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This book is dedicated to our children—Abraham, Sarah, and Sophia Naing—who inspire us to disseminate scientific knowledge that will help to create a better tomorrow.

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Immune System in Action

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 is 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 the crosstalk 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

Immune cells · Cancers · Cytokines · Innate · Adaptive · Checkpoints

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 formation [3]. The innate and adaptive immune sys-

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tems 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 will 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.

1 Innate Immune System

An association between inflammation and tumorigenesis has long been described, but 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 non-specific in nature and 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, plays 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 damage 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].

1.1 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 gives rise to the T and B lymphocytes that are responsible for adaptive immunity and the NK cells, while the CMP gives rise to the cells of the innate immune system, leukocytes (neutrophils, monocytes, basophils, and eosinophils), mast cells, DCs, erythrocytes, and the megakaryocytes.

1.1.1 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 naïve neutrophils. TANs exhibit dual conflicting roles at the molecular level [20]. They either take up 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 development and progression of tumor cells [27]. However, under certain conditions such as TGF-β blockade, TANs assume an N1 phenotype, which is 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 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, remodeling of stromal matrix by producing a variety of matrix metalloproteinases (MMP) such as MMP1 and MMP9, and 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. Though 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]. Currently, several therapeutic strategies such as reducing or depleting TAMs, repolarizing TAMs toward M1-like macrophages, and promoting the phagocytosis of TAMs are under investigation. For example, as binding of colony-stimulating factor 1 (CSF 1) with CSF 1 receptor (CSF 1R) promotes immunosuppression by recruiting TAMs, AMG 820 (CSF 1R inhibitor) was evaluated in a first-in-human phase I study in patients with advanced cancer [49]. Modest antitumor activity was observed in 32% of patients evaluable for response. Another anti-TAM strategy that is under evaluation is the inhibition of binding between CD47 expressed (do not eat me signal) on tumor cells and signal-regulatory protein alpha (SIRPα) on the surface of macrophages. A combination of Hu5F9-G4, a humanized anti-CD47 antibody with monoclonal anti-CD20 antibody rituximab, has demonstrated promising activity in patients with aggressive and indolent lymphoma and in patients with ovarian and fallopian tube carcinomas [50].

Eosinophils Eosinophils are derived from the CMP cells, and they constitute less than 5% of circulating leukocytes [2, 51]. Traditionally, eosinophils are associated with host defense against large, multicellular parasitic helminths and fungi with allergic conditions [52]. Eosinophils express a number of receptors such as chemokine receptors, cytokine receptors, immunoglobulin (Ig) receptors, Toll-like pattern recognition receptors, and histamine receptors [53]. Engagement of these receptors causes the release of highly cytotoxic proteins, such as major basic protein; eosinophil-derived neurotoxin or eosinophil peroxidase (EPO); pro-inflammatory 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), and CXCL5; and lipid mediators (platelet-activating factor and leukotriene C4) from the large, highly cytotoxic, secretory cytoplasmic granules at the sites of allergic inflammation [53, 54].

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 [55], oral squamous

cell carcinoma (SCC) [56], and laryngeal and bladder carcinoma [57]. Though 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]) [58, 59], whereby they function as APCs and initiate antigen-specific immune responses by the T cells [60]. Kinetic studies have demonstrated that chemotactic factors such as eotaxins and damage-associated molecular patterns (DAMPs) and high mobility group box 1 (HMGB1) released by necrotic tumor cells preferentially induce eosinophilic migration to tumors [61, 62] prior to infiltration by CD8+ T cells [63]. Tumor-associated tissue eosinophils in its active form release chemokines such as CCL5, CXCL9, and CXCL10 that attract CD8+ T cells to the tumor [64]. 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 [63]. Eosinophils also exhibit antitumor immune response in a T-cell-independent manner [65]. A tumor-derived alarmin IL-33 mediates intratumoral migration and activation of eosinophils. Subsequent degranulation of eosinophils releases cytotoxic granules that have a direct action on the tumor cells resulting in reduced tumor growth [66]. Though 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 [67].

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 [68]. Basophils travel to the sites of allergic inflammation and microbial assault in response to cytokines and chemokines released locally [68]. 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 [69]. IL-4 and IL-13, released within an hour of stimulation, serve as chemo-attractants for other immune cells and direct the differentiation of naïve T cells toward Th2 phenotype resulting in Th2-(allergic)-type immune responses in an IgE-dependent and IgE-independent manner [70, 71]. 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 [71].

Though the role of basophils in tumorigenesis has not been clearly understood, it is believed that basophils promote neoplastic angiogenesis [72]. 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 crosstalk 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 suggest a role for basophils in tumor development and disease recurrence [73].

1.1.2 Mast Cells

Mast cells are tissue-based inflammatory cells of hematopoietic origin [74]. The origin of mast cell has long been debated. Recently, Qi et al. identified pre-basophil 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 [75]. 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 [75]. 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 [76, 77]. 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" [78]. Mast cells express several surface receptors including KIT IgG receptor and Toll-like receptors (TLRs) [78]. 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 [79]. 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 [79], 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 [76, 78], and their ability to store TNF-α in a preformed state allow mast cells to orchestrate the first response to invading pathogens [74]. 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 [74].

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 [80], lip cancer [81], and diffuse large B-cell lymphoma [82]. 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 [81, 83, 84]. On the contrary, mast cell infiltration has been associated with good prognosis in breast [85], ovarian [86], lung [87], and colorectal cancers [88]. 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 induces apoptosis of endothelial cells; chymase, which inhibits angiogenesis; and tryptase leading to tumor fibrosis [86, 89, 90]. 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].

1.1.3 Dendritic Cells

DCs are professional APCs that are resident in most tissues of the body and concentrated in the secondary lymphoid tissues [91]. In the steady state, they originate from the monocyte and dendritic cell progenitor (MDP) derived from the CMP cells in the bone marrow [92]. The MDPs give rise to monocytes and common DC progenitors (CDPs) in the bone marrow [93]. The CDPs give rise to pre-DCs, which migrate from the bone marrow through the blood to lymphoid and non-lymphoid 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 [94]. Plasmacytoid DCs are example of pre-DCs found in the blood, thymus, bone marrow, and secondary lymphoid tissue, which pro-

duce 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 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, 95]. Shortly thereafter, functional maturation of DCs ensues triggering the antigen-presenting machinery, which is the critical link between innate and adaptive immunity [96]. Endocytosis by the DCs decreases, and expression of MHC-I, MHC-II, and costimulatory molecules increases at the surface possibly due to cessation of ubiquitination of MHC class II molecules [95]. 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 naïve 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 [95]. 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 naïve T cells are activated [95]. They present the antigens in the lymphoid organ to the T cells [94]. 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) [94].

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 [91]. 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 [91]. However, recruitment of DCs in the TME is influenced by tumor cell intrinsic factors [97]. For example, activation of the WNT/β--catenin signaling pathway prevents DC recruitment and inhibits T-cell activation resulting in immune exclusion [98]. On the contrary, tumor infiltrating NK cells recruit and promote survival of DCs in the TME [99]. Hence, initiation of antitumor response by DCs is largely dependent on the immune milieu in the TME.

1.1.4 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. Though 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 [100]. 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 [101]. 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 [100].

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 identify non-self-infected cells and self-cells under stress by recognizing pathogen-associated molecular patterns (PAMPs) [102]. 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 [103, 104]. 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 [105]. Activated NK cells induce cytotoxicity and/or promote cytokine production [105]. 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 [106]. Tumor cells however evolve and evade destruction by NK cells [106]. A common escape mechanism used by tumor cells is the proteolytic shedding of NKG2D ligands [107]. 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 [108, 109]. 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 [110]. NK cells express other activating receptors such as CD16, Fc-γ receptor IIIa (FCGR3A), which binds to the Fc region of Ig [111]. This enables the NK cells to identify antibody coated tumor cells and destroys them by releasing perforins.

At least two functional subsets of NK cells have been described based on the expression of CD56 and CD16 [112]. 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.

Though 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 [113]. The immune memory function of NK cells lasts for several months after the initial exposure and is antigen-specific and transferable to naïve animals [113]. Though NK cells are potent killers with immune memory,

only modest success in clinical setting has been achieved as their effectiveness has been hampered by their limited ability to infiltrate tumor cells [114]. Several approaches to augment NK cell activity are under investigation. In recent years, NK cells have been engineered to express TCRs (TCR-NK-92) that are functional and capable of cytotoxic activity [115]. Based on the demonstrated antitumor activity in preclinical studies and their ability to expand indefinitely, engineered NK cells are being evaluated in patients with refractory/relapsed acute myeloid leukemia and lymphoma. Anti-CD19 chimeric antigen receptor (CAR)-NK cells derived from cord blood produced objective response in 73% of patients with relapsed or refractory CD19-positive cancers (non-Hodgkin's lymphoma or chronic lymphocytic leukemia) [116]. Several ongoing clinical trials are investigating the efficacy of NK cell therapy in solid tumors.

2 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 germline-encoded cell surface receptors, the adaptive immune response is a slower process, 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 and 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.

2.1 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].

2.1.1 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 [117]. As the lymphoid progenitor cells migrate through the cortex, they undergo an education program based on the constant interaction with the thymic epithelial cells [118]. The lymphoid progenitor cells that enter the thymus at the corticomedullary junction do not express TCR or CD4 or CD8 co-receptors and are therefore called CD4/CD8 double-negative (DN) lymphocytes (DN1) [119]. As they move through the cortex from the cortico-medullary 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) [120]. 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 expression of pre-TCRs (DN3) [117]. Subsequently, intense proliferation results in 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 generation of complete αβ TCRs. Then, DP thymocytes interact with TECs, and further development into naïve T cells is dependent on their ability to bind with MHC class I or class II molecules associated with self-peptides (positive selection) [117, 121]. 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 [118, 122].

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 naïve 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 naïve 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, naïve 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 [123]. This results in clonal expansion and differentiation of naïve 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 [124], T-helper 2 (Th2) [125], T-helper 17 (Th17) [126], induced Tregs (iTregs) [127], follicular helper T cell (Tfh) [128], and T-helper 9 (Th9) [129]. 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 [130]. Subsequent exposure to the same antigen results in more rapid and robust immune response. A small fraction of T cells called the gamma delta T cells express unique TCRs composed of one γ-chain and one δ-chain that are encoded by the gamma and delta gene loci [131]. Although these cells express clonally rearranged genes, they exhibit antitumor activity independent of MHC/human leukocyte antigen (HLA) restriction, which is an essential feature of conventional αβ T cells. Additionally, they share many markers associated with NK cells. Due to their shared features with the innate and adaptive immune system, there is a growing interest in developing gamma delta T-cell-based immunotherapy.

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 [132]. 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

[133]. ICOS is another member of the CD28 family [134]. Though 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 [135], OX40 [136], CD40 [137], and GITR [138]. 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 [139]. 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 naïve T cells into FoxP3+ Tregs and depletes tumor-infiltrating Tregs and thus blocks the immune suppressive function of Tregs [140]. 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 [139]. Activation of CD40 induces tumor regression indirectly by licensing of DCs and by promoting macrophage-dependent tumoricidal action [141]. 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 [142–144]. 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 [145]. Unlike CTLA-4, the PD-1 to PD-L1 ligand binding does not interfere with co-stimulation, but downregulates B- and T-cell proliferation and cytokine production by interfering with signaling pathways downstream of TCRs and BCRs [146]. Besides CTLA-4 and PD-1, there are other next-generation co-inhibitory receptors such as such as lymphocyte activation gene-3 (Lag-3), T-cell immunoglobulin-3 (Tim-3), and T-cell immunoglobulin and ITIM domain (TIGIT), which are expressed on distinct lymphocyte subsets that are responsible for differential suppression of immune response [147]. 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 [148]. 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 [149]. 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 naïve CD4 T cells in the periph-

ery in the presence of TGF-β. They exert their immunosuppressive action by the expression of immunosuppressive cytokines such as IL10 and TGF-β [127]. Decreasing the activity of Treg cells enhances both innate and adaptive immune response, which can be utilized to treat cancer [150]. 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 [151].

2.1.2 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 [152]. 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) [153]. 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 non-auto-reactive 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. On the contrary, when immature B cells express a non-auto-reactive BCR with low basal BCR signaling and when immature B cells are strongly self-reactive, they 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 [154].

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 [155]. 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 naïve B cell expressing both IgM and IgG surface receptors. Guided by the strength of BCR signal, naïve 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 [155, 156]. 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 non-protein 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 lym-

phoid organs and are activated by foreign proteins in a T-cell-dependent manner [157]. 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 [158]. 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 [159].

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 [158]. 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 they regulate 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 [159]. When a naïve 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 [160]. 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 [161]. Later, humanized

mAbs that are 85–90% human, where only the CDRs are murine, were developed [162]. Currently, fully human mAbs produced by phage display are available [163]. 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 [164]. 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 regulation of cell-mediated immune response [165]. 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 [166]. In addition, CD40/CD40L ligation on resting B cells induces surface expression of MHC and costimulatory molecules and produces pro-inflammatory cytokines, thus contributing to APC licensing of B cells. Thus, B cells serve as professional APCs. Though 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 as a cancer immunotherapeutic agent in a limited number of clinical trials [165].

3 The Immune System in Action!

3.1 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, naïve lymphocytes, B cells, cytotoxic T cells, helper T cells, memory cells, Tregs, myeloid-derived suppressor cells (MDSCs), and stromal cells [167]. Despite the recruitment of immune effector cells at the site of 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. Though 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 [168]. 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 [169]. 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 [169]. This is the longest phase in the immunoediting process, when the tumor cell variants reside in a latent form before escaping eventually [170].

During the escape phase, tumor cells adopt several mechanisms to evade immunosurveillance [171]. 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 [172]. 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 [173]. 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 [174, 175]. 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.

4 Cancer Immunotherapy

The landscape of cancer treatment has evolved over the years. 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 [176]. However, their use as anti-cancer 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 [177]. 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, non-small cell lung cancer (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 [178]. 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 use of PEGylated IL-10 (Pegilodecakin) enhanced intratumoral infiltration and cytotoxic activity of CD8+ T cells [179]. 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 [180]. The clinical activity of pegilodecakin was extended to non-small cell lung cancer when used in combination with a PD-1 inhibitor [181] and to pancreatic cancer when used in combination with FOLFOX [182]. 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 [183]. 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 [180] usually associated with use of immunotherapeutic agents, pegilodecakin is emerging as a potential anti-cancer therapeutic agent worthy of further exploration.

IL-6 is another cytokine overexpressed in several cancers and is associated with aggressive growth and poor prognosis [184]. 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 [185]. 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 cel--induced cytokine-release syndrome, and myelofibrosis/polycythemia vera, respectively. Drugs targeting IL-6/JAK/STAT3 signaling pathway are currently under clinical investigation for treatment of solid tumors.

IL-8 is another cytokine that is overexpressed in various cancers, including breast, colon, cervical, gastric, lung, and ovarian cancer [186]. IL-8 signaling promotes tumor progression, angiogenesis, epithelial-mesenchymal transition, and recruitment of myeloid-derived suppressor cells. Higher levels of IL-8 are associated with advanced stage and grade of the disease and higher tumor burden. Retrospective analysis of data from four phase III trials of immune-checkpoint inhibitor (ICPis) in patients with advanced renal-cell carcinoma, melanoma, or NSCLC indicates that higher baseline serum IL-8 correlates with poor survival across tumor types [187]. This finding suggests that IL-8 may serve as a biomarker of resistance to treatment with ICPis. Agents targeting IL-8 are in clinical development. In a phase I/II study of HuMax-IL8 [188] (BMS-986253; a fully human IgG1 kappa monoclonal antibody) in patients with incurable metastatic or unresectable solid tumors, 11 of 15 patients demonstrated disease control. Combination therapies with IL-8 blockade are ongoing.

