolumab was evaluated in pre-treated ED-SCLC in a phase I/II study (CheckMate 032) in monotherapy or in combination with ipilimumab. The trial initially evaluated nivolumab monotherapy at the dosage of 3 mg/kg ($n = 98$) and the combination nivolumab 1 mg/kg plus ipilimumab 3 mg/kg ($n = 61$), or nivolumab 3 mg/kg plus ipilimumab 1 mg/kg ($n = 54$). Nivolumab monotherapy was associated with an ORR of 11%, whereas the combination achieved a 23% ORR in patients treated with nivolumab 1 mg/kg plus ipilimumab 3 mg/kg, and a 19% ORR in those receiving nivolumab 3 mg/kg plus ipilimumab 1 mg/kg. Tumor responses occurred in patients, irrespective of PD-L1 expression. Durable responses were observed, with a promising 1-year and 2-year OS rate of 27% and 14% for nivolumab 3 mg/kg and 40% and 26% for nivolumab 1 mg/kg plus ipilimumab 3 mg/kg, respectively [82, 83]. Based on these data, a randomized phase II part of the study was launched, comparing nivolumab 3 mg/kg ($n = 147$) and nivolumab 1 mg/kg plus ipilimumab 3 mg/kg ($n = 95$). ORR was in line with those of the non-randomized part of the study (12% for nivolumab and 21% for nivolumab-ipilimumab), regardless of platinum sensitivity, line of therapy, and PD-L1 status [83]. An exploratory analysis evaluated the predictive value of TMB assessed through whole exome sequencing in both patients of the non-randomized and randomized parts of the study. Patients with TMB high (≥248 total missense mutations) were associated with the highest ORR with both nivolumab (4.8% with TMB low, 6.8% with TMB intermediate, and 21.3% with TMB high) and nivolumab-ipilimumab (22.2% with TMB low, 16.0% with TMB intermediate, and 46.2% with TMB high). Furthermore, patients with TMB high experienced the highest OS with both nivolumab (1-year OS rate of 22.1% with TMB low, 26.0% with TMB intermediate, and 35.2% with TMB high) and nivolumab-ipilimumab (23.4% with TMB low, 19.6% with TMB intermediate, and 62.4% with TMB high) [84], further confirming the potential role of TMB as a biomarker for immunotherapy across lung cancers. Based on these preliminary results, in August 2018, FDA approved nivolumab as third-line option in ED-SCLC. Nivolumab is currently being compared with second-line chemotherapy (topotecan or amrubicin) in the phase III randomized trial CheckMate 331 in PD-L1 all comers patents. Primary endpoint of the study is OS.

Similarly, pembrolizumab demonstrated efficacy in pre-treated SCLC in the phase II KEYNOTE-158 study, with 19% ORR and durable activity (6-month PFS rate: 38.9% in PDL1+ and 14.3% in PDL1- patients; 1-year OS rate: 53.1% in PDL1+ and 30.7% in PDL1- patients) [85]. In June 2019, FDA granted accelerated approval of pembrolizumab for the treatment of patients with metastatic SCLC with disease progression on or after platinum-based chemotherapy and at least one other prior line of therapy, based on tumor response rate and durability of response.

Nivolumab 240 mg every 2 weeks and nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks versus placebo were also evaluated as a maintenance strategy in ED-SCLC non-progressing patients after platinum-etoposide in the phase III trial CheckMate 451. The trial did not meet the primary endpoint, without showing a statistically significant advantage in terms of OS with the dual ICB versus placebo (HR 0.92, $p = 0.37$) [86]. PFS and ORR with both nivolumab-ipilimumab and nivolumab alone

Table 4.3 Clinical activity of immune checkpoint inhibitors in phase I-III studies in ED-SCLC

Study	Phase	*n*	Population	Arm(s)	ORR	PFS (mos)	6-mo PFS	OS (mos)	1-yr OS
First-line studies									
Reck et al. [80]	2	130	ED-SCLC	Carboplatin/paclitaxel Carboplatin/paclitaxel + concurrent ipilimumab Carboplatin/paclitaxel + phased ipilimumab	49% 32% 57%	5.2 3.9 5.2	N.A. N.A. N.A.	9.9 9.1 12.9	N.A. N.A. N.A.
Reck et al. [81]	3	954	ED-SCLC	Carboplatin/etoposide + phased ipilimumab Carboplatin/etoposide	62% 62%	4.6 4.4	N.A.	11.0 10.9	40% 40%
IMpower133 [88]	3	403	ED-SCLC	Atezolizumab 1200 mg + carboplatin/etoposide Carboplatin/etoposide	60.2% 64.4%	5.2 4.3	30.9% 22.4%	12.3 10.3	51.7% 38.2%
Maintenance studies									
Gadgeel et al. [87]	2	45	ED-SCLC	Pembrolizumab 200 mg	14.7%	1.4	20%	9.6	37%
CheckMate 451 [86]	3	834	ED-SCLC	Nivolumab 1 mg/kg + ipilimumab 3 mg/kg Nivolumab 240 mg Placebo	N.A. N.A. N.A.	1.7 1.9 1.4	20% 21% 10%	9.2 10.4 9.6	41% 44% 40%
≥2 line studies									
KEYNOTE-028 [90]	1b	24	PD-L1+, ED-SCLC	Pembrolizumab 10 mg/kg	33.3%	1.9	28.6%	9.7	37.7%
KEYNOTE-158 [85]	2	107	ED-SCLC	Pembrolizumab 200 mg	19%	2.0	38.9% (PDL1+) 14.3% (PDL1-)	9.1	53.1% (PDL1+) 30.7% (PDL1-)
CheckMate 032 (non-rand.) [82–84]	1/2	98 61	ED-SCLC	Nivolumab 3 mg/kg Nivolumab 1 mg/kg + ipilimumab 3 mg/kg	11% 23%	N.A. N.A.	N.A. N.A.	4.1 7.8	27% 40%
CheckMate 032 (randomized) [82–84]	1/2	147 95	ED-SCLC	Nivolumab 3 mg/kg Nivolumab 1 mg/kg + ipilimumab 3 mg/kg	12% 21%	N.A. N.A.	N.A. N.A.	N.A. N.A.	N.A. N.A.
Study 1108 [91]	2	21	ED-SCLC	Durvalumab 10 mg/kg	9.5%	1.5	14%	4.8	27.6%
Study 10 (NCT02261220) [92]	1	30	ED-SCLC	Durvalumab 20 mg/kg + tremelimumab 1 mg/kg	13.3%	1.8	16.3%	7.9	41.7%

were modest and in line with the results of a phase II study with pembrolizumab in the same setting [87], suggesting a modest activity of ICIs in the maintenance setting in unselected patients.

The role of ICIs in combination with chemotherapy is being explored in multiple randomized phase III trials, including CASPIAN (durvalumab ± tremelimumab + platinum-etoposide vs. platinum-etoposide), KEYNOTE-604 (platinum-etoposide ± pembrolizumab), and IMpower133 (carboplatin-etoposide ± atezolizumab). The preliminary results of IMpower133 were recently presented and showed, at a median follow-up of 13.9 months, an OS (12.3 vs. 10.3 months; HR 0.70, 95% CI, 0.54–0.91; $p = 0.0069$) and PFS (5.2 vs. 4.3 months; HR 0.77, 95% CI, 0.62–0.96; $p = 0.017$) advantage for chemo-immunotherapy combination. This 2-month OS advantage with addition of atezolizumab was associated with a 13% higher 1-year OS compared with carboplatin-etoposide alone (51.7% vs. 38.2%). The toxicity of atezolizumab plus carboplatin and etoposide was relatively favorable, with no new findings and in line with the safety profile of chemotherapy and atezolizumab alone. Interestingly, an exploratory analysis evaluating the predictive role of blood TMB, assessed through the FoundationOne CDx assay, showed a consistent OS and PFS benefit above and below the prespecified cut-offs of 10 and 16 mutations per megabase [88], questioning the role of TMB as a predictive biomarker to immunotherapy response in SCLC. This is the first trial showing a survival advantage in first-line treatment of ED-SCLC compared with platinum-etoposide after three decades of unsuccessful therapeutic efforts. However, the overall survival benefit is at the moment narrow (only 2 months of absolute OS increase) and it is still unclear whether a combinatorial approach is superior to a sequential strategy, although it is now clear that a maintenance strategy is not effective, at least in unselected patient populations. Furthermore, this schedule seems to be cost-ineffective [89]. The results of ongoing phase III studies with chemo-immunotherapy combinations and CheckMate 331 in second-line versus standard-of-care chemotherapy can provide definitive conclusions on the exact place in therapy of ICIs in ED-SCLC. Moreover, the identification of reliable predictive biomarkers is crucial to overcome the limits of PD-L1 expression (uncertain predictive value, lower expression in SCLC than observed in other solid tumors, including NSCLC) and TMB (conflicting results, tissue availability, and methods' standardization) in this aggressive disease.

On June 27, 2019, AstraZeneca announced that CASPIAN met its primary endpoint, showing a statistically significant and clinically meaningful improvement in OS in combination with etoposide and platinum-based chemotherapy as upfront therapy in patients with ED-SCLC. The full results of the study have not been presented yet and are eagerly awaited.

Conclusions and Future Perspectives

Immunotherapy represented a major breakthrough in lung cancer management and today represents a backbone of treatment in several settings. Although the benefit from this novel therapeutic approach is undeniable, several open questions still remain unanswered. Future clinical trials should define the optimal treatment duration (elective discontinuation after 2 years? Until progression?), efficacy and safety in special populations that are often excluded (patients with viral chronic infections, autoimmune disease, ECOG performance status ≥2, and active brain metastases) or underrepresented in clinical trials (elderly, racial minorities), and novel predictive biomarkers that can better select candidates for immunotherapy. The role of TMB in tissue and/or in liquid biopsy is promising, but is still far from an immediate application in clinical practice. Furthermore, the use of plasma-cell-free-DNA and other circulating biomarkers (exosomes, circulating tumor cells, and cytokines) on liquid biopsy is under active evaluation and might provide useful information that can integrate PD-L1 in the decision-making process. Finally, longer follow-up of clinical trials reported so far and post-approval studies will

provide further details on the long-term safety of ICIs either as single agent or in combination with chemotherapy.

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Immunotherapy in Gastrointestinal Malignancies

5

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Abstract

Gastrointestinal (GI) cancers represent a variety of malignancies, each with a unique interplay between the tumor and local immune microenvironment. The successes that immunotherapy, particularly immune checkpoint inhibition, has brought to various other solid tumors have largely not yielded the same benefits to patients with GI cancers. There are subsets of patients for whom immunotherapy has been FDA approved in recent years. For example, anti-PD-1 therapy is approved for patients with pretreated hepatocellular carcinoma. Additionally, patients with PD-L1-positive gastric cancer are eligible to receive anti-PD-1 therapy in the third line setting. Outside of the rare subset of patients who harbor MSI-H/dMMR tumors, the vast majority of patients with colorectal, anal, biliary tract, and pancreatic cancers have not responded to single-agent immune checkpoint inhibitors. Innovative techniques with thoughtful treatment combinations, adoptive cell therapy, CAR-T cells, as well as novel predictive biomarkers are needed to bring the benefits of immunotherapy to the majority of patients with GI malignancies.

Keywords

Immunotherapy · Immune checkpoint inhibitor · Colorectal cancer · Gastric cancer · Pancreatic cancer · Biliary tract cancer · Hepatocellular carcinoma · Anal cancer · Cancer vaccine · Adoptive cell therapy · CAR-T cells

Introduction

In 2019, over 300,000 individuals in the United States are expected to be diagnosed with a gastrointestinal (GI) cancer, and roughly 50% of that number are expected to die from a GI malignancy [1]. GI cancers represent a wide variety of diseases with distinct histopathologies, oncogenic drivers, and mechanisms of treatment resistance. In order to assess the current role of immunotherapy in GI cancers, one must consider each primary site individually. As a point of illustration, antibodies targeting PD-1 and/or CTLA-4 appear in the National Comprehensive Cancer Network guidelines for the treatment in particular cases of

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gastric, colorectal, and primary hepatic cancers, but they do not currently play a role in the standard of care treatment of virtually any patients with pancreatic cancer [2–5]. There are numerous hypotheses for the variability in response to immunotherapy by disease type in GI cancers. Among these explanations are differences in tumor mutational burden and variation in the presence and makeup of tumor-infiltrating lymphocytes [6–8].

The most significant development in the treatment of GI malignancies with immunotherapy occurred in May 2017 when the United States Food and Drug Administration (FDA) approved the PD-1 monoclonal antibody, pembrolizumab, for any pretreated unresectable solid tumor with microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR) [9]. This approval was based on the results of five early-phase single-arm trials with demonstration of a 39.6% objective response rate and 7.4% complete response rate across all solid tumors in this patient population. Ninety of the 149 patients with MSI-H or dMMR had colorectal cancer with a response rate of 36% in this patient group.

Below, we will assess the state of immunotherapy in GI cancers according to each disease site. We will evaluate the successes and failures and comment on future strategies being utilized to combat resistance to immunotherapy.

Gastroesophageal Cancer

Current Evidence

The expression of programmed death ligand 1 (PD-L1) in gastric cancer had been well established prior to the widespread use of checkpoint inhibitors in clinical practice [10, 11]. Sun et al. described in 2006 the association between PD-L1 expression by immunohistochemistry (IHC) in gastric cancer and poor clinical prognosis, with PD-L1 expressing tumors exhibiting higher rates of lymph node metastasis, larger tumor size, greater depth of invasion and decreased survival. In 2016, the results of the phase 1b KEYNOTE-012 study were published, demonstrating the tolerability and promising efficacy of pembrolizumab in the treatment of 39 patients with recurrent or metastatic PD-L1-positive gastric or gastroesophageal junction (GEJ) cancer [12]. The overall response rate (RR) was 22% and median overall survival (OS) was 11.4 months. The phase 2 KEYNOTE-059 study enrolled 259 patients with previously treated gastric and GEJ cancers, including both PD-L1-positive and -negative tumors [13]. The reported objective RR was 11.6% when including all patients, but was higher at 15.5% in the PD-L1-positive cohort, compared with 6.4% in the PD-L1-negative cohort. Complete responses were seen in patients with both PD-L1-positive and PD-L1-negative tumors. Based on the results of the KEYNOTE-059 study, the FDA granted accelerated approval to pembrolizumab for patients with PD-L1-positive recurrent or metastatic gastric or GEJ cancers. In the phase 3 KEYNOTE-061 trial, 592 patients with gastric or GEJ cancers who had progressed on first-line platinum + fluoropyrimidine chemotherapy were randomized to second-line pembrolizumab or paclitaxel [14]. The initial 489 patients were enrolled regardless of PD-L1 status, but the remaining patients were required to have a combined positive score (CPS) of at least 1, after a protocol amendment. The median OS in the pembrolizumab group was 9.1 months compared to 8.3 months in the paclitaxel group, (hazard ratio [HR] 0.82, one-sided $P = 0.04$). The study authors concluded that pembrolizumab did not significantly improve OS compared with paclitaxel for this population receiving treatment in the second-line. They also noted that protocol-specific and post-hoc subgroup analyses did suggest better efficacy of pembrolizumab in patients with higher levels of PD-L1 expression.

The role of other immune checkpoint inhibitors in patients with gastric or GEJ cancers was assessed in the ATTRACTION-2 trial, performed in East Asia and the CheckMate-032 trial, which studied a Western population [15, 16]. The ATTRACTION-2 trial randomized 493 patients with gastric or GEJ cancers who had received at least two prior lines of systemic therapy to the PD-1 monoclonal antibody, nivolumab or placebo, in a 2:1 ratio. The median OS in the nivolumab group was 5.26 months, compared to 4.14 months in the placebo group (HR 0.63,

P lt; 0.0001). Ten percent of the patients in the nivolumab group experienced grade 3 or 4 toxicity compared with 4% of the placebo group. The phase 1/2 CheckMate-032 trial randomized 160 patients with pretreated metastatic esophageal, gastric, and GEJ cancers to nivolumab alone, nivolumab 1 mg/kg + the CTLA-4 monoclonal antibody, ipilimumab 3 mg/kg or nivolumab 3 mg/kg + ipilimumab 1 mg/kg. Objective RR in each group was 12%, 24%, and 8%, respectively, with 12-month OS rates of 39%, 35%, and 24%.

The PD-L1 antibody, avelumab, has been studied in advanced gastric and GEJ cancers as well. A group of 150 patients with gastric or GEJ cancers were enrolled in the phase 1b JAVELIN Solid Tumor trial, 90 in the first-line maintenance setting and 60 in the second-line [17]. In both groups, the RR was 6.7%. Median PFS in the first-line maintenance group was 2.8 months, compared with 1.4 months in the second-line group. The JAVELIN Gastric 100 study is an ongoing phase III trial that has enrolled patients with advanced gastric and GEJ cancers who have at least stable disease following 12 weeks of first-line oxaliplatin/fluoropyrimidine chemotherapy with randomization to continuation of chemotherapy or avelumab maintenance [18]. The phase III JAVELIN Gastric 300 trial randomized 371 patients with advanced gastric or GEJ cancers to either avelumab or physician's choice chemotherapy in the third-line setting [19]. Median OSs, the primary endpoint, in the avelumab and chemotherapy arms were 4.6 and 5.0 months (HR 1.1, $P = 0.81$), respectively. Median PFS was also shorter in the avelumab arm (HR 1.73, $P > 0.99$).

Future Strategies

Early results using immunotherapy in gastroesophageal cancers have revealed that the majority of patients in the unselected population do not respond to monotherapy with checkpoint inhibitors. Adoptive cell therapy and vaccines have been similarly disappointing in their clinical efficacy. There do, however, appear to be a population of patients who do benefit from immunotherapy, beyond the MSI-H and dMMR patients. Teasing out what are the common characteristics of these patients is the challenge for the next wave of clinical trials with immunotherapy.

Among these populations being studied are patients with a high tumor mutational burden (TMB) and patients with HER-2 amplified tumors. One study being conducted in Japan is a basket study of multiple GI cancers using nivolumab monotherapy for patients with high TMB, as measured by the circulating tumor DNA Guardant360® panel [20]. At the 2019 GI Cancer Symposium, results from a phase II study of 24 patients with HER-2 amplified gastroesophageal cancers treated with pembrolizumab, trastuzumab, and chemotherapy in the first-line setting demonstrated an RR of 83% with three complete responses and a median PFS of 11.4 months [21]. This combination is currently being evaluated further in the phase III KEYNOTE 811 trial [22]. Another study in Japan is evaluating the combination of nivolumab and trastuzumab combined with chemotherapy in patients with HER-2 amplified gastric cancers [23].

One effort to maximize the efficacy of immunotherapy in gastroesophageal malignancies is to optimize the timing of treatment with checkpoint inhibitors. Moving immunotherapy to earlier lines of systemic therapy is one area of focus. Results of the phase III JAVELIN Gastric 100 study are eagerly awaited, in which avelumab is being evaluated as a maintenance therapy in the first-line setting [18]. The phase III KEYNOTE 181 study randomized patients with advanced esophageal or GEJ cancers to pembrolizumab or physician's choice in the second-line setting. While there was no difference in OS in the intention to treat population, patients with a CPS ≥ 10 treated with pembrolizumab were found to have a median OS of 9.3 compared to 6.7 with chemotherapy (HR 0.69, $P = 0.0074$) [24]. Another strategy being assessed in ongoing clinical trials involves the use of checkpoint inhibitors in earlier stages of disease. For example, the combination of perioperative avelumab in combination with chemoradiation in stage II/III esophageal cancer is being studied [25] (Table 5.1).

Table 5.1 Selected active clinical trials with immune checkpoint inhibitors in gastroesophageal cancers

Agent(s)	Patients	Phase	Clinical trial identifier	Notes
SHR1210 (PD-1) ± apatinib ± S1	Neoadjuvant for resectable gastric cancer	II	NCT03878472	Not yet recruiting; China
Multiple combinations: atezolizumab ± chemotherapy ± targeted therapy	Unresectable or metastatic gastric or GEJ cancer	Ib/II	NCT03281369	Recruiting; International – including US
Multiple combinations involving nivolumab and relatlimib (LAG-3)	Advanced gastric or GEJ cancers – first line	II	NCT03662659	Recruiting; International – including US
Nivolumab + ipilimumab + chemoradiation	Perioperative for resectable gastric cancer	I/II	NCT03776487	Recruiting; US
Margetuximab (HER2) + Pembrolizumab	Advanced HER2+ gastric or GEJ cancer	I/II	NCT02689284	Active, not recruiting; International – including US
Pembrolizumab + TS-1 + cisplatin/oxaliplatin	Advanced gastric cancer – first line	IIb	NCT03382600	Recruiting; Japan

Colorectal Cancer

Current Evidence

The subset of patients with colorectal cancer (CRC) who have benefited most from advances in immunotherapy have been those whose tumors are MSI-H or harbor dMMR. MSI-H CRC represents the minority of CRC cases, less than 20%, when all stages are included, though they are associated with a better prognosis compared with microsatellite stable (MSS) CRC, particularly in early-stage disease [26, 27]. Of patients with metastatic CRC, only 4–5% are MSI-H, and the majority of these cases result from sporadic mutations in mismatch repair proteins, rather than being associated with Lynch Syndrome [28]. The immunogenicity of MSI-H tumors has been well-described, with the primary hypothesis being that their high mutational load leads to a higher density of tumor-infiltrating lymphocytes (TIL) and increased expression of checkpoint receptors [29–31].

MSI-H status has subsequently proven to be a powerful predictive biomarker for response to immune checkpoint inhibitors. This was initially demonstrated with the use of pembrolizumab in the phase II KEYNOTE-016 study, which included a cohort of patients with pretreated metastatic dMMR and mismatch repair-proficient CRC [32]. Pembrolizumab significantly increased median PFS (HR 0.10, $P < 0.001$) and median OS (HR 0.22, $P = 0.05$) in the dMMR cohort compared with the mismatch repair-proficient cohort. The KEYNOTE-164 study evaluated pembrolizumab in MSI-H CRC after at least two lines of therapy (cohort A) and at least one line of therapy (cohort B). In cohort A, the RR was 27.9%, and in cohort B the RR was 32% with two complete responses and a 12-month OS rate of 76% [33, 34]. The results of these and other early-phase studies using pembrolizumab in pretreated patients with solid tumors and dMMR led to the 2017 FDA primary site-agnostic approval of pembrolizumab in this setting [9].

The CheckMate 142 study was a phase II clinical trial assessing nivolumab monotherapy or nivolumab in combination with ipilimumab in patients with MSI-H and MSS metastatic CRC [35]. The results from the initial 74 patients with MSI-H metastatic CRC treated with nivolumab monotherapy were published in 2017. The objective RR was 31.1%, all of which were partial responses, and the median duration of response was not reached at the time of publication. Median PFS was 14.3 months, 12 month OS was

73%, and median OS was not reached. The results from the combination nivolumab plus ipilimumab arm were reported in 2018 [36]. There were 119 patients who received combination therapy with an objective RR of 54.6%, including 3.4% with complete responses. Impressively, 83% of responding patients had responses that lasted at least 6 months, with a median duration of response that was not reached. Neither median PFS nor OS were reached in this group, though 12 month PFS and OS were 71% and 85%, respectively. The rate of grade 3–4 treatment-related adverse events (TRAEs) was higher in the combination arm (32%) compared with nivolumab monotherapy (20%), but the rates of any-grade TRAEs were similar (73% vs 70%). Based on the results of the CheckMate 142 study, the FDA granted accelerated approval to nivolumab and combination nivolumab plus ipilimumab for patients with MSI-H or dMMR metastatic CRC [37, 38].

Results from the Canadian Cancer Trials Group (CCTG) CO.26 study were presented at the 2019 Gastrointestinal Cancers Symposium [39]. This phase II trial randomized patients with refractory metastatic CRC 2:1 to the combination of the anti-PD-L1 antibody, durvalumab, plus the anti-CTLA-4 antibody, tremelimumab, or best supportive care. None of the 180 patients enrolled were known to have MSI-H tumors. There was no difference in median PFS between the arms (1.8 vs. 1.9 months), but there was a trend towards increased OS with a median OS of 6.6 months in the treatment arm and 4.1 months in the best supportive care arm (HR 0.72, $P = 0.07$).

Future Strategies

With the promising results of many phase II clinical trials in metastatic CRC, particularly in the MSI-H/dMMR space, a number of phase III have been initiated to confirm the benefits of immunotherapy in this malignancy. Most of these studies are evaluating checkpoint inhibitors in patients with metastatic CRC. KEYNOTE 177 is evaluating MSI-H metastatic CRC patients treated with pembrolizumab compared with standard chemotherapy in the first-line setting [40]. The COMMIT Trial is evaluating the PD-L1 inhibitor, atezolizumab, in a three-arm study in MSI-H metastatic CRC patients in the first-line setting: atezolizumab monotherapy vs. FOLFOX plus atezolizumab plus bevacizumab vs. FOLFOX plus bevacizumab [41]. The strategy of employing immunotherapy in the first-line setting rather than in refractory patients was also evaluated in a cohort of patients in the CheckMate 142 study. Results from this group which evaluated nivolumab plus ipilimumab in MSI-H/dMMR patients with treatment-naive metastatic CRC were presented at the European Society for Medical Oncology (ESMO) 2018 Congress [42]. Forty-five patients received combination checkpoint inhibition with an overall RR of 60%. PFS and OS at 12 months were 77% and 83%, respectively. The results of these studies may significantly alter the current standard of care for front-line therapy in patients with MSI-H metastatic CRC.

Another avenue of exploration in patients with MSI-H CRC is in treatment of these patients with stage III disease. Two ongoing studies evaluating adjuvant checkpoint inhibitors are the ATOMIC and POLEM trials [43, 44]. The ATOMIC trial is evaluating adjuvant FOLFOX with or without atezolizumab. The POLEM trial is evaluating maintenance avelumab for 24 weeks after completion of adjuvant chemotherapy and includes patients with POLE exonuclease domain mutations.

Despite the successes of several checkpoint inhibitors in the treatment of patients with MSI-H metastatic CRC, the vast majority of patients with metastatic CRC have not realized any benefit from treatment with these agents. Strategies aimed at turning these immunologically "cold" cancers into inflamed tumors are desperately being sought. Several ongoing clinical trials combining radiation therapy with immunotherapy are aiming to harness the potential of the "abscopal effect" in treating MSS CRC [45–47]. In this hypothesis, radiation therapy would have a local effect of cell death and surge in inflammatory cytokines. Downstream effects of the cytokine storm include upregulation of tumor

Table 5.2 Selected active clinical trials with immune checkpoint inhibitors in colorectal cancers

Agent(s)	Patients	Phase	Clinical trial identifier	Notes
Avelumab + Cetuximab + Irinotecan	Refractory metastatic MSS CRC	II	NCT03608046	Recruiting; Belgium
Avelumab + chemotherapy	Stage 3 MSI-H or POLE mutant CRC – adjuvant therapy	III	NCT03827044	Recruiting; UK
Chemotherapy ± atezolizumab	Stage 3 dMMR CRC – adjuvant therapy	III	NCT02912559	Recruiting; US
Cabozantinib + atezolizumab	Multiple advanced solid tumors including CRC	Ib/II	NCT03170960	Recruiting; International – including US
Multiple combinations including atezolizumab ± selicrelumab (CD40) ± targeted therapy	Metastatic CRC	Ib/II	NCT03555149	Recruiting; International – including US
FOLFOX + bevacizumab ± nivolumab	Metastatic CRC – First line	II/III	NCT03414983	Recruiting; International – including US
FOLFOX + bevacizumab ± atezolizumab and atezolizumab alone	Metastatic dMMR CRC	III	NCT02997228	Recruiting; US
Nivolumab + Trametinib ± ipilimumab	Refractory metastatic CRC	I/II	NCT03377361	Recruiting; International – including US
Tremelimumab + durvalumab	Metastatic CRC to liver prior to metastasectomy	I	NCT02754856	Recruiting; US
Nivolumab + Relatlimab (LAG-3)	Advanced MSS CRC	II	NCT03642067	Recruiting; US

neoantigen expression and priming of the immune microenvironment, eventually leading to off-target effects of immune activation on other sites of disease. The addition of immune checkpoint inhibitors to cytotoxic chemotherapy, such as FOLFOX, has also been proposed as a mechanism by which to promote an immune response to CRC [48, 49]. Combining immune checkpoint inhibitors with therapies targeting MEK or VEGF has also been studied as a strategy to expand the benefits of immunotherapy to MSS CRC patients with preliminary results indicating some responses in this groups of patients [50, 51]. However, the phase III study, IMblaze370, reported in 2019 that it did not meet its primary endpoint of improved OS with third-line combination atezolizumab and MEK inhibitor, cobimetinib, compared with regorafenib in an almost entirely MSS population [52]. It is clear from these results that significant hurdles still remain in bringing the efficacy of immunotherapy to the majority of patients with CRC (Table 5.2).

Anal Cancer

Current Evidence

Squamous cell carcinoma (SCC) of the anus is a less common malignancy of the GI tract. The pathophysiology of anal cancer resembles other mucosal malignancies caused by the human papillomavirus (HPV), as this infectious agent is associated with the vast majority of cases of anal SCC [53–55]. The safety and efficacy of pembrolizumab was evaluated in the phase Ib multicohort study, KEYNOTE 028 [56]. One cohort of this study included 24 patients with PD-L1-

positive advanced anal SCC. The overall RR was 17% and disease control rate was 58%. 64% of patients experienced TRAEs. The multi-center phase 2 trial, NCI9673, evaluated the clinical benefit of single-agent nivolumab in patients with pretreated metastatic anal SCC [57]. 37 patients received treatment with an RR of 24%, including two complete responses. Immunohistochemistry analysis of tumor samples from patients in this study demonstrated a significantly higher concentration of PD-1 and PD-L1 expression in tumors of those who responded to nivolumab compared with those that did not respond. Authors from both of these studies concluded that given the lack of standard of care treatment for patients with advanced disease, checkpoint inhibitors warrant further investigation as a novel therapeutic option for patients with SCC of the anus.

Future Strategies

Similar to other cancers in which early-phase studies identified evidence of clinical benefit of single-agent PD-1/PD-L1 inhibition, the addition of an anti-CTLA-4 antibody has been proposed to increase clinical activity. An amendment to the NCI9673 study added an additional arm to the phase II study, which will evaluate the combination of nivolumab and ipilimumab in patients with refractory metastatic SCC of the anus [58]. This portion of the study is expected to be completed in early 2020. Pembrolizumab is also being studied as monotherapy in a phase II study in refractory patients with metastatic anal SCC [59]. A phase II study in France will be assessing the efficacy of the combination of atezolizumab and an HPV-directed vaccine, UCPVax, in patients with HPV positive cancers [60]. In an effort to move immunotherapy into earlier stages of anal cancer, a randomized phase II study is evaluating the addition of maintenance nivolumab after combined modality therapy compared to observation for patients with high-risk stage II-IIIB SCC of the anus [61].

Hepatobiliary Cancer

Current Evidence

Hepatocellular Carcinoma

In terms of access to treatment, patients with advanced hepatocellular carcinoma (HCC) have benefited more than any other GI malignancy from the development of immune checkpoint inhibitors. The liver maintains a crucial role in the body's complex system of immune regulation and becomes disrupted during heightened inflammatory states from pre-HCC liver conditions such as chronic hepatitis B and C infections.

Tremelimumab was the first immune checkpoint inhibitor studied in HCC [62]. Of the 20 patients in the initial clinical trial who received treatment, 17 were assessable for response, of whom 17.6% had a partial response. All of these patients had chronic hepatitis C virus infection, and the tolerance of the anti-CTLA-4 antibody was fairly good. In 2017, single-agent nivolumab was granted accelerated approval by the FDA as a second-line agent without any biomarker requirement [63]. This approval was based on the CheckMate 040 study, a phase I/II trial which included 262 total patients, some in the first-line and some having had been previously treated with sorafenib [64]. The safety profile was manageable in this study, and the objective RR was 20% (95% Confidence Interval, 15–26) with nivolumab 3 mg/kg in the dose-expansion phase. The phase II KEYNOTE 224 trial evaluated pembrolizumab in patients with HCC previously treated with sorafenib. Of the 104 patients treated, 18 (17%) experienced a response, with one complete response. OS was 54% at 12 months. Based on the results of KEYNOTE 224, pembrolizumab carries a category 2B recommendation from the NCCN in patients with pretreated HCC [4].

Biliary Tract Cancers

Biliary tract cancers (BTCs) are a rare subset of GI malignancies, comprising cholangiocarcinoma and gall bladder carcinoma. Clinical trials assessing the efficacy of immune checkpoint

inhibitors in patients with BTCs have been largely disappointing. As is the case across the spectrum of solid tumors, the group of patients who have seen clinical benefit are the small population of BTC patients who have tumors with MSI-H or dMMR, a percentage reported as low as 1% and as high as 10% [65, 66]. The phase II KEYNOTE-158 trial was a basket trial that assessed the response to pembrolizumab among several advanced solid tumors. A total of 104 patients with BTC were included, none of whom had MSI-H tumors [67]. The overall RR was 5.8%, with 17 patients (16%) achieving a best response of stable disease. The median PFS was 2.0 months, and the median OS was 9.1 months.

Future Strategies

Novel treatment strategies with immunotherapy in HCC are primarily aiming to introduce immune checkpoint inhibitors in earlier lines of therapy. There is sound biological rationale in this approach, as the immunosuppressive nature of the HCC tumor microenvironment tends to become more pronounced as the disease progresses [68]. The phase III CheckMate 459 study is a randomized control trial comparing first-line sorafenib and nivolumab in patients with advanced HCC [69]. Another intriguing strategy being explored is the combination of oral tyrosine kinase therapy with immune checkpoint inhibitors. For example, two expansion arms have been opened in the CheckMate 040 study which will analyze the effect of cabozantinib plus nivolumab with or without ipilimumab [70]. Whether the potential benefits of increased response to these combinations will outweigh the likely worsened toxicity profile is uncertain.

For patients with BTCs, the role of immunotherapy in the treatment of advanced disease is uncertain. The available evidence thus far suggests that single-agent checkpoint inhibitors will not provide any benefit to BTC patients outside of the minority with MSI-H/dMMR tumors. Other immune targets such as T-cell immunoglobulin and mucin-domain containing 3 (TIM3), lymphocyte activation gene (LAG3), and indoleamine 2,3-dioxygenase (IDO) are currently being studied in various combinations [71]. Outside of immune checkpoint inhibitors, other immunotherapy strategies that have been evaluated in BTCs include vaccines and adoptive cell therapy. Two antigens that are expressed on >80% of BTCs include mucin protein 1 (MUC1) and Wilm's tumor protein 1 (WT1) [71]. In a phase I study of eight BTC patients with gemcitabine and a WT1 vaccine, half of the patients achieved stable disease at 2 months [72]. Another phase I study with a MUC1 vaccine in eight BTC and pancreatic cancer patients yielded an even lower disease control rate [73]. A clinical trial assessing adjuvant adoptive T-cell therapy combined with a postoperative dendritic cell vaccine in resectable intrahepatic cholangiocarcinoma patients, increases in median PFS and OS were seen from 7.7 to 18.3 months and 17.4 to 31.9 months, respectively, when compared to surgery alone [74]. Patients with BTCs will be included in a phase I pilot trial at the University of Texas MD Anderson Cancer Center that evaluates CD8+ T-cell therapy with pembrolizumab in a variety of advanced GI malignancies [75].

Pancreatic Cancer

Current Evidence

Pancreatic ductal adenocarcinoma (PDAC) in many ways represents the quintessential immunologically "cold" tumor. The microenvironment of PDAC tumors is characterized by a low density of CD8+ T-cells, disrupted expression of major histocompatibility complexes (MHC), and immunosuppressive enzymes and cytokines [76, 77]. Several studies have concluded that PD-L1 expression in PDAC is associated with a poor prognosis [78]. In the face of these obstacles, several clinical trials have evaluated the efficacy of immune checkpoint inhibitors in patients with advanced PDAC.

There were 14 patients with PDAC who received single-agent nivolumab in the landmark phase I trial whose results were published in 2012 [79]. However, none of the PDAC patients

achieved an objective response. One patient with PDAC was included in a phase I study of pembrolizumab as a single agent and failed to show a response to treatment [80]. Ipilimumab as a monotherapy at a 3 mg/kg dose was evaluated in a phase II trial for patients with advanced PDAC [81]. None of the 27 patients included in the study achieved an objective response, though one patient continued ipilimumab beyond initial progression and achieved a significant delayed response. In a study at Johns Hopkins, ipilimumab was combined with the GM-CSF cell-based vaccine, GVAX in patients with advanced PDAC. Compared to ipilimumab alone, the combination of ipilimumab and GVAX demonstrated trends towards increased median OS (3.6 vs. 5.7 months, HR 0.51, $P = 0.07$) and 1 year OS (7% vs 27%) [82].

The combination of chemotherapy and immunotherapy was assessed in a phase Ib/II study that evaluated the combination of gemcitabine, nab-paclitaxel, and pembrolizumab in patients with metastatic PDAC [83]. Seventeen patients were treated, with 11 evaluable in the treatment-naïve phase II component. The authors reported three patients with a partial response, with one as long as 15 months, and a disease control rate of 100%. For treatment-naïve patients, median PFS and OS were 9.1 and 15.0 months, respectively.

Single-agent immune checkpoint inhibition is currently only a viable treatment option for patients with MSI-H/dMMR PDAC, a population that may represent as little as <1% of all PDAC patients [84, 85].

Future Strategies

Similar to other cancer types, interest has been shown in the combination of radiation therapy immune checkpoint inhibitors. Results from a recent clinical trial were presented at the 2019 Gastrointestinal Cancers Symposium which include 51 patients with advanced PDAC who were treated with a combination of stereotactic body radiation therapy (SBRT) and durvalumab with or without tremelimumab. The authors reported an overall RR of 9.6%, with two patients having achieved partial responses lasting greater than 12 months. Results from a phase I trial combining hypofractionated radiotherapy with pembrolizumab were recently published [86]. Four patients with advanced PDAC were included, and none of the four demonstrated an objective response by RECIST criteria. A number of other studies are currently ongoing, which include the combination of radiation therapy and immunotherapy in patients with PDAC [87–89].

The targeting of CD40 with an agonist has been demonstrated to reverse immune suppression in PDAC murine models by way of macrophage activation, and combination of a CD40 agonist with gemcitabine led to tumor regression in human PDAC tumors [90]. At the American Association for Cancer Research (AACR) 2019 Annual Meeting, an interim analysis of a phase Ib study was presented that combined gemcitabine, nab-paclitaxel, the CD40 agonist, APX005M with or without nivolumab in patients with treatment-naïve metastatic PDAC [91]. Of the 24 patients with evaluable disease, 20 experienced a reduction in tumor size. Thirteen patients discontinued therapy due to an adverse event. These preliminary results have led to the initiation of a randomized phase II study with these agents.

Adoptive cell therapy and chimeric antigen receptor T-cell (CAR-T) therapy are additional approaches that have gained momentum for future evaluation in PDAC patients. Adoptive transfer of MUC1-specific T-cells has been studied in PDAC mouse models with evidence of anti-tumor effect [92]. An ongoing study at the National Cancer Institute is evaluating adoptive T-cell therapy in a variety of metastatic solid tumors [93]. CAR-T cells have significantly advanced the treatment options of certain patients with relapsed and refractory hematologic malignancies. Attempts to carry these benefits over to patients with solid tumors are in their beginning stages. For patients with PDAC in particular, various CAR-T cells have been engineered to recognize MUC1, carcinoembryonic antigen (CEA), and mesothelin (MSLN) in mouse models [94–96]. There is cautious optimism that CAR-T cell therapy for PDAC may represent a novel immunotherapeutic strat-

egy that could be applicable to a broader population of patients than those who currently benefit from immune checkpoint inhibitors.

Conclusion

The age of immunotherapy is in full effect throughout the field of oncology. The excellent tolerability, high response rates, and, most significantly, durable responses, seen in patients treated initially with checkpoint inhibitors in the field of melanoma, have now been expanded to many patients with lung, urothelial, and kidney cancers, among other solid tumor types. GI malignancies have by and large been noticeably absent from those who have realized the benefits of immunotherapy outside of a few groups of patients. Among these patients who have received FDA approval for treatment with immune checkpoint inhibitors are patients with gastric and GEJ cancers whose tumors are positive for PD-L1 and patients with HCC who have previously received sorafenib. Response rates in these populations remain relatively low, but those who do respond still have the potential to achieve durable clinical benefit. Further research into other predictive biomarkers is being conducted and represents a desperate need in the field of immunotherapy.

For the majority of patients with GI malignancies, including almost all patients with pancreatic, biliary tract, and colorectal cancers, new strategies are needed beyond single-agent checkpoint inhibitors if immunotherapy is going to make its way into the clinic. Novel combination strategies with chemotherapy, radiotherapy, or targeted therapy that are currently being studied may provide an additional immunologic boost that some of these tumors need to overcome resistance to immunotherapy. The next generation of cancer vaccines, adoptive cell therapy, and CAR-T cells for GI malignancies represent additional avenues that may be able to harness the promise of immunotherapy. As each year passes, the knowledge and understanding of susceptibilities and resistance mechanisms of GI cancers to current immune therapies will continue to grow. Optimism remains that at some point the era of immunotherapy will reach the majority of patients with GI cancers, though when and in what form remains to be seen.

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6 Current Landscape of Immunotherapy in Genitourinary Malignancies

Omar Alhalabi, Hind Rafei, Mehmet Asim Bilen, and Amishi Yogesh Shah

Abstract

The past decade has witnessed a revolution of immune checkpoint inhibitors in the treatment of multiple tumor types, including genitourinary cancers. Immune checkpoint inhibitors improved the treatment outcomes of patients with metastatic renal cell carcinoma and metastatic urothelial carcinoma. In prostate cancer, the role of immunotherapy with checkpoint inhibitors is not yet established, but clinical trials investigating their use are ongoing. Other immunotherapeutic approaches that have been explored in these malignancies include cytokines, vaccines, and cellular therapy. Ongoing studies are exploring the use of immunotherapy combinations as well as combination with chemotherapy and targeted therapy in these types of tumors. The use of immunotherapy beyond the metastatic setting is an active area of research. Moreover, there is a great interest in biomarker development to predict response to immunotherapy and risk of toxicity. This chapter is a comprehensive review of the immunotherapeutic approaches, both approved and investigational, for the treatment of renal cell carcinoma, urothelial carcinoma, and prostate cancer.

Keywords

Immunotherapy · Checkpoint inhibitors · Cellular therapy · Cytokines · Vaccines · Renal cell carcinoma · Urothelial carcinoma · Prostate cancer

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Immunotherapy for Renal Cell Carcinoma

Renal cell carcinoma (RCC) represents around 90% of all cancers of the kidney, with clear-cell renal cell carcinoma (ccRCC) being the most common subtype (accounting for approximately 85% of all RCC) [1]. Nearly one third of patients newly diagnosed with RCC have metastatic or

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advanced disease [2, 3]. Risk stratification of patients with newly diagnosed metastatic RCC is essential both to determine prognosis and to plan treatment as a key part of clinical decision-making. One tool for risk assessment for metastatic RCC was established by the International Metastatic Renal Cell Carcinoma Database (IMDC), which integrates six clinical factors that were shown to have an independent prognostic values in a multicenter study of 645 patients [4]. Those criteria include (1) anemia, (2) neutrophilia, (3) thrombocytosis, (4) hypercalcemia, (5) Karnofsky performance status <80, and [6] <1 year from diagnosis to first-line systemic therapy. Patients with none of these factors have favorable disease, while patients with 1–2 factors have an intermediate-risk disease, and patients with more than three factors have poor-risk disease. Another risk assessment tool is the Memorial Sloan Kettering Cancer Center (MSKCC) model in advanced RCC that similarly stratifies patients into favorable, intermediate, or poor risk [5]. Both clinical and laboratory data are included in this model: low Karnofsky performance status, high lactate dehydrogenase, low serum albumin, high corrected serum calcium, and time from diagnosis to systemic treatment [5]. Recently, the model was updated to incorporate genomic data, where the mutation status of *BAP1, PBRM1*, and *TP53* has been shown to have an independent prognostic value in patients with advanced or metastatic RCC treated with first-line tyrosine kinase inhibitors (TKIs) (Table 6.1).

The treatment of ccRCC has witnessed tremendous evolution over the past decade both with the introduction of targeted therapies and with the advent of immunotherapy. Multitargeted TKIs, which inhibit vascular endothelial growth factor receptors (VEGFR) and mammalian target of rapamycin (mTOR), have been standard therapies for the treatment of metastatic RCC (mRCC) [6, 7]. Within the past 3 years, immune checkpoint inhibitors (CPIs) have significantly changed the natural history of metastatic RCC. The combination of ipilimumab with nivolumab has shown significant efficacy in this setting and has been approved for first-line treatment of intermediate- to poor-risk patients with metastatic RCC (further detailed below) [8]. A more intricate understanding of the immune system and its interaction with the tumor microenvironment as well as the different pathways involved in tumorigenesis led to the investigation of new immunotherapeutic modalities in mRCC. Data from clinical trials exploring the combination of immune CPIs with TKIs also show promise for the expansion of available therapeutic options. However, it is important to be mindful of the potential for increased toxicity and cost with these combinations. Other exciting forms of immunotherapies are being investigated, including vaccines, adoptive cell therapy, and newer immunotherapy combinations. These combined efforts will likely continue to transform the field and offer novel options for patients with RCC. Strategies to extrapolate the success of immunotherapy from the metastatic setting to the adjuvant setting are underway. Herein, we present an overview of the various immunotherapies approved and being investigated in the treatment of ccRCC (Fig. 6.1).

Table 6.1 Memorial Sloan Kettering Cancer Center (MSKCC) and International Metastatic Renal Cell Carcinoma Database (IMDC) prognostic tools

Variable	MSKCC	IMDC
Karnofsky performance status	0–1	0–1
Time from diagnosis to systemic treatment <1 year	0–1	0–1
Anemia	0–1	0–1
Neutrophilia		0–1
Thrombocytosis		0–1
LDH > 1.5 × ULN	0–1	
Calcium >10 mg/dL	0–1	0–1

LDH lactate dehydrogenase, *ULN* upper limit of normal

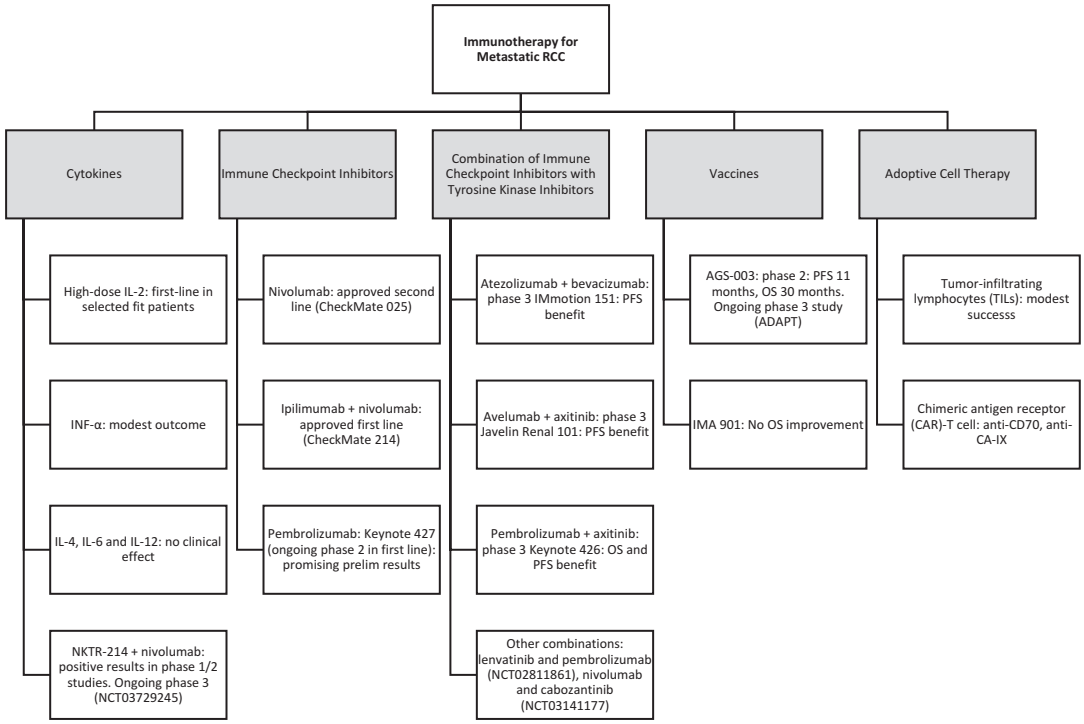

Fig. 6.1 Immunotherapy for the treatment of metastatic renal cell carcinoma. RCC renal cell carcinoma, IL interleukin, INF interferon, Prelim preliminary, PFS progression-free survival, OS overall survival

Rationale for Immunotherapy in RCC

RCC is known to be particularly resistant to chemotherapy, and this could be attributed to many features of this disease. First, RCC is derived from proximal tubules expressing high levels of multidrug-resistant (MDR) P-glycoprotein [9]. Moreover, a number of studies have identified cancer stem cells as a tumor subpopulation that has a self-renewal ability and confers resistance to chemotherapy [10]. However, RCC is exquisitely sensitive to immunotherapy relative to other tumor types. Early observations that removal of the primary tumor can trigger immune responses that could lead to spontaneous regression of metastatic RCC, particularly in the lung, were strong indicators that RCC could be amenable to immunotherapy [11]. Moreover, profuse tumor infiltration with T cells, natural killer (NK) cells, macrophages, and dendritic cells (DC) has been demonstrated in a number of studies, suggesting an inherent role of antitumor immunity [12, 13].

These observations were reinforced by the demonstrated clinical activity of the very first forms of immunotherapies for RCC with interleukin 2 (IL-2) and interferon-alpha (INF-α), although major clinical benefit was seen in only a minority of patients. In 1992, the US Food and Drug Administration (FDA)-approved high-dose intravenous IL-2 for the treatment of RCC [14–16]. This was based on preliminary data showing an overall response rate (ORR) of 15% as well as a 5% complete response (CR) [15]. In a follow-up study, CR was 7% and median duration of response was at least 80 months [17]. Its use, however, was limited by the significant side effect profile as well as the inability to predict response. In an attempt to decrease toxicity, low-dose IL-2 was also investigated and compared to high-dose IL-2, but ORR was much lower with low dose (21% with high dose vs 13% with low dose, $P = 0.048$) [18]. A recent prospective study of 352 patients [19] and another retrospective study of 391 patients [20] suggested an extended clinical

benefit of high-dose IL-2. Stable disease (SD) as a measure of best response was present in 39% and 32% of these cohorts, respectively, and was associated with survival benefit [19, 20]. INF-α, despite being better tolerated and having a broader applicability, had more modest outcomes (overall survival (OS) of 2.5 months greater than placebo) without the durable responses demonstrated with high-dose IL-2 [21].

Until 2005, IL-2 and INF-α were the only two approved therapies for RCC and the median survival was approximately 1 year [22]. Since then, a number of new therapies have been approved that led to a paradigm shift in the treatment of RCC including mTOR inhibitors (everolimus, temsirolimus), VEGF inhibitors (sunitinib, sorafenib, axitinib, pazopanib, cabozantinib, bevacizumab, lenvatinib), and more recently the revolutionary immunotherapies with immune CPIs [23, 24]. The use of high-dose IL-2 as first-line therapy is restricted to well-selected younger patients with a good performance status and without comorbidities.

While harnessing the immune system has long been on interest in the treatment of mRCC, the addition of CPIs to the therapeutic armamentarium was a breakthrough due to the unique immune-editing features they provide, which serve to alter the balance between tumor and immune system [25]. The immune-editing mechanism comprises three phases: elimination, equilibrium, and escape [26]. The elimination phase comprises killing of malignant cells through CD8+ T cells and NK cells. There are some cancer cells that elude the initial host defense mechanisms and survive in a constraint environment in the presence of immune cells in the equilibrium phase. Finally, evasion of the immune surveillance by cancer cells comprises the escape phase [26–28]. Under constant pressure from the immune system, tumor cells thrive through mechanisms that allow them to resist immune cells [29] such as downregulation of antigens, loss of major histocompatibility complex class I (MHC-I) to interfere with antigen presentation, or upregulation of inhibitory pathways and checkpoints such as programmed death-ligand 1 (PD-L1)/programmed death-1 (PD-1) [30–34]. Ongoing efforts to counteract these immune escape mechanisms are driving the scientific research and clinical trials in the exploration of the best treatment modalities for RCC.

Immune Checkpoint Blockade in Locally Advanced or Metastatic RCC (Fig. 6.2)

Nivolumab

Nivolumab is a fully humanized IgG4 anti-PD-1 antibody that blocks the interaction of PD-1 with its ligands PD-L1 and PD-L2 and thus interfering

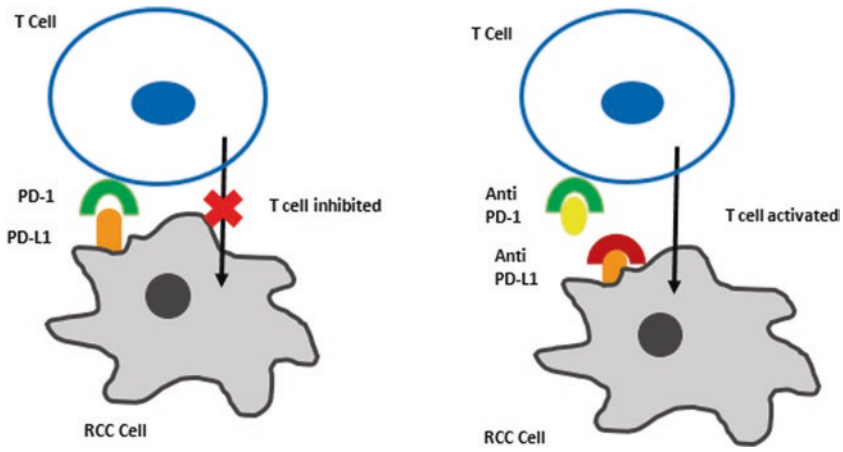

Fig. 6.2 Principle of immune checkpoint inhibition. RCC renal cell carcinoma, PD-1 programmed death 1, PD-L1 programmed death-ligand 1

with the immune response inhibitory pathways [35]. The first sign of efficacy of nivolumab in RCC was demonstrated in two phase 1 trials [36, 37]. A total of 296 patients with various metastatic solid tumors including 34 patients with heavily pretreated metastatic RCC received various doses of nivolumab [37]. At a minimum follow-up of 50.5 months, ORR was 29% and one patient had a CR in the 10 mg/kg cohort. For all doses, the ORR was 29.4%. Among the responders, 30% achieved objective response by 8 weeks (first assessment) and 70% achieved response by 16 weeks (second assessment). Median duration of response was 12.9 months (8.4–29.1). At the time of analysis, 40% of responses were ongoing [36]. These early data were very encouraging for the clinical benefit of immune checkpoint blockade in the treatment of RCC.

The promising activity of the phase 1 trial led to the launching of a phase 2 study of nivolumab in metastatic ccRCC, which consisted of a randomized blinded multicenter clinical trial [38]. Three arms were included in the study with 1:1:1 randomization to three different doses of nivolumab: 0.3, 2, and 10 mg/kg. The randomization was stratified based on the number of prior therapies (1 vs >1 (70%)) and MSKCC risk group (favorable/intermediate vs poor (25%)). The primary endpoint was evaluation of the dose–response relationship as measured by progression-free survival (PFS); secondary end points included ORR, OS, and safety. One hundred sixty-eight patients were enrolled: 60 received nivolumab 0.3 mg/kg, 54 received nivolumab 2 mg/kg, and 54 received nivolumab 10 mg/kg. Median PFS was 2.7 months (80% CI: 1.9–3.0 months), 4.0 months (80% CI: 2.8–4.2 months), and 4.2 months (80% CI: 2.8–5.5 months) for the 0.3, 2, and 10 mg/kg groups, respectively. ORR was 20%, 22%, and 20% in the 0.3, 2, and 10 mg/kg arms, respectively. Continued response beyond 24 months was noted in 14 of the 35 (40%) responders. With a follow-up of at least 24 months, median OS was 18.2 months (80% CI: 16.2–24.0 months) in 0.3 mg/kg arm, 25.5 months (80% CI: 19.8–28.8 months) in the 2 mg/kg arm, and 24.7 months (80% CI: 15.3–26.0 months) in the 10 mg/kg arm. Adverse events (AE) were observed at similar rates between the three arms. The most common treatment-related AE was fatigue (24%, 22%, and 35%, respectively). Nineteen patients (11%) experienced grades 3–4 treatment-related AEs (nausea, arthralgia, and elevation of alanine and arginine transaminases), of which four of these patients were in the 0.3-mg/kg group, 14 patients were in the 1-mg/kg group, and 1 patient was in the 10-mg/kg group [38].

The successful phase 2 again led to the investigation of nivolumab in metastatic ccRCC in a phase 3, multicenter, international, open-label randomized study – CheckMate 025 trial [39]. This study compared the efficacy of nivolumab with everolimus, which is an approved second-line agent for the management of metastatic RCC after progression on an anti-VEGF agent [40]. The primary endpoint was OS rather than PFS, which had been the case in several prior phase 3 trials of new agents in metastatic RCC [41, 42]. This was based on the mechanism of action of nivolumab which enhances inflammation around the tumor causing a radiographic appearance of progression in the absence of true clinical progression, a phenomenon called "pseudoprogression." ORR was higher in the nivolumab group compared to everolimus (25% vs 5%, odds ratio, 5.98 [95% CI: 3.68–9.72]; $P < 0.001$). The median OS was significantly better in the nivolumab group at 25.0 months (95% CI: 21.8 to not estimable [NE]) compared to 19.6 months (95% CI: 17.6–23.1) in the everolimus group. However, the median PFS was not statistically significantly different between the nivolumab arm and the everolimus arm, 4.6 months (95% CI: 3.7–5.4) versus 4.4 months (95% CI: 3.7–5.5), respectively. The clinical benefit of nivolumab encompassed all the MSKCC risk groups. The AEs were similar to those seen in earlier trials.

A separate study investigated the health-related quality of life (HRQoL) in the different treatment groups of CheckMate 025 [43]. HRQoL measures analysis was performed using Functional Assessment of Cancer Therapy-Kidney Symptom Index–Disease-Related

Symptoms (FKSI-DRS) and European Quality of Life (EuroQol)-5 Dimensions (EQ-5D) questionnaires. More patients had a clinically meaningful (i.e., an increase of at least 2 points from baseline) HRQoL improvement with nivolumab (200 [55%] of 361 patients) versus everolimus (126 [37%] of 343 patients; $p < 0.0001$). Median time to HRQoL improvement was shorter in patients given nivolumab (4.7 months, 95% CI 3.7–7.5) than in patients given everolimus (median not reached, NE-NE) [43]. Based on the positive results of the CheckMate 025 study, the FDA approved nivolumab for the management of advanced metastatic RCC after progression on first-line therapy, on November 23, 2015. Limited data exist on the role of nivolumab monotherapy in the frontline treatment of advanced RCC.

Nivolumab Plus Ipilimumab

The increased effectiveness seen in advanced melanoma with the combination of nivolumab and ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) CPI, led to the investigation of this combination in RCC as well. The phase 3 CheckMate 214 trial established the efficacy and safety of ipilimumab and nivolumab combination in metastatic clear-cell RCC [8]. Previously untreated patients with advanced or metastatic clear-cell RCC were randomized to either sunitinib (50 mg per day for 4 weeks out of every 6-week cycle) or the combination of ipilimumab (1 mg/kg) and nivolumab (3 mg/kg) given every 3 weeks for four doses and were then followed by nivolumab (3 mg/kg). At a median follow-up of 25 months, OS was significantly higher in the combination group as opposed to the sunitinib group in the intention-to-treat population (median not reached with the combination vs 32.9 months in the sunitinib group, HR 0.68, 99.8% CI 0.49–0.95). The ORR was also significantly higher with ipilimumab and nivolumab (39% vs 32%), but there was no difference in PFS (median 12.4 vs 12.3 months, HR 0.98).

In the subgroup of 847 patients with intermediate- or poor-risk disease, the OS was significantly higher with the combination of ipilimumab and nivolumab compared to sunitinib (median not reached vs 26 months, HR 0.63, 95% CI 0.44–0.82). The ORR was also significantly higher in the combination group as opposed to sunitinib (42% vs 27%). The disease control rate (DCR) was 72%. While the median PFS was increased with the immunotherapy combination, statistical significance was not attained (11.6 vs 8.4 months, HR 0.82, 95% CI 0.64–1.05). However, PFS and response benefit appeared to be increased in patients with PD-L1 expression ≥1% (214 patients). More pronounced benefit was seen in patients with intermediate- or poor-risk disease as well as PD-L1 expression ≥1% (ORR 58% vs 25%, median PFS 22.8 vs 5.9 months, HR 0.48, 95% CI 0.28–0.82). The CR rate in this group was 16%. On the other hand, in the group of patients with intermediate- or poor-risk disease and PD-L1 expression <1% (562 patients), only OS was significantly increased (median not reached for either group, HR 0.73, 95% CI 0.56–0.96), while there was no significant difference between the combination and sunitinib in either the ORR (37% for the combination vs 28% for sunitinib) or median PFS (11 months for the combination vs 10.4 months for sunitinib, HR 1.0, 95% CI 0.74–1.36). While the study was underpowered to draw significant conclusions from the favorable-risk disease group, exploratory analyses showed that the response rate was lower with the ipilimumab-plus-nivolumab combination compared with sunitinib (29% vs 52%), and PFS was shorter (median 15.3 vs 25.1 months, HR 2.17, 95% CI 1.46–3.22). Survival data are not yet available for the favorable-risk group; however, the maturing data suggest that the nivolumab–ipilimumab combination has better outcomes in the favorable-risk group than initially presented [44].

The toxicity profile of the combination of nivolumab and ipilimumab was consistent with that observed with the use of the combination for other indications and favored the combination group over sunitinib. Grade 3 or 4 AEs occurred in 46% of patients in the immunotherapy combination group versus 63% in the sunitinib group. The most common grade 3 or 4 AEs on the immunotherapy combination group were increased lipase

(10%), diarrhea (4%), and fatigue (4%). The most common AEs in the sunitinib group were hypertension (16%), palmar-plantar erythrodysesthesia (9%), and increased lipase (7%). Immune-related AEs of any grade occurred in 80% of patients who received ipilimumab with nivolumab, among those 35% received high-dose corticosteroids. It is important to note, however, that the treatment was discontinued due to treatment-related AEs in 22% of the patients who received the immunotherapy combination and in 12% of patients who received sunitinib. Moreover, death due to treatment-related AEs occurred in eight patients in the ipilimumab and nivolumab group (causes of death in each patient were pneumonitis, bronchitis, pneumonia and aplastic anemia, lower gastrointestinal hemorrhage, hemophagocytic syndrome, sudden death, lung infection, and liver toxicity) and in four patients in the sunitinib group (two due to cardiac arrest, one due to heart failure, and one due to multiorgan failure).

A separate study reported on patient-reported outcomes (PROs) from the CheckMate 214 study [45]. PROs were assessed according to three measurement tools: the Functional Assessment of Cancer Therapy–Kidney Symptom Index-19 (FKSI-19), which is validated for kidney cancer; Functional Assessment of Cancer Therapy-General (FACT-G), which is validated for cancer in general; and EuroQol Five-Dimensional, Three-Level (EQ-5D-3L), which is validated for general health status. Patients in the immunotherapy combination arm reported better PROs than those who received sunitinib for the two of the three assessment tools, from the start of treatment through about 2 years. The average change in the overall FKSI-19 score between baseline and 103 weeks was 4.00 (95% CI 1.91–6.09) for the combination arm compared with −3.14 (95% CI –6.03 to −0.25) for the sunitinib arm ($P < 0.0001$) and the average change in overall FACT-G score was 4.77 (95% CI 1.73–7.82) for the combination arm versus −4.32 (95% CI −8.54 to −0.11) for the sunitinib arm ($P = 0.0005$). EQ-5D-3L scores, however, were not significantly different between treatment groups.

Based on the results from the CheckMate 214 clinical trial, the combination of ipilimumab and nivolumab was approved by US FDA for the treatment of previously untreated patients with intermediate- to poor-risk advanced or metastatic RCC, on April 16, 2018.

Pembrolizumab

Pembrolizumab, a humanized anti-PD1 IgG4 antibody, is being investigated as single-agent CPI for advanced or metastatic RCC in the Keynote 427 phase 2 trial [46]. Preliminary results from cohort A of this trial were presented at the 2018 American Society of Clinical Oncology (ASCO) annual meeting. One hundred ten patients with previously untreated advanced or metastatic clear-cell RCC were enrolled and received pembrolizumab 200 mg every 3 weeks for 2 years or until confirmed progressive disease, unacceptable toxicity, or patient's decision to withdraw. At a median follow-up of 12.1 months (range 2.5–16.8), pembrolizumab demonstrated an ORR of 38.2% (95% CI 29.1–47.9), with a CR rate of 2.7% and a partial response (PR) rate of 35.5%. The DCR was 59%. The median time to response was 2.8 months, and 74.8% of patients had responses lasting for 6 months or more. Median PFS was 8.7 months (95% CI 6.7–12.2), and the 6-month PFS rate was 60.2%. OS was not reached, and the 6-month OS rate was 92.7%. In the subgroup of 69 patients with intermediate- and poor-risk disease, ORR was 42% (95% CI 30.2–54.5) compared to 31.7% (95% CI 18.1–48.1) in the subgroup of 41 patients with favorable-risk disease. In an analysis based on PD-L1 expression, ORR was 50% (95% CI 34.9–65.1), the CR rate was 6.5%, and the PR rate was 43.5% in the subgroup of 46 patients with tumors overexpressing PD-L1 (combined positive score (CPS) ≥1; tumor and immune cell PD-L1 expression) compared to an ORR of 26.4% (95% CI 15.3–40.3) and all responses being partial in the 53 patients who had low tumor expression of PD-L1 (CPS < 1).

The safety profile of pembrolizumab was consistent that seen in pembrolizumab used for other indications. Treatment-related grade 3–5 AEs

occurred in 22.7% of patients. The most common treatment-related AEs were pruritus (27.3%), fatigue (24.5%), diarrhea (19.1%), rash (15.5%), arthralgia (12.7%), and hypothyroidism (10%). The most common immune-mediated AEs of any grade were hypothyroidism (10.9%), pneumonitis (4.5%), hyperthyroidism (4.5%), colitis (2.7%), hepatitis (1.8%), severe skin reaction (1.8%), and myositis (1.8%). Treatment-related AEs led to the discontinuation of treatment in 12 patients, and treatment-related death due to pneumonitis occurred in 1 patient.

Combined Antiangiogenic Plus CPI Immunotherapy in Locally Advanced or Metastatic RCC

Pembrolizumab with Axitinib

The combination of immune checkpoint blockade with pembrolizumab and VEGF receptor tyrosine kinase inhibition with axitinib has shown antitumor activity in patients with previously untreated advanced RCC [46, 47]. This was confirmed in a phase 1b trial of the combination in the front-line setting of metastatic RCC with ORR of 73% (95% CT 59–84) [48].

The phase 3 Keynote-426 trial demonstrated an OS and PFS benefit of the combination of pembrolizumab and axitinib in the front-line treatment of advanced or metastatic RCC [49]. This study included 861 patients who were randomly assigned to oral sunitinib once daily or to combination therapy. Pembrolizumab was given every 3 weeks along with oral axitinib twice daily. At a median follow-up of 12.8 months, the median OS was not reached in either arm, and the 12-month survival rates were 90% in the combination arm versus 78% in the sunitinib arm (HR for death 0.53, 95% CI 0.38–0.74). Median PFS was 15.1 months in the pembrolizumab plus axitinib arm versus 11.1 months in the sunitinib arm (HR for progression or death 0.69, 95% CI 0.57–0.84), and ORR was 59% versus 36%, respectively. The DCR with the immunotherapy combination was 83.8%. The benefit of the combination of pembrolizumab with axitinib was observed irrespective of the PD-L1 expression or the disease risk category. Grade 3 or higher AEs of any cause occurred in 75.8% of patients in the pembrolizumab–axitinib group and in 70.6% in the sunitinib group. Based on the results of this trial, the combination of pembrolizumab with axitinib was recently FDA approved as a first-line treatment in advanced RCC on April 19, 2019, regardless of IMDC risk score or PD-L1 status. This recent approval poses interesting considerations in the frontline treatment of mccRCC. As compared to historic data in mccRCC, the data from CheckMate-214 and Keynote-426 suggest that OS is the new benchmark for approval of frontline therapies. Furthermore, endpoints such as CR rate, DCR, and treatment-free survival (TFS) may nuance the choice of which therapy to choose in case-specific circumstances. The role of PD-L1 status yet remains indeterminate in therapy selection in mccRCC.

Avelumab with Axitinib

Another combination of antiangiogenesis inhibition with immunotherapy composed of avelumab and axitinib showed promising results in phase 3 study. The Javelin Renal 101 phase 3 trial involved 886 treatment-naive patients with advanced clear-cell RCC, and the patients were randomly assigned to the combination of avelumab and axitinib versus sunitinib [50]. In the group of patients with PD-L1-positive tumors (560 patients), the median PFS was 13.8 months with avelumab with axitinib compared to 7.2 months with sunitinib (HR for progression or death 0.61; 95% CI 0.47–0.79; $P < 0.001$), and ORR was 55.2% compared to 25.5%, respectively. In the overall population, the DCR with the avelumab and axitinib arm was 81%. The median PFS was higher in the combination arm at 13.8 months compared to 8.4 months (HR 0.69; 95% CI, 0.56–0.84; $P < 0.001$). At a median follow-up for OS of 11.6 months and 10.7 months in the two groups, 37 patients and 44 patients had died,

respectively; the role of the regimen in the treatment landscape of mccRCC will become clearer as OS data mature. AEs during treatment occurred in 99.5% of patients in the avelumab and axitinib group and in 99.3% of patients in the sunitinib group. Grade 3 or higher AEs were similar between the two groups, occurring in 71.2% and 71.5% of patients, respectively.

Atezolizumab with Bevacizumab

Positive results of the phase 2 trial of bevacizumab and atezolizumab [51] led to a phase 3 trial of this combination in 915 untreated patients with metastatic RCC (IMmotion151). Patients were randomized to either receive atezolizumab with bevacizumab or sunitinib [52]. Median PFS was longer in the combination arm as opposed to the sunitinib arm (11.2 vs 8.4 months, HR 0.83, 95% CI 0.70–0.97), ORR was 37% and 33%, and CR rates were 5% and 2%, respectively. In the PD-L1-positive population, median PFS was longer with atezolizumab with bevacizumab than with sunitinib (11.2 vs 7.7 months, HR 0.74, 95% CI 0.67–0.96). ORR was 43% (9% CRs) compared with 35% (4% CRs) in the combination and the sunitinib groups, respectively. OS data are immature to analyze in both the overall intention-to-treat and the PD-L1-positive populations.

Other Combinations

Other phase 3 trials are currently ongoing that investigate different combinations including a trial comparing three arms: the combination of lenvatinib and pembrolizumab versus the combination of lenvatinib with everolimus versus sunitinib (NCT02811861). Another phase 3 trial is comparing the combination of nivolumab and cabozantinib with sunitinib (NCT03141177). Other combination studies of sunitinib in combination with nivolumab and pazopanib in combination with either nivolumab or pembrolizumab were stopped early because of increased toxicity with synergistic fatigue and liver toxicity [53, 54]. Table 6.2 summarizes phase 3 combination trials.

Other Immunotherapy Approaches in Locally Advanced or Metastatic RCC

Vaccines

The use of vaccines to enhance the immune recognition of tumor has been investigated in RCC. AGS-003 is an autologous immunotherapy prepared from fully matured and optimized monocyte-derived DCs, which are co-electroporated with amplified tumor RNA from nephrectomy specimens plus synthetic CD40L RNA. AGS-003 was evaluated in combination with sunitinib in an open-label phase 2 study of 21 patients with intermediate and poor risk, treatment-naive metastatic RCC [55]. The median PFS was 11 months (95% CI 6.0–19.4), and the median OS was 30 months (95% CI 9.4–57.1). These results lead to the currently ongoing phase 3 ADAPT study (NCT01582672) where patients with metastatic RCC undergoing debulking nephrectomy are randomly assigned to either sunitinib with AGS-003 or sunitinib alone. AGS-003 was given as eight intradermal injections in the first year followed by boosters every 3 months.

Another cancer vaccine IMA901 that is based on tumor-associated peptides was administered in the front-line setting to patients with metastatic RCC who were positive for HLA-A∗02 antigen and have positive results in a phase 2 study [56]. A phase 3 study, IMPRINT, investigated its addition to sunitinib [57]. Three hundred thirty-nine patients were randomly assigned to sunitinib or sunitinib plus IMA901. The vaccine was given as an intradermal injection in conjunction with 75 μg of granulocyte macrophage colony-stimulating factor (GM-CSF) for up to 10 doses. There was no improvement in median OS, the primary endpoint of the study, with the addition of the vaccine (33.2 months vs not reached, HR 1.34, 95% CI 0.96–1.86, $P = 0.08$).

Other Cytokines

Multiple interleukins have been studied for the use in RCC, including IL-4 [58], IL-6 [59], and IL-12 [60, 61], but their antitumor activities were modest or toxicities of some were concerning.

Table 6.2 Phase 3 trials of the combination of immune checkpoint inhibitors with tyrosine kinase inhibitors in metastatic renal cell carcinoma

Trial name/ clinical trial number	Treatment arm	Control arm	Primary end point	Treatment arm vs control arm					Grades 3–4 adverse events
				PFS (months)	OS (months)	CR	ORR	DCR	
Clear/ NCT02811861	Lenvatinib/ pembrolizumab vs everolimus/ lenvatinib	Sunitinib	PFS	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
IMmotion 151/ NCT02420821	Bevacizumab/ atezolizumab	Sunitinib	PFS in PD-L1 positive; OS in all patients	11.2 vs 8.4 PD-L1 positive: 11.2 vs 7.7	Immature to analyze	5% vs 2% PD-L1 positive: 9% vs 4%	37% vs 33% PD-L1 positive: 43% vs 35%	75% vs 72% PD-L1 positive: 75% vs 69%	Grades 3–4: LFT abnormalities (3%) in treatment arm vs LFT abnormalities (4%) in control arm Notably 16% of patients on treatment arm received systemic steroids
Javelin Renal 101/ NCT02684006	Axitinib/avelumab	Sunitinib	PFS	13.8 vs 8.4 PD-L1 positive: 13.8 vs 7.2	11.6 vs 10.7	3.4% vs 1.8% PD-L1 positive: 4.4% vs 2.1%	51.4% vs 25.7% PD-L1 positive: 55.2% vs 25.5%	81% vs 71.2% PD-L1 positive: 81.8% vs 68.6%	irAEs: 38.2% in treatment arm Grades 3–4: 4% in treatment arm (HTN, HFS, diarrhea, increase ALT) vs 7% in control arm (fatigue, thrombocytopenia, anemia)
Keynote 426/ NCT02853331	Axitinib/ pembrolizumab	Sunitinib	PFS and OS	15.1 vs 11.1 (no difference in PD-L1 expression or risk category)	Not reached in either arm	5.8% vs 1.9%	59.3% vs 35.7%	83.8% vs 75.1%	Grades 3–4: diarrhea (9%), HTN (22%), HFS (5%), increased ALT (13%), increased AST (7%) in treatment arm vs diarrhea (5%), HTN (19%), fatigue (7%) in control arm
CheckMate 9ER/ NCT03141177	Cabozantinib/ nivolumab	Sunitinib	PFS	Ongoing	Ongoing	Ongoing		Ongoing	Ongoing

vs versus, *PFS* progression-free survival, *OS* overall survival, *CR* complete response, *ORR* objective response rate, *DCR* disease control rate, *LFT* liver function tests, *irAE* immune-related adverse events, *HTN* hypertension, *HFS* hand–foot syndrome, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

The combination of IL-2 and IL-12 was shown to be efficacious in preclinical studies, but this was not reproduced in human clinical trials [62].

A novel prodrug of pegylated IL-2, NKTR-214, has gained recent interest due to promising results. NKTR-214 preferentially binds to CD122 on the surface of immune cells and stimulates their proliferation. In both preclinical and clinical studies, NKTR-214 was shown to result in the expansion of these cells and mobilization into the tumor microenvironment [63]. The PIVOT phase 1/2 study is currently evaluating the combination of nivolumab with NKTR-214 in advanced solid malignancies. The preliminary results were presented at the ASCO 2018 annual meeting [64] and reported safety, efficacy, and biomarker data for patients enrolled in the phase 1 dose-escalation stage of the study and for the first patients consecutively enrolled in select dose expansion cohorts in phase 2. In metastatic treatment-naive RCC, prespecified efficacy criteria were met for ORR in stage 1 with 7/11 (64%) patients achieving a PR. Median time on study for 26 patients in stage 2 was 5.6 months. ORR was 46%. ORR in 17 patients with PD-L1-negative tumors was 53% and in 7 patients with PD-L1-positive tumors was 29%. One of two patients (50%) with unknown PD-L1 baseline status experienced a PR. The most common treatment-related AEs in the overall population including 283 patients with various solid malignancies were flu-like symptoms (58.7%), rash (44.5%), fatigue (42.0%), and pruritus (31.4%). Grade 3 or higher AEs occurred in 14.1% of patients, and treatment was discontinued in 2.1% of patients due to treatment-related AEs. Treatment-related immune-mediated AEs occurred in 3.5% of patients. One nivolumab-related grade 5 pneumonitis was reported.

The positive results of the phase 1/2 study led to phase 3 studies including a clinical trial comparing the combination of NKTR-214 with nivolumab to oncologist choice of either sunitinib or cabozantinib for the front-line treatment of metastatic RCC (NCT03729245). Work is also being done to evaluate the role of triplet therapy with nivolumab, ipilimumab, and NKTR-214 in mRCC (NCT02983045).

Adoptive Cell Therapy

The generation and adoptive transfer of tumor-infiltrating lymphocytes (TILs) has demonstrated durable complete responses in metastatic melanoma [65], but the success rates of this strategy are much lower in other cancers [66]. A number of studies have shown that the tumor microenvironment in RCC harbors tumor-reactive T cells [66, 67], but the magnitude and quality of responses generated by these cells and compared to other tumor types remain to be determined. Only modest success was elucidated with TIL therapy in RCC in previous clinical trials [68]. It is important to note, however, that these early trials did not use current advanced methods of TIL harvest and expansion and preoperative chemotherapy regimens, opening the horizon to revisit TIL therapy in RCC. This is especially true with the tremendous success achieved with immunotherapy in RCC, proving that immunologic control of this disease is feasible.

The use of chimeric antigen receptor (CAR)-T cells was also investigated in preclinical and clinical studies. CAR-T cells are generally T cells isolated from the patient and engineered to target TAAs [69]. Second- and third-generation CARs are engineered to express a co-stimulatory molecule, such as CD28, 4-1BB, CD27, ICOS, or OX40, to increase the antitumor effect, proliferation, and survival of CAR-T cells [70]. The greatest challenge in solid tumors is the identification of antigen targets. Many TAAs are also expressed at low level on healthy tissue so that an immune response could have serious toxicities. Carboxy-anhydrase-IX (CA-IX) expression in metastatic RCC was exploited for CAR-T cell therapy [71]. CA-IX is a metalloprotease that is considered a tumor-associated antigen (TAA) in RCC. However, it is also expressed on several normal tissues, such as the epithelium of the gastric mucosa, small intestine, duodenum, and biliary tree [72, 73]. Preclinical studies of first generation of CA-IX-directed T cells in RCC showed a robust cytokine production and cytotoxic activity was demonstrated [74]. Lamers et al. treated three patients with CA-IX-positive

metastatic RCC with first-generation anti-CA-IX CAR-T cells along with IL-2 administration but no prior lymphodepletion [75]. Two of these patients developed grades 2–4 liver toxicity, and liver biopsies showed T-cell infiltration around bile ducts causing cholangitis. CA-IX was overexpressed on the biliary ductal epithelium. Antibodies against the murine-derived scFv were detected in all three patients. In a subsequent study, the investigators preadministered unmodified antibody from which scFv was derived to saturate the liver before CAR-T cell administration and abrogate liver toxicity [71]. With this approach, no hepatotoxicity was observed in all four patients who received antibody pretreatment. No human anti-mouse antibodies against the cellular product were detected in patients who received the pretreatment, suggesting that the inflammation caused by the cholangitis possibly contributed to the generation of human anti-mouse antibodies. Unfortunately, no meaningful clinical responses were seen despite CAR-T cell persistence for 3–5 weeks.

Other antigens are being investigated for the exploitation of corresponding CAR-T cells including CD70 that is significantly overexpressed in RCC. Preclinical evaluation of CD70-targeting CD27-containing CAR in CD70-expressing tumors including RCC supported its safety and efficacy [76]. A clinical trial of anti-CD70 CAR in CD70-expressing solid tumors including RCC is currently recruiting (NCT02830724).

Multiple mechanisms are involved in T-cell suppression and are mediated via myeloid-derived suppressor cells (MDSCs) [56, 77], through arginase-mediated downregulation of the T-cell receptor ζ chain [78] as well as circulatory regulatory T cells (Tregs) [79, 80]. Sunitinib is a multikinase inhibitor for the treatment of metastatic RCC, and it has been shown to decrease MDSCs [81], enhance type-I INF responses, and decrease Treg function [82]. It would be intriguing to investigate the role of VEGFR-TKI in preconditioning and maintenance after CAR-T cell therapy in RCC [83].

Adjuvant Immunotherapy

The success of immunotherapy in advanced and metastatic RCC led to its investigation as adjuvant therapy. Adjuvant IL-2 and INF-α in locally advanced, nonmetastatic RCC following nephrectomy were investigated in multiple clinical trials. A randomized phase 3 study compared INF-α to observation following nephrectomy for pT3–4 M0 and/or pathologically lymph node-positive disease and involved 283 patients [84]. At a median follow-up of 10.4 years, OS was 7.4 years in the INF arm compared to 5.2 years in the observation arm, but this difference was not statistically significant ($P = 0.09$). There was also no difference in recurrence-free survival (RFS) between the two arms (3 vs 2.2 years, $P = 0.33$). The treatment-related toxicity was prominent in this study with 12% of patients experiencing grade 4 AEs (most commonly neutropenia and myalgias). No treatment-related deaths occurred.

Another phase 3 trial was conducted by the Cytokine Working Group which randomized patients to either receive single administration of high-dose bolus IL-2 or observation following complete resection of pT3–T4 Nx or pTany N1–3, and/or M1 RCC [85]. The study was stopped after a per protocol interim analysis showed no improvement in disease-free survival (DFS), which was initially anticipated to be 30% improved in the IL-2 group, despite full accrual. Again, IL-2 toxicity was severe. Eighty-eight percent of patients experienced at least grade 3 or 4 AEs, most commonly hypotension (52% required vasopressor support).

Vaccines were also investigated as potential adjuvant immunotherapeutic agents. Reniale®, an autologous RCC tumor vaccine derived from a lysate of a patient's own renal tumor, has been investigated in the adjuvant setting. A phase 3 trial randomized 379 patients with suspected RCC undergoing nephrectomy to either receive the tumor vaccine or observation postoperatively if the disease was high risk (pT2-T3b, pN0–3) [86]. The vaccine was administered every 4 weeks for a total of six doses. There was a mod-

est 5-year PFS improvement in the vaccine arm (77.4% vs 67.8%, $P = 0.02$). The survival benefit was more pronounced in pT3 tumors. Despite the positivity of this phase 3 trial, concerns about its applicability arose as the pathologic staging was based on the 1993 UICC classification, the lack of blinding, the fact that patients in the control arm did not receive placebo injections, and the exclusion of a large number of patients (179 patients) after randomization due to non-RCC histology, loss to follow-up within 6 months, and other reasons.

Vitespen (HSPPC-96) is a vaccine derived from heat shock protein-peptide complex from autologous tumor [87]. Its use in the adjuvant setting was investigated in a multicenter phase 3 randomized trial of patients with cT1b-T4N0M0 or TanyN1-2M0 RCC and planned to undergo curative nephrectomy [88]. The vaccine was administered weekly for 4 weeks and then every 2 weeks as long as the Vitespen supply lasted or until disease progression. There was no statistically significant difference in RFS or OS between the experimental and control groups. Preplanned and post hoc subgroup analyses suggested that vitespen improves RFS in patients with lower stage (T1b-T2) high-grade tumors. Therapy was well tolerated and no grade 3 or 4 AEs occurred.

Immune checkpoint blockade is also being actively investigated in the adjuvant setting. The PROSPER trial (NCT03055013) is currently exploring nivolumab in both the neoadjuvant and adjuvant settings. Patients with cT2–T4 and/or cN+ disease are randomized to observation or to two courses of nivolumab prior to radical or partial nephrectomy, followed by 9 months of adjuvant nivolumab. This design took advantage of the robust antitumor immune responses elicited in the presence of the primary tumor and, hence, allows for nivolumab administered neoadjuvantly to amplify its efficacy in the adjuvant setting.

The IMmotion 010 (NCT03024996) phase 3 trial is evaluating the efficacy of atezolizumab in the adjuvant treatment of RCC. Patients with pT2 Fuhrman grade 4, pT3a Fuhrman grade 3 or 4, and pT3b-4, or any N+ disease were included. The study is limited to clear-cell or clear-cell component RCC and RCC with or without sarcomatoid dedifferentiation. Primary endpoint is DFS.

Additional clinical trials of other immune CPIs in the adjuvant setting are ongoing, including pembrolizumab (KEYNOTE-564, NCT03142334) and the combination of ipilimumab with nivolumab (CheckMate914, NCT03138512). To date, there are no data on the use of CPIs in the adjuvant setting in RCC.

Biomarkers for Response

The research of biomarkers to predict response to immunotherapy, in general, and in RCC, in particular, is critical but remains challenging. Different trials of immune CPIs in RCC used different assays for the assessment of tumor expression of PD-L1. The CheckMate 025 and 214 trials used Dako PD-L1 IHC 28-8 pharmDx test to assess for PD-L1 expression. While nivolumab efficacy was not affected by PD-L1 expression in CheckMate 025, patients with tumor expressing PD-L1 more than 1% showed a worse OS suggesting rather a prognostic more than a predictive role of PD-L1 [39]. On the other hand, CheckMate 214 showed that PFS benefit was more pronounced in patients expressing PD-L1 (more or equal to 1%) [8]. OS was maintained in all categories. Results from the two trials suggest that PD-L1 IHC expression is not a predictor of response in patients with metastatic RCC receiving immune CPIs. Not only did different trials use different tests for the detection of PD-L1 expression with varying results, but the inconsistencies seen in results across trials make PD-L1 a challenging marker to rely on in predicting response in RCC. Intratumoral heterogeneity of PD-L1 expression was demonstrated by a multisite tumor sampling strategy [89], which identified a greater number of positive cases than those detected by current sampling protocols as the same tumor exhibited multiple regions with positive and negative expressions.

Another biomarker used in other diseases to predict response to immunotherapy is tumor

mutational burden (TMB) and nonsynonymous expression where higher tumor expression of neoantigens was linked to a favorable response to immunotherapy [90, 91]. In RCC, immunotherapy was shown to be effective in higher risk categories where tumor mutational load is high, which warrants additional investigation of the role of TMB as a biomarker of response with immunotherapy [92]. In CheckMate-214, subgroup analysis showed significantly better results of the combination of ipilimumab with nivolumab in the intermediate- to poor-risk disease category, which could be partly related to higher TMB and abundance of neoantigens in these worse risk categories [8]. Contrary to these thoughts, however, TMB across different IMDC or MSKCC prognostic criteria was not shown to be different [92]. Moreover, TMB did not differ between clear-cell and sarcomatoid components of different tumor samples, suggesting that TMB is not associated with worst clinical features, although this hypothesis needs to be further investigated [93]. Another study carried out whole exome and transcriptome sequencing of nine patients with metastatic RCC receiving nivolumab [94]. They found out that RCC had relatively few nonsynonymous mutations and neoantigens. Interestingly, among the nivolumab-treated patients, neoantigen load was significantly higher in nonresponders compared to responders ($P = 0.048$), but nonsynonymous mutation load was not. An exceptional responder who experienced CR (PFS > 30 months) had outlying higher expression of selected immune-related genes compared to the eight other patient samples ($P < 0.05$ for PD-L1, PD-L2; $P < 0.01$ for CTLA4, PD-1, PRF1; $P < 0.001$ for GZMA, BTLA, CD8A) and was in the top 1–5% of expression of these genes among all The Cancer Genome Atlas (TCGA) data. While the sample size of this study is too small to draw a generalizable conclusion, this study could suggest that TMB role in predicting response to immunotherapy is RCC is different from that seen in other tumor types.

Other biomarkers are being actively investigated. An analysis of the phase 3 IMmotion151 trial identified gene signatures in RCC that correlate with improved PFS in patients treated with atezolizumab plus bevacizumab compared to sunitinib [95]. These findings were presented at the European Society for Medical Oncology (ESMO) 2018 Congress. In the study by Rini et al., a group of patients with a gene signature showing high expression of T-effector cells had improved PFS with the combination of atezolizumab and bevacizumab compared with sunitinib (12.45 vs 8.34 months). On the other hand, in patients with low expression of T-effector cell genes, a smaller increase in PFS was seen with the combination compared to sunitinib (9.72 vs 8.41 months). Moreover, they studied a signature of angiogenesis-associated genes and found that in the group of patients with low expression of these genes, median PFS was higher in patients treated with the combination of atezolizumab with bevacizumab as opposed to sunitinib (8.94 vs 5.95 months). The improvement in PFS in the group of patients with high expression of angiogenesis-associated genes was not as robust in patients treated with the combination compared to sunitinib, 12.45 versus 10.2, respectively. They also demonstrated that in the sunitinib-treated group of patients, sunitinib was associated with higher PFS in the high versus low expression of angiogenesis-related genes (10.12 vs 5.95 months, respectively).

Other markers are being explored including PD-L2 expression, the gastrointestinal microbiome composition, and others. This is an active area of research, and the future, perhaps, involves a combination of biomarkers used together to predict response.

Future Directions for Immunotherapy in RCC

Current immunotherapeutic indications in advanced RCC include nivolumab monotherapy after prior antiangiogenic use in metastatic RCC, the combination of nivolumab and ipilimumab in the frontline setting of intermediate- to poor-risk disease metastatic RCC, and the combination of pembrolizumab and axitinib in frontline mRCC. More recent trials of immunotherapy-based treatment approaches combining CPIs

with antiangiogenesis agents show promise and could be approved soon to add to the current immunotherapy landscape. Many other ongoing trials will help elucidate more therapeutic options. No data currently exist on the role of immunotherapy in the adjuvant setting after curative nephrectomy, but this is an area of current investigation. Other immunotherapeutic strategies in the management of RCC are being investigated, including vaccines, adoptive cell transfer, cytokines, etc.

The breakthrough of immunotherapy in RCC is promising, but it is essential to realize that maximal clinical benefit will be hard to achieve without continuous efforts to optimize immune-related toxicities that have been shown to hinder the widespread use and applicability of these treatments. A multidisciplinary approach with assistance from specialists such as pulmonologists, endocrinologists, cardiologists, gastroenterologists, and others is necessary. Moreover, evidence-based and algorithmic approaches in handling toxicity need to be standardized in the management of immune-related toxicities. More research is required in the field of stratifying and prioritizing patients who will draw maximum gain from the use of immunotherapies as well as those who are predisposed to higher toxicities. The discovery and development of newer ways to manipulate the immune system so to potentiate T-cell and immune cell responses in the presence of immune CPIs or other immunotherapies will lead to increase in the scope of benefit from these breakthrough treatments.

Immunotherapy for Urothelial Carcinoma

Bladder cancer is the sixth most common cancer with an estimate of 80,470 new cases to be diagnosed in the United States in 2019 and 17,760 deaths during the same year [96]. Urothelial carcinoma (UC) is the most common subtype in the United States and Europe [97, 98]. Bladder cancer is most frequently diagnosed among people aged 65–74 [99]; therefore, it is important to factor other medical comorbidities into treatment choices. Approximately, 75% of new cases are nonmuscle invasive and characterized by a tendency to recur [100, 101]. On the other hand, muscle-invasive disease (extension past the basement membrane) and metastatic UC represent the other 25% and have a significantly worse outcome [102]. Despite the effectiveness of platinum-based therapies, metastatic UC still has a modest median OS of around 15 months [100, 103]. Similarly, second-line chemotherapies provide a suboptimal OS [104, 105]. CPIs flipped the equation for both platinum-refractory and platinum-ineligible patients [106–113]. Actionable genetic alterations, which are found in >50% of high-grade UCs, are gaining interest as well especially fibroblast growth factor receptor (FGFR) alterations [114]. Additionally, several TAAs in UC are attractive targets for antibody drug conjugate (ADC) development, which are being studied alone and in combinations with CPIs [115, 116]. Here, we describe the FDA-approved immune-oncology (I-O) modalities and the prominent investigational strategies for early or advanced stage UC.

Rationale for Immunotherapy in UC

In 1976, immune modulation was found to be helpful in the management of nonmuscle-invasive bladder cancer (NMIBC) with the use of Bacillus Calmette–Guerin (BCG) [117]. Forty years later, genomic studies showed that bladder cancer ranks third after melanoma and non-small-cell lung cancer in terms of somatic mutation rate [118, 119]. This high mutational burden and genomic instability seem to determine sensitivity to immunotherapy [120, 121]. Genomic alterations are translated into foreign proteins that could be recognized by cytotoxic T cells and potentiate cancer cells response to CPI [122]. However, infiltrating CD4+ and CD8+ T cells expresses high levels of PD-1 in UC [123], rendering them ineffective at eradicating tumors. Furthermore, expression of PD-L1 on UC cells is associated with higher grade, stage, rate of postoperative recurrence, and risk of death after cystectomy [123–125]. These findings provide

the rationale for using anti-PD1 and anti-PD-L1 immunotherapies to treat patients with UC.

Immunotherapy for NMIBC

Following endoscopic removal of tumors, size, multifocality, grade, and other risk factors help determine the further steps of management of NIMBC. Risk of recurrence determines the type and duration of intravesicular therapy or even cystectomy if needed [126].

BCG Vaccine

The first trial to show the benefit of BCG in NMIBC was done by Lamm et al. in 1980 and showed reduction in tumor recurrence [127]. This was followed by the FDA approval for this indication in 1990 [128]. In terms of reducing recurrences, BCG post resection of high-grade NMIBC is superior to observation and superior to intravesicular chemotherapy [129–131]. Based on SWOG8507, BCG is commonly given as an induction phase (6 weekly instillations) followed by maintenance (BCG each week for 3 weeks given 3, 6, 12, 18, 24, 30, and 36 months) [132]. BCG failure can be classified into BCG refractory disease (persistence of high-grade tumors after induction and one maintenance course) and BCG-relapsing disease (reappearance of disease after a disease-free state). Understanding the mechanism of BCG immune response is essential to develop strategies for BCG refractory disease. BCG is thought to invade the urothelium inducing an innate immune response followed by a T helper 1-based adaptive immune response that prevents tumor recurrence. It is unclear if this immune response is tumor specific or BCG specific with a side effect of antitumor activity [128]. A combination of intravesicular pembrolizumab + intravesicular BCG is being investigated in BCG naive high-risk NIMBC and BCG-relapsing NIMBC (NCT02808143).

BCG Refractory Population

Several years prior to anti-PD-1/PD-L1 clinical use in UC, Inman et al. reported that PD-L1 expression was abundant in the BCG-induced bladder granulomata in 11 of 12 patients failing BCG treatment. SWOG1605 (NCT02844816) is a phase 2 trial based on the reported efficacy of atezolizumab in metastatic UC and the known expression of PD-L1 expression in NMIBC after BCG therapy. This trial will evaluate the activity of atezolizumab in BCG-unresponsive high-risk NMIBC [133]. Two similar ongoing clinical trials with pembrolizumab + BCG (NCT02324582) and nivolumab + BCG (CheckMate 9UT; NCT03519256) in BCG-refractory patients are aiming to address this question as well.

Immunotherapy for Muscle-Invasive Bladder Cancer (MIBC)

In addition to the resection of MIBC, most patients require further treatment with cystectomy, partial cystectomy, neoadjuvant, adjuvant therapy, or a combination of these modalities [134, 135]. Neoadjuvant cisplatin-based chemotherapy prior to cystectomy for MIBC patients who are resectable provides 5% improved 5-year OS and 9% improved 5-year DFS [136]. Therefore, neoadjuvant chemotherapy followed by radical cystectomy is a category 1 recommendation for MIBC.

Neoadjuvant Immunotherapy in Cisplatin-Ineligible Patients

Patients with hearing loss, neuropathy, poor performance status, and cardiac or renal insufficiency are typically deemed cisplatin ineligible. It is estimated that 50% of patients are cisplatin ineligible [137, 138]. Neoadjuvant therapy with anti-CTLA-4 showed a measurable immunologic effects, consisting of an increased frequency of CD4 + $ICOS^{hi}$ T cells in tumor tissues and the systemic circulation [139]. PURE-01 (NCT02736266) is an open-label, single-arm, phase 2 study that assessed pembrolizumab in the neoadjuvant setting for MIBC for cisplatin-eligible patients. Fifty patients were enrolled, all underwent cystectomy and 42% had pathological complete response (PCR). A TMB of 15 mutations/Mb was significantly correlated with higher

likelihood of PCR [140]. Atezolizumab is being studied in a similar fashion (ABACUS; NCT02662309). Interim analysis showed that 39% of patients underwent downstaging. However, 10% did not undergo cystectomy [141]. An ongoing trial (NCT02812420) at M. D. Anderson Cancer Center, Houston, TX, is evaluating neoadjuvant durvalumab (anti-PD-L1) plus tremelimumab (anti-CTLA-4) in patients with MIBC who are ineligible for cisplatin-based neoadjuvant chemotherapy. Preliminary data show that of the six patients who underwent cystectomy, three had PCR [142]. DUTRENEO (NCT03472274) is comparing the durvalumab plus tremelimumab combination to cisplatin in the neoadjuvant setting for cisplatin-eligible patients. CPI plus cisplatin chemotherapy is also being investigated (NCT02690558).

Immunotherapy in Combination with Radiotherapy for Localized Bladder Cancer

Several trials are assessing combining radiotherapy with CPIs alone for cisplatin-ineligible MIBC (NCT02891161, NCT03419130) or radiotherapy with CPIs plus chemotherapy for MIBC cisplatin-eligible patients (NCT02662062, NCT03170125, NCT02621151). Of these studies, NCT02621151 gains particular interest as it is a pilot study for MIBC patients who either wish for bladder preservation or are ineligible for cystectomy. This trial is expected to take 2 years to accrue planned 30 patient enrollment [143].

Adjuvant Immunotherapy in High-Risk Patients

Following standard neoadjuvant therapy and cystectomy, in patients with pT3, pT4 disease, or positive nodes, there is an unclear role for additional adjuvant chemotherapy. CheckMate 274 (NCT02632409) is a randomized phase 3 trial comparing nivolumab as adjuvant treatment versus placebo in patients with high-risk invasive UC of the bladder, ureter, or renal pelvis post resection. The IMvigor010 (NCT02450331) and AMBASSADOR (NCT03244384) are similar randomized phase 3 adjuvant trials studying atezolizumab and pembrolizumab, respectively (Table 6.3). NIAGARA (NCT03732677) is a phase 3 study of neoadjuvant durvalumab + cisplatin-based chemotherapy followed by durvalumab adjuvant therapy.

Immunotherapy for Advanced Stage UC

To date, the US FDA has approved five CPI agents as a frontline or second-line treatment for patients with advanced bladder cancer who are either ineligible or progressed after cisplatin [106–113].

Platinum Ineligible

Pembrolizumab

KEYNOTE-052 is the phase 2 trial that studied pembrolizumab as first-line treatment for cisplatin-ineligible patients with metastatic UC [112]. Overall, ORR was 24% (CR 6%), but it was higher at 38% (CR 13.3%) in patients with ≥10% CPS. KEYNOTE-361 trial (NCT02853305) is the phase 3 study for frontline pembrolizumab in metastatic UC. Arms of treatment are pembrolizumab monotherapy, pembrolizumab plus cisplatin-based chemotherapy, or chemotherapy alone [144, 145]. Cisplatin was replaced by carboplatin in cisplatin-ineligible patients. Based on KEYNOTE-052 results, the US FDA approved the use of pembrolizumab for cisplatin-ineligible population in 2017. However, in June 2018, the FDA announced that treatment-naive patients with <10% CPS have lower OS with the use of pembrolizumab as monotherapy compared to carboplatin chemotherapy. Therefore, the FDA changed the prescribing label for pembrolizumab to include cisplatin-ineligible patients with CPS ≥ 10% by an FDA-approved test. If patients are cisplatin and carboplatin ineligible, then pembrolizumab is still indicated regardless of PD-L1 status (Fig. 6.3).

Atezolizumab

The phase 2 IMvigor210 trial included two cohorts (treatment-naive and previously treated patients). Cohort 1 studied atezolizumab in treatment-naive cisplatin-ineligible metastatic

Table 6.3 Ongoing phase 3 trials studying adjuvant checkpoint therapy for invasive UC

NCT identifier (trial)	Intervention	Phase	Population	Estimated sample	Results
NCT02632409 (CheckMate 274)	Nivolumab	3	Adjuvant therapy high-risk MIBC	640	NR
NCT02450331 (IMvigor010)	Atezolizumab	3	Adjuvant therapy high-risk MIBC	800	NR
NCT03244384 (AMBASSADOR)	Pembrolizumab	3	Adjuvant therapy high-risk MIBC and locally advanced UC	739	NR

MIBC muscle-invasive bladder cancer, *NR* not reported

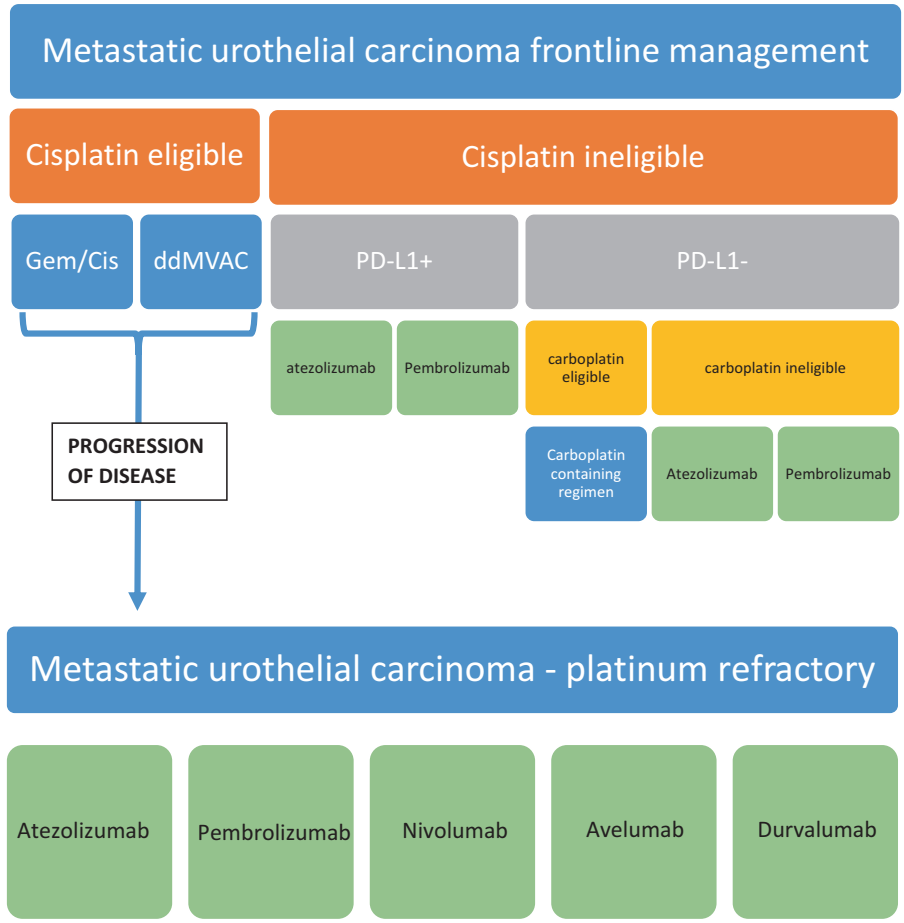

Fig. 6.3 Current treatment algorithm for metastatic urothelial carcinoma

UC patients [146]. This cohort had a different breakdown of patients deemed cisplatin ineligible: 70% had renal impairment; 20% had ECOG PS 2, and 14% had hearing loss. They were stratified based on PD-L1 expression on immune cells (IC) into IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%). ORR in unselected patients was 23%, and in contrast to prior results, ORR did not correlate with PD-L1 expression. Similar to pembrolizumab, the FDA approved atezolizumab in 2017 as first line for cisplatin-ineligible patients. IMvigor130 is an ongoing phase 3 trial randomizing treatment-naive patients to three arms: atezolizumab

plus platinum-based chemotherapy, atezolizumab alone, and chemotherapy alone [147]. Stratification is similar to the IMvigor210. Similar to pembrolizumab, in June 2018, the FDA announced that treatment-naive patients with IC0/1 PD-L1 status have lower OS with the use of atezolizumab compared to carboplatin chemotherapy. Therefore, the FDA changed the prescribing label for atezolizumab to include cisplatin-ineligible patients with IC2/3 by an FDA-approved test. If patients are cisplatin and carboplatin ineligible, then atezolizumab is still indicated regardless of PD-L1 status (Fig. 6.3).

Platinum Refractory

Five agents nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab, with the first two being PD-1 antibodies and the last three being PD-L1 antibodies, demonstrated clinical activity following platinum in metastatic UC with ORRs ranging from 15% to 25% [106–111].

Pembrolizumab

Pembrolizumab for UC was first studied in the phase 1b KEYNOTE-12 trial [148], which required ≥1% PD-L1 expression. ORR was 26% in unselected patients with good tolerance, that is, only 15% with grade ≥3 AEs. The phase 3 KEYNOTE-45 compared pembrolizumab to second-line chemotherapy in platinum-refractory UC [113]. Control arm was investigator's choice of chemotherapy with paclitaxel, docetaxel, or vinflunine. Pembrolizumab had a survival advantage over chemotherapy (10.3 vs 7.4 months) and a better response rate (21% vs 11%). These results showed for the first time in 30 years an agent that improves survival in the second-line setting. The FDA approved pembrolizumab (May, 2017) for metastatic UC progressing during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. For pretreated UC, several trials are attempting combinations of pembrolizumab plus chemotherapy (NCT02437370).

Atezolizumab

Atezolizumab was the first FDA-approved CPI for locally advanced or metastatic UC patients who progressed on platinum therapy. In a phase 1 trial, which enrolled 68 patients with previously treated metastatic UC, atezolizumab had an ORR ranging from 11% to 43% [110]. The higher ORR was seen in tumors expressing high levels of PD-L1, defined as ≥5% in tumor cells or tumor-infiltrating immune cells. Cohort 2 (previously treated) from the abovementioned IMvigor210 had an ORR in all-comers of 15% versus historical control of ORR with second-line cytotoxic chemotherapy of 10%. However, ORR was 27% for IC2/3 and 18% for IC1/2/3 [108]. This provided the basis for the FDA to approve atezolizumab as second-line in May 2016. IMvigor211 was the phase 3 trial that randomized patients who progressed after platinum therapy to receive either atezolizumab or chemotherapy (physician's choice between taxanes or vinflunine). Similar to IMvigor210, PD-L1 on ICs was used to stratify patients. The primary endpoint of OS was tested in hierarchical fixed-sequence procedure: in the IC2/3 population, followed by IC1/2/3, followed by the intention-to-treat. Statistical significance was required at each step before formal testing of the subsequent population. The IC2/3 population failed to show improved survival; therefore, the other populations were not evaluated [141]. Nonetheless, atezolizumab is approved by the FDA for post platinum therapy of metastatic UC based on improvement of ORR in comparison to historic rates for second-line chemotherapy.

Nivolumab

Nivolumab was first studied in the CheckMate 032, which was a phase 1/2 single-arm trial. The trial showed an ORR of 24.4% in patients with locally advanced or metastatic UC who progressed after platinum-based therapy. PD-L1 high (≥1% on tumor cells) and PD-L1 low (<1% on tumor cells) had similar responses (24% vs 26%). However, PD-L1 high median OS was longer (16.2 months vs 9.9 months) [109]. CheckMate 275 was the phase 2 study to

verify these findings [149]. The primary endpoint was ORR in all treated patients and used slightly different stratification for tumor PD-L1 expression (≥5%, ≥1%, and <1%). ORR was 19% for unselected patients. However, when analyzed by tumor PD-L1 expression, ORR was 28.4% in PD-L1 of ≥5%, 23.8% in PD-L1 of ≥1%, and 16.1% in PD-L1 of <1%. Nivolumab was well tolerated with 18% of grade ≥3 AEs. The FDA approved nivolumab in 2017 for use in metastatic UC as second-line post cisplatin therapy.

Avelumab

Avelumab has the additional ability (beside checkpoint inhibition) to lyse PD-L1 expressing tumor cells by an antibody-dependent cell-mediated cytotoxicity [150]. In a phase 1b trial, avelumab showed an ORR of 18.2% in post platinum UC and tolerable profile with only 6.8% grade ≥3 AEs. In a pooled analysis post platinum cohort from the phase 1 dose-expansion JAVELIN Solid Tumor study, avelumab had an OR of 17%. Patients in the JAVELIN trial were not selected based on PD-L1 expression. Maintenance avelumab compared to supportive care in patients with metastatic UC that did not progress after 4–6 cycles of platinum-based chemotherapy is the focus of the JAVELIN Bladder 100 phase 3 trial (NCT02603432) [151]. GCISAVE (NCT03324282) is a phase 2 study that is studying the safety and efficacy of gemcitabine, cisplatin (GC) +/− avelumab in first-line treatment for locally advanced or metastatic UC patients.

Durvalumab

A phase 1 trial of durvalumab in platinum-resistant UC showed an ORR of 46.4% in the PD-L1-positive (defined as ≥25% of tumor cells or tumor-infiltrating immune cells) subgroup and 0% in the PD-L1-negative subgroup [152]. A phase 1/2 trial for metastatic UC patients followed and 95.3% of enrolled patients had failed platinum therapy [107]. ORR was 17.8% across all patients, 27.6% for PD-L1 high, and 5.1% for PD-L1 low. These results led the FDA to grant accelerated approval in 2017 to durvalumab in the second-line setting after failing cisplatin.

Predictive Biomarkers for Response and Resistance

As detailed above, only a minority of patients respond to CPIs. Therefore, several efforts are aimed at identifying biomarkers that predict response. As detailed previously, PD-L1 expression in UC is associated with higher grade of tumor [123], worse clinical outcomes, and less postoperative survival [124]. Intuitively, PD-L1 was predicted as a potential predictive biomarker for CPI therapy. In the IMvigor210 trial, higher PD-L1 expression was associated with an increased response [108]. In contrast, the CheckMate 275 showed nivolumab responses irrespective of tumor PD-L1 expression [149]. Using PD-L1 as a predictive marker faces several critiques. First, staining PD-L1 by immunohistochemistry assays is not yet reproducible. For example, the IMvigor210 used the Ventana SP142 assay to measure PD-L1 on tumor-infiltrating ICs, the durvalumab trial utilized the Ventana SP263 assay to measure PD-L1 on both tumor cells and ICs, and the CheckMate 275 used the Dako PD-L1 28-8 pharmDx kit to measure PD-L1 on tumor cells only [108, 149, 152]. Second, the cutoffs used to define low or high expression are not universal. Third, PD-L1 expression is dynamic, and a single biopsy is unlikely to provide a complete assessment of PD-L1 status for the entire duration of disease [153]. In the CheckMate 275, a 25-gene interferon-γ (IFN-γ) signature was associated with response PD-L1 expression [149]. Genomic defects in IFN-γ pathway genes are linked to anti-PD-1 and anti-CTLA-4 resistance [154–158]. An exploratory subgroup analysis of IMvigor210 Cohort II showed a significant increase in TMB in responding patients relative to nonresponding patients (12.4 mutation/megabase vs 6.4 mutation/megabase) [108]. Smoking status and TCGA subtype did not correlate with TMB. Unified depth of sequencing, comprehensive sequencing panels, and silencing of germline

variants are among the challenges to clinical use of TMB. Other possible biomarkers include the four mRNA subtype clusters I–IV (luminal I, luminal II, basal I, and basal II) elucidated by TCGA project [119]. Sampling the primary tumor, lymph nodes, or metastatic lesions for TCGA subtyping may lead to inappropriate tumor classification, and this limits its utility as a marker. TCGA subtype has not proven to be a strong predictive biomarker for immunotherapy at this time.

Future Directions and Ongoing Trials

Although CPI offers an effective alternative option in a disease that had very few treatment options, objective responses with CPI remain low and more than 75% of patients do not respond. Unfortunately, the majority of patients with UC do not have an elevated PD-L1 expression [159], and many patients in the front line are also cisplatin ineligible [137]. Thus, additional therapies are necessary, and research is ongoing to investigate combinations of CPIs along with other agents that target the immune microenvironment [144].

Combination of Anti-PD-L1 + Anti-CTLA4

DANUBE (NCT02516241) is an ongoing phase 3 trial of durvalumab as monotherapy or combined with tremelimumab versus standard-of-care (SOC) chemotherapy for patients with metastatic or unresectable UC. OS is the primary endpoint for this three-arm trial. CheckMate 901 (NCT03036098) is a similar phase 3 trial evaluating nivolumab + ipilimumab and nivolumab + SOC chemotherapy versus SOC chemotherapy in treatment-naive patients with metastatic UC [160].

Combination of CPI + Chemotherapy (Table 6.4)

It is unclear if CPI therapy will replace current chemotherapy or add a synergetic effect. Currently, IMvigor130 (NCT02807636), KEYNOTE-361 (NCT02853305), and CheckMate 901 (NCT03036098) are addressing whether combination of immunotherapy and chemotherapy will be more effective than immunotherapy alone [144, 145, 147, 160]. Interestingly, cohort 2 of the IMvigor210 study demonstrated high PD-L1 expression corresponded with higher ORR, while in cohort 1, there was no correlation between PD-L1 expression and ORR. The major difference between cohorts was the exposure of cohort 1 patients to chemotherapy prior to receiving atezolizumab [108]. This suggests that prior chemotherapy can modulate the immune microenvironment and expression of PD-L1. Indeed, a recent retrospective study demonstrated that PD-L1 tumor expression was significantly higher on postneoadjuvant chemotherapy specimens than in matched preneoadjuvant specimens, supporting this hypothesis [161].

Table 6.4 Ongoing phase 3 studies assessing frontline CPIs conbined with chemotherapy in patients with metastatic or unresectable UC

NCT identifier (trial)	Intervention	Comparator	Phase	Primary outcome	Results
NCT02516241 (DANUBE)	Durvalumab as monotherapy or combined with tremelimumab	Standard-of-care (SOC) chemotherapy	3	OS	NR
NCT02807636 (IMvigor130)	Atezolizumab plus platinum-based chemotherapy or atezolizumab alone	Platinum-based chemotherapy	3	PFS, OS, AEs	NR
NCT02853305 (KEYNOTE-361)	Pembrolizumab plus cisplatin-based chemotherapy or pembrolizumab alone	Platinum-based chemotherapy	3	PFS, OS	NR
NCT03036098 (CheckMate 901)	Nivolumab + ipilimumab or nivolumab + SOC chemotherapy	SOC chemotherapy	3	PFS, OS	NR

OS overall survival, *PFS* progression-free survival, *AEs* percentage of patients with adverse events, *NR* not reported

Other Combinations

Several trials are investigating immunotherapy with novel agents including other I-O drugs, ADCs, FGFR inhibitors, and others. Frontline combination trial (EV-103) of enfortumab vedotin (ADC against nectin-4) combined with pembrolizumab for cisplatin-ineligible patients with locally advanced or metastatic UC has been launched (NCT03288545). On April 12, 2019, the FDA granted erdafitinib approval for metastatic platinum-refractory UC with susceptible fibroblast growth factor receptor (FGFR) 2 or 3 genetic alterations. The promising results with FGFR-targeted therapies led to the investigation of using them in combination with immunotherapy. FORT-2 (NCT03473756) is a phase 1b/2 trial of the FGFR inhibitor rogaratinib plus atezolizumab in untreated FGFR-positive metastatic UC. FIERCE-22 (NCT03123055) is a phase 1/2 study for combination of FGFR3 inhibitor vofatamab plus pembrolizumab in platinum refractory UC. M7824 is a novel first-in-class bifunctional fusion protein consisting of the extracellular domain of the human transforming growth factor beta (TGFβ) receptor 2, which functions as a "trap" for all three TGFβ isoforms, covalently linked to the C terminus of the heavy chain of the anti-PD-L1 antibody derived from avelumab [162]. Preliminary data from a phase 1 dose-escalation study suggest that M7824 has clinical activity and manageable safety profile in patients with heavily pretreated advanced solid tumors [163]. This is being further explored in UC. NKTR-214, a CD122-preferential IL-2 pathway agonist, is being studied in combination with nivolumab in the phase 1/2 PIVOT-2 (NCT02983045) for cisplatin-ineligible patients. Siefker-Radtke et al. presented promising data during the GU malignancy symposium 2019 showing ORR of 48% in 27 evaluable patients [164].

Cellular Therapy

Cellular therapy for bladder cancer is still in its infancy. NCT02153905 was a phase 1 trial using autologous T-cell receptor immunotherapy targeting MAGE-A3 for patients with metastatic solid tumor who are HLA-A∗01 positive. However, trial was terminated early. NCT03389438 is a phase 1 study with autologous central memory T cells for metastatic bladder UC treated with first-line gemcitabine plus cisplatin. NCT02457650 is an ongoing phase 1 T-cell receptor-transduced T cells targeting NY-ESO-1 for treatment of patients with NY-ESO-1-expressing malignancies.

Future Directions in Immunotherapy for UC

Metastatic UC has a poor prognosis, and immunotherapy was a significant advancement that offered new treatment options to patients with metastatic UC. However, response rates from CPI monotherapy remain low, and it is important to understand mechanisms of resistance, identify biomarkers to choose potential responders, and develop more effective combination therapies. Immunotherapy, currently being investigated in the perioperative setting, offers the promise of improving outcomes by reducing the risk of recurrence.

Immunotherapy for Prostate Cancer

Prostate cancer (PC) is the most common cancer expected to be diagnosed in men in 2019 accounting for nearly one in five new diagnoses. In the United States, it is estimated that PC will still be the second leading cause of death from cancer in men in 2019 [96]. PC deaths have been increasing from an estimate of 26,739 in 2017 and 29,430 in 2018 to 31,620 in 2019 [165, 166]. Perhaps, this could be explained by the recommendations against screening and as a result an increased rate of distant metastases at diagnosis [167, 168]. Androgen deprivation therapy (ADT), commonly using medical castration, remains the current standard of care for initial treatment of patients with metastatic PC [169]. More recently, in February 2018, the FDA approved abiraterone

with prednisone to be added to ADT for newly diagnosed castration-sensitive PC (CSPC) [170, 171]. Additionally, chemotherapy (docetaxel) added to ADT (chemohormonal therapy) is also an option for metastatic CSPC based on the CHAARTED and STAMPEDE phase 3 trials [172, 173]. Despite the effectiveness of the previously mentioned therapies, eventually, all CSPC patients will progress to castrate-resistant PC (CRPC) [170–173]. Per the National Comprehensive Cancer Network (NCCN) guidelines, CRPC patients can be considered for microsatellite instability/mismatch repair (MSI/MMR) testing. Furthermore, they can be considered for mutational testing of homologous recombination genes in germline and tumor tissue [174]. This information is useful for counseling families at increased risk of malignancy, utilizing platinum early in the course of the disease, or guiding enrollment in targeted and immunotherapeutic clinical trials. Current approved therapies for metastatic CRPC include abiraterone, enzalutamide, radium-223, sipuleucel-T, and chemotherapy including docetaxel and cabazitaxel (Fig. 6.4) [175–182]. For men with metastatic CRPC, the median survival in recent phase 3 studies has ranged from 12.2 to 21.7 months [175–181]. The inevitable resistance to hormonal and chemotherapy indicates the need to develop novel therapeutic approaches [183] such as immunotherapies. Here, we discuss the basic immune biology of PC. We then highlight approved and investigational immunotherapy approaches that have advanced to later stage clinical trials.

Rationale for Immunotherapy in PC

Several reasons make immunotherapy an attractive option to target PC. In the 1990s, PC cells were reported to express specific TAAs such as the prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA) [184–186]. These unique proteins to the prostate can serve as immunogenic antigens toward which the immune system can attack. The slow-growing nature of PC and its expression of TAAs allow the immune system time to mount a response [187, 188]. In fact, effector T cells responsive to PC TAAs have been identified in the peripheral blood of patients with PC especially those with CRPC [189, 190]. Preclinical data showed that antiprostate immune responses can exclusively target normal as well as cancerous prostate tissues without affecting other tissues that lack PC TAAs [191–193]. Additionally, histological evaluation of PC tissue

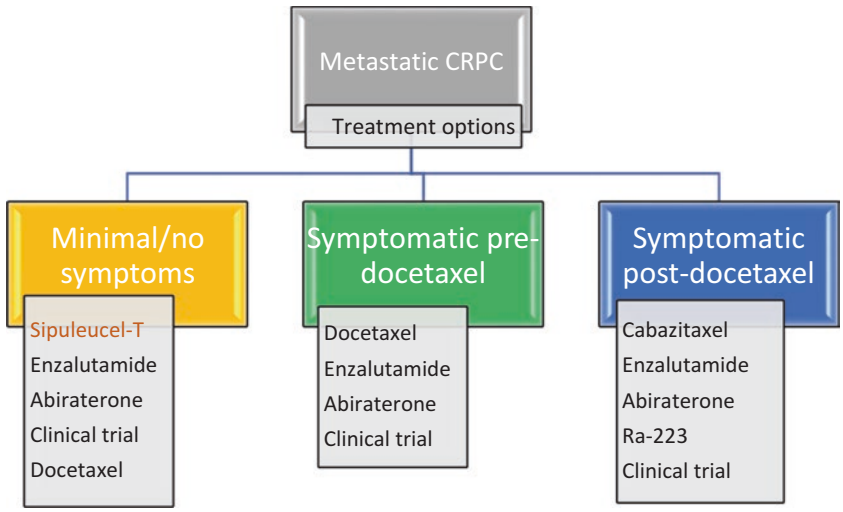

Fig. 6.4 Current treatment options for metastatic CRPC including the only approved immunotherapy sipuleucel-T

has identified infiltrating CD4+ and CD8+ lymphocytes (TILs) that are oligoclonally expanded, suggesting that their presence is due to specific antigenic stimulation [194]. Treatment with ADT modulates the immune microenvironment by inducing infiltration of CD8+ TILs as well as CD68+ macrophages into prostate tumors [195, 196]. CD68+ macrophages seem to be associated with increased risk of biochemical recurrence [196], indicating the complex nature of immune changes driven by ADT. Despite the clonal expansion of TILs, the high expression of PD-1 makes them likely incapable of mounting an effective immune response [194]. Coinhibition of TILs, generated mainly by the interaction between the B7 family and their receptor CD28 family, is another principal immune evasion pathway for PC [197]. Based on these findings, effective immunotherapy strategies against PC, especially CRPC, have focused on training the immune system against PC TAAs (via therapeutic vaccines) [198] and antagonizing immune checkpoints.

Vaccines

"Vaccines" is the broad term for mechanisms designed to stimulate the immune cells to ultimately target specific TAAs and destroy PC cells. Vaccines for PC can be divided into ex vivo processed (e.g., sipuleucel), vector-based (e.g., PROSTVAC), and whole tumor-cell vaccines (e.g., GVAX) [199]. Ex vivo processed vaccines are usually personalized (i.e., generated from the patient's own tumor-reactive immune cells), such as sipuleucel-T. Conversely, vector-based and whole tumor-cell vaccines are commonly generic (i.e., created or engineered to deliver selected TAAs known to be immunogenic) [200]. Several vaccines were developed to target PC, but they failed to show clinical efficacy [201]. We will be discussing agents that reached FDA approval or a late-stage clinical trial.

Sipuleucel-T

Sipuleucel-T is an example of personalized, cell-based, ex vivo processed DC vaccine against PC. Patient's peripheral blood mononuclear cells including antigen presenting cells (APCs) are activated ex vivo with recombinant fusion protein (PAP fused to GM-CSF) and reinfused into the patient (Fig. 6.5). D9901 was a placebo controlled phase 3 of 127 men with metastatic CRPC showed a survival advantage of 4.5 months but no significant delay in time to progression (TTP), which was the intended primary outcome [202, 203]. D9902A was an identical study that showed a trend toward increased survival with sipuleucel-T, although it was not statistically significant with no advantage in the primary outcome, TTP [202]. D9902B or the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT)

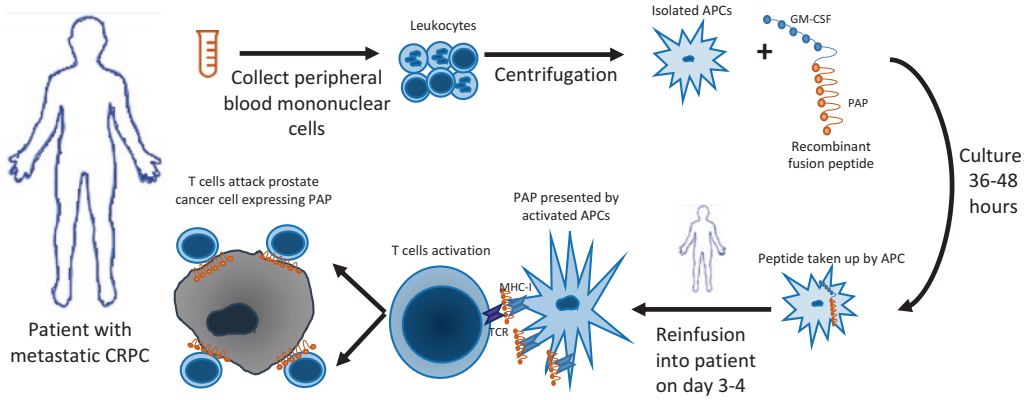

Fig. 6.5 The manufacturing process and proposed mechanism of action for sipuleucel-T

trial was a larger phase 3 that made OS its primary outcome. A total of 512 men with metastatic CRPC were randomized to either sipuleucel-T or placebo. There was a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs 21.7 months in the placebo group) but again no effect on TTP [179]. Based on these findings, sipuleucel-T was the first anticancer immunotherapy to be approved by the FDA. Despite sipuleucel-T approval, the IMPACT is critiqued as two thirds of the cells harvested were lost and not reinfused in the placebo arm. This large cell loss could provide an alternative explanation for the survival improvement [204]. However, these concerns were not credited during the FDA review due to the careful consideration given to the leukapheresis procedures in the placebo arm [205]. Sipuleucel-T is being studied in different combinations with other vaccines, antiandrogens, chemotherapy, cytokines, or CPIs. Examples of added agents include a DNA vaccine encoding PAP (NCT01706458) [206] after sipuleucel-T; however, PAP-specific T-cell responses, median TTP, and median OS were not statistically different from giving sipuleucel-T alone. STRIDE (NCT01981122) is a study that compared concurrent versus sequential enzalutamide with sipuleucel-T in metastatic CRPC, but it is not powered enough for difference in OS or PFS [207]. STAMP (NCT01487863) is a similar study to STRIDE using abiraterone instead of enzalutamide, and it is not powered to report differences in clinical outcomes as well [208]. Combinations of sipuleucel-T with chemotherapy were either terminated or withdrawn (NCT01420965, NCT02793765, and NCT02793219). On the other hand, NCT01804465 is a phase 2 study comparing immediate versus delayed addition of ipilimumab to sipuleucel-T and is still recruiting as of April 2019. Finally, it is worth mentioning that radiographic or PSA progression does not accurately reflect survival with sipuleucel-T, and finding an immune biomarker that can accurately reflect clinical benefit is urgently needed [209]. The absence of objective parameters to judge whether or not sipuleucel-T is benefitting the patients poses a major difficulty in determining when to consider sipuleucel-T ineffective and switch treatment.

GVAX

GVAX is an off-the-shelf allogeneic whole-cell vaccine that is made from irradiated PC lines and is genetically transduced to express GM-CSF. Two phase 1/2 studies established the safety of GVAX in CSPC and CRPC and suggested clinical response by reducing PSA [210, 211]. However, phase 2 and phase 3 trials are so far not promising. NCT00771017, a phase 2 combination with ADT trial for nonmetastatic biochemically relapsed PC was withdrawn. VITAL-1 (NCT00089856) was a phase 3 trial comparing GVAX to docetaxel in chemo-naive metastatic CRPC, but was terminated based on futility analysis showing <30% chance of meeting primary endpoint. VITAL-2 (NCT00133224) was another phase 3 trial with GVAX combined with docetaxel that was terminated due to an independent data monitoring committee recommendation reporting excess deaths in the experimental arm [201].

PROSTVAC

PROSTVAC is a recombinant vaccinia virus, modified to express PSA. It is safe and can induce stable PSA levels in half of treated patients, but it was not effective in inducing enough PSA-specific T-cell population [212, 213]. Therefore, PROSTAVAC-*VF* was developed as a prime/boost strategy using *v*accinia (primer) and *f*owlpox (booster) recombinant viral vectors. The vectors were engineered to express three co-stimulatory molecules (CD80, CD54, and CD58), hence, the name PROSTVAC-VF/TRICOM. Despite showing 8.5-month OS benefit, the phase 2 trial with this vaccine failed to show PFS benefit in metastatic CRPC which was its primary endpoint [214]. Consequently, the phase 3 trial, PROSPECT, was conducted to further investigate these findings but failed to show the benefit in OS. In fact, the trial was stopped early after meeting criteria for futility [215, 216]. Nonetheless, combination trials with

PROSTVAC-VF are underway. For example, the phase 2 trial NCT03315871 an anti-PD-L1 antibody (avelumab) with TGF beta-Trap molecule is added to PROSTVAC. Additionally, PROSTVAC is being studied in combination with other CPIs (NCT03532217, NCT02933255), enzalutamide (NCT01867333, NCT01875250), and chemotherapy (NCT02649855).

CPIs

CPIs have revolutionized the management of solid tumors in the past few years [217, 218]. Unfortunately, CPIs have not been as successful in PC perhaps due to its multifaceted and pleotropic immune tumor microenvironment [219]. Particularly, the sole use of CPIs has shown limited evidence of antitumor activity, likely due to the immunologically "cold" nature of the tumor and low PD-L1 expression on tumor cells. However, if existing PC treatments can trigger an adaptive immune response, attracting infiltrating immune cells and increasing tumor PD-L1 expression, there is a rationale for combinations improve outcomes [220] (Tables 6.5 and 6.6).

Anti-CTLA-4 for Metastatic PC

Ipilimumab blocks the T-cell-negative regulator CTLA-4 allowing CD28 and B7 interactions, which result in T-cell activation, proliferation, tumor infiltration, and ultimately, cancer cell death. In a phase 1/2 study (NCT00323882), escalating doses of ipilimumab (3–10 mg/kg) were used with and without radiation for metastatic CRPC. The 10 mg/kg with radiation cohort suggested activity and had similar rate of irAEs to the previously reported rates [221]. Therefore, 10 mg/kg was the dose chosen for phase 3 trials. NCT00861614 was a phase 3 trial in post docetaxel CRPC that involved bone-directed radiotherapy followed by randomization to either ipilimumab or placebo [222]. NCT01057810 was the second phase 3 trial that randomized patients with chemotherapy-naive metastatic CRPC without visceral metastases to ipilimumab alone versus placebo [223]. In both studies, ipilimumab did not improve OS, and when given alone, it increased PFS and had a higher PSA RR, suggesting antitumor activity in a patient subset. A small phase 2 trial using ipilimumab plus chemotherapy did not show any improvement in the activity of ipilimumab [224]. Another phase 2 trial evaluated ipilimumab combined with ADT early on for CSPC and established the safety of the combination [225]. Combination trials of ipilimumab with abiraterone (NCT01688492), ADT (NCT01194271, NCT01377389, NCT00170157), and sipuleucel-T (NCT01832870) are ongoing.

Anti-PD-1 in Metastatic PC

Pembrolizumab is another CPI that blocks the interaction of PD-1 and its ligand PD-L1, leading to T-cell activation and antitumor activity in PD-L1-positive mCRPC based on the phase 1b KEYNOTE-028 trial ($n = 23$) [226]. PD-L1 positivity was defined as expression in ≥1% of tumor or stromal cells. ORR was 17.4% with a median duration of response of 13.5 months. KEYNOTE-199 was a phase 2 that enrolled 258 patients with docetaxel-refractory mCRPC in cohorts 1 through 3 (C1–3). A total of 131 patients had measurable PD-L1+ disease (C1), 67 patients had measurable PD-L1- disease (C2), and 60 patients had nonmeasurable, bone-predominant disease (C3). Chemotherapy-naive subjects with mCRPC either having failed or showing signs of failure with enzalutamide in Cohorts 4 and 5 received pembrolizumab monotherapy in addition to their current regimen of enzalutamide. ORR ranged from 3% to 5%, and DCR lasting ≥6 months was 11%. ORR was not different between C1 and C2, indicating antitumor activity and disease control regardless of PD-L1 status. The RR was numerically higher in patients with somatic BRCA1/2 or ATM mutations (12%), supporting further investigation in patients with homologous recombination defects (HRD) [227]. A small phase 2 single-arm clinical trial demonstrated activity of pembrolizumab + enzalutamide in CRPC patients after progression with enzalutamide. Of the 10 patients enrolled,

Table 6.5 Later stage clinical trials for checkpoint inhibitors combined with oral therapies for metastatic CRPC (mCRPC)

NCT identifier (trial)	Phase	Outcome measures	Intervention	Population	Anticipated sample size	Preliminary results
NCT01688492	1/2	Primary: safety, PFS Secondary: PSA kinetics, bone scan changes	Ipilimumab + abiraterone + prednisone	Chemotherapy-naive mCRPC	57	NR
NCT02861573 (KEYNOTE-365)[a]	1b/2 umbrella trial	Primary: PSA RR, AEs, discontinuation rate Secondary: DCR, OS, DOR, ORR, rPFS (PCWG3 RECIST)	Cohort A: Pembrolizumab + olaparib	Post docetaxel mCRPC	70	Enrolled 41 patients. DCR ≥ 6 months: 29%. ORR: 7% (28 evaluable patients) [248]
			Cohort C: Pembrolizumab + enzalutamide	Post abiraterone acetate but prechemotherapy mCRPC	70	Enrolled 69 patients. DCR ≥ 6 months: 33%. ORR: 20% (25 evaluable patient) [235]
			Cohort D: Pembrolizumab + abiraterone + prednisone	Post enzalutamide acetate but prechemotherapy mCRPC	70	NR
NCT02787005 (KEYNOTE-199: cohorts 4 and 5)	2	Primary: ORR Secondary: DCR, PSA RR, AEs, discontinuation rate	Pembrolizumab + enzalutamide	Chemotherapy-naive mCRPC	370 (for all cohorts)	Results for single-agent pembrolizumab in postdocetaxel cohorts 1, 2, and 3 show ORR 3–5% [227]
NCT03338790 (CheckMate-9KD)[b]	2	Primary: ORR, PSA RR Secondary: rPFS, TTR, DOR, TTP-PSA, OS, AEs.	Arm A: Nivolumab + rucaparib	Post docetaxel mCRPC	330 (for all arms)	NR
			Arm C: Nivolumab + enzalutamide	Post abiraterone acetate but prechemotherapy mCRPC		NR
NCT03016312 (IMbassador 250)	3	Primary: OS Secondary: rPFS, PSA RR, TTP PSA, AEs, ORR	Atezolizumab + enzalutamide	Post abiraterone acetate and failure of taxane in mCRPC	730	NR
NCT03330405 (JAVELIN PARP MEDLEY)	2	Primary: toxicity, ORR Secondary: avelumab and talazoparib kinetics, TTR, DOR, PFS	Avelumab + talazoparib	mCRPC	242 (for all arms)	NR
NCT02484404	1/2	Primary: RP2D, ORR	Durvalumab with olaparib and/or cediranib	mCRPC	384 (for all arms)	17 patients enrolled; 47% had PSA responses >50% [240]

PFS progression-free survival, *PSA RR* prostatic-specific antigen response rate, *AE* adverse events, *ORR* objective response rate, *DCR* disease control rate, *OS* overall survival, *DOR* duration of response, *rPFS* radiographic progression-free survival, *PCWG3 RECIST* Prostate Cancer Working Group 3 modified RECIST 1.1, *NR* not reported, *TTP-PSA* time to prostate-specific antigen progression, *TTR* time to tumor response

[a]Cohort B of the KEYNOTE-365 is pembrolizumab + docetaxel + prednisone

[b]Arm B of the CheckMate-9KD is nivolumab in combination with docetaxel

Table 6.6 Selected combination trials of vaccines with checkpoint inhibitors for prostate cancer

NCT identifier (trial)	Phase	Status	Outcome measures	Intervention	Population	Anticipated sample size	Preliminary results
NCT02933255	1/2	Recruiting	Primary: safety Secondary: peripheral PSA-specific T cells, Treg prostate infiltration, PSA/PD-L1/MRI changes	PROSTVAC-VF + nivolumab	Chemotherapy-naive mCRPC	29	NR
NCT03315871	2	Recruiting	Primary: PSA RR Secondary: AEs, PSA slope over time	PROSTVAC-VF + avelumab linked with TGF beta-Trap molecule	Biochemically recurrent prostate cancer	34	NR
NCT03532217	1	Recruiting	Primary: safety, neoantigen-reactive T cells among other correlatives Secondary: PSA RR, OS, rPFS	PROSTVAC-VF + ipilimumab + nivolumab + neoantigen DNA vaccine	mCSPC	20	NR
NCT01832870	1	Completed	Primary: PAP/PA2024-specific immune response. Secondary: PSA, radiographic, clinical response, T-cell activity	Sipuleucel-T + ipilimumab	Chemotherapy-naive mCRPC	Actual enrollment of 9	Increased PAP/PA2024-specific immune response [249]
NCT01804465	2	Recruiting	Primary: PAP/PA2024-specific immune response. Secondary: PSA, radiographic, clinical response, T-cell activity	Sipuleucel-T + immediate or delayed ipilimumab	Chemotherapy-naive mCRPC	54	NR

mCRPC metastatic castrate-resistant prostate cancer, *mCSPC* metastatic castrate-sensitive prostate cancer, *PFS* progression-free survival, *PSA RR* prostatic-specific antigen response rate, *OS* overall survival, *DOR* duration of response, *rPFS* radiographic progression-free survival, *PCWG3 RECIST* Prostate Cancer Working Group 3 modified RECIST 1.1, *NR* not reported

three experienced a biochemical response and two a radiological response. Genetic analysis revealed markers of MSI in one patient [228]. MSI has been shown to be a predictive factor for response to pembrolizumab [229].

Pembrolizumab in High MSI

The prevalence of MMR deficiency in metastatic CRPC is estimated at 2–5% [230, 231]. In one series from MSKCC, 20 of 839 PC patients (2.4%) were found to have MSI-H/dMMR tumors, defined as an MSI sensor score of ≥3 and TMB of ≥10, confirmed by IHC and mutational signature analysis. Of 13 of 20 MSI-H patients who consented to germline analysis, 3 of 13 (23%) had a germline MMR gene mutation. In total, 10 patients with MSI-H tumors received a PD-1/PDL-1-targeting agent. Of 10 patients, five had radiographic PR or PSA decline of >60%, one had SD for 6 months, and four had no response or were inevaluable [232]. In fact, pembrolizumab is FDA approved for a variety of advanced solid tumors (including CRPC) that are MSI-H or dMMR, after progressing on a prior treatment, and no satisfactory alternative treatment options are available.

Combination of Anti-CTLA-4 Plus Anti-PD-1

At the 2019 Genitourinary Cancers Symposium, Sharma et al. presented a preplanned interim efficacy/safety analysis for nivolumab + ipilimumab in patients with mCRPC from the phase 2 CheckMate 650 [233]. Asymptomatic/minimally symptomatic patients with mCRPC were divided into pretaxane therapy (cohort 1) and after taxane (cohort 2). Treatment was nivolumab 1 mg/kg + ipilimumab 3 mg/kg Q3W for four doses, and then nivolumab 480 mg every 4 weeks. Coprimary endpoints were ORR and radiographic PFS per PC working group 2 [234]. Sixty-two patients were enrolled, and ORR was 26% and 10% in cohorts 1 and 2, respectively. Higher activity in the chemotherapy-naive cohort is consistent with data from other immunotherapy modalities such as sipuleucel-T. In both cohorts, ORR was higher in patients with PD-L1 ≥ 1%, DNA damage repair (DDR), HRD, or above-median TMB. Careful interpretation is recommended, given the small number of subgroups. Grade 3–4 TRAEs occurred in 39% and 51% of patients in cohorts 1 and 2, respectively.

CPIs Plus Enzalutamide

KEYNOTE-365 is a phase 1b/2 umbrella trial [235] that is based on the activity seen with pembrolizumab in KEYNOTE-199 and following reports of adding enzalutamide [227, 228]. This study is assessing different combinations of pembrolizumab, either with olaparib (poly ADP ribose polymerase [PARP] inhibitor) (cohort A), docetaxel (cohort B), enzalutamide (cohort C), or abiraterone (cohort D). Cohort C enrolled a total of 69 patients and had a DCR ≥ 6 months of 33%. ORR was 20% in 25 evaluable patient, that is, having measurable disease [235]. CheckMate 9KD (NCT03338790) is another phase 2 umbrella trial evaluating nivolumab in combination with rucaparib (PARP inhibitor), docetaxel, or enzalutamide [220]. IMbassador 250 (NCT03016312) is a phase 3 multicenter trial evaluating atezolizumab with enzalutamide versus enzalutamide alone for CRPC [236].

Other Ongoing Immunotherapeutic Trials in PC

CPIs Plus PARP Inhibitors

Data suggest that 25–30% of sporadic mCRPC patients have somatic or germline defects in DNA repair pathways, which may confer sensitivity to PARP inhibition (PARPi) [174]. Data from the above-mentioned CheckMate 650, KEYNOTE-199, and other reports suggest that there may be improved activity in CRPC with DDR mutations when treated with CPIs [227, 233, 237]. NCT02484404 is phase 1/2 trial based on the hypothesis that increased DNA damage by olaparib will complement antitumor activity of the anti-PD-L1 durvalumab, in part due to increased signaling through STING

(stimulator of interferon (INF) genes) pathway and enhanced IFN production [238]. Of 17 CRPC patients, eight (47%) had PSA responses >50%, and six of the eight responders had mutations in the DDR pathways [239, 240]. This was the first study to demonstrate activity for the PARPi+CPI combination in PC patients without having to have defects in DDR genes. While this study is limited by a small patient cohort, the 12-month PFS of 51.5% in a taxane-refractory population is promising. As mentioned above, the KEYNOTE-199 and CheckMate 9KD are aiming to answer this question.

PSMA Radioligand Therapy and Combinations with Immunotherapy

PSMA's expression is upregulated in dedifferentiated and CRPC making it an attractive target for therapy [241]. ^{177}Lu-PSMA-617 is composed of the therapeutic radionuclide Lutetium-177 attached to the high-affinity PSMA ligand called PSMA-617. ^{177}Lu-PSMA-617 has shown a promising activity in metastatic CRPC based on a meta-analysis that included 455 patients [242]. PSMA-lutetium Radionuclide Therapy and ImmuNotherapy in Prostate CancEr (PRINCE) is an Australian phase 1/2 trial (NCT03658447) that is assessing the safety and efficacy of pembrolizumab in conjunction with ^{177}Lu-PSMA-617. NCT03805594 is a similar study conducted in the United States.

Chemokine Receptor 2 (CXCR2) Antagonist in Combination with Enzalutamide

ACE (NCT03177187) is a phase 1/2 study studying AZD5069 (CXCR2 antagonist) + enzalutamide in metastatic CRPC to reverse enzalutamide resistance. CXCR2 antagonism is reported to stop recruitment of MDSCs to the premetastatic niche and, as a result, reduce the chance of developing cancer metastasis [243].

Cellular Therapy

In CRPC, two groups reported developing a CAR construct targeting PSMA [244, 245]. NCT01140373 is a phase 1 trial that started in 2010 using PSMA CAR T cell and has not reported results yet. A major concern is the immune suppressive microenvironment; therefore, TGFβ-insensitive PSMA-directed CAR-T cells were developed. This newer construct resulted in increased proliferation, enhanced cytokine secretion, resistance to exhaustion, and long-term in vivo persistence in a human PC mouse models [246]. NCT03089203 is a phase 1 clinical trial conducted at the University of Pennsylvania to assess the safety and preliminary efficacy of this lentivirally transduced PSMA-directed/TGFβ-insensitive CAR-T cells in men with metastatic CRPC [247].

Future Directions for Immunotherapy in PC

PC has evident potential to induce immune responses, and clinical data have proven the principle that immune modulation can prolong survival [179]. However, developing immunotherapies for PC has faced several challenges. Perhaps, immunotherapies may be most effective when used earlier in the course of disease or in a combinatorial fashion. Identifying the beneficial combinations of hormonal therapy, chemotherapy, CPIs, and vaccines is the current goal of several clinical trials (Fig. 6.6). Another important consideration for immunotherapy is identifying patients who are most likely to benefit from therapy. Most intriguing is the possibility of identifying patients with high-risk, localized PC with a preexisting antitumor immune response and treating them with immunotherapy in a neoadjuvant or adjuvant setting to maximize the benefit. There is currently substantial evidence that immunotherapy may be active and beneficial in PC, and continued evaluation of this treatment is surely warranted.

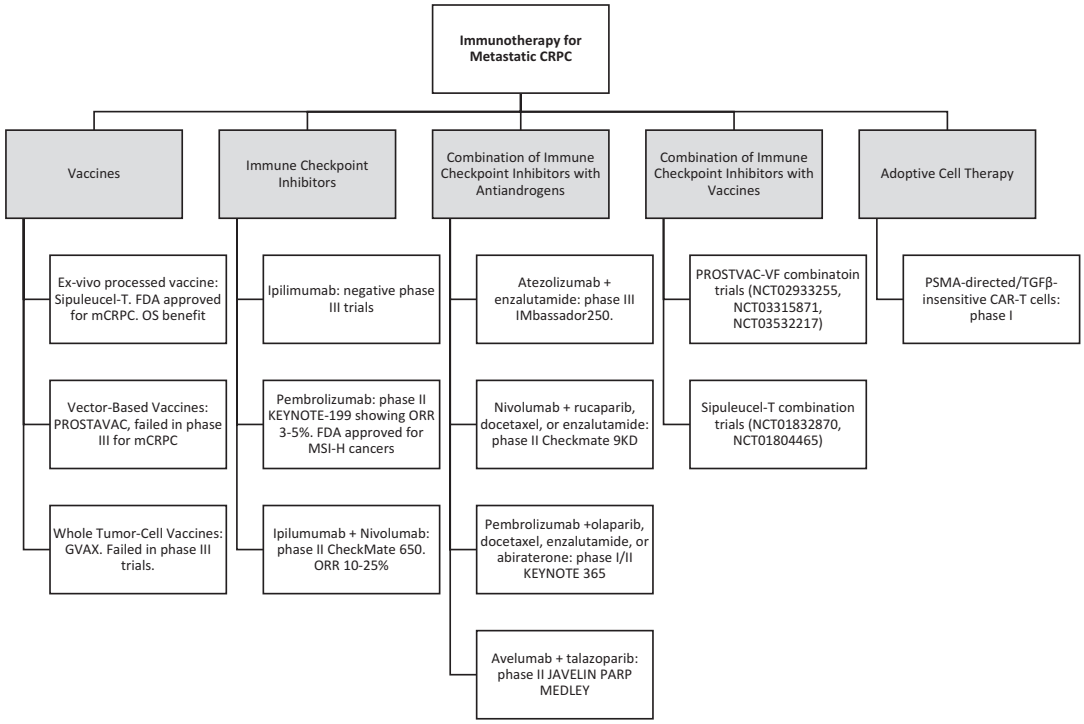

Fig. 6.6 Selected categories of current immunotherapy landscape for prostate cancer. Sipuleucel-T remains the only FDA-approved agent

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Immuno-oncology for Gynecologic Malignancies

7

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Abstract

Patients with advanced and/or recurrent gynecologic cancers derive limited benefit from currently available cytotoxic and targeted therapies. Successes of immunotherapy in other difficult-to-treat malignancies such as metastatic melanoma and advanced lung cancer have led to intense interest in clinical testing of these treatments in patients with gynecologic cancers. Currently, in the realm of gynecologic oncology, the FDA-approved use of immune checkpoint inhibitors is limited to microsatellite instable cancers and PD-L1-positive cervical cancer. However, there has been an exponential growth of clinical trials testing immunotherapy approaches, both alone and in combination with chemotherapy and/or targeted agents, in patients with gynecologic cancers. This chapter reviews some of the major reported and ongoing immunotherapy clinical trials in patients with endometrial, cervical, and epithelial ovarian cancer.

Keywords

Endometrial cancer · Cervical cancer · Ovarian cancer · Immunotherapy · Immune checkpoint inhibitors · Cancer vaccines · Adoptive cell transfer

Introduction

Management of advanced and/or recurrent gynecological malignancies has been a challenge, because conventional therapy is often of limited and transient benefit [1–3]. In the search for more effective alternatives, attention has shifted more towards targeted and immune therapies. Recent immunotherapy trials have demonstrated significantly improved response rates in non-gynecologic cancers that were historically seen to be difficult to treat, such as metastatic melanoma and non-small cell lung carcinoma [4, 5]. Essential to protect the human body against foreign pathogens, the immune system also plays an integral role in eliminating cancerous cells through the process of immune surveillance [6]. Malignant cells may evade the immune system by several mechanisms which include activation of immune checkpoint pathways involving programmed cell death protein-1 (PD-1)/programmed cell death ligand (PD-L1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4),

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and various immunosuppressive cytokines. These mechanisms serve to suppress T-cell activity, thus promoting tumor tolerance and growth [7]. Treatment modalities in immunotherapy serve to augment the host's antitumor immune response and/or inhibit the immunosuppressive signals in the tumor microenvironment [6]. We will begin this chapter with a brief review of various immunotherapy approaches in use and under investigation for the treatment of gynecologic cancers including immune checkpoint inhibitors, cancer vaccines, and adoptive cell transfer (ACT) [8]. We will then summarize some of the major findings detailing outcomes of immunotherapy and ongoing clinical trials targeting different gynecologic cancers.

Immune Checkpoint Inhibitors

Regulated by a balance of co-stimulatory and inhibitory signals, immune checkpoints help the human immune system respond effectively to foreign pathogens while preventing overactivation that could result in autoimmunity or collateral tissue destruction [7]. At the initial antigen recognition by the T-cell receptor (TCR), CTLA-4 mitigates the amplitude of TCR-mediated signaling in cytotoxic T lymphocytes (CTLs) via counteracting CD28 co-stimulatory activity. Specifically, CTLA-4 sequesters CD80 and CD86 from binding to CD28 in CTLs while enhancing the immune-suppressive activity of regulatory T-cells. While CTLA-4 primarily acts on newly activated T-cells, PD-1 receptor activation via PD-L1 and PD-L2 functions to limit activation of CD-8+ effector T-cells mainly in peripheral tissue (due to the wide expression pattern of PD-1 ligands on a variety of normal and malignant cell types) to prevent collateral tissue damage. Tumor cells may overexpress PD-L1 either in response to inflammatory signals in the tumor microenvironment (adaptive immune resistance) or via upregulation through oncogenic signaling (innate immune resistance). In either situation, PD-1 downregulates effector T-cell response, and with chronic antigen exposure from tumor cells, this can result in T-cell anergy and self-tolerance.

Thus, immune checkpoint blockade via anti-CTLA-4 antibodies (e.g., ipilimumab, tremelimumab) and/or anti-PD-1/PD-L1 antibodies (e.g., pembrolizumab, nivolumab, durvalumab, avelumab, atezolizumab, and others) serve as potential therapeutic options to augment the antitumor activity of adaptive immunity.

Cancer Vaccines

The general principle of cancer vaccines is to elicit the host's adaptive immune response to target malignant cells and can be given either in the prophylactic or therapeutic setting [9, 10]. For prophylactic vaccines, these are typically given prior to exposure to the neoplastic-inducing antigen to prevent pre-malignant and malignant cellular transformation. One classic example is administration of the human papilloma virus (HPV) – vaccine series containing L1 virus-like particles specific high-risk carcinogenic HPV types (e.g. 16 and 18) to teenagers and adults in order to reduce HPV infection rates in order to reduce the incidence of cervical dysplasia or cervical cancer. In contrast, therapeutic vaccines consisting of tumor-specific antigens (as peptides or antigen-activated dendritic cells) are administered in patients with cancer in order to enhance the host's antitumor immune response [9]. As well, whole tumor antigen vaccines prepared via several approaches (including but not limited to free-thaw lysates, tumor cells treated with ultraviolet irradiation, RNA electroporation, or hypochlorous oxidation) is a novel technique that can potentially allow for a broad and stronger immune response given a higher number of tumor-associated antigens as opposed to a single antigen [11].