IL-12 is a pro-inflammatory cytokine produced by APCs with potent pleiotropic activity [189]. However, despite its potent immune stimulation potential and profound antitumor activity in preclinical studies, systemic use of IL-12 was limited due to dose-limiting toxicities and limited efficacy at tolerable doses [190, 191]. Currently, several early-phase clinical trials are investigating novel localized IL-12 delivery strategies that would enhance IL-12 concentrations in the tumor. Notable among them are immunocytokines, NHS-IL-12, which is a fusion protein engineered to contain IL-12 and tumor binding antibody. Though transient lymphopenia and elevated liver transaminases were observed, it was well tolerated. Despite the increase in NK cells and broadening the TCR diversity of TILs, no objective response was observed [192]. NHS-IL-12 in combination with avelumab, an ICPi, is also under investigation. Other approaches include intratumoral delivery of genetic material encoding IL-12 using plasmids, mRNA, viruses, transduced cells and controlled release of recombinant IL-12 through a delivery system directly implanted in the tumor. In a phase II study that explored intratumoral injection of plasmid DNA encoding IL-12 (pIL-12) followed by electroporation in patients with in-transit or M1a melanoma, 33% had objective response including 11% with complete response [193]. Importantly, there were no treatment-related grade 3 or 4 adverse event. In the same study, in patients with advanced melanoma, 35.7% had objective response including 17.9% with complete response [194]. IL-12 based combination therapies as a neoadjuvant and adjuvant to chemotherapy, radiation, and ablation are in early stages of

development. The synergistic activity of IL-12 in combination with ICPis is promising. In a phase II study of intratumoral injection of pIL-12 followed by electroporation in combination with pembrolizumab, an ICPi, in patients with non-infiltrated melanoma, 41% had objective response including 36% with complete response [195].

IL-27 is an IL-12 family cytokine with structural similarities to IL-6 family [196]. While IL-27 directly inhibits tumor cell proliferation, survival, and angiogenic and invasive properties, it also promotes the development of NK cells and cytotoxic T lymphocytes, thereby contributing to antitumor immunity through several mechanisms [197]. Further, by augmenting NK cell-mediated killing of tumor cells, IL-27 enhances APC access to tumor antigens. Thus, IL-27 serves a critical link between the innate and adaptive arms of the human immune system. Unlike IL-12, IL-27 is less toxic in preclinical studies as the anti-angiogenic effects of IL-27 are independent of IFN-γ. However, IL-27-induced expression of PD-L1, TIM3, and IDO can downregulate tumor-specific T-cell responses and dampen the antitumor activity of the molecule. In mouse models, administration of IL-27–expressing recombinant adeno-associated virus (AAV–IL-27) caused rapid depletion of Tregs and significantly inhibited tumor growth in a broad spectrum of cancer types [198]. In combination with anti-PD-1 treatment, IL-27 gene therapy also produced complete tumor rejection in two models, suggesting a potential role in anti-cancer therapy.

IL-15 is a proinflammatory cytokine that has several functions in common with IL-2. While both cytokines stimulate the proliferation of cytotoxic CD8 T cells and NK cells leading to enhanced antitumor responses [199], IL-15 has no major effect on Tregs and is secreted in small quantities. As IL-15 demonstrated superior antitumor activity in preclinical studies, first-in-human trials of recombinant human IL-15 (rhIL-15) by bolus, subcutaneous, and continuous intravenous infusions were conducted [200]. When rhIL-15 was administered as bolus, severe toxicities precluded further investigation. With other dosing strategies, although IL-15 produced significant expansions of CD8+ T cells and NK effector cells in circulation and intratumorally, the response was modest at best due to induction of checkpoints TIGIT, TIM3, IL-10, and PD-1 on CD8 T cells and the lack of tumor-specific targeting by NK cells [201]. To overcome this challenge, IL-15-based combination trials with intralesional agonistic anti-CD40, checkpoint inhibitors, anti-CTLA-4 and anti-PD-L1, and cancer-directed monoclonal antibodies are ongoing [202].

IL-18 is a member of the IL-1 cytokine family. Although IL-18 is upregulated in TILs, administration of recombinant IL-18 in melanoma patients did not produce the expected response [203]. It was because a "decoy receptor," IL-18BP, that binds with IL-18 with extremely high affinity was produced at very high levels in tumors, which curtailed the capabilities of IL-18 to elicit an immune response [204]. Currently, an engineered "decoy-resistant" IL-18 variant (DR-18) that retains full signaling capacity through the IL-18 receptor is being evaluated in patients with solid tumors.

Cytokines have been implicated in the pathogenesis of autoimmune diseases. As mechanisms underlying immune-related adverse events associated with immunotherapy are thought to be driven by autoimmunity [205], blockade of cytokines is being investigated for management of these toxicities. Blockade of IL-17 and tumor necrosis factor in the management of immunotherapy-induced cutaneous (psoriasiform) and gastrointestinal toxicity respectively is promising [206, 207].

Several mAbs have also been used in the treatment of cancer [208] 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) [209]. However, 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 [210]. With the discovery that activated T cells express CTLA-4, which on binding with B7 molecules on the APC blocks co-stimulation of T cells and produces immune suppression, a series of experi-

ments 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 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 [211]. 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 [212–216]. As a result of durable responses and survival benefits produced in several tumor types, FDA granted accelerated approval of several ICPis as monotherapy (Table 1) [217].

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 [218]. 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 [219]. Further, as these co-inhibitory receptors have non-redundant signaling pathways, a combined blockade of these mechanistically different pathways may be synergistic in restoring T-cell-mediated immune response [147]. 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 [220]. 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 suggests that such combinations may potentially be synergistic [221]. A list of FDA-approved ICPi-based combination regimens is provided in Table 2. Similarly, radiation-induced immunomodulatory changes provide local control and prolong survival, but are insufficient to shift the balance of the immunosuppressive TME to achieve tumor rejection [222]. To overcome this limitation, clinical studies evaluating the combination of radiotherapy and ICPis are currently underway [223, 224]. Furthermore, blockade of next-generation co-inhibitory receptors Lag-3, Tim-3, and TIGIT are under active investigation [147].

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 [225]. Several IDO inhibitors such as INCB024360 [226, 227], indoximod [228], IDO peptide vaccine [229], BMS-986205 [230], and NLG919 [231] 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 [232].

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, 4-1BB, CD40, or GITR (glucocorticoid-induced tumor necrosis factor receptor), provides a potent "go" signal that actively promotes the optimal "killer" CD8 T-cell responses [233]. 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 [234]. 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

Table 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
		Esophageal squamous cell carcinoma
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
		Cutaneous squamous cell carcinoma
		Triple negative breast cancer
		Tumor mutational burden-high (TMB H) [≥10 mutations/megabase (Mut/Mb)] solid tumors
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
Cemiplimab-rwlc	PD-L1	Cutaneous squamous cell carcinoma
		Basal cell carcinoma
		Non-small cell lung cancer

[a]List of FDA-approved immune checkpoint inhibitors as of March 17, 2021, 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

was found to be safe. Antitumor activity with monotherapy has been modest at best in patients with solid tumor [233]. However, improved response rates have been observed when T-cell agonists were used in combination with ICPis. An ORR of 26.1% has been reported with utomilumab (4-1BB agonist) plus pembrolizumab (ICPi) [235], 50% with urelumab (4-1BB agonist) plus nivolumab [233], 19% partial response and 52% stable disease in patients with pancreatic cancer treated with CP-870,893 (CD 40 agonist) plus gemcitabine, and 20% partial response and 40% stable disease in patients with solid tumor treated with CP-870,893 (CD 40 agonist)

Table 2 FDA-approved immune checkpoint inhibitor-based combinations and indications[a]

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
		Hepatocellular carcinoma
		Malignant pleural mesothelioma
		Non-small cell lung cancer
Nivolumab with Ipilimumab and two cycles of platinum-doublet chemotherapy	PD-1 and CTLA-4	Non-small cell lung cancer
Nivolumab with cabozantinib	PD-1	Renal cell carcinoma
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	Non-squamous, non-small cell lung cancer
Atezolizumab with carboplatin and etoposide	PD-L1	Small cell lung cancer
Atezolizumab paclitaxel protein-bound and carboplatin	PD-L1	Non-squamous, non-small cell lung cancer
Atezolizumab with bevacizumab	PD-L1	Hepatocellular carcinoma
Atezolizumab with cobimetinib and vemurafenib	PD-L1	Melanoma
Avelumab with axitinib	PD-L1	Renal cell carcinoma
Durvalumab with etoposide and either carboplatin or cisplatin	PD-L1	Extensive-stage small cell lung cancer

[a]List of FDA-approved immune checkpoint inhibitors as of March 17, 2021, 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

plus paclitaxel and carboplatin. In a neoadjuvant study, 9 of 10 patients with pancreatic cancer treated with urelumab (4-1BB agonist) plus nivolumab plus GVAX vaccine were disease-free after a median follow-up of 12 months [236].

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 [237]. 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 was investigated in a colorectal cancer tumor bearing mice [238]. Response was optimal when OX40 agonist antibody was delivered immediately after radiation therapy during the post-radiation window of increased antigen presentation [238], 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 [239].While some TLRs on cancer cells favor tumor progression [240, 241] and promote resistance to chemotherapy, most TLRs on immune cells serve as sensors [239]. 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 [242, 243]. 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 [243]. 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 [244]. 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 promotes cross-priming of T cells by the DCs resulting in initiation of adaptive immune response [245]. 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 [246–248]. Macrophages are cells of the innate immune system that serve as a double-edged sword in response to cytokines in the TME [249]. 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 engages 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 [49, 250] and reprogramming TAMs to acquire an antitumor M1-like phenotype using bioconjugated manganese dioxide nanoparticles [251] or ferumoxytal nanoparticles [252] or concurrent CSF-1R blockade and CD40 agonism [253] 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 [254]. However, in the TME, increased MDSCs express arginase I that metabolizes L-arginine to L-ornithine and urea [255]. 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 [256].

5 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 [257]. Some of

the important biomarkers of response are further discussed.

5.1 PD-L1 Expression

Cell surface expression of PD-L1 in pretreatment tissue samples is currently the most widely used validated biomarker to preselect patients for treatment with PD-1/PD-L1 inhibitors [258].The FDA has approved three PD-L1 IHC assays for use in conjunction with specific therapeutic agents. They are Dako 22C3 for selecting NSCLC patients for treatment with pembrolizumab [259]; Ventana SP142 for atezolizumab in patients with urothelial carcinoma, triple-negative breast cancer, or NSCLC; and Dako 28–8 for the combination of ipilimumab and nivolumab in patients with NSCLC. 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 [260]. 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 [261]. There is also marked heterogeneity in PD-L1 expression between samples from the primary and metastatic sites in the same individual [262]. 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 cut-off values and scoring patterns [263]. As a result, there is lack of defined criteria to determine PD-L1 status of the patient. The above findings suggest that though 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 [260, 264].

5.2 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 [265–267]. On the contrary, infiltration of the tumor tissue by Tregs is associated with poor survival in ovarian, breast cancer, and hepatocellular carcinoma [268–270]. 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 [271]. 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 [272]. 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, in the presence of markers for Th1 polarization, cytotoxic and memory cells were predictive of low recurrence rate.

Baseline expression of TILs may not always suggest response to immune checkpoint blockade. TILs may not always predict response to ICPIs. For example, CD8+ T cells at the IM were positively associated to response with pembrolizumab in patients with metastatic melanoma [273], but not in patients with unresectable stage III/IV melanoma treated with ipilimumab [274]. However, on treatment, increase in the levels of tumor infiltrating T cells at the CT and IM was predictive of response to treatment with ICPi in several studies [273–275]. 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 [273].

Based on T-cell landscape within the tumor, solid tumors have been classified as hot (highly infiltrated and inflamed), altered-excluded (T cells only at the invasive margin), altered-immunosuppressed (some infiltration, but not inflamed), and cold (very low infiltration and not inflamed) [276]. Each subtype is characterized by a specific immune signature and differed in their 2-year risk of relapse. Based on these findings, immunoscore was developed.

Other predictive models have been proposed based on PD-L1 expression and TILs [277]. For example, four subtypes of TME have been identified, namely, type I (PD-L1+ with TILs driving adaptive immune resistance), type II (PD-L1– with no TILs, indicating immune ignorance), type III (PD-L1+ with no TILs, indicating intrinsic induction), and type IV (PD-L1– with TILs, indicating the role of other suppressors in the promotion of immune tolerance). Classification of a cancer into one of the four categories could potentially identify therapies that may be beneficial. For example, patients with type I TME may benefit from ICPis, while patients with type II TME might require priming and may not benefit from use of ICPis.

5.3 Immunoscore

Immunoscore is a methodology by which in situ immune infiltrate is quantified. This supersedes the TNM classification of tumors used for estimation of the degree of progression of the tumor to make informed treatment decisions [272]. 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 plays an important role in immune escape and progression of the tumor, immune contexture discussed above is a better prognostic indicator than TNM classification [278]. 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 [279]. Recently, the consensus immunoscore was validated in a study conducted by an international consortium of centers in 13 countries [280]. 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, 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.

5.4 T-Cell Receptor Sequencing

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 undergoes clonal expansion. Thus, characterization and estimation of TCR repertoire diversity by next-generation sequencing of complementarity determining region 3 (CDR3) 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 [281]. 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 [282]. In 10 patients with metastatic melanoma treated with nivolumab [283], 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 [273]. TCR sequencing of pre- and posttreatment samples showed the number of clones that had expanded was 10 times more in the responders than in non-responders. 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.

Tumor antigen-specific T cells may provide a direct assessment of tumor immunogenicity. Novel technologies to predict the antigen specificity of a TCR are being developed. It was recently reported that in a cohort of 22 cancer patients treated with CTLA-4 inhibitor, TCR convergence evaluated using Oncomine TCRB-LR assay was elevated in those who had an objective response to CTLA-4 blockade ($p = 0.033$), and it discriminated responders from non-responders [284]. The prediction of response improved further when a combination of convergence and clonality was used ($p = 0.001$) compared to models using either convergence or clonality as sole predictor of response.

5.5 Single-Cell Sequencing

As intratumoral heterogeneity may influence response to immune checkpoint blockade, an in-depth characterization of tumor and immune cells in the TME is critical to understand the players responsible for response or resistance to treatment. With continued development of next-generation sequencing, several approaches are now available for immune repertoire sequencing. Notable among them are single-cell sequencing technologies, wherein a single-cell genome or transcriptome is sequenced to obtain genomic, transcriptome, or other multi-omics information [285, 286]. They offer a powerful, sensitive, and unbiased approach to study cellular heterogeneity that is often masked while using traditional bulk sequencing methods.

5.6 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 neoepitomes generated by somatic mutations that function as neoantigens and elicit a brisk immune response [287]. In several clinical trials, higher clinical benefit rate and longer progression-free survival have been reported in patients with high mutation burden treated with ICPis [287–289]. 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 [290] [291]. 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 [288]. 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. Recently, the FDA approved the use of pembrolizumab for patients with tumors with a high TMB, defined as ≥10 mutations/Mb using the Foundation OneCDx Platform [258]. In addition, molecular alterations in the PI3K pathway may promote tumor immune evasion through constitutive expression of PD-L1 [292]. 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 [266].

5.7 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 [289]. 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 [293]. On further evaluation, more refined immune signatures were found to produce similar results in patients with HNSCC and gastric cancer [294]. The 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 [295]. 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 [296]. 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 [289]. The IPRES signature was also found in non-responsive 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.

5.8 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 interac-

tion between the tumor and the immune cells in the TME of the patient [297]. 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), and (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 [298]. 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 germline 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.

5.9 Serum Biomarkers

Several routinely available peripheral blood parameters have been evaluated as a biomarker of response to treatment with ICPis [275, 299–306]. 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 [302, 303]. Though 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 [302–306], 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 [302] and was an independent predictor of response in patients with melanoma [307]. On the other hand, elevated level of LDH at baseline was an independent predictor of poor survival [302, 308]. Despite the association between these peripheral blood parameters and treatment outcomes, there is no validated biomarker available for use in the clinic.

5.10 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 ICPis. 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 [309]. 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 [310]. 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.