Adoptive Cell Transfer

In adoptive cell transfer (ACT), autologous T-cells are extracted (either from tumor tissue itself or from the peripheral blood) and are

subsequently expanded ex vivo, with or without genetic modification, and then re-infused back into circulation [12, 13]. Clinically used categories of ACT include tumor-infiltrating lymphocytes (TIL), genetically engineered T-cell receptors (TCR), and chimeric antigen receptor (CAR) T cell therapies [12, 13]. TIL therapy consists of several steps including surgical extraction of tumor tissue to gain access to a heterogeneous population of T lymphocytes that presumably recognize tumor-specific antigens [13, 14]. Isolation of TIL is subsequently followed by ex vivo cellular expansion, preconditioning lymphodepletion, TIL infusion, and adjuvant IL-2 to aid with in vivo TIL expansion and maintenance [14, 15]. Lymphodepletion is thought to be critical and improve the therapeutic responses to TIL immunotherapy through the elimination of both the endogenous T-lymphocytes that may compete with TIL for stimulatory cytokines/IL-2 and the regulatory T-cells that serve to inhibit the T-cell activity [13, 16]. In contrast to TIL (which are naturally occurring group of polyclonal T-lymphocytes with varying recognition of and affinities towards tumor-associated antigens), genetically engineered TCR and CAR T-cells are T-lymphocyte populations modified with the same high affinity tumor recognition moiety that are obtained from the peripheral blood [12, 13]. Following leukopheresis, the peripheral blood-derived T-lymphocytes are genetically modified (frequently via the use of retroviral vectors), to render specificity against a tumor-specific antigen, then subsequently expanded and re-infused back into the patient [12, 13]. These genetically modified T-cell approaches also frequently involve preconditioning using lymphodepleting chemotherapy. Important distinctions between CAR- and TCR-engineered T-cell therapies include the fact that TCR-modified T-cells recognize tumor-specific antigens in the context of a specific major histocompatibility complex (MHC) – 1 [12, 13]. Therefore, one of the limitations TCR T-cells in their utility is that the treatment is restricted to patients with common HLA types (typically HLA-A∗0201) used in engineering the TCR. Another limitation is the possibility of tumors downregulating the MHC protein expression and thereby decreasing tumor recognition. CAR T-cells address this limitation as these cells are genetically modified with an antigen-recognition moiety fused to intracellular T-cell signaling domains. This allows tumor antigen recognition by CAR T-cells to be independent of MHC proteins [17]. However, the major limitation of the CAR T-cell approach is the need for tumor antigen to be present on the cell surface.

In an era of precision medicine, immunotherapy represents one of the promising therapies that may be used to improve oncologic outcomes in gynecologic cancers. The following text will review the published, ongoing, and upcoming clinical trials in endometrial, ovarian, and cervical cancer.

Endometrial Cancer

Following the published results by the Cancer Genome Atlas Research Network, contemporary classification of endometrial cancer has shifted away from the traditional two histologic types (endometrioid vs. non-endometrioid; sometimes referred to as type I and type II cancers) and towards four types based on genomic sequencing: DNA polymerase epsilon (POLE) ultramutated, microsatellite instability hypermutated (MSI-H), copy-number low, and copy-number high [18]. Microsatellites are repeated sequences of DNA that become sites of DNA replication errors with "microsatellite instability" occurring in the setting of defects in the DNA mismatch repair (MMR) pathway. Defects of MMR function result in MSI in approximately 20–30% of endometrial tumors [18, 19]. Loss of MMR function is typically due to sporadic hypermethylation of the MLH1 promotor and less frequently due to germline mutations (i.e., hereditary nonpolyposis colon cancer (HNPCC) syndrome, also known as Lynch syndrome) [18, 20]. MMR-deficient and POLE-mutant endometrial tumors display a high number of tumor-infiltrating lymphocytes as well as a high neoantigen load (due to high somatic

tumor DNA mutational burden) giving the potential to elicit a strong antitumor immune response [18, 21–23].

Immune Checkpoint Inhibitors in Endometrial Cancer

There has been growing interest in the use of immune checkpoint inhibitors in MSI-H endometrial tumors since the landmark publication by Le and colleagues [24]. In this phase II study of MMR-deficient colorectal cancers and non-colorectal solid tumors and MMR-proficient colorectal cancers treated with pembrolizumab (anti-PD-1 antibody), patients with MMR-deficient cancers had clinically significant objective response rates (ORR) of 30–70% and an improved progression-free survival (PFS). Among the colorectal cancer patients, those with MMR-proficient tumors demonstrated no responses [24]. Although this cohort predominantly consisted of colorectal cancer patients, there were two MMR-deficient endometrial cancers that demonstrated favorable responses (one had a partial response and the other a complete response) [24]. In another study, Le and colleagues expanded their evaluation of pembrolizumab (10 mg/kg every 2 weeks) by examining the response in a cohort of 86 patients with 12 different cancer types with MMR deficiency who had progressive disease on at least one prior treatment (Table 7.1) [25]. Among the 15 endometrial cancer patients, there was a 53% ORR (three complete and five partial responses) with a 73% disease control rate (DCR) (20% had stable disease) [25]. MSI-H tumors display a higher expression of PD-L1 compared to microsatellite stable (MSS) tumors, and this expression appears to be correlated with improved response to PD-1 and PD-L1 inhibitors [23, 33]. In another trial, Fader et al. reported a 56% ORR with four partial responses and one complete response to pembrolizumab in MMR-deficient recurrent or persistent tumors as well as a DCR of 88.9% [26]. Given the above results, pembrolizumab was awarded US Food and Drug Association (FDA) approval for the use in treatment of MMR-deficient solid tumors following recurrence or progression on standard therapy in May 2017.

Another PD-1 inhibitor under investigation is nivolumab. In a Japanese, phase II multicenter study, nivolumab (240 mg IV every 2 weeks) was administered to mixed cohort of patients including advanced uterine cancer patients (clinical trial JapicCTI-163,212) [27]. In their preliminary results, Hasegawa and colleagues found an ORR of 22.7% in 23 uterine cancer patients with acceptable drug safety profile [27]. ORR was similar regardless of presence or absence of PD-L1 expression (25% vs. 21.4%, respectively) with all patients with MSI-H tumors experiencing partial responses [27]. For another PD-1 inhibitor, investigators administered dostarlimab (TSR-042) at 500 mg IV every 3 weeks for the first 4 cycles, then 1000 mg IV every 6 weeks in recurrent or advanced endometrial cancer patients (NCT02715284) [28]. In the preliminary results of 94 evaluable patients, the ORR was 27.7% (50% in MSI-H tumors and 19.1% in MSS tumors) and a DCR of 48.9%. The treatment-related adverse event (TRAE) rate was 61.8% with 11.8% with grade 3 or higher with the most common being increased aspartate aminotransferase [28].

Other studies have shown more limited benefit of immune checkpoint inhibitors in patients with endometrial cancers. As an ongoing, open-label phase Ib trial, KEYNOTE-028 is evaluating the safety and efficacy of pembrolizumab on PD-L1-positive advanced solid tumors [29]. In this study, a cohort of 24 patients with advanced endometrial cancer and PD-L1 positivity were treated with pembrolizumab 10 mg/kg every 2 weeks for up to 24 months (or until progression or unacceptable toxicity) after failing 2 prior lines of therapy [29]. The DCR was 25% (n = 6) including 12.5% (n = 3) with partial responses. Progressive disease occurred in 54.2% (n = 13) and 20.8% (n = 5) could not be assessed. Of note, 19 of the 24 tumor samples were evaluable for MSI-H status with the sole patient with an MSI-H tumor having progressive disease. One of the three patients with a partial

Table 7.1 Reported immune checkpoint inhibitors studies in endometrial cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
PD-1 inhibitors						
Le et al. 2017 [25]	Phase II	15	MMR-deficient endometrial cancer with progressive disease	Pembrolizumab (10 mg/kg IV q2 weeks)	ORR 53% (5 PR/3 CR) DCR 73%	Overall*: 74% (mainly rash/pruritus, fatigue, diarrhea/colitis). Grade 3–4: 20% (diarrhea/ colitis, pancreatitis, hyperamylasemia)
Fader et al. 2016 [26]	Phase II	9	Recurrent/ persistent MMR-deficient cancers	Pembrolizumab (10 mg/kg IV q2 weeks)	ORR 56% (4 PR/1 CR) DCR 88.90%, 12-month OS 89%	Mainly Grade 1–2; no TRAE higher than 3
Hasegawa et al. 2018 [27]	Phase II	23	Advanced/ recurrent EC	Nivolumab 240 mg IV q2 weeks	ORR: 23% (similar regardless of PD-L1 status), 3.4 month PFS, 12-mo OS 48.5%	Overall*: 56.3%, Grade 3–4 toxicities: 12.5% (mainly pruritus, increased lipase, diarrhea)
Oaknin et al. 2018 [28]	Phase I/II	94	Recurrent or persistent EC	Dostarlimab 500 mg IV q3 weeks for 4 cycles, then 1000 mg IV q6 weeks	ORR 27% (50% in MSI-H/19.1% in MSS). DCR 48.90%	Overall: 61.8%; Grade 3+ 11.8%, most common grade 3+ TRAE = AST increase
Ott et al. 2017 [29]	Phase IB	24	Locally advanced or metastatic PD-L1 positive with progression after standard therapy	Pembrolizumab (10 mg/kg q2 weeks) up to 24 months	ORR 12.5% (3 PR/0 CR), DCR 25%, PFS 1.8 months, 6- & 12-month PFS rate: 19.0% & 14.3%, 6- & 12-month OS rate: 67.0% & 51.0%	Overall: 54.2% (most common fatigue, pruritus, pyrexia, decreased appetite), Grade 3: 16.7% (asthenia, back pain; anemia, hyperglycemia, hyponatremia; chills and pyrexia, diarrhea)
PD-L1 inhibitors						
Fleming et al. 2017 [30]	Phase IA	15	Advanced and recurrent EC	Atezolizumab 1200 mg IV (or 15 mg/kg) q3 weeks	ORR 13% (2 PR/0 CR), DCR 26% mPFS 1.7 months, mOS 9.6 months	Overall: 47% (mainly grade 1–2)
Combination therapy						
Makker et al. 2019 [31]	Phase II	53	Metastatic endometrial cancer	Pembrolizumab 200 mg IV q3 weeks and Lenvatinib 200 mg po qday	ORR 39.6% (20 PR/ 1 CR) DCR 86.80% PFS 7.4 months	Overall: 94% (common: hypertension, diarrhea, fatigue, hypothyroidism), grade 3: 68%, serious TRAE: 30% with 1 death due to intracranial hemorrhage

(continued)

Table 7.1 (continued)

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Rubinstein et al. 2019 [32]	Phase II	28 per arm	Persistent or recurrent endometrial carcinoma and endometrial carcinosarcoma	Durvalumab 1500 mg IV q4 weeks vs. Durvalumab 1500 mg IV q4 weeks and Tremelimumab 75 mg IV q4 week	Monotherapy: ORR 14.8% (3 PR/1 CR), 24-week PFS 13.3% Combination: ORR 11.1% (1 PR/2 CR), 24-week PFS 18.5%	Grade 3 (7% vs. 32%, respectively) Grade 4 (4% vs. 11%, respectively)

AST aspartate aminotransferase, *DCR* disease control rate = stable disease + partial response + complete response rates, *mOS* median overall survival, *CR* complete response, *IV* intravenous, *MMR* mismatch repair, *mPFS* median progression-free survival, *MSI-H* microsatellite instability high, *MSS* microsatellite stable, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *PO* oral, *q* every, *PR* partial response, *TRAE* treatment-related adverse events
*Includes other non-endometrial cancers

response was found to have a POLE-mutant tumor [29]. The high expression of a large set of immune-related genes and increased neoantigen load may explain the favorable response to immune checkpoint inhibitors in POLE-mutated tumors [18, 34]. Additionally, POLE-mutated tumors demonstrate a higher expression of PD-L1/PD-L2 proteins as well as a higher extent of T lymphocytic infiltration than MSI and MSS endometrioid tumors [18, 22, 23, 34]. Another PD-L1 inhibitor currently being investigated in endometrial cancer is atezolizumab. In a phase Ia study, atezolizumab (1200 mg IV every 3 weeks) was administered in advanced or recurrent endometrial cancer patients (NCT01375842) [30]. In their preliminary results of 15 patients, the ORR was 13% with 2 patients having partial response and a DCR of 26% without significant TRAE [30]. Response appeared to be higher in tumor PD-L1 expression and tumor lymphocytic infiltration [30].

The combination of immune checkpoint inhibitors and multi-tyrosine kinase inhibitors has been reported to result in higher response rates. In a phase Ib/II study, lenvatinib (inhibitor of vascular endothelial growth factor 1–3, fibroblast growth factor receptor 1–4, and other kinases) and pembrolizumab were administered a mixed cohort of MSI-H/MSS advanced endometrial cancer patients (NCT02501096) [31]. Among 53 evaluable patients, tumors were primarily MSS (85%) with an overall ORR of 39.6% (1 complete response and 20 partial responses) at 24 weeks of treatment and DCR of 86.8%. Although impressive tumor responses were seen, the TRAE rate was high (94%) with grade 3 TRAE rate of 68% (most common being hypertension and diarrhea) [31]. Serious TRAE occurred in 30% of patients with one treatment-related death due to intra-cranial hemorrhage) [31]. A phase III trial investigating lenvatinib/pembrolizumab versus physician's choice is currently underway (NCT03517449). At the 2019 American Society of Clinical Oncologists Meeting, the preliminary results of a phase II trial of durvalumab (PD-L1 inhibitor) with or without tremelimumab (CTLA-4 inhibitor) in persistent/recurrent endometrial cancer were presented (NCT03015129) [32]. Twenty-eight patients were enrolled in each treatment arm. The durvalumab monotherapy group had an ORR of 14.8% (1 complete response and 3 partial responses) with PFS of 13.3% at 24 weeks [32]. The combination group had an ORR of 11.1% (2 complete responses and 1 partial response) with a PFS of 18.5% at 24 weeks [32]. Grade 3 and 4 TRAE were 7% and 4% in the monotherapy group and 32% and 11% in the combination group, respectively [32]. Numerous ongoing trials utilizing combination therapy are shown in Table 7.2.

Table 7.2 Ongoing studies for immune checkpoint inhibitors in endometrial cancer

Study	Design	Patient population	Therapy	Endpoints	Study status
MK-3475-158/ KEYNOTE-158 (NCT02628067)	Phase II	Advanced (unresectable and/or metastatic) disease that have progressed on standard of care therapy	Pembrolizumab	Primary: ORR	Recruiting
NCT02549209	Phase II	Advanced or recurrent disease	Pembrolizumab + carboplatin + paclitaxel	Primary: ORR, AE	Recruiting
NCT02899793	Phase II	Persistent, recurrent, or metastatic POLE-mutation and/or MMR-deficient endometrial tumors with prior treatment	Pembrolizumab	Primary: ORR, frequency and severity of AE Secondary: PFS, OS	Recruiting
NCT02982486	Phase II	Locally advanced non-operable or metastatic endometrial carcinoma with somatic-deficient MMR with at least 1 prior failed systemic therapy	Nivolumab + ipilimumab	Primary: ORR Secondary: PFS, OS	Not recruiting yet
NCT02912572	Phase II	POLE-mutated, MSS, and MSI-H persistent or recurrent tumors with prior therapy	Avelumab +/− talazoparib	Primary: Drug activity Secondary: PFS, OS, TRAE, immune-related objective response	Recruiting
KEYNOTE-775 (NCT03517449)	Phase III	Advanced, recurrent, or metastatic with at least 1 failed prior line of systemic therapy	Pembrolizumab + lenvatinib vs. investigator's choice of chemotherapy	Primary: PFS, OS Secondary: ORR, HRQoL, AEs	Recruiting
NCT03526432	Phase II	Advanced, recurrent, or persistent with at least 1 prior platinum-based chemotherapy regimen	Bevacizumab + atezolizumab	Primary: ORR Secondary: OS/ PFS, safety, Immune related response	Recruiting

AE adverse event, *HRQoL* Health-related quality of life, *IV* intravenous, *MMR* mismatch repair, *MSI-H* microsatellite instability high, *MSS* microsatellite stable, *q* every, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *POLE* DNA polymerase epsilon, *TRAE* treatment-related adverse events

Vaccines in Endometrial Cancer

One of the identified tumor-associated antigens that have been utilized, as a target for therapeutic vaccinations, is a product of the Wilm's tumor gene: WT1 [35, 36]. Classically categorized as a tumor-suppressor gene, WT1 may instead perform oncogenic functions in many malignancies and is highly expressed in multiple cancers including gynecologic malignancies [36]. In a phase II clinical trial, Ohno et al. utilized a WT1 peptide vaccine on 12 patients with HLA-A∗2402-positive gynecologic cancers resistant to standard therapy (Table 7.3) [36]. Two of endometrial cancer patients (carcinosarcoma and endometrioid adenocarcinoma histologic subtypes) both had progressive disease after 3 months but the treatment was otherwise well tolerated [36]. In another phase I/II study, a mixed cohort of end-stage serous endometrial carcinoma ($n = 3$) and leiomyosarcoma ($n = 3$) patients received four weekly vaccines of autologous dendritic cells electroporated with WT1 mRNA [37]. Although all three serous endometrial carcinoma patients (two HLA-A2 positive and one HLA-A2 negative)

Table 7.3 Reported vaccine therapy trials in endometrial cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Ohno et al. 2009 [36]	Phase II	2	HLA-A∗2402-positive endometrioid adenocarcinoma and carcinosarcoma resistant to standard therapy	Intradermal injections of 3.0 mg of HLA-A∗2402-restricted adjuvant modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant administered q week for 12 weeks	ORR 0%, DCR 0%	Mild erythema at injection site with no grade 3–4 toxicities
Coosemans et al. 2013 [37]	Phase I/II	3	Advanced uterine cancer	4 weekly vaccines of autologous dendritic cells electroporated with WT1 mRNA	ORR 0%, DCR 0%, Increase in WT1-specific T-cells and NK cells in HLA-A2 positive endometrial cancers	Mild erythema at injection site with no grade 3–4 toxicities
Jager et al. 2006 [38]	Phase I	1	Advanced NY-ESO-1 cancers	2 vaccinations with rV-NY-ESO-1 at a dose of 3.1×10^7 pfu followed by 2 vaccinations with rV-NY-ESO-1 at a dose of 7.41×10^7 pfu at 4-week intervals	ORR = 0%, DCR = 0%, humoral and cellular responses increased as indicated by NY-ESO-1-specific antibody production and CD4/CD8 response	Mild erythema at injection site with no grade 3–4 toxicities
Kaumaya et al. 2009 [39]	Phase I	2	Recurrent and/or metastatic disease	Combination vaccines of a mixture of two B-cell epitopes of HER2 fused to a T-cell epitope with nor-muramyl-dipeptide (n-MDP) adjuvant emulsified in Montanide ISA 720 at 0.25 or 0.5 mg IM q3 weeks × 3, additional vaccinations given later based on if there were toxicity	ORR 50% (1 PR / 0 CR), DCR = 50%	Grade 3*: 12.5%, (diarrhea, pain, hyperglycemia)

(continued)

Table 7.3 (continued)

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Jackson et al. 2017 [40]	Phase I/IIa	Treatment group ($n = 6$) Controls ($n = 3$)	Endometrial cancer patients at risk of recurrence with HLA-2+ patients after primary treatment	HLA-A2 restricted, FBP-derived peptide (1.5 ml) vaccine administered at several doses: 100 mcg/0.5 ml, 500 mcg/0.5 ml, 1000 or mcg/0.5 ml + 250 mcg/1.0 ml GM-CSF intradermally	2-year DFS rate 43% vs. 33.6% ($p = 0.36$); for 1000 mcg dosage: 2-year DFS 85.7% vs. 33.6% ($p = 0.02$), recurrence rate 41.4 vs. 54.6% ($p = 0.35$); for 1000 mcg group 13.3% vs. 54.6%, $p = 0.01$	Most common: induration at injection site, erythema, and pruritus; 1 grade 3 toxicity but no grade 4 or 5

CR complete response, *DCR* disease control rate = stable disease + partial response + complete response rates, *DFS* disease-free survival, *FBP* Folate-binding protein, *HLA* human leukocyte antigen, *IV* intravenous, *NK cells* natural killer cells, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *Pfu* plaque-forming units, *PR* partial response, *q* every, *TRAE* treatment-related adverse events, *WT1* Wilm's tumor gene
*Includes other non-endometrial cancers

demonstrated disease progression, some immunological activity was present in the HLA-A2 positive patients as noted by an increase in WT1-specific T-cells and NK cells [37]. However, the two HLA-A2 positive leiomyosarcomas demonstrated some disease control (one with stable disease but eventually progressed and another had a mixed response prior to progression) [37].

Another targeted epitope is associated with NY-ESO-1, which is classified as a "cancer germline antigen" (an antigen expressed in the germ cells and multiple different types of malignancies). In a series of 36 patients with various stage III/IV NY-ESO-1 expressing malignancies, the patients were administered a recombinant vaccinia/fowlpox-NY-ESO-1 vaccine series [38]. In the only endometrial cancer patient, the vaccine mounted both humoral and cellular responses indicated by NY-ESO-1-specific antibody production and CD4/CD8 response although the patient ultimately had progressive disease [38].

Human epidermal growth factor-2, HER2, is overexpressed in many epithelial-derived cancers (often with breast cancers) and has been the target for vaccination in other malignancies [39]. In a phase I clinical study, patients with various metastatic cancers received combination vaccines of a mixture of two B-cell epitopes of HER2 fused to a T-cell epitope [39]. Of the 24 patients enrolled, two endometrial cancer patients had received the vaccines after 2 failed chemotherapy treatments with 1 of the patients demonstrating high antibody production and partial response [39].

Folate-binding protein (FBP) is another immunogenic protein overexpressed in endometrial (as well as ovarian) cancer [41]. In the interim analysis of a phase I/IIa trial by Jackson and colleagues, a mixed cohort of 51 patients with either endometrial or ovarian cancer received an HLA-A2 restricted, FBP-derived peptide vaccine to prevent recurrence (NCT01580696) [40]. The vaccine was well tolerated and resulted in a lower risk of recurrence in the higher dosage treatment group (1000 mcg) compared to the control group (13.3% vs. 55%, respectively; $p = 0.01$), and a higher estimated 2-year disease-free survival (85.7% vs. 33.6%, respectively; $p = 0.021$) [40].

ACT in Endometrial Cancer

Although there are no reported studies discussing TIL, TCR-T, or CAR-T therapy in endometrial cancer at the time of this chapter's preparation, another ACT therapeutic option involves lymphokine-activated killer (LAK) cells. This process involves collection of peripheral blood containing mononuclear cells that are stimulated in vitro with IL-2 to become LAK cells [42]. These LAK cells are re-infused into the patient and are capable of lysing tumor cells without MHC restriction while sparing normal tissue [42]. In a study by Steis et al., they selected patients with various cancers that had metastatic disease restricted to the peritoneal cavity [43]. These patients received IL-2 (100,000 U/kg IV every 8 hours) for 3 days, followed by leukapheresis for 5 days [43]. LAK cells were expanded in vitro by incubating the peripheral blood mononuclear cells in IL-2 for 7 days, then administered IP for 5 days with IL-2 (25,000 U/kg IP every 8 hours) [43]. In the cohort, there was only one endometrial cancer patient but that patient failed to respond to therapy with the therapy overall having multiple side effects including intraperitoneal fibrosis [43]. In another study, Santin et al. observed stable disease in a patient with endometrial cancer with unresectable, chemo-resistant liver metastases who was treated with infusion of peripheral T-cells stimulated with tumor lysate-pulsed autologous dendritic cells [44].

Cervical Cancer

The carcinogenesis of cervical cancer evokes great interest in immunotherapeutic options. Chronic HPV infection is attributed as the etiologic agent for the development of cervical cancer in nearly all cases. Although the majority of HPV-infected people do not develop cervical cancer (due to HPV clearance by a competent immune system), chronic HPV infections results in the expression of oncoproteins E6 and E7 that bind and inactivate the TP53 and Rb tumor suppressor gene product, respectively. Immunotherapeutic options for cervical cancer will be reviewed.

Immune Checkpoint Inhibitors in Cervical Cancer

Several studies have demonstrated relatively high PD-1/PD-L1 expression on cervical tumors (as high as 95% in cervical intraepithelial neoplasia and 80% of squamous cell carcinomas) and thus these cancers are potential targets for immune checkpoint inhibitors [45–47]. In KEYNOTE-028, the cervical cancer subgroup consisted of 24 patients with advanced disease and PD-L1-positive tumors that had progressed on prior standard therapy [48]. Following the administration of pembrolizumab (10 mg/kg every 2 weeks up to 24 months), the subgroup had an ORR of 17% (4 patients with partial response) with a DCR of 17% (Table 7.4) [48]. In an interim analysis in the KEYNOTE-158 phase II, open-label trial, 98 cervical cancer patients received pembrolizumab (200 mg every 3 weeks), including 83.7% of patients who had PD-L1-expression in their tumors and 78.6% who had prior lines of chemotherapy for recurrent or advanced disease (NCT02628067) [49]. Among these patients, the ORR was 12.2% (9 had a partial response and 3 had a complete response) with responders all having PD-L1-positive tumors (including one patient with adenocarcinoma). The DCR was 30.6% including 15 of the 18 (83.3%) patients with stable disease who had PD-L1-positive tumors [49]. Since June 2018, the FDA has approved pembrolizumab in advanced cervical cancer expressing PD-L1 with disease progression during or after chemotherapy.

Another PD-1 inhibitor reported in the cervical cancer literature is nivolumab and has demonstrated promising results. For neuroendocrine cervical cancer known to be an aggressive cervical cancer subtype, two case reports have demonstrated complete response to nivolumab monotherapy (despite being PD-L1 negative) and a near complete response (95% resolution of target lesions) when nivolumab was combined with

Table 7.4 Reported immune checkpoint inhibitors studies in cervical cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Monotherapy						
Frenel et al. 2017 [48]	Phase IB	24	PD-L1 advanced cervical cancer that progressed on prior therapy	Pembrolizumab 10 mg/kg q2 weeks up to 24 months	ORR 17% (4 PR/ 0 CR) DCR 17%, mPFS = 2 months, 6- and 12-month PFS = 21% & 4%, mOS = 11 months, 6- and 12-month OS = 67% & 40%	Overall: 75%, mainly rash and pyrexia Grade 3: rash and proteinuria
Chung et al. 2019 [49]	Phase II	98	Previously treated advanced cervical cancer	Pembrolizumab 200 mg q3 weeks for 24 months	ORR 12.2% (9 PR/ 3 CR) DCR 30.60%, mPFS = 2.1 months, 6-month PFS = 25%, mOS 9.4 months, 6- and 12-month OS 75.2% and 41.4%	Overall: 65.3%, most common being hypothyroidism, decreased appetite, and fatigue Grade 3–4: 12.2%
Hollebecque et al. 2017 [50]	Phase I/II	19	Recurrent or metastatic cervical cancer with up to two failed systemic therapies	Nivolumab 240 mg q2 weeks	ORR 26.3% (4 PR/ 1 CR) DCR 70.8%*, mPFS 5.5 months	Overall 70.8% Grade 3–4 12.5%
Santin et al. 2018 [51]	Phase II	25	Persistent or recurrent cervical cancer	Nivolumab 3 mg/kg every 2 weeks for up to 46 doses over 92 weeks	ORR 4% (1 PR/0 CR) DCR 40%, 6-month PFS rate 16%, 6-month OS rate 78.4%	Overall: 84% Grade 3: 24% Grade 4: 8%
Lheureux et al. 2018 [52]	Phase I/II	42	Metastatic or recurrent cervical cancer	Ipilimumab 3 mg/kg q3 weeks × 4 cycles or (10 mg/kg q3 weeks for 4 cycles and 4 cycles of maintenance q12 weeks)	ORR 2.9% (1 PR/ 0 CR) DCR 32.40% mPFS 2.5 months mOS 8.5 months	Grade 3 TRAE: 11.7% (mainly diarrhea and colitis)
Combination therapy						
Mayadev et al. 2017 [53]	Phase I	19	Stage IB2 - IVA cervical cancer with node positive disease undergoing chemoradiation	Cisplatin (40 mg/M^2) q week × 6 + extended field radiation, then sequential ipilimumab was given at 3 mg/kg, 10 mg/kg, and expansion cohort of 10 mg/kg	DCR 74% DFS survival of 74% at 1 year	Mostly grade 1–2 (most common being GI distress, rash & endocrinopathies). Grade 3: 16%, transient which resolved (lipase, neutropenia, and rash)
Friedman et al. 2019 [54]	Phase II	10	Recurrent, persistent, or metastatic cervical cancer	Atezolizmab 1200 mg IV q3 weeks and bevacizumab 15 mg/kg IV q3 weeks	DCR 50% mPFS 2.9 months mOS 9 months	TRAE 3: 23% (arachnoiditis, sensorineural hearing loss, lower extremity weakness, thrombosis, rectal bleed)

AE adverse event, *CR* complete response, *ORR* objective response rate, *DCR* disease control rate = stable disease + partial response + complete response rates, *DFS* disease-free survival, *IV* intravenous, *mOS* median overall survival, *mPFS* median progression-free survival, *OS* overall survival, *PFS* progression-free survival, *PR* partial response, *q* every, *TRAE* treatment-related adverse events

*Includes 5 vaginal and vulvar cancers

stereotactic body radiation [55, 56]. In a larger study, nivolumab (240 mg every 2 weeks) was tested in 5 HPV-associated malignancies including cervical, vulvar, and vaginal cancers that previously had up to two failed prior systemic therapies (CheckMate358; NCT02488759) [50]. In the preliminary results of this ongoing phase I/II multicohort study, the majority of the cohort consisted of cervical cancer patients (19 of 24) with the rest having vaginal or vulvar cancer. The overall ORR was 20.8% with a DCR of 70.8% (15 of 24) and was well tolerated [50]. Response to therapy was only noted in the cervical cancer patients (ORR 26.3%) with one complete and four partial responses, regardless of PD-L1 status [50]. In the preliminary phase II results of another trial with nivolumab (NRG-GY002; NCT02257528), the agent was demonstrated to have poor response rate with an ORR of 4% (1 partial response) with a DCR 40% in a cohort of 25 cervical cancer patients with persistent or recurrent disease who failed at least one prior line of systemic therapy [51].

Another immune checkpoint inhibitor under investigation in patients with cervical cancer is ipilimumab (CTLA-4 inhibitor). In the phase I study (GOG 9929), ipilimumab was administered after chemoradiation for patients with stage IB2-IIB or IIIB-IVA cervical cancer with node positive disease (NCT01711515). Preliminary results in the 19 evaluable subjects demonstrate a 1-year disease-free survival of 74% with tolerable side effects [53]. In another phase I/II clinical trial, 42 patients with metastatic cervical cancer (squamous cell or adenocarcinoma) with progression on at least 1 line of platinum chemotherapy received ipilimumab [52]. Among the 34 evaluable patients, the ORR was 2.9% (1 partial response) with DCR of 32.4% and a median PFS and OS of 2.5 months and 8.5 months, respectively [52]. Expression of CD3, CD4, CD8, FoxP3, indoleamin 2,3-dioxygenase, and PD-L1 expression did not predict benefit [52].

In a phase II study by Friedman et al., atezolizumab (1200 mg IV every 3 weeks) and bevacizumab (15 mg/kg IV every 3 weeks) were administered to patients with recurrent, persistent, or metastatic cervical cancer (NCT02921269) [54]. There were 10 evaluable patients with no confirmed responses and a DCR of 50% [54]. The median PFS was 2.9 months and overall survival was 9 months with 23% of patients having grade 3 TRAE [54]. A number of ongoing trials are testing the efficacy of immune checkpoint inhibitors as a part of various combinations regimens (Table 7.6).

Vaccines in Cervical Cancer

Given the role of chronic HPV infection in the carcinogenesis of cervical cancer, and the success of prophylactic HPV vaccines for prevention of dysplasia and cervical cancer, there is great interest in development of therapeutic HPV vaccines that typically target the E6 and E7 oncoproteins. In a phase II study, amalimogene filolisbac (ADXS11-001) (live, attenuated Listeria monocytogenes (Lm) vaccine containing the HPV-16 E7 oncoprotein) was administered by random assignment with or without cisplatin to 109 recurrent or treatment-refractory cervical cancer patients in India. The response rate was similar between both groups (17.1% vs. 14.7%) with comparable survival rates but the combination group experienced more adverse events that were not related to the study drug [57]. ADXS11-001 was also examined in the GOG/NRG0265 phase II study (NCT01266460) (Table 7.5) [58]. In the preliminary results of the trial, ADXS11-001 was administered as monotherapy to 50 patients with persistent or recurrent metastatic cervical cancer who progressed on at least one prior line of systemic chemotherapy [58]. The 12-month OS was 38% with a ORR of 2% (1 complete response) and DCR of 32% [58]. TRAE occurred in 96% of patients with the most frequent being fatigue, chills, anemia, and nausea; grade 3 and 4 TRAE were present in 39% and 4% of patients, respectively [58]. Another phase I/II study examined the safety and efficacy of durvalumab (anti-PD-1 inhibitor) with or without ADSX11-001 in previously treated recurrent or metastatic cervical cancer and other HPV-related squamous

Table 7.5 Reported vaccine therapy and adoptive cell therapy trials in cervical cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Basu et al. 2018 [57]	Phase II	Mono ($n = 35$) combo ($n = 34$)	Recurrent or treatment-refractory	ADXS11-001 1 cycle (3 infusions) (1×10^9 CFUs as an 80-mL IV infusion over 15 minutes on day 1, 29, and 57) and combo therapy = ADX-011 (day 1 only) + cisplatin weekly (40 mg/m^2) post-vaccine 4 weeks × 5 weeks, then 1 cycle of ADS11-011 (3 infusions)	ORR: 17.1% (3 PR/3 CR) [mono] vs. 14.7% (2 PR/3 CR) [combo], mPFS 6.08 months (mono) vs. 6.44 months (combo), mOS 8.28 (mono) vs. 8.78 (combo) months	More TRAE in the combination group (46.3% in combo group and 36.4% in mono group). Most common were chills and pyrexia
Huh et al. 2017 [58]	Phase II	50	Persistent or recurrent metastatic cervical cancer	ADXS11-001 (1×10^9 CFU) q3 weeks × 3 doses for stage 1 or until 1 year for stage 2 of the trial	2% (0 PR / 1 CR) 12-month OS was 38%	Overall: 96% (most frequent being fatigue, chills, anemia, and nausea) Grade 3 & 4: 39% and 4%, respectively
Slomovitz et al. 2016 [59]	Phase I/II	5	Recurrent or metastatic cervical cancer	ADXS11-001 q4 weeks and durvalumab (3 mg/kg or 10 mg/kg) q2 weeks	ORR 40% (1 PR/1 CR)	*Overall: 91%. The most frequent was chills/rigors, fever, nausea, hypotension, diarrhea, fatigue, tachycardia, and headache. Grade 3 & 4: 27% and 9%, respectively
Stevanovic et al. 2019 [60]	Phase II	18	Metastatic cervical cancer after standard therapy	Single infusion of E6 and E7 reactive TILs following lymphodepletion chemotherapy	ORR 28% (3 PR/2 CR)	*Grade 3–4: conditioning agent (myelosuppression and infection)
Jazaeri et al. 2019 [61]	Phase II	27	Recurrent, metastatic, or persistent squamous cell/adenosquamous or adenocarcinoma	After non-myeloablative lymphodepletion, patients were infused with their autologous TIL (LN-145) followed by IL-2 administration	ORR 44% (11 PR/1 CR) DCR 89%	TRAE generally consistent with the underlying advanced disease and the profile of the lymphodepletion and IL-2 regimens
Lu et al. 2017 [62]	Phase I	3	Metastatic or locally advanced/recurrent cancer. HLA-DPB1∗0401 positive and with tumors that contained 50% MAGE-A–positive tumor cells	Non-myeloablative chemotherapy preparative regimen followed by a single intravenous infusion of autologous TCR-transduced CD4+ T cells. A cell dose escalation starting at 10^7 total cells and escalating at half-log increment (highest 10^{11} cells). Post-infusion high-dose IL-2 intravenously at 720,000 IU/kg every 8 hours to physiologic tolerance	ORR 33% (0 PR/1 CR)	Transient grade 3 and from chemotherapy and high-dose IL-2. Prolonged high fever (39.0–40.0 °C) after cell infusion

AE adverse event, *CFU* colony-forming units, *Combo* combination therapy, *CR* complete response, *DCR* disease control rate = stable disease + partial response + complete response rates, *IV* intravenous, *Mono* monotherapy, *OS* overall survival, *mOS* median overall survival, *mPFS* median progression-free survival, *ORR* objective response rate, *PFS* progression-free survival, *PR* partial response, *q* every, *TRAE* treatment-related adverse events

*Includes other cancers

Table 7.6 Ongoing trials in cervical cancer

Study	Design	Patient population	Agent and dosing	Endpoints	Study status
KEYNOTE-826 (NCT03635567)	Phase III	Persistent, recurrent, or metastatic cervical cancer without treatment with systemic chemotherapy and is not amenable to curative treatment with surgery and/or radiation	Pembrolizumab + investigator's choice of chemotherapy vs. placebo + investigator's choice chemotherapy	Primary: PFS, OS Secondary: ORR, DOR, 12-month PFS, AE	Recruiting
BEATcc (NCT3556839)	Phase III	Persistent, recurrent, or metastatic cervical cancer is not amenable to curative treatment	Cisplatin + paclitaxel + bevacizumab vs. cisplatin + paclitaxel + bevacizumab + atezolizumab	Primary: OS Secondary: RFS, ORR, DOR, TRAE	Recruiting
NCT03614949	Phase II	Persistent, recurrent, or metastatic cervical cancer	Stereotactic body radiation therapy + atezolizumab	Primary: ORR Secondary: PFS, OS	Recruiting
NCT03508570	Phase Ib	Cervical cancer with metastatic peritoneal carcinomatosis and recurred after or progressed on frontline and 1–2 second line standard treatments	IP nivolumab +/− IP ipilimumab	Primary: MTD, RP2D Secondary: PK, toxicities, and IrAE, ORR	Recruiting
NCT02164461	Phase I/II	Persistent, metastatic, or recurrent cervical cancer	ADXS11-001 × 10^{10} CFU	Primary: MTD, AE Secondary: Changes in clinical immunology in serum and ORR	Awaiting results
AIM2CERV (NCT02853604)	Phase III	High-risk locally advanced cervical cancer	ADXS11-001 q3 weeks × 3 doses for the first 3 months in the adjuvant setting following chemoradiation	Primary: DFS; safety and tolerability	Active but not recruiting

ADXS11-001 axalimogene filolisbac, *AE* adverse events, *CFU* colony-forming units, *DOR* duration of action, *DCR* disease control rate = stable disease + partial response + complete response rates, *IrAE* Immune-related adverse events, *MTD* maximum tolerated dose, *OS* overall survival, *ORR* objective response rate, *PFS* progression-free survival, *PK* Pharmacokinetics, *RFS* recurrence-free survival, *RP2D* recommended phase II dose, *TIL* tumor-infiltrating lymphocytes, *TRAE* treatment-related adverse events

cell carcinomas of the head and neck (NCT02291055) [59]. In the phase I portion of the trial, combination therapy was examined with 8 cervical cancer patients treated [59]. Among the 5 evaluable patients, the ORR and DCR was 40% (1 partial and 1 complete response) with TRAE present in 91% of patients and grade 3 and 4 TRAE present in 27% and 9%, respectively. The most frequent TRAE were chills/rigors, fever, nausea, hypotension, diarrhea, fatigue, tachycardia, and headache. Additional studies examining ADSX11-011 use are currently under investigation (Table 7.6).

ACT in Cervical Cancer

In their phase II study, Stevanovic and colleagues administered a single infusion of E6 and E7 reactive TIL following lymphodepletion chemotherapy in patients with metastatic HPV-associated cancers following at least one prior

standard chemotherapy or chemoradiotherapy regimen [60, 63]. In the cervical cancer subcohort, the ORR and DCR was 28% (5 out of 18) including two patients who had complete responses after 22 and 15 months of treatment with no evidence of disease after 67 and 53 months, respectively (Table 7.5) [60, 63]. The proportion of HPV-reactive T-cells in peripheral blood post-infusion was positively correlated with improved clinical response [63]. Interestingly, analysis of the tumor antigens targeted by the TIL administered in patients who had complete objective responses demonstrated persistence of TIL that recognized neoantigens and cancer germline antigens in addition to the expected HPV viral antigens [64]. Given these promising results, there is another ongoing phase II, multicenter study to evaluate TIL therapy in patients with recurrent, metastatic, or recurrent cervical cancer (NCT03108495). The preliminary results of this trial presented at 2019 annual American Society of Clinical Oncology Meeting showed an ORR of 44% (1 complete and 11 partial responses) with a DCR of 89%, but with a short follow-up period (median follow-up of 3.5 months) [61].

Using ACT with genetically modified T-cells, Lu and colleagues administered dose-escalating autologous purified CD4+ T-cell therapy using an MHC class II-restricted, TCR that recognizes the cancer germline antigen, melanoma-associated antigen-A3 (MAGE-A3) to a cohort of 17 patients with various cancers [62]. In the preliminary results, although two of the three cervical cancer patients did not demonstrate a response to therapy, one of the patients who received 2.7×10^9 cells had a complete objective response at 29 months [62].

Ovarian Cancer

Immunotherapy represents a potentially promising alternative therapy in ovarian cancer for several reasons. PD-L1 expression appears to be highly prevalent in ovarian cancer compared to other malignancies with high expression associated with worse survival [65]. Furthermore, with a high prevalence of TIL and select groups with high neoantigen load, ovarian tumors are potential targets for therapeutic vaccines and ACT as well [66, 67].

Immune Checkpoint Inhibitors in Epithelial Ovarian Cancer

In a multicenter phase I trial, Brahmer et al. administered an anti-PD-L1 antibody to a heterogeneous cohort of advanced cancers, including 17 ovarian cancer patients [68]. In the ovarian cancer cohort, the ORR was 6% (1 partial response) with a DCR of 23.5% (Table 7.7) [68]. In an open-label, phase II trial, Hamanishi and colleagues administered up to 6 cycles of nivolumab to advanced or recurrent, platinum-resistant ovarian cancer [69]. In a cohort of 20 patients, nivolumab demonstrated an ORR of 15% (1 partial and 2 complete responses) and DCR of 45%. The median PFS was 3.5 months and median OS was 20 months [69]. In KEYNOTE-028, 26 patients with PD-L1-positive advanced, metastatic ovarian cancer received pembrolizumab with the majority of patients having at least 3 prior lines of systemic therapy [70]. The ORR was 11.5% (2 partial and 1 complete response) with a DCR of 38.5% and acceptable side effect profile [70]. In KEYNOTE-100 study, 376 patients with advanced, recurrent ovarian cancer were administered pembrolizumab and divided into two cohorts (A, $n = 285$ or B, $n = 91$) based on the history of number of prior lines of systemic therapy and treatment-free interval [71]. The ORR in cohort A was 7.4% (16 partial and 5 complete responses), while in cohort B it was 9.9% (7 partial and 2 complete responses) while the DCR was 37.2% and 37.4%, respectively. Higher PD-L1 expression (as measured as combined positivity score (CPS) ≥ 10) appeared to be correlated with higher clinical response (ORR 17.1% vs. 5.2% vs. 5.0% for CPS ≥ 10, 1–10, <1, respectively) [71].

The JAVELIN trials have investigated the use of avelumab in epithelial ovarian cancer. In the phase IB JAVELIN Solid Tumor study, avelumab was administered to 125 patients with advanced,

recurrent, or refractory ovarian cancer [72]. The ORR was 9.6% (including 1 complete and 11 partial responses) and DCR of 52% [72]. The 1-year PFS rate was 10.2% with a median OS was 11.2 months and acceptable side effect profile [72]. The study authors did not find an association between PD-L1 nor BRCA status and treatment response [72]. In JAVELIN Ovarian 200, 566 platinum-resistant/refractory ovarian cancer patients were randomized to one of 3 treatment arms: avelumab alone, pegylated liposomal doxorubicin alone, or both (NCT02580058) [74]. Preliminary results demonstrated that avelumab monotherapy resulted in the worst PFS, and there was no additional benefit with the combination of avelumab to pegylated liposomal doxorubicin (1.9 vs. 3.5 vs. 3.7 months, respectively). Similar results were seen with OS (11.8 vs. 13 vs. 15.7 months) [74]. However, subgroup analyses demonstrated that PD-L1 positivity was associated with slight clinical benefit with combination therapy in terms improved PFS (3.7 vs. 3.0 months; HR 0.65, 95% CI 0.46–0.92) with a trend towards improved OS (17.7 vs. 13.1 months; HR 0.72, 95% CI 0.48–1.08) [74]. Grade 3 TRAE were highest in the combination arm (42.9%) followed by PLD alone (31.6%) and avelumab alone (16.0%) [74].

In a phase I study by Infante and colleagues, atezolizumab was administered to 12 patients with advanced ovarian cancer with the majority having at least 2 prior lines of therapy [73]. In preliminary results of the 9 patients with an evaluable response, there was a 22% ORR and DCR (2 patients with partial response) [73].

Combination Therapy: Immune-chemotherapy

Given the strength immunosuppressive tumor microenviroment and modest response to single-agent immune checkpoint inhibitor therapies, interest has grown to utilize combination therapy in ovarian cancer. Wenham and colleagues presented their preliminary findings at the 2018 International Gynecologic Cancer Society Meeting where platinum-resistant recurrent ovarian cancer patients were treated with weekly paclitaxel and pembrolizumab (NCT02440425) [75]. In the 37 evaluable patients, the ORR was 51.4% (all partial responses) with DCR of 86.5%. The 6-month PFS rate was 64.5% and median PFS 7.6 months with a median OS of 13.4 months [75].

Combination Therapy: Immune-targeted Therapy

In a phase I study by Lee and colleagues, durvalumab was administered with either olaparib (poly-ADP-Ribose inhibitor) or cediranib (vascular endothelial growth factor receptor 1–3 inhibitor) to 26 patients with various cancers, the majority of which was ovarian (73%) [76]. In the 10 evaluable recurrent ovarian cancer patients who received durvalumab and olaparib, the ORR was 20% (two partial responses) with a DCR of 90% [76]. Durable responses in this treatment group were not explained by homologous recombination DNA repair pathway defects and none of the patients had germline BRCA mutations (two patients with somatic BRCA mutations had stable disease). For the 6 evaluable patients who received durvalumab and intermittent cediranib and were assessed for response, the ORR was 50% (all partial responses) and had a DCR of 83% [76]. Although the doublets overall had an acceptable safety profile, daily dosing cediranib treatment was not tolerated due to recurrent grade 2 and non-dose limiting toxicity grade 3 and 4 TRAE [76]. A biomarker analysis of a subset of the tumors demonstrated some clinical benefit correlated with tumoral PD-L1 expression [82]. In a larger cohort of recurrent, platinum-resistant ovarian cancer patients (majority consisting of BRCA wild types), Lee and colleagues found that durvalumab and olaparib had an ORR of 14.7% (5 partial responses; 2 with germline BRCA mutated and 3 with BRCA wild type) and DCR of 52.9% (NCT02484404) [77]. In another durvalumab/olaparib study, Drew et al. administered olaparib followed by maintenance olaparib and durvalumab therapy in platinum-sensitive ovarian cancer patients with germline BRCA mutations (MEDIOLA study; NCT02734004)

Table 7.7 Reported immune checkpoint inhibitors studies in epithelial ovarian cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Monotherapy						
Brahmer et al. 2012 [68]	Phase I	17	Progressive disease with advanced or metastatic ovarian cancer	Anti-PD-L1 3 mg/kg or 10 mg/kg up to 16 cycles	ORR 6% (1 PR/0 CR), DCR 23.50% (DCR only seen at 10 mg/kg dose)	∗Overall: 61% (fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache) Grade 3 or 4 TRAE in 9%
Hamanishi et al. 2015 [69]	Phase II	20	Platinum-resistant ovarian cancer	Nivolumab 1 or 3 mg/kg q2 weeks up to 6 cycles	ORR 15% (1 PR/ 2 CR), DCR 45% mPFS 3.5 months, mOS 20 months	Most common: increased serum AST, hypothyroidism, lymphocytopenia, decreased albumin, fever, increased serum ALT, maculopapular rash, arthralgia, arrhythmia, fatigue, and anemia Grade 3–4: 40%
Varga et al. 2019 [70]	Phase IB	26	Advanced ovarian cancer with failure of previous therapy and PD-L1 positivity	Pembrolizumab 10 mg/kg q2 weeks for up to 24 months	ORR 11.5% (2 PR/ 1 CR), DCR 38.50% mPFS 1.9 months, mOS 13.8 months	Overall: 73.1% (most commonly arthralgia, nausea, pruritus). One grade 3 TRAE
Matulonis et al. 2019 [71]	Phase II	Cohort A ($n = 285$ Cohort B ($n = 91$)	Advanced recurrent ovarian cancer. Cohort *A* = 1–3 prior lines of treatment & TFI 3–12 months; cohort *B* = 4–6 prior lines of therapy and TFI of at least 3 months	Pembrolizumab 200 mg IV q3 weeks up until 2 years	ORR Cohort A: 7.4% (16 PR/5 CR); Cohort B: 9.9% (7 PR/2 CR), DCR 37.2% vs. 37.4%. CPS ≥ 10 is correlated with higher clinical response, mPFS 2.1 months for both; mOS not reached for cohort A and 17.6 months for cohort B	Overall: 73.1%. Grade 3–5: 19.7% (Most common fatigue 2.7%, 2 deaths due to Stevens-Johnson syndrome and one hypoaldosteronism). Ir-AEs: 22.6% with most common being hypo/ hyperthyroidism, grade 3–5 severity: severe skin reaction and colitis
Disis et al. 2019 [72]	Phase IB	125	Platinum-resistant ovarian cancer	Avelumab 10 mg/kg q2 weeks until progression or withdrawal	ORR 9.6% (11 PR/1 CR), DCR 52% mPFS 2.6 months, 6- and 12-month PFS rate 16.1% and 10.2%, respectively, mOS 11.2 months, 12-month OS rate 47% PD-L1 status nor BRCA status was associated with response	Overall: 68.8% Grade 3–4: 7.2%

(continued)

Table 7.7 (continued)

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Infante et al. 2016 [73]	Phase 1A	9	Advanced, recurrent ovarian cancer	Atezolizumab 0.3 mg/kg, 10 mg/kg, or 15 mg/kg q3 weeks	ORR 22.2% (2 PR/0 CR) DCR 22.20% mPFS 2.9 months, mOS 11.3 months	Overall: 91.7% and mainly grade 1–2 fatigue and pain. Grade 3: 17% (autoimmune hepatitis and maculopapular rash)
Combination therapy: immune-chemotherapy						
Pujade-Lauraine et al. 2019 [74]	Phase III	566	Platinum-resistant/ refractory ovarian cancer patients	Randomized 1:1:1 ARM#1: avelumab alone ARM#2: PLD alone ARM#3: avelumab + PLD	ORR 3.7% vs. 4.2% vs. 13.3% PFS 1.9 vs. 3.5 vs. 3.7 months OS 11.8 vs. 13 vs. 15.7 months	Grade 3 or more: highest in ARM#3 (42.9%) followed by ARM#2 (31.6%) and ARM#1 (16.0%). PPE syndrome (9.9%), neutropenia & rash (9.3% each), fatigue (7.1%), and stomatitis (5.5%)
Wenham et al. 2018 [75]	Phase II	37	Recurrent EOC platinum resistant, at most 3 prior therapies	Weekly paclitaxel (80 mg/ m^2) with pembrolizumab 200 mg IV q3 weeks	ORR 51.4% (PR only), DCR 86.50%, 6-month PFS 64.5%, mPFS 7.6 months, mOS 13.4 months	Most common = anemia, fatigue, neutropenia, nausea, edema, diarrhea, dyspnea, leukopenia, neuropathy, vomiting, abdominal pain, lymphopenia, cough, hypomagnesemia. Grade 3 or 4 AE: leukocytosis, anemia, neutropenia, lymphopenia; neutropenia, glucose intolerance, hyponatremia
Combination therapy: immune-targeted therapy						
Lee et al. 2017 [76]	Phase I	Arm#1 ($n = 10$) Arm#2 ($n = 9$)	Eligible patients had recurrent or metastatic ovarian cancer	Dose escalation ARM#1: Durvalumab (10 mg/kg q2 weeks – 1500 mg q4 weeks) + olaparib (200–300 mg BID) ARM#2 Durvalumab (10 mg/kg q2 weeks – 1500 mg q4 weeks) + cediranib (30 mg qday or 20 mg with 5 days on/2 days off)	ORR: ARM#1 20% (2 PR/0 CR); ARM#2 50% (3 PR /6 CR) DCR: ARM#1 90%; ARM#2 83%	ARM#1 grade 3 included anemia and lymphopenia. ARM#2: grade 3 fatigue and grade 4 hypertension. Daily cediranib treatment was not tolerated due to recurrent grade 2 and non-dose limiting toxicity grade 3 and 4 AE
Lee et al. 2018 [77]	Phase II	35	Recurrent, platinum resistant ovarian cancer	Durvalumab 1500 mg IV q4 weeks and olaparib 300 mg BID	ORR 14.7% (5 PR/ 0 CR), DCR 52.90%	Grade 3 or 4: anemia, lymphopenia. Olaparib dose reduction due to anemia, atrial fibrillation, and nausea refractory to supportive care

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Drew et al. 2018 [78]	Phase II	32	gBRCAm platinum-sensitive relapsed ovarian cancer	Olaparib 300 mg po BID × 4 weeks, then olaparib 300 mg po BID + durvalumab 1.5 g IV q4 weeks	ORR 63% (14 PR/ 6 CR) DCR 81%	Grade 3 AE = anemia, increased lipase, increased amylase, and neutropenia
Konstantinopoulos et al. 2019 [79]	Phase I/II	60	Recurrent ovarian cancer	Pembrolizumab 200 mg q3 weeks + niraparib 200 mg q day	ORR 18% (8 PR/ 3 CR), DCR 65%, mPFS 3.4 months, 6- and 12-month PFS 31% & 12%. ORR consistent across platinum-based chemo sensitivity, previous bevavcizumab, somatic BRCA mutations or HRD biomarker status	Most common: fatigue, nausea, anemia, constipation. Grade 3: myelosuppression
Liu et al. 2018 [80]	Phase II	38	Platinum sensitive and resistant ovarian cancer	Bevacizumab 10 mg/kg and nivolumab 240 mg every 2 weeks until progression	ORR 26.3% (10 PR/0 CR), DCR 34.2%, mPFS 9.4 months	Most common = fatigue, AST/ALT elevation, myalgia, and skin changes
Combination therapy: immune-immunotherapy						
Burger et al. 2018 [81]	Phase II	Arm#1 ($n = 49$) Arm#2 ($n = 51$)	Recurrent ovarian cancer	ARM#1: nivolumab 3 mg/kg IV then q2 weeks × 4 then maintenance 3 mg/kg IV q2 weeks for up to 42 doses. ARM#2: nivolumab 3 mg/kg IV + ipilimumab 1 mg/kg q3 weeks × 4 then maintenance nivolumab 3 mg/kg IV q2 weeks	ORR 31.4% vs. 12.2%, mOS 28.1 months vs. 21 months	AE: more frequently from ARM#2 vs. ARM#1 Grade 3 or more: 67% (ARM#2) vs. 55% (ARM#1)

AE adverse events, *CPS* combined positivity score, *CR* complete response, *DCR* disease control rate = stable disease + partial response + complete response rates, *gBRCAm* germline BRCA mutated, *IrAE* Immune-related adverse events, *mOS* median overall survival, *mPFS* median progression-free survival, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *PPE* palmar-plantar erythrodysesthesia, *PR* partial response, *RFS* recurrence-free survival, *TRAE* treatment-related adverse events

[78]. In the 32 patients, there was an ORR of 63% (14 partial and 6 complete responses) with a DCR of 81% at 12 weeks and tolerable safe profile [78]. In TOPACIO/KEYNOTE-162, the investigators examined another PARPi/immune checkpoint inhibitor combination in a different patient population consisting of recurrent, platinum-resistant ovarian cancer patients with enrollment regardless of BRCA mutational status [79]. In this phase I/II study, niraparib and pembrolizumab was given to a cohort of 67 patients with ovarian or triple-negative breast cancer [79]. In the 60 evaluable ovarian cancer patients, the ORR was 18% (8 partial and 3 complete responses) and the DCR was 65% with acceptable treatment side effect profile [79]. The ORRs were seen to be consistent regardless of platinum-based chemotherapy sensitivity, previous bevacizumab, somatic BRCA tumor mutation, or homologous recombination defect biomarker status [79].

In another combination doublet study, Liu and colleagues tested nivolumab plus bevacizumab in a mixed cohort of platinum-sensitive and platinum-resistant ovarian cancer patients [80]. In the preliminary analyses of 38 patients, there was an ORR of 26.3% (10 partial responses with the majority in platinum-sensitive patients) with a DCR of 34.2% and tolerable side effect profile (NCT02873962) [80].

Combination Therapy: Immune-immunotherapy

Immunotherapy doublet therapy for ovarian cancer is currently being investigated in the phase II NRG-GY003 trial (NCT02498600) [81]. Burger and colleagues presented their preliminary findings at the 2018 International Gynecologic Cancer Society Meeting where 100 recurrent ovarian cancer patients were randomized to either nivolumab alone or nivolumab/ipilimumab followed by maintenance nivolumab [81]. Although the trial was not powered to detect a difference in overall survival (median OS 28.1 months vs. 21 months, respectively), ORR at 6 months was higher in the combination group than the monotherapy group (31.4% vs. 12.2%, respectively; OR 3.28, $p = 0.034$) [81]. Adverse events were higher in the combination group than the monotherapy group but were overall well tolerated [81]. There are a plethora of ongoing studies utilizing immune checkpoint inhibitors in combination with other agents in ovarian cancer (Table 7.10).

Vaccines in Epithelial Ovarian Cancer

Vaccines have been a point of interest in ovarian cancer to target tumor-associated antigens. NY-ESO-1 is expressed in >40% of advanced epithelial ovarian cancers and is one of the tumor-associated antigens of interest for vaccine therapy [83] (Table 7.8). In a study by Diefenbach et al., high-risk ovarian cancer patients with HLA-A∗0201 positivity had the administration of a NY-ESO-1b peptide and Montanide vaccination series following primary debulking and chemotherapy [84]. In the 9 patients evaluated, the vaccine series was overall well tolerated and appeared to mount a T-cell immunity response regardless of tumor expression of NY-ESO-1; 3 patients with NY-ESO-1 negative tumors having clinical remission at 25, 38, and 52 months [84]. In another phase I study, the addition of NY-ESO-1 vaccine and decitabine (DNA methylation inhibitor) following doxorubicin chemotherapy for 10 patients with recurrent epithelial ovarian cancer demonstrated increased antibody production and T-cell responses with an ORR of 10% (1 partial response) and DCR of 60% [85]. A phase I trial by Sabbatini et al. demonstrated that vaccine adjuvants to NY-ESO-1 such as Montanide-ISA-51 preparation and toll-like receptor ligand poly-ICLS (polyinosinic-polycytidylic acid-stablized by lysine and carboxymethylcellulose) can generate a stronger immune response in terms of antibody and CD8+ activity [86].

Dendritic cell vaccines have also been used in several trials. In a phase I/II trial, 11 ovarian cancer patients in their first or second clinical remission received monocyte-derived dendritic (DC)

loaded with Her2/neu (highly expressed in ovarian cancers), human telomerase reverse transcriptase, and pan-DR peptide antigens with or without cyclophosphamide chemotherapy prior to administration [87]. Overall 3-year survival was 90% with a trend towards survival in those who received cyclophosphamide therapy prior to vaccination [87]. In a phase I/II study, Baek et al. administered autologous dendritic-cell vaccination with IL-2 consolidation following debulking and chemotherapy and demonstrated good tolerability in 10 patients [88]. Three patients had maintenance of complete remission after vaccination for 83, 80.9, and 38.2 months and one patient had complete response for 50.8 months [88]. Increased immune response and reduced immune-suppressive factor secretion was also evident [88]. Another study compared autologous dendritic cell vaccine with chemotherapy to chemotherapy alone for recurrent platinum-sensitive ovarian cancers and demonstrated a trend towards improved ORR (87.5% vs. 62.5%, respectively) for the vaccine cohort (NCT02107950) [89]. A European multicenter, phase II study found that sequential administration of dendritic vaccines following primary cytoreductive surgery and chemotherapy had a trend of improved PFS compared with concomitant administration with adjuvant chemotherapy (24.3 vs. 18.3 months, $p = 0.05$) (NCT02107937) [90].

Kuwano et al. investigated the use of personalized vaccination based on HLA-type and pre-existing host immunity (by IGG response levels to tumor-associated antigens) and have demonstrated some disease stabilization with good tolerability [91]. Personalized vaccine generated by autologous dendritic cells pulsed with oxidized autologous whole-tumor cell lysate also demonstrated broad antitumor immune response activity [92].

In the DeCidE trial, DPX-Survivac (vaccine containing mix of HLA class I peptides against survivin antigen), low dose cyclophosphamide, and epacadostat (selective inhibitor of indoleamine 2,3-dioxygenase 1) were administered to stage IIC-IV recurrent ovarian cancer patients (NCT02785250) [93]. Preliminary results in the 10 evaluable patients demonstrated an ORR of 30% (3 partial responses) and DCR of 60% with good treatment tolerability [93].

Clinical trials utilizing autologous whole tumor vaccines are currently underway for high-risk stage III/IV ovarian cancer patients as adjuvant therapy (NCT01309230) or maintenance therapy (NCT02346747) (Table 7.10).

ACT in Epithelial Ovarian Cancer

Multiple trials have examined ACT in ovarian cancer. The first trial was by a 1991 study by Aoki et al. who examined TIL therapy without IL-2 infusion in advanced or recurrent ovarian cancer with or without cisplatin-containing combination chemotherapy [94]. In the TIL group without chemotherapy, there was an ORR of 71.4% (1 complete and 4 partial responses) while the group with both TIL and chemotherapy had a 90% ORR (7 with complete response and 2 with partial responses) where 4 of the 7 patients with complete responses did not have recurrence for >15 months of follow-up (Table 7.9) [94]. Another study by Ikarashi et al. demonstrated that TIL therapy may also induce increased cytotoxic T-cell and natural killer cell activity [95]. Another study by Fujita and colleagues compared patients with EOC following primary debulking and chemotherapy who were treated with TIL therapy without IL-2 infusion compared to controls. In their small study, they found that those who received TIL therapy had a better 3-year overall survival (100% vs. 65.5%) and PFS (82.1% vs. 54.5% respectively) rate compared with the control group [96]. In contrast to the above previous 3 studies, Pedersen et al. utilized an IL-2 infusion following TIL therapy in 6 patients with progressive platinum-resistant disease [97]. The DCR was 100% with 5 patients who had a reduction in size of target lesions (but did not meet partial response criteria) and antitumor reactivity seen in the TIL infusion products

Table 7.8 Reported vaccine therapy trials in epithelial ovarian cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Diefenbach et al. 2008 [84]	Phase I	9	HLA-A*0201, positive and high-risk epithelial ovarian cancer (defined by suboptimal initial debulking surgery, failure to normalize CA-125 after 3 cycles of chemotherapy, or positive second-look surgery)	HLA-A*0201–specific NY-ESO-1b peptide with Montanide-ISA-51 vaccine q3 weeks × 5 doses	ORR 33.33% (0 PR /3 CR. DCR 33.33% T-cell immunity in both NY-ESO-1 positive and negative tumors	Fatigue, anemia, pruritus, myalgias, and hyper- or hypothyroidism. No grade 3–4 AE
Odunsi et al. 2014 [85]	Phase I	10	Women with relapsed EOC, who normally receive doxorubicin as salvage therapy for recurrent disease.	Decitabine, doxorubicin, & vaccination (NY-ESO-1 peptide + Montanide-ISA-51 + GM-CSF) × 4 cycles	ORR 10% (1 PR/0 CR) DCR 60%	Mainly injection side reactions. Grade 3 or 4: neutropenia, injection site reactions
Sabbatini et al. 2012 [86]	Phase I	Arm#1 ($n = 4$) Arm#2 ($n = 13$) Arm#3 ($n = 11$)	Stage II to IV histologically documented epithelial carcinoma arising in the ovary, fallopian tube, or peritoneum in 2nd or 3rd remission	ARM#1: NY-ESO-1 OLP only ARM#2: NY-ESO-1 OLP + Montanide-ISA-51 ARM#3: NY-ESO-1 OLP + Montanide-ISA-51 + Poly-ICLC	NY-ESO-1–specific Antibodies and CD8 + T cells: Undetectable after vaccination in ARM#1, 46% & 62%, respectively, with ARM#2, 91% & 91%, respectively, with ARM#3. Montanide ISA-51 increased NY-ESO-1–specific CD4 + T cells frequency and polyclonality. Poly-ICLC accelerated the induction of immune responses	Injection site reactions and fatigue that were definitely or possibly related, respectively
Chu et al. 2011 [87]	Phase I/II	5 per ARM	HLA-A2+ stages II–IV disease with no clinical evidence of disease after primary debulking surgery and chemotherapy, or those with stages I–IV disease with no clinical evidence of disease after secondary surgical treatment for a first recurrence diagnosed after a progression-free interval of at least 2 years	ARM#1: Mature autologous Dendritic cells pulsed with HLA-A2-restricted hTERT 988Y, Her2/neu 369V2V9, Her2/neu 689, and PADRE peptides (PolyPeptide laboratories, San Diego, CA) with cyclophosphamide ARM#2: as above without cyclophosphamide	PFS 40% (ARM#1) vs. 80% (ARM#2) ($p = 0.17$) ARM#2 had no change in total lymphocytes or regulatory cells. Modest T-cell response to vaccine but less than normal response to control vaccine (diphtheria conjugate protein CRM197)	Most common: erythema, induration, pruritus, and pain at the site of injection, fever and fatigue. No grade 3–4 toxicities

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Baek et al. 2015 [88]	Phase I/II	10	DC vaccination was introduced as a consolidation therapy in patients initially treated with debulking surgery and chemotherapy	Autologous monocyte-derived DCs pulsed with autologous tumor lysate and KLH at 4-week intervals	DCR 50%, PFS 21.7 months, OS 43.8 months, Increased NK activity, Interferon-gamma secreting T cells, immune-stimulatory cytokine secretion and reduced immune-suppressive factor secretion	Most common: flu-like symptoms
Cibula et al. 2018 [89]	Phase II	32 per ARM	Platinum-sensitive recurrent advanced stage EOC	ARM#1: Chemo + DCVAC. DCVAC (1×10^7 DCs/dose). ARM#2: Chemo only	ORR 87.5% vs. 62.5%, mPFS 10.9 vs. 9.4 months for ARM#1 and #2, respectively	Most AE: related to chemo. No grade 3 based on vaccines
Rob et al. 2018 [90]	Phase II	Arm#1 ($n = 34$) Arm#2 ($n = 34$) Arm#3 ($n = 31$)	Stage III EOC (serous, endometrioid, or mucinous), PS 0–2, post-PDS with <1 cm maximal residuum and no prior systemic therapy	ARM#1: combo DCVAC (1×10^7 DCs/dose) + chemo, ARM#2: Sequential chemo then DCVAC ARM#3: Chemo alone	mPFS 18.3 vs. 24.4 vs. 18.6 months; gain in PFS in ARM#2 ($p = 0.05$) and similar trend in OS	No grade 3 TRAE related to DCVAC
Kawano et al. 2014 [91]	Phase II	42	Platinum-sensitive and platinum-resistant recurrent ovarian cancer	Personalized vaccine based on peptides selected in consideration of the HLA-type and pre-existing host immunity, as assessed by IgG levels against each of the 31 different vaccine candidates + Montanide ISA-51 (Seppic, Paris, France) +/− chemo (if tolerable by patient)	ORR 2.3% (0 PR /1 CR), DCR 7.10% mOS 19.1 months	Mainly grade 1 or 2 dermatological reaction at the injection sites except one grade 3 leg infection. Severe adverse events associated with chemotherapy, rather than directly associated with the vaccinations
Tanyi et al. 2018 [92]	Phase I	25	Platinum-treated, immunotherapy-naïve, recurrent ovarian cancer patients	ARM#1: OCDC only ARM#2: OCDC + bevacizumab ARM#3: OCDC + bevacizumab + cyclophosphamide	ORR 0% vs. 10% vs. 10% DCR 30% vs. 50% vs. 70% Vaccination induced T-cell responses to autologous tumor antigen, which were associated with significantly prolonged survival	Mainly grade 1 or 2 AE, most common being pain

(continued)

Table 7.8 (continued)

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Dorigo et al. 2018 [93]	Phase I/II	10	Subjects with advanced ovarian cancer (stage IIc-IV with evidence of disease progression)	DPX-Survivac (dose escalation) + metronomic CPA + epacadostat	ORR 30% (3 PR/0 CR) DCR 60%	Well-tolerated

AE adverse events, *Chemo* chemotherapy, *Combo* combination, *CR* complete response, *DCR* disease control rate = stable disease + partial response + complete response rates, *DCVAC* dendritic-cell vaccine, *RP2D* recommended phase II dose, *HLA* human leukocyte antigen, *IL-2* interleukin-2, *KLH* keyhole limpet hemocyanin, *mOS* median overall survival, *mPFS* median progression-free survival, *MTD* maximum tolerated dose, *OCDC* oxidized autologous whole-tumor cell lysate injected intra-nodally, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *PR* partial response, *RFS* recurrence-free survival, *TRAE* treatment-related adverse events

Table 7.9 Reported trials in adoptive cell therapy in epithelial ovarian cancer

Study	Design	*N*	Patient population	Therapy	Results	TRAE
Aoki et a. 1991 [94]	Phase I	TIL only ($n = 7$) TIL + chemo ($n = 10$)	Advanced or recurrent EOC	ARM#1: TIL (at least 1×10^{10} cells); no IL-2 infusion ARM#2: cisplatin-containing chemo followed by TIL infusion; no IL-2 infusion	ORR: 71.4% (4 PR/1 CR) (mono) vs. 90% (2 PR/7 CR) (combo) DCR: 85.7% (mono) vs. 100% (combo)	Fever and chills in 30%
Ikarashi et al. 1994 [95]	Phase I	TIL ($n = 12$) Controls ($n = 10$)	Epithelial ovarian cancer of advanced stage (International Federation of Obstetrics and Gynecology Stage II, III, or IV) following PDS	PDS then cisplatin-containing chemo followed by TIL (5×10^8 cells) without IL-2 vs. PDS + chemo	Increased CD8+ cells, cell-mediated immunity, and NK cell activity with CD16 and CD56 APCs)	Toxicity mainly from chemo (nausea/vomiting, alopecia, myelosuppression)
Fujita et al. 1995 [96]	Phase I	TIL + chemo ($n = 13$) Chemo only ($n = 11$)	Epithelial ovarian cancer of advanced stage (International Federation of Obstetrics and Gynecology Stage II, III, or IV) following PDS without residual tumor	PDS then cisplatin-containing chemo Followed by TIL (5×10^8 cells) without IL-2 vs. PDS + chemo	3 year PFS = 82.1% (combo) vs. 54.5% (mono), $p < 0.05$. 3 year OS of disease-free patients = 100% (combo) vs. 67.5% (mono) respectively (= < 0.01)	Toxicity mainly from chemo, e.g., nausea/vomiting, alopecia, myelosuppression
Pedersen et al. 2018 [97]	Phase I	6	Progressive platinum-resistant metastatic ovarian cancer	Standard lymphodepleting chemotherapy followed by TIL therapy and decrescendo IL-2 stimulation	ORR 0%, DCR 100% mPFS 3 months, mOS 10 months, high expression of LAG-3) and PD-1	Mild TRAE; hypophosphotemia, fever, hypokalemia, anemia, lymphocytopenia, thrombocytopenia
Freedman et al. 1994 [98]	Phase I	8	Advanced epithelial ovarian carcinoma, and who were refractory to platinum-based chemotherapy	IP TIL + IP IL-2 infusion	ORR 0% ascites regression (two patients), tumor and CA-125 reduction (one patient), and surgically confirmed stable tumor and CA-125 values (one patient)	Grade 3: anemia and peritonitis

AE adverse events, *CFU* colony-forming units, *Chemo* chemotherapy, *Combo* combination therapy, *CR* complete response, *DCR* disease-control rate = CR rate + PR rate + stable disease rate, *DOR* duration of action, *IL-2* interleukin-2, *IrAE* Immune-related adverse events, *LAG3* Lymphocyte activation gene 3, *Mono* monotherapy, *MTD* maximum tolerated dose, *ORR* objective response rate, *OS* overall survival, *PD-1* programmed cell death protein 1, *PDS* primary debulking surgery, *PK* Pharmacokinetics, *PFS* progression-free survival, *PR* partial response, *PROs* patient-reported outcomes, *TIL* tumor-infiltrating lymphocytes, *TRAE* treatment-related adverse events, *RFS* recurrence-free survival, *RP2D* recommended phase II dose, *WT1* Wilm's tumor gene

Table 7.10 Ongoing clinical trials for epithelial ovarian cancer

Study	Design	Patient population	Agent and dosing	Endpoints	Study status
NCT02839707	Phase II/III	Recurrent, platinum-resistant high-grade ovarian cancer	ARM#1: Atezolizumab + PLD ARM#2: Atezolizumab + Bevacizumab + PLD, ARM#3: PLD + Bevacizumab	Primary: MTD, PFS, OS Secondary ORR, AE, PROs, PD-L1 expression	Suspended for scheduled interim monitoring
PemCiGem (NCT02608684)	Phase II	Recurrent ovarian cancer	Gemcitabine/cisplatin/pembrolizumab then pembrolizumab maintenance	Primary: ORR Secondary: PFS, time to progression, DOR, OS, AE	Active but not recruiting
ATLANTE (NCT02891824)	Phase III	Progressive non-mucinous EOC, fallopian tube, and primary peritoneal cancer (platinum sensitive relapse)	ARM #1: placebo + bevacizumab + platinum-based chemotherapy ARM#2: atezolizumab + bevacizumab + platinum-based chemotherapy	Primary: PFS Secondary: OS, PROs, AE	Recruiting
NCT01928394	Phase I/II	Advanced or metastatic ovarian cancer	Nivolumab +/− Ipilimumab +/− cobimetinib	Primary: ORR Secondary: PFS, TRAE, OS	Active but not recruiting
NCT03026062	Phase II	Platinum-resistant or refractory high-grade epithelial ovarian, peritoneal, or fallopian tube cancer	ARM#1: Sequential tremelimumab then durvalumab ARM#2 combination tremelimumab + durvalumab	Primary: Immune-related progression-free survival Secondary: TRAE, OS, ORR	Recruiting
NCT02726997	Phase I/II	Newly diagnosed Stage III-IV ovarian, primary peritoneal, or fallopian tube cancer	Addition of durvalumab to neoadjuvant and/or adjuvant carboplatin/paclitaxel with maintenance durvalumab monotherapy	Primary: pharmacodynamic changes by treatment Secondary: PFS, feasibility of treatment	Recruiting
NCT02520154	Phase II	Stage III-IV ovarian, primary peritoneal, or fallopian tube cancer	Addition of pembrolizumab to neoadjuvant and/or adjuvant carboplatin/paclitaxel with maintenance pembrolizumab monotherapy	Primary: PFS, Response rate Secondary: feasibility of treatment, AE, OS	Recruiting
NCT02834975	Phase II	Stage III-IV ovarian, primary peritoneal, or fallopian tube cancer	Addition of pembrolizumab to neoadjuvant chemotherapy	Primary: pathologic ORR Secondary: PFS, safety, and tolerability of combination therapy	Recruiting
NCT03038100	Phase III	Stage III-IV ovarian, primary peritoneal, or fallopian tube cancer	Addition of atezolizumab to adjuvant carboplatin, paclitaxel, and bevacizumab following PDS	Primary: PFS, OS Secondary: OR, DOR, PROs, HRQoL, AE	Active but not recruiting
NCT03330405	Phase II	Recurrent platinum sensitive ovarian cancer	Avelumab + talazoparib	Primary: DLT, OR Secondary: PK, ORR, tumor marker levels, PD-L1 biomarker in tumor tissue	Recruiting

Study	Design	Patient population	Agent and dosing	Endpoints	Study status
KEYLYNK-001/ ENGOT-ov43 (NCT03740165)	Phase III	First-line treatment of women with BRCA non-mutated advanced epithelial ovarian cancer	First-line chemotherapy (carboplatin/ paclitaxel) +/− pembrolizumab +/− olaparib (+/− placebo for each or both)	Primary: PFS, OS Secondary: PFS, TRAE, treatment discontinue rate, QoL, pathologic CRR	Recruiting
ENGOT-0 V44 The FIRST Study (NCT03602859)	Phase III	First-line treatment of stage III or IV non-mucinous epithelial ovarian cancer	ARM#1: chemotherapy + dorstarlimab placebo + maintenance placebo ARM#2: chemotherapy + dorstarlimab placebo + maintenance niraparib + maintenance dorstarlimab placebo ARM#3: chemotherapy + dorstarlimab + maintenance niraparib + dorstarlimab	Primary: PFS Secondary: OS, TRAE, ORR	Recruiting
NCT03598270	Phase III	Patients with recurrent ovarian, tubal, or peritoneal cancer and platinum treatment-free interval (TFIp) >6 months	ARM#1: Investigator's choice chemotherapy + atezolizumab + maintenance atezolizumab and niraparib ARM#2: investigator's choice chemotherapy + maintenance niraparib + placebo	Primary: PFS Secondary: OS, TRAE, PROs, HRQoL, ORR, DOR, PK	Recruiting
DUO-O (NCT03737643)	Phase III	Newly diagnosed stage III-IV ovarian, primary peritoneal, or fallopian tube cancer	ARM#1: carboplatin/paclitaxel/bevacizumab + durvalumab placebo + maintenance bevacizumab + placebo maintenance durvalumab & olaparib ARM#2: carboplatin/paclitaxel/bevacizumab + durvalumab placebo + maintenance bevacizumab & durvalumab + placebo maintenance olaparib ARM#3: carboplatin/paclitaxel/bevacizumab + durvalumab placebo + maintenance bevacizumab, durvalumab, & olaparib.	Primary: PFS Secondary: OS, HRQoL, pathological CRR, PK, ORR, DOR, safety and tolerability	Recruiting
ATHENA (NCT03522246)	Phase III	Newly diagnosed advanced (FIGO stage III-IV) epithelial ovarian, fallopian tube, or primary peritoneal cancer	Maintenance rucaparib +/− nivolumab following primary or interval cytoreductive surgery	Primary: PFS Secondary: OS, ORR, DOR, TRAE, AE,	Recruiting

(continued)

Table 7.10 (continued)

Study	Design	Patient population	Agent and dosing	Endpoints	Study status
NCT03508570	Phase Ib	Platinum-resistant or refractory ovarian cancer with metastatic peritoneal carcinomatosis and recurred after or progressed on frontline and 1–2 second-line standard treatments	IP nivolumab +/− IP ipilimumab	Primary: MTD and P2PD Secondary: PK, IrAE, ORR	Recruiting
NCT01309230	Phase II	Stage III/IV papillary serous or endometrioid ovarian cancer following primary debulking and complete response to treatment	Intradermal autologous Vigil™ (1.0×10^7 cells/injection; maximum of 12 vaccinations)	Primary: Time to recurrence Secondary: immune function (surrogate markers, TIL, TAM, safety)	Recruiting
NCT02346747	Phase II	Stage IIIb-IV high-grade ovarian, fallopian tube or primary peritoneal following optimal PDS and chemotherapy	Vigil ® Ovarian (gemogenovatucel-T) engineered autologous tumor cells (EATC): receive 1.0×10^7 cells of gene transfected, irradiated, autologous tumor cells via intradermal injection once a month vs. placebo	Primary: PFS	Active but not recruiting
NCT00562640	Phase I	Recurrent platinum resistant or refractory ovarian cancer	WT1-peptide-sensitized T cells infusion when administered alone or with non-myelosuppressive chemotherapy (Cyclophosphamide) in patients	Primary: safety and tolerability, mean tolerated dose, quantitation of alterations in concentration of peptide-specific T-cells post infusion, response	Active but not recruiting
NCT01174121	Phase II	Chemorefractory ovarian cancer on at least second-line chemotherapy	Arm#1: CD8+ enriched TIL. ARM #2: unselected TIL. ARM#3: unselected TIL + Pembrolizumab prior to administration +3 cycles after ARM#4: unselected TIL + Pembrolizumab at progression for up to 8 cycles	Primary: Response rate Secondary: Frequency and severity of TRAE, safety and efficacy of pembrolizumab following TIL therapy	Recruiting
NCT01883297	Phase I	Recurrent platinum resistant high grade serous ovarian, fallopian tube, or primary peritoneal cancer, with evidence of disease progression from previous line of treatment	Conditioning with cyclophosphamide then re-stimulated TIL and IL-2 infusion	Primary: AE Secondary: clinical response to treatment, immune response	Recruiting
NCT02876510	Phase I	HLA-positive phenotype with advanced/metastatic ovarian cancer with up to 1 previous failed therapy	Conditioning with cyclophosphamide + fludarabine + autologous T-cell products (ACTolog® (IMA101–101)) with IL-2 +/− atezolizumab	Primary: AE; Secondary: feasibility of treatment, peripheral T-cell persistence, T-cell functionality, incidence of clinical responders, OS, PFS	Recruiting

Study	Design	Patient population	Agent and dosing	Endpoints	Study status
NCT03412526	Phase II	Platinum-resistant or platinum refractory disease	Conditioning with cyclophosphamide + fludarabine then total body radiation then unselected or 4-1BB-enriched TIL+ IL-2 infusion	Primary ORR, AE Secondary: OS, response rate, PFS, QoL	Recruiting

AE adverse events, *CFU* colony-forming units, *DOR* duration of action, *HLA* human leukocyte antigen, *IL-2* interleukin-2, *IrAE* Immune-related adverse events, *MTD* maximum tolerated dose, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *PK* Pharmacokinetics, *PROs* patient reported outcomes, *QoL* Quality of life, *RFS* recurrence-free survival, *RP2D* recommended phase II dose, *TIL* tumor-infiltrating lymphocytes, *TRAE* treatment-related adverse events, *WT1* Wilm's tumor gene

[97]. However, they noted that the lack of better therapeutic response may be due to high expression of lymphocyte-activation gene 3 (LAG-3) and PD-1, which are both involved in immune inhibitory signaling when interacting with MHCII and PD-L1, respectively [97]. Another study by Freedman et al. examined the administration of intraperitoneal TIL therapy with IL-2 in 11 patients and found clinical activity in 4 patients: ascites regression (2 patients), tumor and Ca-125 reduction (1 patient), and stable tumor and CA-125 levels in 1 patient [98].

Given the encouraging results, there is a plethora of ongoing clinical trials employing ACT for the treatment of ovarian cancer which are listed in Table 7.10.

Other Gynecologic Malignancies

There are few immunotherapy studies in other gynecologic malignancies. Quéreux and colleagues examined patients with metastatic or unresectable vulvar and vaginal melanomas who received immune checkpoint inhibitors in a retrospective review [99]. In the 6 patients that received ipilimumab, there were 4 patients with progressive disease, 1 stable response, and 1 patient who had a partial response but 89% reduction in tumor volume and a survival of 31 months [99]. In the 8 patients that were treated with nivolumab, there were partial responses in 4 patients [99]. One vaginal melanoma patient had received both ipilimumab and nivolumab and had a partial response [99].

Conclusion

Immunotherapeutic options hold modest but promising results in gynecologic cancers. Although a number of early studies have found limited clinical efficacy of vaccines as a monotherapeutic strategy, therapeutic vaccines may be useful as an adjunct in oncologic treatment as we await future trial results. Demonstrating impressive clinical responses in other solid tumors (e.g., metastatic melanoma), ACT and its utilization in gynecologic cancers are growing, and this approach has demonstrated promising early results in cervical and ovarian cancer. Additionally, immune checkpoint inhibitors have demonstrated durable clinical responses in various clinical trials, and this has resulted in granting approval for select patient population (e.g., pembrolizumab for MMR-deficient or MSI tumors and PD-L1-positive cervical cancers). Although immune checkpoint inhibitors have been the focus of interest in immunotherapy, there has been an explosion of new clinical trials in the recent years to investigate other modalities as well. With the modest results of using one immunotherapeutic agent, combination therapy utilizing agents from various immunotherapeutic/cytotoxic/targeted modalities is being investigated in multiple trials and to determine the optimal treatment regimens for right subset of patients. However, with a wealth of new immunemodulatory drugs, there will need to be a rethinking and innovation of clinical testing and trial design to optimize financial and clinical resources in pursuit of improved oncologic outcomes.

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8 Immunotherapy for Neuro-Oncology

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Abstract

Immunotherapy has changed the landscape of treatment of many solid and hematological malignancies and is at the forefront of cancer breakthroughs. Several circumstances unique to the central nervous system (CNS) such as limited space for an inflammatory response, difficulties with repeated sampling, corticosteroid use for management of cerebral edema, and immunosuppressive mechanisms within the tumor and brain parenchyma have posed challenges in clinical development of immunotherapy for intracranial tumors. Nonetheless, the success of immunotherapy in brain metastases (BMs) from solid cancers such as melanoma and non-small cell lung cancer (NSCLC) proves that the CNS is not an immune-privileged organ and is capable of initiating and regulating immune responses that lead to tumor control. However, the development of immunotherapeutics for the most malignant primary brain tumor, glioblastoma (GBM), has been challenging due to systemic and profound tumor-mediated immunosuppression unique to GBM, intratumoral and intertumoral heterogeneity, low mutation burden, and lack of stably expressed clonal antigens. Here, we review recent advances in the field of immunotherapy for neuro-oncology with a focus on BM and GBM.

Keywords

Glioblastoma · Brain metastases · Checkpoint inhibitors · Immunosuppressive macrophages · Immunotherapy combinations · GBM immune microenvironment · Tumor mutational load · Tumor-infiltrating lymphocytes · Cell therapy · Peptide vaccines · Cell vaccines · Oncolytic viral therapies

Immunosurveillance in the CNS

Early preclinical experiments had demonstrated immunity to skin homografts in mouse brain, cultivating the belief that CNS is an immune-privileged organ [1]. Later, through characterization of immune reactions in multiple sclerosis and encephalitis, the immunologic activity of CNS became apparent [2]. It was only recently discovered that T-cells exist and enter the CNS via lymphatic vessels lining the dural sinuses that connect the CSF to deep cervical lymph nodes [3]. CNS antigens are presented to

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T-cells by antigen-presenting cells (APCs) of the CNS (microglia and dendritic cells) that return to the CNS via perivascular system. The discovery of CNS lymphatic system in the era of immunotherapy advances in cancer was timely and has changed the long-held belief that the CNS is an immune-privileged organ. In addition to trafficking CNS lymphatics, immune cells are able to communicate to the brain parenchyma through a disrupted blood–brain barrier (BBB) as evidenced by gadolinium enhancement on T1-weighted MRI in tumors such as BM and high-grade primary brain tumors.

Immunotherapy for Brain Metastasis

BM is the most common form of intracranial malignancy, and its incidence is on the rise as therapeutic advances are controlling systemic disease leading to longer patient survival [4]. BM occurs as much as ten times more frequently than primary brain tumors occurring in 9–10% of all cancer diagnoses [5]. The incidence has been estimated to be between 11.2 and 14.3 per 100,000 [5]. The three most common primary cancers associated with brain metastasis are lung (20–56%), breast (5–20%), and melanoma (7–16%) [6]. Promising data are emerging on the benefit of checkpoint inhibitors (CPIs) in melanoma and NSCLC brain metastasis [7, 8] suggesting that CNS location of the tumor does not preclude the clinical efficacy of immunotherapy.

CPIs have been at the forefront of immunotherapy advances for the treatment of cancer, and their FDA approvals are on the rise [9]. CPIs are antibodies that bind to T-cell inhibitory signals on T-cells, APC, and tumor cells and stimulate profound immune responses against tumors by activating previously exhausted T-cells and maintaining their effector function. The most widely used CPIs include monoclonal antibodies against CTLA-4 and PD-1 (expressed on T-cells), and PD-L1 (expressed on APCs and tumor cells) [10, 11].

The prognosis of metastatic melanoma was dismal before recent advances in targeted therapy and immunotherapy. One-year overall survival (OS) rate of 25.5% was reported in a 2008 meta-analysis of 42 phase II cooperative group trials in patients with stage IV melanoma [12]. In 2018, there was a report of a 3-year OS rate of 63% in 94 patients with measurable, unresectable stage III or IV melanoma who received ipilimumab (anti-CTLA-4 antibody) and nivolumab (anti-PD-1 antibody) as concurrent therapy in a phase I study [13]. The annual incidence of BM from melanoma is increasing, which may be due to improved survival as a result of novel targeted therapies and immunotherapy for metastatic melanoma and/or more frequent imaging for screening [14]. The current lifetime incidence of BM from metastatic melanoma is estimated to be ≥50% [14, 15]. Conventional treatments such as surgical resection and stereotactic radiotherapy improve local control, but do not impact overall survival. In addition, whole-brain radiation and systemic chemotherapy options (i.e., temozolomide) have limited efficacy for the treatment of melanoma BM [15, 16]. With improved survival of metastatic melanoma patients with the use of CPI, the field moved toward addressing the role of CPI in melanoma with BM.

Initial immunotherapy studies evaluated the combination of CPI and cytotoxic chemotherapy. Di Giacomo and colleagues evaluated the combination of ipilimumab and fotemustine in a single-arm phase II trial of metastatic melanoma that included 20 patients with asymptomatic melanoma BM. In their study, ten patients had complete response (CR), while five had stable disease (SD) with a median progression-free survival (PFS) of 3 months [17]. At a median follow-up of 39.9 months, those with the BM had a 3-year survival rate of 27.8% with a median overall survival (mOS) of 12.7 months [18]. Subsequently, Margolin and colleagues conducted an open-label study of ipilimumab in patients with BM from melanoma. Of the 72 patients in the study, 51 had asymptomatic brain metastases and were not on corticosteroids while 21 had symptomatic BM and were on corticosteroids at the time of receiving ipilimumab. The patients who did not receive corticosteroids had higher response rates of 18% with an OS of 7 months compared to 5%

and an OS of 3.7 months for those who received corticosteroids [19]. The lower response rate and survival in the corticosteroid group might have been because of more advanced disease requiring steroids and/or effect of steroids on CPI efficacy. The above studies were encouraging, but had included patients who had received prior treatment for BM, and therefore, the role of CPI as an upfront treatment for untreated BM was unknown prior to the pivotal study by Tawbi and colleagues.

Recently, Tawbi and colleagues evaluated the efficacy and safety of nivolumab plus ipilimumab in an open-label, multicenter, phase II study in patients with melanoma who had asymptomatic untreated BM and demonstrated clinically meaningful intracranial efficacy. Fifty-seven percent of patients had intracranial benefit defined as stable disease (SD) for at least 6 months after the initiation of treatment, complete response (CR), or partial response (PR) (26% CR, 30% PR, 2% SD). Therapy with nivolumab plus ipilimumab prevented intracranial progression for more than 6 months in 64% of patients [7]. Similarly, Goldberg and colleagues conducted a nonrandomized phase II trial examining pembrolizumab in patients with untreated or progressive BM from NSCLC and melanoma. They reported responses in 6 and 4 out of 18 patients with NSCLC and 18 patients with melanoma, respectively [8]. The success of CPI in BM from these solid cancers is encouraging to the neuro-oncology community as it indicates that the brain is capable of initiating and regulating immune responses and has raised interest in identifying the role of immunotherapy in malignant primary brain tumors. The above trials of immunotherapy for BMs from solid tumors are summarized in Table 8.1.

Glioblastoma

GBM is the most common malignant brain tumor in adults with mOS of 14.6 months with the current standard of care [20]. The standard of care includes maximal safe resection when possible [21] followed by 60 Gy of radiation administered over 6 weeks (2 Gy per fraction × 30 fractions) with concurrent temozolomide (TMZ) at a dose of 75 mg/m2 administered daily over 6 weeks. This is followed by adjuvant TMZ at 150–200 mg/m2 administered on days 1–5 of 28 days cycles for 6–12 cycles. Despite this multimodality treatment, GBM invariably recurs leading to death with a 2-year survival rate of 26.5% [20].

Preclinical studies of CPI in GBM were promising as increased intratumoral CD8+ T-cells and long-term tumor-free survival were observed in mouse models [22, 23]. However, similar antitumor responses were not seen in a large phase III trial of nivolumab versus bevacizumab in recurrent GBM (n = 1 84, nivolumab; n = 185, bevacizumab) [24]. In addition, there was no survival benefit when nivolumab was added to radiation and temozolomide in newly diagnosed MGMT-unmethylated GBM in a phase III study (CheckMate-498) [25]. The reason for the disparity between preclinical studies and human studies is multifold, including the highly clonal nature of the cell lines used as opposed to clonal heterogeneity in GBM [26] and local and systemic immunosuppression unique to GBM in human. Understanding the mechanisms of immunosuppression in GBM is crucial in our efforts to implement immunotherapeutic approaches for the treatment of this deadly disease.

Immunosuppression in Glioblastoma

Unique local and systemic mechanisms of immunosuppression have posed roadblocks to the clinical development of immunotherapy in GBM.

Several factors contribute to local immunosuppression in GBM: tumor-intrinsic factors, tumor immune microenvironment, and the interaction between the two. GBM cells have intrinsic defects in antigen presentation. Tumor antigen presentation by the HLA class I peptide complex to the activated T-cells is needed for the immune system to recognize and destroy cancer cells. Loss of heterozygosity [27] in HLA class I is frequent in adult GBM and is associated with shorter overall survival [28]. In addition, GBM cells

Table 8.1 Select checkpoint inhibitor clinical trials for brain metastases from solid tumors

Title/Setting	Treatments	Phase	*N*	Outcome	Clinical trial identifier	Reference
Asymptomatic melanoma BM	Ipilimumab and fotemustine	II	20	CR: 10 SD: 5 mPFS: 3 mo mOS: 12.7 mo 3-yr survival rate: 27.8%	NCT01654692	[17, 18]
Symptomatic and asymptomatic melanoma BM	Ipilimumab	II	72	51 asymptomatic BM: RR: 18% mOS: 7 mo 21 symptomatic BM + steroids: RR: 5% mOS: 3.7 mo	NCT00623766	[19]
Untreated melanoma BM	Nivolumab and ipilimumab	II	94	Intracranial benefit: 57% CR: 26% PR: 30% SD > 6 mo: 2%	NCT02320058	[7]
Untreated or progressive BM from NSCLC and melanoma	Pembrolizumab	II	18 per cancer type	RR: 22% in melanoma RR: 33% in NSCLC	NCT02085070	[8]

Abbreviations: *CR* complete response, *BM* brain metastasis, *NSCLC* non-small cell lung cancer, *OS* overall survival, *PFS* progression-free survival, *RR* response rate, and *SD* stable disease

overexpress the T-cell inhibitory ligand, PD-L1 [29], which suppresses T-cell activation via T-cell anergy and apoptosis. GBM tumor cells have also been shown to upregulate immunosuppressive signaling pathways such as signal transducer and activator of transcription 3 (STAT-3) and indoleamine 2,3-dioxygenase (IDO) [30, 31]. In addition to tumor-intrinsic factors, the tumor immune microenvironment plays a pivotal role in GBM immunosuppression. GBM immune microenvironment is filled with immunosuppressive macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T-cells (Treg) [32–34]. Furthermore, the primary APC of the CNS, microglia, and cells capable of spontaneous cytotoxicity, national killer (NK) cells, and monocytic cells are nonfunctional in gliomas [35, 36]. Interaction between tumor and immune cells within the tumor immune microenvironment further contributes to local immunosuppression in GBM. GBM cells overexpress FasL which through its interaction with Fas expressed on T-cells leads to T-cell apoptosis [37]. Similarly, direct interactions between GBM cells and NK cells via atypical HLA molecules suppress NK cell activity [38, 39]. Immunosuppressive soluble factors such as TGF-β [40] and IL-10 [41] released by GBM cells, macrophages, microglia, and Tregs further contribute to local immunosuppression in GBM.

Interestingly, despite being a disease confined to the CNS, GBM imparts profound systemic immune suppression in the host. Total T-cell counts are reduced even in treatment naïve GBM patients [42–44]. Peripheral T-cells are thought to be sequestered in the bone marrow due to decreased surface sphingosine-1-phosphate receptor 1 (S1P1) expression which normally regulates T-cell exit from lymphoid organs and their egression from the bone marrow [44]. GBM patients' peripheral blood contains an abundant monocyte population which inhibits T-cell proliferation and lacks the ability to differentiate into mature dendritic cells (DCs) [45]. In addition, circulating monocytes and macrophages isolated from GBM patients have elevated expression of T-cell inhibitory ligand, PD-L1, and have the ability to suppress activation of cocultured T-cells [46]. The systemic immunosuppression in GBM is further exacerbated by lymphotoxic effects of radiation, TMZ, and corticosteroids [43]. Overall, profound local and systemic immunosuppressive mechanisms in GBM have to be targeted for the successful implementation of immunotherapy in GBM.

Checkpoint Inhibitors for the Treatment of GBM

PD-1/PD-L1 Inhibitors

PD-1/PD-L1 axis inhibitors are among the best-studied CPIs in GBM. Responses to anti-PD-1 antibodies, nivolumab and pembrolizumab, have been described in cases of GBM with high mutation burden. Examples include a case report of durable response to nivolumab in two siblings with biallelic mismatch repair deficiency with recurrent multifocal GBM [47] and successful use of pembrolizumab in a patient with germline POLE deficiency and GBM metastatic to the spine [48]. High mutational load and mismatch repair deficiency are known markers of response to CPI in a number of solid tumors [49], but these molecular characteristics are only found in a minority of GBM patients [50] and their associations with clinical response to CPI is unproven. The relevance of hypermutation and response to CPI in GBM is currently being tested in a clinical trial of pembrolizumab in patients with recurrent malignant glioma with a hypermutator phenotype (NCT02658279).

Completed trials of CPI in GBM have been summarized in Table 8.2. CheckMate 143 (NCT 02017717) was the first large randomized trial of PD-1 inhibitors in GBM where nivolumab was compared with bevacizumab in recurrent GBM at first relapse (n = 184, nivolumab; n = 185, bevacizumab). Preliminary results reported as an abstract at the World Federation of Neuro-Oncology Societies meeting in 2017 reported no difference in overall survival [24]. An exploratory phase I cohort within CheckMate 143 assessed nivolumab monotherapy (n = 10) versusvs. nivolumab plus ipilimumab (n = 30). Adverse events leading to discontinuation

Table 8.2 Completed checkpoint inhibitors clinical trials for GBM

Title/setting	Treatments	Phase	*N*	Outcome	Clinical trial identifier number	Reference
CheckMate 143 Recurrent GBM	NIVO versus BEV	III	369	mOS 9.8 mo vs. 10 mo similar use of corticosteroids in above cohorts	NCT02017717	[24]
CheckMate 143 Recurrent GBM	Cohort 1B NIVO +/− IPI	I	40	Adverse event profile superior in NIVO monotherapy than in combination arms	NCT02017717	[51]
CheckMate 143 ND GBM	Cohort 1C: NIVO+TMZ + RT - amp;gt; TMZ (MGMT methylated and unmethylated)	I	55	Neurological adverse events were similar to other trials without immunotherapy	NCT02017717	[52]
	Cohort 1D: NIVO+RT - amp;gt; TMZ (MGMT unmethylated)	I	58			
Recurrent GBM	Pembro versus pembro + BEV	II	80	PFS-6 6.7% versus 26%	NCT02337491	[53]
Recurrent GBM	Neoadjuvant pembro versus adjuvant pembro	II	35	mOS 13.7 mo versus 7.5 mo mPFS 3.3 versus 2.4	NCT02852655	[57]
Recurrent GBM	Neoadjuvant nivo	II	30	mOS 7.3 mo mPFS 4.1 mo	NCT02550249	[58]
Recurrent GBM	Neoadjuvant pembro	II	15	mPFS: 7 mo mOS: not reached at median follow-up of 12 mo 1-yr survival rate: 72%	NCT02337686	[33]

Abbreviations: *BEV* bevacizumab, *GBM* glioblastoma, IPI ipilimumab, *ND* newly diagnosed, *NIVO* nivolumab, *OS* median overall survival, *Pembro* pembrolizumab, *PFS* progression-free survival, *RT* radiation, and *TMZ* temozolomide

occurred more commonly in patients receiving dual immunotherapy [51]. Therefore, the combination therapy with nivolumab and ipilimumab is not being further pursued at this time.

Recurrent GBM is a highly resistant tumor, and therefore, the implementation of CPI clinical trials in the newly diagnosed setting has been pursued. An exploratory cohort of CheckMate 143 assessed the safety and tolerability of nivolumab in combination with radiation +/− TMZ in patients with newly diagnosed GBM and found a similar neurological adverse event as in other trials without CPI in the newly diagnosed setting [52]. However, a phase III trial of nivolumab plus radiation versus. temozolomide plus radiation in MGMT-unmethylated GBM demonstrated no survival benefit [25]. Another phase III trial of nivolumab in combination with radiation and TMZ (standard of care) in MGMT-methylated GBM is currently ongoing (NCT02667587).

Similar to nivolumab, pembrolizumab was shown to have limited monotherapy activity in recurrent GBM. Early results of a phase II study of pembrolizumab or pembrolizumab plus bevacizumab in recurrent GBM at first or second relapse demonstrated that patients receiving bevacizumab had superior PFS at 6 months (26%), as expected given pseudoresponse seen on MRI with bevacizumab. However, PFS6 for pembrolizumab only patients was similar to historical controls for recurrent GBM (6.7%) [53]. In this study, the combination of bevacizumab and pembrolizumab was well tolerated.

Until recently, PD-1 inhibition was mainly used as adjuvant treatment in GBM trials. However, recent successes with the use of neoadjuvant PD-1 blockade in melanoma [54, 55] and respectable lung cancer [56] have raised interest in the use of anti-PD-1 in the neoadjuvant setting with the goal to alter GBM immune microenvironment. Cloughesy and colleagues recently reported on the success of neoadjuvant pembrolizumab in recurrent GBM [57]. They randomized 35 recurrent GBM patients to receive neoadjuvant pembrolizumab followed by surgery and subsequent pembrolizumab monotherapy versus. adjuvant pembrolizumab. They reported a survival benefit in the neoadjuvant versus the adjuvant group (13.7 months vs. 7.5 months; hazard ratio 0.39 neoadjuvant/adjuvant; $P = 0.04$). Treatment with neoadjuvant pembrolizumab was associated with upregulation of T-cells and interferon-γ-related gene expression and downregulation of cell cycle–related genes. These results are encouraging with the caveat that the study was powered for tissue analysis and not survival. Similarly, Schalper and colleagues performed a single-arm phase II clinical trial (NCT02550249) in which they tested a presurgical dose of nivolumab followed by postsurgical nivolumab and demonstrated enhanced expression of chemokine transcripts, higher immune cell infiltration, and augmented TCR clonal diversity among tumor-infiltrative T-cells in resected tumor tissue [58]. In another single-arm neoadjuvant study by de Groot and colleagues, neoadjuvant pembrolizumab was tested in 15 patients with recurrent GBM where mPFS was 7 months and mOS was not reached at median follow-up of 12 months with an estimated 1-year OS rate of 72% (95% CI: 52–99.6%) at the time of reporting the results [33]. GBM tissue treated with pembrolizumab was found to be poorly infiltrated with T-cells and was enriched with distinct CD68+ populations consistent with an immunosuppressive tumor microenvironment. The ability of neoadjuvant PD-1 blockade to alter the tumor immune landscape has challenged the previous dogma that minimum tumor burden is required for effective immune therapy.

Pembrolizumab is currently being tested in the newly diagnosed setting in combination with radiation plus TMZ as monotherapy (NCT02530502). In addition to the above PD-1 inhibitors, 2 PD-L1 inhibitors, atezolizumab and durvalumab, are currently being tested in newly diagnosed GBM patients (NCT03174197 and NCT02336165, respectively).

CTLA-4 Axis Inhibitors

Dual immunotherapy targeting both PD-1/PD-L1 and CTLA-4 pathways has been more successful than monotherapy in melanoma [59]. However, higher rates of adverse events were seen when dual therapy was used in CheckMate 143 GBM

trial [53]. Several combinatorial therapies with CPI and other forms of immunotherapy are going, and dual CTLA-4 and PD-1/PD-L1 blockade are currently being proposed.

Why Is Checkpoint Inhibition More Effective in BM Than in GBM?

The differences between the effectiveness of CPI in brain metastasis and GBM likely lie in low mutation burden in GBM, the overwhelming impact of GBM on local and systemic immunosuppression, and the infiltrative nature of GBM tumor within the brain parenchyma.

Strong associations between clinical response and high mutation burden and/or PD-L1 expression have been described in melanoma and NSCLC, but it is not yet clear how these factors contribute to intracranial responses seen with CPI in the brain metastasis from these solid tumors [7, 8]. Tumor mutation load, which is associated with abundance of antigens and neoantigens leading to increased immunogenicity, is lower in GBM in comparison to cancer types in which CPIs are highly active [60], GBM has a higher expression of the T-cell inhibitory ligand, PD-L1, than BM [61]; however, the role of PD-L1 as a marker of response to CPI in GBM is not clear. Another key difference is that GBM is among the most immunosuppressive of solid tumors despite confinement to the intracranial compartment [62]. In fact, GBM utilizes a variety of immune suppressive mechanisms to prevent its immune detection and eradication [63]. These immunosuppressive mechanisms include infiltration of GBM microenvironment by immunosuppressive T-cells (regulatory T-cells) and macrophages [64] and release of immunosuppressive soluble factors such as TGF-ß and IL-10 [63]. In addition to local immune suppression, systemic immune suppression has been described in GBM patients even prior to the start of radiation and chemotherapy [44]. Local and systemic immunosuppressive mechanisms in GBM are described in detail in section "Introduction".

In addition, GBM tumor cells infiltrate the brain parenchyma and disseminate while in BM, the infiltrative growth is not seen, and parenchymal metastases remain in the perivascular space [65]. The infiltrative nature of GBM is a barrier to the success of drug delivery. Therapeutic monoclonal antibodies in particular tend to accumulate in the necrotic center which has a disrupted BBB rather than the infiltrative edge which has a more intact BBB [66]. Since GBM cells are highly infiltrative with single cells shown to migrate into regions distant from the initial tumor mass, the disease has an extremely high propensity for recurrence making it more challenging for immunotherapy to be as successful [67].

Vaccines

The fundamental notion behind cancer vaccine strategies is the induction of antitumor immune responses that mediate tumor regression through a targeted cytotoxic T-cell effect while sparing normal tissue. Peptide vaccines and cell vaccines comprise the two major types. Peptide vaccines take advantage of tumor-specific antigens which are proteins encoded by mutant genes in the tumor to induce an immune response against the tumor cells. Cell vaccines comprise autologous or allogenic immune cells that trigger antitumor immune responses.

Peptide Vaccines

EGFRvIII (type III epidermal growth factor receptor mutation) is expressed in 20–30% of patients with GBM and has been targeted for treatment of GBM via pharmacological inhibition and a peptide vaccine. EGFRvIII is formed due to the deletion of exons 2-7 of EGFR resulting in an extracellular truncation of EGFR allowing it to be constitutively active in the absence of ligand [68]. The EGFRvIII targeting vaccine PEP-3-KLH (keyhole limpet hemocyanin) (rindopepimut) was studied in a large multicenter, double-arm phase III clinical trial, ACT IV [69]. Seven hundred patients with newly diagnosed GBM were enrolled into two arms: PEP-3-KLH plus TMZ versus KLH plus TMZ (control arm). Though PEP-3-KLH exhibited sufficient safety in the study, it failed to provide a survival benefit. There was no difference in the mOS of patients who received the vaccine compared to

the control group for patients with minimum residual disease (MRD) and all intention-to-treat (ITT) patients (PEP-3-KLH vs. control: MRD: 20.1 months vs. 20 months; ITT: 17.4 months vs. 17.4 months). Interestingly, a post hoc analysis revealed that patients with bulky disease had a survival benefit from PET-3-KLH with a 2-year OS rate of 30% versus 19% for the control arm (P = 0.029) [69]. This finding challenged the dogma that a minimum tumor burden is required for effective immunotherapy. The unsatisfactory efficacy results of the ACT IV phase III trial ended the development of EGFRvIII-targeted peptide vaccines. Remarkably, evidence of loss of EGFRvIII expression was noted in about 60% of the small subset of patients with tumor tissue available at recurrence, although this may be a general evolutionary phenomenon that may have occurred independent of EGFRvIII-targeted vaccination. The lack of stability of EGFRvIII expression may preclude its use as a molecular target for treatment in GBM. GBM is a heterogeneous tumor, and the selection of one molecular target of immunotherapy like EGFRvIII might be insufficient. This may especially be the case if its expression is not stable and not ubiquitous which means that multipeptide vaccines against several targets and non-peptides with higher immunogenicity are likely needed.

Mutations in isocitrate dehydrogenase (IDH) exist in about 80% of low-grade gliomas affecting multiple pathways and metabolisms [70]. The most common of such mutations is the R123H mutation in IDH1 which accounts for approximately 70% of all IDH mutations [70]. Typically, GBM tumors that evolve from low-grade glioma harbor IDH1 mutations while only a small fraction of primary GBM cases harbor mutations in IDH1 [71]. Schumacher and colleagues demonstrated that IDH1 (R132H) contains an immunogenic epitope suitable for mutation-specific vaccination and developed a 15-amino-acid polypeptide targeting IDH1 R132H [72]. They found that peptides encompassing the mutated region were presented on major histocompatibility complexes (MHC) class II and induced mutation-specific CD4+ responses. In a mouse model, IDH1 peptide vaccines were shown to promote improved survival leading to intratumoral downregulation of TGF-β2 and IL-10 and upregulation of granzyme-b, IFN-γ, and perforin-1 [73]. Platten and colleagues tested a mutation-specific peptide vaccine targeting IDH1R132H in patients with newly diagnosed anaplastic astrocytoma and GBM with IDH1R132H mutations in a phase I trial. The trial demonstrated safety and immunogenicity [74]. Currently, an ongoing phase I clinical trial investigates the IDH1 peptide vaccine in recurrent low-grade gliomas (NCT02193347).

To address the challenges of developing peptide vaccines against one antigen, the development of the latest peptide vaccines for brain tumors has now moved toward personalized multipeptide vaccines with activity against several targets. GBM-specific peptide vaccine, IMA950, was developed to target 11 tumor-associated peptides identified on HLA surface receptors in primary human GBM tissue [75]. Rampling and colleagues conducted a phase I trial of IMA950 and found that 20 of the 40 evaluable patients were multi tumor-associated peptide (TUMAP) responders which exceeded their primary endpoint of multi-TUMAP responses in at least 30% of patients [75]. Similarly, a phase I/II trial testing IMA950 adjuvanted with poly-ICLC in HA-A2 + glioma patients observed CD8+ T-cell responses to a single or multiple peptides in 63.2% and 36.8% of patients, respectively [76].

In addition, Keskin and colleagues have demonstrated that the use of multi-epitope, personalized neoantigen vaccination is feasible in GBM despite its relatively low mutation load and immunologically "cold" tumor microenvironment [77]. They conducted a phase I/Ib trial involving ten patients with newly diagnosed GBM. Neoantigens were identified in each individual patient by comparing whole-exome sequencing data from the surgically resected tumor to that of matched normal cells [77]. For each patient vaccine, a pool of 7–20 peptides were selected as actionable neoepitopes predicted to bind to the HLA class I molecules of each patient. The vaccine was safe with no serious adverse side effects. Patients who received corticosteroids to treat side effects did not have a

T-cell response to vaccination. However, the two patients that did not receive dexamethasone had strong antitumor immune responses generating neoantigen-specific T-cells that were able to cross the blood–brain barrier and traffic to the tumor in the brain. The T-cells comprised of both CD8+ and CD4+ T-cells enriched in a memory phenotype [78]. Clonal expansion of neoantigen-reactive T-cells was seen in the tumor identical to circulating T-cells. These correlative results are encouraging, but need to be interpreted with caution as responses were only seen in two patients. These responses were seen in patients who were not on steroids emphasizing the judicious use of steroids in immunotherapy trials.

Similarly, Hilf and colleagues used a similar multi-epitope-based personalized vaccine strategy, but targeted both neoantigens and unmutated tumor-specific antigens to increase the number of actionable epitopes. In this phase I study, 15 patients were enrolled by the multicenter initiative Glioma Actively Personalized Vaccine Consortium (GAPVAC), and two types of vaccines were tested [79]. The results of microarray analysis of the patient transcriptome and mass spectrometry analysis of their HLA immunopeptidome determined the composition of both vaccines. The patients were first vaccinated with APVAC1 which is a pool of nine unmutated peptides derived from a premanufactured library of non-mutated antigens that are overrepresented in GBM tumors. The second vaccine, APVAC2, was preferentially targeted against mutated neoantigens, and if no neoantigens were identified in a patient, then the vaccine was targeted against non-mutated antigens that were not present in the premade library. Both of these vaccines were safe and generated T-cell responses against the proteins in the vaccine with APVAC1 inducing a sustained CD8+ T-cell response and APVAC2 inducing both CD4+ and CD8+ T-cell responses [79]. There is a favorable mOS in this study of 29 months, which suggests a potential clinical benefit compared with historical controls. These two recent first-in-human phase I studies of personalized neoantigen vaccines for patients with GBM have demonstrated that "cold tumors" with a low mutational burden can be infiltrated with antigen-specific T-cells through personalized vaccines.

Another approach in the peptide vaccine has been the development of heat shock protein (HSP) vaccines. HSPs function as intracellular chaperones and have been shown to be involved in the activation of both innate and adaptive immune systems. HSPs are involved in protein folding, protein stabilization, peptide loading onto MHC class I molecules, tumor initiation, and proliferation [80]. Akin to GAPVAC, HSP vaccines do not just target one antigen but rather target a mechanism that is implicated in tumor-specific antigen presentation in GBM. HSP–peptide complexes (HSPPCs) mediate endocytosis and trigger immune responses to tumor-antigenic peptides by antigen presentation [81]. Bloch and colleagues conducted a first phase II clinical trial investigating the HSPCC-96 vaccine in recurrent GBM after gross total resection and administered the vaccine every week for 4 weeks and then every 2 weeks until tumor recurrence. Following the treatment, mOS was 42.6 weeks (95% CI: 34.7–50.5) and OS rate at 12 months was 29.3% (95% CI: 16.6–45.7). The toxicity of the vaccine was also minimal with a single grade 3 event related to the vaccine [82]. Completed peptide and cell vaccine trials are summarized in Table 8.3. Overall, the generation of peptide vaccines for glioma has been feasible with correlative studies indicating biological activity. However, sustained clinical benefit has not been observed indicating that the degree of immune activation may not be sufficient for meaningful clinical response. Combinatorial immunotherapy approaches may aid in improving immune stimulation and clinical benefit.

Cell Vaccines

In addition to peptide vaccines, cell-based vaccines using DCs have been of particular interest in GBM. DCs are the most potent APC of the immune system. In order to produce autologous DC vaccines, DCs are first isolated from the patient, loaded with the tumor antigen, matured via exposure to cytokines, and then reinjected into the patients' body. The very first report of a DC vaccine used in GBM was by Liau and

Table 8.3 Select vaccine clinical trials for GBM

Title/setting	Treatments	Phase	*N*	Outcome	Clinical trial identifier	Reference
ACT IV ND GBM	TMZ + rindopepimut-KLH versus KLH	III	745	MRD mOS: 20.1 months versus 20 months	NCT01480479	[69]
NOA-16 ND GBM and AA (IDH1R132H-mutated)	IDH1 peptide vaccine	I	32	Demonstrated safety and immunogenicity	NCT02454634	[74]
IMA950 ND GBM	GBM multipeptide vaccine IMA950	I	40	Well tolerated with multi-TUMAP responses in at least 30%	NCT01222221	[75]
IMA950 ND GBM and AA HLA-A2 +	IMA950/poly-ICLC vaccine	I/II	GBM = 16 AA = 3	Safe and well tolerated mOS 19 mo for GBM CD8+ T-cell response to multipeptides: 36.8%	NCT01920191	[76]
GAPVAC ND GBM	APVAC1 vaccine plus Poly-ICLC and GM-CSF APVAC2 vaccine plus Poly-ICLC and GM-CSF	I	16	Safe with mOS of 29 mo	NCT02149225	[79]
GP96 heat shock protein–peptide complex vaccine Recurrent GBM	HSPPC-96	I/II	41	mPFS 19.1 weeks mOS 42.6 weeks	NCT00293423	[82]
HGG-2006 ND GBM	DC-based tumor vaccination	I/II	77	mPFS 10.4 months mOS 18.3 months more severe than that of other DC vaccine studies	2006–002881-20	[84]
DCVax-L ND GBM	Adjuvant TMZ plus DCVax-L versus adjuvant TMZ	III	2:1 DCVax-*L* = 232 Control = 99	mOS 23.1 (90% of the ITT received DCVax-L) 2-yr survival rate: 46.2% 3-yr survival rate: 25.4%	NCT00045968	[85]

Abbreviations: *AA* anaplastic astrocytoma, *DC* dendritic cells, *GBM* glioblastoma, *HGG* high-grade glioma, *HSPPC* heat shock protein–peptide complex, *IDH* isocitrate dehydrogenase, *ITT* intention-to-treat, *KLH* keyhole limpet hemocyanin, *MRD* minimal residual disease, *ND* newly diagnosed, *OS* overall survival, *PFS* progression-free survival, and *TMZ* temozolomide

colleagues in 2000, where they treated a patient with recurrent brainstem GBM with autologous DCs pulsed with allogeneic MHC-I matched tumor peptides. A measurable cellular immune response to the allogeneic GBM peptides was seen as demonstrated by increased T-cell infiltration within the intracranial tumor site in the biopsy sample obtained following vaccination. However, improved survival was not observed [83].

On a larger scale, Ardon and colleagues treated 77 patients with newly diagnosed GBM with an autologous DC vaccine. They integrated the vaccination into the Stupp regimen and found a median PFS and OS of 10.4 and 18.3 months, respectively. However, the adverse events were more severe than that of other DC vaccine studies with 38 serious adverse events found in 30 patients and 19 hematological adverse events in 18 patients [84].

Liau and colleagues conducted a phase III trial evaluating the addition of DCVax-L, an autologous tumor lysate-pulsed DC vaccine, to standard therapy for newly diagnosed GBM [85]. In their study, patients were randomized to TMZ plus DCVax-L or TMZ and placebo after surgery and chemoradiotherapy. The primary endpoint was PFS while the secondary endpoint was OS. The median OS was 23.1 months from surgery for the intent-to-treat population with nearly 90% of the ITT population receiving DCVax-L. The 2- and 3-year survival rates were 46.2 and 25.4%, respectively. The addition of DCVax-L to standard therapy is feasible and safe, and may extend survival. Generating DC vaccines that are engineered to target numerous tumor antigens specific to a patient's tumor or to target a common antigen presented by most tumors is time and resource demanding.

Cell Therapy

Another form of immunotherapy is active transfer of immune cells such as CAR T-cells and NK cells to the donor to leverage their antitumor activity. The main challenges in development of cell therapy in GBM are the intracranial location of the tumor, determining the most efficacious route of cell delivery (intravenous vs. intrathecal), and identification of a universal cell surface antigens to target.

CAR T-Cells

Chimeric antigen receptor (CAR) T-cells are engineered T-cells that target a specific target on the tumor cells and mount T-cell-mediated antitumor responses [86]. CAR T-cell therapies are at the forefront of immunotherapy approaches for the treatment of highly clonal neoplasms such as lymphoma and leukemia [87]. Aside from ubiquitously expressing monoclonal antigens, the location of the tumor cells (peripheral blood) make hematological malignancies perfect candidates for CAR T-cell therapies.

CAR T-cell therapies have not been as successful in solid tumors [88]; however, a case report of success in GBM has been promising and has raised interest in the generation of CAR T-cells in GBM. Brown and colleagues treated a 50-year-old male with multifocal GBM with intracavitary injections of IL13Rα2-targeted CAR T-cells into a right temporo-occipital lesion through a catheter placed within the resection cavity [89]. Local tumor control was achieved, but meanwhile, the tumor grew in the leptomeningeal spinal space and the patient received treatments via an intrathecal catheter placed in the lateral ventricles. Complete remission of the spinal tumors and the intracranial tumors were achieved with intrathecal administration of IL13Rα2-targeted CAR T-cells, which was sustained for 7.5 months. The cause of tumor recurrence was thought to be due to decreased expression of IL13Rα2 based on preliminary analysis. This case report best exemplifies the barriers in the successful use of CAR T-cells in GBM: lack of stably expressed antigens and identifying an effective route of administration. The effectiveness of IL13Rα2 CAR T-cells can be attributed to the CSF location of cancer cells and the ease of delivery of CAR T-cells in the intrathecal compartment.

In addition to IL13Rα2, CAR T-cells targeting EGFRvIII and HER2 have been evaluated in clinical trials [90, 91]. O'Rourke and colleagues

treated ten recurrent GBM patients with EGFRvIII mutation with EGFRvIII CAR infusions. They demonstrated transient expansion of CART-EGFRvIII cells in peripheral blood of all patients and increased expression of inhibitory molecules and Treg infiltration in five out of seven patients with available post-treatment tissue. However, despite the promising correlative outcome, mOS of the patients was not improved [90]. Ahmed and colleagues generated HER2-specific T-cells using HER2 positive autologous GBM cells in 2010 and demonstrated their antitumor efficacy in autologous GBM xenografts in the brain of severe combined immunodeficient mice. Phase I trial of HER2 CAR T-cells in recurrent GBM is currently ongoing (NCT03389230).

Several factors contribute to lack of response to CAR T-cells in GBM including lack of stably expressed antigens, intratumoral heterogeneity, impaired CAR T-cell proliferation in a hypoxic environment, and an immunosuppressive microenvironment which leads to ineffectiveness of CAR T-cells. Efforts in altering the tumor microenvironment have focused on combinatorial immunotherapy approaches. For example, increased levels of PD-1 expression on transduced anti-HER2 CD8+ T-cells following antigen-specific stimulation with anti-PD-L1+ tumor cells in mice have been described [92], and combination of EGFRvIII CAR T-cells with pembrolizumab is currently being evaluated in newly diagnosed GBM (NCT03726515).

NK Cells

Decades of failed targeted therapy approaches in GBM and recent failures in immunotherapy targeting specific antigens (checkpoint inhibitors, vaccine peptides, and CAR T-cells) indicate that alternative strategies that are not dependent on tumor antigen presentation are needed in GBM. One such approach would be to leverage the innate immune system which is able to destruct tumor cells without the need for antigen presentation. NK cells are large lymphocytes of the innate immune system capable of lysing infected cells directly via secreting granules and granzymes or via antibody-dependent cellular cytotoxicity [93].

NK cells for the treatment of solid tumors have shown promise [94]. Autologous NK cells have been used in early clinical trials for the treatment of gliomas via a combination of focal and intravenous injections without severe neurological toxicity [95]; however, the generation of autologous NK cells from individual patients is time-consuming and only attainable in specialized centers. Therefore, there has been interest in the generation of allogeneic over-the-shelf. NK cells obtained from cord blood and placenta. Similar to CAR T-cells, the route of administration of NK cells is debated and will be tested in upcoming NK cell trials within our institution. NK cells for the treatment of pediatric medulloblastoma via posterior fossa are currently ongoing at MD Anderson Cancer Center (NCT02271711).

Oncolytic Viral Therapies

Oncolytic viruses have been the subject of intense investigation for the treatment of cancer. Initially, the mechanism of action of oncolytic viruses was thought to be due to direct tumor lysis and cytotoxicity [96]. With the discovery of profound immunosuppression and immune escape by tumor cells, it became apparent that oncolytic viruses may release pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) molecules that alter the tumor immune microenvironment. It is now known that viral infection of tumor cells induces inflammation within the tumor via T-cell priming and facilitates the recognition of cellular antigens by the host immune system [97]. The antitumor effect of viral therapy is likely driven by both cytotoxicity and adaptive immune responses. Several oncolytic viruses have been studied in GBM including polio-, retro-, adeno-, measles, and herpes viruses, and many virus therapy trials in GBM are in early stages. Here, we describe three selected advanced clinical trials of viral therapy in GBM: PVSRIPO (poliovirus), Toca 511 (retrovirus), and DNX2401 (adenovirus). The summary of these trials can be found in Table 8.4.

Table 8.4 Select virus therapy clinical trials for GBM

Title/setting	Route of delivery	Phase	*N*	Outcome	Clinical trial identifier number	Reference
Polio virus (PVSRIPO) Recurrent GBM	Convection-enhanced delivery	I	61	OS rate: 21% at 24 and 36 months	NCT01491893	[98]
Retrovirus Toca 511 (vocimagene amiretrorepvec) Recurrent GBM	Injection of virus into the resection cavity	I	45	mOS: 13.6 mo	NCT02414165	[100]
Adenovirus DNX-2401 Recurrent GBM	Injection of virus into the tumor	I	37	OS rate: 20% at 72 months	NCT00805376	[101]

The recombinant oncolytic poliovirus, PVSRIPO, is a genetically engineered form of poliovirus Sabin type 1 with attenuated neurovirulence. PVSRIPO received breakthrough therapy designation from FDA in 2016 for a phase I study in recurrent GBM (NCT01491893). The results of this trial were published in 2018 by Desjardin and colleagues [98]. They treated 61 patients with recurrent GBM in a dose-escalation study via intratumoral infusion by convection-enhanced delivery. One dose-limiting toxic effect (grade IV intracranial hemorrhage immediately after catheter removal) was observed at dose level number 5 and dose level-1 was selected as the phase 2 dose (5.0×10^7 $TCID_{50}$). The overall survival rate was 21% at 24 months and 36 months. Safety results indicated that the neurovirulence potential of poliovirus was effectively eliminated in PVSRIPO.

Toca 511 is a non-lytic retrovirus and has been engineered to preferentially kill tumor cells by encoding a modified yeast cytosine deaminase that converts the prodrug 5-fluorocytosine (5-FC) to the potent anticancer drug, 5-fluorouracil (5-FU), in an infected tumor cell [99]. In a phase I open-label study, Cloughesy and colleagues treated 45 patients with recurrent or progressive high-grade glioma undergoing resection with intracavitary injections of Toca 511 followed by IV injection of Toca FC, an extended-release form of prodrug 5-FC [100]. Infected cells convert the prodrug 5-FC to 5-FU which leads to cell death via cytosine deaminase that is otherwise not present in normal noninfected humans cells. Toca 511 and Toca FC were well tolerated and demonstrated OS of 13.6 months (95% confidence interval, 10.8–20.0) and OS rate of 29.1% at 2 years. A phase II/III study of this approach is currently ongoing.

DNX-2401 is an oncolytic adenovirus that achieves tumor cell targeting through a 24-base deletion of E1A and insertion of an Arg–Gly–Asp (RGD) motif onto a viral capsid protein. In a phase I trial of DNX-2401 administered via intratumoral injection in recurrent malignant gliomas, 20% of patients were alive >3 years after treatment of their recurrent GBM [101]. Molecular profiling of pre- and post-treated tissue showed tumor infiltration by CD4+ and CD8+ T-cells and reduction of TIM-3 expression indicating that DNX-2401 may be able to overcome some features of T-cell exhaustion. Given immune-mediated anti-glioma response elicited by DNX-2401, it is currently being assessed in a phase I/II clinical trial in combination with pembrolizumab (NCT02798406).

The significance of the survival rate of about 20–30% at 2 years seen in the above viral trials has been questioned [102]. Retrospective analysis and literature review have shown similar survival rates in patients enrolled in other non-viral therapy trials [102, 103]. The patients with longer survival seem to possess favorable biological and/or demographic features [102]. Larger randomized trials that stratify for the favorable diagnostic features, such as IDH mutation and MGMT status, are needed to

determine the efficacy of viral therapy monotherapy and in combination with CPIs.

Combinatorial Approaches

CPIs have been the backbone of immunotherapies in various solid cancers. However, their ineffectiveness in phase III trials in GBM as monotherapy has led to combinatorial immunotherapy trials that combine CPI with other forms of immunotherapy in order to overcome the profound immunosuppression in GBM and increase antitumor effects of CPI. Combinatorial trials have focused on approaches to overcome the potential mechanism of resistance to CPI in GBM including lack of T-cell infiltration, impaired T-cell activation, and augmenting BBB penetration.

Oncolytic viral therapies described above are thought to induce tumor T-cell infiltration, and combinatorial trials with CPI are currently ongoing with DNX2401 (NCT02798406) and an inducible adenoviral vector engineered to express hIL-12 (Ad-RTS-hIL-12) (NCT03636477). In addition, active transfer of CAR T-cells is thought to overcome the lack of T-cell infiltration within GBM tumor microenvironment, and combinatorial trials of CAR T-EGFRvIII and pembrolizumab are currently ongoing (NCT03726515). Another approach to increase intratumoral T-cells is vaccination with DCs [104–106]. Trials of DC vaccines in combination with anti-PD-1 therapy in recurrent GBM are currently ongoing (NCT02529072 and NCT03014804).

Other efforts to alter the GBM microenvironment have focused on overcoming impaired T-cell activation via inhibition of immune-modulating enzymes (IDO1) and cytokines (TGF-β, CSF-1) and immune cell surface molecules (LAG-3).

Indoleamine 2,3-dioxygenase I (IDO1) is the rate-limiting enzyme in conversion of tryptophan into kyneurenine and its by-products [107]. Elevated IDO1 expression is thought to downregulate T-cell activity via depletion of tryptophan and induces T-cell apoptosis via increased levels of kyneurenine and its by-products [108]. Two IDO1 inhibitors, epacadostat (ECHO-204) and INT230–6 (IT-01), are currently in phase I/II clinical trials in combination with nivolumab for advanced cancers to include recurrent GBMs (NCT02327078 and NCT03058289, respectively).

Transforming growth factor-β (TGF-β) is among the most well-established immunosuppressive soluble factors released by GBM cells, TAMs, Tregs, and microglia within the GBM microenvironment [22]. In addition to its role in immunosuppression, TGF-β activates genes that are involved in proliferation, invasion, angiogenesis, and glioma stemness. Multiple TGF-β compounds have been used as monotherapy for the treatment of gliomas including anti-sense oligonucleotides targeting soluble extracellular TGF-βII [109], TGF-β receptor sequestering soluble TGF-β (GC1008) [110], and TGF-βI receptor kinase inhibitor (galunisertib/LY2157299) [111]. These agents have not been shown to be efficacious in treatment of recurrent GBM as monotherapy when compared with chemotherapy [109, 110]. Their lack of effectiveness maybe due to differential expression of TGF-β and the relevance of a particular isoform during GBM evolution. A recent study on differential expression and clinical significance of TGF-β isoforms in GBM suggests that TGF-β expression and its correlation to survival outcome are more relevant in the newly diagnosed setting and that TGF-βI, and not TGF-βII, is the dominant isoform [112]. Galunisertib, a small molecular inhibitor of TGF-β receptor kinase I, is being combined with nivolumab in a phase I/II trial in recurrent GBM (NCT 02423343) in order to prime the tumor microenvironment to augment CPI effectiveness.

Another growth factor that has been implicated in GBM immunosuppressive microenvironment is colony stimulating factor-1 ligand (CSF-1). CSF-1 ligand interaction with its receptor (CSF-1R) has been shown to induce generation of immunosuppressive M2 macrophages and enhances glioma cell progression [113]. Similar to TGF-β inhibitor monotherapy trials, the CSF-1R and KIT inhibitor, PLX3397, did not show efficacy in recurrent GBM despite its ability to readily cross the BBB [114]. Combinatorial

trials of CSF-1R in combination with two PD-1 antibodies, spartalizumab and nivolumab, are currently ongoing in two distinct trials in advanced cancers to include gliomas (NCT02829723 and NCT02526017).

Lymphocyte-associated globulin-3 (LAG-3) is a surface molecule expressed on activated T-cells, B-cells, and NK cells [115], and was shown to be present in perivascular niche of the tumor in six of nine of human GBM samples tested [116]. In preclinical mouse models, dual anti-PD-1 and anti-LAG-3 was superior to either treatment alone in improving survival of glioblastoma bearing mice [116]. A phase I/II study of nivolumab with anti-LAG3 antibody or urelumab in recurrent GBM is currently ongoing (NCT02658981). Urelumab is a fully humanized IgG4 monoclonal antibody targeting CD137 or 4-1BB, an inducible receptor-like protein expressed in both cytotoxic and T-helper cells, which upon cross-linking with anti-CD3-stimulated T-cells results in enhancement of T-cell proliferation [117].

CPIs are also being tested in combination with blood–brain barrier (BBB) disruption methods with the goal to increase the exposure of intratumoral antigens to immune cells and their access to tumor microenvironment. The phase I and II trials of pembrolizumab in combination with MRI-guided laser ablation (MLA) in recurrent GBM are currently enrolling patients (NCT02311582).

Continued efforts at stepwise multimodality immunotherapy strategies are needed to overcome immunosuppressive mechanisms in GBM for successful implementation of immunotherapy in GBM.

Conclusion

Immunotherapy advances in solid cancers such as melanoma and NSCLC are promising and raise the interest in implementing immunotherapy for the treatment of GBM. CPIs have been at the forefront of immunotherapy advances in various solid cancers; however, phase III clinical trials of CPI in GBM have been disappointing. Window-of-opportunity trials of CPIs in recurrent GBM have been instrumental in improving our understanding of the GBM microenvironment, potential reasons for lack of clinical efficacy, and a potential novel mechanism to enhance the efficacy of these agents through a neoadjuvant approach. Through these studies, we have learned that the GBM microenvironment lacks cytotoxic T-cells and contains abundant immunosuppressive macrophages and myeloid-derived suppressor cells. Current combinatorial immunotherapy trials aim to overcome the immunosuppressive GBM microenvironment via approaches that address lack of T-cell infiltration (oncolytic viral therapies, vaccine peptides, dendritic cell vaccines, and CAR T-cells), lack of success with targeting one antigen in GBM (GAPVAC vaccine and NK cells), increase in T-cell activation (antibodies against T-cell stimulatory ligands and pro-inflammatory cytokines), and maintenance of T-cell activation (CPI and TGF-B inhibition). Given the success of immunotherapy for the treatment of BM from melanoma and NSCLC, we now know that successful treatment of intracranial neoplasms with CPI is possible and that the CNS location of GBM does not preclude antitumor immune responses. Continued efforts at conducting well-designed window-of-opportunity clinical trials with a focus on successful activation and maintenance of tumor-specific responses are needed to improve the clinical development of immunotherapy in GBM.

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Immunotherapy and Radiation

9

Lauren E. Colbert and Anuja Jhingran

Abstract

Radiation is an essential tool in cancer therapy, both in the definitive and palliative setting. Radiation therapy can drive the cancer immune cycle via several mechanisms, but it also has immune suppressive effects that might be overcome via radiation/immunotherapy combination approaches. Understanding this underlying biology will lead to improved combination therapy approaches. Although clinical evidence of radiation and immunotherapy combination approaches in the metastatic setting to induce an abscopal response is limited, combination approaches in the oligometastatic and definitive setting are extremely promising.

Keywords

Radiation · Radiotherapy · Immunotherapy · Abscopal response

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Basic Radiobiology

Radiation therapy is an essential tool in cancer therapy, yet its interactions with immunotherapy and the interplay of radiation therapy with the immune system are still not entirely understood. For many diseases, radiation therapy can replace surgical resection of the tumor, but in the setting of immunotherapy, the remaining tumor cells may play a key role in creating an "in-situ vaccine" to drive immune response that a surgically resected tumor cannot provide. In addition, radiation to nearby lymphoid organs may also affect the immune response to cancer therapy.

Radiation-induced cancer cell death is primarily mitotic death caused by DNA strand breaks that interfere with mitosis. Because of this, tumor killing by ionizing radiation may not occur for weeks to months. The classical "Four R's" of radiobiology are the underpinning of our current understanding of how dose and fractionation, treatment time, and cellular factors lead to the biologic effect of a given radiation treatment. Gray (Gy) is the unit of measurement for delivered radiation and is equivalent to 1 Joule/kilogram of absorbed radiation in a given material.

Repair is in reference to the DNA repair that generally results between given fractions of a radiation treatment, usually via nonhomologous end joining and homologous recombination pathways. In vitro experiments suggest that this sublethal injury repair requires approximately 6 hours

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between fractions. Allowing this interval will allow normal tissue repair but also leads to a need for a higher total dose for equivalent tumor kill when radiation treatment is fractionated. For example, a stereotactic radiation of 20 Gy is roughly equivalent to 50 Gy if delivered in 25 fractions.

Repopulation refers to the cell populations that proliferate during the overall treatment delivery time. This is very histology dependent, as it primarily depends on the doubling time of individual cell types. For example, treatment outcomes for squamous cell histologies are highly dependent on overall treatment time. This is due to rapid cell doubling time of these squamous cancers, requiring fractions to be delivered in an overall shorter duration.

Redistribution refers to the portions of the cell cycle where cells are particularly vulnerable to DNA damage. This is generally during the late G2 phase but can vary. Because cell cycles are not synchronized during the moment that an individual dose of radiation is delivered, some cells may be more sensitive to DNA damage than others. Allowing some time for cells to continue through the cell cycle before the next fraction means that different cells may be in more sensitive parts of the cell cycle at that time. In this way, fractionation actually leads to higher overall tumor kill.

Reoxygenation is important because fully oxygenated cells are much more sensitive to ionizing radiation than the same cells when anoxic. In addition to direct DNA damage, radiation also induces oxygen radicals that can cause secondary DNA damage. Under hypoxic conditions, these are not present and cellular repair is much quicker, resulting in less cell kill. Allowing time for reoxygenation to occur will allow for greater cell killing.

The Role of Radiation in the Cancer Immunity Cycle

The accumulated DNA damage caused by ionizing radiation results in "cellular debris" that can stimulate the cancer immunity cycle by release of cancer cell antigens. The antigens produced by these genomic abnormalities are captured and presented by dendritic cells and can then be bound to and presented by major histocompatibility type I complexes (MHC-1) to cytotoxic CD8+ T cells [1, 2]. Proinflammatory cytokines are necessarily released during this step in order to avoid immune tolerance to these presented immunogens. In the absence of tolerance, these antigens are presented by MHC-1 and MHC-2 molecules to T cells, leading to priming and activation. Activated cytotoxic T cells must then be trafficked to the tumor, primarily through cytokines and infiltrating blood vessels, and into the tumor microenvironment. The activated CD8+ T cells must then recognize and bind to the cancer neoantigen and in turn lead to cell killing. Increased cell death can then lead to additional antigen release and a continuation of the cancer immunity cycle.

This stimulation of the cancer immunity cycle can happen in the absence of immunotherapy but rarely leads to durable immunity alone. This can be due to regulatory immune factors (checkpoints) in lymph nodes and in the tumor bed, including PD-L1, PD-1, CTLA-4, and others. These immune regulatory factors control regulatory T cells and dendritic cells to restrict presentation or response to these cancer neoantigen-MHC1 complexes. Many of these immune suppressive factors are stimulated or enhanced by radiation in the absence of immunotherapy. Thus, the immune response to radiation is a delicate balance, which is also affected by many factors including dose, fractionation, timing and dose of combination agents, host, and environmental factors. If combined thoughtfully, radiation and immunotherapy can work together to take advantage of the immune stimulatory effects while overcoming some of the immune suppressive effects of radiation therapy.

Immune Stimulatory and Suppressive Effects

The complex interaction of radiotherapy with immune response is a delicate balance between both immunosuppressive and immune-activating

effects, particularly related to T-cell response. Ionizing radiation imparts effects on a wide variety of immune cells, including macrophages, cytotoxic T cells 1–4, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and dendritic cells (DCs) that act as antigen presenting cells (APCs). Ionizing radiation results in an inflammatory, pro-immune state by initiating double-stranded DNA breaks, activation of apoptotic cascades, release of DNA and RNA from the nucleus to the cytoplasm, and eventually cell death. This activation of cell death results in activation of inflammatory cytokines including danger-associated molecular patterns (DAMPs) [3–10], calreticulin [11–14], ATP, and high mobility group protein B (HMGB1), in addition to traditional inflammatory cytokines such as fibroblast growth factor (FGF), transforming growth factor β (TGF-β), tumor necrosis factor (TNF), and other inflammasome-activating factors [15–17]. Radiation also induces dendritic cells (DC's) in the tumor microenvironment leading to increased tumor antigen presentation by MHC II APCs [17, 18].

Although all of these factors are immune-stimulating, radiation also initiates an immunosuppressive response. Many studies have demonstrated increases in Tregs, a cell type critical to regulation of self-immunity, via secretion of immunosuppressive cytokines such as IL-10 in response to radiation [17, 19–21]. These Tregs are inherently radioresistant and have a fast regeneration rate, which can lead to an imbalance of immunosuppressive Tregs proportionate to other T-cell types. Additionally, radiation can drive Treg migration into nearby draining lymph nodes and increase systemic expression of immunosuppressive TGFβ and CTLA-4 [22–25]. Finally, MDSCs, which play a suppressive role and are also key for tumor vascularization, are extremely radioresistant and are recruited to tumor stroma immediately following local radiation [26, 27].

On the converse side, radiation also has immune suppressive effects. Regulatory T cells (Tregs) are stimulated by radiation and can lead to immune tolerance and prevent activation of CD8+ T cells. There are multiple potential mechanisms for this T cell activation, including some evidence for involvement of TGFB pathways, but it has not been well described as of yet.

Radiated Tumor as an "In-Situ Vaccine"

Although vaccine strategies are hypothetically interesting in generating additional neoantigen to drive antigen presentation and T cell priming and activation, cancer vaccine development is challenging. This is in part due to difficulty in identifying neoantigen(s) of interest and in part due to heterogeneity between patients. The construction of a single vaccine is an enormous undertaking, particularly for the potential benefit of only one individual or a small population of patients. Multi-antigen vaccines are of interest, and less likely to be susceptible to immune editing, but configuration of multi-antigen vaccines is an even more daunting challenge. Genetically modified autologous T cells are also interesting as a potential approach to bypass the antigen presentation step altogether, and again this requires knowledge of a particular target of interest.

For this reason, approaches that might turn the tumor into its own vaccine by driving release of and presentation of antigen are intriguing. Radiation appears to be very effective in this, although the optimal dose and fractionation to do so without driving regulatory T cell and immunosuppressive mechanisms is unknown. Radiation can also upregulate uptake and presentation of tumor DNA delivery by APCs in multiple ways. RT can lead to DNA release and in turn type I interferon (IFN-I) release, that activates the STING pathway and promotes priming of T cells [28, 29]. RT also upregulates calreticulin, which is a cell surface signal for uptake of dying cancer cells [12–14]. This occurs via translocation of an ER protein complex between calreticulin and disulfide isomerase ERp57 to the plasma membrane [30]. This in turn promotes activation of dendritic cells and macrophages to uptake dying cancer cells. CD47 is a related integrin-associated protein that can counter these calreticulin signals and protect cells from macrophage and dendritic cell phagocytosis. If

expression of CD47 is lost and calreticulin is translocated to the cell surface, APC uptake will ensue. Radiosensitivity can be increased by inhibiting CD47 in mouse tumors [30]. Radiation also triggers cyclic guanosine monophosphate adenosine monophosphate synthase (cGAS) and stimulator of interferon (STING) genes, which work together to increase type I IFN signaling with dendritic cells. This ramps up the adaptive immune response. Radiation-damaged tumor cells can also drive maturation of dendritic cells to mobilize adaptive immunity, via release of damage-associated molecular pattern molecules (DAMPs). These DAMPs signal pattern recognition receptors that lead to this maturation.

Nuclear protein high-mobility box-1 (HMGB1) is also a promotor of antigen cross-presentation that is promoted by RT [31]. HMGB1 is a histone-chromatin binding protein that binds to TLR4 and TLR9, which in turn activate pathways leading to NF-κB pathways and release of its associated cytokines. HMGB1 also seems to be strongly associated with antigen-specific T cell responses in patients undergoing RT [32].

Interestingly, this antigen release can happen very quickly, even within minutes, and at very low doses of radiation. A standard fraction dose of radiation is 1.8–2 Gy, but even doses as low as 0.5 Gy can stimulate this antigen presentation. Evidence suggests that other pathways may be responsible for this rapid response, primarily via cytoplasmic ATP channels. Extracellular ATP is detected by P2X7 which turn leads to secretion of several pro-inflammatory cytokines, and also leads to costimulatory ligands CD80 and CD86 which induce activated T-cells in response to antigen presenting cells. These low doses of radiation also seem to be associated with reprogramming tumor-associated macrophages (TAMs) into an M1 phenotype, which can affect vascular tumor vasculature and help with intratumoral migration of T cells [33].

Radiation can also have effects on priming and activating T cells. Although radiation can also increase regulatory T cell functions, it also increases MHC-1 expression [34], increasing recognition by activated cytotoxic CD8+ T cells [35], and upregulates ligands responsible for tumor killing NK T cells [36]. This upregulation of NK cells may become especially valuable when T cell homeostasis is affected and activated CD8+ T cells are either not present or unable to recognize antigen- MHC-1 complexes. Radiation can also assist in the tumor microenvironment to allow T cell migration into tumor. Cytokines induced by radiation and an associated "inflamed" microenvironment can increase effective recruitment of effector T cells and in turn tumor destruction by cytotoxic CD8+ T cells. Endothelial cell signaling that also contributes to a "friendly" tumor microenvironment including intercellular adhesion molecue-1 (ICAM-1) also helps draw activated T cells into the tumor microenvironment.

Clinical Fractionation and the Immune Response

Over the past 30 years, little has changed in regard to the fractionated administration of therapeutic radiation. Dose fractionation attempts to take advantage of the ratio of sensitivity of tumor tissues and normal tissues to sublethal damage and potentially lethal DNA damage caused by ionizing radiation (referred to as the α/β ratio). By administering repeated single doses of 1.8–2 Gy over several weeks, normal tissues with a lower α/β ratio are allowed time to repair and repopulate, while tumor tissues with a higher α/β ratio are unable to repair induced DNA double-strand breaks and undergo cell death. This fractionation scheme also allows for reoxygenation of tumors and cell cycle reassortment, resulting in increased cell kill for hypoxic cells and cell kill in more sensitive phases of the cell cycle, respectively. In the modern era, techniques such as hypofractionation (giving larger daily doses) and hyperfractionation (giving more frequent, smaller doses) have been employed in attempts to take advantage of biologic variation between tumor types. Increasingly, there is evidence to suggest that choice of fractionation may play a role in defining the immunogenic equilibrium as a result of ionizing radiation. Although preclinical and increasingly clinical

data suggest a synergy between radiation and checkpoint inhibitor therapies that might result in abscopal responses, results of recent clinical trials of combination with traditional fractionation have been dismal. Recently, investigations into stereotactic doses in combination with checkpoint inhibitor therapy have been undertaken, without improved results.

Although primarily limited to laboratory studies, evidence suggests that local immune response in the setting of checkpoint inhibitor therapy can be optimized with a single, large dose of radiation, while conversely systemic immune response may be optimized with fractionated radiation. Combining these approaches may optimize both. Most T-cells, besides Tregs, have a higher α/β ratio and are exquisitely sensitive to fractionation, indicating that repeated dosing may cause greater lymphopenia than a large, single dose. Filatenkov et al. [37] recently demonstrated in mouse colon cancer models that a single 30 Gy dose versus a 10 × 3 Gy regimen resulted in significantly increased CD8+ T-cell infiltration into tumors and decreased release of MDSCs and other immunosuppressive cytokines by the tumor into nearby lymph nodes. Similarly, Verbrugge et al. [38] found that in a mouse orthotopic breast cancer model, a single 12 Gy dose resulted in higher levels of functionally active CD8+ T-cells with PD-1 expression and in turn higher response to PD-1 therapy.

Conversely, systemic immune activation via release of DNA and RNA into the cytoplasm and subsequent release of chemotactic cytokines may be improved with fractionated radiation, particularly when combined with anti-PD1, anti-PD-L1, or anti-CTLA-4 therapy. CTLA-4 expression is increased in Treg cells following total body irradiation [39], and thus anti-CTLA-4 therapy following radiation may decrease migration of radioresistant Tregs into lymph nodes and eventually systemic circulation. Dewan et al. suggested this mechanism led to increased abscopal effects for a more fractionated schedule of 5 × 6 Gy as compared to a single, 20 Gy dose (Dewan et al. 2009 [40]). Zeng et al. also demonstrated that high, single dose radiation combined with anti-PD-1 therapy resulted in improved survival versus either radiation alone or anti-PD-1 therapy alone in mice. Similarly, work by Vanpouille-Box et al. demonstrated that induction of DNA exonuclease Trex1 by large dose radiation is a causative factor in immunogenic attenuation, suggesting smaller doses may be more likely to lead to systemic abscopal responses [41].

Timing is also an important consideration in immunotherapy and radiation therapy combinations. A large retrospective review of 750 patients found that of patients who received radiation and immunotherapy in combination, those who received RT concurrently with immunotherapy had an improved overall survival over those who received them sequentially. Patients who had also received the immunotherapy for a longer timepoint prior to RT (>30 days) also had improved survival. This supports hypotheses that the immune priming effect is essential to radiation response. This is supported by preclinical data with anti-PDL1 [42], but preclinical studies with anti-CTLA4 and anti-OX40 therapies [43] suggest that maximum efficacy with different inhibitors may vary.

Clinical Combinations: Safety and Toxicity

Many studies of combination immunotherapy and radiation approaches in the palliative setting suggest that this approach does not significantly increase toxicity risk over immunotherapy alone. There are case reports of potentially worse rates of colitis and radiation necrosis following abdominal or brain irradiation. As treatment doses escalate and chemotherapy is potentially added, however, these risks should be considered in trial design. In definitive settings such as head and neck cancer, where chemoradiation timing affects outcomes, delay of radiation completion by addition of immunotherapy is a real risk. GORTEC 2015-01 is a phase II randomized controlled trial comparing cetuximab-based chemoradiation with pembrolizumab-based radiation therapy for locally advanced head and neck squamous cell carcinomas. Ninety-two percent of

patients completed the full treatment course, with no increase in rates of adverse effects. The only potentially worsened risk was a higher rate of thyroiditis in the pembrolizumab arm (18% vs 6%) [44]. Other studies support this finding.

Clinical Combinations: The Abscopal Response

Although a pivotal case report in the *New England Journal of Medicine* describing the phenomenon of a single area of irradiation causing regression of metastatic sites garnered clinical attention, the term "abscopal" was originally described by [45]. The term "ab" meaning "far" in Latin and "scopus" meaning "target" describes this concept that irradiation of one site can release antigen, stimulate the immune response particularly with the aid of checkpoint inhibitor therapy, and cause complete response of distant sites. Unfortunately, clinical evidence of such a response as a common occurrence is lacking. A recent systematic review identified 94 cases in 52 articles, including 2 phase I trials, 1 retrospective series, 1 letter to the editor, and 48 case series [46]. The majority of these cases were melanoma and non-small cell lung cancer patients, and RT dose and fractionation, timing with immunotherapy, method of delivery, and target sites varied widely. Several randomized trials are beginning to mature, evaluating this abscopal response in the randomized setting. Theelen et al. [47] evaluated patients with advanced non-small cell lung cancer who had received second line treatment, combining pembrolizumab with stereotactic radiation at a dose of 24 Gy in three fractions delivered within the week before the first cycle of pembrolizumab. This study showed no significant difference in overall response rate between patients receiving pembrolizumab alone (21% vs 39%; $p = 0.28$), no difference in progression-free survival (2.8 months vs 7.1 months; $p = 0.08$), or overall survival (7.6 months vs. 19.2 months; $p = 0.1$). Another study by McBride et al. Evidence suggests [47] in metastatic head and neck squamous cell carcinoma, randomizing radiation in combination with nivolumab similarly showed no difference in ORR (30.8% vs 25.9%; $p = 0.93$), progression-free survival (28% vs 16%; $p = 0.89$), or overall survival (46% vs 54%; $p = 0.46$) to its occurrence.