5.11 Microbiome Assessment

Growing body of evidence suggests that alterations in the gut microbiome may be associated with cancer development, progression, response to treatment with PD-1 inhibitors, and even cancer-related symptoms [311]. Alpha diversity of gut microbiomes in fecal samples was significantly higher in patients with metastatic melanoma responding (CR/PR/SD $\geq$6 months) to treatment with PD-1 inhibitors [312]. 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 non-responders. In addition, patients with abundance of *Faecalibacterium* genus in Clostridiales order had significantly longer PFS compared to patients with abundance of Bacteroidales. In another study in melanoma patients, analysis of baseline stool samples demonstrated a significant association between commensal microbial composition and response to treatment with ICPis [313]. Bacterial species *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* were more abundant in responders. Thus, favorable gut microbiome may enhance antitumor response in patients treated with ICPis. In another study in 88 patients with advanced, metastatic, unresectable cancers [314], fatigue was measured using the MD Anderson Symptom Inventory – Immunotherapy. *Eubacterium hallii* was negatively associated with fatigue severity scores, whereas *Cosenzaea* was positively associated with fatigue scores suggesting a possible association between microbiome composition and fatigue in patients with advanced cancers.

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 [264]. 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 [212, 315]. Therefore, identification of a single immunologic biomarker may not be predictive of response [264]. 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 [316]. For example, the 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 [257]. In a large meta-analysis [317] of whole-exome and transcriptomic data from more than 1000 patients across seven different tumor types treated with ICPis, clonal TMB was identified as the strongest predictor of response to treatment with ICPis, followed by total TMB and CXCL9 expression. Copy-number analysis identified that 9q34 (TRAF2) loss was associated with response

and CCND1 amplification was associated with resistance.

6 Conclusion

Seminal studies have described the different components of the innate and adaptive immune system. Though they are two distinct arms of the human immune system, they are intricately organized in time and space and are critically dependent upon 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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Resistance to Immunotherapy: Mechanisms and Means for Overcoming

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Abstract

Immune checkpoint blockade transformed cancer therapy during the last decade. However, durable responses remain uncommon, early and late relapses occur over the course of treatment, and many patients with PD-L1-expressing tumors do not respond to PD-(L)1 blockade. In addition, while some malignancies exhibit inherent resistance to treatment, others develop adaptations that allow them to evade antitumor immunity after a period of response. It is crucial to understand the pathophysiology of the tumor-immune system interplay and the mechanisms of immune escape in order to circumvent primary and acquired resistance. Here we provide an outline of the most well-defined mechanisms of resistance and shed light on ongoing efforts to reinvigorate immunoreactivity.

Keywords

Malignancy · Immunotherapy · Resistance · Checkpoint · Pathway · Antigen · Effector · Regulatory · Suppressor

Abbreviations

B2M	beta-2 microglobulin
CAF	cancer-associated fibroblast
CAR	chimeric antigen receptor
CCR	chemokine receptor
CR	complete response
CRC	colorectal carcinoma
CSF	colony-stimulating factor
CSF1R	colony-stimulating factor 1 receptor
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
DC	dendritic cell
EGFR	epidermal growth factor receptor
FasL	Fas ligand
FcγR	Fcγ receptor
FDA	US Food and Drug Administration
HIF-1	hypoxia-inducible factor 1
ICAM	intercellular adhesion molecule
ICB	immune checkpoint blockade

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ICI	immune checkpoint inhibitor
IDO	indoleamine 2,3-dioxygenase
IFN-γ	interferon-gamma
iRECIST	immune response evaluation criteria in solid tumors
iRs	immune downregulating checkpoints
ITIM	immunoreceptor tyrosine-based inhibitory motif
JAK	Janus kinase
LAG-3	lymphocyte-activation gene 3
LAIR-1	leukocyte-associated immunoglobulin-like receptor 1
mAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
MDSC	myeloid-derived suppressor cell
MHC	major histocompatibility complex
MICA-B	MHC-I-related chain B
M-MDSC	monocytic subtype of myeloid-derived suppressor cell
MMR	mismatch repair
MPR	major pathologic response
MSI-H	microsatellite instability high
NK	natural killer
NSCLC	nonsmall cell lung cancer
OS	overall survival
PBMC	peripheral blood mononuclear cell
PD	progressive disease
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
PFS	progression-free survival
PI3K	phosphatidylinositol 3-kinase
PR	partial response
PTEN	phosphatase and tensin homolog
RCC	renal cell carcinoma
RECIST	response evaluation criteria in solid tumors
SD	stable disease
STAT	signal transducers and activators of transcription
STING	stimulator of interferon genes
TAM	tumor-associated macrophage
Teff	effector T cell
TGF-β	transforming growth factor beta
Th	T-helper cell
TIGIT	T-cell immunoreceptor with Ig and ITIM domains
TIL	tumor-infiltrating lymphocyte
TIM-3	T-cell immunoglobulin 3
TKI	tyrosine kinase inhibitor
TLR	toll-like receptor
TMB	tumor mutational burden
TME	tumor microenvironment
TNBC	triple-negative breast cancer
TNF-α	tumor necrosis factor alpha
Treg	regulatory T cell
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

1 Introduction and Definitions

Immune checkpoint inhibitors (ICIs) are a class of immunotherapeutics that have scored a remarkable breakthrough across a large spectrum of malignant tumors. Distinct from other modalities, such as chemotherapy and small molecules, which induce temporal apoptosis of tumor cells, immunotherapeutics attempt to re-recruit effector immune cells and create a response that employs immune memory in an effort to produce long-lasting antitumor effects. This class of agents can produce rapid, deep, and, most significantly, durable responses. Still, a large proportion of patients do not respond to treatment, or develop progression of malignancy after a variable period of benefit. Furthermore, since the publication of the first phase III ipilimumab trial, which showed an improvement in overall survival (OS) but not in progression-free survival (PFS), it has been recognized that tumors under the effect of ICI may not always follow the same pattern of response seen in other types of therapy [1].

Several unique issues have emerged since the widespread adoption of ICIs in the treatment of cancer. Unfamiliar patterns of delayed tumor response, initial and late resistance to treatment, oligoprogression, lymph node-only progression, and pseudoprogression have all surfaced. To address these issues and to avoid misinterpretation of tumor response, the Society for Immunotherapy of Cancer assembled a taskforce to create consensus guidelines that would provide a consistent definition for different types of resistance. The recommendations aim to stan-

dardize tumor assessments in patients who are receiving anti-PD-(L)1 (programmed cell death protein 1/programmed death-ligand 1) therapy, and to help investigators in designing clinical trials for drugs being developed in this field. In addition, they identify patients who are unlikely to derive benefit from an initial or more prolonged exposure to anti-PD(L)1, and reduce the chance of mislabeling patients' responses to treatment. In the setting of a clinical trial, these standards are expected to reduce the chance a response is mistakenly attributed to a subsequent line of therapy [2].

The SITC taskforce recognized three different patterns exhibited by tumors progressing in the context of ICI therapy: primary resistance, secondary resistance, and off-treatment progression.

Primary Resistance is applicable to patients experiencing either initial progressive disease (PD), or stable disease (SD) lasting less than 6 months. In addition, to make a reasonably accurate assessment of treatment benefit, a minimum drug exposure of 6 weeks is required. The panel acknowledges that some indolent tumors may need to be evaluated over a longer period of time. In the absence of rapid tumor growth or clinical deterioration, a confirmatory scan, or clinical evaluation for clinically detectable disease (e.g., skin lesions), should be carried out at 4–12-week intervals after first suspicion for PD (Table 1). This would ensure late responders to PD-(L)1 treatment are not removed from therapy inappropriately. Clinical judgment is required in case of a clinical deterioration attributable to PD, as continuing anti-PD-(L)1 therapy in these patients may not be safe.

Secondary/Acquired Resistance Patients receiving PD-(L)1 therapy who demonstrate an initial clinical benefit such as complete response (CR), partial response (PR), or SD for a minimum of 6 months but whose tumors progress while on therapy are classified as having secondary resistance. This was defined with the main goal of aiding in clinical trial design by guiding eligibility and stratification for subsequent analysis. As with primary resistance, a confirmatory evaluation is recommended 4–12 weeks after initial PD, and should demonstrate progression in ≥2 sites in patients with multiple metastases (Table 1). In addition, to be categorized as secondary resistance, lymph node-only progression requires tissue confirmation. Again, patients with disease-related clinical deterioration or rapid disease progression do not require confirmatory radiologic evaluation.

Off-treatment Progression A third scenario is PD after treatment discontinuation due to patient preference, toxicity, or other reasons such as a predetermined finite number of cycles, as in (neo) adjuvant treatment. Mechanisms of resistance in this scenario may or may not resemble those seen in other types of resistance. The taskforce recommends that patients with PD < 12 weeks from the last dose of anti-PD-(L)1 therapy can be considered to have primary resistance (or early relapse). Relapse ≥12 weeks is considered "late relapse", as it is difficult to label this as resistance. A treatment rechallenge is warranted in patients with late relapse, especially if occurring >6 months. In both of these scenarios, a biopsy is required, rather than a confirmatory scan, to confirm progression/recurrence (Table 1).

Noting that macroscopic disease is present in the case of neoadjuvant therapy, and in anticipation of increased utilization of this approach, the definitions of primary and secondary resistance mentioned above can be applied here. However, the unique advantage of having histologic evaluation of residual tumor in this setting allows for further classification based on pathologic response. Patients who achieve a major pathologic response or better (CR, near CR, or major PR) with a subsequent relapse down the road are thought to fit into the secondary resistance category; while those not achieving a major pathologic response fit into the primary resistance category [2]. Notably, some neoadjuvant trials have defined major pathologic response as ≤10% of residual viable tumor [3, 4].

Progression after treatment discontinuation in the metastatic setting can be classified based on attained benefit and interval from last anti-PD-(L)1 treatment. Patients who have not previously achieved PR/CR are considered to have primary resistance; while patients who achieved PR/CR and relapsed after ≤12 weeks are considered to have secondary resistance. Late progression is considered when a patient who achieved PR/CR experiences a relapse >12 weeks from last dose. However, it is difficult to classify this scenario as resistance since these patients have a > 5% chance of responding to rechallenge, regardless of intercurrent treatment.

Caveats These definitions are designed to address anti-PD-(L)1 monotherapy, and may or may not necessarily be applicable to combination ICIs or to chemo-immunotherapy. Indolent tumors that are slowly progressing despite therapy, but not enough to call PD per RECIST, represent a group that may need a longer period of exposure than suggested intervals, and the taskforce urged investigators to use clinical judgment. The definitions are applicable to most but not all solid tumors, especially in cases where conventional response criteria are not commonly used, such as in glioblastoma, hepatocellular carcinoma, and prostate cancer, among others. If feasible, biopsy confirmation should be considered in cases of oligoprogression, especially if involving the lung or lymph nodes. Criteria can generally be applied to patients in clinical trials. In clinical practice, however, local therapy to sites with oligoprogression may be reasonable if deemed appropriate by the treating physician. Finally, it is noteworthy that the taskforce did not reach a unanimous agreement whether to use RECIST 1.1 vs iRECIST for clinical trial eligibility criteria [2, 5, 6] (Table 1).

2 Functional Categorization of Resistance Mechanisms

Multiple classifications of resistance have been suggested, some are based on response phenotype, such as primary and secondary; while others pertain to the type of response exhibited by the immune system, such as innate and acquired. Nevertheless, significant mechanistic overlap exists between tumor resistance to innate immunity and to immunotherapy, and between primary and acquired tumor resistance; therefore, we have elected to propose a functional classification based upon the role of different key players.

2.1 Defective Immune Cell Recognition

2.1.1 Impaired Immunogenicity and Neoantigen Alteration

Neoantigens are novel protein epitopes expressed via major histocompatibility complexes (MHCs) and result from emerging mutations and genomic instability in the tumor genome. The resulting new peptide sequences are immunogenic and are considered cornerstone elements in immune recognition by cytotoxic T lymphocytes (CTLs). There are essentially two types of tumor antigens: tumor-specific antigens (TSA) and tumor-associated antigens (TAA). TSAs are usually present only in tumor cells and are created by two main mechanisms, emerging mutations in tumor genomes, and viral incorporation into cell genomes enforcing the creation of oncoviral neoantigens. TAAs are present both in the tumor and in some other nonmalignant cells to which T cells have developed tolerance [7].

The neoantigen burden is related to the number of mutations present in a specified area of the tumor genome, also known as tumor mutational burden (TMB). Although point mutations are significantly more common, frameshift insertions/deletions, exon skipping, and protein fusions are all events that create proteins which are structurally more altered [8]. This process occurs in a random fashion, and because a large proportion of mutations is not shared among different patients, they can be considered patient-specific [9].

Tumors with germline or somatic deficiencies in DNA repair mechanisms appear to exhibit improved responsiveness to ICIs. Mismatch repair-deficient (dMMR) tumors with high mic-

Table 1 SITC taskforce definitions of resistance [2]

On-treatment progression – advanced/metastatic disease			
Type of resistance	Minimum drug exposure	Best RECIST response	Confirmatory evaluation[a]
Primary resistance	6 weeks	PD SD < 6 months	Required 4–12 weeks after RECIST PD
Secondary resistance	6 months	CR, PR, or SD > 6 months	Required 4–12 weeks after RECIST PD[bc]
Off-treatment progression – Adjuvant settings			
Type of resistance	Last dose of anti-PD-(L)1	Confirmatory biopsy required	Confirmatory evaluation[a]
Primary resistance (early relapse)	< 12 weeks	Yes	Not required
Late relapse[d]	≥ 12 weeks	Yes	Not required
Neoadjuvant settings			
Type of resistance	MPR (defined as CR, near CR, or major PR) achieved?		
Primary resistance	No		
Secondary resistance	Yes		
Off-treatment progression in advanced/metastatic disease			
Type of resistance	End of treatment CR/PR	Time from last dose	Confirmatory evaluation[a]
Primary resistance	No	n/a	Not required
Secondary resistance	Yes	≤ 12 weeks	Required
Late progression	Yes	> 12 weeks	Required

[a]Imaging or clinical for clinically measurable lesions (skin)
[b]Unless clinical deterioration due to PD
[c]Interval depends on tumor biology and rate of growth
[d]Relapse ≥ 6 months may warrant a rechallenge

rosatellite instability (MSI-H) leading to the formation of thousands of neoantigens exhibit a significantly higher response rate to ICIs compared to MMR-proficient tumors across a vast variety of tumors. Thus, in a first tissue-agnostic approval of its kind, the US Food and Drug Administration (FDA) authorized the use of pembrolizumab in dMMR tumors after increased response rates were seen in several different solid tumor types spanning both colorectal cancer (CRC) and non-CRC, with dMMR or MSI-H [10, 11].

Tumor histologies that tend to develop higher TMBs, such as melanoma, nonsmall cell lung cancer (NSCLC), and MSI-H CRC, have shown greater response rates to ICIs, suggesting a predictive role for high TMB as a biomarker of response [10, 12, 13]. This led to another FDA tissue-agnostic approval of pembrolizumab in solid tumors with high TMB, which was ultimately defined as ≥10 mutations/megabase [10].

Some immunologically cold tumors such as pancreatic and breast carcinomas exhibit low response rates to ICB, due in part to the low TMB and low antigen load resulting in poor immunogenicity. These tumors have generally shown disappointing results with ICIs and appear to commonly exhibit patterns of primary resistance [14–16]. On the other hand, different neoantigens exhibit different levels of immunogenicity; hence, a high-quality neoantigen is one that is potently immunogenic. For example, pancreatic ductal carcinomas demonstrate a high level of primary resistance to ICIs due to low neoantigen load and less immunogenic (low-quality) antigens, among other factors [17].

Because a multitude of factors contributes to immunogenicity and immune response, not all TMB-high tumors respond to ICIs. Likewise, some tumors with low TMB respond well to ICIs. Merkel cell carcinomas, for example, respond well to first-line ICIs even when TMB is low.

TMB-low Merkel cell carcinomas were found to be mostly polyomavirus-related, suggesting that viral-associated antigens in tumor cells are highly immunogenic [18]. A similar observation was noted in human papillomavirus-associated head and neck and cervical cancers, which demonstrated higher response rates in virus-positive tumors compared to virus-negative ones [19]. This observation was not universal across all viral-associated malignancies, such as hepatocellular carcinoma, possibly due to different mechanisms of carcinogenesis. Likewise, in renal cell carcinoma (RCC), no association between TMB and clinical benefit from atezolizumab was found in the exploratory molecular analysis of the IMmotion150 randomized phase II trial [20].