Although anecdotal evidence exists, prospective data of the abscopal effect remains lacking. We currently still have an inadequate understanding of the mechanism of the abscopal effect in radiation, which makes thoughtful design of combination trials with this end goal difficulty. Interestingly, the abscopal effect does not occur in p53 deficient mice, which suggests that mechanisms of cell death that rely on p53 may be essential. P53 is also essential to NK cell killing and co-stimulation of cytotoxic CD8+ t cells, both of which could play a role in the abscopal response. PD-1 and PD-L1 upregulation in the tumor microenvironment are also seen in patients who experience a radiation induced antitumor immune response but who have no abscopal effect, and may represent tumors in which there is an ability to prime antitumor T cells but an inability to activate them [28]. This suggests that better timing of immunotherapy and radiation combinations may improve likelihood of an abscopal effect.

Clinical Combinations: Oligometastatic Disease

There is also data to suggest that although radiation to a single site may improve development of an adaptive immune response, this antigen release might also increase risk of hyperprogression, and thus worse outcomes after combination radiation and immunotherapy studies. It is also hypothesized that a lower burden of gross disease might increase the ability of an adaptive immune response to attack other sites of disease. In lung cancer, several randomized studies have now shown a survival benefit for definitive treatment of all metastatic sites with radiation therapy, and studies in other disease sites are ongoing. The estimated 2-year progression-free survival rate is approximately 77.9% and overall survival rate approximately 53.7% [48].

Clinical Combinations: Definitive Setting

The landmark trial of radiation and checkpoint inhibitor therapy in the definitive study is the PACIFIC trial in non-small cell lung cancer [49]. This study evaluated the anti-PDL1 antibody, durvalumab, in combination with platinum-based chemoradiotherapy. Platinum chemotherapy is a strong radiosensitizer and also enhances antigen release via DNA damage that may stimulate the cancer immune cycle. In this study, 713 patients were randomized to standard-of-care chemoradiation versus chemoradiation followed by adjuvant durvalumab. The study showed an impressive near doubling of PFS and OS. The median OS was not reached in the durvalumab arm, with only 6.5% of patients having grade 3+ pneumonitis. A secondary analysis of the KEYNOTE-001 study for patients with locally advanced or metastatic NSCLC also demonstrated that patients who had previously undergone radiation therapy similarly had a significant near doubling of both PFS and OS [50]. There are multiple ongoing trials examining the role of checkpoint inhibitor therapy in the definitive setting in head and neck squamous cell carcinoma, but early reported data are promising [51]. There are also several studies in HNSCC and NSCLC exploring immunotherapy as a neoadjuvant approach preoperatively.

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CAR T-Cells

10

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Abstract

CAR-T (chimeric antigens receptor-T) cell therapy is a breakthrough therapy of the twenty-first century for the management of different malignancies including lymphomas and leukemias. Numeral trials are underway to understand the optimal CAR-T cell design and dose to maximize efficacy and mitigate toxicity. Currently two CAR-T cell therapy products, axicabtagene ciloleucel and tisagenlecleucel, are approved by the US Food and Drug Administration, which have shown excellent responses in otherwise poor prognostic lymphomas and leukemias. The favorable outcomes achieved of this therapy were noted to be durable during long-term follow-up. Understanding the challenges associated with manufacturing and the reasons for T cell failure including poor T cell expansion, persistence, and tumor resistance are critical for its wide-scale application in order to attain the full potential of this novel therapy. Here we review the salient features of the different CAR-T products and discuss the pivotal trials that led to its approval.

Keywords

CAR T-cell · Axicabtagene ciloleucel · Tisagenlecleucel · Lisocabtagene maraleucel · Diffuse large B-cell lymphoma · Follicular lymphoma · Acute lymphoblastic leukemia · Immunotherapy · Cytokine-release syndrome · Immune effector cell-associated neurotoxicity syndrome · Tocilizumab · Siltuximab

Introduction

In 1891, Dr. William B. Coley, an American surgeon, made a compelling observation that the immune system can be triggered to shrink tumors. The quest to exploit the power of immunotherapy, however, was forestalled by an era of chemotherapy that ensued. During World War II, the accidental sinking of a US naval ship led to a group of sailors developing pancytopenia due to poisoning from mustard gas (nitrogen mustard). The observation prompted a wide-scale screening of these chemical compounds with cytotoxic potential; further clinical trials led to the first Food and Drug Administration (FDA) approval of a chemotherapy drug, nitrogen mustard. The immunotherapy field took further impetus, not until the last 2 decades, due to our deeper understanding of the immune system, the cellular and molecular pathways leading to tumor

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development. Two groundbreaking therapies that have shown great promise in this field involve "taking the breaks off" or "pushing the pedal" of the immune system. These therapies, namely, immune checkpoint inhibitors and adoptive cell therapy, have been successful in a variety of malignancies, while the former mostly in solid tumors and the latter in hematological malignancies.

Adoptive cell therapy includes both genetically engineered T-cell receptor (TCR) therapy and chimeric antigen receptor (CAR) T-cell therapy. The former requires antigen presentation by innate T-cells, while the latter has receptors transduced in T-cells which offer antigen-presenting cell (APC) independent effector T-cell function and antigenic specificity.

Adoptive T-Cell Therapy Adoptive T-cell therapy such as allogeneic hematopoietic cell transplantation and donor lymphocyte infusion (DLI) has been clinically utilized for greater than three decades. These immunotherapeutic strategies use T-cells in the crudest of forms, with varying degrees of success, and have become the treatment of choice for many relapsed refractory hematological cancers due to lack of more effective or less toxic options. However, due to its nonselective nature (Human leukocyte antigen (HLA) disparity) and off-tumor toxicity, allogeneic transplantation comes with significant treatment-related morbidity and mortality, both acute and long term.

TCR and CAR T-cell therapies emerged to mitigate this nonspecific alloreactivity, further bypass immune tolerance and enhance effector function. Antigen recognition by the αβ moieties on T-cell receptor surface is cardinal for TCR therapy and binds both intracellular and/or extracellular peptides in a major histocompatibility complex (pMHC)-dependent presentation by antigen-presenting cells. The αβTCR activation requires concerted effects of receptors CD4 and CD8. TCR lacks an intrinsic intracellular signaling moiety and, thus, once activated triggers its binding to CD3 complex, and through a complex mechanism, yet to be elucidated, leads to an optimal cytotoxic anticancer T-cell activity.

Transfection of T-cells with virally inserted chimeric antigen receptors not only retains the extracellular antigen specificity but also is able to function in an MHC and co-receptor independent manner. The technology was pioneered by Dr. Gideon Gross and Dr. Zelig Eshhar 30 years ago [20]. Dr. Carl H. June and Dr. Bruce Levine furthered the CAR therapeutic strategy from bench to bedside by treating patients with relapsed acute lymphoblastic leukemia. Its unparalleled therapeutic efficacy in this devastating disease leads the way to an explosion of CAR T-cell therapies in clinical trials. A brief summary of CAR T-cell evolution is shown in Fig. 10.1. In this chapter, we will review the various aspects of CAR T-cell, their efficacy, toxicity, and management in different tumors presented in recent clinical trials and their future potential.

Chimeric Antigen Receptor Structure and Function

The simplest level of CAR structure consists of an extracellular domain, hinge, transmembrane domain, and an intracellular signaling domain (Fig. 10.2). The CAR T-cell ectodomain recognizes the extracellular tumor antigen and initiates downstream signal transduction, which channels through the hinge, transmembrane, and costimulatory domains leading to a complex cascade of CAR T-cell activation, transcription factor expression, cell proliferation, survival, and cytokine release resulting in cytotoxic activities.

Ectodomain or Extracellular Domain (ECD) The extracellular target-binding site in a CAR structure is the single most important factor that serves as a lock and key for target antigen specificity. The ECD is directed against a well-documented target on the cancer cell's surface, which can be a carbohydrate, protein, or glycolipid structures. An ECD against an appropriate tumor-associated antigen (TAA) is the most crucial component of a CAR T-cell (Table 10.1). The

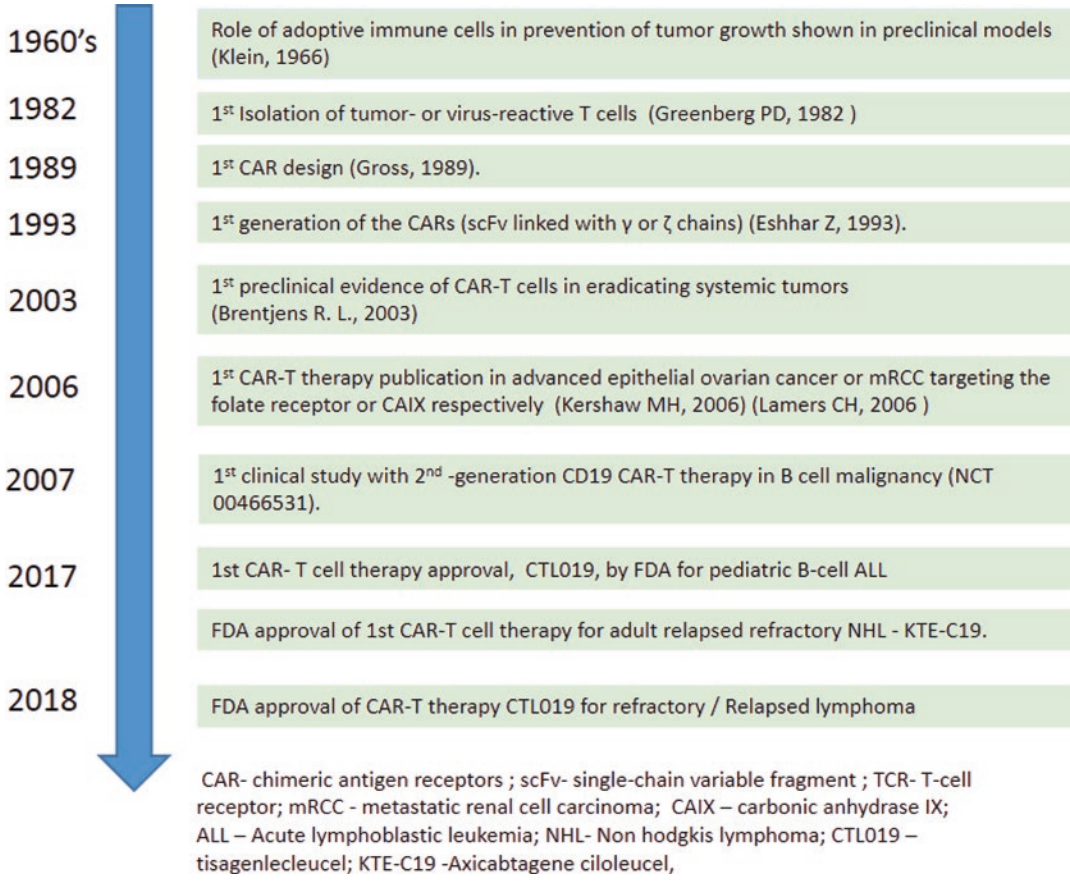

Fig. 10.1 Timeline of progress in the development of CAR T-cell therapies

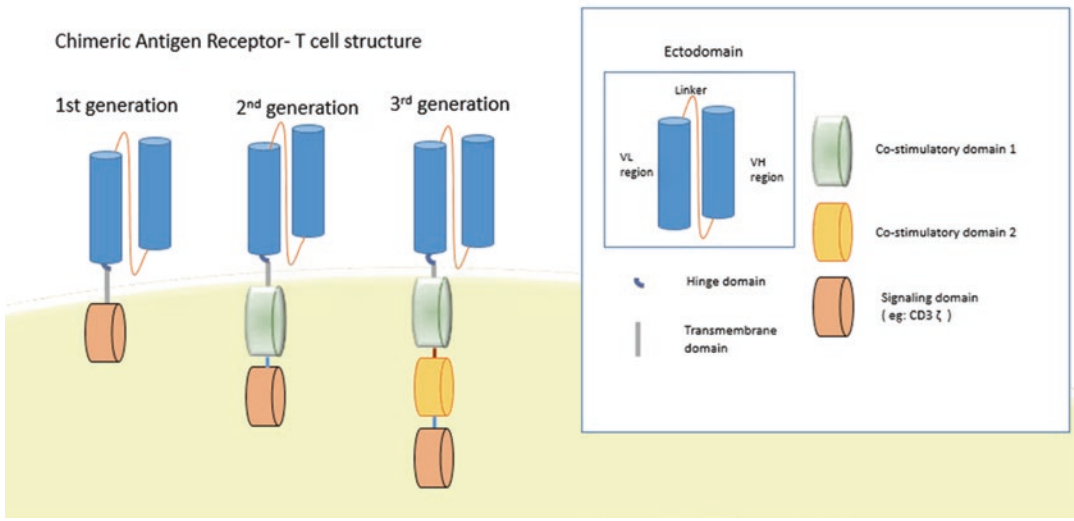

Fig. 10.2 Chimeric antigen receptor- T cell structure

Table 10.1 TAAs that are actively investigated in clinical trials

Cancer type	TAA
Colorectal carcinoma	CEA EGP-40
Liver	CEA GPC3
Breast cancer	CEA Mesothelin ROR1 erb-B 2,3,4
CNS tumors	EGFRvIII EphA2 (glioblastoma) EGFR GD2 (neuroblastoma) CD171 (neuroblastoma) IL13-Rα2 (glioblastoma) Her-2/ErbB2 (medulloblastoma)
Lung cancer	EGFR GPC3 Mesothelin (mesothelioma) ROR1
Renal	VEGFR-II CAIX CD70
Gynecological cancers	FR-α MUC1 MUC16 FBP (ovarian) CD44v7/8 (cervical cancer) CD70 (ovarian cancer)
Mesothelioma	FAP
Prostate	PSMA PSCA
Pancreatic cancer	Mesothelin CD70 CD24 FAP HER2 Prostate stem cell antigen MUC1
Hematological	CD19, CD20 and CD22, CD38, κ-light chain (NHL) CD30 (Hodgkin lymphoma) CD33 (AML) BCMA, NY-ESO-1, NKG2D ligands, SLAMF7 (CS1), CD138 (syndecan-1) (myeloma)

CEA carcinoembryonic antigen, *EGP-40* colon cancer-associated Ag, *GPC3* glypican-3, *ROR-1* receptor tyrosine kinase-like orphan receptor 1, *CD* cluster of differentiation, *EGFRvIII* epidermal growth factor receptor vIII, *ErbB* erythroblastosis oncogene B, *EphA2* EPH receptor A2, *FAP* fibroblast activation protein alpha, *GD2* ganglioside, *HER2* human epidermal growth factor receptor 2, *VEGFR* vascular endothelial growth factor receptor, *iCas9* inducible caspase-9 (safety switch), *IL13Rα2* interleukin-13 receptor subunit alpha-2, *CA IX* carbonic anhydrase IX, *FR-α* folate receptor-alpha, *MUC* mucin 1, cell surface associated, *FBP* folate binding protein, *FAP* fibroblast activation protein, *BCMA* B-cell maturation antigen, *NY-ESO* New York esophageal squamous cell carcinoma 1, *NKG2D* natural killer ligand, *SLAMF7* self-ligand receptor of the signaling lymphocytic activation molecule

selection of the target TAA is essential and ideally will be universally expressed on the targeted cancer cells, infrequently lost in refractory disease, and not expressed on nonessential normal tissue. The most commonly used ectodomain is derived from the single-chain variable fragment (scFv) of a tumor antigen-reactive murine monoclonal antibody. The scFv is formed by a light chain and heavy chain (which in general are antigen-binding regions of a B-cell monoclonal antibody), connected by a flexible peptide linker which enhances the affinity of the CAR to target antigens. The scFvs (Fig. 10.1) are synthesized from one of the various expression strategies either from murine or humanized antibodies. The scFv obviates the need for tumor antigen processing and MHC class restriction to lock the target, unlike TCR gene therapy which requires peptide procession and major HLA restriction. The ECD is connected to intracellular domains by an extracellular hinge region and a transmembrane (TM) region.

Hinge (Spacer) This is generally derived from the constant Fc portion of IgG subclass immunoglobulins (such as IgG1 and IgG4), IgD or CD8 domains, and connects the antigen recognition part, scFV, with the transmembrane domain. The hinge, though inconspicuous in the overall structure, has a significant impact on the overall function and cytokine signature during T-cells expansion [1]. Though the length of the hinge region affects the flexibility of the scFv, it can increase Fc vulnerability for interaction with off-target FcR receptors and has the potential to nullify CAR efficacy by unintentional CAR and/or innate immune response activation. Research is underway to improve CAR T-cell persistence and antitumor efficacy by improved hinge structure through point mutations which can optimize the aforementioned interactions [26].

Transmembrane Domain Between the hinge and the signaling endodomains lies the transmembrane domain. This forms an integral part of the CAR structure and spans across the cell membrane and functions as a signal gateway to the intracellular compartment. This is usually derived from CD3-ζ, CD4, CD8, or CD28 molecules.

Intracellular Domain The first-generation CAR design consisted of only Fcγ (the γ-chain from FcεRI) or CD3ζ (ζ-chain of the TcR complex) intracellular domain. Thus, the modified T-cell activation was dependent on exogenous IL-2, which although was shown to have impressive tumor killing in a preclinical model, the effect could not be translated in vivo due to poor T-cell expansion, less stability, and antitumor activity due to absent interaction with the TCR and costimulatory receptors. Subsequently, costimulatory domains were added to the CAR constructs to create the second (CD28 or 4-1BB) and the third generation (combinations of CD28, ICOS, OX40/CD134, and 4-1BB/CD137) CARs. The addition is shown to be more therapeutically effective due to enhanced persistence, less differentiation, less exhaustion, prolific expansion, cytotoxicity, memory, and efficacy over the first generation.

More novel designs of CARs are under development. Bivalent CARs, targeting two distinct TAA in the same CAR molecule, are generated by coupling two different single-chain fragment variables. Tandem CARs (Tan CARs) generated through cotransduction, generating a pool of T-cells containing two or more CAR T-cells, appear to be successful in preclinical models and, theoretically, develop synergistic responses due to multiple targets and reduced likelihood of antigen-loss relapses [22, 53]. The fourth-generation CARs which have a functional modification in addition to its structural change, the so-called T-cells redirected for universal cytokine-mediated killing (TRUCK), use T-cells as vehicles to produce and release tumoricidal cytokines inside the targeted tumor tissue. This causes direct killing and also a second wave of immune recruitment [11]. To deliver the pleiotropic effects of CAR T-cells in a controlled manner, preclinical tests are ongoing with the so-called "smart T-cells", which are furnished with one of

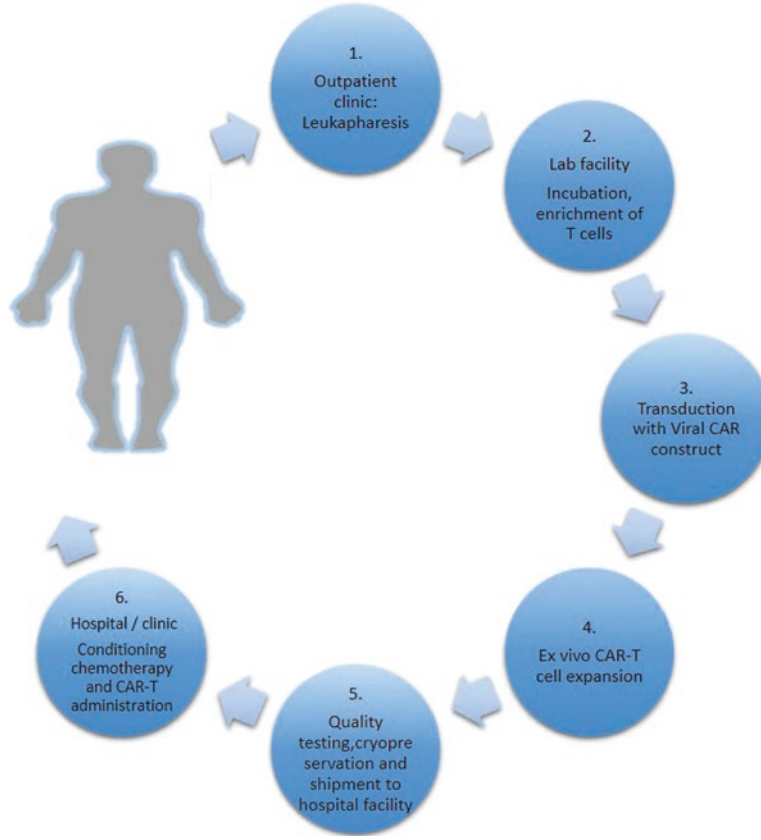

Fig. 10.3 CAR T-cell therapy in different cancer types

the different technologies including a presence of suicide gene, switchable dual-antigen receptors, or synthetic control devices (using inducible caspase-9 (iCasp9) and synthetic Notch (synNotch) receptors) [74].

Manufacturing and Treatment

Building autologous CAR T-cells requires a series of well-organized steps (Fig. 10.3). The process starts with the collection and enrichment of CD3+ lymphocytes through the process of leukapheresis. The principal of leukapheresis is the same as that for peripheral blood stem cell (PBSC) collection in hematopoietic stem cell transplant. The collection process in CAR T-cell patients, however, presents unique challenges. Apart from the target cells for collection being small, mature lymphocytes (in contrast to stem cell collection which targets large, immature CD34+ stem cells), potential CAR-T recipients often have active disease, cytopenias, and poor T-cell function due to multiple prior therapies. Factors that have shown to adversely impact T-cell collection include older age, pre-collection thrombocytopenia, multiple prior cancer treatments, non-mobilized lymphocytes, presence of circulating blasts, and natural killer cells [3, 68]. The success has shown to be influenced by the nature of the T-cells collected (naïve or early memory phenotype elicit a greater antitumor potential) [17, 28]. A minimum absolute peripheral blood lymphocyte count greater than 100–200 cells/mL is expected to result in a successful T-cell collection [47, 59].

Leukapheresis This is the process of filtering blood from the donor for the purpose of T-cell collection, originally pioneered by Freireich and colleagues. Leukapheresis, usually well tolerated and safe, is an outpatient procedure involving placing a

dependable venous access (central or peripheral), removing blood, and filtering the peripheral blood mononuclear cells [64]. The remainder of the blood is returned to the circulation. In CAR T-cell patients, adverse events are reported in <15% during apheresis and can manifest as hypotension requiring fluid bolus, agitation, vomiting, fevers, and procedure-related pain. Severe side effects in the form of syncope, citrate toxicity, and vascular injuries are uncommon, described to occur in less than 0.5% in incidence [3, 8].

FDA approved instruments are available to perform extraction of T-cells from the blood that is withdrawn, which involves elutriation, a technique that relies on the application of centrifugal force to the continuous or semicontinuous flow of anticoagulated whole blood. This results in the separation of cell layers based on its density. The mononuclear cell layer (both monocytes and lymphocytes) is sandwiched between the dense polymorphonuclear cell/red blood cell (RBC) layers and the less dense platelets. This is followed by purification of the T-cell from other blood cells by a complex process of washing and antibody–bead conjugate selection [56]. The extracted apheresis product is shipped to the lab, either as a fresh or frozen product depending on the planned manufacturing procedure, where T-cells are incubated and genetically modified with a viral vector encoding the CAR and expanded. There are three major types of stable gene expression vectors used for clinical applications: gamma retroviral vectors, lentivirus vectors, and the transposon/transposase system. Lentivirus vectors, which have a safer integration site profile than gamma retroviral vectors, are commonly used in clinical practice of generating CAR T-cell therapies. Other methods of gene transfer are currently being investigated. Viral transduction is followed by the expansion of modified T-cells before the cells are cryopreserved. The cryopreserved cells are transferred back to the hospital center for administration.

Conditioning Chemotherapy Conditioning chemotherapy is a part of most of the CAR T-cell protocols and has shown to improve outcomes. The most commonly utilized regimen is fludarabine and cyclophosphamide, but other regimes such as bendamustine have also been utilized. The impact of the conditioning chemotherapy on the cancer to cause an objective tumor response in patients with chemotherapy-resistant cancers is hypothesized to be very low [5, 13, 31, 49, 69, 71]. The conditioning helps to create a less competitive environment for the adoptive transferred T-cells by promoting host lymphocyte depletion, more supportive cytokine milieu, and decreased immunosuppressive cells such as regulatory T-cells and myeloid-derived suppressor cells [27, 73].

CAR T-Cell Infusion Once the cryopreserved product is received by the treating center and the patient deemed ready for infusion, the staff thaws the cells at the bedside, confirms the patient's identification, and infuses the cells via gravity over approximately 30 minutes. Though the infusion of CAR T-cells is generally safe, the ensuing toxicity of the treatment varies by the type of product, dose, disease burden, and patient characteristics. Hence, the site of administration of CAR T-cell infusion can be both inpatient and outpatient. Given the toxicities of the currently approved products (axicabtagene ciloleucel and tisagenlecleucel) require early identification and specific medical interventions, including transfer to intensive care for successful outcome, these are often administered in the inpatient setting although acute infusion reactions are rare. A portion of patients with tisagenlecleucel and lisocabtagene have been infused as outpatients; however, this requires intensive monitoring, education of staff, and coordination of care. Patients are often premedicated with antipyretics and antihistamines. Systemic steroids including hydrocortisone are generally avoided due to concerns about lymphotoxicity and arrested expansion. After the CAR T-cells are infused, patients require close monitoring while they are at risk for the development of cytokine-release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). In ZUMA-1 trial,

patients could be discharged at day 7 posttreatment in the absence of any sign of CRS or ICANS, while in ELIANA and JULIET trial, patients could be discharged same day [47, 62, 44]. Patients are also instructed to have a caregiver present 24 hours a day and stay locally within 2 hours for at least 4 weeks following CAR T-cell infusion that allows prompt access to hospital that is equipped to manage CAR T-cell toxicities.

Hematological Malignancies

Diffuse Large B-Cell Lymphoma (DLBCL) Patients with chemotherapy-refractory DLBCL have a dire prognosis, with no curative treatment options available until recently [12, 16]. The majority of second-line patients are not eligible for hematopoietic stem cell transplant due to chemotherapy-refractory disease, age, and/or comorbidities. The international, multi-cohort retrospective non-Hodgkin lymphoma research (SCHOLAR-1) study retrospectively evaluated outcomes in patients with refractory DLBCL. Refractory was defined as progressive disease or stable disease as best response at any point during chemotherapy (after four cycles of first-line or two cycles of later-line therapy) or relapsed within 12 months of autologous stem cell transplantation. The objective response rate noted in this group was a dismal 26% (with CR at 7%) to the next line of therapy, and the median overall survival was 6.3 months. Only 27% of patients were alive at 2 years. Outcomes were consistently poor across all patient subgroups.

The clinical efficacy of CAR T-cell therapy in this refractory group of patients in pivotal CAR T-cell trials is gratifying with impressive response rates and sustained durability. There are currently only two CAR T-cell products FDA approved as of 2019, tisagenlecleucel (CTL019, Kymriah) and axicabtagene ciloleucel (KTE-19, Yescarta). Tisagenlecleucel was approved for the treatment of pediatric relapsed and/or refractory B-cell precursor acute lymphoblastic leukemia on August 30, 2017; the same product was further approved in relapsed or refractory large B-cell lymphoma. Axicabtagene was approved for use in relapsed or refractory large B-cell lymphoma including primary mediastinal large B-cell lymphoma, on October 18, 2017 [47].

Axicabtagene The CAR T-cell construct (CD28 costimulatory domain) is derived from the initial NCI designed CAR construct. The same CAR vector construct was further used in the pivotal ZUMA-1 trial, which included patients with refractory diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, or transformed follicular lymphoma (TFL).

Patients achieved an objective response rate (ORR) of 83%, with a complete response (CR) rate of 58%, and 42% of the patients continued to have a response, with 40% continuing to have a CR with a median follow-up of 27 months [39]. The molecular subgroups of DLBCL did not have an impact on the response rate; ORR was 88% (CR 57%) and 76% (CR 59%) in germinal enter B-cell and activated B-cell DLBCL subgroups, respectively [33, 47].

Tisagenlecleucel The 4-1BB costimulation domain used in this product is known to be associated with longer persistence of CAR T-cells and less T-cell exhaustion. Schuster et al. reported a 57% CR rate in the pilot study of 28 patients with refractory B-cell lymphomas treated with this construct (CTL019). Among refractory DLBCL, the CR rate was 43%. This included three double-hit lymphoma patients (one histologic transformation) all who had complete responses. The JULIET study was built upon the aforementioned study and included relapsed/refractory DLBCL and transformed follicular lymphoma, with ORR of 52% with 40% achieving CR and 14% achieving PR. At 6 months from infusion, the ORR was 37% with a CR rate of 30%. The median duration of response was not reached with 26 months of median follow-up [62, 63].

Lisocabtagene Maraleucel In TRANSCEND NHL 001, a multicenter, pivotal trial, which started as a phase 1 first-in-human study of JCAR017, used a defined composition CAR T-cell product CD19-directed CAR T-cell (4-1BB costimulatory domain). This was administered with an equal ratio of CD4+ and CD8+ CAR T-cells. The trial has not yet been published, but preliminary reports describe that an ORR was 74% for the entire patient population, with a CR rate of 52%. At the 6-month analysis, the ORR was 35% and the CR rate was 31%. There was a dose–response relationship shown in this trial, where different doses were used in different cohorts. The core group, which had patients with high-grade B-cell lymphoma (double/triple hit), DLBCL NOS de novo, or TFL (treated with 5×10^7 cells in a single dose), had an overall response rate of 76% and a CR rate of 47%. In comparison, those treated with higher dose (1×10^8 cells in a single dose) had an overall response rate of 80% and a CR rate of 63%. Among 16 double/triple hit patients, the best ORR was 81%, and the 3-month CR rate was 60%. In those who relapsed within 12 months of a stem cell transplant, the ORR was 85%. This product is not yet FDA approved [25].

Indolent Lymphoma An indolent B-Cell lymphoma can have ominous clinical features, either manifesting as high follicular lymphoma international prognostic index (FLIPI) scores, early relapse after therapy, or by transformation histologically to DLBCL. These features have been consistently associated with poor outcomes. Relapse of follicular lymphoma (FL) after first-line treatment with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) within 2 years defines a unique category of patients at substantially high risk of death from lymphoma. The first patient treated on a phase I trial at the NCI with a second-generation CD19-targeted CAR-T (CD28 costimulatory domain) was a patient with advanced relapsed/refractory FL who received lymphocyte-depleting regimen with cyclophosphamide and fludarabine. The day after the last fludarabine dose, the patient received 1×10^8 anti-CD19 CAR-Ts intravenously, followed by 3×10^8 anti-CD19 CAR-Ts the next day. After the second CAR-T infusion, the patient received 720,000 IU/kg IL-2 intravenously every 8 hours, for a total of eight doses. The patient achieved a PR for 32 weeks after anti-CD19 CAR-T therapy. A follow-up trial from the NCI group was conducted in patients with FL or marginal zone lymphoma (MZL). In this trial, patients (four FL and one MZL) were treated with a single infusion of CAR-transduced T-cells. IL-2 was also administered intravenously 3 hours after the CAR-T infusion at a dose of 720,000 IU/kg every 8 hours; doses of CAR-Ts ranged from 0.3×10^7 to 3.0×10^7 CAR-Ts/kg bodyweight. Results from this trial showed that three of four patients with FL achieved PR, with a follow-up between 8 and 17 months, and the one patient with MZL achieved PR, with a follow-up of 12 months.

The NCI trial included two patients with FL who both achieved CR; however, one patient developed myelodysplastic syndrome requiring treatment after a remission lasting of 19 months. The second patient has an ongoing CR for 11+ months at the time of the report [33].

Refractory FL (14 patients) who relapsed within 24 months of initial diagnosis and/or remained refractory to least two lines of therapy were treated in the University of Pennsylvania trial using CTL019. At the time of the most updated report, the 3-month ORR and CR rate were reported as 79% and 50%, respectively. The results looked very promising for this high-risk group of patients, defined by prior multiple therapies (median number 5), relapsed post-autologous/allogeneic, with a median progression-free survival (PFS) that was not reached. Seventy percent of patients were disease-free after a median follow-up of 29 months. It remains unclear if responding patients will have durable responses, and/or potential cure, or if the disease will eventually relapse as happens with many indolent lymphoma therapies.

Turtle et al. showed the use of 1:1 ratio CD4/CD8 CAR-T in five patients with FL resulted in an ORR of 80% with a CR rate at 40%. CRs were only seen in the cohort that received fludarabine/cyclophosphamide conditioning chemotherapy (2/3 at 67%) with none in the cyclophosphamide alone conditioning arm (0/2 at 0%) [69].

In CLL, CAR T-cells have produced responses ranging from 57% to 74%, with CRs ranging from 21% to 29% [49, 58]. In patients who attained a CR, responses were deep (with minimal residual disease negative) and very durable suggesting the potential of cure in these patients with advanced CLL. There was evidence of long-term persistence of CTL019 cells as detected by flow cytometry or quantitative polymerase chain reaction [32, 51, 52]. The group at the NCI also reported the data on 20 patients treated with allogeneic anti-CD19 CAR T-cells in patients with different B-cell malignancies who progressed after allogeneic hematopoietic stem cell transplantation (alloHSCT). T-cells obtained from each recipient's alloHSCT donor source were used for the engineered T-cell production. In this study, five patients had CLL with one patient achieving a complete response and one with partial response. A durable CR (>30 months) was reported in a patient with chronic lymphocytic leukemia. There was no new reported graft versus host disease (GVHD) related to the allogeneic CAR T-cell infusion. This clinical benefit was seen in patients even despite prior DLI failure showing the potential superiority of the engineered T-cells [7]. Based on preclinical models suggesting synergy, a clinical trial is evaluating anti-CD19 CAR T-cells combined with the BTK inhibitor ibrutinib, which to date has achieved an almost 90% minimal residual disease (MRD) and negative marrow CR is observed in patients with high-risk, TP53 positive relapsed CLL. Though this is a small study with short follow-up, it shows that a combinatorial approach would enhance the potency of CAR T-cells [19]. Several studies are currently ongoing to prove this concept on a wider population cohort [23].

Mantle Cell Lymphoma: Eight patients with mantle cell lymphoma (four of them receiving Cy/Flu conditioning) were included in the study at Fred Hutchinson Cancer, with no CRs reported and only two PRs in the cohort of MCL [69]. The phase I TRANSCEND study has included patients with MCL; however, the results reported are primarily for patients with relapsed large B-cell lymphomas. The NCI trial (NCT00924326) included 22 patients with relapsed/refractory advanced-stage lymphoma; there was only one patient with MCL who experienced a CR and had ongoing response +17 months [33]. Building upon the success of the NCI trial, the CD19-targeted CAR T-cell product axicabtagene is currently being investigated in patients with relapsed/refractory MCL in the ongoing ZUMA-2 trial (NCT02601313).

Hodgkin's and T-Cell Lymphoma In HL, the treatment decision regarding a combined modality approach and duration of chemotherapy is mainly based on the stage and presence of poor prognostic features. Despite the high cure rates, relapses occur in approximately 10–15% of patients with localized Hodgkin's disease and approximately a third of those with advanced-stage disease. Around 10–15% of patients will have refractory disease to first-line therapy. With the advent of hematopoetic stem cell transplant (HSCT), anti-CD30 antibody, and checkpoint inhibitors, a major proportion of these patients are salvageable. The patients who fail these therapies comprise the major unmet need in Hodgkin's lymphoma. The immunosuppressive tumor environment and the relative paucity of the malignant RS cells make it challenging to seek an appropriate target to be explored in the CAR T-cell platform. In addition, despite the B-cell origin of the lymphoma, CD19 is generally absent in RS cells. The two main targets that are currently explored are CD123 (expressed in RS cells and other immune cells in tumor microenvironment) and CD30 antigen (expressed in RS and some activated T-cells in the tumor microenvironment). In T-cell lymphomas, targeting CD30 with CAR T-cells does appear to be an attractive therapeutic option; however, this TAA is not uni-

versal and thus has been tested mostly in anaplastic large cell lymphoma (ALCL).

A phase I dose-escalation study using CAR T-cells targeting CD30 included patients with relapsed/refractory CD30+ Epstein–Barr virus-negative HL ($n = 7$) or ALCL ($n = 2$). Three dose levels (DL) were investigated: two patients received 2×10^7 CAR+ cells/m2 (DL1), two patients received 1×10^8 CAR+ cells/m2 (DL2), and five patients received 2×10^8 CAR+ cells/m2 (DL3). The responses reported to date include two out of seven complete responses (CR), three out of seven stable disease (SD), and two progressive disease (PD) in patients with relapsed/refractory HL. Of two patients with ALCL, one had a CR that persisted 9 months after the fourth infusion of CD30. The modest response from anti-CD30 CAR T-cells is likely due to two main reasons: one due to the heavy microenvironmental T-cell suppressive infiltrate in Hodgkin lymphoma and second, which was common to these trials, was the absence of conditioning therapy. Currently, a phase I dose-escalation trial RELY-30 (NCT02917083) is currently ongoing using cyclophosphamide/fludarabine conditions to create lymphodepletion for adequate CAR T-cell expansion in these group of patients.

Acute Lymphoblastic Leukemia Acute lymphoblastic leukemia (ALL) is the most common cancer in children and adolescents in the United States with an annual incidence of over 3000 cases [72], with 10-year overall survival reaching almost 80% [72]. Achieving a CR in relapsed patients occurs in about a third of patients [15, 48]. The prognosis is grim for patients with primary refractory disease, and relapse post-allogeneic hematopoietic stem cell transplantation (HSCT) results in median overall survival of 3–6 months.

CAR T-cells have shown to be very promising in these groups of patients with the induction of remission rates as high as 70–90% seen across multiple trials with different CAR T-cell constructs (scFv and costimulatory domains). Majority of patients in these trials are heavily pretreated including prior CD19 targeted therapies (e.g., blinatumomab) and/or HSCT. Remission is also seen in the Philadelphia chromosome-positive (Ph+) disease and in Down syndrome-associated ALL [35, 41].

Tisagenlecleucel is the only FDA approved autologous CD19-targeted CAR T-cell product for the treatment of R/R B-cell ALL in patients under 25 years old. The multicenter international trial that led to its approval reported an ORR rate of 81%. The rates of event-free survival and overall survival were 73% and 90%, respectively, at 6 months and 50% and 76% at 12 months. The median duration of remission was not reached. Tisagenlecleucel has been found to have an ongoing persistence of at least 20 months at the time of the report.

In the NCI trial, in ALL patients treated with CD19 CAR T-cell with a CD28 costimulatory domain, three-quarters of MRD-negative responders proceeded to HSCT. The relapse rate was significantly higher in subjects who did not have an HSCT after CAR therapy (6/7; 85.7%) compared to those who did (2/21; 9.5%) ($p = 0.0001$). It is challenging to generalize the findings across different consults and once MRD-negative status is achieved, where to consolidate with HSCT, especially for transplant-naïve patients, is currently an open question [41].

Myeloma BCMA (CD269), a tumor necrosis family receptor superfamily member (TNFRSF17.4), which is unique to the mature B-cell lineage cells including post-germinal center B-cells, plasmablasts, and normal plasma cells, is currently the main target being tested in CAR T-cell trials in myeloma. In the first-in-human clinical trial of BCMA-specific CAR T-cell therapy conducted at the NCI (CD28 costimulatory domain), ORR as high as 81% was obtained with some patients achieving a stringent CR and MRD undetectable disease in bone marrow [2, 6]. Bluebird Bio's bb2121 cell therapy product (4-1BB costimulatory domain) has further set the benchmark in multiple myeloma in a multicenter phase 1 dose-escalation trial (NCT02658929) in patients with relapsed/refractory myeloma who have received ≥3 prior regimens, double-refractory, with an ORR of 86%, a response independent of the

degree of BCMA expression [55]. Nanjing Legend Biotech in China recently reported updated results from phase 1, LCAR-B38M CAR T-cell trial (4-1BB costimulatory domain). Patients on this trial had fewer lines of prior therapy and achieved an ORR of 88% with CR in 68% of patients [75]. Other BCMA CAR-T trials with different products are currently ongoing with data preliminary at this point [50]. BCMA CAR-Ts hold great promise with high efficacy and mild and manageable cytokine-release syndrome. Other targets being explored in myeloma are listed in Table 10.1.

Solid Tumors

CAR T-cells for solid cancers have not yet been able to reproduce the success of their hematological counterparts. Solid tumors present a more complex array of surface proteins, and trials so far have shown an inefficient homing of CAR T-cells to tumor locations. Apart from the low persistence after infusion, the ability of T-cells to survive through the immunosuppressive microenvironment in solid tumors (T_{reg} cells, MDSCs, TAMs, tumor-associated neutrophils, and immature DCs) has been equally challenging. There are several ongoing trials worldwide, with different targets under investigation (Table 10.1).

Toxicity and Management

The unique and major toxicities of CAR-T treatment include cytokine-release syndrome (CRS), and neurotoxicity most recently coined as immune effector cell-associated neurotoxicity syndrome (ICANS). CRS and ICANS are completely reversible in most instances, and early recognition is paramount. Less common side effects include B-cell aplasia, hemophagocytic lymphohistiocytosis (HLH)/macrophage-activation syndrome (MAS), anaphylaxis, and tumor lysis syndrome (TLS).

CRS, an inflammatory syndrome observed not just solely with CAR-T but also with other immune effector cell therapies, involves a constellation of symptoms that range in severity from mild to being fatal. Symptoms tend to occur early with CD28 costimulatory domain CARs than in those treated with 4-1BB costimulatory domain CARs. The median time to onset was 2 days (range: 1–12 days) in axi-cel and 3 days (range: 1–51) in tis-cel. Symptoms include fever, rigors, hypotension, tachycardia, hypoxia, capillary leak, in severe cases cardiac dysfunction, respiratory failure, renal failure, hepatic failure, and disseminated intravascular coagulation. T-cell and tumor cell interaction releases a massive amount of cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α, and interleukins (IL-6, IL-8, IL-10, IL-15, IFN-g, and MCP-1). This leads to monocytes and macrophage activation, which further triggers a pro-inflammatory cascade of cytokines and unrestrained progression of CRS. There also exists a deregulated endothelium (due to increased Ang2:Ang1 ratio and VWF), which plays a role in triggering concurrent ICANS. The incidence of CRS was reported in 93% of patients (grade ≥ 3 in 13%) in ZUMA-1 (axi-cel), 58% of patients (grade ≥ 3 in 22%) in JULIET trial (tis-cel), and 37% of patients (grade ≥ 3 in 1%) in TRANSCEND NHL 001 trial (liso-cel). Factors that predict severe CRS included high tumor burden, high bone marrow involvement, high baseline inflammatory state, rising IL6, baseline thrombocytopenia, and therapy-related factors such as the use of high-intensity lymphodepletion with cyclophosphamide and fludarabine, higher CAR T-cell dose, and type of costimulatory domain (e.g., CD28 > 4-1BB).

There are considerable difference and overlap in the management of these toxicities across grades, across clinical trials, and different institutions. The American Society of Blood and Marrow Transplantation (ASBMT) recently came up with a consensus grading system for CRS and neurotoxicity associated with effector cell therapies for use across clinical trials and for approved therapies [38]. Organ toxicity associated with CRS is graded according to CTCAE v5.0. Most patients have a compromised immune system or have ongoing neutropenia, the symptoms mimic sepsis syndrome, and clinical management needs a con-

certed effort from the CAR-T specialist and infectious disease team. Sepsis guidelines should be followed with blood cultures, imaging, and empiric broad-spectrum antibiotics.

Early CRS with grade 1 can be managed with supportive measures including antipyretics, antiemetics, intravenous fluids, and empiric antibiotics as appropriate. Grade 2 is defined in the presence of fever (≥38.0 °C) with hypotension not requiring vasopressors and/or hypoxia requiring the use of oxygen delivered by low-flow nasal cannula (≤6 L/minute) or blow-by. In addition to fluid bolus, IL6 blocking agents (tocilizumab or siltuximab) should be considered if deterioration to require vasopressors or to grade 3. Consider shifting patient for more intensive care in critical care unit. Dexamethasone is reserved if hypotension persists despite IL6 blockade or fluid boluses or if there is a high risk for severe CRS (high tumor burden). Grade 3 is defined as fever (≥38.0 °C) with hypotension requiring one vasopressor) (with or without vasopressin) and/or hypoxia requiring high-flow nasal cannula (>6 L/minute), facemask, non-rebreather mask, or venturi mask not attributable to any other cause [38]. IL6 blocking agents should be used immediately if not used before and should be managed in critical care unit. Steroids (dexamethasone preferred over methylprednisolone due to better central nervous system penetration) are often needed in cases refractory to IL-6 blockade. Dexamethasone is dosed 10–20 mg every 6 hours for grade 3 and up to methylprednisolone 1000 mg/day for grade 4. If clinical improvement noticed, consider keeping the duration of steroids as minimum with short taper due to the theoretical possibility of abrogating T-cell efficacy. The median time to CRS resolution ranges from 7 days (axicabtagene ciloleucel) to 8 days (tisagenlecleucel).

Refractory cases of CRS are rare and are associated with high mortality. Other agents being used and considered investigational include anti-TNFα (etanercept), IL-1R inhibitor (anakinra), T-cell depleting alemtuzumab, and ATG, cyclophosphamide, ibrutinib, and GM-CSF inhibition [38, 57, 66, 67] (Table 10.2).

ICANS, a unique neurotoxicity syndrome, is the second most common adverse event that can occur concurrently with or after the resolution of CRS or in the absence of CRS. The incidence in clinical trials was reported in 64% (grade ≥ 3 in 32%) of patients in ZUMA-1(axi-cel), 39% (grade ≥ 3 in 12%) of patients in JULIET trial (tis-cel), and 19% (grade ≥ 3 in 12%) of patients in TRANSCEND NHL 001 (liso-cel) trial. Though there is a similarity in the pathophysiology to CRS, the exact mechanism is still elusive. Severity seems to correlate with high tumor burden and a more severe CRS [21, 61]. An analysis showed higher levels of cytokines, which are usually associated with systemic inflammation (i.e., IL-6, IL-10, and IFN-γ), in patients who develop severe ICANS indicating a correlation between systemic inflammation and ICANS. Some of the earliest signs can be subtle and can often be missed during the routine assessment. This includes diminished attention, impaired handwriting which can deteriorate quickly to language disturbance, confusion, disorientation, agitation, aphasia, somnolence, and tremors. Severe cases of ICANS are associated with motor weakness, seizures, incontinence, mental obtundation, increased intracranial pressure, papilledema, and cerebral edema.

The manifestation of CRES can be biphasic; the first phase occurs concurrently with CRS (more common) and a second phase after CRS resolves or in the absence of CRS. The management involves a multidisciplinary approach, close hemodynamic monitoring, aggressive medical and supportive care, and use of specific drugs with IL6 blocking agents: tocilizumab, siltuximab, or steroids [46]. Though IL-6 blockade can reverse CRES during the first phase, it is found to be suboptimal by itself during the second phase, likely due to decreased blood–brain barrier (BBB) permeability in the absence of an inflammatory phase. Corticosteroids should be considered as a first-line treatment during this second phase. Similar to CRS, ASBMT guidelines for ICANS were proposed to harmonize the neurological toxicity grading and utilize the assessment of five neurological domains (Table 10.3). A 10-point immune effector cell-associated encephalopathy (ICE) score is assessed across these five domains, which include elements for assessing orientation,

Table 10.2 American society for transplantation and cellular therapy (ASTCT) CRS consensus grading and management

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Temperature ≥ 38 °C	Temperature ≥ 38 °C	Temperature ≥ 38 °C	Temperature ≥ 38 °C
		with		
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		and/or†		
Hypoxia	None	Requiring low-flow nasal cannula or blow-by	Requiring high-flow nasal cannula, facemask, non-rebreather mask, or venturi mask	Requiring positive pressure (e.g., CPAP, BiPAP, intubation, and mechanical ventilation)
Management	Antipyretics Antiemetics IV fluid Sepsis workup Growth factors and antibiotics if neutropenic	Conservative measures as in grade 1 IL-6 blockade +/− corticosteroids Supplemental oxygen as needed	Transfer to intensive care unit Conservative measures as in grade 1 Vasopressors for hypotension + Corticosteroids Supplemental oxygen as needed	Transfer to intensive care unit Conservative measures as in grade 1 Vasopressors for hypotension +Corticosteroids Supplemental oxygen as needed

Anti-IL6	Tocilizumab	Siltuximab
Origin	Humanized monoclonal antibody	Human–murine IGκ chimeric monoclonal antibody
Target	IL-6 receptor antagonist	Binds to soluble IL-6
FDA	Approved by the for the management of severe CRS	Off-label use
Dose and frequency	Minimum interval of 8 hours to a maximum total of four tocilizumab doses 4–8 mg/kg (max 800 mg)	One dose in 3 weeks 11 mg/kg IV

†CRS grade is determined by the more severe event

naming, command-following, writing, and attention. Other neurological domains assessed for ICANS grading include the level of consciousness, seizures, motor weakness, and raised intracranial pressure/cerebral edema.

Hemophagocytic lymphohistiocytosis (HLH)/macrophage-activation syndrome (MAS) is an uncommon event (1% incidence with CAR-T therapies) characterized extreme immune activation, cytokine release, lymphohistiocytic tissue infiltration, multiorgan failure, and even death if not recognized early. HLH can mimic events of T-cell therapy such as fevers, cytopenias, hyperferritinemia, and elevated C-reactive protein (CRP), and rarely can have an overt presentation with rapid splenomegaly, or evidence of hemophagocytosis. Traditional diagnostic criteria of HLH are unreliable due to symptom overlap with CAR-T adverse events. Clinical expertise and judgment on a case-by-case basis is paramount, and in the majority of cases, HLH/MAS is managed in same way as for CRS and resolve with CRS resolution [38].

B-cell aplasia is an on-target off-tumor effect of CAR T-cell and uncommonly can persist for years in patients, leading to hypogammaglobulinemia [43, 54, 60]. Hypogammaglobulinemia can occur as early as 9 weeks after CAR T-cell infusion, and immunoglobulin replacement has shown to lower the risk of infections in such cases [30, 31, 43, 51]. GVHD is a concern with allo-HSCT CAR-T products; however, the risk has been fairly low in early clinical trials mostly due to the dampening of the natural alloreactivity from the CAR-T generation process [7, 9, 29]. Other toxicities rarely associated with CAR

Table 10.3 ASBMT guidelines for ICANS

ICE
Orientation: orientation to year, month, city, and hospital: 4 points
Naming: ability to name three objects (e.g., point to clock, pen, button): 3 points
Following commands: ability to follow simple commands (e.g., "Show me two fingers" or "Close your eyes and stick out your tongue"): 1 point
Writing: ability to write a standard sentence (e.g., "Our national bird is the bald eagle"): 1 point
Attention: ability to count backward from 100 by 10: 1 point

ASBMT ICANS grade	Defining features of grade	Management
Grade 1	ICE score 7–9 and/or depressed level of consciousness but awakens spontaneously No seizures, motor weakness, or raised ICP/cerebral edema	Aspiration precautions and IV hydration Seizure prophylaxis with levetiracetam EEG Imaging of brain Consider tocilizumab if there is concurrent CRS
Grade 2	ICE score 3–6 and/or depressed level of consciousness but awakens to voice No seizures, motor weakness, or raised ICP/cerebral edema	Supportive care as in grade 1 Consider dexamethasone or its equivalent of methylprednisolone
Grade 3	ICE score 0–2 and/or depressed level of consciousness but awakens to tactile stimulus Any clinical seizure focal or generalized that resolves rapidly, or nonconvulsive seizures on EEG that resolve with intervention No motor weakness Focal/local edema on neuroimaging	Supportive care as in grade 1 Dexamethasone 10–20 mg IV q 6 hours or its equivalent of methylprednisolone Control seizures with benzodiazepines (for short-term control) and levetiracetam +/− phenobarbital and/or lacosamide High-dose methylprednisolone 1000 mg/day for focal/local edema
Grade 4	ICE score 0 and patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse or stupor or coma Life-threatening prolonged seizure (>5 min), or repetitive clinical or electrical seizures without return to baseline in between Deep focal motor weakness such as hemiparesis or paraparesis Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad	Supportive care as in grade 1 High-dose methylprednisolone 1000 mg/day Control seizures with benzodiazepines (for short-term control) and levetiracetam +/− phenobarbital and/or lacosamide Imaging of spine for focal motor weakness Lower ICP by hyperventilation, hyperosmolar therapy with mannitol/hypertonic saline, and/or neurosurgery consultation for ventriculoperitoneal shunt in patients with cerebral edema

Abbreviations: *ASBMT* American Society for Bone Marrow Transplant, *CRS* cytokine-release syndrome, *EEG* electroencephalogram, *ICANS* immune effector cell-associated neurotoxicity syndrome, *ICE* immune effector cell-associated encephalopathy, *ICP* intracranial pressure, *IV* intravenous

T-cell therapy include pneumonitis, fatal infections, anaphylaxis, and tumor lysis syndrome. Due to the potential risk of insertional mutagenesis with CAR-T generation and with the use of conditioning chemotherapy, the long-term adverse events with this therapy are currently unclear and would need to be careful calibration in the future years to assess the overall safety.

Resistance Pathways

The prognosis of patients after failure of CAR-T is poor. The resistance of the tumor and the cause of T-cell failure are an area of active research; some potential mechanisms include loss of target, genetic reprograming and T-cell exhaustion. In the international trial which included young

adults and pediatric patients with acute lymphoblastic leukemia, around a third of the relapses were with CD19-negative variants [34, 43]. The same phenomenon was also observed in two of the patients treated in the NCI trial for children and young adults with refractory B-cell malignancies with CD19-CAR T-cells [37]. There are several mechanisms postulated for this escape mechanism including alternative splicing, CD19 gene deletion, or mutation. The loss of target has also been shown in treatment with other immunotherapeutic agents including rituximab leading to CD20 negative relapses. A phenomenon called trogocytosis or shaving has been used to explain this mechanism with monoclonal antibodies, where the receptor drug complex is removed by the monocytes and macrophages expressing Fcγ which can bind the drug bound to the CD receptor of the cell. This leads to drug clearance and also leads to the selection of target negative tumor cells. This leads to drug clearance and also leads to the selection of target negative tumor cells It could also be the presence of a sub-detection level presence of a CD19 negative clone [65, 70]. Selection pressure, with genetic reprograming and lineage switch, has been demonstrated as another uncommon mechanism of relapse. Multiple groups have shown the emergence of relapses with a myeloid phenotype and loss of expression of B lymphoid lineage antigens, in ALL patients treated with anti-CD19 CAR-T [18, 24]. T-cell exhaustion, a fundamental phenomenon seen with T-cells, was first described in chronic viral infections in mice, exposed to chronic recurrent or repetitive antigens. This was subsequently reported in human chronic viral infections and cancer [4, 45]. This would incapacitate T-cells functionality, proliferative potency, and cytokine release with subsequent limitation of lytic capability. Consequent to this, there is upregulation of multiple inhibitory receptors/immune checkpoints (PD1 and PDL-1) that bind to their ligands expressed by tumor cells and antigen-presenting cells in the tumor microenvironment (TME) [10]. It is been established that the absence of costimulatory domain can pave the way to tumor resistance, and the presence of costimulatory domain protects against PD-1 upregulation and other mediators of resistance in tumor microenvironment. CD19 CAR T-cells incorporating the 4-1BB costimulatory domain were shown to be more persistent than those incorporating CD28 in clinical trials showing clues regarding the role of costimulation domain. The 4-1BB costimulation has shown to abrogate the persistent exhaustion induced by CAR signaling [14, 40]. Trials are underway using different combinatorial approaches of using costimulation domains in CAR T-cell.

Despite these early interpretations, our knowledge of the resistance phenomenon in CAR-T is still in infancy, and a clear understanding of these pathways is critical to build upon the early success of CAR-T.

Future Directions

CAR-T holds great promise in the treatment of hematological and solid malignancies. It is clear that the scope of this engineered T-cell product is something beyond the scope of our current understanding. Future trials are currently underway to identify and optimize CAR structure (include multispecific CAR T-cells; tandem CARs or Tan CARs) and reduce the toxicity of treatment by using suicide switch technology (caspase-9 (iCasp9), synthetic Notch (synNotch) receptors). Allogeneic off-the-shelf CAR T-cell therapy is underway with minimal GVHD, reduced wait times, can meet the high demand of relapsing patients, and avoids the use of heavily pretreated autologous T-cells. CAR T-cells with dissociated signaling domains and switch receptors, which have the potential to combat tumor antigen resistance, with improved efficacy and durability of response, are underway [11, 36, 42]. As we learn more about the technology that allows heightened efficacy, safety, proliferation, expansion, and inflammatory cell recruitment, there would be more customizable CAR designs and therapies to tailor to a personalized approach for our patients.

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Skin Reactions to Immune Checkpoint Inhibitors

11

Anisha B. Patel and Omar Pacha

Abstract

Due to the novelty of immune checkpoint inhibitors, their cutaneous adverse events (AEs) have only been recently characterized. This, along with the substantial rate of cutaneous reactions, has left many clinicians without sufficient familiarity to diagnose and treat cutaneous AEs. Pruritus and rash are among the top five immune-related AEs reported in clinical trials for this class of therapy. Incidence varies between 35 and 50% for cutaneous AEs among the eight FDA-approved drugs. Although only 2% are reported as grade 3 or 4 events, the impact on quality of life can be significant for these patients and is best described and most severe in ipilimumab trials. Of ipilimumab patients, 43.5% have a cutaneous AE and, at our institution, 20% of them had a dose interruption as a result. This means potentially 9% of patients have dose interruption of ipilimumab because of their cutaneous AEs. In the following chapter, we review the categories of these drugs, common cutaneous effects, their grading, and management options.

Keywords

Immune checkpoint inhibitors · Dermatitis · Ipilimumab · Nivolumab Anti-PD-1 · Anti-CTLA-4 · Dermatitis · Rash · Immunotherapy · Pruritus

The novelty of immune checkpoint inhibitors has only recently led to the characterization of cutaneous adverse events (AEs). This, along with the substantial rate of cutaneous reactions, has left many clinicians insufficiently familiar with diagnosis and treatment. Pruritus and rash are among the top five immune-related AEs reported in clinical trials in this class of therapy. Incidence varies between 35 and 50% for cutaneous AEs among FDA-approved drugs. Although only 2% are reported as grade 3 or 4 events, the quality of life impact can be significant for these patients and is best described in ipilimumab trials. Of ipilimumab patients, 43.5% have a cutaneous AE and, at our institution, 20% of them had a dose interruption as a result. This means potentially 9% of patients have dose interruption of ipilimumab because of their cutaneous AEs [1]. In the following chapter, we review the categories of these drugs, common cutaneous effects, their grading, and management options.

In general, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade and the drugs that

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bind the programed death receptor-1 (PD-1) have similar reactions, although PD-1 receptor inhibitors are usually better tolerated than CTLA-4 inhibitors with fewer reported skin AEs (43.5% and 18%, respectively) [1]. Additionally, it appears that both the reactions tend to be delayed, with anti CTLA-4s causing a rash after about a month of therapy and anti PD-1s slightly later [1]. Programmed death-ligand 1 (PD-L1) inhibitors and a second-generation CTLA-4 inhibitors are now being used in clinical trials, and these drugs are increasingly being used in combination therapies; however, large population AE data is not yet available. Both of these drug classes appear to have the same milieu of cutaneous AEs as their first-generation counterparts, possibly with lower severity overall. Interestingly, skin toxicities have been associated with improved responses and paradoxically, if well managed, can be an indicator of a good prognosis [2–4].

Common Cutaneous Adverse Events Seen with Immune Checkpoint Inhibitors

This class of medication is not *immune* to the typical cutaneous drug reactions seen with other classes of medications. Histologically, these reactions present a spectrum with morbilliform drug eruptions on the mild end and Stevens Johnson's Syndrome (SJS)/Toxic Epidermal Necrolysis (TEN) on the severe end [5].

Morbilliform drug eruption (commonly identified as "maculopapular") clinically presents with erythematous macules and thin nonscaling papules coalescing into blanchable patches and thin plaques that start on the trunk and spread peripherally to the extremities. Histology shows a superficial perivascular infiltrate with variable vacuolar change, dyskeratosis, and eosinophils. Patients are usually asymptomatic and occasionally pruritic. If painful or if there is progression to vesicles, one should consider early erythema multiforme (EM) or SJS/TEN. EM presents with targetoid erythematous thin papules often involving the acral and mucosal skin. The papules can become centrally dusky and vesiculate. When the distribution is more diffuse and mucosal surfaces are involved, but body surface area (BSA) remains below 10%; this is SJS. When the BSA is greater than 30%, this is called TEN, which can rapidly progress. For morbilliform eruptions, topical steroids with drug continuation are often sufficient. For EM, depending on the severity, oral or IV steroids can be used with drug cessation. For SJS and TEN, drug cessation and supportive care are critical, possibly with the addition of intravenous steroids or intravenous immunoglobulin therapy.

Urticaria is also a common type I drug reaction that can be seen with immune checkpoint inhibitors. Histology demonstrates minimal epidermal change with an edematous papillary and superficial reticular dermis with an infiltrate of lymphocytes, eosinophils, and variable neutrophils. Onset is within days, and the erythematous pruritic wheals can usually be controlled with oral antihistamines and drug cessation. Biologic therapies, such as anti-IgE monoclonal antibodies, could also be considered.

Cutaneous Adverse Events Shared by Anti-CTLA-4 and Anti-PD-1 Therapies

"Rash" is one of the most commonly reported cutaneous AEs, second only to pruritus, and has an 11% incidence in trials for pembrolizumab and nivolumab and a 19% incidence in trials for ipilimumab. This nonspecific description encompasses a variety of inflammatory skin diseases, including psoriasiform, eczematous, lichenoid, and morbilliform drug eruptions. Compared to anti-CTLA-4 antibodies, the anti-PD-1 antibodies have a lower incidence of rash; however, the incidence of severe (grade 3 and 4) cutaneous AEs is the same (2.4% and 2.6%, respectively). Eczema, pruritus, and vitiligo are seen with both classes of immune checkpoint inhibitors [6–12].

It is important to distinguish between the inflammatory skin reactions as they have different treatment options for the more severe presentations. Although mild presentations may be treated with topical steroids, diffuse presentations

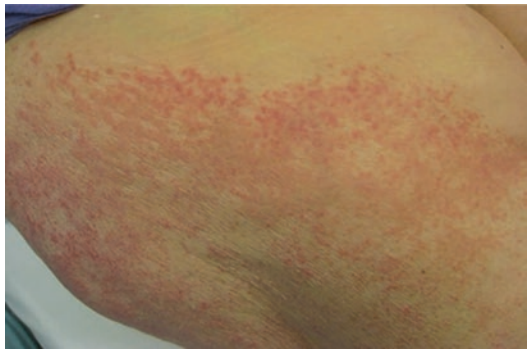

Fig. 11.1 Eczema, erythematous papules coalescing into plaques that are rough and have minimal scale

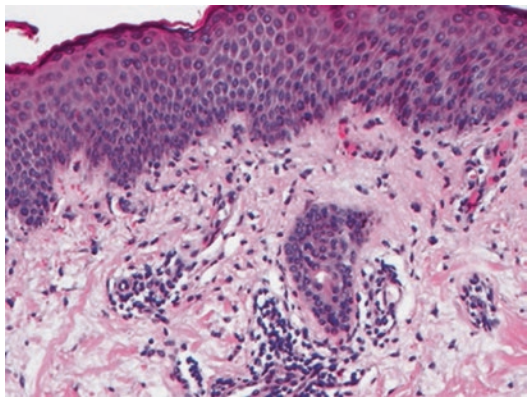

Fig. 11.2 Eczema, spongiotic dermatitis with dermal eosinophils

require systemic treatments, some of which are specific to the type of inflammatory reaction (Figs. 5.1 and 5.2).

Eczema appears as pruritic, ill-defined, edematous, and erythematous papules coalescing into plaques occasionally with vesicles in exuberant cases. As it evolves, the plaques are rough, erythematous, and have visible excoriation. Distribution is diffuse, affecting the trunk and extremities more than the face with a flexural predominance, as is typical with atopic dermatitis. Scalp and genital areas are often involved in diffuse presentations. Plaques are very pruritic with pain in areas of microfissures or superinfection. The histology shows prominent spongiosis and the variable presence of eosinophils [13]. Treatment consists of topical steroids, usually mid-strength creams, such as triamcinolone 0.1%, to begin with and graduating to super-potent formulations, such as clobetasol 0.05% cream. The face, axilla, and groin are usually treated with mild and low-potency steroids, such as hydrocortisone 2.5% or desonide 0.05% creams. Patients can be effectively controlled with a regimen of topical steroids involving twice daily application for flares and twice weekly application for maintenance. Supplementation with first-generation oral antihistamines, such as diphenhydr- amine or hydroxyzine, is a mainstay. In the author's experience, the addition of second-generation nonsedating antihistamines, such as cetirizine or loratadine, in the morning is also beneficial. In patients with grade 3 AEs, involving >30% of BSA, and refractory to topical therapies, the addition of oral steroids, such as prednisone at 1 mg/kg, is usually effective and can be slowly tapered. The slow taper is often effectively weaned with topical steroid maintenance.

Preliminary literature does not show a change in treatment efficacy with the use of oral steroids, making this the first choice systemic therapy in patients who are resistant to topical steroids [14, 15].

As the rash duration for severe grade cutaneous AEs can be prolonged, lasting months after therapy cessation, steroid-alternatives are needed. Biological therapy for atopic dermatitis targeting interleukin-4 receptor alpha subunit (IL-4Ra) is a potential treatment option for severe refractory eczema in patients requiring continuing therapy with immune checkpoint inhibitors.

For pruritus without rash, clinical presentation is variable. Most often patients have normal-appearing skin, although they can have skin changes secondary to manipulation masquerading as a primary rash. Geometric erosions and ulcerations, prurigo nodules, and linear erosions are secondary to pruritus. Prurigo nodules are ill-defined, discrete, erythematous, hyperpigmented acanthotic papules often with central erosion. Histology shows fibrosis and vertically oriented blood vessels in the superficial dermis with an overlying acanthotic epidermis. The first step in management is to eliminate a primary inflammatory condition. For primary pruritus, a stepwise approach depending on severity is best. For mild

cases, a first- generation antihistamine is oftentimes sufficient, with the added benefit of sedation that can help patients sleep when pruritus is usually most severe—right before bed. As the intensity increases, the addition of tricyclic antidepressant doxepin nightly and GABA agonists like gabapentin at increasing doses have been effectively used.

Vitiligo presents as depigmented well-demarcated macules coalescing into patches, occasionally preceded by erythema and pruritus, exclusively reported in melanoma patients (Fig. 11.3). Incidence is about 2% for anti-CTLA-4 and anti-PD-1 therapies [3]. Histology shows loss of melanocytes at the dermal–epidermal junction (Fig. 11.4). Patients are usually asymptomatic, but can have occasional preceding pruritus. Treatment for vitiligo includes a combination of topical steroids and ultraviolet (UV) light therapy; however, in melanoma patients with this drug-induced side effect, treatment is not usually undertaken because of the risk of further skin cancers with increased UV exposure.

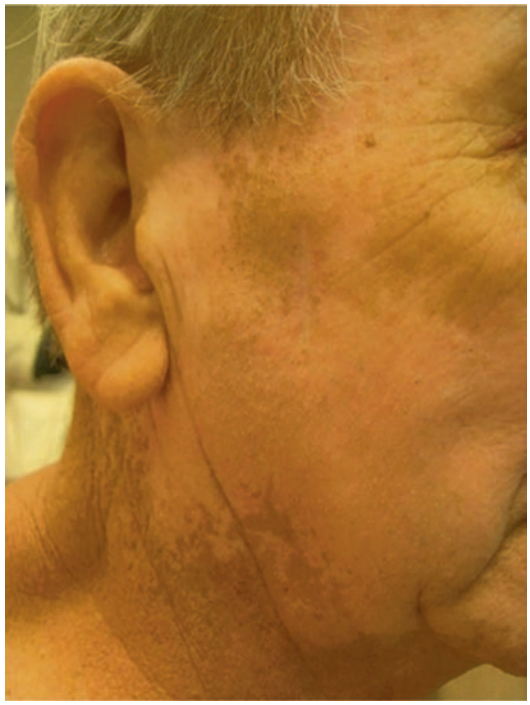

Fig. 11.3 Vitiligo, depigmented patches of head and neck

The unmasking of rheumatologic disease, with or without cutaneous involvement, can be seen as well. Although less common than inflammatory rashes, these AEs can be seen with both classes of checkpoint inhibitors and include large-vessel vasculitis, dermatomyositis (with or without muscle involvement), lupus erythematosus, and Sjogren's disease. [16, 17] It is unclear if these AEs are being unmasked or induced by the drug. In cases such as dermatomyositis, which is also a paraneoplastic disease, careful evaluation of the time course is necessary to determine the most likely correlation. [18]

Common Cutaneous Adverse Events for Anti-CTLA-4

The most commonly reported adverse events in patients receiving ipilimumab are "rash" from one quarter to more than one half of patients and

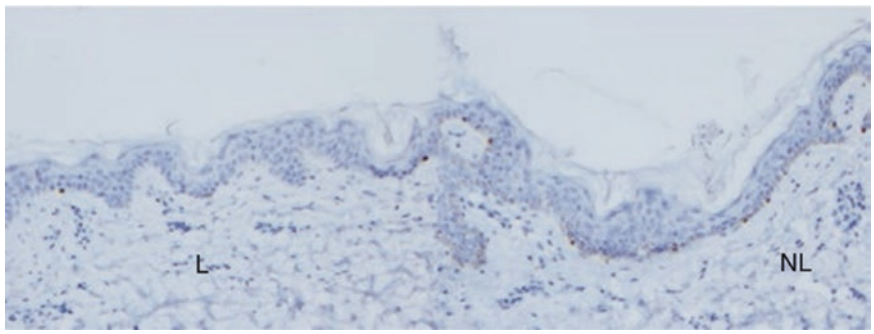

Fig. 11.4 Vitiligo-MART1 immunostain in lesional skin (L) showing decreased melanocytes at the dermal–epidermal junction compared to MART1 immunostain of nonlesional (NL) skin

pruritus from a quarter to one-third [19]. The type of rash varied from mild eczema to toxic epidermal necrolysis [20], with the majority experiencing a more traditional morbilliform drug eruption or an eczematous atopic dermatitis-like eruption [19]. The onset of rash has been reported to appear at about 3 weeks and then usually resolves around 2.5 months [19]. Although in our institutional review, complete resolution was usually not obtained for most patients until drug cessation (unpublished data Patel). The most common CAEs seen with this class of medication are discussed above. Less frequent eruptions include acneiform eruption [12] and granulomatous dermatitis [21].

Its mechanism of action through the activation of T cells by the prevention of T cell blockade leads to an upregulation of the body's immune system and therefore its antitumor activity as described elsewhere in this text. It appears that the cutaneous AE is independent of dosing with those on 10 mg/kg developing similar CAEs as those on 3 mg/kg. Fortunately, high-grade rash as defined by the common terminology criteria as grade 3 or higher was substantially lower at 2.4% [22].

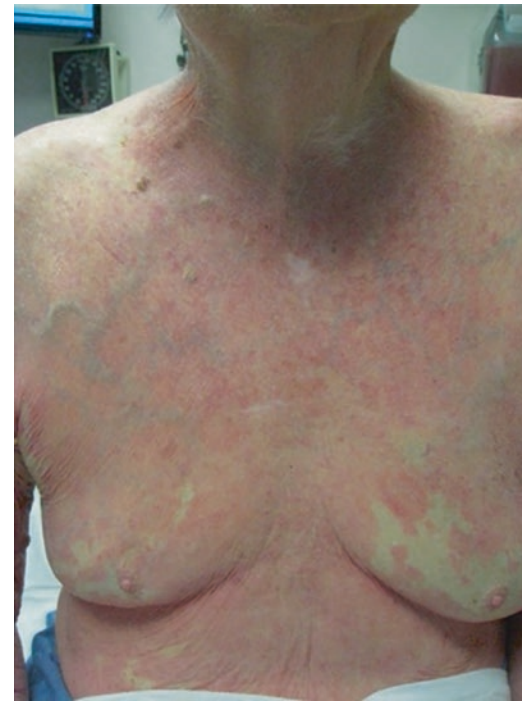

Fig. 11.5 Psoriasiform dermatitis, erythematous well-demarcated plaques with fine adherent scale

CAE in Anti-PD-1

In addition to the shared inflammatory skin reactions discussed earlier, psoriasis [23, 24], lichenoid dermatitis [25] and bullous pemphigoid have been induced by anti-PD-1 antibodies [26, 27]. More recently, eruptive keratoacanthomas has been reported in patients receiving anti-PD-1 therapy [28] (Figs. 5.5 and 5.6).

Psoriasiform dermatitis can appear clinically as classic psoriasis vulgaris with well-demarcated erythematous slightly indurated plaques with adherent fine scale and areas of sparing in a focal to diffuse distribution. It is often worse on extremities than trunk and has a predilection for the scalp. It can also present in inverse distribution with prominence in intertriginous areas [24] or in the pustular variant [29]. It can be pruritic or painful, induce microfissures, and contribute to edema of extremities. Histology shows a spongiotic psoriasiform dermatitis with subcorneal pustules with variable eosinophils. The authors have found psoriasis to be more resistant to treatment than eczema, making distinguishing between the two a prognostic indicator of rash outcome. Treatment should start with topical steroids with antihistamines, if indicated. Escalation of treatment includes oral acitretin, oral apremilast, ultraviolet-B (UV-B) therapy, or oral steroids. Biological medications such as interleukin-17 (IL-17) inhibitors are a potential therapy for refractory cases and have been used anecdotally with success [29].

Lichenoid dermatitis is a pruritic papular eruption mimicking lichen planus. Treatment should start with topical steroids, and can include oral acitretin, methotrexate, or steroids. Bullous pemphigoid is an antibody-mediated bullous disorder presenting with tense bullae. The bullae vary in size, are filled with serous fluid, and are extremely pruritic. Histology shows a subepidermal vesicular dermatitis with prominent eosinophils in the superficial dermis and within the

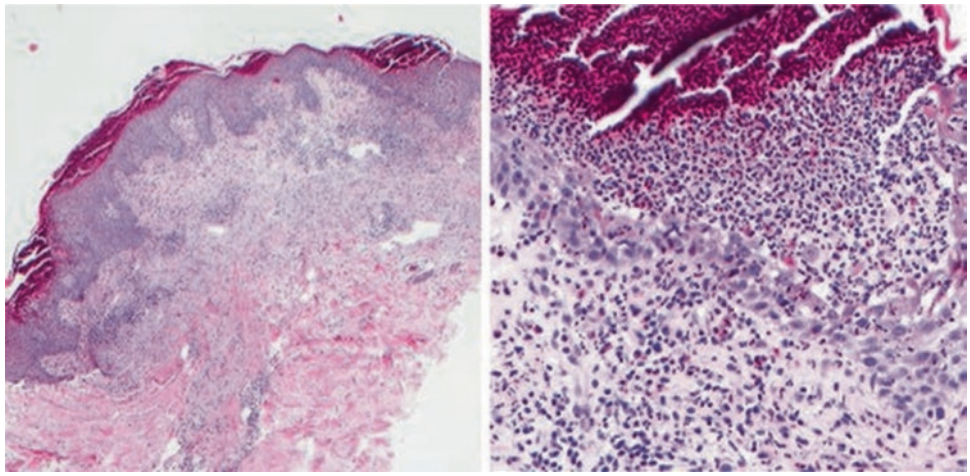

Fig. 11.6 Spongiotic psoriasiform dermatitis with subcorneal pustules, irregular acanthosis, and numerous eosinophils

bullae. The dermal–epidermal split is cleaved and the epidermal roof is intact. Dyskeratosis is not a feature. Direct immunofluorescence high- lights IgG deposition at the dermal–epidermal junction. Topical and oral steroids as well as rituximab have been used successfully in this slow-to-appear cutaneous AE [30].

Eruptive keratoacanthoma appears to be relatively well-demarcated and a low grade of squamous cell carcinoma. They were treated conservatively in this report without treatment interruption for the patients [28].

Combination Therapies

Combination checkpoint inhibitor therapies are being used more frequently with loading doses of anti-CTLA4 and antiPD-1/PD-L1 therapies, followed by maintenance anti-PD-1/anti-PD-L1. Although the cutaneous AEs are predominantly eczema, psoriasis, pruritus, and vitiligo, the incidence numbers are approximately 50% in our institutional database, which includes both clinical trials and standard-of-care patients. Dose impact appears to be less than with monotherapy as patients have systemic toxicities that are dose-limiting, minimizing the effects of the CAE.

Grading

Grading has nearly been universally based upon the Common Terminology Criteria for Adverse Events and more recently a modified version produced by the American Society of Clinical Oncology as their "Practice Guideline," which focuses on symptoms and quality of life rather than extent of involvement. This appears to be a more useful measure as relatively small body surface area involvement can still be dose limiting (Table 11.1 and Fig. 11.7).