Downregulation, epitope modification, loss, and shedding of neoantigens are some examples of how tumors evade ICI therapy. Loss of neoantigens via genomic alteration, commonly deletion, has been shown to play a role in a cohort of NSCLC patients whose disease progressed after initial response [21]. Alternative splicing leading to loss of the CD19 epitope accounts for some relapses after chimeric antigen receptor (CAR) T-cell-based immunotherapy [22]. Whole-exome sequences of paired tumor samples before ICI treatment and after progression revealed a change in the somatic mutation landscape that included both gains and losses. However, several tumor-specific neoantigens were found to have been lost in the resistant clones, compared to the pretreatment tumor, due to genomic alteration as well as elimination of some tumor subclones. This process of therapy-induced immunoediting eliminated antigens that were recognized by circulating T cells.

2.1.2 Dysfunctional Antigen-Processing Machinery

Defective antigen presentation has been described in a study of melanoma patients with tumors that became refractory to ICIs after initial response. The development of a frameshift deletion in the beta-2 microglobulin (B2M) component of MHC-I was noted in one of four patients, and resulted in the loss of outer membrane localization of MHC-I without affecting production, as evidenced by persistent intracellular staining by immunohistochemistry. MHC-I is essential for T-cell recognition, and the loss of surface localization impairs immune destruction in both treatment-naïve and ICI-treated patients [23, 24]. Defective antigen presentation through mutations in B2M was also demonstrated in 29% of metastatic melanoma patients with PD after treatment with ICIs. Threefold enrichment in B2M gene loss of heterozygosity was noted in patients who did not respond to treatment with anti-PD1 and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy compared to responders [25].

Shedding of surface antigens has long been recognized as a potential resistance mechanism to monoclonal antibodies (mAb) and immunoconjugates [26]. The role of antigen shedding in mediating resistance to ICI remains undefined. However, the combination of anti-PD-(L1) with antibody-drug conjugates has yielded encouraging results in urothelial carcinomas, as an example [27].

Other alterations in MHC-I have been reported. For instance, shedding of natural killer (NK)-activating ligands on MHC-I has been shown to play an important role in tumor immune escape. Proteolytic shedding of MHC-I-related chain A/B (MICA/B), NKG2D activators, is undertaken by tumors to evade cytotoxic destruction [28]. Invigorating the antitumor response through generation of polyclonal anti-MICA antibodies has promising results in preclinical in vivo studies [29].

2.1.3 Immunoediting

Immunoediting is the process through which the immune system both prevents and promotes tumorigenesis through immunogenic "sculpting." Once a tumor cell survives self-correction mechanisms, it is believed to go through three phases of immunoediting: elimination, equilibrium, and escape [30]. Elimination is the phase in which the immune system detects and destroys tumor cells before they become clinically apparent.

Equilibrium is characterized by tumor dormancy. In the escape phase, the immune system fails to restrict tumor growth, resulting in disease progression. This process is described in the pathogenesis of tumor development in treatment-naïve conditions. However, it appears to greatly overlap with primary and acquired resistance to immunotherapy [7] (Fig. 1). Some tumors appear to revert to a state of equilibrium in response to treatment with ICIs, with or without tumor regression. However, later in the course of treatment, less immunogenic clones survive and reenter the escape phase. This phenomenon is usually accompanied by an increase in the number of tolerant immune cells. Interestingly, tumor subclones with immune tolerance-promoting mutations in CDKN2A gene and nearby interferon (IFN)-γ gene were selected for subsequent growth as demonstrated in a cohort of melanoma patients with PD after nivolumab treatment. Therefore, tumor evolutionary selection of less immunogenic clones is considered an important mechanism of resistance following ICI therapy [7, 31].

2.1.4 Tumor Heterogeneity

The degree of PD-L1 expression can differ spatially and temporally within a single patient. This may account, at least in part, for differences in response rates among patients with seemingly similar tumor characteristics [32]. In the same tumor, expression of PD-1 or PD-L1 can vary considerably among different regions. A gene expression signature analysis of 35 tumor regions belonging to 10 NSCLC tumor samples revealed intriguing intertumoral and intratumoral heterogeneity. Furthermore, a heterogeneous tumor microenvironment (TME) was noted using gene expression analysis of stromal and immune cells [33]. Additionally, remarkable differences in PD-L1 expression were observed between primary tumors and metastatic lesions and between coexisting metastatic sites [33, 34]. It should be noted that these differences in expression patterns could be attributed in part to inter-assay variability [35, 36].

3 Barriers to Immune Cell Trafficking into Tumor

Barriers to T-cell trafficking into the tumors have been described as a potential etiology by which tumors escape immunosurveillance. The tumor endothelium establishes a kind of a physical barrier that restricts T-cell infiltration into the tumor nest, possibly established by overexpression of the endothelin B receptor, which limits T-cell adhesion to the endothelium. In ovarian cancer samples, overexpression of endothelin B receptor was found to be strongly associated with lack of tumor-infiltrating lymphocytes (TILs) and with shorter survival [37]. Other proangiogenic growth factors, such as vascular endothelial growth factor A (VEGF-A), also impair T-cell adhesion to endothelium by dysregulating vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells. VEGF therefore appears to play an important role in impeding effector T-cell (Teff) trafficking into the TME. Furthermore, the VEGF-A gene was found to be downregulated in patients responding to PD-1 blockade, compared to nonresponders, which corresponded to lower VEGF-A levels [38]. These findings provide a rationale for the therapeutic combination of anti-VEGF plus anti-PD-(L)1 agents, which has shown significant improvement in both response rate and PFS in patients with RCC [39].

Fas ligand (FasL, CD95L), a homeostatic mediator of T-cell apoptosis, has been shown to be upregulated by immunosuppressive and pro-angiogenic factors in the TME, and expression of FasL was associated with absence of intratumoral CD8+ T cells [40].

Epigenetic inactivation of the cGAS-STING pathway is believed to be responsible, in part, for decreased immune cell trafficking into the tumor nest. Among other functions, the STING pathway appears to facilitate CTL trafficking and infiltration into tumor tissue. Several tumor types have been found to have defects in the cGAS-STING pathway, including ovarian cancer, colon cancer and melanoma [41, 42]. An intratumoral STING agonist, MK-1454, is being tested in

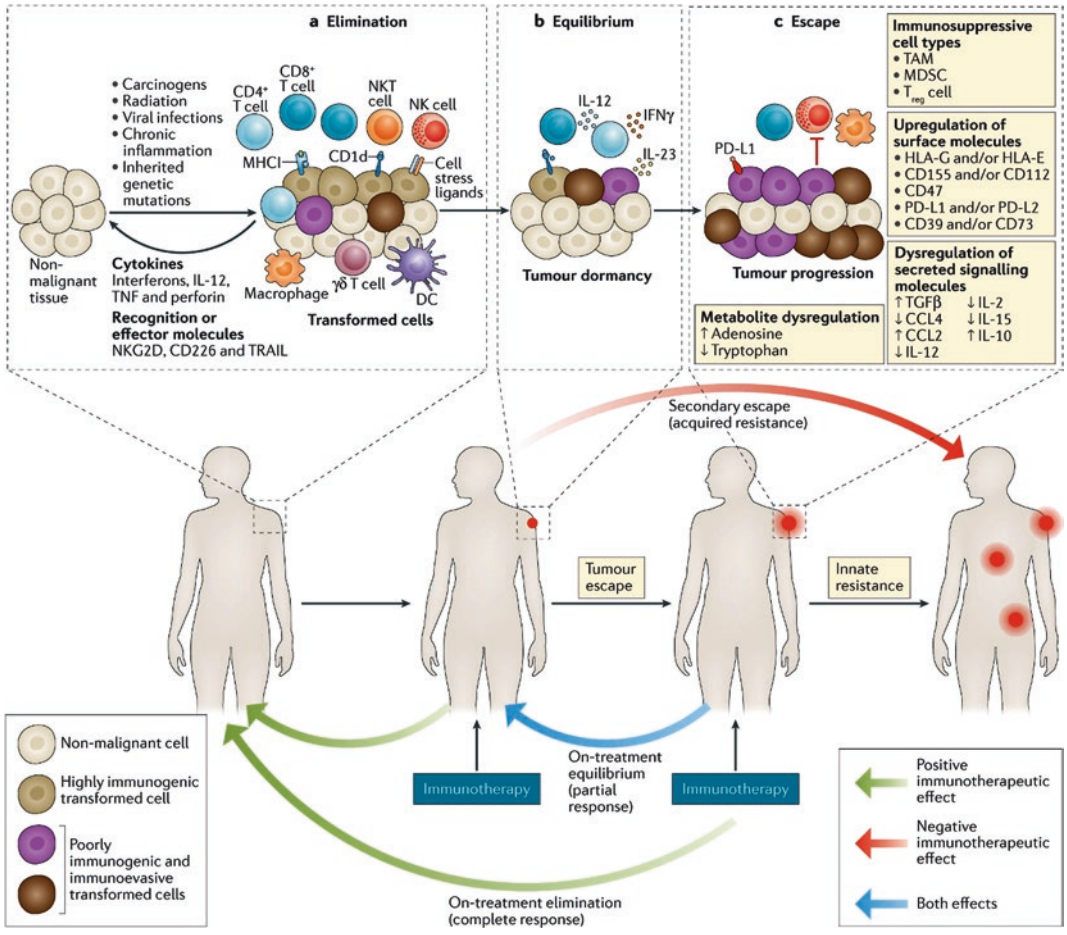

Fig. 1 Cancer immunoediting phases. a. Elimination: transformed cells that have escaped tumor suppressors are recognized and eliminated by innate and acquired immunity. b. Equilibrium: surviving cells enter a state of quiescence or limited growth where their immunogenicity is edited by the adaptive immunity. c. Escape: activation of immunosuppressive pathways allows unrestrained growth of tumors. Complete response occurs when immunotherapy is successful in overcoming immunosuppressive mechanisms and restoring anti-tumor immunity, i.e., reverting tumors to elimination phase. Incomplete reversal of tumor-induced immunosuppression results in tumors reverting to a state of on-treatment equilibrium that lasts until tumor subclones become capable of restoring immunosuppression and regrow resulting tumor progression and acquired resistance. Innate tumor resistance occurs as a result of immunotherapy failure to significantly restore anti-tumor immunity. Abbreviations: DC, dendritic cell; MDSC, myeloid-derived suppressor cell; MHCI, MHC class I; NK cell, natural killer cell; NKT cell, natural killer T cell; PD-L1, programmed cell death 1 ligand 1; TAM, tumor-associated macrophage; Treg cell, regulatory T cell. Adopted with permission from O'Donnell et al, Nat Rev Clin Oncol. 2019;16(3):151–67. [7]

combination with an anti-PD-1 agent in clinical trials (NCT04220866, NCT03010176).

Intratumoral injection of various immunotherapeutics has shown promising synergistic efficacy with PD-(L)1 blockade, inducing abscopal responses in noninjected tumors. Oncolytic and non-oncolytic viruses, myeloid dendritic cells (DCs), encapsulated mRNA (mRNA-2752), bifunctional fusion protein targeting CD47 checkpoints (SL-172154, TTI-621), cell-based inflammatory DCs (ilixadencel, immune primer), STING-activating agonist (MIW815), and others are being tested in combination with ICIs to enhance T-cell trafficking into the tumor bed and

to enhance antitumor activity by bypassing both physical and chemokine barriers [43–46] (NCT04502888).

Lastly, an interesting preclinical study of the intratumoral administration of seasonal flu vaccine in mice was successful in converting immunologically inert tumors into hot tumors and in increasing T-cells and DCs infiltration into tumors. In addition, this treatment enhanced the effect of PD-L1 blockade and re-sensitized resistant tumors to such therapy [47].

4 Dysfunctional Effector Immune Cells within the TME

Teffs are produced from naïve T cells upon acute antigen exposure. Once the antigen is cleared, the majority of Teffs undergo apoptosis while a minority change into memory T cells that are normally present in small numbers that can sharply increase upon antigen re-exposure. However, in cases of prolonged and/or repetitive exposure to the involved antigen, such as in chronic infections and in cancer, an immune-tolerant state ensues as T cells undergo transcriptional and epigenetic changes under the effects of inhibitory cytokines rendering them less functional and less reactive to the antigen in question. Upregulation of the inhibitory checkpoint PD-1 on T cells has been shown to occur as a result of chronic exposure to an antigen [48]. Dysfunctional T cells have low proliferative activity and are believed to exist in three forms: anergic, senescent and exhausted. Anergic T cells form in response to suboptimal stimulation and inadequate antigen exposure, and have low or no effector function. Senescent T cells arise from repetitive stimulation and have good effector functions but low proliferative properties. Exhausted T cells arise due to persistent overstimulation, have a high expression of inhibitory receptors, and are believed to have a mechanism of evolution in cancers that is distinct from that in chronic infections [49]. Several factors contribute to the development of dysfunctional T cells, including upregulation of inhibitory receptors, production of suppressive cytokines in an immunosuppressive TME, as well as the epigenetic and transcriptional dysregulation of T cells [49] (Fig. 2). Moreover, deficient immunologic memory is a hallmark of T-cell exhaustion resulting from chronic antigen exposure [50]. PD-(L)1 blockade, despite its ability to reinvigorate T cells, frequently falls short of efficiently restoring long-lasting memory, especially with continued high antigen exposure [51].

4.1 Co-Expression of Inhibitory Receptors on T Cells

Dysfunctional T cells are characterized by increased expression of multiple immune down-regulating checkpoint receptors (iRs) such as PD-1, CTLA-4, TIM-3, LAG-3, TIGIT, LAIR-1, and others (Fig. 3). In general, the more iRs expressed, the more significant the dysfunction.

Immunotherapy-induced upregulation of alternative checkpoints with Teff-repressive functions is now well-described in several tumor types (Table 2). Thirty-two NSCLC tumors were analyzed for iRs expression. Compared to circulating T cells from healthy donors, which had virtually no expression, TILs from patient samples were found to express PD-1 (43.5%), CTLA-4 (~25%), and LAG-3 (~12%). The study also demonstrated that the expression of checkpoints increased with tumor progression, providing an important proof of concept for the dynamicity of T-cell dysfunction as a progressive process. Treatment with PD-1 blockade restored Teff functions, as evidenced by increased IL-2, IFN-γ, and tumor necrosis factor (TNF)-α production in some, but not all, tumor samples. Failure of PD-1 blockade to restore effector function correlated with high PD-1 expression, and was also associated with upregulation of TIM-3, CTLA-4, and LAG-3 [52]. This observation was also reported in other tumor types where blocking a single checkpoint such as PD-1, LAG-3, or CTLA-4 in a murine model of ovarian cancer produced a compensatory upregulation of the other iRs. In this study, combination checkpoint blockade elicited superior tumor control compared to monotherapy inhibition [54].

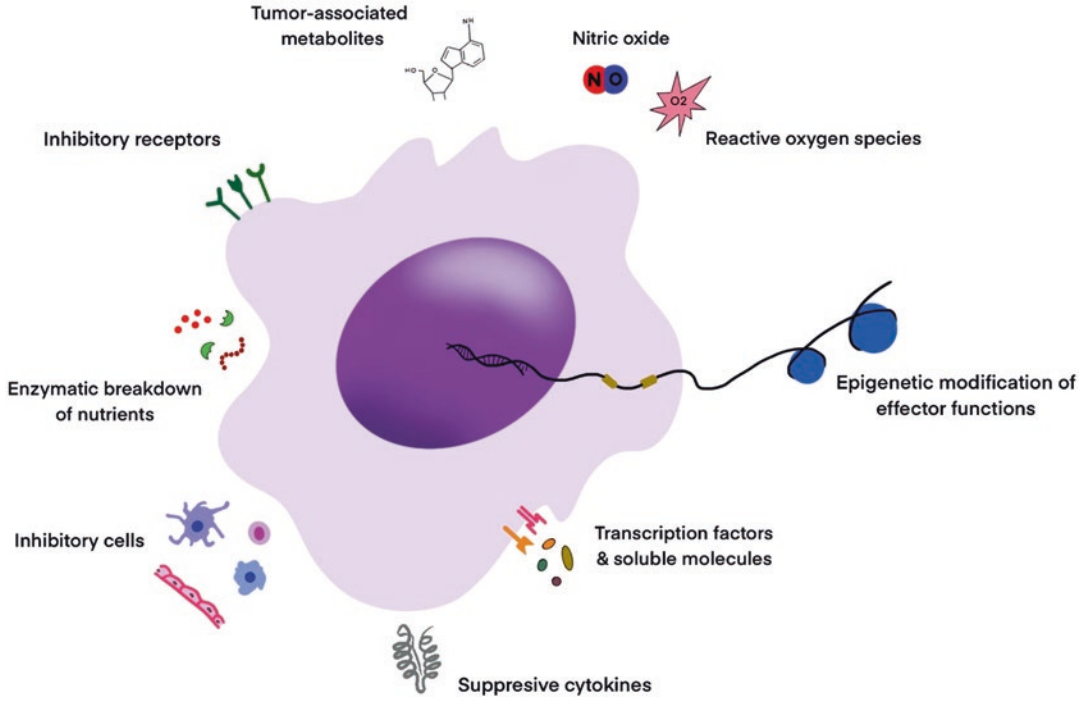

Fig. 2 Illustration of factors in the TME that are implicated in T-cell dysfunction. For instance, the upregulation of inhibitory receptors on immune cells, the production of suppressive cytokines and transcription factors by inhibitory cells, the generation of tumor-associated metabolites, NO and ROS, and the epigenetic dysregulation of inflammatory cells and cytokines are all elements that contribute to dysregulation of effector T-cell functions [49, 52, 53]

From a therapeutic standpoint, reversing/overcoming T-cell dysfunction can be achieved by either combining multiple ICIs that target different checkpoints or combining an ICI with a T-cell costimulatory agonist. The former has been successfully applied in the clinical setting as dual inhibition of PD-1 and CTLA-4 has shown enhanced efficacy in tumors like melanoma, NSCLC, and malignant pleural mesothelioma, albeit with increased immune-related adverse events [59–61]. Results of other ICI combinations such as anti-TIGIT mAb are starting to be reported [62, 63].

LAIR-1 is an inhibitory receptor expressed by a wide variety of immune cells, including NK cells, monocytes, DCs, and T and B cells, among others. LAIR-1 can inhibit NK and CTL cytotoxicity by binding to its ligands, collagen, C1q complement component and surfactant protein D, or by cross-linking with monoclonal antibodies . The LAIR-2 protein is highly homologous to the extracellular component of LAIR-1 and, when binding to the common ligands, can antagonize LAIR-1's inhibitory function [57]. The experimental drug NC410, a dimeric LAIR-2 bound to an Fc receptor, can serve as a decoy for the LAIR-1 ligands, thereby it helps decrease the inhibitory signal. It is currently being tested in a clinical trial in advanced solid malignancies (NCT04408599).

Potentiating T-cell function by using an agonist mAb to costimulatory receptors is another method of restoring function of exhausted T cells (Table 3). Utomilumab is an agonist of the costimulatory receptor 4-1BB (CD137), and has shown clinical activity as a single agent and in various combinations with anti-PD-1 and anti-chemokine receptor-4 (CCR4) agents [64–66]. Other costimulatory receptors, such as OX40, CD40, GITR, and ICOS may also become potential targets of agonist-based therapeutic interventions [67].

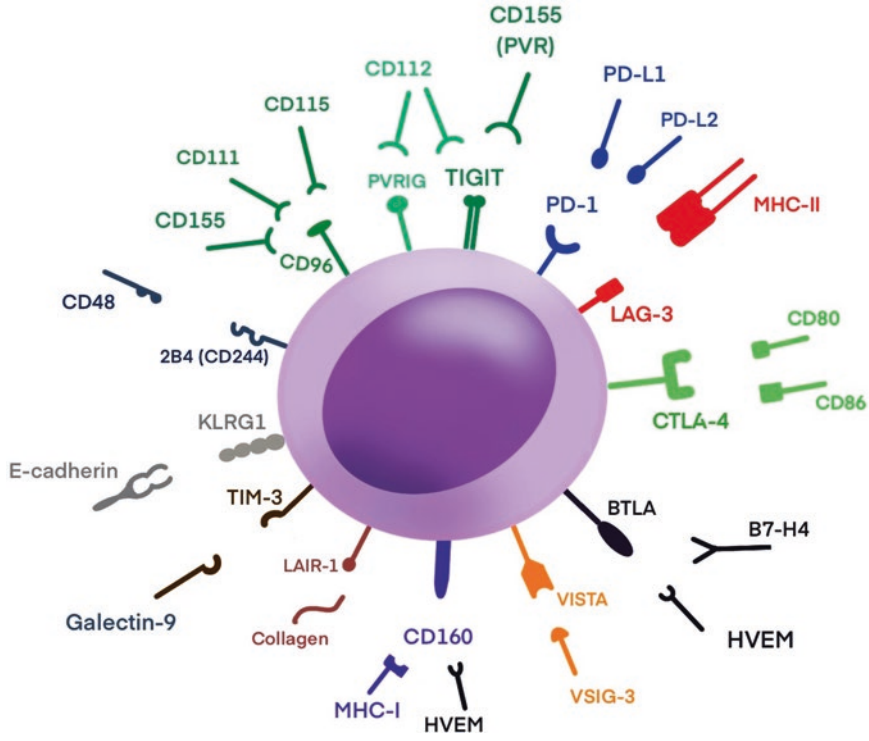

Fig. 3 Illustration of known inhibitory receptors and checkpoints and their ligands on T cells
Abbreviations: *BTLA* B- and T-lymphocyte attenuator, *CTLA-4* cytotoxic T-lymphocyte-associated protein 4, *HVEM* herpes virus entry mediator, *ITIM* immunoreceptor tyrosine-based inhibitory motif, *KLRG1* killer cell lectin-like receptor G1, *LAG-3* lymphocyte-activation gene 3, *LAIR-1* leukocyte-associated immunoglobulin-like receptor 1, *MHC* major histocompatibility complex, *PD-1* programmed cell death-1, *PD-L1* programmed cell death-ligand 1, *PVR* poliovirus receptor, *PVRIG* PVR-related immunoglobulin domain containing, *TIGIT* T-cell immunoreceptor with Ig and ITIM domains, *TIM-3* T-cell immunoglobulin 3, *VISTA* V-domain immunoglobulin suppressor of T-cell activation, *VSIG-3* V-set and immunoglobulin domain containing 3 [54–58]

Table 2 Illustration of T-cell inhibitory receptors with examples of targeting drugs

Inhibitory receptors on T cell [54]	Targeting drugs
PD-1	Pembrolizumab, nivolumab, pidilizumab [68], cemiplimab [69]
CTLA-4	Ipilimumab, tremelimumab [70]
TIGIT	Tiragolumab (NCT04300647, NCT04294810, NCT04513925)
LAG-3	Relatlimab (NCT04552223, NCT04095208, NCT04080804)
TIM-3	TSR-022, MBG453, LY3321367, Sym023 [71]
BTLA	JS004, TAB004 (NCT04278859, NCT04137900)
CD160	ELB01101 [72]
LAIR-1	NC410 (NCT04408599)

Table 3 Examples of T-cell stimulatory receptors with potential targeting drugs

Stimulatory receptors on T cell	Drugs
OX40	Pogalizumab, IBI101 [73], PF-04518600 (NCT03092856), BMS-986178 (NCT03831295), MEDI6469 (NCT02205333)
CD40	Selicrelumab, APX005M, ChiLob7/4, JNJ-64457107, SEA-CD40, CDX-1140H, ABBV-428, dacetuzumab [74], LVGN7409 (NCT04635995)
GITR	BMS-986156 [75], INCAGN01876 (NCT03277352), ASP1951 (NCT03799003)
ICOS	GSK3359609 (NCT04128696), MEDI-570 (NCT02520791)
4-1BB (CD137)	Utomilumab [64]

4.2 Immunosuppressive Cells in the TME

The TME is a complex interactive tumor cell-extrinsic system of cellular components, paracrine and autocrine factors, soluble molecules in the extracellular matrix, and vasculature. In some tumors, the TME cell composition can be a hostile milieu for Teffs, resulting in various degrees of dysfunction. Inhibitory cells interact with Teffs by several mechanisms, the most important of which is activation of iRs and secretion of inhibitory cytokines. Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), cancer-associated macrophages, cancer-associated fibroblasts (CAFs), adipocytes and endothelial cells have all been shown to have an important role in fostering T-cell exhaustion [49].

4.2.1 Regulatory T Cells

The FoxP3+ CD4+ subgroup of infiltrating T cells, Termed Tregs, are the main inflammatory downregulators in the TME. Tregs play an important role in promoting immune tolerance, and are found in abundance in many tumors. Their abundance has been linked to shorter OS in several tumor types including melanoma, hepatocellular carcinoma, RCC, gastric cancer and breast carcinomas, among others [76]. Tregs are chemotaxed into the TME via complex processes, most notably through chronic antigen exposure and the subsequent production of multiple Treg-upregulating cytokines by other immunosuppressive cells. The role of Tregs is important in mediating tumor resistance to both innate immunity and immunotherapeutics. Treatment with ICIs has been shown to increase the Teff/Treg ratio. However, it has also been shown that, in some cases of treatment refractoriness, ICI treatment may lead to further recruitment of Tregs to the TME, which plays a role in mediating resistance. This was shown to be the case in a murine model of claudin-low breast cancer that is generally known to be resistant to ICB [77]. In melanoma murine models, tumors with a higher Teff/Treg ratio were shown to be more responsive to ICB, which further highlights the role played by Tregs in mediating resistance to therapy [78, 79].

4.2.2 Myeloid-Derived Suppressor Cells

Treg proliferation and attraction to the TME is orchestrated by a network of immune and stromal cells that produce immunomodulatory cytokines and soluble molecules. MDSCs are increasingly recognized as a major player in the tumor evasion of innate immunity and also in mediating resistance to ICB. MDSC expansion and activation are controlled by various soluble factors such as IL-6, colony-stimulating factors, IL-10, VEGF, and toll-like receptors (TLRs) [80]. In addition, preclinical models suggest a role for CCL2 and CCL5 in their migration to the tumor niche through binding to receptors such as CCR2, CCR4, and CCR5 [80, 81]. Other molecules such as CXC chemokine ligand (CXCL)3 appear to also play a role in MDSC recruitment to the tumor bed by binding CXC chemokine receptor (CXCR)2 on MDSCs [80, 82]. IL-8 has also been shown to play a role in recruiting MDSCs to the TME [83]. The monocytic subtype of MDSC (M-MDSC) contributes to T-cell dysfunction via antigen-specific and antigen-nonspecific mechanisms; these include the production of reactive oxygen species and nitric oxide, the production of immunosuppressive transcription factors and cytokines such as transforming growth factor (TGFβ) and IL10, the production of arginase and other enzymes that degrade nutritionally important amino acids, and the production of ADAM17 which disrupts the ability of T cells to home to activation sites [81, 84]. Further evidence suggests that accumulating MDSCs within the tumor bed limits the efficacy of ICIs [85]. Clinical response to CTLA-4 blockade in melanoma patients was associated with lower frequencies of M-MDSCs by flow cytometry of circulating peripheral blood mononuclear cells (PBMCs) [86]. In addition to this predictive biomarker role, MDSCs' role in resistance is also suggested by the finding that higher circulating M-MDSCs frequency was associated with reduced tumor-specific T-cell activation and expansion and was independently associated with inferior survival in

a cohort of melanoma patients [87]. Overcoming MDSCs' effects and restoring sensitivity to ICIs can be achieved through several mechanisms, including decreasing frequency, blocking recruitment, and even directly neutralizing MDSCs [80].

4.2.3 Tumor-Associated Macrophages

M-MDSCs give rise to another type of regulatory cells, the TAMs, which are the most abundant immune cells in the TME. Although not completely understood, the differentiation of MDSCs to M2-phenotype TAMs appears to be promoted through hypoxia-induced production of HIF1α which leads to pSTAT3 downregulation. Therefore, hypoxic conditions within the tumor milieu appear to shift MDSC differentiation toward the immunosuppressive phenotype M2-TAM, rather than the effector phenotype M1-TAM [81, 88, 89]. The M1/M2 subtypes represent a continuum of phenotypes determined by upregulation/downregulation of stimulatory and inhibitory chemokines and receptors; polarization of TAMs toward M2 has been shown to be an important mechanism of resistance to therapy [90]. TAMs interact directly with naïve T cells by inhibiting their proliferation and function, and indirectly by preventing T-cell interaction with MHC, with consequential tumor progression [91]. TAMs can express several immune checkpoint ligands, including PD-L1 and the co-inhibitory receptor B7-H4, which plays a role in inhibiting the antitumor response of T cells. Production of IL-10 and other suppressors of CD8+ T-cell activation is another important role of M2-TAMs [90]. Using in vivo imaging, Arlauckas and colleagues demonstrated that anti-PD-1 mAbs are swiftly captured from the T-cell surface by PD-1-negative TAMs minutes after administration [92]. The role of TAMs in mediating resistance to anti-PD-1 therapy is also suggested by the finding of increased TAMs relative to CTLs in the pretreatment tumor samples of nonresponding melanoma patients, whereas responders were found to have an abundance of CTLs relative to TAMs which correlated with improved survival. Co-inhibition of colony-stimulating factor 1 receptor (CSF1R) and PD-1 induced complete regression of all BRAF-mutant cell-line tumors via effective elimination of TAMs [93]. Likewise, targeting TAMs via CSF1R blockade appears to be a promising strategy by which resistance to ICIs may be overcome. In a preclinical mouse model of pancreatic cancer, the combination of PD-1 or CTLA-4 inhibition with CSF1R blockade greatly enhanced antitumor effects compared to monotherapy with either ICI [94]. ARRY-382 is a CSF1R inhibitor that is currently being tested in solid tumor clinical trials as monotherapy and in combination with a PD-1 inhibitor (NCT02880371). B7-H4 is a co-inhibitory receptor upregulated by IL-6 and IL-10 that is expressed on TAMs as well as various tumors and plays an important role in T-cell inhibition [95]. FPA150 is an anti-B7-H4 mAb that is currently being tested in trials in combination with anti-PD-1 therapy (NCT03514121). Inhibition of phosphatidylinositol 3-kinase (PI3K)-γ, which is highly expressed on myeloid cells, including both M-MDSCs and TAMs, has been shown to inhibit the immunosuppressive phenotype polarization of TAMs from M1 toward M2 and promote CTL-mediated tumor killing, thus reversing myeloid-mediated ICI resistance [96]. An ongoing phase 1 clinical trial is currently evaluating the combination of nivolumab with IPI-549 (eganelisib) in solid tumors (NCT02637531).

4.2.4 Gamma-Delta (γδ) T Cells

γδ T cells represent a small proportion of tissue-dwelling lymphocytes and less than 5% of circulating lymphocytes [97, 98]. This MHC-nonrestricted subset of lymphocytes play an important role in innate immunity against both infections and tumors directly through the swift production of soluble cytotoxic molecules such as granzymes and perforin, as well as indirectly through the production of inflammatory cytokines such as TNF-α and IFN-γ; hence, these cells contribute to innate and adaptive immunity, and are not typically considered inhibitory cells. However, a small subset of γδ T cells has been shown to play an immunosuppressive and pro-tumorigenic role. IL-17-producing γδ T cells

enhance the recruitment of MDSCs and immunosuppressive neutrophils, restrain αβ T-cell activation, promote angiogenesis, and may directly induce apoptosis of effector immune cells [98–102].

While conventional CAR αβ T-cell therapy has proven effective in the treatment of B-cell hematologic malignancies, its efficacy against solid tumors remains very limited [103]. Taking advantage of their natural residence in the TME of solid tumors and their antigen-presenting properties, CAR-transduced γδ T cells, particularly the Vδ1 and Vδ2 subsets, appear to be an appealing therapeutic approach with enhanced antitumor efficacy [104].