CAE as Prognostic Indicators

Vitiligo is a relatively innocuous adverse event as it is largely asymptomatic and untreated. It is, however, associated with increased progression free survival and tumor response when occurring in patients on immune checkpoint inhibitors. Vitiligo is widely believed to be an underreported side effect as it can be easily missed if a full body skin exam is not performed. Vitiligo has only been reported in patients being treated with melanoma [2, 3, 33, 34]. Incidence of rash was also associated with increased survival and tumor response [2].

Table 11.1 Common terminology criteria for adverse events [31]

Grade	1	2	3	4	5
Rash	Macular or papular eruption covering <10% BSA with or without symptoms (e.g., pruritus, burning, tightness)	Macular or papular eruption covering 10–30% BSA with or without symptoms (e.g., pruritus, burning, tightness) and limiting of instrumental ADL	Macules/ papules covering >30% BSA with or without associated symptoms and limiting of self-care ADL	Generalized exfoliative, ulcerative, or bullous dermatitis	Death
Alopecia	Hair loss of up to 50% of normal for that individual that is not obvious from a distance but only on close inspection; a different hairstyle may be required to cover the hair loss, but it does not require a wig or hairpiece to camouflage	Hair loss of >50% of normal for that individual that is readily apparent to others; a wig or hairpiece is necessary if the patient desires to completely camouflage the hair loss or if loss is associated with psychosocial impact			
Hypopigmentation	Hypopigmentation or depigmentation covering <10% BSA, with no psychosocial impact	Hypopigmentation or depigmentation covering >10% BSA or with associated psychosocial impact			
Pruritus	Mild or localized, relieved spontaneously or by local measures	Intense or widespread, relieved spontaneously or by systemic measures	Intense or widespread, and poorly controlled despite treatment		

1.0 Skin Toxicities	
1.1 Rash/inflammatory dermatitis	
Definition: Erythema multiforme minor (a targetoid reaction in the skin and mucous membranes usually triggered by infections, such as herpes simplex viruses, but can be associated with an immune-related drug eruption and if progresses to erythema multiforme major, it and can be a harbinger of SCAR, such as SJS), lichenoid (resembling the flat-topped, polygonal, and sometimes scaly or hypertrophic lesions of lichen-planus), eczematous (inflammatory dermatitis characterized by pruritic, erythematous, scaly, or crusted papules or plaques on the skin, which is vulnerable to superinfection, psoriasiform [resembling the well-demarcated, erythematous, and scaly papules and plaques of psoriasis], morbilliform [a nonpustular, nonbullous measles-like exanthematous rash of the skin often referred to as "maculopapular" and without systemic symptoms or laboratory abnormalities, excluding occasional isolated peripheral eosinophilia, palmoplantar erythrodysesthesia [hand-foot syndrome; redness, numbness, burning, itching, and superficial desquamation of the palms and soles], neutrophilic dermatoses [eg, Sweet syndrome], and others)	
Diagnostic work-up Pertinent history and physical examination Rule out any other etiology of the skin problem, such as an infection, an effect of another drug, or a skin condition linked to another systemic disease or unrelated primary skin disorder If needed, a biologic checkup, including a blood cell count and liver and kidney tests Directed serologic studies if an autoimmune condition is suspected, such as lupus or dermatomyositis: a screening antinuclear antibody test, SS-A/Anti-Ro, SS-B/Anti-La if predominantly photodistributed/photosensitivity, antihistone, double-stranded DNA, and other relevant serologies. Consider expanding serologic studies or diagnostic work-up if other autoimmune conditions are considered based on signs, symptoms Skin biopsy Consider clinical monitoring with use of serial clinical photography Review full list of patient medications to rule out other drug-induced cause for photosensitivity	
Grading	**Management**
Grading according to CTCAE is a challenge for skin. Instead, severity may be based on BSA, tolerability, morbidity, and duration.	
G1: Symptoms do not affect the quality of life or controlled with topical regimen and/or oral antipruritic	Continue ICPi Treat with topical emollients and/or mild-moderate potency topical corticosteroids Counsel patients to avoid skin irritants and sun exposure
G2: Inflammatory reaction that affects quality of life and requires intervention based on diagnosis	Consider holding ICPi and monitor weekly for improvement. If not resolved, interrupt treatment until skin AE has reverted to grade 1 Consider initiating prednisone (or equivalent) at dosing 1 mg/kg, tapering over at least 4 weeks In addition, treat with topical emollients, oral antihistamines, and medium- to high-potency topical corticosteroids
G3: As G2 but with failure to respond to indicated interventions for a G 2 dermatitis	Hold ICPi therapy and consult with dermatology to determine appropriateness of resuming Treat with topical emollients, oral antihistamines, and high-potency topical corticosteroids Initiate (methyl)prednisolone (or equivalent) 1-2 mg/kg, tapering over at least 4 weeks
G4: All severe rashes unmanageable with prior interventions and intolerable	Immediately hold ICPi and consult dermatology to determine appropriateness of resuming ICPi therapy upon resolution of skin toxicity and once corticosteroids are reduced to prednisone (or equivalent) ≤ 10 mg Systemic corticosteroids: IV (methyl)prednisolone (or equivalent) dosed at 1-2 mg/kg with slow tapering when the toxicity resolves Monitor closely for progression to severe cutaneous adverse reaction Should admit patient immediately with direct oncology involvement and with an urgent consult by dermatology Consider alternative antineoplastic therapy over resuming ICPis if the skin irAE does not resolve to G1 or less; if ICPIs are the patient's only option, consider restarting once these adverse effects have resolved to a G1 level
1.2 Bullous dermatoses	
Definition: Including bullous pemphigoid or other autoimmune bullous dermatoses, bullous drug reaction	
Diagnostic work-up Physical examination Rule out any other etiology of the skin problem, such as an infection, an effect of another drug, or a skin condition linked to another systemic disease If needed, a biologic checkup, including a blood cell count, liver, and kidney tests; consider serum antibody tests to rule out bullous pemphigoid or, under the guidance of dermatology, sending patient serum for indirect immunofluorescent testing to rule out other autoimmune blistering diseases Referral to dermatology for blisters that are not explained by infectious or transient other causes (eg, herpes simplex, herpes zoster, bullous impetigo, bullous insect bite, friction or pressure blister) Consider skin biopsy (both hematoxylin and eosin evaluation of lesional skin and direct immunofluorescence evaluation of perilesional skin)	

Fig. 11.7 Management of skin irAEs in patients treated with ICPIs [32]

Grading	Management
G1: Asymptomatic, blisters covering < 10% BSA and no associated erythema	If blisters are < 10% BSA, asymptomatic, and noninflammatory (such as the case with friction blisters or pressure blisters), cessation of ICPi is not necessary, and only observation and/or local wound care is warranted. When symptomatic bullae or erosions, which are deroofed vesicles or bullae, are observed on the skin or mucosal surfaces, the cutaneous irAE is by definition considered at least G2 See G2 management recommendations
G2: Blistering that affects quality of life and requires intervention based on diagnosis not meeting criteria for grade > 2 Blisters covering 10%-30% BSA	Hold ICPi therapy and consult with dermatology for work-up and to determine appropriateness of resuming Attention given to general local wound care, which includes plain petrolatum ointment and bandages or plain petrolatum ointment gauze and bandage over any open erosions, which are left over on the skin after the blister has popped or if the roof of the blister easily sloughs off Counsel patients to avoid skin irritants and overexposure to sun, wear protective clothing, use sunscreens Work-up for autoimmune bullous disease as above Initiate class 1 high-potency topical corticosteroid (eg, clobetasol, betamethasone or equivalent) and reassess every 3 days for progression or improvement Low threshold to initiate treatment with prednisone (or equivalent) at 0.5-1 mg/kg dosing and taper over at least 4 weeks Monitor patients with G2 irAEs closely for progression to involvement of greater BSA and/or mucous membrane involvement. Consider following patients closely using serial photography Primer on monitoring for complicated cutaneous adverse drug reactions: • Review of systems: Skin pain (like a sunburn), fevers, malaise, myalgias, arthralgias, abdominal pain, ocular discomfort or photophobia, sores or discomfort in the nares, sores or discomfort in the oropharynx, odynophagia, hoarseness, dysuria, sores or discomfort in the vaginal area for women or involving the meatus of the penis for men, sores in the perianal area, or pain with bowel movements • Physical examination: Include vital signs and a full skin examination specifically evaluating all skin surfaces and mucous membranes (eyes, nares, oropharynx, genitals, and perianal area). Assess for lymphadenopathy, facial or distal extremity swelling (may be signs of DIHS/DRESS). Assess for pustules or blisters or erosions in addition to areas of "dusky erythema," which may feel painful to palpation. To assess for a positive Nikolsky sign, place a gloved finger tangentially over erythematous skin and apply friction parallel to the skin surface. Nikolsky sign is positive if this results in detached or sloughing epidermis demonstrating poor attachment of the epidermis to the dermis, which is the case in some autoimmune disorders (eg, pemphigus) and SJS/TEN
G3: Skin sloughing covering > 30% BSA with associated pain and limiting self-care ADL	Hold ICPi therapy and consult with dermatology to determine appropriateness of resuming Administer IV (methyl)prednisolone (or equivalent) 1-2 mg/kg, tapering over at least 4 weeks If bullous pemphigoid is diagnosed, it may be possible to avoid long-term use of systemic corticosteroids and treat with rituximab, as an alternative approach to treating the irAE Seek infectious disease consultation if patient might have secondary cellulitis or if patient has other infection risk factors, such as neutropenia, etc.
G4: Blisters covering > 30% BSA with associated fluid or electrolyte abnormalities	Permanently discontinue ICPi Admit patient immediately and place under supervision of a dermatologist Administer IV (methyl)prednisolone (or equivalent) 1-2 mg/kg with tapering over at least 4 weeks when the toxicity resolves If bullous pemphigoid is diagnosed, it may be possible to avoid long-term use of systemic corticosteroids and treat with rituximab as an alternative approach to treating the irAE Seek infectious disease consultation if patient might have secondary cellulitis or if patient has other infection risk factors, such as neutropenia, etc

Fig. 11.7 (continued)

1.3 SCARs, including SJS, TEN, acute generalized exanthematous pustulosis, and DRESS/DIHS

Definition: Severe changes in either structure or functions of skin, the appendages or the mucous membranes due to a drug

Diagnostic work-up

- Total body skin examination with attention to examining all mucous membranes as well as complete review of systems
- Rule out any other etiology of the skin problem, such as an infection, an effect of another drug, or a skin condition linked to another systemic disease
- A biologic checkup, including a CBC with differential test, and liver and kidney function tests, including urinalysis, in addition to the blood work; if the patient is febrile, blood cultures should be considered as well
- Skin biopsies to assess for full-thickness epidermal necrosis, as is seen in SJS/TEN, as well as other possible etiologies like paraneoplastic pemphigus or other autoimmune blistering dermatoses or other drug reactions, such as acute generalized exanthematous pusulosis
- Consider following patients closely using serial clinical photography
- If mucous membrane involvement or blistering is observed on the skin, consider early admission to a burn center for further monitoring and management
- Primer on monitoring for complicated cutaneous adverse drug reactions:
  - Review of systems: Skin pain (like a sunburn), fevers, malaise, myalgias, arthralgias, abdominal pain, ocular discomfort or photophobia, sores or discomfort in the nares, sores or discomfort in the oropharynx, odynophagia, hoarseness, dysuria, sores or discomfort in the vaginal area for women or involving the meatus of the penis for men, sores in the perianal area, or pain with bowel movements
  - Physical examination: Include vital signs and a full skin examination specifically evaluating all skin surfaces and mucous membranes (eyes, nares, oropharynx, genitals, and perianal area). Assess for lymphadenopathy, facial or distal extremity swelling (may be signs of DIHS/DRESS). Assess for pustules or blisters or erosions in addition to areas of "dusky erythema," which may feel painful to palpation. To assess for a positive Nikolsky sign, place a gloved finger tangentially over erythematous skin and apply friction parallel to the skin surface. Nikolsky sign is positive if this results in detached or sloughing epidermis demonstrating poor attachment of the epidermis to the dermis, which is the case in some autoimmune disorders (eg, pemphigus) and SJS/TEN

All grades	In cases of suspected SJS or any mucous membrane involvement, discontinue ICPi treatment and monitor closely for improvement, regardless of grade
G1: NA	For SCARs, there is no G1 category; if lower BSA is involved with bullae or erosions, there should remain a high concern that this reaction will progress to G3 or G4
G2: Morbilliform ("maculopapular") exanthem covering 10%-30% BSA with systemic symptoms, lymphadenopathy, or facial swelling	Hold ICPi and monitor patients closely every 3 days with G2 irAEs for progression to involvement of greater BSA and/or mucous membrane involvement Consider following patients closely using serial photography Initiate therapy with topical emollients, oral antihistamines, and medium- to high-strength topical corticosteroids Consider initiation of prednisone (or equivalent) 0.5-1 mg/kg tapered over at least 4 weeks
G3: Skin sloughing covering < 10% BSA with mucosal involvement associated signs (eg, erythema, purpura, epidermal detachment, mucous membrane detachment)	Hold ICPi therapy and consult with dermatology Treat skin with topical emollients and other petrolatum emollients, oral antihistamines, and high-strength topical corticosteroids; dimethicone may also be offered as an alternative to petrolatum Administer IV (methyl)prednisolone (or equivalent) 0.5-1 mg/kg and convert to oral corticosteroids on response, wean over at least 4 weeks Admit to burn and/or consult wound services with attention to supportive care, including fluid and electrolyte balance, minimizing insensible water losses, and preventing infection Given the immune mechanism of action of these medicines, use of immune suppression is warranted and should be offered For mucous membrane involvement of SJS or TEN, appropriate consulting services should be offered to guide management in preventing sequelae from scarring (eg, ophthalmology; ear, nose, and throat; urology; gynecology; etc, as appropriate)
G4: Skin erythema and blistering/sloughing covering ≥ 10% BSA with associated signs (eg, erythema, purpura, epidermal detachment, mucous membrane detachment) and/or systemic symptoms and concerning associated blood work abnormalities (eg, liver function test elevations in the setting of DRESS/DIHS)	Permanently discontinue ICPi Admit patient immediately to a burn unit or ICU with consulted dermatology and wound care services Consider further consultations based on management of mucosal surfaces (eg, ophthalmology; urology; gynecology; ear, nose, and throat surgery; etc) Initiate IV (methyl)prednisolone (or equivalent) 1-2 mg/kg, tapering when toxicity resolves to normal IVIG or cyclosporine may also be considered in severe or corticosteroid-unresponsive cases Consider pain/palliative consultation and/or admission in patients presenting with DRESS manifestations

Additional considerations: The usual prohibition of corticosteroids for SJS is not relevant here, as the underlying mechanism is a T-cell immunodirected toxicity. Adequate suppression is necessary with corticosteroids or other agents and may be prolonged in cases of DRESS/DIHS

All recommendations are expert consensus based, with benefits outweighing harms, and strength of recommendations are moderate

Abbreviations: ADL, activities of daily living; BSA, body surface area; CTCAE, Common Terminology Criteria for Adverse Events; DIHS, drug-induced hypersensitivity syndrome; DRESS, drug reaction with eosinophilia and systemic symptoms; G, grade; ICPi, immune checkpoint inhibitor; ICU, intensive care unit; irAE, immune-related adverse event; IV, intravenous; IVIG, intravenous immunoglobulin; NA, not applicable; SCAR, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TENS, toxic epidermal necrolysis.

Fig. 11.7 (continued)

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Gastrointestinal Tract Adverse Events

12

Hamzah Abu-Sbeih and Yinghong Wang

Abstract

Immune checkpoint inhibitors (ICIs) have shown significant benefit in cancer patients. Their success, however, is associated with immune-related adverse events (irAEs), which commonly affect the gastrointestinal tract, resulting in diarrhea and colitis. IrAEs range from mild self-limiting to severe life-threatening diseases and potentially limit the use of these medications. Diagnosis of ICI-induced enterocolitis is based on clinical symptoms, physical examination, stool tests, endoscopic and histologic evaluation, and/or imaging. Current management strategy is mainly anti-diarrheal agents for mild symptoms and immunosuppressants (e.g., corticosteroids, and infliximab or vedolizumab) for more severe diseases.

Keywords

Immune checkpoint inhibitors · Immunotherapy · Colitis · Diarrhea · Enterocolitis · Gastrointestinal adverse events

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Enterocolitis

Incidence

ICI-induced enterocolitis is reported in 15–25% of patients receiving cytotoxic T lymphocyte antigen-4 (CTLA-4) inhibitors [1–3]. Blockade of programmed death protein-1 and its ligand (PD-[L]1) is associated with lower rate of enterocolitis, up to 10% [4]. However, when combined, the risk of enterocolitis becomes as high as 30% [5]. Alternatively, diarrhea can occur in up to 54% of patients receiving ICI therapy, especially the combinatorial approach [4]. Moreover, diarrhea grade 3 and 4 is the most common serious adverse event leading to ICI discontinuation, occurring in 10% of patients receiving ICIs [3, 6]. Colonic perforation has been reported in ~2% of patients treated with ICI therapy [2, 7]. The occurrence of ICI-induced enterocolitis has been proposed to correlate with favorable response of cancer to ICI therapy, reflected by improved survival rates in these patients compared with those who did not develop ICI-induced enterocolitis [8, 9]. Nonsteroidal anti-inflammatory drugs and fecal microbiome are speculated to play a role in the development of ICI-induced enterocolitis [1, 10–12].

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Clinical Presentation

ICI-induced gastrointestinal toxicities most commonly present as watery diarrhea, followed by abdominal pain, blood or mucous with stool, abdominal distension, nausea and vomiting, and fever [1, 2, 13]. Weight loss might occur in patients with protracted severe ICI-induced enterocolitis [1]. Many patients often have only nonbloody self-limiting diarrhea without the other associated enterocolitis symptoms [14, 15], whereas severe enterocolitis may result in colonic perforation and death [16–18]. The severity of diarrhea and colitis is graded based on the Common Terminology Criteria for Adverse Events *version 5.0* [19]. Enterocolitis generally occurs around 5–10 weeks following initiation of ICI treatment [17, 20]. However, the onset can range from immediately after the first dose to more than 6 months after the last infusion of ICI [13, 21, 22]. Enterocolitis onset can be acute or gradual. A mild self-limiting transient diarrhea might occur after the first few doses of ICI therapy; this diarrhea should not be confused with immune-mediated enterocolitis.

Diagnosis

Patients on ICI treatment who develop gastrointestinal symptoms should be evaluated for other etiologies first [18]. Infectious stool workup should include bacterial (e.g., *Clostridium difficile*), viral (e.g., CMV), parasitic, or fungal infections [23, 24]. Of note, in some cases, ICI-induced enterocolitis and gastrointestinal infections can coexist, which makes it difficult to distinguish between both [25]. Blood tests, such as complete blood count and comprehensive metabolic panel, can assess in the exclusion of other etiologies and the severity of the disease. Additionally, workup for celiac disease, fecal elastase for pancreatic insufficiency, and TSH for thyroid dysfunction should be performed to rule out these etiologies of diarrhea.

Currently, there are no available specific serologic or fecal markers for ICI-induced enterocolitis [26]. Nonetheless, fecal calprotectin and lactoferrin are stool inflammatory markers that have been widely used in the clinical practice for patients with inflammatory bowel disease. Given the similarities between both entities, these markers have also been studied and shown to be of value in the evaluation of ICI-induced enterocolitis [2, 7]. Fecal lactoferrin might be used to determine who should undergo endoscopic evaluation, and fecal calprotectin might be used to monitor for response of enterocolitis to treatment [27, 28].

Cross-sectional abdominal imaging (i.e., computerized tomography (CT) or magnetic resonance imaging (MRI)) can assist in the evaluation of colonic inflammation. Moreover, abdominal imaging might be helpful to assess for colonic perforation, obstruction, and toxic megacolon, which might complicate ICI-induced enterocolitis. Features of ICI-induced enterocolitis on imaging include diffuse wall thickening, mesenteric vessel engorgement, peri-colic fat stranding, and mucosal enhancement [2, 29]. Free intraperitoneal air indicates the presence of bowel perforation [30]. Nevertheless, the sensitivity of imaging to detect enterocolitis is approximately 50%, and therefore, endoscopy is considered the gold standard for the evaluation of enterocolitis [28, 31].

For patients with ≥ grade 2 diarrhea or colitis symptoms or with persistent grade 1 but positive lactoferrin, colonoscopy with biopsy is highly recommended to evaluate the severity and extent of ICI-induced enterocolitis, as it was reported that severe endoscopic presentation correlates with response of enterocolitis to treatment [7, 32]. Furthermore, endoscopy should be performed as soon as possible following enterocolitis onset to guide treatment options; this approach has been proven to be effective in preventing unfavorable outcomes [7]. Endoscopic manifestations often reveal erythema, edema, exudates, granularity, loss of vascular pattern, erosions, ulcerations, and bleeding (Fig. 12.1) [31–33]. Most commonly, enterocolitis will be extensive, involving the entire colon and ileum, followed by isolated left colon inflammation [31]. Isolated right colon and ileal inflammation has been reported in 10–15% of patients [7]. Therefore, initial endos-

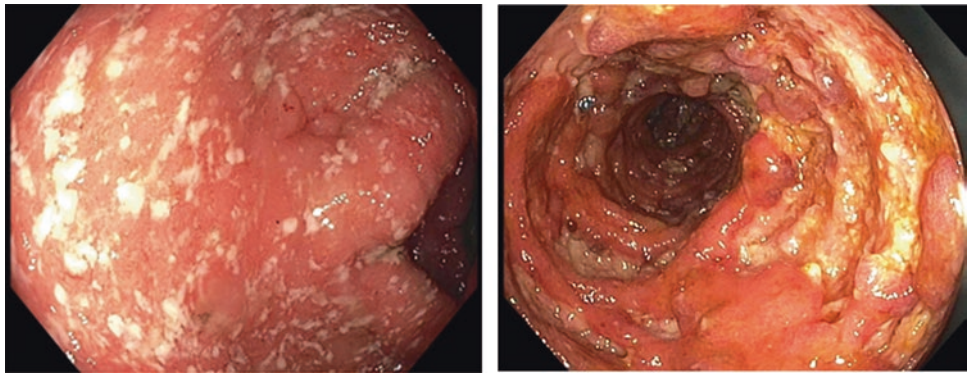

Fig. 12.1 Endoscopy images of immune checkpoint inhibitors–related colitis showing diffuse erythema, edema, inflammatory exudate, loss of vascular pattern, and deep large ulcerations

Table 12.1 Mayo Score for endoscopic presentation

Score	Disease status	Endoscopic features
0	Normal	Normal colon
1	Mild	Erythema, altered vascularity, mild friability
2	Moderate	Diffuse erythema, absent vascular pattern, marked friability, erosions
3	Severe	Spontaneous bleeding and mucosal ulcerations

copy evaluation with full extent colonoscopy is preferred over flexible sigmoidoscopy to detect colonic inflammation proximal to the left colon [34]. Follow-up endoscopy can be sigmoidoscopy if isolated left colon involvement is confirmed initially. Endoscopic inflammation pattern of ICI-induced enterocolitis can be focal, segmental, patchy, or diffuse circumferential [34]. Routine biopsy is recommended even with normal endoscopic evaluation to investigate for a subtype of enterocolitis that mimics microscopic colitis [13, 35]. Mayo Score for endoscopic presentation used in patients with ulcerative colitis can be also used to assess for the severity of ICI-induced enterocolitis (Table 12.1) [36].

Microscopic findings of ICI-induced enterocolitis are categorized into acute, chronic, and microscopic inflammation. Acute inflammation features are the most common and include neutrophil and/or eosinophil infiltration, epithelium apoptosis, cryptitis and crypt microabcesses; chronic inflammation features include crypt architectural distortion, basal lymphoplasmocytosis, granuloma, and Paneth cell metaplasia; and microscopic features are rare and can be either lymphocytic infiltration in the epithelium or subepithelial collagen band deposition [31, 32, 37]. Chronic histologic features are similar to those of Crohn's disease and ulcerative colitis. Active histologic features can be used as a surrogate marker for severe diseases with worse outcomes [7]. Thus, the identification of active features should indicate early initiation of aggressive immunosuppressive therapy. In addition, the status of cytomegalovirus infection on the histopathological examination of the colon tissue should be evaluated [2].

Treatment

Current treatment recommendations of ICI-induced enterocolitis depends on the clinical severity only [38]. For patients with grade 1 toxicity, usually conservative management with adequate oral hydration, diet modification, and close follow-up monitoring is recommended. Antimotility agents can be used after exclusion of infectious etiology but are often not recommended [28]. Mesalamine (i.e., 5-ASA) has been reported to be effective in mild grade diarrhea [39]. Usually ICI therapy can be continued in grade 1 disease. If conservative management fails or symptoms progress to higher grade, more aggressive treatment strategy is required.

For a grade 2 and higher toxicity, ICI therapy should be halted, temporarily for grade 2 and 3 and permanently for grade 4 [27, 28, 40]. The mainstay treatment for grade 2 and higher ICI-induced enterocolitis is immunosuppressive therapy to hamper the inflammation. These include corticosteroids and other more potent immunosuppressants (e.g., infliximab and/or vedolizumab) [3, 41, 42]. The recommended oral corticosteroid for ICI-induced enterocolitis is prednisone or equivalent with a dose of 1–2 mg/kg. Intravenous corticosteroid (e.g., methylprednisolone) is indicated in patients with grade 3–4 enterocolitis. Corticosteroids should be tapered over 4 weeks after symptoms resolution, as steroid treatment for less than 30 days has been shown to be associated with less frequent infectious events [31]. The use of steroid enema and budesonide was reported in case studies but are not standard practice, especially for grade 3–4 enterocolitis [20, 23, 39, 43].

In cases of refractory to corticosteroid treatment, infliximab (anti-TNF) and vedolizumab (anti-integrin) are recommended [3, 41, 44]. Screening for HIV, tuberculosis, and hepatitis B and C should be performed before initiating these agents. Early use of infliximab is reported to be associated with shorter duration of immunosuppressant treatment and improved clinical outcome [41, 42, 45]. Contraindications for infliximab include bowel perforation and active infection, especially sepsis [17]. Response to infliximab therapy is usually within 1–3 days [13], while some patients may need more than one dose [39]. The stated response rate to infliximab is as high as 85% [2]. Vedolizumab is a potential substitute for infliximab with encouraging clinical outcomes, comparable efficacy, and favorable safety profile [46]. Currently, it is recommended as third-line agent after failure of infliximab or if infliximab is contraindicated. Early introduction of potent immunosuppressive therapy (i.e., infliximab or vedolizumab) improves the outcomes of ICI-induced enterocolitis regardless of steroid response, especially in patients with severe disease presentation [47]. Reports have shown that mycophenolate mofetil can be used in the treatment of ICI-induced enterocolitis [42]. Recently, fecal microbiota transplantation has been proposed to be effective in patients with ICI-induced enterocolitis that is refractory to available immunosuppressive therapy [48].

After resolution of enterocolitis to grade 1 or less, ICI therapy might be resumed, especially PD-(L)1 inhibitors [17]. Enterocolitis symptoms can recur after weeks to months from resolution of the initial episode to mimicking inflammatory bowel disease. Recurrence of gastrointestinal symptoms requires comprehensive evaluation for the ICI-enterocolitis with similar approach to the first episode [23]. Repeat immunosuppressant treatment may be needed. Of note, in patients with high suspicion of bowel perforation or toxic megacolon, surgical consultation is warranted [17, 42, 49, 50].

Conclusion

The recognition of ICI-induced enterocolitis is increasing with the wide use of ICI therapy in the past few years. The diagnosis and the severity measures of ICI-induced enterocolitis are based on multiple evaluation modalities, such as laboratory tests, abdominal imaging, and endoscopic assessment. The cornerstone treatment of ICI-induced enterocolitis is corticosteroid therapy, followed by infliximab and vedolizumab. The ultimate goal is to provide appropriate treatment to keep the enterocolitis in remission while continuing ICI treatment. Further prospective studies are still needed to improve the management strategy of ICI-induced enterocolitis.

Gastroenteritis

Although nausea and vomiting are frequent symptoms in cancer patients. In patients treated with ICI therapy, it has been reported that nausea and vomiting, especially if severe enough, might be a consequence of immune-mediated gastritis [27, 51]. The body of evidence regarding this disease is very limited. After ruling out other etiologies, esophagogastroduodenoscopy with biopsy can help to establish the diagnosis of ICI-

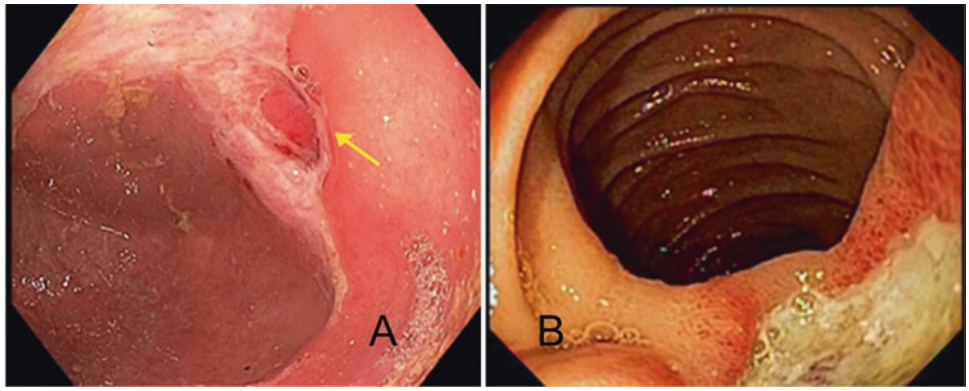

Fig. 12.2 Endoscopy findings of gastritis related to immune checkpoint inhibitors. Endoscopy images demonstrating deep, large, mucosal ulceration in the stomach (**a**) and bleeding mucosal ulceration the duodenum (**b**) [54]

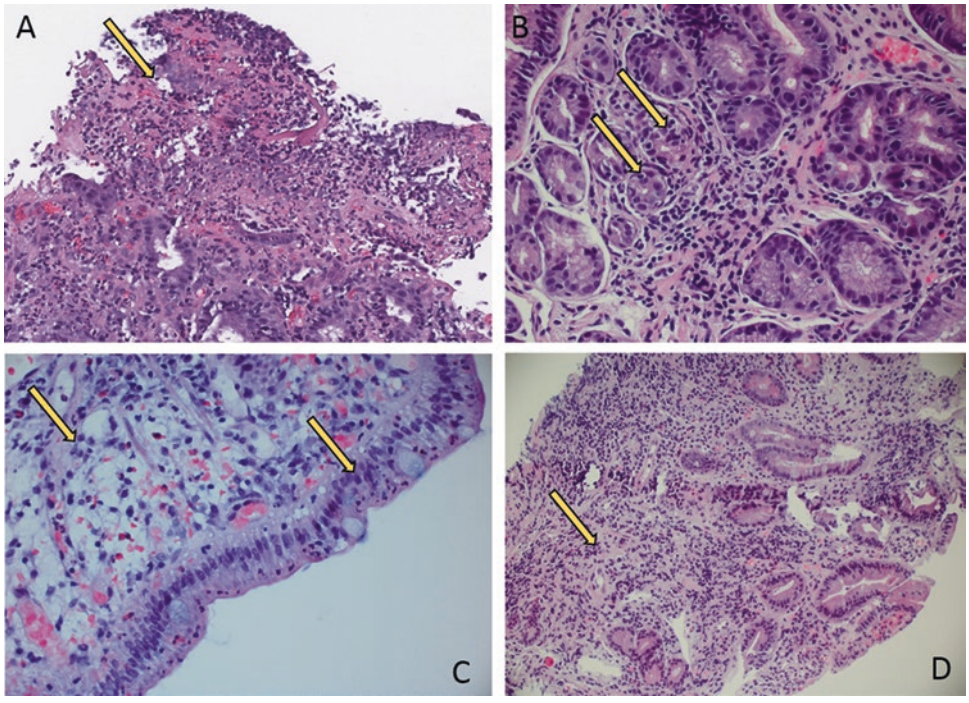

Fig. 12.3 Histologic features of immune checkpoint inhibitor–related upper gastrointestinal injury: (**a**) denudation of epithelium with fibro-inflammatory exudates, (**b**) active gastritis with neutrophils in epithelium; chronic gastritis with lymphocytes in lamina propria, (**c**) increased intraepithelial neutrophils, lamina propria edema and increased neutrophils in lamina propria, villous blunting, and (**d**) duodenum: shows altered architecture and gland drop out [54]

related gastroenteritis. Endoscopically, erythema, edema, friability, erosions, and ulcerations might be observed (Fig. 12.2). Histologically, commonly described features in the gastric mucosa are lamina propria expansion and intraepithelial neutrophilic infiltration. In duodenal biopsies, villous blunting, lymphoplasmacytic lamina propria expansion, as well as plasma cells and eosinophils infiltrates, neutrophilic cryptitis, and/or villitis have been reported (Fig. 12.3) [52, 53]. The appropriate treatment for such toxicity still is unclear. The reported

medical treatments include proton pump inhibitors, H_2 blockers, corticosteroids, and vedolizumab [54]. The role for these treatment modalities still needs further investigation.

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Immune-Related Adverse Events: Pneumonitis

13

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Abstract

Checkpoint inhibitors are part of the family of immunotherapies and are increasingly being used in a wide variety of cancers. Immune-related adverse events pose a major challenge in the treatment of cancer patients. Pneumonitis is a rare immune-related adverse event that presents in distinct patterns. The goal of this chapter is to instruct readers on the incidence and clinical manifestations of pneumonitis and to offer guidance in the evaluation and treatment of patients with pneumonitis.

Keywords

Checkpoint inhibitors · Immune-related adverse event · Pneumonitis · Thoracic imaging · Organizing pneumonia · Nonspecific interstitial pneumonia · Hypersensitivity pneumonitis · Diffuse alveolar damage

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Introduction

The prevalence of cancer is rising in parallel with increasing life expectancy [1]. Recurrent and refractory cancers pose major therapeutic challenges for clinicians, and new strategies are necessary to counter the evolving landscape of cancer [2]. Immunotherapy is one such strategy where the immune system can be weaponized against cancers to induce a potentially durable reduction in tumor burden [3–5]. Common targets of immunotherapy agents include the programmed cell death protein 1 (PD-1) pathway and the cytotoxic T-lymphocyte-associated protein-4 pathways (CTLA-4), which we discuss in detail below [6]. Tumor cells can suppress the natural antitumor activity of T-cells through several mechanisms, including expression of PD-L1 (a ligand for PD-1) and CTLA-4 [7]. Inhibitors of the PD-1 and CTLA-4 pathways boost antitumor immune responses by preventing homeostatic downregulation of T-lymphocyte activity, which normally occurs during chronic infection to prevent excessive tissue injury [8, 9]. However, a reinvigorated immune system may lead to disturbances in

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normal immune self-tolerance and, as a result, may induce off-target immune-related adverse events (irAEs), which may affect numerous organs. In this chapter, we focus on pulmonary irAEs that occur after immunotherapeutic agents.

Inhibition of T-Lymphocyte Function by the PD-1 and CTLA-4 Pathways

PD-1 is a monomeric transmembrane protein in the immunoglobulin superfamily that is found on the surface of macrophages and T- and B-lymphocytes [10–12]. PD-1 is primarily expressed in mature T-cells and appears within 24 h of T-cell activation as a mechanism to regulate T-cell activity to prevent injury to healthy tissue [13]. PD-1 binds primarily to two ligands, PD-L1 and PD-L2. PD-L1 is broadly expressed by hematopoietic cell lineages and various epithelial and endothelial cells, while PD-L2 is expressed primarily by dendritic cells and B-lymphocytes [10]. Several inflammatory cytokines can induce PD-L1 expression on the surface of lymphocytes and on nonimmune cells [11]. The interaction of PD-1 with its ligands causes the recruitment of phosphatase Src homology protein 2 (SHP2), which leads to subsequent inactivation of the PI3K/AKT signaling [14, 15]. In T-lymphocytes, activation of the PD-1 pathway blocks proliferation, impairs inflammation, and decreases survival [16]. Binding of PD-1 to PD-L2 decreases T-lymphocyte cytokine production, but does not inhibit proliferation [17]. Furthermore, activation of the PD-1 pathway induces the differentiation of naïve T-lymphocytes into T-regulatory lymphocytes, which induce immune tolerance [18, 19]. Cancer cells harness the inhibitory functions of PD-1 activation by expressing PD-L1 and PD-L2, which limits antitumor immune responses [20]. PD-1 can also be expressed on tumor-associated macrophages, which may lead to a tumor microenvironment that is conducive to cancer progression [21].

Optimal T-lymphocyte activity requires binding of costimulatory molecules such as CD28, expressed on the T-lymphocyte cell surface, to its receptors B7-1 (CD80) and B7-2 (CD86), expressed on antigen presenting cells [22, 23]. CTLA-4 is a CD28 homolog that has a higher affinity for B7 than CD28, but does not produce a stimulatory signal. CTLA-4 has a 36-amino acid cytoplasmic tail that lacks enzymatic activity, but also has an immunoreceptor tyrosine-based inhibitory motif that has inhibitory functions [24, 25]. Activation of CTLA-4 induces signals that inhibit T-lymphocyte function [23, 26–29], decrease T-lymphocyte proliferation, and impair secretion of interleukin-2 [22, 23, 26, 27, 30]. In health, CTLA-4 is mainly expressed by T-regulatory cells and CTLA-4 activation is an important mechanism to promote peripheral tolerance [31]. Loss of CTLA-4 function leads to fatal autoimmunity in mice [32, 33]. Similarly, cancer cells express CTLA-4 on the tumor surface, which leads to impaired T-cell function and survival [34, 35].

Immune Checkpoint Inhibition as a Therapeutic Strategy in Cancer

Cancer cells harness checkpoint activation through the PD-1 and CTLA-4 pathways to induce energy in antitumor lymphocytes. Inhibition of these pathways can lead to tumor regression. In this section, we will briefly discuss the CTLA-4 inhibitor: ipilimumab, the PD-1 inhibitors: nivolumab and pembrolizumab, and the PD-L1 inhibitors: atezolizumab, avelumab, and durvalumab. Ipilimumab is the only CTLA-4 inhibitor approved by the Food and Drug Administration (FDA) at this time. Ipilimumab binds to the front β-sheet of CTLA-4 and interferes with the formation of CTLA-4:B7 complexes [36]. Another CTLA-4 inhibitor, tremelimumab, is in development, but not yet approved by the FDA and is beyond the scope of this chapter. Inhibitors of the PD-1 pathway broadly fall into two categories: inhibitors of PD-1 function and inhibitors of PD-L1 function. Nivolumab and pembrolizumab bind competitively to PD-1 to form PD-1: monoclonal antibody complexes [37]. These two drugs bind to PD-1 in slightly different orientations. Atezolizumab, avelumab, and durvalumab bind

to PD-L1 in different orientations and interfere with the formation of PD-L1 and CD-80:B7.1 complexes, without inhibiting the PD-L2/PD-1 pathway. The FDA has approved several PD-1 and PD-L1 inhibitors to treat many tumor types and several more trials of ICI therapy are underway. Further details about current FDA-approved immune checkpoint inhibitors and their indications can be found in chapter 1.

Clinical and Radiologic Patterns of Pneumonitis

In the following section, we discuss presentations of pneumonitis after immune checkpoint inhibitor (ICI) therapy. Pneumonitis is a rare irAE after ICI therapy that presents as an interstitial lung disease [38]. Pneumonitis after ICI therapy presents in four patterns: organizing pneumonia (OP), nonspecific interstitial pneumonia (NSIP), hypersensitivity pneumonitis (HP), and diffuse alveolar damage (DAD).

For the purposes of this chapter, we will combine NSIP and HP into one category, due to similarities in presentation and in therapeutic approaches. Table 13.1 summarizes the clinical, radiological, and pathological features associated with each pattern of pneumonitis, and Fig. 13.1 shows characteristic images from chest computed tomography (CT) scans. A more complete discussion of the clinical features and pathophysiology of various ILDs is available elsewhere [39, 40].

OP OP is a common manifestation of pneumonitis after ICI therapies [41]. OP primarily affects distal bronchioles, respiratory bronchioles, alveolar ducts, and alveolar walls [42]. Symptoms of OP may include low-grade fever, malaise, and cough, and the onset of symptoms in idiopathic cases is often subacute [43–46]. Respiratory infections are often associated with the development of OP, though the mechanism remains unclear [47]. Thoracic CT imaging of patients with OP primarily appears as ground-glass or consolidative opacities which are more predominant in the lung periphery in subpleural regions [48]. The reverse halo sign, which is characterized by ground-glass opacities surrounded by denser consolidative opacities, can be seen in OP but is not pathognomonic [49]. The extent of radiological involvement can vary substantially from case to case. The histology of OP is characterized by excessive proliferation of plugs of granulation tissue (Fig. 13.2) in distal airspaces with infiltration by lymphocytes and plasma cells [48]. These plugs consist of loose collage, fibroblasts, and myofibroblasts. Bronchoalveolar lavage (BAL) is often performed in OP to rule out infection, though a BAL inflammatory signature is not sufficient to diagnose OP [48]. The treatment of OP depends upon the severity of the disease. We recommend use of the Common Terminology Criteria for Adverse Events (CTCAE, Table 13.2) to grade the severity of pneumonitis [50]. Mild cases (Grade 1) of OP may resolve spontaneously, but close monitoring for early signs of pulmonary impairment is imperative [51]. Patients with pneumonitis of grade 2 or higher should be treated with corticosteroid therapy. Corticosteroids are highly efficacious in OP, and treatment doses typically start at 0.5–1 mg/kg/day of prednisone or equivalent for 3–6 months. Interruptions in corticosteroid treatment may result in relapse of OP [52].

Noncorticosteroid therapies, such as cyclosporine, rituximab, and macrolides, have been associated with anecdotal success in small case series of steroid-refractory patients but are not typically used [53–56]. Current guidelines recommend immunosuppressive agents, such as infliximab, cyclophosphamide, mycophenolate mofetil, and intravenous immunoglobulin, for treatment of pneumonitis that does not improve with corticosteroid therapy, but these recommendations are also based on case reports or small case series [115, 132–134]. Infliximab has been reported to be effective in severe pneumonitis, but this requires validation in a prospective study [41]. Tocilizumab, an interleukin-6 receptor antagonist, may be a viable option for treatment of steroid-refractory pneumonitis. For example, in a single center study, of the 87 patients who were treated with nivolumab, 34 were given tocilizumab for high-grade immune-related adverse events that were refractory to corticoste-

Table 13.1 Clinical, radiological, and histopathological features of common patterns of pneumonitis

Type	Clinical features	Radiological features	Histopathological features	Treatment
Organizing pneumonia (OP)	Nonproductive cough, dyspnea, weight loss, usually for less than 2 months	Patchy areas of consolidation or ground-glass opacities which are often seen in the periphery. Multiple alveolar opacities, solitary opacities, or infiltrative opacities can be seen	Proliferation of granulation tissues in the distal bronchus and alveoli along with mild to moderate infiltration of plasma cells and lymphocytes	Mild OP with no pulmonary function Impairment – resolution can occur spontaneously, but requires close monitoring of respiratory symptoms, imaging, and/or pulmonary function. Progressive and/or persistent symptoms with evidence of pulmonary function Impairment – corticosteroid therapy with doses usually starting at 0.5–1 mg/kg/day of prednisone or equivalent for 3–6 months
Nonspecific interstitial pneumonia (NSIP)	Nonproductive cough, dyspnea, which develops over weeks to months. Bibasilar crackles are also heard in majority of patients	Reticular markings, traction bronchiectasis, and ground-glass opacities are seen mostly in lower zones	Fibrosis with diffuse inflammatory cell infiltration and uniform and diffuse thickening of alveolar walls, but without loss of alveolar structural integrity	Patients with minimal symptoms and no change in pulmonary function-observation Moderate symptoms or impairment in pulmonary function test- corticosteroid therapy (0.5–1 mg/kg/day of prednisone or equivalent) for 8–12 weeks Steroid-refractory disease – Therapy with intravenous corticosteroids and/or cytotoxic therapies
Diffuse alveolar damage (DAD)	Rapid onset of progressive dyspnea and cough over days to weeks	Widespread airspace opacities may be more prominent in the dependent areas of the lung	Alveolar thickening with hyaline membrane deposition and infiltration with inflammatory cells	Supportive therapies for patients with respiratory failure and intravenous high-dose corticosteroids

roid therapy. Of those, 27 patients (around 80%) showed clinical improvement and the median time to discharge was 4 days [128]. Anakinra is an interleukin-1 receptor antagonist used for the treatment of inflammatory disorders, such as rheumatoid arthritis. Anakinra inhibits interleukin-1 signaling by competitively binding to IL-1R and blocking both IL-1α and IL-1β activity. Using anakinra to block the interleukin-1 pathway may be another viable option for treatment of steroid-refractory pneumonitis. Further randomized clinical trials exploring these immunosuppressive therapies are needed. In general, at least temporary cessation of ICI therapy is recommended to allow for resolution of pneumonitis.

NSIP NSIP is a rare ILD that is often associated with autoimmune diseases or human immunodeficiency virus infection, and along with OP,

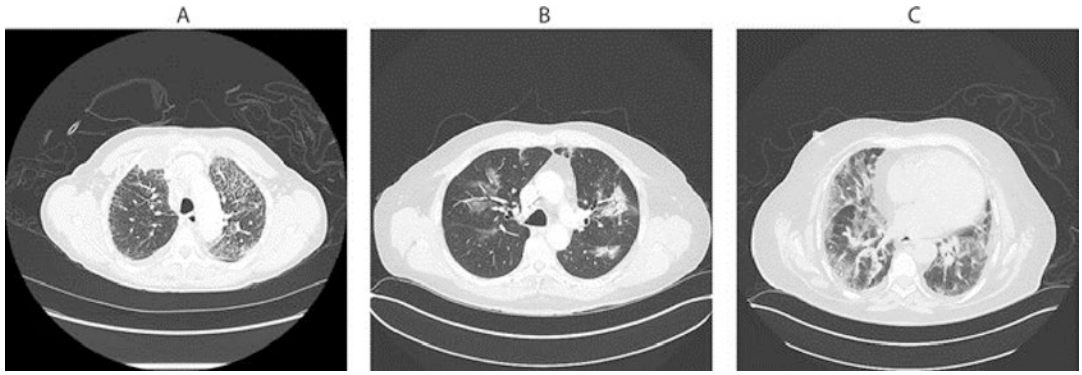

Fig. 13.1 Representative images of (**a**) nonspecific interstitial pneumonitis, (**b**) organizing pneumonia, and (**c**) diffuse alveolar damage in patients receiving precision oncology therapies

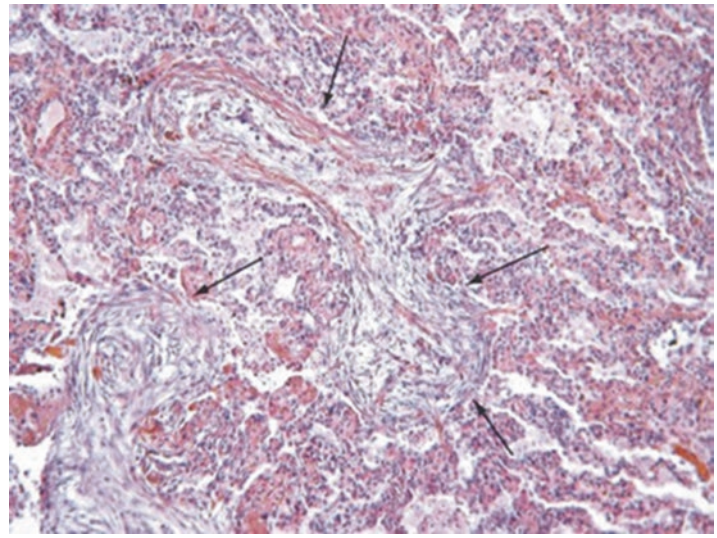

Fig. 13.2 Buds of granulation tissue (*arrows*) in the lumen of alveoli. (Reproduced with permission from *Clinical Respiratory Medicine*, Cottin V. and Cordier J., 2012, Elsevier Publishing)

is a common manifestation of pneumonitis after ICI therapy [57]. NSIP typically presents with nonspecific symptoms of cough and dyspnea, though the duration of symptoms may vary from case to case. Thoracic CT imaging of NSIP typically reveals ground-glass opacities, reticular infiltrates, and traction bronchiectasis [58–60]. Subpleural sparing of lung infiltrates may help distinguish NSIP from idiopathic pulmonary fibrosis [61]. The HP variant of ICI-related pneumonitis may be characterized by air trapping on expiratory chest CT imaging [62]. However, unlike HP, which occurs in the general population, there is no clear link to pulmonary exposures, such as aerosolized molds [63] or toxic chemicals [64]. Histologically, NSIP is characterized by dense fibrosis with diffuse inflammatory cell infiltration and uniform and diffuse thickening of alveolar walls, but unlike idiopathic pulmonary fibrosis, there is no loss of alveolar integrity [65]. Fibroblastic foci may be present, but are less common in cases of NSIP [66]. The HP variant of pneumonitis may be characterized by poorly formed noncaseating granulomas [62]. In general, patients who develop NSIP after ICI therapy require corticosteroid therapy (0.5–1 mg/kg/day of prednisone or equivalent) for 8–12 weeks. Steroid-refractory disease is more commonly seen in NSIP than in OP and may require further therapy with intravenous corticosteroids and/or cytotoxic therapies [51]. For ICI-related NSIP, interruption of ICI therapy is generally recommended [67].

Table 13.2 Grading of pneumonitis as outlined by the Common Terminology Criteria for Adverse Events v5.0

Grade	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Symptoms	Asymptomatic	Symptomatic, limiting instrumental activities of daily living	Severe symptoms, limiting self-care activities of daily living	Life-threatening respiratory compromise	Death
Intervention required	Clinical or diagnostic observations only; intervention not indicated	Medical intervention indicated	Medical intervention and oxygen are indicated	Urgent medical intervention is indicated (e.g., tracheostomy or intubation)	

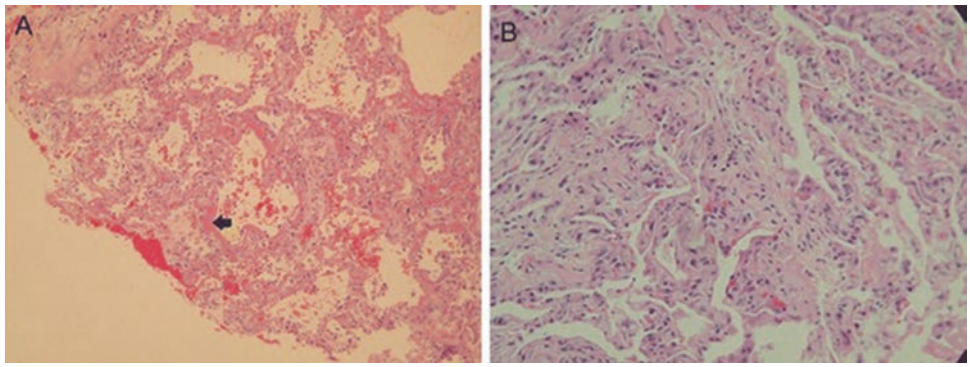

Fig. 13.3 Pathological findings of diffuse alveolar damage. (**a**) Diffuse alveolar damage in the acute phase. The interstitium is edematous. Hyaline membrane (arrow) is seen lining the alveolar ducts (hematoxylin and eosin stain, ×100). (**b**) Diffuse alveolar damage in the organizing phase. The interstitium is thickened with organizing connective tissue. Prominent type 2 pneumocyte hyperplasia is seen (hematoxylin and eosin stain, ×200) [71]

DAD DAD is a severe form of pneumonitis caused by widespread alveolar injury that results in severe capillary leak and noncardiogenic pulmonary edema [67, 68]. Clinically, the presentation is similar to the acute respiratory distress syndrome (ARDS), characterized by tachypnea, severe hypoxemia, and widespread alveolar infiltrates. Typically, this occurs more rapidly than OP or NSIP, with the onset of symptoms rapidly progressing in days. The presence of DAD in the histological examination may not always correlate with ARDS. For example, only one-half of patients that had DAD were clinically diagnosed with ARDS in several open lung biopsies or autopsy studies [71, 122, 124–126]. Though histology is difficult to obtain due to the severity of illness, the histopathologic appearance of diffuse alveolar damage (DAD) is characterized by the formation of thickened alveolar membranes, hyaline membrane deposition, and infiltration with inflammatory cells (Fig. 13.3) [69, 70]. The acute phase of DAD is characterized by inflammation and edema of alveolar structures, while the organizing phase is characterized by the deposition of collagen by fibroblasts [71]. Thoracic CT images of DAD show widespread airspace opacities, which may be more prominent in the dependent areas of the lung [72–74]. Other diseases may mimic drug-induced DAD and should be ruled out. Pulmonary infections and eosinophilic pneumonias may be ruled out by analysis of BAL fluid, while congestive heart failure should be ruled out with a thorough clinical examination, echocardiography, and potentially right heart catheterization. Supportive therapies, including noninvasive or invasive mechanical ventilation, are often necessary to treat respiratory failure associated with DAD. Early initiation of high-

dose systemic corticosteroids is generally recommended, although data supporting this practice is very limited. Mortality rates remain high despite aggressive therapy [75].

Clinical Approach to the Evaluation of ICI-Related Pneumonitis

Because symptoms of pneumonitis may be subtle and masked by other comorbid symptoms associated with the underlying cancers (e.g., large lung cancers or widespread pulmonary metastases), we advise clinicians that evaluate and treat patients who are on ICI therapies have a low threshold for initiating a thorough evaluation for pneumonitis. Symptoms such as dyspnea, cough, fever, and chest pain should raise the suspicion for pneumonitis [76, 77]. We recommend thoracic imaging and pulmonary function testing. Chest radiography is not sufficiently sensitive to detect subtle findings of pneumonitis; therefore, symptomatic patients should be referred for thoracic CT imaging [78]. Radiation doses associated with thoracic CT are low with modern scanners, making serial thoracic imaging a safe and effective method to evaluate progression or resolution of pneumonitis [79]. Pulmonary function testing should be performed at the time of evaluation, as early impairment in pulmonary function may herald the onset of pneumonitis [80]. Furthermore, in patients with confirmed pneumonitis, pulmonary function should be monitored serially to evaluate for progression or resolution of pneumonitis. Early consultation with pulmonary experts is recommended, and bronchoscopy with BAL should be performed early in the course of the evaluation of patients who are suspected of having ICI-related pneumonitis in order to rule out alternative diagnoses, such as infectious pneumonia. Surgical biopsies of the involved lung parenchyma should be considered in select patients to evaluate the histopathological features of pneumonitis. Transbronchial biopsies are generally not recommended due to poor sensitivity for the detection of ILD [81].

Incidence and Clinical Characteristics of Pneumonitis After ICI Therapy

The incidence of pneumonitis varies with the specific agent. For example, in clinical trials, pneumonitis rates have been reported in about 1% of patients treated with ipilimumab, while the incidence with PD-1 and PD-L1 inhibitor monotherapy has been reported in about 3–5%. The incidence of pneumonitis with combination therapy with PD-1 or PD-L1 inhibitors and CTLA-4 inhibitors is reported to be as high as 10% [82–86]. In general, the median onset of pneumonitis is about 3 months [41, 87–89]. Pneumonitis after ICI therapy generally presents as OP or NSIP, but may rarely present as DAD and can have a fulminant course. In this section, we discuss incidence rates and specific forms of pneumonitis that occur with each FDA-approved ICI therapy.

CTLA-4 Inhibitors

Ipilimumab is the only CTLA-4 inhibitor approved by the FDA at the time of this writing. The incidence of pneumonitis with ipilimumab is low, with pneumonitis of any grade occurring in 1.3% of treated patients, and high-grade (grades 3 or 4) pneumonitis occurring in 0.3% of treated patients [90]. The median time from treatment initiation to the onset of pneumonitis has been reported to be around 2.3 months, and the most common pattern of pneumonitis is OP [91]. While some irAEs are more common with CTLA-4 inhibitors than PD-1 or PD-L1 inhibitors [92, 93], pneumonitis is less common, though the mechanism for this difference is unclear [94]. Pneumonitis occurs at about one-third the rate in patients treated with ipilimumab for melanoma treatment as compared to those being treated for renal cell cancer or non-small cell lung cancer [94]. One possibility for this may be the presence of lung disease from cigarette smoking, as has been described in other ILDs [95].

PD-1 and PD-L1 Inhibitors

In this section, we will discuss the PD-1 inhibitors: nivolumab and pembrolizumab, and the PD-L1 inhibitors: atezolizumab, avelumab, and durvalumab. Pneumonitis after PD-1 inhibition occurs as much three times more frequently as compared to conventional chemotherapy regimens across several types of cancers [96].

Recent studies show the incidence for all-grade pneumonitis for PD-1 inhibitors in clinical trials is around 3%, with most studies reporting incidence rates of 3–5% [82, 84, 96]. The incidence of high-grade (grade 3 or higher by CTCAE criteria) pneumonitis for PD-1 inhibitors in clinical trials is around 1–1.5% [82, 84, 96]. However, the pneumonitis rate seems to vary between different tumor types. For example, the rate of any-grade pneumonitis and high-grade pneumonitis in renal cell cancer (any: 4.4%, high: 1.7%) and non-small cell lung cancer (any: 4.3%, high: 2.0%) are higher than in studies of melanoma (any: 1.4%, high: 0.9%) [96].

Similar to ipilimumab, the incidence of pneumonitis after PD-1 inhibition seems to be higher in smoking-related cancers. In a case-control study of patients who developed pneumonitis after PD-1 inhibitor therapy, smoking status was not associated with the risk of pneumonitis, but a history of COPD or lung radiotherapy was predictive of pneumonitis [97]. However, there does not appear to be any difference in the incidence of pneumonitis by PD-1 inhibitor dosage, suggesting that irAEs are not directly tied to these therapies in a dose-dependent fashion [96]. This is consistent with our observation that pneumonitis after PD-1/PD-L1 axis inhibition appears to be an idiosyncratic phenomenon.

Rates of pneumonitis may be higher when considering patients being treated outside the controlled context of clinical trials. In a single center study of 204 patients that included both clinical-trial-enrolled and non-clinical-trial-enrolled patients with NSCLC, the incidence of any-grade pneumonitis was 19% and high-grade pneumonitis was 11% [130]. The median time of progression to pneumonitis was 6.3 months after starting immunotherapy. Furthermore, data from the same group showed that the development of pneumonitis is associated with impaired survival in NSCLC patients [129]. In this cohort, patients with adenocarcinoma who developed pneumonitis had higher mortality than those with nonadenocarcinoma histology (squamous or other). Similarly, a retrospective study from a large, comprehensive cancer center reported that patients who presented to the emergency department for PD-1/PD-L1 pneumonitis were associated with poor overall survival compared to patients who developed other irAEs, such as colitis [123].

Concurrent treatment with ICI and conventional therapies may also result in higher rates of pneumonitis. In a phase III randomized trial exploring durvalumab after concurrent chemoradiotherapy in stage III non-small cell lung cancer (NSCLC), the pneumonitis rate, which included pneumonitis from an irAE or secondary to radiation pneumonitis or as a consequence of combination of both, was reported as 34%, compared to 25 % in placebo arm. Pneumonitis was the most frequent adverse event leading to the discontinuation of the trial regimen (4.8% of patients in the durvalumab group and in 2.6% of those in the placebo group) [121].

Recent studies suggest that pneumonitis after PD-L1 inhibitor therapy may occur less frequently than after PD-1 inhibitor therapy. For example, in a pooled analysis of data from phase 1 and phase II trials, the overall incidence of any-grade pneumonitis for avelumab in patients with advanced solid tumors was around 1.2% [131]. Similarly, Pillai et al. and Khunger et al. both reported that the incidence of any-grade pneumonitis was higher in NSCLC patients treated with PD-1 inhibitors as compared to PD-L1 inhibitors (PD-1 vs PD-L1: around 4% vs around 2%) [83, 127]. There are several caveats that could cause these results to be prone to bias. Both randomized and single-arm, open-label trials with varying doses of PD-1/PD-L1 inhibitors were included. Additionally, patients included in these trials were not always similar. For example, some trials enrolled treatment-naïve patients, while the majority of the trials enrolled previously treated patients, which could influence the tolerability of

the treatment. In addition, there is limited data from randomized, controlled trials that directly compare the toxicities of PD-1 and PD-L1 inhibitors. Further studies are needed to better understand the incidence of pneumonitis, particularly as these therapies are approved for new cancers.

Combination Therapy with PD-1/PD-L1 Inhibitors and CTLA-4 Inhibitors

By inhibiting both the CTLA-4 and PD-1 pathways, it is possible to achieve greater immune activation, which may increase antitumor responses in certain cancers [98]. However, this also increases the risk for irAEs, including pneumonitis. Compared to monotherapy, the incidence of pneumonitis with combination therapy may be as high as 10% and the time to onset is usually sooner [84]. Naidoo et al. found that the median time to pneumonitis onset was 2.7 months in patients receiving combination ICI therapy as opposed to 4.6 months in those receiving ICI monotherapy [84]. Wu et al. found a similarly higher incidence of pneumonitis with combination ICI therapy as compared to ICI monotherapy. In combination ICI therapy, the incidence of pneumonitis was almost 7% and the incidence of high-grade pneumonitis was almost 2% [96]. This suggests that when compared to ICI monotherapy, combination ICI therapy results in a higher risk for any-grade and high-grade pneumonitis and a faster onset to pneumonitis in patients in whom this develops. ICI therapies often have durable effects due the induction of immunologic memory [99]. As a result, sequential treatment with PD-1/PD-L1 inhibitors and CTLA-4 inhibitors may have a similar increase in the risk of pneumonitis as with combination ICI therapy, where both PD-1/PD-L1 inhibitors are given at the same time. In a small study of 40 patients who received nivolumab or pembrolizumab followed by ipilimumab, Bowyer et al. found that 8% of patients experienced high-grade pneumonitis [100]. This finding needs to be confirmed in a larger study cohort, but suggests that when ICI therapies are given sequentially, the risk of pneumonitis is similar to combination therapy.

Radiologic Patterns of Pneumonitis After ICI Therapy

Pneumonitis after ICI therapy typically presents as NSIP or OP. In clinical practice, in a cohort of 915 patients who received ICI monotherapies or combination therapies, the most common pattern of pneumonitis was NSIP (18/27), followed by OP (5/27). Others have shown that OP is more common after PD-1 [41] or CTLA-4 inhibitor therapy [91]. DAD reactions are rarer and typically have a more severe clinical course, but may still be managed with prompt initiation of immunosuppression.

Other manifestations of pulmonary irAEs have been described in the literature. Airway inflammation with bronchiolitis has been described in a patient who was receiving nivolumab for non-small cell lung cancer [101]. Rapidly recurrent pleural and pericardial effusions were reported in two patients within 8 weeks of initiating nivolumab therapy [102]. An increased incidence of pleural effusions was also noted in the early clinical trials of nivolumab therapy in patients with non-small cell lung cancer, although these effusions could not be definitely attributed to nivolumab, as opposed to progression of disease [103]. ICI-related pleural and pericardial fluid accumulation may be a form of irAE or a form of pseudoprogression. Drug interruption and management of pleural/pericardial drainage procedures are the primary focus of treatment. Initiation of immunosuppressive therapy for recalcitrant effusions is reasonable, although the role of steroids in this setting has not been established.

Sarcoid-like reactions have been observed with ipilimumab [91, 104, 105] and with PD-1 inhibition [106, 107]. Sarcoid-like reactions are rare irAEs, and the manifestations vary from case to case. Presentations may include mediastinal lymphadenopathy, pulmonary infiltrates, skin rashes, and renal disease. While these reactions may resemble sarcoidosis clinically, the

immunology is not necessarily identical to sarcoidosis, which occurs in the general population [104, 108]. However, inhibition of immune checkpoint pathways may increase the population of Th17 cells, which are thought to be involved in non-ICI-related sarcoidosis [109, 110]. Therefore, there is a plausible biological basis for the incidence of sarcoid-like reactions in patients treated with ICI inhibitors. Treatment includes interruption of ICI treatment and systemic steroids. Further work is necessary to understand the incidence of sarcoid-like reactions after ICI therapies.

Areas of Uncertainty

Rechallenge with ICI Therapies After the Occurrence of Pneumonitis

A key question in patients receiving ICI therapy is whether the onset of irAEs, such as pneumonitis, may indicate a more favorable response to treatment. Some groups have found that patients who experience irAEs have a better treatment response [89, 111], while others have not [112]. Therefore, rechallenge with ICI therapies after the occurrence of ICI-related pneumonitis may be desirable. Several groups have reported the safety of resuming ICI therapy after irAEs [113, 114]. Additionally, the overall incidence of irAEs is higher upon drug rechallenge, with about half of patients experiencing any-grade irAEs. Furthermore, about 20% of patients experience irAEs which are different from the initial irAE [114]. In other words, patients who develop pneumonitis after ICI therapies may experience a nonpneumonitis irAE upon drug rechallenge. Generally, these events are treatable with corticosteroids and are not fatal [89], though rare fatalities have been reported [114]. However, it is not clear whether ICI rechallenge is of sufficient clinical benefit to warrant the risk of recurrent irAEs [35]. The Society for Immunotherapy of Cancer recommends that drug rechallenge can remain an option in patients with grade 2 pneumonitis, which has resolved completely, as well as in select patients with grade 3 pneumonitis, which has resolved completely and in whom the benefits of ICI therapies outweigh the risks of recurrent irAEs [115]. Patients with grade 4 pneumonitis should not undergo rechallenge with ICI therapies. Further work in this area is necessary to guide practice algorithms.

Biomarkers to Identify Patients at Risk for Pneumonitis

As noted earlier in this chapter, certain patients may be at higher risk for the initiation of pneumonitis. In particular, patients with preexisting lung injury from smoking or from radiation may bear a higher risk for ICI-related pneumonitis. Recent advances in imaging techniques have allowed thoracic CT images to be analyzed at the voxel level to detect textural features which are associated with disease or health [116]. A similar approach led to the development of a radiomic-based algorithm, which predicted the onset of pneumonitis from pretreatment thoracic CT scans of patients who underwent ICI therapies [117]. These findings need to be externally validated but highlight the power of imaging as a biomarker of disease risk.

Interleukin-17 is an inflammatory cytokine that is upregulated in many autoimmune diseases, including inflammatory bowel disease [118]. Elevated serum IL-17 levels were predictive of colitis in patients with melanoma treated with ipilimumab [119]. Similarly, in patients with leukemia, Th1/Th17 cells are expanded in bronchoalveolar lavage fluid from patients with leukemia who developed pneumonitis after ICI therapy as compared to control patients with leukemia who had not received ICI therapy [120]. Further work is necessary to identify inflammatory biomarkers in the blood or in the bronchoalveolar lavage fluid that can help predict the onset of pneumonitis after ICI therapy.

Conclusions

Pneumonitis is a rare but serious irAE that occurs after therapy with PD-1, PD-L1, and CTLA-4 inhibitors. Pneumonitis should be recognized promptly if patients have new pulmonary symptoms, such as cough or shortness of breath. The workup in patients with suspected pneumonitis should include pulmonary function testing, thoracic CT imaging, and bronchoscopy with bronchoalveolar lavage to rule out infection. Treatment with corticosteroids is generally effective and results in prompt resolution of symptoms. However, untreated pneumonitis can be fatal. Further work is needed to identify which patients are at the highest risk for the development of pneumonitis after ICI therapies.

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Hepatobiliary Adverse Events

14

Hamzah Abu-Sbeih and Yinghong Wang

Abstract

Immune checkpoint inhibitors (ICIs) are increasingly used for multiple cancer types. Hepatotoxicity is a reported adverse event of ICI treatment. It can present as asymptomatic elevation of aspartate transaminase and alanine transaminase or symptomatic hepatitis with fever, malaise, and even death in rare cases. The diagnosis of ICI-induced hepatitis is made after exclusion of other etiologies based on medical history, laboratory evaluation, and imaging and histological findings. Treatment of ICI-induced hepatitis consists of ICI discontinuation and immunosuppression in severe cases. Pancreatic injury as asymptomatic lipase elevation or acute pancreatitis-like disease with abdominal pain and evidence on imaging has been documented as a toxicity of ICI therapy. Appropriate treatment of pancreatitis still needs further investigation. Few cases, reports, and series documented cholecystitis and cholangitis as possible adverse events related to ICI therapy as well.

Keywords

Immune checkpoint inhibitors · Immunotherapy · Hepatitis · Transaminitis · Pancreatitis · Corticosteroids · Cholecystitis · Liver injury

Liver Toxicity

Incidence

Immune checkpoint inhibitor (ICI)-induced hepatotoxicity occurs in 5–30% of patients [1, 2]. The incidence of hepatotoxicity in patients treated with anti-PD-(L)1 agents is 5–10%. On the other hand, in patients treated with CTLA-4 blocking antibodies, the risk of hepatotoxicity can be up to 15% [3, 4]. This risk increases up to 30% in patients receiving combination therapy of both [3–5]. The most common pattern of ICI-induced hepatic injury is hepatocellular and panlobular [5–12]. Grade 3–4 hepatitis has been reported in 1–3% of patients receiving ICI monotherapy and in 8–14% of patients treated with a combination anti-PD-1 and anti-CTLA-4 therapy [5, 7–10, 13–16].

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Clinical Presentation

ICI-induced hepatotoxicity develops through an immune-mediated mechanism which manifests as either hepatocellular or cholestatic injury [14, 17–19]. The presentation of ICI-induced hepatotoxicity remains highly heterogeneous, ranging from complete asymptomatic with mild rise in aminotransferases to death as a consequence of liver failure [6, 20, 21]. Although hepatotoxicity is commonly an incidental finding on routine laboratory screening, clinical signs and symptoms of ICI-induced hepatotoxicity can occur rarely, which include fever, malaise, abdominal pain, jaundice, and changes of stool color [17, 22]. Increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin are the commonly recorded biomarkers of ICI-induced hepatotoxicity, without major differences according to ICI classes [13, 17, 20, 23]. ICI-induced hepatitis can occur at any time, but often becomes clinically evident 8–12 weeks after initiation of ICI therapy [16, 20, 24]. The onset of hepatotoxicity is usually earlier with anti-CTLA-4 therapy as compared with anti-PD-(L)1 therapy. Patients with delayed onset of hepatitis tend to have milder disease [14, 25]. It should be noted that the sudden onset of fulminant hepatitis can occur despite the patient having tolerated long-term ICI treatment [26].

Diagnosis

The exclusion of other causes of liver injury, such as concomitant medications, autoimmunity hepatitis, viral infection, and alcohol, is the initial approach for the diagnosis of ICI-induced hepatitis [13, 27]. In addition to hepatic function test, the evaluation for other etiologies by diagnostic laboratory and imaging studies is a critical step to guide the appropriate treatment. In rare instances, liver biopsy might be needed to definitely exclude other etiologies [28].

Diagnostic laboratory biochemistry should include complete liver function test, including ALT, AST, total bilirubin, and alkaline phosphatase. Abdominal imaging might be considered, such as computerized tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US), although findings of ICI-induced hepatitis usually are nonspecific [29]. Nonetheless, imaging modalities can be of value to detect other etiologies that lead to abnormal liver enzymes, for example, liver metastasis and thromboembolic event [17, 30]. Reported radiological features of ICI-induced hepatitis include periportal edema, hepatomegaly, periportal MRI T2-hyperintensity, attenuated liver parenchyma, and enlarged periportal lymph nodes on CT and MRI in severe hepatitis [17, 25, 31]. ICI-induced hepatitis usually has normal appearance of the liver on imaging [17, 32].

Liver biopsy should be reserved for severe persistent cases of ICI-induced hepatitis, or when the diagnosis is uncertain. Histological examination of ICI-induced hepatitis demonstrated nonspecific features of panlobular hepatitis and bile duct injury, including fibrin ring granulomas, central vein endothelitis, prominent sinusoidal lymphohistiocytic infiltrates, and endothelialitis involving central veins (Fig. 14.1) [20, 22, 33, 34]. The histology of anti-PD-(L)1-induced hepatitis is different from that of anti-CTLA4 [35]. PD-(L)1 antibody-induced hepatitis causes lobular nongranulomatous hepatitis [16], whereas CTLA-4 antibody-induced hepatitis causes granulomatous hepatitis with fibrin deposits [16]. In addition, ICI-induced hepatitis has increased numbers of $CD3^+$ and $CD8^+$ lymphocytes and decreased $CD20^+$ B cells and $CD4^+$ T cells compared with autoimmune hepatitis and drug-induced liver injury [34].

Treatment

The severity of hepatotoxicity is graded according to the Common Terminology Criteria for Adverse Events version 5.0 [36]. These grades are used to determine the appropriate treatment of hepatotoxicity [37, 38]. After the detection of hepatotoxicity, liver function tests should be monitored weekly for grade 1 and 2 hepatotoxicity and every other day or daily for grade 3–4 toxicity. For grade 1 hepatitis,

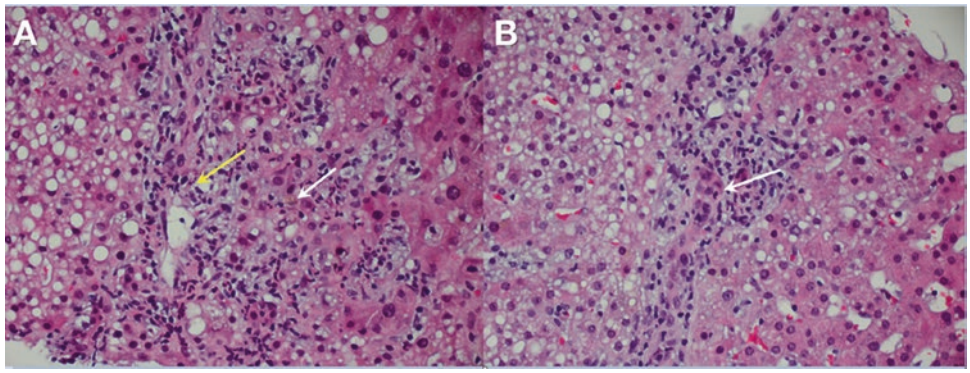

Fig. 14.1 Liver biopsy histology. (Panel **a**) (Hematoxylin and eosin stain, 20×) Portal mixed inflammation (yellow arrow) and periportal cholestasis (white arrow). (Panel **b**) (Hematoxylin and eosin stain, 20×) Bile ductular injury or cholangitis. (*Journal for Immunotherapy of Cancer*, 2019 Feb 18;7(1):47)

expectant management with close laboratory monitoring is recommended [39]. ICI can be continued in these cases. For grade 2 and higher, after other apparent causes are excluded, corticosteroid should be initiated, and ICI should be withheld. The dosage of corticosteroids that has been recommended ranges from 0.5 to 2 mg/kg, followed by a 4–6 weeks of taper [11, 13]. ICI can be resumed when corticosteroid has been tapered to 10 mg/day (toxicity grade $\leq$ 1) for grade 2. Permanent discontinuation of ICI and corticosteroids treatment is recommended for grades 3 and 4 hepatitis [39].

Usually, corticosteroids lead to the normalization or improvement of liver enzymes in most patients [20, 26, 34]. Some patients might need multiple cycles of corticosteroid treatment [17]. The median time from corticosteroids initiation to resolution is approximately 8 weeks [40]. In clinical practice, spontaneous improvement of liver biochemistry following ICI cessation without any corticosteroid therapy has been reported [16]. Patients with ICI-induced hepatitis that is refractory to high dose corticosteroids may need a trial of mycophenolate mofetil based on a few case studies [6, 21]. Because of potential hepatotoxic effect, infliximab is not recommended for the treatment of ICI-induced hepatitis [22, 24]. Anti-thymocyte globulin therapy was also reported as an alternative treatment in the event of corticosteroid intolerance [21].

ICI-induced hepatotoxicity treatment has been reported to improve hepatomegaly and periportal lymphadenopathy on imaging [17]. Liver function panel should be monitored as some patients may have rebound elevation of AST and ALT even after completion of corticosteroids therapy and clinical resolution [20].

Conclusion

ICI-induced hepatotoxicity has been increasingly encountered given the wide use of ICIs in the past few years. ICI-induced hepatitis often occurs 5–15 weeks after the initiation of ICI therapy. The presentation of ICI-induced hepatitis is usually asymptomatic elevations of AST, ALT and total bilirubin, but may be accompanied with fever, malaise, and even death in rare cases. The diagnosis of ICI-induced hepatitis is usually made after the exclusion of other etiologies of hepatitis. When the diagnosis of ICI-induced hepatitis is made, ICI treatment should be discontinued, and corticosteroids should be started. Resumption of ICI therapy might be considered in patients with grade 1 or 2 hepatotoxicity. Future studies are still required to further improve the management of ICI-induced hepatitis.