4.2.5 Cancer-Associated Fibroblasts

CAFs are another type of TME regulatory cell that plays an important role in T-cell dysfunction, in addition to desmoplasia promotion. Considered one of the most abundant cells in the stroma of most tumors, CAFs play a bidirectional signaling role between tumor cells and other immune cells, including TILs and TAMs [105]. In addition to altering the extracellular matrix, CAFs produce angiogenic factors like VEGF that contribute to metastasis and neoangiogenesis. They also cross talk with tumor cells via the production of amino acid metabolites that act as a diverse fuel source promoting proliferation and aggressiveness [106]. However, like TAMs, there appears to be phenotypic heterogeneity in CAFs, as some types appear to impede tumor progression [107]. More recently, CAFs have emerged as a major mediator in the immunosuppressive TME. CAFs contribute to T-cell dysfunction through multiple mechanisms, most importantly by impairing T-cell trafficking and recruitment to the tumor milieu, and secondly by repressing the cytotoxic function of CD8+ T cells [108]. These effects are mediated through the production of several CAF-derived molecules and ligands, including TGF-β, CXCL12, CXCL5, IL-6, collagen, and fibronectin, and through the upregulated expression of immune checkpoint ligands including PD-L1, PD-L2, and FasL. The production of collagen by CAFs traps immune cells and creates high interstitial pressure within the tumor, which promotes progression of metastases [109]. CAFs also promote a DC phenotype that is unable to interact with and present antigens to CTLs [105]. Furthermore, CAF-mediated CXCL-1 and CXCL-2 have been shown to promote the growth and recruitment of MDSCs and Tregs to tumor stroma, as well as polarize TAMs toward the M2 phenotype [110–112]. Chakravarthy et al. identified a poor prognosis phenotype of CAFs that is upregulated in many cancer types and is driven mainly by TGF-β signaling. More importantly, this phenotype was associated with resistance to PD-1 blockade in melanoma and bladder tumor samples [113]. The combined inhibition of TGF-β and PD-L1 using a bidirectional fusion protein has shown enhanced antitumor activity in preclinical mouse models [114]. In mouse models of hepatocellular carcinoma, increased infiltration of CAFs was associated with resistance to PD-1 blockade. More interestingly, inhibiting activated CAFs rescued the antitumor effects of anti-PD-1 treatment in orthotopic immune competent models [115]. Galunisertib, a novel TGF-β inhibitor, in combination with nivolumab, is currently being investigated in an early-phase clinical trial in solid tumors with focus on NSCLC and hepatocellular carcinoma (NCT02423343).

4.2.6 Dendritic Cells

Through antigen presentation and T-cell priming, DCs are frequently the initial inducers of inflammatory response, and they conceivably play a pivotal role in the tumor-immunity cycle. Although several phenotypes have been identified, the function of DCs is largely context-dependent in that it can be skewed toward a stimulatory or an inhibitory phenotype. The conventional DC1 subtype is the principal primer of T cells after antigen exposure, consequently promoting effector function [116]. DC1s produce stimulatory cytokines like CXCL9/CXCL10 which help recruit and locally activate CD8+ T cells in the TME [117, 118]. The type 2 conventional DCs (DC2) interact with CD4+ T cells, while the plasmacytoid DCs produce IFN. Monocyte-derived DCs are effective in antigen uptake but less efficient in activation of T cells [116]. DC functions are context-dependent and therefore can be skewed toward an inhibitory

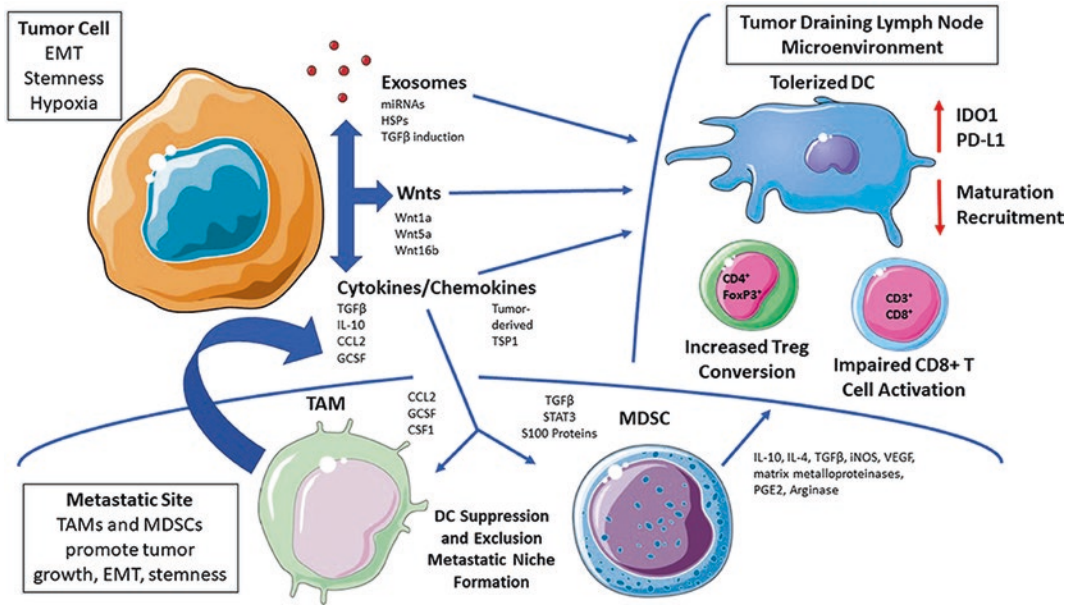

Fig. 4 Mechanisms of DC Tolerization. DCs residing within the tumor are functionally tolerized in the TME by immunosuppressive cells, inhibitory cytokines, and tumor exosomes. Tolerized DCs suppress T cell effector functions and enhances Treg differentiation, thus promoting tumor growth and metastasis. Abbreviations: EMT, epithelial-mesenchymal transition. TAM, tumor-associated macrophage; MDSC, myeloid-derived suppressor cell; IDO, indoleamine 2,3-dioxygenase; RA, retinoic acid; Arg, arginase; TSP1, thrombospondin-1. Adopted with permission from DeVito et al, Front Immunol. 2019;10:2876 [120]

phenotype upon tumor progression through a mechanism which is not fully defined [119]. IFN-γ produced by activated T cells in turn upregulates PD-L1 expression on DC1s, which plays a key role in limiting T-cell activation. Upregulation of PD-L1 on DCs occurs after antigen uptake as a mechanism to shield DCs from the cytotoxicity of activated T cells. However, this also suppresses tumor-directed immunity by contributing to T-cell dysfunction [119]. Furthermore, it has been shown that tumors subvert DCs by promoting a tolerization phenotype. This occurs via multiple mechanisms, including tumor-derived soluble molecules (IL-10, TGF-β, VEGF), tumor-derived exosomes (promoting a pre-metastatic niche), and the recruitment of other inhibitory cells in the TME (MDSCs, TAMs, Tregs) [120] (Fig. 4). PD-L1 expression on DCs appears to be indispensable for the efficacy of PD-L1 blockade therapy as the antitumor effect is completely lost in DC/PD-L1 knockout mice [119]. Targeting DCs is appealing and has been achieved through several novel mechanisms with variable success. The first DC-based vaccine, sipuleucel-T, was FDA-approved in 2010 and relies on ex vivo activation of and antigen delivery to DCs. Single-agent use of this form of immunotherapy yielded limited antitumor activity [121]. However, the combination of CTLA-4 blockade with sipuleucel-T resulted in remarkable activity in a small trial in patients with castration-resistant prostate cancer, and is currently being tested in a larger cohort with anti-CTLA-4 and anti-PD-L1 agents [122, 123] (NCT01804465).

Nanovaccines represent another modality that can target TLR signaling on DCs using insoluble nanoparticles that directly deliver peptide antigens to DCs with promising preclinical efficacy in vivo [124]. Ex vivo culture, activation, and antigen-loading of autologous myeloid-derived DCs followed by administration in patients' lymph nodes is another DC-based immunotherapeutic strategy with promising clinical activity in small cohorts of patients with melanoma [125, 126].

Toll-like Receptors

TLRs are receptors that play a role in innate and acquired immunity, and in antitumor immune response. They are either expressed on the cell surface and bind proteins and lipids (TLR1, TLR2, TLR4, TLR5, TLR6), or are expressed intracellularly on the endosomal membrane and bind nucleic acid (TLR3, TLR7, TLR8, TLR9). They can be expressed by several immune cells, particularly antigen-presenting cells including DCs and macrophages, and several types of tumors [127, 128]. Pathogen- and damage-associated molecular patterns bind to TLRs on DCs and other antigen-presenting cells inducing their maturation and initiating the immune response cycle. Foreign antigens, including cancer neoantigens, are then presented to T cells, leading to their activation [127].

TLR targeting has gained considerable interest over the past decade, as TLR agonists were found to exert an antitumor effect when administered locally. Single-agent use of TLR agonists has been implemented in different scenarios (e.g., bacillus Calmette-Guerin vaccine binding TLR2/TLR4 approved for superficial bladder cancer and topically applied imiquimod for actinic keratosis), but efficacy has been modest at best. The use of TLR agonists as an adjunct to DC-based vaccines has yielded promising results by enhancing immunogenicity in a cohort of patients with melanoma, the majority of whom had high-risk nonmetastatic disease [129]. Intratumoral TLR9 agonists are currently being tested in advanced stages of clinical development, after earlier phase trials showed promising activity in both injected and noninjected tumors. Injection with tilsotolimod, a TLR9 agonist, in combination with ipilimumab yielded a 38% response rate and a 71% disease control rate in a cohort of patients with anti-PD-1-refractory melanoma [130]. In another phase Ib trial, the combination of intratumoral TLR9 agonist CMP-001 with pembrolizumab yielded clinical responses in anti-PD-1-refractory patients, serving as a proof of concept of the ability to reverse resistance to ICI [131]. Other intratumoral TLR agonists are being tested in various clinical trials, such as the TLR4 agonist GLA-SE in CRC (NCT03982121), the TLR7 agonist imiquimod in breast cancer (NCT01421017), the TLR7 agonist DSP-0509 in combination with pembrolizumab for advanced solid tumors (NCT03416335), and MEL60 in combination with long-peptide vaccine in resected melanoma (NCT02126579).

4.2.7 Endothelial Cells

Transmigration of circulating T cells into the tumor nest is mediated through chemotactic cytokines and the upregulated expression of adhesion molecules and ligands on activated endothelial cells. However, constitutive activation of the tumor vasculature by proangiogenic factors in the TME can paradoxically lead to dysfunctional endothelial cells that impair leukocyte adhesion and transendothelial migration [132]. Dysfunctional endothelial cells express ligands that greatly reduce immune cell permeability. The FAS antigen ligand (FasL), under the effect of IL-10 and prostaglandin E, can induce apoptosis of CTLs but not Tregs [40]. Dysfunctional tumor vasculature is known to represent an efficient barrier for recruitment of Teffs and thus pose a challenge toward effective immune checkpoint blockade (ICB) [133]. Suppression of VEGF-A has been shown to increase CD8+ T-cell influx into tumors [40]. Treatment strategies that harness the crosstalk between tumor angiogenesis and the immune system can restore the antitumor effects of ICIs. Several proangiogenic molecules have been found to effectively contribute to immunosuppression. VEGF has been shown to impair DC functional maturation; thus, anti-VEGF treatment was successful in restoring the differentiation of monocytes into DCs [134, 135]. In addition, VEGF contributes to T cell exhaustion by enhancing PD-L1 expression on DCs and suppressing antigen presentation [136]. Direct VEGF binding to the VEGFR2 receptor on Teffs suppresses proliferation and upregulates PD-1 expression, while binding to the same receptor on Tregs and MDSCs enhances their infiltration into the tumor milieu [137]. VEGF-mediated modulation of VCAM-1 and ICAM-1 adhesion molecules creates a barrier that is impermeable

to effector immune cells, precluding homing of T cells to tumors [138]. Consequently, it is postulated that vascular normalization via the use of VEGF inhibitors has the potential to augment anti-PD-(L)1 therapy and enhance antitumor response. Moreover, treatment with VEGF/VEGFR inhibitors has been shown to upregulate PD-L1 on tumor cells, and the combined blockade of PD-L1 and VEGF showed synergistic antitumor effect in pancreatic neuroendocrine and breast cancer mouse models [139]. This combination has demonstrated clinical efficacy across a variety of tumor types in phase III trials and is already FDA-approved in RCC, NSCLC, hepatocellular carcinoma, and endometrial carcinoma [140] (Fig. 5).

4.2.8 Tumor-Derived Pericytes

Pericytes are perivascular cells that play an important role in vascular structure and integrity. However, in tumor beds, pericytes may frequently lose close attachment to endothelial cells in the tortuous, erratic tumor vessels, causing aberrant permeability and dysfunctional, leaky flow [141]. Besides their role in tumor angiogenesis, the type 2 pericytes appear to play an important interactive role with other cells and chemokines in the TME. In vitro studies have shown that pericytes may contribute to some immunological functions like phagocytosis and antigen presentation [142]. Pericytes can produce several types of cytokines, growth factors, and adhesion molecules, and are considered an important component of the immunologic shield [141, 143]. Promoting pericyte maturation has been show to restore vasculature function and improve CD8+ T-cell transmigration into the tumor niche which resulted in improved antitumor immunity in mouse models [144]. Tumor-derived pericytes express PD-L1, which has a known role in CD8+ T-cell dysfunction, and Rgs5, which prompts anergy of CD4+ T cells. These effects contribute to shielding of tumor cells from immune-mediated destruction, a finding that suggest pericytes may be an appealing target for immunomodulation. Needless to say that therapeutic approaches should focus on normalizing pericyte functionality rather than elimination [145].

4.3 Cytokines and Other Soluble Molecules in T-Cell Dysfunction

As a critical component of autocrine and paracrine signaling, cytokines are involved in all pathways leading activation and trafficking, as well as to the dysfunction and exhaustion, of T cells. Many cytokines are receptor-pluripotent in that they can bind several receptors on a cell surface. Receptors, likewise, may bind different types of ligands. Manipulating cytokine production, or receptor binding, can potentiate the effectiveness of ICB by preventing the development of resistance [146]. In this context, it is noteworthy that the use of cytokines such as IL-2 for RCC and melanoma, and IFN-γ for myeloproliferative neoplasms, was one of the earlier forms of immunotherapy implemented in clinic, albeit with limited success [147]. Among the cytokines that seem to have a great impact on T cell functions are the C-X-C motif ligands 9 and 10 (CXCL9 and CXCL10). CD8+ Teffs, NK cells, and type 1 helper T cells (Th1) all express CXCR3, which binds ligands CXCL9 and CXCL10 produced by Th1. This binding results in the chemotaxis and infiltration of effector cells into tumors, which in turn is correlated with improved clinical outcomes in response to PD-(L)1 blockade [148, 149]. Epigenetic silencing of CXCL9 and CXCL10 leads to poor Tcell infiltration into tumors; and treatment of colon cell lines with a histone methylation inhibitor leads to higher CXCL9 and CXCL10 expression and more efficient T-cell migration toward tumors [150]. The reversal of epigenetic silencing of CXCL9 and CXCL10 was also synergistic with PD-L1 blockade therapy in ovarian cancer xenografts [151]. This has raised interest in epigenetic reprogramming as a method to improve T-cell trafficking to the TME and therefore improve response to ICIs. Combination therapies of anti-PD-(L)1 with a hypomethylating agent are currently being evaluated in clinical trials in a variety of liquid and

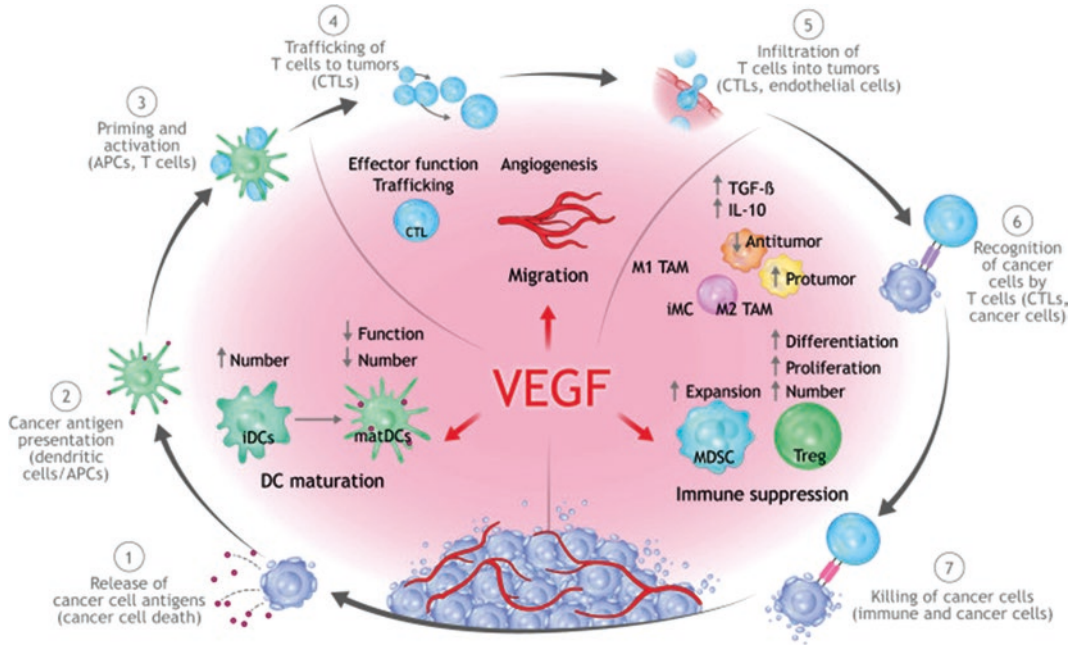

Fig. 5 VEGF-mediated immunosuppression in the TME. VEGF-induced constitutive activation of tumor vasculature leads to endothelial cell dysfunction and vascular aberration. VEGF is also implicated in reduced T cell permeability, increased inhibitory cytokines and regulatory cells, and impaired DC maturation. Abbreviations: APC, antigen-presenting cells; CTLA, cytotoxic T lymphocyte associated; DC, dendritic cell; MHC, major histocompatibility complex; PD-1, programmed cell death 1 protein; PD-L1, programed cell death ligand 1; PlGF, placental growth factor; TME, tumor microenvironment; TCR, T-cell receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Reproduced with permission from Hack et al, Front Immunol. 2020;11:598877 [140]

solid malignancies (NCT03233724); and some trials started to report outcomes [152].