Pancreatic Toxicity

Incidence and Diagnosis

Among different ICI classes, the reported incidence of ICI-induced pancreatic injury is 0.6–4% [41–43]. ICI-induced pancreatic injury is usually an incidental finding of elevated lipase, and rarely it become symptomatic with abdominal pain and vomiting. Of note, abdominal pain and vomiting can be due to toxicities involving other parts of the gastrointestinal tract, which can coexist with pancreatic injury. Amylase can also be elevated; however, amylase elevation is nonspecific. Lipase and amylase elevations are usually recorded after a median of 3 months from ICI therapy initiation [44]. The most important step in the diagnosis of ICI-induced pancreatic injury is the exclusion of other etiologies for elevated lipase, such as alcohol, hypertriglyceridemia, and pancreatic metastasis. Cross-sectional abdominal imaging with CT scan or MRI can help to establish the diagnosis of ICI-induced pancreatitis and to evaluate for short- and long-term adverse events of pancreatitis, for example, diabetes mellitus, pancreatic cyst, features of chronic pancreatitis. Commonly observed features of ICI-induced pancreatitis are segmental hypoenhancement, peripancreatic fat stranding, and pancreatic enlargement with heterogeneous enhancement (Fig. 14.2) [42]. Adverse consequences can occur in up to 10% of patients with ICI-induced pancreatitis [42].

Treatment

Given the similarities between ICI-induced pancreatitis and classic acute pancreatitis, ICI-induced pancreatitis should be managed in a similar fashion to classic acute pancreatitis [45]. This approach consists of intravenous fluid administration and analgesic medications, where intravenous fluids were reported to be protective from adverse pancreatitis consequences. The role of corticosteroids and other immunosuppressive agents in such patients is not well-established [37, 42]. It is not recommended to follow up lipase and amylase values after initiation of pancreatitis therapy. Nonetheless, it is important to monitor pancreatitis adverse consequences, especially with early onset pancreatitis or with smoking or hyperlipidemia history as these were revealed to be associated with increased risk of pancreatic injury [42, 46, 47]. ICI therapy can be resumed after resolution of ICI-induced pancreatic injury to optimize cancer treatment.

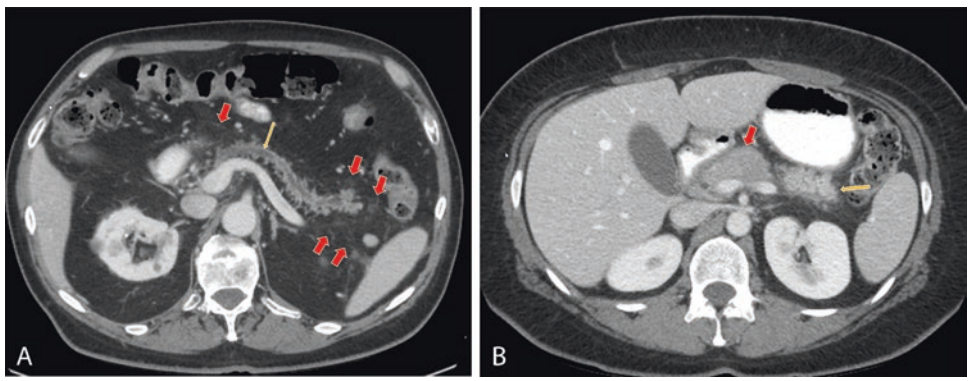

Fig. 14.2 (**a**) The peripancreatic fat stranding (short block arrows) are suggestive of pancreatitis. Pancreatic duct dilatation (long arrows) is due to metastasis in the pancreatic head. (**b**) The segmental hypoenhancement of pancreatic head and proximal pancreatic body (short block arrow) versus normal enhancement of distal pancreatic body and pancreatic tail (long arrow) is suggestive of acute pancreatitis. (*Journal For Immunotherapy of Cancer* 2019;Feb 6, 7(1):31)

Gall Bladder Injury

Recognition and management of rare adverse events of ICI therapy is essential to maintaining effective cancer treatment. Acute cholecystitis with or without cholangitis has been reported in case studies and case series [48–50]. The incidence of acute acalculous cholecystitis is documented to be 0.6%, which is higher than the incidence among cancer patients without ICI exposure, and to be higher in CTLA-4 inhibitors [48]. The median time to cholecystitis is 6 months after ICI therapy. Very limited data are available regarding ICI-related cholecystitis and cholangitis. Their treatment should follow recommendations from classic non-ICI ones. Gall bladder wall perforation and sepsis have been reported with ICI-related cholecystitis [48].

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Immune Checkpoint Inhibitors (ICIs)-Related Cardiotoxicity

15

Abdulrazzak Zarifa, Juan Lopez-Mattei, Nicolas Palaskas, Cezar Iliescu, Jean-Bernard Durand, and Peter Y. Kim

Abstract

The growing success of immune checkpoint inhibitors (ICIs) has led to effectively treating several types of cancers. Even though their use has been associated with the development of cardiac adverse effects, which may decrease the overall survival in cancer patients. These cardiac toxicities are thought to be the result of targeting specific checkpoint proteins on normal myocardial cells leading to over stimulation of the immune system as well as secondary downstream off-target effects on normal tissue.

Although cardiotoxicities related to immunotherapy are reportedly rare, they can be severe and associated with life-threatening conditions such as fulminant myocarditis, hemodynamic instability, and cardiac arrest.

We will review the most commonly reported cardiovascular toxicities associated with ICIs and their management.

Keywords

Immune checkpoint inhibitors · Anti-CTLA4 · Anti-PD1 · Anti-PDL1 · Cardiotoxicity · Myocarditis

Introduction

Immune checkpoint inhibitors (ICIs) have increasingly become a target of interest for pharmacologic blockade with demonstrable antitumor effects across a broad spectrum of tumor types [1, 2]. Potential short- and long-term cardiac toxicities are emerging as use of these agents is increasing which necessitates a holistic analysis of immune-related adverse events (irAEs) such as myocarditis.

Overall cardiac toxicity with this group of drugs has been reportedly low in early trials, although prespecified endpoints for myocarditis were not established. Specifically, ipilimumab had a very low reported risk of cardiac toxicity (<0.1% based on 1498 patients) and pembrolizumab had no reported cardiac toxicity when it was administered at the recommended dosage of 2 mg/kg every 3 weeks [3].

Nonetheless, cardiovascular toxicities are considered to be among the least common

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adverse events experienced, however, these events generally result in severe cardiac toxicities [4–8].

Suggested Mechanism of Cardiotoxicity

Cardiotoxicities induced by immunotherapies are most likely caused by inhibition of the CD-28 family regulatory molecules: cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1), which are important in suppressing T cell response in the heart [9]. Normally, these molecules prevent inflammation in the tissue and protect against cardiac muscle injury associated with the inflammation [10]. Data from animal models suggest that modulation of the PD-1 pathway can lead to immune-mediated cardiovascular toxicity, primarily in the form of autoimmune myocarditis. Knockout of the PD-1 receptor in mice causes severe dilated cardiomyopathy characterized by high levels of immunoglobulin G autoantibodies that react specifically to cardiac troponin [11]. Massive infiltration of CD4(+) and CD8(+) T cells and myeloid cells was found in the hearts of MRL PD-1 deleted mice concomitant with the production of high-titer autoantibodies against cardiac myosin. This is in contrast to CTLA-4 deleted mice in which most of the CD4(+) T cells are nonspecifically activated and invade various organs, suggesting that myocarditis in PD-1 deficiency is mediated by antigen-specific autoimmune response [12].

In CTLA-4 deficient mice, multi-organ lymphoproliferative diseases develop within few weeks of life, including T cell-mediated myocarditis [13]. Induction of tolerance and upregulation of regulatory T cells (Treg) could be a pharmacologic approach to preventing autoimmune myocarditis [14]. Thus, the ICI-induced cardiotoxic effects could be explained by lowering the threshold for activation of T cells specific for self-antigens in the heart [15].

Johnson et al. also described the cases of two metastatic melanoma patients who developed lethal myocarditis while being treated with ipilimumab and nivolumab combination therapy [16]. They performed T cell receptor sequencing on biopsies from the tumor, heart, and skeletal muscles focusing on the highly variable complementarity-determining region 3 (CDR3). There was elevated expression of muscle-specific transcripts in patient tumor specimens and high-frequency T cell receptor sequences, which were shared between the tumor, heart, and skeletal muscles suggesting that these T cells might be responding to a common antigen possibly resulting in the development of autoimmune myocarditis and myositis [16]. In addition, there are some preclinical data to suggest the expression of PD-1 and PD-L1 receptors in cardiac tissue, which could lead to inflammation from ICI therapy [5] (Fig. 15.1).

Clinical Spectrum of Immune-Mediated Cardiotoxicity

Myocarditis/Cardiomyopathy

Myocarditis was rarely observed in early clinical trials; however, given the increasing use of immune checkpoint inhibitors, there has been a growing number of case reports of ICI-induced myocarditis. Only one case of myocarditis was reported in a multicenter phase I trial testing intravenous anti-PD-L1 antibody at escalating doses from 0.3 to 10 mg per Kg of body weight administered to patients with selected advanced cancers [17]. The first published report of PD-1 inhibitor-associated myocarditis was reported by Laubli et al. in 2014 involving a case of acute heart failure in a 73-year-old woman with metastatic melanoma of the uvea due to autoimmune myocarditis after institution of pembrolizumab. The patient developed severe acute heart failure with echocardiographic evidence of severely impaired left ventricular function with dyssynchrony. Viral titers were negative, but myocardial biopsy showed lymphocytic infiltration with a predominance of CD8 positive cells. Management consisted of corticosteroids and guideline-driven heart failure medications. The patient subsequently improved with recovery of left ventricular function [8].

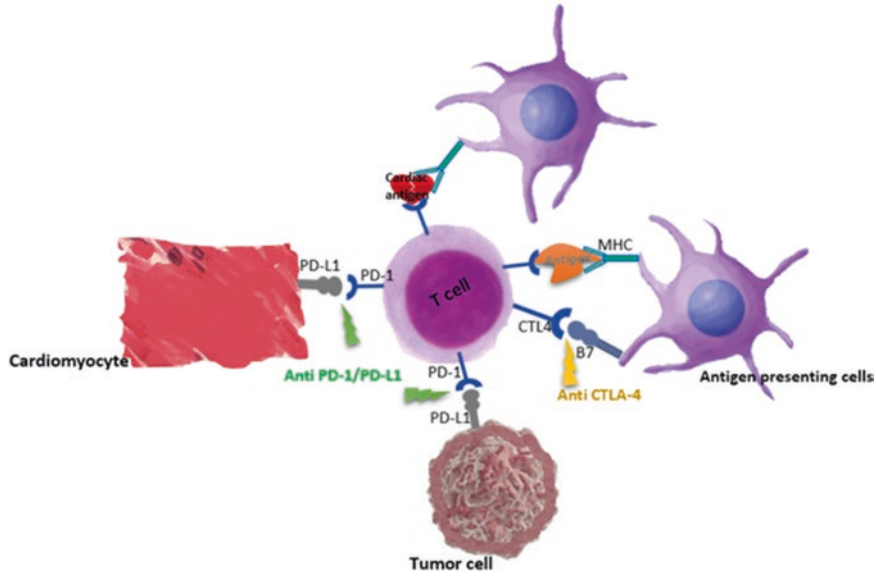

Fig. 15.1 Mechanism of cardiotoxicity of immunotherapy. Immune checkpoint inhibitors mechanism of action MHC: major histocompatibility complex, TCR: T cell receptor, CTLA-4: cytotoxic T-lymphocyte-associated protein 4, PD-1: programmed cell death 1, PD-L1: programmed cell death ligand 1. PD-L1 expression on injured cardiomyocytes likely representing a protective mechanism for cardiac tissue during inflammation

In a multicenter phase II clinical trial, patients with advanced Merkel cell carcinoma, who had received no previous systemic chemotherapy, were given pembrolizumab which resulted in myocarditis in 1 patient after the first dose requiring glucocorticoids as treatment [18].

Geisler et al. reported on the effects of ipilimumab on the cardiac muscles in an 83-year-old woman treated for metastatic melanoma. The patient had suspected acute coronary syndrome without a culprit lesion on cardiac angiography. The echocardiogram revealed apical ballooning, hyperdynamic basal wall motion, and systolic anterior motion of the mitral valve with associated severe left ventricular outflow obstruction. When a positron emission tomography–computed tomography (PET/CT) was repeated as part of restaging, it revealed increased uptake in the cardiac apex. They suggested that autoimmune myocarditis following ipilimumab treatment could cause a takotsubo cardiomyopathy-like syndrome [19].

Further reports emerged thereafter of autoimmune myocarditis as a result of combination ICI therapy. A 68-year-old woman with metastatic melanoma was treated with ipilimumab/nivolumab combination therapy. One day after the second infusion, she developed left arm pain, chest pain, fever, and malaise. A cardiac MRI demonstrated subepicardial and midwall delayed hyperenhancement involving the lateral wall, suggestive of myocarditis. Her immunotherapy was discontinued, and she was treated with steroids [20].

In a more extensive case series among six clinical cancer centers with substantial experience in the administration of immune checkpoint blocking antibodies, eight cases of immune-related cardiotoxicity after ipilimumab and/or nivolumab/pembrolizumab were identified. Among these cases, seven out of eight cases were diagnosed by endomyocardial biopsy/cardiac MRI, while one case only had the presumptive diagnoses of myocarditis based on clinical characteristics but were unable to be confirmed by tissue characterization due to patient decompensation [15]. In this case series, severe myocarditis has been reported to be more frequent during combination treatment as the ejection fraction EF dropped significantly with combination treatment.

Johnson et al. also reported the adverse cardiac events of Bristol-Myers Squibb safety database, in the nivolumab arm, 10 (0.06%) patients reported myocarditis versus eight (0.27%) in the combination arm. Also, fatal events occurred more frequently in the combination arm versus the nivolumab arm, five (0.17%) versus one (<0.01%), respectively [16].

Pericarditis/Pericardial Effusion

Pericardial disease has been reported to be an associated immune-related adverse effect. Nesfeder et al. [21] described a 64-year-old male, with stage IIIB adenocarcinoma of the lung, who was being treated with nivolumab and developed a pericardial effusion with tamponade physiology. The patient was admitted with initial diagnosis of atrial fibrillation, during which a transthoracic echocardiogram showed a small pericardial effusion. After his ninth round of nivolumab during a second hospitalization for pneumonia, there was a progressively enlarging moderate sized pericardial effusion seen on repeat imaging. The management plan at the time was to continue monitoring with serial echocardiograms. One week later, he presented with chest pain and was found to have an enlarging circumferential pericardial effusion with mild collapse of the right and left atria. Cytology of the pericardial fluid failed to reveal a secondary cause of the effusion including malignant cells or infection. However, they concluded that due to the temporal relationship to treatment, the most likely cause of the pericardial effusion was an immune-related side effect of nivolumab. A second case involves a 67-year-old male with metastatic squamous cell carcinoma of the lung, who developed a pericardial effusion after his fifth cycle of nivolumab. He developed rapid respiratory decline requiring mechanical ventilation and was found to have a large pericardial effusion causing tamponade. Sampling of the pericardial fluid showed leukocytes without malignant cells or infectious organisms. Given his rapid response to steroids and onset of symptoms with treatment, this was also thought to be nivolumab induced [22].

There have been multiple case reports, involving the use of ipilumumab (anti-CTLA-4), showing a late-onset pericardial effusion 3–4 months after completing therapy. Dasanu et al. describe a 65-year-old woman, with BRAF positive melanoma, who underwent treatment with standard dose ipilimumab 3 mg/kg IV every 3 weeks for four doses. Of note, during her treatment she developed multiple immune-mediated side effects which all improved after systemic steroid treatment. Four months following treatment, the patient presented to the emergency department with progressive shortness of breath and chest discomfort. A CT scan of the chest showed a large pericardial effusion which required urgent pericardiocentesis. Fluid pathology showed lymphocytic pericarditis and reactive mesothelial cells without evidence of malignancy. Autoimmune and infectious serologies were also negative. She was treated with IV methylprednisolone and had rapid clinical improvement. The authors believed that these late-onset immune-mediated adverse events could be related to a delayed immune cell proliferation that occurred over several months following the initial treatment [23]. Another case of late-onset pericardial disease was reported 12 weeks following treatment with ipilimumab. The patient presented with hypotension, and a metabolic work up was consistent with hypothyroidism and adrenal insufficiency. There was also found to be large pericardial with fibrinous pericarditis and pleural effusions. After initiation of high-dose steroids patient's hypothyroidism, adrenal insufficiency, and pericarditis improved [24]. Another more recent combination phase 1b trial of durvalumab (anti-PDL-1) with tremelimumab (anti-CTLA-4), in patients with non-small cell lung cancer, showed that one of the three treatment-related deaths was secondary to cardiac tamponade [25].

In addition to the case reports of pericardial effusions while on checkpoint inhibitors, a single-center study reported the prevalence of hemodynamically significant pericardial effusions requiring pericardiocentesis while on ICI to be 0.38% (15/3966) [26]. While uncommon, when compared those requiring pericardiocentesis who were not on ICI the relative risk was 3.1 which suggests that the ICI was contributing the devel-

opment to the effusion [26]. Nivolumab had the highest prevalence of 0.61% followed by pembrolizumab (0.19%) and atezolizumab (0.32%) [26].

While ICI-related pericardial effusions are rare, they have the potential for delayed development, can be associated with other immune-mediated side effects, and can pose a life-threatening condition. It is important to be aware of pericardial disease as a potential complication of immune checkpoint inhibitor therapy.

QTc Prolongation/Arrhythmia/Heart Blocks

Immune-mediated effects on the cardiac conduction system have also been reported in case series. Nivolumab has been reported to be associated with advanced heart blocks. In one report, a 63-year-old male with metastatic uveal melanoma developed a troponin I positive and autoantibody positive myocarditis and myositis after a second infusion with nivolumab. A few days later, he was noted on ECG to have a new-onset third-degree atrioventricular block. It was assessed to be most likely because of an autoimmune-induced myocarditis, causing a cardiac conduction defect [27].

QTc prolongation is a common concern with new biologic therapies. The effect of ICIs on the QT interval has been mixed in the literature. Agrawal et al. examined the risk of QTc prolongation in ICIs in a randomized multicenter phase 2 trial of patients receiving nivolumab for advanced clear cell renal cell carcinoma. Electrocardiograms were obtained at baseline, predose, at end of infusion, and 3 hours post infusion during multiple cycles of treatment. They concluded that no patient had QTc changes characterized as borderline or prolonged >480 milliseconds at doses up to 10.0 mg/kg [4]. However, in a small phase 1 trial with a cohort of 12 Japanese patients undergoing treatment with ipilimumab and paclitaxel for non-small cell lung cancer, QTc prolongation was seen in 50% of the patients. The degree of prolongation and timing of the ECGs were not reported [28]. There remains a need for further study of ICIs and their potential risk of QTc prolongation.

Dysrhythmias, such as atrial fibrillation, have also been reported with ICIs. Atrial fibrillation was observed with use of tremelimumab in phase II trials. Tarhini et al. observed that 1 of 37 patients developed atrial fibrillation during combination immunotherapy of Interferon Alfa-2b and tremelimumab for treatment of stage IV melanoma [29]. In another phase two trial using tremelimumab for the treatment of metastatic gastric and esophageal carcinoma, 2 out of 18 patients develop atrial fibrillation. Both patients lacked a clear precipitant for atrial fibrillation and occurred near the end of treatment [30]. It is unclear if the occurrence of atrial fibrillation was secondary to myocarditis or occur through a different mechanism. Cardiac rhythm monitoring should be continued during ICI therapy to identify and treat for potential conduction abnormalities and dysrhythmias.

Hypertension

Elevated blood pressure has been reported with the use of ICIs. A phase II clinical trial examining tremelimumab as a second line treatment in patients with metastatic gastric and esophageal adenocarcinoma, observed three patients with infusion-related hypertension. One patient required antihypertensive medications, and the others resolved spontaneously [30]. Another phase II trial evaluating atezolizumab (PD-L1 Inhibitor) following treatment with platinum-based chemotherapy in metastatic urothelial carcinoma, showed three episodes of grade 3–4 adverse hypertensive events [31]. Given the limited data, it is difficult to ascertain whether these elevated blood pressures were a direct causal relationship to ICI therapy, but warrant continued monitoring and further investigation.

Incidence, Risk Factors, and Timeline of Immune-Mediated Cardiotoxicity

With the rising use of ICIs, there is growing data demonstrating the incidence of cardiac side effects. The Bristol-Myers Squibb corporate safety databases of 20,594 patients who received

nivolumab, ipilimumab, or both. Severe adverse events of myocarditis were reported in 0.09% of these patients. The combination of nivolumab and ipilimumab induced more frequent and severe myocarditis than single-agent nivolumab (0.27% vs. 0.06%; $P < 0.001$; five fatal events versus one event) [16].

In a small case series of 17 patients with ICI-associated myocarditis, no obvious cardiac or cancer specific clinical features were identified to predispose patients to severe myocarditis [16]. However, in a separate case series of eight patients who developed myocarditis after receiving a combination of ipilimumab and nivolumab, five patients had preexisting cardiac disease or peripheral arterial disease but all were symptom-free prior to starting ICI therapy. The latency of developing myocarditis was 17 days after the first treatment (range, 13–64 days) [15]. However, these events can develop within 2–32 weeks after starting treatment [32].

Monitoring and Prevention

Since immune-mediated myocarditis has an early onset after receiving immunotherapy and a fulminant progression, a monitoring strategy is suggested especially when receiving combination therapy [16]. A baseline ECG and weekly testing of troponin levels during weeks 1–3 for patients receiving combination immunotherapy is one suggested approach [16]. Nonetheless, upon the development of symptoms, a more extensive workup is necessary and could be directed by a consultant cardiologist [33]. Initial workup would including ECG, troponin, brain natriuretic peptide (BNP), echocardiogram, and a chest X-ray. Additional testing to be guided by cardiology may include stress test, cardiac catheterization with endomyocardial biopsy, and cardiac MRI [33]. ICI-related myocarditis can present with biventricular dilatation and systolic dysfunction; however, about 50% of cases may have LVEF ≥50% [5]. In cardiac MRI, the presence of late gadolinium enhancement and increased T2 signal intensity are suggestive of underlying myocarditis [34] (Fig. 15.2).

Management

The Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group and the American Society of Clinical Oncology (ASCO) have developed clinical practice guidelines that were published in 2017 and 2018, respectively [33, 35]. Contrary to the general notion with majority of ICI-related AEs where toxicity can be closely monitored if it is grade 1, the recommendation is to hold ICI for grade 1 cardiac AE and permanently discontinue if beyond grade 1. The cornerstone of management recommended is high-dose corticosteroids (1–2 mg/kg of prednisone) initiated rapidly (oral or IV depending on symptoms) in the inpatient

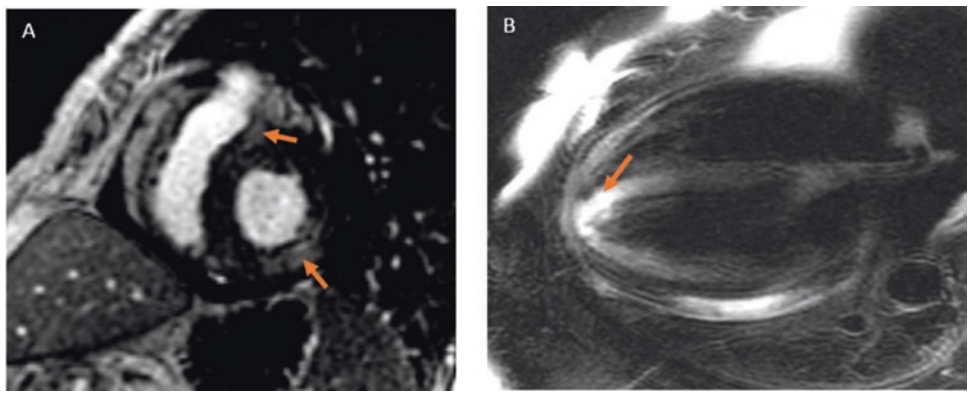

Fig. 15.2 Cardiac MRI findings showing myocarditis. (**a**) Image showing late gadolinium enhancement within the myocardium. (**b**) Increased T2 signal showing edema

setting. This is likely secondary the majority of reported myocarditis cases in the literature receiving corticosteroids [5]. Cardiac symptoms should be managed according to American College of Cardiology (ACC)/AHA guidelines and with individualized guidance from a cardiologist familiar with immune-related cardiac side effects.

Critically ill patients or those with the clinical characteristics for fulminant cardiac decompensation such as those with extremely elevated troponin or significant conduction abnormalities may require immediate transfer to a coronary care unit for further management including ECMO or ventricular support devices, or mechanical support such as intra-aortic balloon pulsation. In patients without an immediate response to high-dose corticosteroids, escalation to cardiac transplant rejection doses of corticosteroids (methylprednisolone 1 g every day) and/or the addition of either biologic agents such as mycophenolate, infliximab, or antithymocyte globulin (ATG) may be considered. Once treated, rechallenging such patients with ICI is not recommended given the high risk for recurrence [33] (Table 15.1).

Prognosis

ICI-related cardiac toxicities are often described to be fulminant and progress to death. Major adverse cardiovascular events including a cardiovascular death, cardiac arrest, cardiogenic shock, and hemodynamically significant complete heart block have been reported in 46% of patients with ICI myocarditis [5]. However, prompt recognition, immunosuppressive therapy, and holding of ICIs can improve cardiac contractility and conduction abnormalities [36]. The overall paucity of reports on these adverse events limit the ability to predict progression or recurrence.

Table 15.1 Common cardiac toxicities of ICIs

Cardiac toxicity	Time to onset	Management
Myocarditis	2–32 weeks	High-dose corticosteroids (1–2 mg/kg of prednisone) initiated rapidly Mycophenolate, infliximab, or antithymocyte globulin (ATG)
Pericarditis	6–15 weeks	
Arrhythmia	2–8 weeks	Standard treatment can be followed per AHA/ACC guidelines
Hypertension	17–22 weeks	

Conclusion

ICIs have shown great promise in prolonging overall survival in various cancers through specific immune mechanisms. Although rare, the cardiac adverse effects of immunotherapy can lead to serious complications and increased mortality.

Myocarditis is the most common and often potentially fatal complication of immunotherapy, which can present clinically with cardiomyopathy and conduction abnormalities. These toxicities may present as early as 2 weeks or as late as 36 weeks after starting treatment. The early identification and treatment of cardiac immune toxicities is critical to limit fulminant complications. Multidisciplinary care involving both oncologists and cardiologists is recommended to provide optimal care of patients affected by immune-related cardiac effects.

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Renal Toxicity

16

Maen Abdelrahim and Ala Abudayyeh

Abstract

With the increasing use of immunotherapy, there has been an associated increased survival in many cancers but has also resulted in unregulated organ-specific toxicities. In this chapter, we discuss the renal toxicities associated with a checkpoint inhibitor (CPI) from the typical acute tubulointersitial nephritis to glomerulonephritis, their proposed mechanisms, and treatments. We also discuss the use of CPI and reactivation of preexisting autoimmune diseases and focus on renal cell cancer in setting of Chronic kidney disease (CKD). Transplant rejection in the setting of CPI use is yet to be further studied, and available data is presented in this chapter.

Keywords

Acute interstitial nephritis · Autoimmune disease induction · Organ transplant rejection · Renal cell cancer · Immune-related adverse events

Introduction

With the advent of the era of immunotherapy, there has been a marked increase in survival in several cancers, such as advanced melanoma, renal cell carcinoma, non-small cell lung cancer (NSCLC), urothelial carcinoma, and head and neck cancers. Harnessing the immune system against tumor by releasing the breaks off the regulators of the immune system, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and the other targets, the programmed cell death protein 1 (PD-1) and its ligand (PD-L1), has also resulted in unregulated organ-specific toxicities. The expansion in the use of checkpoint inhibitors has gained great momentum, being used in solid tumors to hematological malignancies and widely tested in clinical trial. The recognition of increasing adverse events associated with checkpoint inhibitors has created the terminology immune-related adverse events (IRAEs). The adverse events have been associated with poorer survival outcomes [1]. Autoimmune colitis, hepatitis, endocrinopathies, and cutaneous IRAEs were the most frequently reported adverse irAEs, with renal toxicity comprising 3.8%, based on a meta-analysis evaluating case reports [1]. A study by Cortazar et al. looked at the incidence of acute

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kidney injury (AKI) in 3695 patients on clinical trials treated with a checkpoint inhibitor (CPI); the overall incidence of AKI was 2.2%. The incidence of grade III or IV AKI and need for dialysis was 0.6% [2]. AKI occurred more frequently in patients who received combination therapy with ipilimumab and nivolumab (4.9%) than in patients who received mono-therapy with ipilimumab (2.0%), nivolumab (1.9%), or pembrolizumab (1.4%) [2]. When defining AKI based on AKI network criteria in a population of 99 patients, incidence of AKI was reported to be a low as 9.9% and as high as 29% [3]. In this chapter, we address renal toxicity associated with checkpoint inhibitors and its implication on the development of chronic kidney diseases, which can affect the overall survival, especially, in renal cell carcinoma patients.

Renal Toxicity AIN

The most commonly associated renal toxicity with CPI has been acute interstitial nephritis (AIN), with some reports of granulomatous interstitial nephritis [2, 4–6]. AKI has been noted to occur from 1 to 8 months, with a reported median time of 3 months for development since starting treatment [2]. Patients often present pyuria, subnephrotic proteinuria, with rare cases of eosinophilia, rash, or fevers, which are typical of AIN [7]. Since CTLA-4 activity is in the lymphoid organs regulating peripheral tolerance, it has been demonstrated in CTLA-4-deficient mice, a lymphoproliferative disease develops with multiorgan lymphocytic infiltration and tissue destruction [8, 9]. PD-1 regulates tolerance primarily at the level of target organs. In mice models PD-1, PD-L1 are important inhibitory regulators of CD8(+) T cells in tubulo-interstitial inflammation and provide protection from ischemic reperfusion injury [10, 11]. The mechanism associated with CPI and renal injury is yet to be elucidated; however, what has become evident is the delayed response after exposure to CPI, which is not typical of AIN. It has been suggested that due to the disruption of CTLA-4 and PD-1 signaling, there is loss of self-tolerance and leads to migration of autoreactive T cells to the kidney, leading to a significant inflammatory response with a predominance of T cells. There have been further studies indicating PD-L1 acts as a protective molecule against CD8+ CTL activation in renal parenchymal immune [12], which would support a possible mechanism where the activated T cells against possible drugs such as antibiotics and proton pump inhibitors are no longer exhausted when you inhibit PD-1 and therefore mount an immune response [4, 13]. The presence of autoreactive T cells that have escaped the negative selection process in the thymus could also potentially be activated in the presence of CPI and lead to tissue inflammation [14, 15].

Auto-Immune Induction and Preexisting Auto-Immune Disease

Interestingly, IRAE has included induction of autoimmune diseases after use of CPI such as sarcoidosis, lupus, psoriasis, diabetes, and polymyalgia rheumatic/arteritis, among others. Not all patients develop autoimmune diseases but likely the ones with genetic predisposition and nongenetic or environmental factors, such as infections, vitamin D level, smoking, microbiota, and changes in the T-cell receptor repertoire [16, 17]. A possible mechanism is that the treatment with CPIs may result in the unveiling of underlying "silent" autoimmunity, resulting in chronic, persistent inflammatory disease that is treated as a primary autoimmune disease [18]. Rheumatologists have appreciated the autoimmune induction post CPI and have advocated for questionnaires for patients on CPI and autoimmune serology screening [19]. Autoimmune diseases have not escaped the kidney: there have been case reports of lupus nephritis, minimal change disease, and thrombotic microangopathy after CTLA-4 antibodies treatment [20, 21]. Interestingly, there is evidence that PD-1 is involved in autoimmune diseases as demonstrated in PD-1 knock-out mice models who develop lupus and severe arthritis [22]. A recent abstract has reported on membranous

nephropathy, ANCA vasculitis, IgA nephropathy, C3 glomerulopathy, AA type amyloid, and the typical AIN after CPI [23]. One of the cases in the series with AIN had aggressive T cell infiltration, with CD4+ and CD8+ T cell infiltration, and further demonstrated in another case in the literature [15, 23]. The glomerulonephritis (GN) noted in these biopsies presented with either CTLA-4 antibodies or PD-1 inhibitors treatments [23]. Patients with GN after CPI have been treated as de novo GN with some success. Another interesting notion is the higher likelihood of patients with preexisting autoimmune disorders to develop irAE on CPI. There are limited data available about management of these patients. In a recent met-analysis by Abdel-Wahab et al., among 123 patients, 92 (75%) had irAEs, of which 50 patients (41%) had exacerbation of their current autoimmune symptoms, 31 (25%) had new irAEs, and 11 (9%) had both. Interestingly, two cases had preexisting autoimmune nephritis (IgA nephropathy and IgM nephropathy) [24]. In a prospective study of 45 patients with cancer and preexisting autoimmune or inflammatory disease, treatment with anti-PD-1 antibodies demonstrated that patients with preexisting autoimmune disease were more likely to have irAE. Overall survival in the group with autoimmune disease versus the group without was no different [25].

Kidney Transplant and CPI

There is an increased incidence of melanoma of 2.4 times higher in solid organ recipients compared to the general population, with renal or liver transplant recipients having a higher risk [26]. Treatment protocols and management of possible organ rejection is an *unmet* need especially in kidney transplant patients. This has been highlighted in published case reports. Cases by Lipson et al. initially reported successful treatment of melanoma in kidney transplant patients using ipilumumab; however, more recently, cases of acute rejection were published [27, 28]. More cases have displayed the prevalence of increased risk of rejection of organs after CPI treatments. Based on publications, there were six cases of kidney transplant patients who underwent CPI treatment, with four patients developing rejection, leading to the conclusion that the patients treated with PD-1 inhibitors and combination therapy of ipilimumab and PD-inhibitors were more likely to develop rejection [29–31]. PD-1 and PD-L1 interactions might participate in the induction of allograft tolerance. PD-L1 can limit effector T cell function and expansion as well as induce regulatory T cells, allowing for increased graft tolerance. There is also evidence of upregulation of PD-1 on T cells and PD-L1 on hematopoietic and organ transplant cells, which limits allo-specific T cell activation and proliferation against the allograft [32, 33]. Using PD-1 as a target for therapeutic strategy to improve graft survival has been further investigated by enhancing the expression of PD-1 or PD-L1 [34].

A recent comprehensive review work further supports that PD-1 antibodies may be more likely to lead to rejection. In a recent study by Abdel-Wahab et al., 39 patients with allograft transplant were identified from both institutional and literature review of case reports. Fifty-nine percent had prior renal transplantation with a median time to CPI initiation after solid organ transplant (SOT) was 9 years (range 0.92–32 years). Allograft rejection occurred in 41%. There was no difference in rejection rates in anti-CTLA-4 and anti-PD-1. Median time to rejection was 21 days (95% confidence interval (CI):19.3–22.8 days). There were no associations between frequency, timing, or type of rejection and time interval since SOT. Graft loss occurred in 81%. Death was reported in 46% [35, 36].

Renal Toxicity in RCC

Chronic kidney disease (CKD) and cancer have a bidirectional relationship. This is evident in the observations that cancer and/or its treatments can lead to CKD and that CKD is a risk factor for cancer development. A number of observational studies have shown the high prevalence of CKD in patients with solid tumors [37–40]. RCC account for 2.4% of adult malignancies, the vast

majority being clear cell histology:ccRCC [41]. Evaluating data from the Fox Chase Cancer Center, Canter et al. [42] showed that 22% of 1114 RCC patients had CKD stage 3 or higher before nephrectomy, and this percentage increased to 40% for patients older than 70 years [42]. Therefore, many patients with RCC are likely to have CKD before the use of systemic therapy. Two decades ago, the initial treatments for RCC involved targeting the immune system using interleukin 2 (IL-2) and interferon alpha (IFN-α). Following the VHL/HIF/VEGF underlying biology understanding, targeted therapies such as anti-vascular endothelial growth factor (VEGF), tyrosine kinase inhibitors (TKIs), and mTOR inhibitors became the mainstay treatments with clear benefit in progression-free survival [43]. These VEGR TKI have long been associated with renal toxicity.

PD-L1 is expressed in about 20–25% of ccRCC tumor cells and was independently associated with metastatic cancer progression (RR, 3.46; $P < 0.001$) and death from RCC (RR, 4.13; $P < 0.001$) [44]. RCC patients with tumor PD-L1 expression are at significant risk of rapid cancer progression and accelerated rates of mortality. Clinical trials using Nivolumab in metastatic ccRCC was the first of its class to be approved for the treatment of metastatic, in 2014, after randomized, open-label, phase 3 study compared nivolumab with everolimus (CheckMate 025 study) in patients who had failed prior VEGF inhibition. The median overall survival was 25.0 months with nivolumab and 19.6 months with everolimus (HR 0.73; 98.5%CI [0.57–0.93], $p = 0.0018$) [45]. In CheckMate 025, Motzer et al. reported 8% of the RCC patients had an elevation in creatinine and reported as grade 3 or 4 toxicity [45, 46].

More recently, in first-line setting, the doublet ipilimumab plus nivolumab further demonstrated improved overall survival benefit over standard-of-care sunitinib in the intermediate and poor-risk population. Median OS was not reached for the immuno-oncology combination (95% CI [28.2-NR]) versus 26 months for sunitinib (95% CI [22-NR]) (HR 0.63, 99.8%CI [0.44–0.89]) [47]. Data of renal toxicity specifically are not available yet. Clinical trials are now investigating using combination therapy of anti-VEGF and IO based on high response rate with combination approach in phase I [48, 49]. These combinations of VEGFR/TKI and PD-1/PD-L1 inhibitor will require a great focus on renal toxicity when phase III will be presented. The first combination of VEGF inhibition plus PD-L1 inhibition to have been reported in phase III, is the IMmotion 151 trial of atezolizumab plus bevacizumab compared with sunitinib in first line setting (Motzer, ASCO GU 2018). The grade 3–4 proteinuria and hypertension rates reported in this study were in line with the use of bevacizumab, and this combination presented a favorable safety profile when compared to sunitinib.

Management of Renal Toxicity

The mainstay treatment for renal toxicity associated with CPI has been steroids, as is typically done with other organ irAEs [50]. However, it has become evident that biomarkers for organ toxicity associated with CPI is much needed to understand novel treatments [51]. For example, Interleukin-17 has been noted to be high in patients treated with ipilimumab [52], and therefore use of infliximab at a dose of 5 mg/kg once every 2 weeks is started in patients that fail to respond to steroids after 3 days [53]. There is yet more to be done in the renal realm, and staining renal tissue for cytokines and T cell subtypes from patients with irAEs would further help understand novel approaches. The basic approach with AKI after CPI-use would be a nephrology consult, lab and urine analysis. Also, a kidney biopsy would be indicated to delineate if the patient has AIN versus a glomerular process, which may require more than steroids. Based on case reports and CKIN (Cancer and Kidney International Network Workgroup on Immune Checkpoint Inhibitors), steroids is the mainstay treatment with AIN, starting at 1 mg/kg and tapering over 1–2 months with a close follow-up [46]. Any glomerular disease present would be treated with steroids and would consider further immunosuppressive agents, such as rituximab or cellcept, based on the renal biopsy pathology. This would be in conjunction of holding the checkpoint inhibitor. Possible rechallenge

would be reasonable if all possible contributors to AIN have been discontinued, such as nonsteroidal anti-inflammatory drugs (NSAIDS) and proton pump inhibitors(PPI). Monitoring creatinine closely every 2 weeks would be important to ensure improvement.

As far as kidney transplant recipients are concerned, there is still lacking data in management and the recommendations are based on case reports. Kidney transplant patients treated with CPIs need to have both an oncologist and transplant nephrologist in close communication for possible organ rejection. Close monitoring of renal function especially after immunosuppression is reduced with the diagnosis of cancer. One case in the literature suggests, switching tacrolimus to sirolimus and a higher dose of steroids may have been of benefit in preventing organ rejection while on immunotherapy [54].

Although there has been a concern in the use of steroids and the hampering of antitumor effects of CPI, it has been demonstrated by Horvat et al. in 298 patients treated with ipilimumab, where 85% has irAE, where one-third required systemic steroids with no impact on survival or time to treatment failure [55].

Conclusion

Given the wide use of CPI across tumor types, physicians should be trained to detect renal complications. The large majority of cases present either with creatinine level impairment of renal parenchyma damage, the most common being acute interstitial nephritis. Prompt identification and management are needed to prevent chronic kidney disease.

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Immune-Related Oral, Otologic, and Ocular Adverse Events

17

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Abstract

Emerging immunotherapy agents, such as immune checkpoint inhibitors, have shown remarkable promise in the treatment of various malignancies. These drugs selectively target different steps in the immune response cascade to upregulate the body's normal response to cancer. Due to the novelty of these therapeutic agents, their toxicity profile is less well understood.

Meta-analysis results reveal that the overall prevalence of oral mucositis, stomatitis, and xerostomia is lower with checkpoint inhibitors compared to conventional chemotherapy, and head and neck radiation therapy. However, the widespread use of immunotherapy reveals new oral mucosal barrier adverse events, including bullous pemphigoid, mucous membrane pemphigoid, and lichenoid mucositis. Audiovestibular dysfunction can occur from autoimmune-mediated pathways of immunotherapy (adoptive cell) with limited treatment options. Such auditory complications can lead to speech recognition deficits and sensorineural hearing loss. Ocular toxicities are among the most common adverse events resulting from the use of these agents. The majority of ocular immune-related adverse events (irAEs) are mild, low-grade, non-sight threatening, such as blurred vision, conjunctivitis, and ocular surface disease. Serious and sight-threatening events, including corneal perforation, optic neuropathy, and retinal vascular occlusion, can occur but are infrequent. In this chapter, we review the current evidence on the clinical manifestations of oral, audiovestibular, and ocular immune-related adverse events (i.e., irAEs).

Keywords

Oral adverse events · Hearing loss · Ocular adverse events · Immune-related ocular toxicities · Immune-related otologic toxicities · Immune-related oral toxicities · Checkpoint inhibitors · Ipilimumab · Pembrolizumab · Nivolumab · Anti-PD-1/PD-L1 · CTLA-4 · Atezolizumab

Emerging immunotherapeutic agents, including immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death protein ligand 1 (PD-L1), have revolutionized cancer treatment. The first immune checkpoint inhibitor (ipilimumab), an

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anti-CTLA-4, was approved in 2011. Since then, the U.S. Food and Drug Administration (FDA) has approved more than half a dozen immune checkpoint inhibitors to treat various malignancies. These agents are part of a broader class of chemotherapy agents termed immunotherapy, which selectively target different steps in the immune response cascade to upregulate the body's normal response to cancer. While the effects of traditional chemotherapy are well known, the toxicity profile of emerging immune therapies is not fully elucidated. They have been associated with atypical side effects labeled collectively as immune-related adverse events (irAEs).

Many of these events are related to the same immunologic mechanisms responsible for their therapeutic effects. Among the hypothesized mechanism is a breakdown of peripheral tolerance and induction of organ specific inflammatory process leading to immune dysregulation. Ocular toxicities are among the more common adverse events resulting from these agents with a large spectrum in type and severity [1, 2]. Other common irAEs include dermatologic, endocrine, gastrointestinal, hematologic, renal, and neurologic manifestations of disease. Less understood, perhaps owing to its rarity are audiovestibular irAEs. Similarly, severe oral adverse events are limited to a few case reports.

Immunotherapy and Oral Toxicities

Mucositis and xerostomia are two of the most common oral toxicities encountered with systemic chemotherapy, radiation therapy to the head and neck, and hematopoietic stem cell transplantation (HSCT) [3–5]. The term oral mucositis (OM) refers to ulcerative and erythematous lesions resulting from cytotoxic chemotherapy/radiation therapy-induced mucosal injury [6]. OM is an acute regimen-limiting complication of cancer therapy as the lesions are often painful and lead to compromised nutrition, oral hygiene, and risk for local and systemic infections [3]. The exact pathophysiology of mucositis is not known but is believed to be a result of a complex series of biological cellular events in the submucosal epithelium and connective tissue, which precede epithelial damage [4, 7]. The incidence of oral mucositis/stomatitis, irrespective of severity, has been reported to range from 59.4% to 100% in head and neck cancer patients receiving radiation/chemotherapy, between 70% and 86.6% in HSCT patients, and 14.4–81.3% in patients receiving chemotherapy for solid tumors [8].

Xerostomia, which is the subjective sensation of dry mouth, is an acute but persistent oral toxicity of external radiation therapy to the head and neck resulting from reduced secretory capacity of damaged salivary glands [9, 10]. Patients with reduced salivary secretions have an increased risk of oral infections, carious lesions of teeth, oral mucosal discomfort/pain, declined oral functioning and nutritional state, and an overall poorer quality of life [10]. During radiation therapy, xerostomia has been reported to affect 93% of treated individuals with a slight decrease to 85.3% prevalence 2 years postradiation therapy [10]. Chemotherapy-induced xerostomia has been shown to be much less severe and often reversible at the end of the treatment [11].

Prevalence of Mucositis and Xerostomia with Immunotherapy: A Meta-Analysis

A systematic review and meta-analysis of immunotherapy-based clinical trials registered on clinicaltrials.gov reporting prevalence of mucositis and xerostomia was carried out. A systematic search was conducted on February 2, 2019, and data were extracted from all completed trials (Phases 1, 2, and 3) with reported adverse events data. Oral toxicity data, irrespective of toxicity grading, primary tumor, or drug dosage, were extracted from study arms with administration of a single immunotherapy drug. All adverse events from combination therapies, including chemotherapy, radiation, stem cell transplantation, and other immunotherapy agents, were excluded. The proportion of each oral morbidity along with the 95% confidence

intervals (CIs) was plotted using forest plots. A fixed continuity correction of 0.5 was added to studies where the proportions were 0% or 100% [12]. The studies' heterogeneity was assessed using the I^2 statistic which measures the percentage of total variation that is due to heterogeneity rather than chance. If a statistically significant percentage of the total variation was found to be due to heterogeneity, then the combined proportion from the studies in the meta-analysis was estimated using a random effects model in which each study was weighted equally. Detailed methodology and interpretation are published elsewhere [13, 14].

A total of 20 clinical trials (Table 17.1) were identified, which reported immunotherapy-associated oral toxicities including mucositis, stomatitis, xerostomia, and rare oral adverse events such as dysgeusia, dysphagia, decreased appetite, oropharyngeal or oral pain/discomfort, cheilitis, osteomyelitis, oral candidiasis, and other oral infections. Nine studies reported OM with a weighted prevalence of 5% (95% confidence interval: 2–8%; Fig. 17.1). A higher OM prevalence (10%) was noted with CTLA-4 compared to PD-1 (6%) and PD-L1 (4%) inhibitors. Twelve studies reported stomatitis as a separate entity and yielded a weighted prevalence of 3% (95% confidence interval: 2–4%; Fig. 17.2). PD-1 inhibitors showed a higher prevalence of stomatitis (6%) compared to CTLA-4 (2%) and PD-L1 (3%) inhibitors. Similarly, a higher proportion of individuals taking PD-1 inhibitors had xerostomia (11%) compared to CTLA-4 (2%) and PD-L1 (5%) inhibitors. The overall weighted pooled prevalence of xerostomia was estimated to be 5% (95% confidence interval: 3–7%) based on 10 clinical trials (Fig. 17.3).

Other Immunotherapy-Related Oral Adverse Events: Case Reports

Owosho et al. reported on a 52-year-old male with a history of stage IV, metastatic melanoma of unknown primary with metastases to the left iliac region and pancreatic head, who developed osteonecrosis of the right mandible following administration of ipilimumab at 3 mg/kg intravenous (230 mg) every 3 weeks for a total of 4 doses [15]. The patient presented with a gingival swelling on the lingual aspect of the right mandibular molars following administration of the second dose of ipilimumab. On clinical examination, the patient had localized bleeding on probing, mild discomfort, and a small amount of purulent discharge from the gingival sulcus.

Cases with lichenoid reaction involving the oral mucosa, bullous pemphigoid, and mucous membrane pemphigoid cases have been reported. Naidoo et al. reported 2 cases of patients who developed bullous pemphigoid blisters in the oral cavity [16]. An 80-year-old male previously treated with ipilimumab (3 mg/kg) for metastatic melanoma was treated with second-line nivolumab every 2 weeks. After several dermal lesions, he developed erosions and vesicles on the buccal mucosa after 26 doses of nivolumab. Bullous pemphigoid ELISA was positive, and the oral lesions were treated with oral tacrolimus ointment and dexamethasone swish/spit, while nivolumab was withheld. Another 78-year-old female with metastatic melanoma, treated with first-line ipilimumab (3 mg/kg) with no previous adverse events, developed bullous pemphigoid on her buccal mucosa after a year of durvalumab as second-line therapy. Resolution was achieved with topical steroids alone.

Jour et al. reported another case of a 63-year-old male with a history of recurrent metastatic squamous cell carcinoma of the tongue who was initiated on treatment with nivolumab after progression on the previous radiation, chemotherapy, and erlotinib (150 mg) treatment [17]. The patient developed mucosal blisters that supported a finding of bullous pemphigoid on clinical, histologic, direct immunofluorescence, and immunohistochemistry. Initial management included withholding nivolumab treatment and initiation of topical corticosteroid cream with moderate resolution. Patient developed new oral erosions once he was rechallenged with nivolumab after 21 days. Complete resolution of lesions was achieved with oral prednisolone (10 mg) and cessation of nivolumab.

Table 17.1 Summary of included trials

NCT number	Immunotherapy	Title	Malignancy	Trial phase
Anti-PD-1 checkpoint inhibitors				
NCT02007070	Pembrolizumab	Study of pembrolizumab (MK-3475) in participants with advanced non-small cell lung cancer (MK-3475-025/ KEYNOTE-025)	Non-small cell lung cancer	Phase 1
NCT02179918	Pembrolizumab	A study of 4-1BB agonist PF-05082566 plus PD-1 inhibitor MK-3475 in patients with solid tumors (B1641003/ KEYNOTE-0036)	Advanced solid tumors	Phase 1
NCT02180061	Pembrolizumab	Study of pembrolizumab (MK-3475) in participants with advanced melanoma (MK-3475-041/KEYNOTE-041)	Melanoma	Phase 1
NCT00441337	Nivolumab	A study of MDX-1106 in patients with selected refractory or relapsed malignancies	Non-small-cell lung, malignant melanoma, colorectal, renal, prostate cancer	Phase 1
Anti-CTLA-4 checkpoint inhibitors				
NCT00920907	Ipilimumab	Comparison of Ipilimumab manufactured by two different processes in participants with advanced melanoma	Advanced melanoma	Phase 1
NCT01820754	Ipilimumab	Evaluation of circulating T cells and tumor infiltrating lymphocytes (TILs) during/after Presurgery chemotherapy in non-small cell lung cancer (NSCLC)	Non-small cell lung cancer	Phase 2
NCT01990859	Ipilimumab	Phase 2 study of ipilimumab in Japanese advanced melanoma patients	Melanoma	Phase 2
NCT00162123	Ipilimumab	A companion study for patients enrolled in prior/parent Ipilimumab studies	Melanoma	Phase 2
NCT00094653	Ipilimumab	MDX-010 antibody, MDX-1379 melanoma vaccine, or MDX-010/ MDX-1379 combination treatment for patients with unresectable or metastatic melanoma	Unresectable or metastatic melanoma	Phase 3
NCT01585987	Ipilimumab	An efficacy study in gastric and gastroesophageal junction cancer comparing Ipilimumab versus standard of care immediately following first-line chemotherapy	Locally advanced (unresectable) or metastatic adenocarcinoma of the gastric and gastro-esophageal junction	Phase 2
NCT00623766	Ipilimumab	Evaluation of tumor response to ipilimumab in the treatment of melanoma with brain metastases	Melanoma	Phase 2
NCT00796991	Ipilimumab	Drug–drug interaction—3 arm—carboplatin/paclitaxel, dacarbazine	Advanced melanoma	Phase 1
NCT01057810	Ipilimumab	Phase 3 study of immunotherapy to treat advanced prostate cancer	Prostate cancer	Phase 3
NCT00323882	Ipilimumab	Study of MDX-010 in patients with metastatic hormone-refractory prostate cancer	Metastatic prostate cancer	Phase 1lphase 2

(continued)

Table 17.1 (continued)

NCT number	Immunotherapy	Title	Malignancy	Trial phase
Anti-PD-L1 checkpoint inhibitors				
NCT02008227	Atezolizumab	A study of atezolizumab compared with docetaxel in participants with locally advanced or metastatic non-small cell lung cancer who have failed platinum-containing therapy	Non-squamous non-small cell lung cancer	Phase 3
NCT02031458	Atezolizumab	A study of atezolizumab in participants with programmed death-ligand 1 (PD-L1) positive locally advanced or metastatic non-small cell lung cancer	Non-small cell lung cancer	Phase 2
NCT02302807	Atezolizumab	A study of atezolizumab compared with chemotherapy in participants with locally advanced or metastatic urothelial bladder cancer [IMvigor211]	Bladder cancer	Phase 3
NCT01846416	Atezolizumab	A study of atezolizumab in participants with programmed death-ligand 1 (PD-L1) positive locally advanced or metastatic non-small cell lung cancer (NSCLC) [FIR]	Non-small cell lung cancer	Phase 2
NCT01903993	Atezolizumab	A randomized phase 2 study of atezolizumab (an engineered anti-PD-L1 antibody) compared with docetaxel in participants with locally advanced or metastatic non-small cell lung cancer who have failed platinum therapy–"POPLAR"	Non-small cell lung cancer	Phase 2
NCT02558894	Durvalumab	Phase II study of MEDI4736 monotherapy or in combinations with tremelimumab in metastatic pancreatic ductal carcinoma	Metastatic pancreatic ductal adenocarcinoma	Phase 2

Zumelzu et al. reported a case of mild mucous membrane pemphigoid in an 83-year-old patient after administration of pembrolizumab therapy for metastatic melanoma [18]. The patient developed erosions and blisters 6 months after discontinuation of the pembrolizumab therapy that was administered for 10 months. Complete remission of the oral lesions was achieved with minimal doxycycline therapy.

Schaberg et al. reported a case of a 69-year-old male with history of metastatic urothelial carcinoma refractory to multiple lines of chemotherapy who was started on PD-L1 inhibitor therapy [19]. After 11 weeks of treatment, the patient developed a burning sensation on the tongue, gingiva, and buccal mucosa. Intraoral examination showed symmetric reticulated thin white plaques consistent with Wickham's striae, histopathologically confirmed as lichenoid mucositis with pseudoepitheliomatous hyperplasia and reactive spongiosis. No other contributing factors to a lichenoid reaction could be found. Symptomatic improvement was achieved with a dexamethasone elixir swish and spit.

Immunotherapy and Hearing Loss

Hearing loss is a well-known consequence of cancer treatment. Both radiation therapy and certain chemotherapeutic agents have demonstrated the ability to injure a patient's native inner ear function. Radiation, in the setting of treatment of head and neck malignancies, is known to damage both the inner ear and cause middle ear dysfunction—resulting in both sensorineural and conductive hearing loss, respectively. Traditional chemotherapy modalities, such as carboplatin

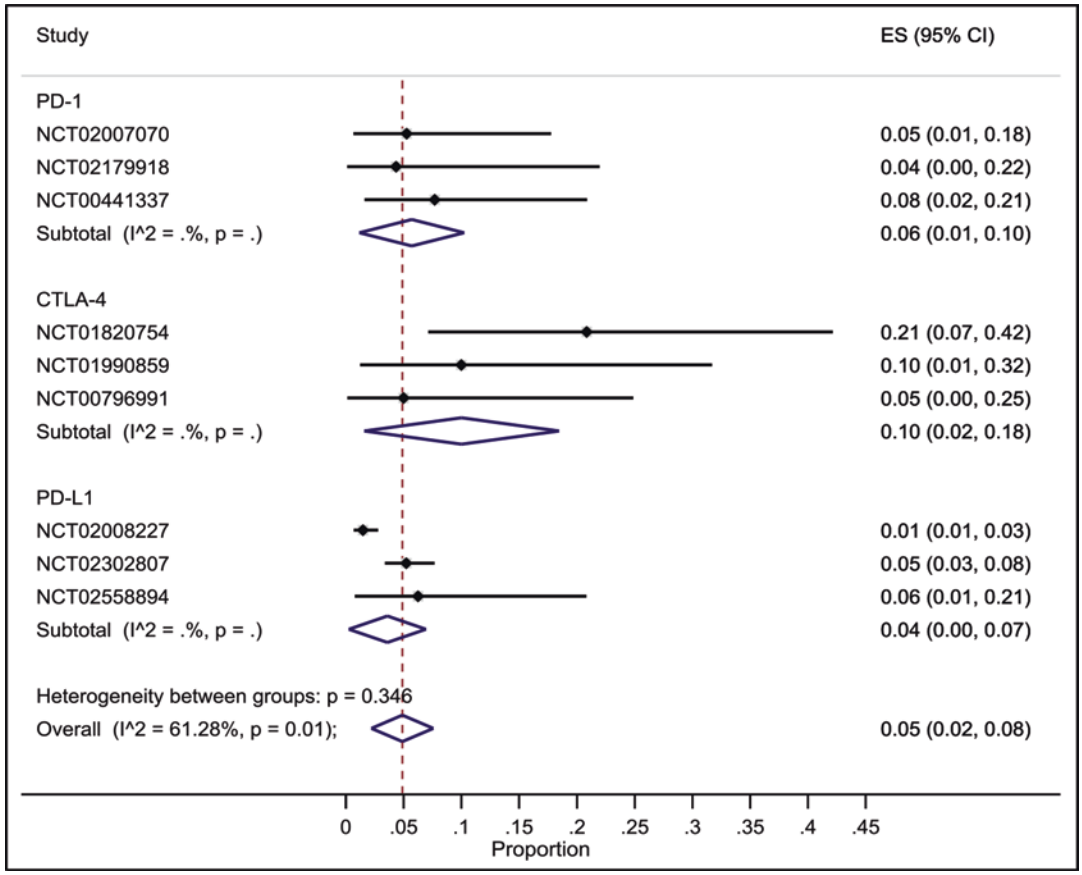

Fig. 17.1 Forrest plot for meta-analysis of prevalence of oral mucositis

and cisplatin, also have well-known and well-studied ototoxicity profiles.

Adoptive Cell Immunotherapy

Autoimmune-mediated complications leading to audiovestibular dysfunction has been previously described in adoptive cell immunotherapy (ACI). In 2009, Johnson and colleagues reported on a series of 36 patients undergoing adoptive cell immunotherapy for metastatic melanoma [20]. Highly reactive T-cell receptors (TCRs) with high anti-melanoma/melanocyte activity were identified via screening of human or murine lymphocytes. Genes encoding these TCRs were then implanted into retroviral vectors and amplified ex vivo prior to transfusion into recipients. All patients underwent baseline audiogram evaluation. While tumor regression was seen in 30% and 19% of human and mouse TCR, respectively, audiometric evaluations demonstrated hearing loss in 10 of 20 patients. This began approximately 1 week following initiation of therapy and was postulated to be related to an inflammatory cytokine surge detected in patients beginning 3–6 days following transfusion. Of those with hearing loss, 70% underwent intratympanic steroid injection with all patients experiencing improvement. Overall, 25% of patients undergoing therapy developed dizziness related to inner ear dysfunction.

Similarly, Seaman and colleagues reported on their experience with 32 patients undergoing ACI with TCRs targeting either gp-100 or MART-1 for metastatic melanoma [21]. All patients underwent pre-intervention audiogram testing for baseline hearing levels. Seventeen of 32 patients

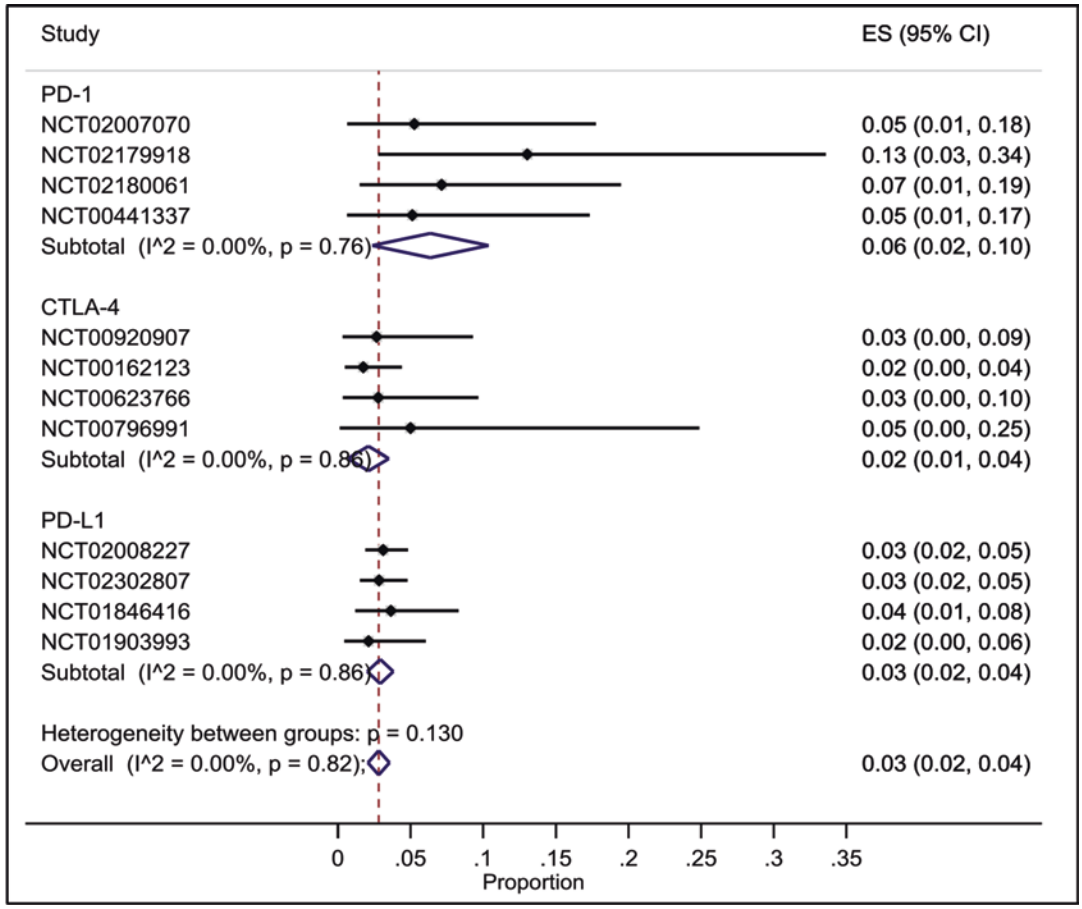

Fig. 17.2 Forrest plot for meta-analysis of prevalence of stomatitis

(53%) showed hearing loss, manifesting an average of 9.5 days following initiation of therapy. Three patients reported dizziness.

In both of the above studies, the proposed mechanism of audiovestibular dysfunction involved aberrant cross reactivity of TCRs to the melanocytes within the stria vascularis of the inner ear. The stria vascularis, a thin, vascularized tissue bed, forms the inner sidewall of the cochlea. It creates and maintains endocochlear ion gradients to provide the electrochemical basis of hearing. Melanocytes, or intermediate cells as they are known in the stria vascularis, are essential contributors to the maintenance of this gradient [22]. Intermediate cells maintain the potassium ion rich milieu of the endolymph within the scala media of the cochlea. It is the electrochemical gradient between the potassium rich endolymph and the potassium poor perilymph within the cochlea that creates the endocochlear potential. This potential is produced by the hair cells in response to the mechanical displacement of the basilar membrane [23]. Absence or dysfunction of stria melanocytes results in sensorineural hearing loss (SNHL). The most common form of non-syndromic, congenital sensorineural hearing loss involves genetic mutations coding for connexin-26, a gap junction protein essential to intermediate cells' ability to recirculate potassium ions [24]. Multiple syndromic causes of congenital hearing loss affect the function of intermediate cells including Tietz Albinism-Deafness Syndrome [25], Craniofacial-deafness-hand syndrome [26, 27], and Waardenburg syndrome [28, 29]. The essential role played by the intermediate cells in hearing supports the hypothesis that their dysfunction or

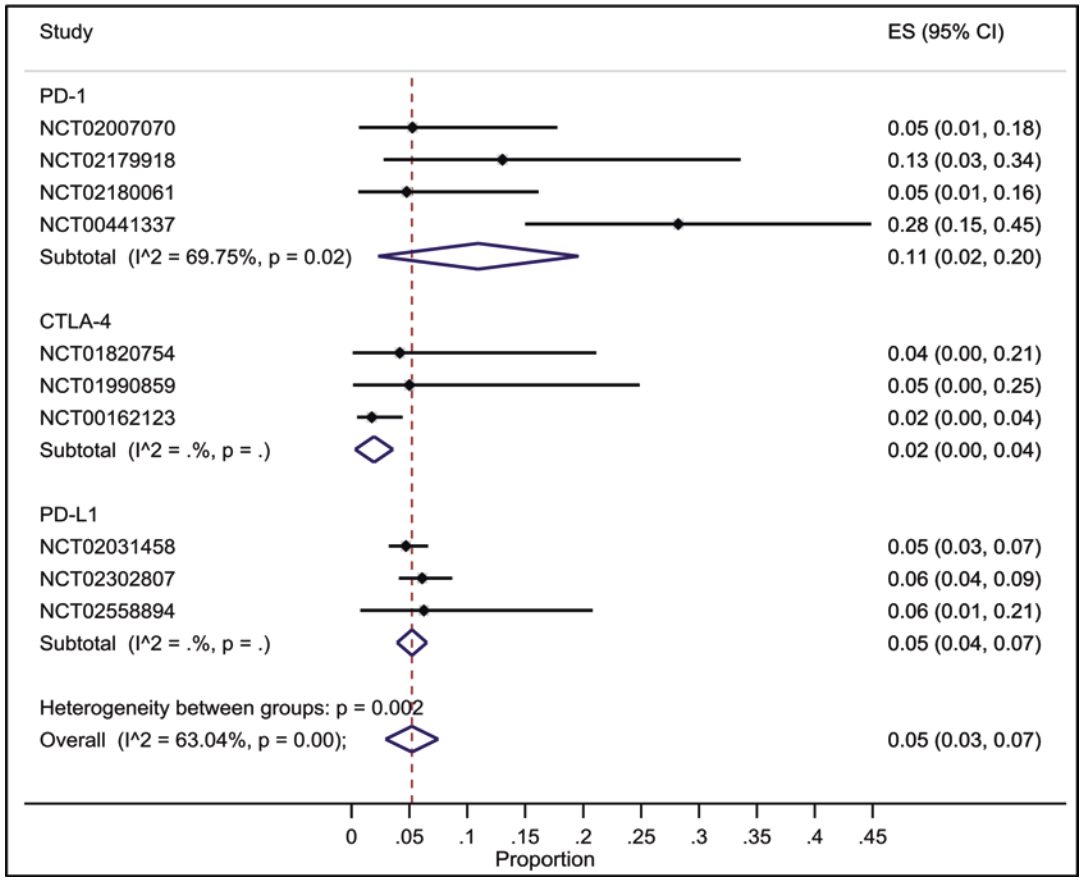

Fig. 17.3 Forrest plot for meta-analysis of prevalence of xerostomia

destruction is the underlying cause of hearing loss following ACI.

Vogt-Koyanagi-Harada Syndrome

Melanocyte destruction within the inner ear has an autoimmune analog in Vogt-Koyanagi-Harada (VKH) syndrome. VHK is a constellation of symptoms including bilateral posterior uveitis, vitiligo, central nervous system deficits, and sensorineural hearing loss. This is thought to be T cell-mediated autoimmune destruction of melanocytes [30]. This condition is more frequently seen in patients with darker skin tone, women, and those aged 20–50 years old. Aggressive treatment with corticosteroids or immunomodulators is the preferred treatment for this disease. Those with uveitis may require intravitreous steroid injection. In the above cases of hearing loss related to adoptive immune therapy, multiple patients also experienced rash, gastrointestinal upset, and changes in visual acuity.

Case Reports

Immune-related adverse events have been reported with the use of ICIs. However, hearing loss appears to be rare and limited to sporadic case reports and to individual patients within larger cohorts of patients with reported irAEs. No clinical trials have evaluated the impact of ICIs on hearing. Only one animal study looked at the impact of anti-PD-1 therapy on a murine animal model [31]. In this study, hearing thresholds were largely unaffected in the group that received immunotherapy alone. When the anti-PD-1 agent

was added to cisplatin, it resulted in minor worsening of hearing compared to the group receiving cisplatin alone.

Case #1

Zibelman et al. reported on an 82-year-old man with metastatic mucosal melanoma who underwent initial treatment with ipilimumab (3 mg/kg), a CTLA-4 inhibitor, before switching to pembrolizumab, a PD-1 inhibitor (2 mg/kg every 3 weeks), due to disease progression [32]. Following his second dose of pembrolizumab, the patient noted bilateral hearing loss.

Audiometry confirmed a mild to moderately–severe symmetric sensorineural hearing loss (SNHL) with word recognition scores (WRSs) of 48% and 44% in the right and left ears, respectively. The patient had not experienced any episodes of meningitis, taken ototoxic chemotherapy agents, or experienced any other obvious etiology for his hearing loss. He underwent intratympanic dexamethasone injections (10 mg/mL), 6 injections on the right and 4 on the left and subjectively noted complete recovery of his hearing. Postinjection audiogram showed recovery of low-frequency hearing thresholds but still with moderate-to-severe SNHL in the higher frequencies. His word recognition scores improved to 88% and 84%. He continued his pembrolizumab therapy and had no further audiovestibular symptoms.

Case #2

Diamantopoulos et al. reported a case of an 81-year-old woman with stage IIIb (T2aN1bM0) cutaneous melanoma who presented 8 months after her initial diagnosis with metastatic lesions to the skin of her left breast and axillary lymph nodes [33]. Imaging showed an additional metastatic pulmonary lesion. She was started on encorafenib 300 mg daily, and binimetinib at 45 mg twice daily as part of a phase III clinical trial.

Six months after initiation of therapy, the patient experienced a 10-day course of headaches, light sensitivity, and worsening visual acuity. She underwent a detailed ophthalmological exam, which revealed bilateral panuveitis. In addition to her ocular symptoms, the patient also experienced bilateral sudden hearing loss with elevation of pure tone thresholds to 60 dB in the right and 40 dB in the left consistent with an asymmetric bilateral SNHL. The patient did not have a pre-intervention audiogram for comparison. Other causes of sudden onset SNHL, including infectious and autoimmune etiologies, were excluded based on testing.

Encorafenib and binimetinib were both immediately discontinued, and the patient was started on 64 mg of methylprednisolone daily for 7 days along with dexamethasone eye drops. Her vision gradually improved; however, no data are given regarding resolution of her hearing loss.

Case #3

Tampio et al. reported a case of a 67-year-old man with a history of sarcoidosis with widely metastatic melanoma [34]. Testing revealed BRAF and PDL-1 markers and it was decided to proceed with nivolumab monotherapy with a plan for 12 cycles of 240 mg administration. Approximately 2 months after starting therapy, the patient presented to the emergency department for bilateral light sensitivity. He was seen the following week in the Ophthalmology Clinic and was noted to have findings consistent with intraocular inflammation. Concern for an autoimmune reaction to his current immunotherapy regimen led to a cessation of ICI therapy and initiation of corticosteroid eye drops.

Approximately 2 weeks after the above events, the patient noticed bilateral ear fullness, subjective hearing loss, and brief episodes of vertigo with head movement. Audiogram showed a bilateral mild to severe sloping, high-frequency SNHL with word recognition scores of 100% bilaterally. Because of the bilateral sudden SNHL and bilateral panuveitis, this presentation was felt to be part of broader, ICI agent-induced autoimmune reaction, and a 60 mg daily prednisone burst was initiated and tapered over 5 weeks. The patient had received 4 cycles of nivolumab, and repeat MRI and PET/CT at this time showed resolution of neoplastic disease. At 6 weeks follow-up, the patient noted completely resolved ocular symptoms and improved hearing. Repeat audio-

gram at the 4 months follow-up showed normalization of the speech reception thresholds.

Immunotherapy and Ocular Toxicity

The majority of described ocular irAEs are mild, low-grade, non-sight threatening, such as blurred vision, conjunctivitis, and ocular surface disease (dry eye). Serious and sight threating events such as corneal perforation, optic neuropathy, and retinal vascular occlusion can occur but are infrequent. Knowledge and awareness of ocular side effects is imperative to guide the proper treatment plan. A multidisciplinary approach between the medical and ocular oncologist is essential in the identification and management of these events [1, 35, 36].

Fu et al. conducted a study of ocular toxicities associated with all FDA approved oncologic immune therapies through March 2015. The review included 32 independent reports that met the inclusion criteria. The severity of ocular events was graded according to common terminology criteria for adverse events (CTCAE) grade (Version 4.0). The study concluded that the most commonly reported events were conjunctivitis and blurred vision; reported in nine (19.6%) and ten (21.7%) agents of the total reviewed. Imatinib was found to have the highest incidence of grade 3 or higher toxicity. Overall imatinib and crizotinib had the highest incidence of any ocular events. Acute serious and sight threating ocular events were rare, and accounted for <1% including retinal vascular occlusion, retinal pigment epithelial detachment, corneal ulceration and perforation, and blindness. Devastating vision-threatening ocular irAEs were reported with only five classes of agents (10.9%): EGFR inhibitors (erlotinib and gefitinib), MEK inhibitors (trametinib), V600E mutated BRAF inhibitors (vemurafenib), anti-CTLA4 inhibitors (ipilimumab), and targeted antibodies [37–43].

Abdel-Rahman et al. conducted a systematic review to assess the incidence of ocular irAEs. Eleven prospective trials were analyzed included one trial for ipilimumab and tremelimumab, three for nivolumab, five for pembrolizumab, and one comparing pembrolizumab to ipilimumab. The incidence of uveitis ranged from 0.3% to 6%, whereas the incidence of dry eyes ranged from 1.2% to 24.2%. Among the four randomized studies comparing immune checkpoint inhibitors agents versus nonimmune checkpoint inhibitors, the pooled analysis for odds ratio of all grade is 3.40 [95% CI: 1.32–8.71; $P = 0.01$]. This suggests that these toxicities are more common with immune checkpoint inhibitors compared to control [44–46].

Antoun et al. conducted a systematic review to evaluate ocular and orbital irAEs of checkpoint inhibitors. They suggested that irAEs may occur as early as 1 week after initial dose with the median occurrence of 2 months after initiation of therapy. Common ocular events included peripheral ulcerative keratitis (PUK), uveitis, and Vogt-Koyanagi-Harada (VKH) syndrome. Peripheral ulcerative keratitis, severe peripheral infiltration, and ulceration were reported with ipilimumab. In addition uveitis has been reported with nivolumab and bilateral uveitis and papillitis with pembrolizumab. Vogt-Koyanagi-Harada syndrome has been reported in one case with combination of ipilimumab and anti-PD1 inhibitors [47, 48].

Bitton et al. reviewed 745 patients from a single center and national registry between June 2014 and March 2018, identifying patients with moderate-to-severe ocular toxicity following anti-PD-L1 administration. Dry eye was the first and most frequently reported event. In total, three patients had moderate-to-severe ocular events, with an overall prevalence of 0.4% and an incidence of 0.7 per 1000 patient-months of treatment. In addition to the cases reported through the national registry, five presented with intraocular inflammation, two with ocular surface disease, and one with orbital myopathy; five (62.5%) developed exophthalmos [49].

Fang et al. looked at the association between immune checkpoint inhibitors and ophthalmic adverse effects using data from U.S. FDA's Adverse Events Reporting System (FAERS) database from 2003 to 2018. The study identified 113 ocular events including dry eye, uveitis, ocular myasthenia, and "eye inflammation." Nivolumab showed the highest number of ocular

events. It also had the highest association with ocular myasthenia followed by pembrolizumab. Atezolizumab had the highest association with "eye inflammation," while ipilimumab had the highest association with uveitis. Nivolumab was also associated with these two toxicities. No cases were reported for other checkpoint inhibitors including avelumab, cemiplimab, and durvalumab [36, 40, 50].