In contrast, the interaction of CXCL12, a cytokine produced by stromal cells particularly CAFs, with its receptor on T cells, CXCR4, has been shown to play a role in recruiting and retaining FoxP3 + CD4+ Tregs in tumors like basal-like breast cancer and epithelial ovarian carcinoma [153, 154]. Moreover, high CXCR4 expression predicts a more advanced stage and lower survival in other tumors like gastric cancer [155]. CXCL12/CXCR4 blockade by a CXCR4 antagonist or by oncolytic virotherapy has been shown to reduce tumor growth and improve survival in immunocompetent murine models of ovarian cancer [154, 156]. Dual blockade of PD-(L)1 and CXCL12-CXCR4 has been shown to be synergistic in thwarting immunosuppression in the TME and enhancing antitumor immunity in preclinical models [157]. This combination is being tested in early-phase trials (NCT04177810).

The monocyte chemoattractant protein 1 (CCL2) is produced by immune cells and implicated in the migration of monocytes. In addition, it is also produced by some tumors and implicated in the migration of other cells like Tregs and endothelial cells to sites of inflammation [158]. Other cytokines, including CCL3 and CCL5, are also involved in immune cell migration to the TME, particularly neutrophils and macrophages. Inhibition of these cytokines has been shown to reduce invasive potential and neoangiogenesis in preclinical models of breast and ovarian cancers [159, 160]. Blocking CCL2 reduces immunosuppression and enhances the antitumor activity of an adenoviral vector expressing IFN-α [158].

As a major stimulator of the T-cell adaptive response, DCs produce a wide array of cytokines that are involved in immune response. Among these, CCL17 and CCL22 produced particularly by monocyte-derived DCs, among other immune cells, as well as by some tumors, appear to play an important role in Treg recruitment to tumors. Blocking CCL17 and CCL22 in monocyte-derived DCs using RNA interference reduces the frequency of Treg recruitment and increases CD8+ T cells in human breast cancer xenografts [161, 162]. Moreover, the CCL17/CCL22 receptor, CCR4, is expressed by Th2 cells and by some of the most terminally differentiated and immunosuppressive tumor-infiltrating FoxP3-high Tregs [163]. CCR4 expression has been found in several tumors, especially T-cell malignancies. In addition to its efficacy related to antibody-dependent cytotoxicity in T-cell neoplasms, anti-CCR4 mAb was effective in inducing FoxP3-high Treg depletion [162, 164]. Signaling of CCL17/CCL22-CCR4 is implicated in tumor resistance to ICIs, as upregulation of both ligands has been shown to occur as a result of ICI therapy in vivo. More interestingly, CCR4 inhibition had a synergistic antitumor effect with anti-CTLA-4 therapy [165].

The transmigration of MDSCs into the TME is mediated through the CXCR2 receptor, which binds CXCL1, CXCL8, CXCL5, and CXCL7, among others. Elevated levels of CXCR2 ligands, CXCL1 and CXCL8, was detected in pediatric sarcoma patients, and appear to confer worse prognosis. Mice reconstituted with CXCR2-negative hematopoietic cells showed enhanced antitumor activity when exposed to PD-1 blockade [166]. In addition to its role in promoting angiogenesis and epithelial-to-mesenchymal transition, CXCL8 (IL-8) plays an immunosuppressive role in the TME. Produced by many tumors, CXCL8 recruits both types of MDSCs. Furthermore, high CXCL8 levels were found to predict poor outcome in patients treated with immunotherapy [83]. Anti-IL-8 mAbs can abolish signaling through both receptors, CXCR1 and CXCR2. Preclinical studies in claudin-low breast cancer showed this strategy to be highly effective in reducing MDSCs and increasing immune-mediated cytotoxicity [167]. Early reduction of IL-8 levels was shown to be strongly correlated with tumor response to anti-PD-1 therapy in two cohorts of NSCLC and melanoma patients [168]. Single-agent anti-IL-8 mAb therapy yielded modest antitumor activity in pretreated patients with a variety of solid tumors [169]. Studies with combined PD-1/IL-8 blockade are underway to evaluate clinical activity (NCT03400332, NCT03689699, NCT04050462).

In addition to its role in cell growth, proliferation, differentiation, and cell matrix formation, TGF-β appears to play a key role in driving immune evasion. In patients with CRC, elevated TGF-β levels was associated with lack of T-cell infiltration, low Th1 activity, reduced cytotoxicity, and poor clinical outcome. In genetically reconstituted low TMB, MS-stable, T-cell-excluded colon cancer metastases, PD-(L)1 inhibition produced limited antitumor efficacy, as would be expected; however, the subsequent blocking TGF-β signaling produced a potent cytotoxic T-cell response and restored sensitivity to anti-PD-(L)1 therapy. This suggests an important role for TGF-β in promoting T-cell exclusion and blocking the Th1 effector phenotype in the TME [170]. Likewise, TGF-β signaling was found to be one of the main determinants of clinical outcome in a cohort of patients with urothelial carcinoma. Lack of response to anti-PD-L1 therapy was associated with a TGF-β signaling signature in fibroblasts. Furthermore, co-blockade of PD-L1 and TGF-β enhanced T-cell trafficking into tumors and produced a more profound antitumor effect [171]. Consistent with these findings, an elevated plasma level of TGF-β was also found to be a significant predictor for poor treatment outcome in a cohort of patients with hepatocellular carcinoma treated with anti-PD-1 therapy [172]. Several TGF-β inhibitors have been developed, including small molecule inhibitors and mAbs. Some of these agents have shown activity as monotherapy or in combination in early-phase trials [173–175]. Trials evaluating the combined inhibition of PD-(L)1 and TGF-β in a variety of solid tumors are underway (NCT02423343, NCT04390763).

Bintrafusp alfa is bifunctional fusion protein composed of the extracellular domain of TGF-β receptor 2, linked to the heavy chain segment of the anti-PD-L1 antibody. Bintrafusp alfa functions as a trap to all isoforms of TGF-β while simultaneously mitigating immunosuppression. Preclinical data have demonstrated the ability of bintrafusp alfa to increase T-cell trafficking and cytotoxicity in cell lines and mouse models [114, 176]. In addition, PD-L1 binding allows for concentration within a PD-L1-positive tumor; and preclinical studies showed that up to 27% of the injected dose concentrate in the tumor with a peak tumor/blood ratio of 58:1 [177]. Promising clinical activity have been noted in a cohort of patients with heavily pretreated advanced solid tumors in a phase 1 trial [178] and in several solid tumor indications [179–181].

IL-10, previously termed "cytokine inhibitory factor," is one of the first inhibitory factors to be identified. IL-10's immunosuppressive role is considered a key component in limiting excessive inflammatory response. IL-10 is produced by many immune cells including CD4+ and CD8+ T cells, TAMs, and DCs, as well as tumor cells. It plays a role in the downregulation of Th1 inflammatory cytokines, namely, IL-2, TNF-α, and IFN-γ, and inhibits MHC-II expression on activated monocytes. Nevertheless, it is currently believed that IL-10 may in fact possess a bifunctional role, as it has also been shown to have an immune-stimulatory role by inducing tumor-dwelling CD+ T-cell activation and expansion [182–184]. An elevated level of IL-10 has been identified as an adverse prognostic indicator in several tumor types, including both hematological and solid malignancies [185]. In vivo inhibition of IL-10 enhances cytotoxic T-cell function and the antitumor activity of PD-L1 blockade [186]. In contrast, pegilodecakin, a pegylated recombinant IL-10, has been tested in clinical trials and demonstrated activity in pretreated advanced RCC (NCT02009449).

As discussed above, VEGF is another important mediator of immunotherapy resistance. In addition to its role in disrupting normal vasculature, VEGF impairs CTL proliferation and trafficking, and inhibits DC maturation and antigen processing [140].

IFN-γ is believed to play a role in innate antitumor immunity by enhancing antigen presentation through upregulation of MHC-I. However, it can also promote an immunosuppressive TME through activation of the JAK/STAT pathway, resulting in increased expression of PD-L1 in what represents a negative feedback loop [187]. The efficacy of combining IFN-γ and ICIs is being evaluated in early-phase trials in a variety of solid and liquid tumors (NCT02614456, NCT03063632).

IFN-α is a pleiotropic cytokine with antineoplastic properties and has been in clinical use for adjuvant therapy of high-risk melanoma. The immunomodulatory effects of IFN-α include stimulating CXCL10 secretion, which in turn enhances CD8+ T-cell trafficking and effector activity within the TME [188]. In vivo IFN-α treatment of a murine colon cancer cell line increased PD-1 expression on TILs. Co-inhibition of PD-1 and IFN-α increased CD4+ and CD8+ TILs and reduced tumor growth more than IFN-α alone [189]. Several studies are underway evaluating this combination in humans in metastatic and adjuvant settings (NCT02506153, NCT02174172).

Another cytokine implicated in immunomodulation is the IL-6, which is produced by tumor cells and tumor-infiltrating immune cells. Elevated circulating IL-6 levels was noted in several tumor types, and correlated with advanced tumor stage and reduced response to therapy [190, 191]. Both IL-6 and IFN-γ were shown to upregulate PD-L1 expression on antigen-presenting cells; and this process appears to be mediated by activation of the Janus kinase/signal transducers and activators of transcription 3 (JAK/STAT3) signaling pathway [187, 191]. A positive autocrine feedback loop then forms as STAT3 enhances IL-6 gene expression, which contributes to the development of an immunosuppressive TME in epidermal growth factor receptor (EGFR)-mutant NSCLC. In addition, STAT3 hyperactivation in immune cells in the TME has been shown to upregulate both MDSCs and Tregs [191, 192]. In vivo silencing of the

STAT3 pathway led to downregulation of PD-L1 expression and reduced metastatic potential in a murine mouse model of breast cancer [193]. Single targeting of either IL-6 or STAT3 has generally yielded dismal results and limited antitumor activity in early-phase trials [194–196]. However, the use of a combination strategy with ICB may prove more promising, and is being investigated in a variety of solid tumors (NCT04191421, NCT04691817).

5 Oncogenic Signaling Pathways

Oncogenic alterations in the tumor cell genome, both gain- and loss-of-function mutations, have been implicated in promoting an immunosuppressive TME. Oncogene addiction is not an exclusive cell-intrinsic process; rather, it is greatly influenced by crosstalk with an immune-permissive TME composition [197]. Advances in molecular technologies have shed light on the interaction between the immune system and the tumor's driver mutations; consequently, several aberrations have been identified as potential mechanisms for tumor resistance to innate immunity and immunotherapy.

5.1 JAK/STAT Mutations

Inactivation of the IFN-JAK1/JAK2 pathway resulting from an emerging loss-of-funcion mutation has been described in melanoma patients who developed secondary resistance to ICIs. Tumor cells appear to resort to abrogation of IFN-mediated signaling as a potential way to evade treatment with anti-PD-1 therapy. As discussed above, IFN signaling leads to an adaptive increase in PD-L1 expression. Eliminating this pathway is postulated to decrease therapeutic target receptors, rendering treatment ineffective [23]. Activation of the JAK/STAT pathway through the amplification of chromosome 9p24.1 region, which encodes for JAK2 and PD-L1/L2, has been described in a subset of triple-negative breast cancers (TNBC) and was linked to poor prognosis. This PDJ amplicon leads to an IFN-induced increase in PD-L1 expression by 5- to 38-fold. Subseqently, JAK2 knockdown in TNBC cell lines completely blocked inducible PD-L1 expression [198]. An ongoing phase I trial is evaluating the combination of JAK2 inhibitor with ICIs in TNBC patients [199].

5.2 Mutations in the Ras-Mitogen-Activated Protein Kinase (Ras-MAPK) Pathway

Activation of the Ras-MAPK pathway has been shown to correlate with reduced TIL in a subset of TNBC patients who failed to achieve pathologic CR after neoadjuvant therapy. In addition, activation of this pathway may suppresses MHC expression and upregulates PD-L1, an effect possibly mediated through IFN-γ signaling. A similar finding was reported in human melanoma cell lines. The process is believed to play an important role in tumor evasion of innate immunity, as well as in MAPK-activated tumor resistance to ICB [200–202]. The synergy of MEK inhibitors and PD-(L)1 blockers was demonstrated in syngeneic murine models of triple-negative and HER2-positive breast cancer [200, 201]. In early-phase trials, the combination of dual MAPK pathway inhibitors with an anti-PD-(L)1 agent led to increased immune infiltration into tumors and yielded promising activity [203, 204].

5.3 Loss of Phosphate and Tensin Homolog (PTEN) Tumor Suppressor

Loss of PTEN, with subsequent PI3K-AKT-mTOR signaling activation, is not only oncogenic but is also implicated in mediating resistance to immunotherapy. PTEN loss induces VEGF and immunosuppressive cytokines production, reduces T-cell trafficking and cytotoxicity, and promotes MDSCs in the TME [96, 205, 206]. For instance, acquired PTEN loss was shown to confer primary resistance to anti-PD-1

therapy in patients with uterine leiomyosarcoma [205]. Treatment with PI3K inhibitors improved the antitumor efficacy of ICIs in murine models [96, 206]. A phase I trial of a PI3K-γ inhibitor in combination with an anti-PD-1 agent reported favorable outcomes and early signs of clinical activity [207]. Several trials are underway evaluating the safety and efficacy of combined inhibition of PI3K and PD-1 (NCT04193293, NCT03711058).

5.4 Activation of the Wnt/β--Catenin Signaling Pathway

The role of Wnt signaling in oncogenesis and tumor propagation has been documented in several tumor types, including CRC, mammary carcinoma, hematologic malignancies, and melanoma, among others. The effect of aberrant Wnt/β-catenin signaling extends beyond tumor cells to include the TME [208]. For example, in metastatic melanoma, activation of the Wnt/β--catenin pathway impaired T-cell priming and activation by tolerizing DCs, and was correlated with reduced TILs [120, 209, 210]. A novel β-catenin inhibitor is being combined with an anti-PD-1 agent in a phase I clinical trial in solid tumors (NCT02521844).