Management

Many mild ocular toxicities are managed with topical corticosteroids and/or lubrication. Severe side effects may require systemic corticosteroids and/or termination of the drug. The decision regarding continuation or withdrawal of treatment should be evaluated on a case-by-case basis, depending on the severity of toxicity and the response to treatment. Detailed recommendations with clinical practice guidelines based on evidence from a rigorous systematic review, published medical literature and expert consensus for management of ocular (irAEs) have been recently published. In general immunotherapy should be continued with close monitoring for grade 1 toxicities, with few exceptions. Therapy may be held or reduced for grade 2 toxicities. For grade 3 toxicities or above, treatment should be held and high-dose corticosteroids considered. Rechallenge can be considered with extreme precaution after a grade 3 toxicity. Permanent discontinuation should be considered in all grade 4 cases [51–54].

Summary

Immune-based cancer therapy has revolutionized the treatment of various malignancies. Clinicians should be familiar with likely adverse events associated with immune therapies. Ocular toxicities are among the most common adverse events resulting from the use of these agents. The majority are mild, and not sight threating; however, serious events can occur and lead to blindness. Acute visual changes always necessitate an immediate ophthalmologic assessment.

The overall prevalence of commonly encountered oral toxicities, including oral mucositis, stomatitis, and xerostomia, was found to be lower with checkpoint inhibitors compared to conventional chemotherapy and head and neck radiation therapy. However, the widespread use of immunotherapy reveals new oral mucosal barrier adverse events, including bullous pemphigoid, mucous membrane pemphigoid, and lichenoid mucositis. Auditory and vestibular dysfunctions have also been reported in patients treated with immunotherapy directed toward melanocytes.

A multidisciplinary approach with good communication is crucial for prompt referral and management of such complications. At present, there is a lack of standardized surveillance guidelines for all patients potentially at risk. Establishing an ophthalmic, otolaryngology and audiology, and oral surveillance protocol with baseline screening is ideal. The specific frequency and exam parameters may be dependent on the agent and its toxicity profile.

Further research is needed to establish prevalence/incidence of immunotherapy-induced oral, ocular, and audiovestibular toxicities as well as their pathophysiology and management.

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Cancer Imaging in Immunotherapy

18

Mira Ayoub, Yousra Eleneen, and Rivka R. Colen

Abstract

Immune therapeutics are revolutionizing cancer treatments. In tandem, new and confounding imaging characteristics have appeared that are distinct from those typically seen with conventional cytotoxic therapies. In fact, only 10% of patients on immunotherapy may show tumor shrinkage, typical of positive responses on conventional therapy. Conversely, those on immune therapies may initially demonstrate a delayed response, transient enlargement followed by tumor shrinkage, stable size, or the appearance of new lesions. New imaging response criteria, such as the immune-related Response Evaluation Criteria in Solid Tumors (irRECIST) and immune-related Response Criteria (irRC), are being implemented in many trials. However, FDA approval of emerging therapies, including immunotherapies, still relies on the current RECIST criteria. In this chapter, we review the traditional and new imaging response criteria for evaluation of solid tumors and briefly touch on some of the more commonly associated immunotherapy-induced adverse events.

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Keywords

Cancer imaging · irRC · Immune imaging criteria · irRECIST · Immunotherapy

Introduction

Cancer immunotherapy has caused a plethora of new and important radiographic features that are imperative to understand when assessing tumor response and immune-related adverse events [1–3]. Immunotherapy, which is an approach to treat cancer by augmenting or generating an immune response against cancer cells, causes radiographic responses distinct from conventional cytotoxic chemotherapies [2, 3].

Objective imaging response criteria as measured by the World Health Organization (WHO) and Response Evaluation Criteria in Solid Tumors (RECIST) criteria were originally created to assess the effects of cytotoxic chemotherapy and are dependent on tumor shrinkage and absence of new lesions; however, these criteria do not perform well in assessing the effects of drugs with other mechanisms of action, such as antiangiogenic therapies or immune therapies [1–4]. Evaluation of tumor response to cytotoxic chemotherapy depends on tumor shrinkage within a few weeks of initiating treatment. In fact, in addition to the appearance of new lesions and increased tumor size, stable disease was at

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one point considered a treatment failure [4]. On the other hand, new tumor therapies with recombinant cytokines, cancer vaccines, and immunomodulatory monoclonal antibodies may demonstrate a delayed response, transient enlargement (transit flair-up phase) followed by tumor shrinkage, stable size, or the appearance of new lesions [4] . Unique challenges associated with immunotherapy reflect delays in response and therapy-induced inflammation. Cancers after immunotherapy demonstrate confounding radiographic appearances with only 10% showing regression [4]. Typically, these tumors initially demonstrate a delay in response, including none or slow decrease in tumor size, increase in tumor size, and/or the appearance of new lesions, which over time become stable, decrease, or resolve without further treatment (Fig. 18.1). Over the years, there have been many modifications to the different assessment criteria by combining changes in size and inclusion of metabolic features of specific tumors to overcome the limitations of the traditional criteria [5]. However, these modifications have caused difficulties in assessing treatment efficacy since standardization of response assessments among those clinical trials lacks. It is critical to distinguish as early as possible between patients who are responding to a particular treatment and those who are not in order to maximize the effectiveness of patient care [5]. In addition, it is important to understand immunotherapy-induced side effects as in some cases treatment might be changed or halted. In this chapter, we discuss the use of a variety of traditional and new immunotherapy criteria for the evaluation of tumor response in patients who are undergoing immunotherapy. We also briefly discuss some of the immunotherapyinduced adverse events.

Conventional Imaging Response Criteria (Table 18.1)

The WHO and the RECIST criteria were the first criteria developed to assess tumor responses to traditional cancer treatment, which included cytotoxic chemotherapy, radiation therapy, or surgical resection. These criteria depend on changes in tumor size and do not take into consideration

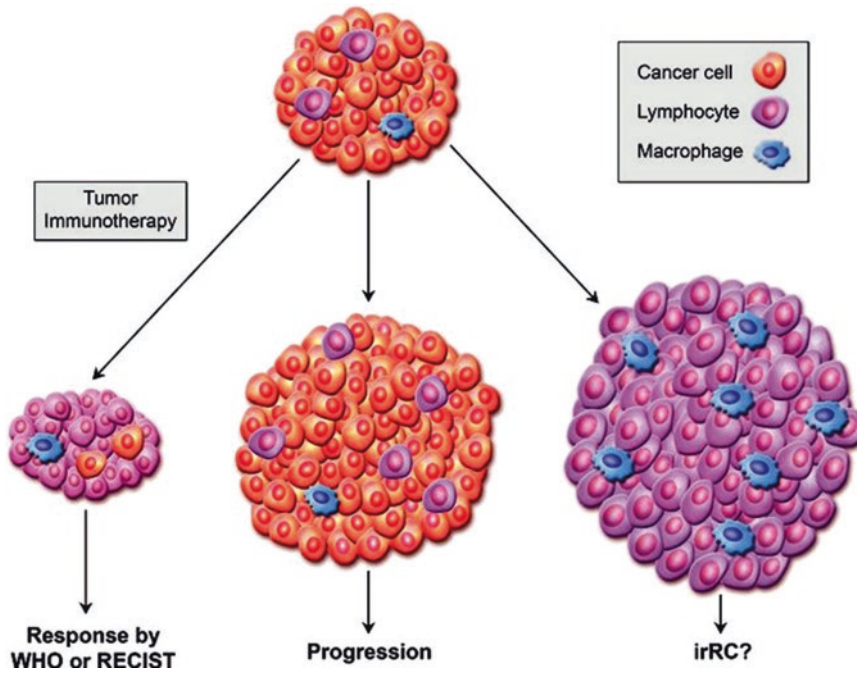

Fig. 18.1 Cancer imaging in immunotherapy

Table 18.1 Comparison between the basis of WHO, RECIST 1.0, RECIST 1.1, irRC, and irRECIST criteria [1, 2, 4]

Criterion	WHO	RECIST 1.0	RECIST 1.1	irRC	irRECIST
Method of measurement	SPD	Longest diameter	Longest diameter (except in lymph nodes)	SPD	Single longest diameter (except in lymph nodes)
Measurable lesions	Should be measurable in two dimensions, no minimum lesion size	Minimum size = 10 mm at spiral Computed tomography (CT), 20 mm at conventional CT	Minimum size = 10 mm at CT	Minimum size of the lesion is 5 mm × 5 mm	Minimum size = 10
Number of lesions measured	No assessment	Ten lesions (≤5 in any one organ)	Five lesions (≤2 in any one organ)	Ten lesions (≤5 in any organ)	Five lesions (≤2 in any one organ)
Progressive disease	≥25% increase in SPD	20% increase in SLD or new lesions, unequivocal progression considered to indicate progressive disease	>20% increase in SLD, ≥5-mm increase in size, new lesions, detailed description of unequivocal progression	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart
Lymph nodes	Unspecified	Unspecified	Short axis: target lesions ≥15 mm, nontarget lesions = 10–15 mm, nonpathologic lesions <10 mm	Unspecified	Short axis: target lesions ≥15 mm, nontarget lesions = 10–15 mm, nonpathologic lesions <10 mm
New lesions	No assessment	No assessment	Provides guidance as to when a lesion is considered new (i.e., representative of progressive disease)	Does not constitute progressive disease in itself, but is rather added to the SPD and contributes to progression	Does not constitute progressive disease in itself, but is rather added to the sum of longest diameter and contributes to progression
Guidance for imaging studies	No assessment	CT, MRI, chest radiography	CT, MRI, FDG PET	CT, MRI, chest radiography, FDG PET	CT, MRI, chest radiography, FDG PET

appearance of new lesions when evaluating responses that may be related to treatment [4].

WHO Criteria

In 1981, the WHO published the first tumor response criteria, thus establishing a standard assessment metric and nomenclature to evaluate treatment response [6]. The WHO criteria introduced the concept of assessing tumor burden using the sum of the products of diameters (SPD) (i.e., longest overall tumor diameter and longest diameter perpendicular to the longest overall diameter) and determining response to therapy by evaluating the changes from baseline during

treatment [6]. These criteria were categorized into four tumor response groups: complete response (tumor not detected for at least 4 weeks), partial response (≥50% reduction in the SPD from baseline, also confirmed at 4 weeks), progressive disease (≥25% increase in tumor size in one or more lesions), and no change (stable) in disease (neither partial response, complete response, nor progressive disease) (Table 18.1) [7] . However, the WHO has a few major pitfalls (discussed below), in particular, because tumor measurements are based on SPD, small increases in tumor size may result in a sufficiently overall increase in tumor size (≥25% increase) to consider it as progressive disease [5].

RECIST 1.0, 1.1, and mRECIST Criteria

RECIST 1.0

In 2000, the RECIST 1.0 criteria was established and addressed some of the pitfalls of the WHO criteria. Of these, the key features of RECIST 1.0 included a clear definition of measurable disease, number of lesions to be assessed, and the use of unidimensional (i.e., longest dimension) rather than bidimensional tumor measurements (Table 18.1) [6].

RECIST 1.1

In 2009, RECIST 1.1 was developed. RECIST 1.1 addressed multiple questions regarding the assessment of lymph nodes, number of lesions to be assessed, and use of new imaging modalities, such as multidetector computed tomography (MDCT) and magnetic resonance imaging (MRI) [8]. In RECIST 1.1, the number of target lesions is reduced; target lesions can reach a maximum of five lesions (up to two lesions in any one organ) and must be measured in their longest dimension (should be at least 10 mm in the longest diameter to be considered measurable), except for lymph nodes, which use the shortest diameter (must be at least 15 mm in the short axis to be considered pathological). In coalescing lesions (nonnodal lesions), its portions should be added together (as lesions coalesce) and its longest dimensions measured [8]. Furthermore, if a lesion cannot be reliably measured, the next largest lesion that can be reproducibly measured should be selected. In addition, if any target lesions (including lymph nodes) become too small to be measured, these should also be recorded and taken in assessment of response and reassessed in the follow-up examination to determine if they represent a new lesion [5] (Table 18.1).

Modified RECIST (mRECIST)

Modified RECIST (mRECIST) was created to measure the response rate in hepatocellular carcinoma (HCC). Similar to RECIST 1.0 and 1.1, mRECIST uses tumor size as an index of tumor response; however, in contrast, mRECIST takes into account treatment-induced tumor necrosis, and changes in size are determined by assessing for viable tumor, referred to an uptake of contrast agent in the arterial phase on CT or MRI [9, 10]. For example, a complete tumor response is defined as the disappearance of arterial phase enhancement in all target lesions which should be classified as a measurable lesion according to the RECIST criteria [5]. Tumors in malignant portal vein thrombosis are considered as nonmeasurable disease since the bland thrombus formed during the course of treatment can obscure the tumor.

Choi Response Criteria

The Choi criteria was initially proposed for assessment of gastrointestinal stromal tumors (GIST) on imatinib, a tyrosine kinase receptor inhibitor. This study found that GISTs on treatment may initially increase in size due to internal hemorrhage, necrosis, or myxoid degeneration. Some may show a minimal decrease in tumor size but not sufficient enough to be classified as having a positive response to therapy according to RECIST criteria [11]. The Choi criteria focuses on changes in density (Hounsfield units on CT) rather than tumor shrinkage to assess response. A decrease in tumor density on CT is often seen in these tumors responding to imatinib and is related to tumor necrosis or myxoid degeneration. There are two main limitations of the Choi criteria: it

cannot be applied to MRI and there is lack of sufficient validation in other tumors.

EORTC

The European Organization for Research and Treatment of Cancer (EORTC) criteria has formalized the concept of assessing tumor response via quantifying the changes in fluorodeoxyglucose (FDG) uptake. Criteria standardization and rules were proposed on patient preparation, timing of [18F]-FDG positron emission tomography (PET) scans, attenuation correction and dose of [18F]-FDG, methods to measure [18F]-FDG uptake, tumor sampling, reproducibility, and definition of [18F]-FDG tumor response [12, 13].

The criteria follows the model of RECIST in terms of defining four response categories with similar names as RECIST. Complete metabolic response (CMR) would be the complete resolution of [18F]-FDG uptake within the tumor volume so that it is indistinguishable from surrounding normal tissue. Partial metabolic response (PMR) would be classified as a reduction of a minimum of 15–25% in tumor [18F]-FDG SUV after one cycle of chemotherapy, and greater than 25% after more than one treatment cycle. Stable metabolic disease (SMD) would be classified as an increase in tumor [18F]-FDG SUV of less than 25% or a decrease of less than 15% and no visible increase in extent of [18F]-FDG tumor uptake (20% in the longest dimension). Progressive metabolic disease (PMD) would be classified as an increase in [18F]-FDG tumor SUV of greater than 25% within the tumor region defined on the baseline scan, visible increase in the extent of [18F]-FDG tumor uptake (20% in the longest dimension) or the appearance of new [18F]-FDG uptake in metastatic lesions [12, 13].

PERCIST Criteria

Based on the premise that newer cancer therapies are more cytostatic than cytocidal, tumor response can manifest with a decrease in metabolism without a notable tumor size reduction [14]. In 2009, the PET response criteria for solid tumors (PERCIST) was proposed and is based mainly on FDG uptake to evaluate tumor response [15]. PERCIST focuses on the percentage of change in metabolic activity from baseline and the number of weeks from initiation therapy. The standardized uptake value (SUV) corrected for lean body mass (SUL) is used for the assessment of tumor response. The SUL peak is measured within a spherical region of interest of 1.2 cm in diameter (or 1 cm^3 for volume) within the area of highest uptake in the tumor [5]. PERCIST defines four metabolic response categories. In brief, according to these criteria, complete response means disappearance of all metabolically active tumors while partial metabolic response is defined as a 0.8-unit (>30%) decline in SUL peak between the most intense lesion before treatment and the most intense lesion after treatment. Of note, the lesion at follow-up may be a different lesion than previously measured since the most active lesion needs to be followed. Progressive disease is defined as an increase (>30%) in SUL peak or the appearance of a new metabolically active lesion [5]. It is likely that PERCIST will replace the EORTC criteria in the same way that RECIST has replaced the WHO criteria [12].

RANO Criteria

The Revised Assessment in Neuro-Oncology (RANO) criteria was proposed to overcome the significant limitations in the Macdonald criteria for response assessment in high-grade gliomas. The Macdonald criteria didn't take into account, for example, pseudoprogression, pseudoresponse observed with antiangiogenic agents, and the inability to capture recurrence in the nonenhancing component of the lesion, due to using only the contrast-enhancing component of the tumor in it [13].

Similar to the Macdonald criteria, the RANO criteria uses two-dimensional tumor measurements; however, the RANO criteria also accounts for changes in the nonenhancing T2/FLAIR signal abnormality. Measurable disease is defined

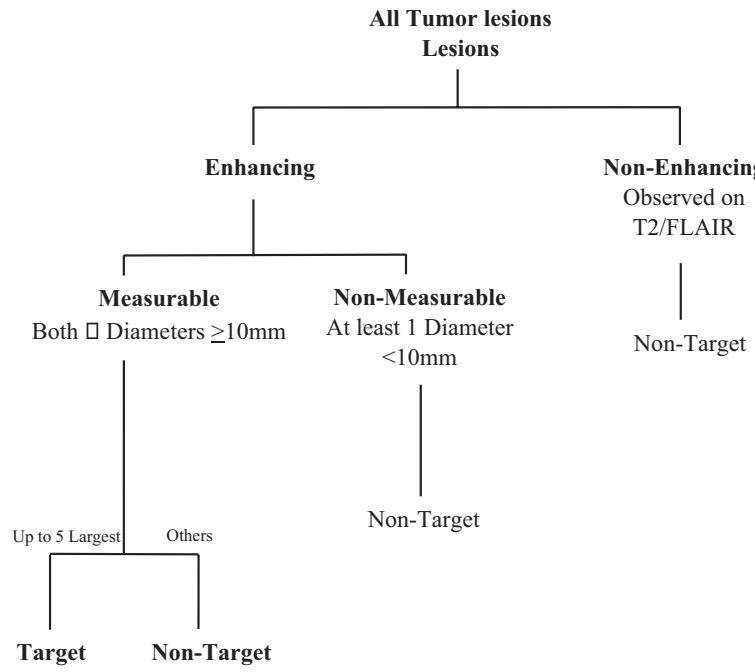

Fig. 18.2 Algorithm for identifying measurable and target lesions [16]

Table 18.2 RANO criteria for response assessment in high-grade gliomas [16, 17]

Criterion	CR	PR	SD	PD
T1-Gd + (bidimensional product)	None	≥50% ↓	<50% ↓ to <25% ↑	>25% ↑[a]
Estimated volumetric change	100% decrease	≥65% decrease	<65% decrease to <40% increase	≥40% increase
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or↓	↑[a]
New lesion	None	None	None	Present[a]
Corticosteroids	None	Stable or ↓	Stable or ↓	NA[b]
Clinical status	Stable or ↑	Stable or↑	Stable or↑	↓[a]
Requirement for response	All	All	All	Any[b]

[a]Progression occurs when this criterion is met

[b]Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration

as two perpendicular diameters of at least 10 mm (visible on two or more axial slices being preferably not more than 5 mm apart with 0 mm skip) and allows selection of a total of five target lesions (Fig. 18.2). RANO criteria addressed pseudoprogression and pseudoresponse. The RANO criteria for high-grade glioma are summarized in Table 18.2 [16, 17].

In RANO, the postradiation examination as the baseline for response assessment instead of the postsurgical MRI scan can be used. Progressive disease is defined by at least two sequential scans separated by at least 4 weeks, both showing >25% increase in the sum of products of perpendicular diameters or > 40% increase in total volume of enhancing lesions. If the follow-up scan exhibits SD or PR/CR, then the first scan that showed "preliminary PD" is noted at pseudoprogression. Pseudoprogression is also considered if imaging showed PD and the follow-up scan >4 weeks apart showed SD, CR, PR or the lesions became nonmeasurable; if the latter, the scan that showed "preliminary PD" is noted as "pseudoprogression" [16]. On the other hand,

if imaging demonstrated preliminary PR/CR and the follow-up scans exhibited PD with respect to the "preliminary CR/PR" scan, then the response isn't sustained and is noted as pseudoresponse. Pseudoresponse can also be noted in tumors that show regression in size of their enhancing component whilst their nonenhancing component show progression [16].

RANO-BM

The Response Assessment in Neuro-Oncology Brain Metastases working group initially convened in 2011 and proposed response assessment on the basis of literature review and consensus opinion [18]. RANO-BM adopted features from RECIST and RANO-HGG to be able to meet the specific needs of patients with brain metastases, where response assessment in RANO-BM is being based on the sum diameter of one-dimensional measurements, corticosteroid dosing and clinical status (Table 18.3) [17].

Cheson Response Criteria for Malignant Lymphomas

Tumor assessment criteria have been developed specifically for lymphoma. In lymphoma, masses often don't regress in size completely after therapy because of the presence of residual fibrosis and necrotic debris; thus, reporting whether the tumor is viable or not viable does not depend solely on the stability of the tumor's size. The Cheson response criteria analyzes the size and the metabolic activity of the tumor during the course of treatment. The revised version of the Cheson criteria in 2007 replaced gallium scintigraphy with PET and included the evaluation of flow cytometry and immunohistochemistry as mentioned in Tirkes et al. (Table 18.4) [5].

Immunotherapy Imaging Response Criteria

Evaluating tumor responses during immune therapy in solid cancers remains a challenge [5, 20]. The mechanism of action in immunotherapy differs substantially from cytotoxic agents; thus a well-tailored set of criteria to capture accurate and exact response to this new line of therapeutic agents is needed [4, 5, 20]. To this end, Wolchok et al. presented a set of criteria to evaluate immune-related responses, adopting a bidimensional approach similar to the WHO criteria and measuring a maximum number of five lesions per organ (Table 18.5) [4]. Although these criteria were widely accepted, it still harbors some

Table 18.3 RANO-BM criteria for response assessment in brain metastases [17]

Criterion	CR	PR	SD	PD
Target lesions	None	≥30% decrease in sum LD relative to baseline	<30% decrease relative to baseline, but <20% increase in sum LD relative to nadir	≥20% increase in sum LD relative to nadir[a]
Nontarget lesions	None	Stable or improved	Stable or improved	Unequivocal PD[a]
New lesion(s)[b]	None	None	None	Present[a]
Corticosteroids	None	Stable or decreased	Stable or decreased	NA[c]
Clinical status	Stable or improved	Stable or improved	Stable or improved	Worse[a]
Requirement for response	All	All	All	Any[c]

LD longest dimension
[a]Progression occurs when this criterion is met
[b]New lesion = New lesion not present in previous studies and visualized in at least two projections
[c]Increase in corticosteroids dose alone will not be considered to determine progression in the absence of persistent clinical deterioration

Table 18.4 Cheson response criteria definitions [19]

Table response definitions for clinical trials				
Response	Definition	Nodal masses	Spleen, liver	Bone marrow
CR	Disappearance of all evidenceof disease	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
PR	Regression of measurable disease and no new site	≥50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	≥50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if positive prior to therapy; cell type should be specified
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT		
Relapsed disease or PD	Any new lesion or increase by ≥50% of previously involved sites from nadir	Appearance of a new lesion(s) 1.5 cm in any axis, ≥50% increase in SPD of more than one node, or ≥ 50% increase in longest diameter of a previously identified node 1 cm in short axis. Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy	>50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

Abbreviations: *CR* complete remission, *FDG* [18F] fluorodeoxyglucose, *PET* positron emission tomography, *CT* computed tomography, *PR* partial remission, *SPD* sum of the product of the diameters, *SD* stable disease, *PD* progressive disease

Table 18.5 Summary of immune-related response criteria (irRC) [4]

Method of assessment of lesion	The largest bidimensional diameters are used to evaluate each lesion.
Total tumor burden evaluation	The total tumor burden is the sum of the products of diameters (SPD) of target lesions and new lesions.
New target lesions	If the new lesions fulfill the criteria of target lesion assessment, the two diameters are determined and the product of these diameters is incorporated into the SPD and contributed to the evaluation of total tumor burden.
New nontarget lesions	If the new lesions fail to fulfill the criteria of target lesions, they do not contribute to total tumor burden. However, complete remission of such lesions is essential for establishing a complete response.
Imaging modalities	Almost all current imaging modalities could be used to assess tumors in a longitudinal manner. These include CT, MRI, and PET-CT.
Target lesions criteria	Target lesions should measure at least 5 × 5 mm. A maximum of five cutaneous lesions and 10 visceral lesions could be selected. No more than five lesions could be selected per organ.
Time-point response assessment	The growth kinetics of target and new lesions are determined. Percentage change of tumor growth is then calculated referencing baseline assessment as well as the smallest reported tumor burden (nadir).
Types of overall response	Complete response (irCR), partial response (irPR), stable disease (irSD), and progressive disease (irPD)
Complete response (irCR)	irRC requires for complete response the total (100%) remission of all target, nontarget, and new lesions for two consecutive evaluations at least 4 weeks apart.
Partial response (irPR)	irRC requires for partial response a decrease of at least 50% of the tumor burden compared to the baseline. This percentage change must be confirmed by a consecutive scan after no less than 4 weeks
Progressive disease (irPD)	irRC requires a total increase of tumor burden of at least 25% from the smallest reported tumor burden (nadir). However, irRC advice against the evaluation of progressive disease after just one cycle of immunotherapy as immune response requires more duration to establish a true and measurable antitumor effect. Also, immune response might mimic tumor flare and exaggerate the target lesion diameters, thus enhancing the percentage Increase.
Stable disease (irSD)	If percentage change shows an increase less than 25% from the smallest -recorded tumor burden (nadir) or a decrease less than 50% from baseline, patient status is recorded as stable disease and patient is usually followed for several cycles.
Limitations	No specific description on how to assess nodal disease. Bidimensional assessment reproducibility is lower than unidimensional assessments.

challenges. For instance, assessing a relatively large number of lesions per organ could be relatively time-consuming in cases of extreme tumor burdens [2, 21]. Furthermore, evaluation of excessive number of lesions impacts the reproducibility of the results [2, 21]. As such, Nishino et al. proposed a modification to the immune-related response criteria (irRC) in the light of RECIST 1.1 guidelines [2, 8, 21]. With regard to brain tumors, the Immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria is a set to tumor metrics to assess brain tumors in patients undergoing immune therapies.

Immune-Related Response Criteria

Arising from the heightened awareness by national and international communities as to the unique radiographic response patterns seen with vaccines and immunotherapeutics, modifications were made to the WHO and RECIST criteria in 2004 and 2005. In 2009, the immune-related Response Criteria (irRC) was published by Wolchok et al., based on the observed patterns in treatment response from phase II clinical trials in advanced melanoma patients who were receiving ipilimumab, a human monoclonal antibody that blocks cytotoxic T lymphocyte antigen–4 (CTLA-4). In this study [4], four patterns of treatment responses were recognized: (1) a decrease in the size of the lesion and without new tumors, similar to what is seen after conventional cytotoxic therapy; (2) stable disease after completion of treatment; (3) a delay in tumor response to therapy after an initial increase in total tumor burden; (4) the appearance of new lesions that precede tumor shrinkage.

In contrast to the WHO and RECIST criteria, irRC takes into account both the index and new measurable lesions to assess the "total tumor burden," a new concept from prior criteria, and compare to the baseline scan [4]. The irRC was derived from the WHO criteria and, therefore, the thresholds of response remain the similar. However, the irRC response categories have been modified from those of the WHO criteria [4]. According to the irRC, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated at the baseline. At every time point, the index lesions and any new measurable lesions are added together to accurately measure the total tumor burden (TTB) [(TTB = SPDindex lesions + SPDnew, measurable lesions)]. This is a major difference from the WHO criteria, which considers all new measurable lesions as progressive disease [5]. Further, a confirmatory examination at least 4 weeks from the initial scan documenting progression is required by the irRC prior to declaring progressive disease, as there can be a delay in response in patients on immunotherapy. In addition, decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The overall response according to the irRC is derived from time-point response assessments based on tumor burden, as described in Table 18.5.

The irRC does not mention the use of specific imaging modalities in the assessment of tumor response, although CT and MRI are typically used. However, research on novel PET radiotracers that incorporate amino acids, nucleotides, choline, and s-receptor to detect cell proliferation or cell death is being carried out [22]. Further, immune-related adverse effect can be sometimes identified with FDG-PET/CT and metabolic changes can be noted before the clinical symptoms to allow early change of the immunotherapy [1].

Immune-Related RECIST Criteria

The newly proposed irRECIST (Table 18.6) and adopted irRC [4] set thresholds for determining different possible responses, including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [2, 21]. Nishino et al. demonstrated that such changes did not result in any statistically significant variation of the response evaluation in melanoma patients receiving immunotherapy [2, 21]. They also demonstrated that irRECIST measurements were relatively more reproducible than the more involved bidimensional irRC measure-

ments [2, 21]. In 2017, the RECIST working group published the immune-RECIST (iRECIST) based on RECIST 1.1, where the definition of pseudoprogression was introduced. It is noteworthy, iRECIST criteria was used for response assessment to immunotherapy in trials for patients with brain metastases, by discerning between intra- and extracranial responses [24]. The criteria are summarized in Table 18.7 [25].

Immunotherapy Response Assessment for Neuro-oncology Criteria

The iRANO criteria is used to assess brain lesions in patients undergoing immunotherapy [3]. In order that misclassification of patients with stable or increasing tumor size and new lesions as progressive disease does not occur when the therapy is actually effective and the patient is receiving clinical benefit, the iRANO criteria was published. In brief, the iRANO follow the same guidelines as the RANO criteria. However, in those cases of appearance of disease in the absence of clinical deterioration within 6 months of immunotherapy, continuation of immunotherapy and repeat assessment in 3 months is recommended (Table 18.8). As with all current imaging assessment criteria, the iRANO guidelines will require future amendments, including the possible incorporation of volumetrics, advanced imaging sequences, and other types of imaging analytics. A recent study by our group demonstrated that radiomics can discriminate between patients who have pseudoprogression versus true tumor progression with high sensitivity (97%), specificity (79%), and accuracy (95%) in patients with glioblastoma [26]. The iRANO criteria also added specific guidance for the determination of progressive disease in patients with brain metastases undergoing immunotherapy. The criteria for iRANO-BM is summarized in Table 18.9 [3].

It's crucial for clinicians to indicate and conclude an underlying tumor progression during the course of immunotherapy. It has been shown that early radiographic progression in patients who ultimately derive clinical benefit actually stabilize or even improve within 3 months. The

Table 18.6 irRECIST response criteria [23]

Complete response (CR)	Complete resolution of nonnodal lesions and < 10 mm short-axis for lymph nodes. No confirmation necessary
Partial response (PR)	≥30% decrease in tumour burden
Stable disease (SD)	Does not meet criteria for irCR/irPR/irPD
Progressive disease (PD)	≥20% increase in tumor burden relative to nadir and a minimum absolute increase of 5 mm; new lesions Confirmation of PD via a subsequent scan ≥4 weeks later to detect delayed responses is required

Table 18.7 iRECIST response criteria [25]

Type of response	Definition
Complete response (iCR)	Total remission of all target and nontarget lesions, including the lack of appearance of new lesions, confirmed by a consecutive imaging evaluation performed ≥4 weeks after the first one
Partial response (iPR)	A decrease of at least 50% in the total tumor burden compared to baseline, confirmed by a consecutive investigation performed after ≥4 weeks
Stable disease (iSD)	The change of the total tumor burden is reduced to less than 50% when compared with baseline, or increased to less than 20% when compared with nadir.
Unconfirmed progressive disease (iUPD)	Increase in the total tumor burden of at least 20% compared to nadir.
	The term "unconfirmed" refers to the initial dimensional increase that can be detected after 1 cycle of immunotherapy; further confirmation at imaging is needed.
Confirmed progressive disease (iCPD)	Increase in the total tumor burden of at least 20% when compared to nadir. A further increase in the tumor burden (≥5 mm) or a further increase of nontarget lesions or the appearance of new target or nontarget lesions must be noted in the next assessment after the examination in order to confirm disease progression.

Nadir: The smallest value of the sum of the longest diameters of target lesions recorded during therapy

Table 18.8 Summary of Immunotherapy Response Assessment in Neuro-Oncology (iRANO) [3]

Method of assessment of lesion	Bidimensional assessment of the longest perpendicular diameters of all enhancing lesions.
Total tumor burden evaluation	Sum of product of the longest diameters of all target lesions
New target lesions (appearing more than 6 months after initiation of immune therapy)	Target lesions appearing more than 6 months after the initiation of therapy are considered a sign of true tumor progression.
New target lesions (appearing less than 6 months after initiation of immune therapy)	Target lesions appearing less than 6 months with no associated tumor-related clinical decline of patient should be followed for at least 3 more months taking in reference the time point at which progression was initially reported.
Imaging modalities	MRI is the gold standard in evaluation of intracranial neoplasms; however, the criteria could be also used to evaluate CT scan with relative restrictions.
Target lesions criteria	Target lesions should measure at least 10 × 10 mm. A maximum of five target lesions could be selected.
Time-point response assessment	The growth kinetics of target and new lesions are determined. Percentage change of tumor growth is then calculated referencing baseline assessment as well as the smallest reported tumor burden (nadir).
Types of overall response	Complete response (CR), partial response (PR), minor response (MR), stable disease (SD), and progressive disease (PD).
Complete response	Requires 100% decrease in tumor burden, including total remission of all enhancing and nonenhancing lesions for two consecutive scans at least 4 weeks apart. With no new lesions, no clinical decline and no more than the physiological dose of steroids.
Partial response	Requires a decrease of at least 50% or more in tumor burden of enhancing lesion, with stable nonenhancing lesions and T2FLAIR lesions for two consecutive scans at least 4 weeks apart. With no new lesions, no clinical decline and a stable or decreased dose of steroids.
Minor response	Only considered in assessment of low grade gliomas, requires 25–49% decrease in the sum of the product of bi-perpendicular diameters of T2FLAIR lesions. With no new lesions, no clinical decline and stable or decreased dose of steroids.
Progressive disease	In case of malignant and low grade gliomas at least a 25% increase in the tumor burden putting in reference the smallest recorded tumor burden (nadir) while in case of brain metastases at least a 20% increase in the tumor burden, putting in reference the smallest recorded tumor burden (nadir). Also, appearance of new lesions after 6 months of start of immune therapy, remarkable clinical decline, or remarkable worsening of T2FLAIR lesions.

Table 18.9 summary of immune therapy response assessment in brain metastases (iRANO-BM) [3]

Complete response	Disappearance of all the enhancing target and nontarget lesions for ≥4 weeks; no new lesions; no steroids; clinically stable or improved
Partial response	≥30% decrease in the sum of the longest diameters of all target lesions for ≥4 weeks; no new lesions; stable or decrease steroid dose; clinically stable or improved
Minor response	NA
Stable disease	Does not qualify for complete response, partial response or progressive disease
Progressive disease	≥20% increase in the sum of the longest diameters of target lesions; or unequivocal progression of enhancing nontarget lesions; or new lesions; or substantial clinical decline

iRANO working group has come up with an algorithm to guide assessment of progressive disease in neuro-oncology patients undergoing immunotherapy to decrease the likelihood of prematurely stating progressive disease in patients with PsP or delayed response (Fig. 18.3) [3].

Future Directions for Immune Therapy Imaging Assessment

Although irRECIST and irRC represent an improvement over the conventional WHO criteria and RECIST to evaluate tumor response in immunotherapy, there remains limitations and challenges and further refinements are warranted

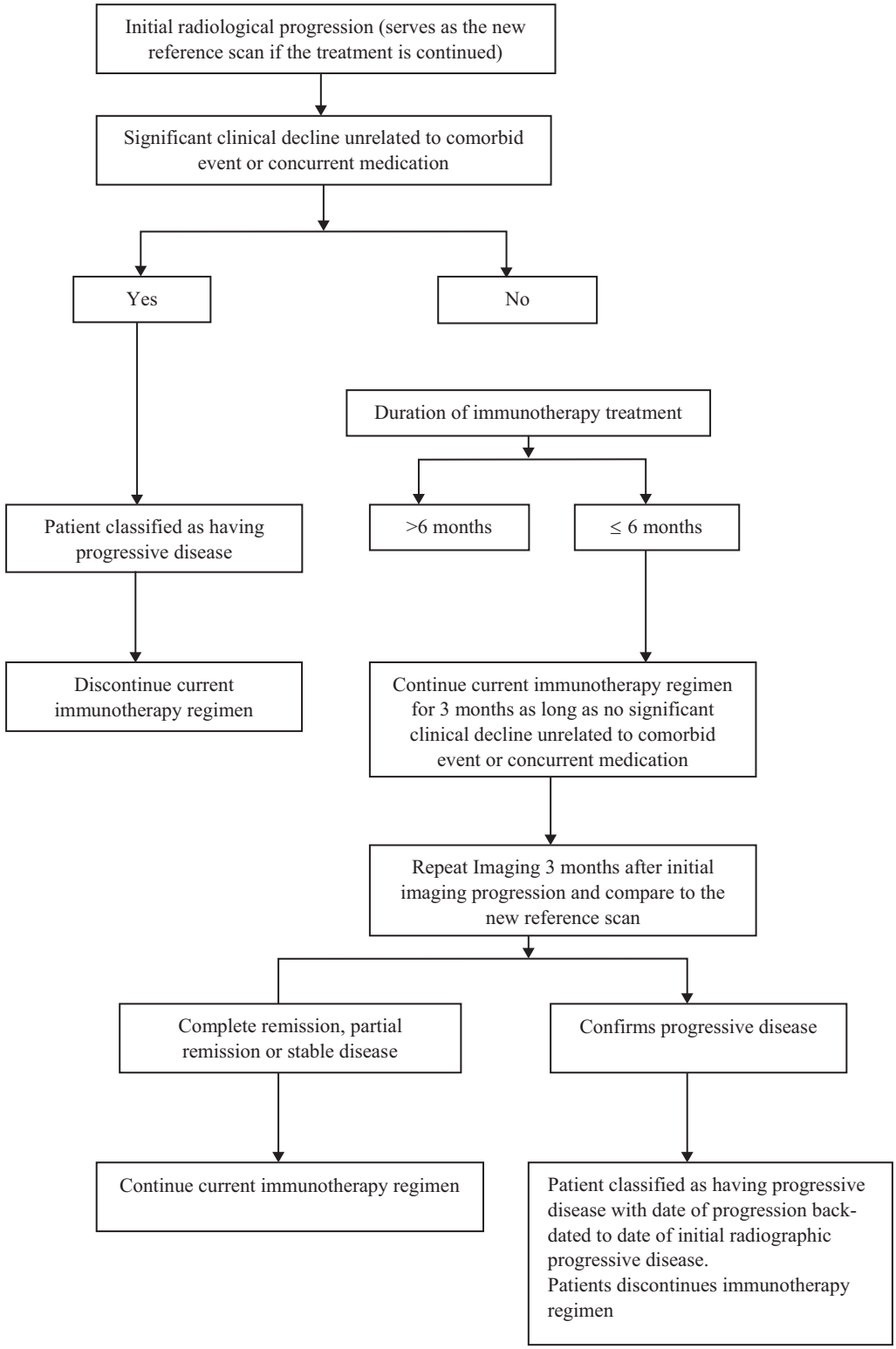

Fig. 18.3 iRANO treatment algorithm for the assessment of progressive imaging findings in neuro-oncological malignancies [3]

[4]. Plans for improving imaging response criteria include volumetric (3D) imaging, dynamic contrast imaging, and functional (molecular) imaging. Radiomics is a more recent developing field within imaging that can help in more precise tumor assessments that are unrelated to tumor size or burden. Radiomics has the potential to be a noninvasive digital biopsy technique that is spatially guided and that can quantify T-cell infiltration of tumors and reflect the entire tumor burden by providing information on each cancer lesion, in contrast to the traditional biopsy that represents only a sample of the tumor. Quantitative imaging biomarkers can support personalized design of immunotherapy interventions and longitudinally monitor and assess immune checkpoint blockade response [27, 28]. Radiomics can be the key to help discriminate between pseudoprogression and true progression, which are significantly difficult to differentiate radiographically. Multiple studies conducted by our group demonstrated 5 texture features were able to robustly predict whether a GBM patient had pseudoprogression or true progression [29–32]. Roger Sun et al. reported on an eight-feature radiomics-based signature of CD8 cell expression, which they developed by use of CT images. The radiomics signature was also shown to be associated with clinical outcomes in patients treated with anti-PD-1 or anti-PD-L1 immunotherapy in an independent cohort [28]. Further, radiogenomics, the linkage between imaging phenotypes and tumor genomics, might help develop more robust stratification and end-point imaging biomarkers for immunotherapy and molecular targeted clinical trials.

Imaging in Immune-Related Adverse Events

Immune-related adverse events (irAE) can represent a serious complication and can be challenging for any imager. Thus, it is important to be aware and take into consideration the possibility of its occurrence so that early management is undertaken [33]. Treatment of adverse events is typically based on published guidelines and includes delaying treatment dosing, administering corticosteroids, or terminating therapy depending on the severity of the event. However, success in outcome lies heavily on correctly identifying and interpreting these complications.

Severe colitis has the highest mortality and worst outcome associated with irAE [33]. Because of the possibility of misdiagnosis of autoimmune colitis, the patient can take antibiotic therapy instead of corticosteroid therapy, which can result in a delayed diagnosis and complication by colonic bowel perforation [33]. Other common immune adverse events are sarcoid-like adenopathy and pancreatitis. It is important to recognize and accurately diagnose these events in order to avoid misdiagnosis for metastatic disease [1]. There are also many other events which can occur as a result of immunotherapy, for example, autoimmune hepatitis, pneumonitis, thyroiditis, myocarditis, pericarditis, temporal arteritis, conjunctivitis, sarcoid-like reaction such as lymphocytic vasculitis, organizing pneumonia, and fasciitis [34, 35]. Autoimmune hepatitis may be seen as periportal edema and hypoattenuation of the edematous liver parenchyma in CT. However, these finding are not specific to autoimmune hepatitis and can be seen in the setting of cancer immunotherapy [1].

Immunotherapy-induced pneumonitis is an uncommon yet potentially fatal irAE that requires clinical suspicion and early detection. A recent study by our group demonstrated that specific radiomic imaging features (extracted from baseline CT scans) were able to predict those patients that will subsequently develop pneumonitis prior to the initiation of immune therapy (Fig. 18.4). This study highlights the ability of imaging to identify those patients that might be most susceptible to irAE before the irAE even occurs [36].

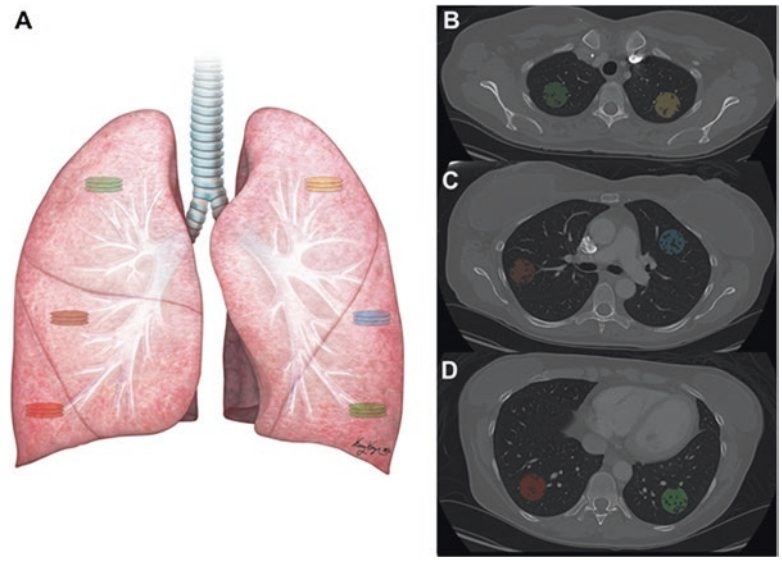

Fig. 18.4 (**a**) An illustration of the outlined regions of interest (ROIs) in the lungs. An ROI containing three consecutive slices, taken in each lobe in the right lung and ROIs outlined in the left lung correspond to the same level as the right lung ROIs. Postcontrast lung CT images depicting the segmented ROIs in upper (**b**), middle (**c**), and lower, (**d**) sections of the right and left lungs. Each ROI is outlined with a different label. Contrast-enhancing vessels from the ROIs were subtracted. Radius of the ROI ranged between 14 and 15 mm

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The Microbiome in Immuno-oncology

19

Joseph Toker, Reetakshi Arora, and Jennifer A. Wargo

Abstract

The field of cancer therapy has been revolutionized through the use of immunotherapy, and treatment with these therapies now spans from early to late stage, and even into prevention. However, there are still a significant proportion of patients who do not derive long-term benefit from monotherapy and even combined therapy regimens, and novel approaches are needed to enhance therapeutic responses. Additionally, ideal biomarkers of response to immunotherapy are lacking and are critically needed. An emerging area of interest in immuno-oncology (IO) is the microbiome, which refers to the collection of microbes (and their genomes) that inhabit an individual and live in symbiosis. There is now evidence that these microbes (particularly those within the gut) impact host physiology and can impact responses to immunotherapy. The field of microbiome research in immuno-oncology is quickly emerging, with the potential use of the microbiome (in the gut as well as in the tumor) as a biomarker for response to IO as well as a therapeutic target. Notably, the microbiome may even have a role in toxicity to therapy. The state of the science in microbiome and IO are discussed and caveats and future directions are outlined to provide insights as we move forward as a field.

Keywords

Microbiome · Checkpoint blockade · Immunity

Introduction

Along with the rise of immunotherapy and next-generation sequencing, the microbiome has recently emerged as a promising frontier in oncology. Influenced by genetics, geography, diet, and medication, the microbiome is the genetic network composed of trillions of microbes that coexist within living organisms [1]. The human intestines alone are occupied by thousands of different microbial taxa, most of

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which are symbiotic, rather than pathogenic [1]. These microbes include archaea, fungi, viruses, protozoa, and bacteria [2]. Gastrointestinal microbes help metabolize toxic and complex compounds, and they contain critical enzymes that synthesize vitamins B and K [3, 4]. Substantial evidence, made possible by next-generation sequencing, also indicates that the microbiome is involved with regulating the immune system [3].

History of the Microbiome

During the fourth century, the Chinese scholar Ge Hong indirectly acknowledged the clinical significance of the microbiome [1]. In the 1680s, Antonie van Leewenhoek observed the profound diversity of the human microbiome by comparing oral and fecal samples from healthy and sick patients [5]. For his work in immunology, the 1908 Nobel Prize in Physiology or Medicine was awarded to Élie Metchnikoff, who postulated that microbes could confer clinical benefits [6]. And in the 1950s, Ben Eiseman famously used fecal retention enemas to treat fulminant pseudomembranous enterocolitis diarrhea [7]. Fecal material transplants (FMTs) application to cancer is much more contemporary, though several clinical trials are currently employing FMT to study the interface between the microbiome and response to cancer therapy, most notably checkpoint-blockade immunotherapy. These trials investigate modulation of the gut microbiome in cancer and cancer therapy, as well as modulation of the gut microbiome to prevent cancer treatment–related toxicity.

Characterizing the Microbiome

Several approaches can be used in studying the microbiome, with inherent advantages and disadvantages to each approach (Fig. 19.1). The largest proportion of microbes within the body reside within the gut, and the "gut" microbiome is most commonly profiled by obtaining fecal samples or rectal swabs. In addition to studying the gut microbiome, microbiota in other "niches" in the body may also be assessed, including in tumors of patients with cancer, where biopsies and surgical resection samples may be profiled to characterize the intratumoral microbiome [8]. A frequent approach to characterizing the microbiome utilizes sequencing of the 16S rRNA gene, which exists only in prokaryotic cells. All bacteria possess a full-length 16S sequence that contains extremely variable regions known as V-regions. These regions are amplified using PCR, sequenced, and compared to reference databases, such as Greengenes, SILVA, and the Ribosomal Database Project [9]. V4 is particularly variable, and its sequence can enable precise discrimination across most bacterial domains [10]. While the 16S sequencing technique is relatively fast and low-cost, it is subject to copy number variations and PCR primer and amplification biases that limit the precision of taxonomic identification [11]. 16S sequencing also does not shed light on the functional roles of bacteria within the microbiome [11], but it does usefully generate data that can determine alpha and beta diversity [12].

A more comprehensive means of characterizing the microbiota involves whole genome sequencing (or metagenomic sequencing). This affords the opportunity to query not only bacteria, but viruses, fungi, protozoa, and other microbes within a given sample [12]. Metabolomics, a powerful molecular method to understand the dynamics and metabolic relationships between the host and the microbiota, quantifies the metabolites produced by microbes [13] in contrast to metaproteomics, which quantifies protein and peptide expression [14]. These methods have helped formulate the Human Microbiome Project, an NIH initiative that aims to elucidate the interface between the microbiome and human health [1]. Culturing of specific microbes from samples is also an approach that is being utilized widely.

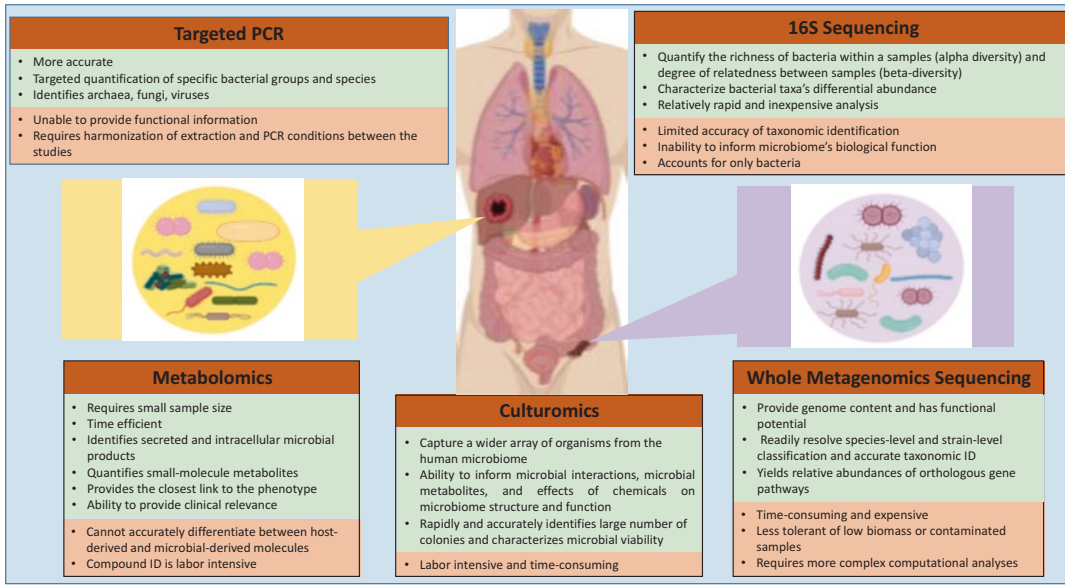

Fig. 19.1 Microbiome profiling: Describes several approaches that can be used in studying the microbiome, with inherent advantages ( ) and disadvantages ( ) to each approach

The Microbiome in Disease States

Disruption of the homeostasis of microbial communities is termed dysbiosis and may be associated with a decrease in the diversity of the microbes within a particular niche. Dysbiosis has been linked to numerous disease states, including neurological, metabolic, cardiovascular, and gastrointestinal diseases [15–19]. Malnutrition can lead to dysbiosis of the gut microbiome, which can weaken vaccine responses and predispose individuals to infection [20–22]. Inflammatory diseases, such as type I diabetes, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease (IBD), are also associated with dysbiosis of the gut microbiota [23]. Recent large case-control studies additionally demonstrate a connection between dysbiosis and cancer (such as through repeated antibiotic use), with the development of both gastrointestinal (GI)-tract and non-GI-tract tumors [19]. We do not yet have a comprehensive understanding of the mechanistic underpinnings behind this.

The Role of Gut and Tumor Microbes in Carcinogenesis

Though gastrointestinal commensal microbes are critical to normal physiology, pathogenic microbes may contribute to the development of cancer via a number of different processes. Within the liver and biliary tracts, microbes produce secondary bile acids that may damage DNA and affect NKT cell function, thereby leading to tumorigenesis [24, 25]. In the breast, microbiota can adversely affect the balance in estrogen metabolism [26]. Within the colon, dysbiosis might alter signaling pathways and thereby induce inflammation or impairment of the immune system. The systemic action of microbial metabolites and cytokines may combine with the interactions between pathogen associated molecular patterns (PAMPs) and pattern-recognition receptors (PRRs) to increase antitumor immune function [27, 28].

Several intestinal bacteria are known to be associated with oncogenesis. Among others, they

include *Salmonella typhi* in biliary cancer [29] and *Helicobacter pylori* in gastric cancer [30]. Most of these bacteria tend to induce chronic inflammation that leads to carcinogenesis, but others, such as *H. pylori*, are genotoxic and thereby affect intracellular signaling related to the growth and proliferation of mucosal cells [31]. *H. pylori* is implicated in both MALT lymphoma and gastric adenocarcinoma; the World Health Organization lists it as a class I carcinogen [30]. *Campylobacter jejuni* and *E. coli* have been associated with carcinogenesis in murine models by virtue of their production of certain metabolites: cytolethal distending toxin [32] and colibactin [33–35], respectively. The abundance of both *F. nucleatum* and *C. difficile* is strongly associated with colorectal cancer (CRC) [36]. *F. nucleatum* promotes the growth of CRC cells by activating β-catenin–Wnt signaling pathway and inducing oncogenic gene expression through FadA adhesion virulence factor [37].

In addition to the gut, microbes have been found to occupy tumors in lung, colon, breast, gastric, ovarian, and prostate cancers [38–43]. Tumors in the respiratory system, enteric tract, and reproductive tract are normally exposed to microorganisms, and bacterial translocation from the GI tract to other organ systems can occur in both healthy and sick humans [44]. Rodent models have demonstrated that systemically administered bacteria, particularly anaerobes and facultative anaerobes, can infiltrate and thrive within the tumor microenvironment [41, 45]. There is also growing evidence suggesting that these microbes can influence response to cancer therapy [46–49].

The Influence of Microbes on Host Immunity and Anticancer Responses

Microbes at different sites may influence antitumor immunity, including microbes that exist within tumors themselves as well as microbes within the gut (Fig. 19.2). As noted previously, intratumoral microbes have been identified across cancer types and have the capacity to influence host immunity (and antitumor immunity).

The mechanism through which microbes enter tumors is incompletely understood; however, it is plausible that inflammation within the gut could influence mucosal permeability resulting in translocation of microbes into the bloodstream and ultimately into the tumor microenvironment, where clearance of microbes may be impaired in the setting of hypoxia/altered metabolism and immune-excluded tumors [50]. These intratumoral microbes can further induce an immunosuppressive microenvironment via recruitment of myeloid-derived suppressor cells (MDSCs) and the production of immunosuppressive cytokines [41, 51]. In addition to influencing antitumor immunity, intratumoral microbes may have direct effects by altering the impact of chemotherapy on tumor cells themselves [52, 53].

Preclinical models suggest that by targeting these intratumoral microbes, one may sensitize tumors to treatment with immune checkpoint blockade [41], and clinical trials interrogating co-targeting of intratumoral microbes and antitumor immune responses are currently under development. However, the presence of intratumoral microbes may also be associated with enhanced responses to therapy in some cases [54, 55], and additional studies are needed to better understand these interactions and are likely to be context dependent. An example of this is in virally driven tumors, such as Merkel cell carcinoma, HCV, and HBV-associated hepatocellular carcinoma [54], where enhanced responses are noted likely owing to an element of tumor-foreignness and recognition by the immune system.

In addition to intratumoral microbes, microbes within the gut may profoundly impact overall host immunity as well as antitumor immune responses. This is fairly intuitive, as all along the vast surface area of the gastrointestinal tract, trillions of microbes interact with a rich network of infiltrating immune cells and intestinal epithelial cells (IECs) just on the other side of the gastrointestinal mucosa. It is becoming apparent that changes in gut microbiota composition and density can impact local immune responses that can

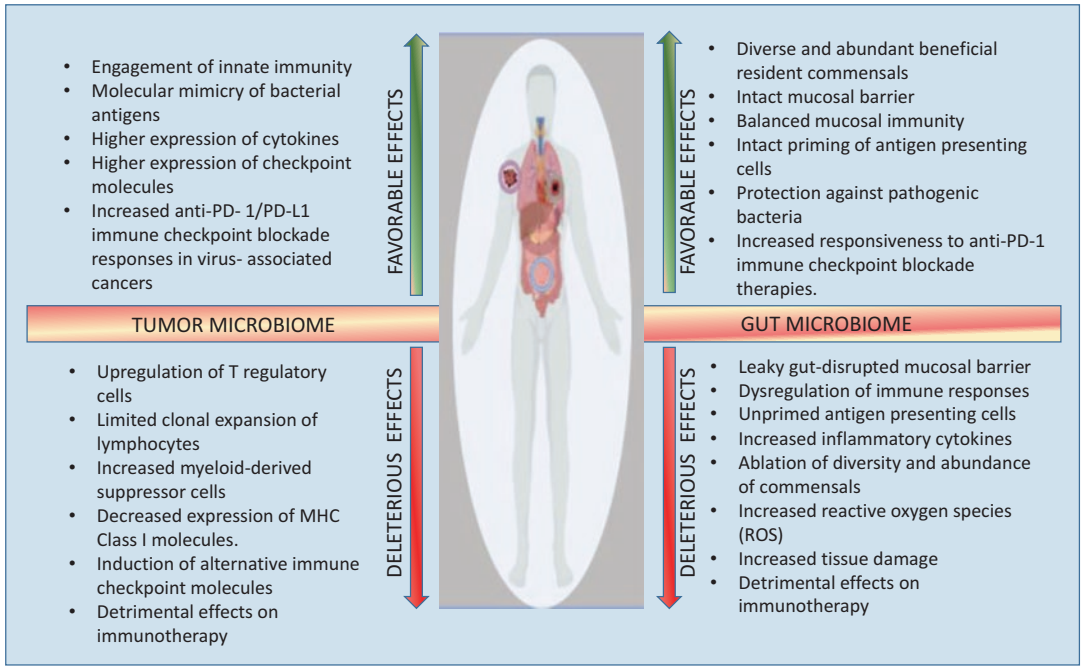

Fig. 19.2 Positive and negative impact of tumor and gut microbiota. Tumor and Gut microbiome may impact systemic immunity and responses to cancer therapies. Complex interplay of immune response and favorable or detrimental effects skew the balance in different cancer types and outcome of cancer therapies

also alter the immunity and inflammation in distal organs [56].

Several mechanisms exist through which microbe host interactions may shape immunity. Commensal bacteria influence the systemic innate immunity via bacteria-derived molecules, including microbe-associated molecular patterns (MAMPs) and PAMPs. These are recognized by PRRs on innate immune cells initiating a cascade of MyD88-dependent pathway [57]. Activation of these receptors may suppress inflammatory responses and promote immunological tolerance to normal microbiota components and may also facilitate recognition of different general MAMPs to trigger innate intestinal immunity [58, 59] resulting in a signal cascade that in turn can activate variety of genes coding for chemokines, cytokines, acute phase proteins, and effectors of humoral immune response [5, 60]. Toll-like receptors (TLRs) from the membrane of epithelial and lymphoid cells of the small intestine are crucial to educate the immune system regarding differentials recognition of commensal versus pathogenic microbes. Bacterial metabolites, such as short-chain fatty acids (SCFAs), the products of dietary fiber fermentation by the microbiota, have been implicated in augmentation of the systemic immunity [61].

The interaction of immune cells and these commensals at the level of the gut is facilitated by intraepithelial lymphocytes and IECs containing Paneth cells, which secrete antimicrobial peptides. Underneath these is the lamina propria, which hosts immune cells, including antigen-presenting cells, innate lymphoid cells, CD4+ and CD8+ T, and B cells. Adaptive immune responses are further shaped by the intricate interaction between the gut microbes and the gut-associated lymphoid tissues (GALT) and mesenteric lymph nodes (mLNs). The mLN serves as the site for the differentiation of naïve T cells. Antigen-presenting cells (APCs) like dendritic cells travel to mLNs once activated, via interdigitation of dendrites through mucosal layer or transphagocytosis, where they interact and stimulate naïve T cells to form CD4+ T regulatory cells

(Tregs) and T helper 17 (Th 17) cells or directly stimulating CD8+ T cells. Once primed, these effector T cells then enter the systemic circulation to facilitate immune responses at distant sites or at the site of the tumor. There is increasing evidence that dysbiosis of the microbiota within the gut and their metabolites might skew the balance of anti-flammatory and pro-inflammatory cytokines and disrupt the ratios of regulatory T cells and T-helper 17 cell subsets [62, 63], thereby affecting systemic susceptibility to infections, altered responses to vaccines, and antitumor immunity.

Evidence Regarding the Role of the Gut Microbiome in Response to Immunotherapy

Some of the earliest work interrogating the impact of gut microbes on response to immunotherapy was performed in preclinical models [64, 65]. In one of these studies, investigators demonstrated that identical mouse strains purchased from different vendors (Taconic Farms and Jackson Laboratories) exhibited distinct microbiome and responded differentially to treatment with immune checkpoint blockade targeting the programmed death receptor 1 (PD-1) to treat melanoma tumors [64]. Parallel studies were performed interrogating response to immune checkpoint blockade targeting cytotoxic lymphocyte antigen 4 (CTLA-4) [65]. In these studies, mice with "favorable" gut microbiome had more functional APCs, such as dendritic cells, facilitating priming of antigen-specific T cell responses [64].

These two preclinical studies laid the groundwork for subsequent observational studies in clinical cohorts. Several groups interrogated the relationship of gut microbes and response to immune checkpoint blockade in patients with melanoma and other cancers [47–49, 66, 67]. In these studies, distinct microbial signatures were observed in the gut microbiota of responders vs nonresponders to immune checkpoint blockade across a range of cancer types [47, 48, 67]. Furthermore, it was noted that treatment of patients with antibiotics administered at the time of checkpoint blockade initiation impaired success of anti-PD-1-based therapy [68], suggesting that evidently disrupting the gut microbiota (dysbiotic gut) may negatively affect therapeutic responses. Notably, several of these studies incorporated fecal microbiota transplant (FMT) from responding versus nonresponding patients into germ-free mouse models, demonstrating that the phenotype could be recapitulated and that gut microbes could be manipulated to enhance responses to therapy. However, despite enthusiasm over the identification of such signatures in responders versus nonresponders, there was little overlap between the specific microbial taxa associated with response across the cohorts [69], highlighting that additional analyses (and studies) will be required for validation and ultimately to help guide composition of optimal microbial consortia.

In addition to associations with response, differential gut microbiota "signatures" have also been demonstrated in patients with immune checkpoint-associated colitis [66, 70] as well as toxicity to other forms of treatment, such as graft-versus-host disease in the setting of stem cell transplant [71–74].

Modulating the Microbiome to Enhance Responses in Immuno-oncology

Based on these findings, there is tremendous enthusiasm in modulating the microbiome in hopes to enhance responses to immunotherapy. Several different approaches may be employed for microbiome modulation (Fig. 19.3) and have been/are being used in the treatment of other diseases, though optimal strategies in the setting of treatment for cancer are yet unknown.

Numerous factors may influence gut microbes, including host genetics, diet, medications, and immunity (Fig. 19.3). Accordingly, any of these modifiable factors may be manipulated to modulate the composition of the gut microbiota to influence disease states, including cancer. This

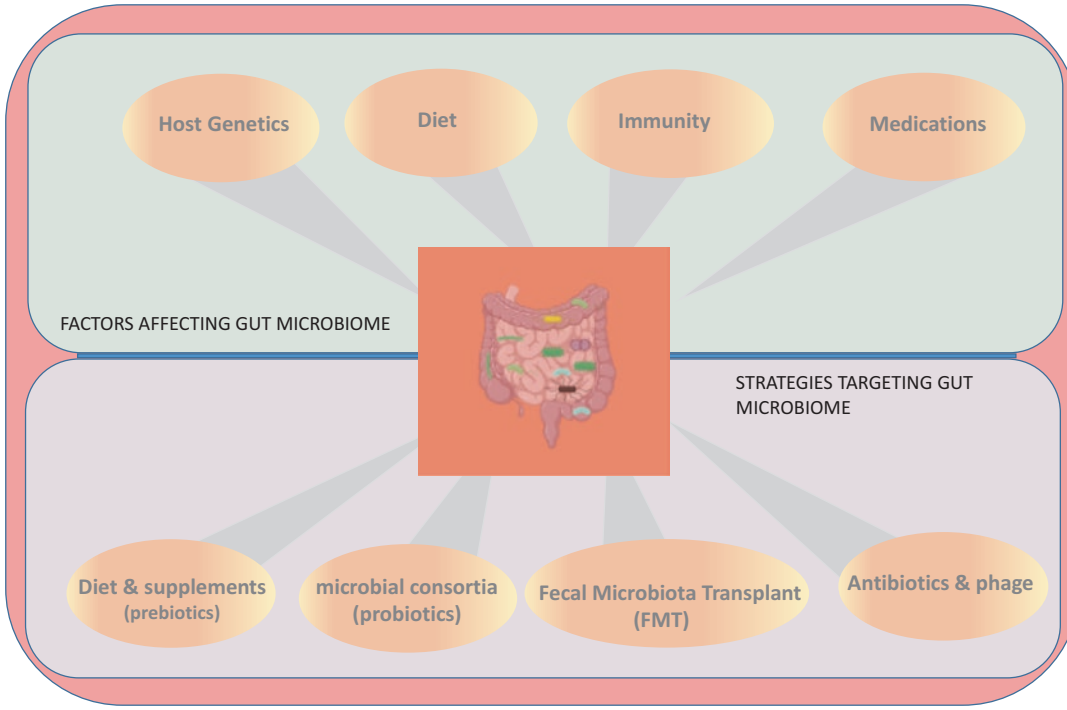

Fig. 19.3 Factors affecting the gut microbiome and strategies to target gut microbiota. Various factors like host genetics, dietary lifestyle, immune state, and medications can immensely impact gut microbiome. Understanding of these factors and interaction with gut microbiota facilitated different strategies to modulate it via FMT, the administration of probiotic or bacterial consortia, varying dietary habits, using tailored antibiotic therapies, or bacteriophages

includes the use of dietary manipulation and administration of prebiotics, microbial consortia, targeted antibiotic/phage approaches, and administration of donor fecal material (Fig. 19.3).

Each of these approaches have been tested in noncancer indications with variable success in impacting disease states [1, 75–80], and they are now being employed to modulate gut microbes in the setting of cancer therapy. Clinical trials incorporating modulation of gut microbes that are currently underway utilize strategies such as fecal material transplant (FMT) from complete responders to immune checkpoint blockade (NCT03643289, NCT03595683, NCT03341143, NCT03772899, NCT02600143, NCT03353402) as well as several trials using limited microbial consortia (NCT03595683, NCT03637803). Early signals exist regarding potential activity of such approaches [81]; however, there are numerous considerations in the design and implementation of such studies which must be considered [82].

Conclusions and Future Directions

The age of the microbiome is upon us, and microbes within the tumor and gut may profoundly impact overall physiology, carcinogenesis, and response to cancer immunotherapy. As we move forward as a field, we must consider microbes and their contributions, as well as their interactions with the host. Though initial studies are underway exploring manipulation of the microbiome, we must work together as a global team to learn how optimal strategies enhance responses to therapy (and ultimately to prevent cancer altogether).

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Conflict of Interest J. Wargo is an inventor on a US patent application (PCT/US17/53.717) submitted by the University of Texas MD Anderson Cancer Center that covers methods to enhance immune checkpoint blockade responses by modulating the microbiome. He reports compensation for speaker's bureau and honoraria from Imedex, Dava Oncology, Omniprex, Illumina, Gilead, PeerView, Physician Education Resource, MedImmune, and Bristol-Myers Squibb. He serves as a consultant/advisory board member for Roche/Genentech, Novartis, AstraZeneca, GlaxoSmithKline, Bristol-Myers Squibb, Merck, Biothera Pharmaceuticals, and Microbiome DX. J. Wargo also receives research support from GlaxoSmithKline, Roche/Genentech, Bristol-Myers Squibb, and Novartis.

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New Developments in the Use of Patient-Reported Outcomes in Cancer Patients Undergoing Immunotherapies

20

Tito R. Mendoza

Abstract

In a previous chapter, how best to measure symptoms was discussed, the desirable properties of a psychometrically valid symptom assessment tool were listed, available symptom assessment tools were reviewed, methods to assist in the interpretation of patient-reported outcomes (PRO) data were provided, and the current use of PROs in immunotherapy was described. Two areas for further research were also identified. These two areas were (1) deciding on the frequency of administration of symptom assessment and (2) determining the adequacy of the chosen symptom list to cover both known and unknown effects of immunotherapy. This brief update provides new developments on these two critical issues that are of significant concerns to researchers and clinicians who are investigating the use of immunotherapies either singly or in combination in cancer patients.

Keywords

Patient-reported outcomes · Symptoms · Immunotherapy · Cancer · Recall period · Qualitative research

Introduction

Standard clinician-graded toxicity ratings made during clinical trials often do not correlate well with patient report of symptomatic adverse events [1]. Because symptoms are subjective reports, patients are the best source of information. Patient-reported outcomes (PRO) provide patients the opportunity to describe what he or she is experiencing during and after treatment. Those who use validated PRO measures in clinical trials to obtain the patient's assessment of the severity and impact of treatment-related symptoms (e.g., clinicians, patients, regulators, and payers) increasingly find that capturing the patient's experience of the effects of new therapies adds critical information for evaluating these therapies and for judging the value of one therapy versus another. This is especially true when new therapies provide only small increases in overall survival or time to progression or are effective for only a modest percentage of the patients who receive them. PROs are considered an essential component of oncology drug development, with-

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out which clinicians and regulators have an incomplete picture of how patients are affected by a new agent [2].

Immune checkpoint inhibitors are a new class of immunotherapeutic agents that remove the inhibitory signal provided to immune T cells so they can launch a cytotoxic attack on tumor cells. Checkpoint inhibitors such as ipilimumab, nivolumab, pembrolizumab, and atezolizumab can be given as monotherapy or combined with other immune checkpoint inhibitors, with targeted therapies, or with standard cancer treatments such as radiotherapy or chemotherapy [3]. Whereas much is known about the adverse events (AEs) of standard-of-care therapies for cancer, much less is known about the symptomatic toxicities associated with immune checkpoint inhibitors (immune-related adverse events, or irAEs) either singly or as combination therapies. Because symptomatic toxicities are irAEs that are best known through patient reporting, it follows that PROs if possible should be included in every immunotherapeutic clinical trial. However, incorporating PROs in these studies requires some thoughtful considerations. In a previous paper, two areas that needed additional guidance were identified. These two areas were (1) deciding on the frequency of administration of symptom assessment and (2) determining the adequacy of the chosen symptom list to cover known and unknown effects of immunotherapeutic agents or their combinations. This brief update provides new developments on these two critical issues that are of significant concerns to researchers and clinicians who are investigating the use of immunotherapies in cancer patients.

How Often Should We Ask Patients About Their Symptoms?

Addressing the frequency of symptom assessments requires a delicate balance between maximizing symptom information and minimizing patient burden. Often, patients are asked about their symptoms as frequently as possible but with the consequential result of decreased adherence. Closely related to the issue of deciding on the frequency of assessment is the recall period of a particular PRO. For example, there are PROs, such as the European Organization for Research in Treatment of Cancer Quality of Life (EORTC-QLQ) [4], that ask patients about their symptoms and quality of life over "the past week." On the other hand, there are PROs, such as the MD Anderson Symptom Inventory (MDASI) [5], that have versions with both recall period of "the past 24 hours" and "the past week."

If there is a PRO with "the past 24 hours" recall period, should it be administered on a daily basis? How about administering a PRO with "the past week" on a weekly basis? One factor to consider is the quality of the data. Specifically, how do ratings of a set of symptoms using the validated patient-reported outcome version of the Common Terminology Criteria – Adverse Events (PRO-CTCAE) [6] administered daily compared to ratings made by the same patient but based on patient's recall of the past week, the past 2 weeks, or the past 3 weeks? One metric to use is to compare, for example, the maximum daily symptom rating made over 7 days with the symptom rating using "the past week" recall. Similarly, the maximum daily symptom rating made over 14 days can be compared with the symptom rating using the "past 2 week" recall period. Mendoza et al. [7] reported that daily ratings made over 7 days were comparable with ratings over the past week. However, there were differences found when daily ratings made over 14 days were compared with symptom ratings using the "the past 2 week" recall period. Likewise, there were differences found when daily ratings made over 21 days were compared with symptom ratings using the "the past 3 week" recall period. Similar results were seen when comparing daily ratings made over 28 days with symptom ratings using the "the past 4 week." In summary, longer recall periods of 2, 3, or 4 weeks may be biased in the direction of underestimating the true worst level of symptomatic adverse events.

The most critical factor to consider in determining the frequency of symptom assessments is the objectives of a research study or the context

of a clinical study. For example, Aloia et al. [8] administered the Health Outcomes Recovery Survey, a PRO that measures quality of recovery and patient satisfaction to surgical inpatients daily for five postoperative days. Clearly, symptoms of surgical inpatients, specifically pain, should be frequently tracked.

Daily assessments may not be necessary to understand the pattern of patient-reported symptoms during radiation therapy and concurrent chemotherapy for patients with head and neck cancer. Rosenthal et al. [9] followed a cohort of patients who completed the head and neck module of the MDASI weekly during the course of radiation therapy–based treatment. With weekly assessments, the study was able to identify the pattern of both local and systemic symptoms. The study found that the degree of symptom interference with daily activities was temporally distinct and was marked by increased magnitudes and shifts in individual symptom rankings, as well as identifiable symptom clusters. Wang et al. [10] administered the multiple myeloma module of the MDASI to patients weekly for up to 6 months beginning 3 months post transplant. The study was able to identify a group of patients with consistently higher symptom burden over that time period.

In summary, if the disease-related or treatment-related effects on symptoms are immediate, a more frequent assessment may be needed. On the other hand, if the disease-related or treatment-related effects on symptoms change more slowly, assessment should be less frequent.

How Do We Know Which Symptoms to Ask?

Once we have decided on the frequency of assessment, another critical consideration is to determine which symptoms to ask patients. Many of the symptomatic toxicities patients are asked originated from the list of immune-related adverse events (irAEs) associated with immunotherapeutic agents. However, we cannot be sure that we have adequately covered known and unknown effects of immunotherapeutic agents or their combinations from this list of irAEs. These irAEs are what clinicians have observed and there may be some symptomatic toxicities that may not be readily apparent solely by observation.

Qualitative or cognitive interviewing [11] is considered to be the gold standard in uncovering a set of symptoms associated with new immunotherapeutic agents or their combinations. As recommended in the FDA PRO Guidance, conducting qualitative interviews on patients with the target disease is a necessary first step to establish the content validity of a PRO. For example, although the Lung Cancer Symptom Scale – Mesothelioma (LCSS -Meso) is a validated PRO to measure the symptoms of malignant pleural mesothelioma (MPM) [12], Williams et al. [13, 16] found additional symptoms via qualitative interviewing that are considered important for patients with MPM. It should be noted that the original LCSS-Meso was not developed via qualitative interviews. However, with the emergence of qualitative interviewing as gold standard, Gelhorn et al. [14] revisited the content validity of the LCSS-Meso via qualitative interviewing. As another example, a validated measure of treatment-related neuropathy assessment (TNAS) [15] was later modified after qualitative interviewing showed several items needed revision and removal [13, 16].

Performing qualitative interviews is expensive and requires time and effort. However, this is the only method that can uncover the unknown effect of a new immunotherapeutic agent. Asking patients in a questionnaire whether they have additional symptoms not asked typically does not produce the desired results. As another option, many PROs, such as the MDASI, EORTC QLQ and the PRO-CTCAE, maintain symptom item libraries. Another reasonable approach is to evaluate and consider the known effect of an immunotherapeutic agents and match them with items from these symptom item libraries. Basch, Rogak, and Dueck [17] suggested that a PRO questionnaire for a cancer trial can be populated with items based on common and expected symptoms for an agent under consideration.

Conclusions

In this update to a previous paper on symptom assessment in immunotherapy, the focus was on two important issues, namely, the frequency of administration and adequacy of the chosen symptom list to cover known and unknown effects of immunotherapy.

Determining the frequency of administration depends upon the study objectives of research studies or the clinical context in clinical practice. Several examples to demonstrate when to perform daily assessments and when to incorporate weekly administration of PROs were presented.

To uncover the unknown effects of immunotherapeutic agents either as single or in combination as cancer therapy, the preferred method is to perform qualitative interviewing on patients with the target disease. Another approach is to match the list of irAEs associated with an immunotherapeutic agent with the symptom items from the library of widely used PROs, such as the MDASI, EORTC QLQ, and the PRO-CTCAE.

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