5.5 KRAS Mutation

KRAS is one of the most altered genes in human malignancies, and is known to play several critical roles in the immune composition of the TME. KRAS mutation in NSCLC appears to be associated with increased tumor C8+ T-cell infiltration, inflammed TME phenotype, and increased responsiveness to ICB [211, 212]. In contrast, KRAS-mutated CRC and pancreatic adenocarcinoma exhibit an immunosuppressive TME, which was also associated with lower response rate to ICB [82, 213, 214].

KRAS mutations cause upregulation of PD-L1 through activation of the PI3K/AKT/mTOR pathway [215]. In addition, MAPK/ERK signaling was shown to contribute to stabilization of PD-L1 mRNA [216, 217]. Activation of the MEK-ERK pathway in KRAS-mutated lung cancer can modulate the TME through increased secretion of IL-10 and TGF-β. Further, in vivo inhibition of KRAS in a KRAS-driven lung tumorigenesis model significantly reduced Treg infiltration, which provides a proof of concept of the cell-extrinsic activity of this mutation [218]. Furthermore, through the suppression of interferon regulatory factor 2, KRAS leads to increased CXCL3 expression and binding to CXCR2 on MDSCs prompting their migration to the TME [82]. Lastly, the occurrence of STK11/LKB1 co-mutation in KRAS-mutated NSCLC has been shown to significantly reduce response rate to PD-1 inhibition [214, 219].

Inhibition of the KRAS downstream pathway through MEK inhibitors, or through a KRAS mRNA vaccine, in combination with anti-PD-(L)1 therapy is currently being studied in early-phase trials (NCT03948763, NCT03681483, NCT03299088).

5.6 Epidermal Growth Factor Receptor Mutation

An immune-tolerant TME is a hallmark of EGFR-mutant NSCLC, which is known to exhibit reduced responsiveness to anti-PD-(L)1 therapy [220–222]. Despite some controversy, it appears that both lower PD-L1 expression and lower TMB in these tumors lead to the relative refractoriness to ICB [223, 224]. The EGFR signaling pathway promotes an uninflamed TME through enhanced Treg migration and through skewing DCs toward a tolerant phenotype [223, 225]. Phosphorylation of STAT3, a downstream signaling transducer of EGFR, increases expression of indoleamine 2,3-dioxygenase (IDO) which in turn promotes the expansion of MDSCs and enhances their immunosuppressive effect [226]. In an interesting study by Huang and colleagues, most exosomes purified from biopsies of lung tumors were found to contain large quantities of EGFR protein. When captured by DCs, these EGFR-laden exosomes promote DC to differentiate to a tolerogenic phenotype

that promotes Tregs and suppresses tumor-specific CD8+ T cells [225]. Tyrosine kinase inhibitor (TKI) therapy with EGFR inhibitors has been shown to revive some of the inflammatory aspects of the TME and increase CD8+ T-cell infiltration [227]. The combination of anti-PD-L1 and EGFR-TKI yielded a response rate of 43% in a cohort of patients previously resistant to TKI monotherapy, albeit with increased incidence of interstitial lung disease [228]. There are several ongoing trials evaluating different TKI-ICI combinations (NCT02364609, NCT03082534, NCT04017650).

BCA101 is a first-in-class bifunctional antibody that targets both TGF-β and EGFR. It is being tested in combination with anti-PD1 therapy in EGFR-driven tumors in a phase I trial (NCT04429542).

6 Tumor-Associated Enzymatic Activity and Metabolites

Enzymatic activity in the TME impacts the innate and adaptive immune response by catabolizing important immune cell amino acid nutrients, creating inhibitory metabolic byproducts, and playing a role in intracellular signaling pathways.

6.1 Indoleamine 2,3-Dioxygenase-1 (IDO-1)

IDO-1 is a versatile enzyme, mainly induced by IFN-γ, that has been shown to regulate immune response by reducing uncontrolled activation in inflammatory conditions. It has gained attention due to its notable role in modifying antitumor immune response and the potential for targeting in clinic. In response to immune activation, IDO catalyzes the metabolism of tryptophan, thus depleting an essential element for effector T cell function. The metabolic product of this process is kynurenine, which is the ligand for the aryl hydrocarbon receptor. Kynurenine promotes the differentiation of FoxP3+ Tregs and enhances their immunosuppressive effects [229, 230]. More interestingly, a distinct intracellular signaling role of IDO-1 was identified as it was found to promote a regulatory phenotype in plasmacytoid DCs under the effect of TGF-β [231]. Upregulation of IDO-1 has been shown to occur in some tumors as a response to ICI therapy. A phase I/II trial revealed an encouraging response rate for the combination of pembrolizumab and IDO-1 inhibitor, epacadostat, in a variety of tumor types [232]. However, in a larger cohort of patients, this combination failed to produce significant benefit over single-agent pembrolizumab in a randomized double-blind phase III trial [233].

6.2 Adenosine

CD73 (ecto-5′-nucleotidase) is a cell surface enzyme implicated in purinergic signaling by mediating the breakdown of adenosine monophosphate to adenosine. CD73 is upregulated by many tumor types and has key functions in regulating tumor proliferation, invasiveness, angiogenesis, and immune-evasion. The metabolic product, adenosine, promotes cancer cell survival and progression, and plays an important immunosuppressive role in the TME [53, 234, 235]. CD73 can be expressed on neoplastic cells of several tumor types, as well as on Tregs, MDSCs, and endothelial cells. TGF-β plays an important role in sustaining CD73 expression on CD8+ T cells. Adenosine binds to receptors A2AR/A2BR on lymphocytes, suppressing their effector function and downregulating the inflammatory response. Moreover, adenosine has been shown to inhibit DC maturation, thus impairing antigen presentation. The adenosinergic immunosuppressive role of CD73 is an appealing target to revive antitumor immunity [234, 236–238]. In addition to conferring an adverse prognosis, CD73 expression is associated with reduced ICI efficacy [239, 240]. Targeting CD73 has been achieved through direct antibody blockade or by blocking the adenosine receptor. The anti-CD73 mAb MEDI9447 in combination with durvalumab demonstrated some clinical activity in the treatment of refractory CRC and pancreatic carcinoma [241]. AZD4635, a small molecule inhibitor of A2AR, rescued antitumor immunity

in DCs in vitro, and inhibited tumor growth in syngeneic mouse models [238]. AZD4635 yielded notable antitumor activity as a single agent and in combination with durvalumab in a phase I trial [242].

7 Impact of Anatomical Site

While immunotherapy achieved remarkable milestones in malignancies like melanoma and NSCLC, it yielded disappointing results in other tumors like luminal-type breast cancer and pancreatic adenocarcinoma. Tissue-specific differences in immune infiltrate composition and function are plausibly implicated in these differences, especially the tissue-dwelling myeloid cells and DCs. Zagorulya and colleagues proposed a phenotypic classification of DCs that infiltrate different anatomic sites and correlated this with the likelihood of successful ICB. For instance, lung tissue appears to skew DCs toward a stimulatory phenotype that is efficient in antigen presentation and T-cell activation, leading to a more inflammatory TME and higher ICB success rate. This is in contrast to immune-desert tumors, like pancreatic ductal carcinomas, which are infiltrated with rare DCs that are skewed toward an inhibitory phenotype [116]. On the other hand, some metastatic sites appear to be particularly less responsive to ICB. For example, liver metastases exhibit lower response rates to ICB even if they originate from primary tumors known to respond to such therapy [243]. Several mechanisms have been found to account for the immune-tolerant TME in liver tissue. Tolerogenic DCs with a weak antigen-presenting phenotype predominate in the liver and produce IL-10 and TGF-β, resulting in Treg induction and Teff inhibition. In addition, Kupffer cells in the liver appear to display an immunosuppressive macrophage phenotype. Despite their ability to prime CD8+ T cells, the resulting cells are largely dysfunctional in that they produce low levels of IFN-γ and have poor effector capabilities [116, 244].

8 Hyperprogression Phenomenon

In discussing mechanisms of immune evasion, one cannot overlook the few instances where ICI therapy may paradoxically enhance tumor growth and cause accelerated progression. Hyperprogression is a distinct entity that has been noted to occur in several tumor types in response to treatment with ICIs. Depending on the criteria used to define it, the estimated incidence ranges between 4% and 29% of treated patients [245, 246]. A definition for hyperprogression has not been unanimously agreed upon, but some authors suggest using the combined findings of RECIST progression on first evaluation scan plus a twofold volumetric tumor growth rate, where volume is calculated as $V = 4\,\pi\,R^3/3$, R being the radius is half the sum of maximum dimensions of target lesions, assuming a spherical tumor shape [247]. Others have proposed using a more than 50% increase in monthly tumor growth rate, or a twofold increase in tumor growth rate between the pretreatment and first evaluation scans [248, 249]. Lastly, Lo Russo and colleagues suggested criteria that take into consideration clinical deterioration and shortened time to treatment discontinuation [250]. The biochemical and molecular basis of hyperprogression is not fully understood, but resistance mechanisms discussed earlier are plausibly implicated. More interestingly, however, a role for the anti-PD-(L)1 Fc region interaction with the Fc receptor (FcγR) on TAMs has been suggested. This Fc-FcγR interaction was shown in human lung cancer-derived xenografts to cause significant tumor growth in mice treated with nivolumab. Using an anti-PD-1 agent that lacks the Fc region [F(ab)2] did not lead to tumor growth. This paradoxical tumor growth in response to ICI treatment occurs as a result of macrophages reprogramming toward a tumor-promoting M2 phenotype in response to the Fc-FcγR binding [250]. Immune-mediated dedifferentiation of breast cancer models was described by Stein and colleagues who demon-

strated how tumor cells' interaction with nonlytic CD8+ T cells induced a stem cell-like phenotype in the tumor [251]. Another group compared pretreatment and posttreatment gastric cancer tissue samples from a patient with hyperprogression and showed that anti-PD-1 therapy may have caused a significant increase in proliferation and activation of PD-1+ tumor-infiltrating effector Tregs, a finding that was not seen in patients without hyperprogression. Treg suppression, e.g., by targeting OX40, could prove critical in preventing hyperprogression for at-risk patients [246].

9 Conclusion

ICI therapy fundamentally altered the way we treat many solid tumors due to the rapid, deep, and durable responses seen. While this promise has led to functional cures in some patients, only a proportion of patients with solid tumors treated with immunotherapy have a sustained response, with the majority manifesting primary resistance. Patients who initially respond and subsequently progress may have completely different underlying biology of their tumors than those with primary resistance. Thus, subsequent trials of immunotherapy approaches in these patients should take this into account. For instance, if the patient initially had a response to PD-1 inhibition, it is likely that there are tumor-directed T cells that could be further induced by effectively addressing other negative regulatory influences in the tumor. However, in a patient with a TMB-low cancer, with no viral antigens that have primary resistance, a strategy that includes generating a T-cell response (such as a vaccine, oncolytic virus, or tumor-targeted cytokine) or delivering a T-cell response (CAR-T, bispecific antibody, or T-cell receptor-engineered cells) would be a rational approach. Thus, understanding the immune-relevant biology of the tumor is important when considering immunotherapy, especially in tumors resistant to front-line single-agent immunotherapy.

Combination immunotherapy approaches for patients with common underlying deficiencies in the tumor immunity cycle offer the best way to move the field forward to better therapeutic options. These approaches include addressing the need to generate tumor-targeting effector cells, to expand their numbers, and to allow them to be functional in the often hostile TME. Immunotherapeutic drugs that can address multiple mechanisms with one agent could prove critical in these strategies, especially if they have a targeting component to enrich the agent in the TME.

The explosion of omics approaches (including single-cell RNA-Seq) and the added context gained with multiplexed multispectral imaging and spatial transcriptomics offer many opportunities to better understand the underlying biology of the tumor and to gain insights into rational combination approaches as we seek to make functional cures a reality for people with solid tumors.

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Immunotherapy for Melanoma

Justin T. Moyers and Isabella C. Glitza Oliva

Abstract

Melanoma is the leading cause of death from skin cancer and is responsible for over 7000 deaths in the USA each year alone. For many decades, limited treatment options were available for patients with metastatic melanoma; however, over the last decade, a new era in treatment dawned for oncologists and their patients. Targeted therapy with BRAF and MEK inhibitors represents an important cornerstone in the treatment of metastatic melanoma; however, 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 brief overview of the current research efforts in the field of immuno-oncology for melanoma.

Keywords

Metastatic melanoma · Immunotherapy · Checkpoint inhibitors · Adjuvant · Neoadjuvant

1 Introduction

Melanoma represents the malignant transformation and proliferation of melanocytes, which are primarily found in the skin, but may also occur in the uvea, gastrointestinal mucosa, genitourinary mucosa, as well as meninges/central nervous system (CNS) [1]. While it only comprises about 1% of all skin cancer cases, it is accountable for the majority of skin cancer deaths. Furthermore, the annual incidence has been increasing worldwide. Based on data from the American Cancer Society, 106,110 new cases of melanoma will be diagnosed in 2021 in the United States alone, with 7180 people expected to die of the disease [2]. Melanoma can affect anyone, and some of the rise may be attributed to increased skin cancer awareness and detection of earlier stage tumors, but exposure to ultraviolet radiation (sun exposure, tanning beds) has been contributing to the incidence [3]. Other risk factors include fair skin, history of blistering sunburns in early age, dysplastic or atypical nevi, 50 or more of small nevi, and familial dysplastic nevus syndrome [4]. It is important to note that although melanoma can transform from preexisting nevi, about 70% of

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cases can develop de novo (i.e., not from a preexisting pigmented lesion)[3]. Prognosis is related to many factors; late stage, depth (thicker than 4 mm), advanced age, male sex, location (chest and back), and ulceration are associated with poorer prognosis [5]. The survival rate depends primarily on the stage, with 99% 5-year survival for stages I and II and 66% for stage III, and it decreases to 27% for stage IV [6]. However encouragingly, mortality has been improving steadily in the years since checkpoint inhibitor (CPI) approval [6].

Treatment for early-stage melanoma is surgery, which is highly curable. Based on thickness of the primary melanoma and presence of ulceration, initial surgical management may include sentinel node biopsy for staging. For patients with advanced and unresectable disease, systemic therapy most often represents the backbone of therapy. Encouragingly, new immunotherapeutics and targeted therapies for metastatic melanoma since 2011 show improving outcomes for many patients. However, access to immunotherapy is not universal for melanoma patients in the USA and is dependent upon patient sociodemographic factors [7].

While this chapter focuses primarily on immunotherapy, featuring a concise summary of its past, present, and anticipated future use, it should be mentioned that we also have seen remarkable results with the use of targeted therapies in melanoma. The RAS/RAF/MAPK signaling pathway is known to be involved in melanoma transformation [8, 9]. BRAF mutations, specifically BRAF V600 E or K, are observed in up to 50% of cutaneous melanoma, but to a significant lesser degree in acral lentiginous and mucosal melanomas [10]. Combinations of BRAF and MEK inhibitors (inhibiting the RAS/RAF/MAPK pathway) are very effective in *BRAF* mutated melanoma, and a selected overview of clinical trials of regulatory approved therapies is shown below (Table 1).

As both the indications for targeted and immunotherapies have broadened, trials investigating the BRAF/MEK inhibitors and immunotherapy combinations and their most efficacious sequencing in locally advanced and metastatic disease are currently ongoing (e.g., NCT02902029, NCT03235245, NCT02631447, NCT02968303, NCT02910700, NCT03554083, NCT02224781, NCT02967692, NCT04310397, NCT04375527, NCT03554083, and NCT03178851).

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

2.1 High-Dose Interleukin-2

Interleukin- 2 (IL-2) is a T-cell growth factor, which stimulates T-cell proliferation and cytotoxic activity [16]. 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 10%) [17]. Importantly, in patients with an ongoing response at 30 months, no further progression events were noted, supporting the proof of concept that immunotherapy can lead to long-term responses. Toxicity was significant with 2.2% of patients (n = 6) experiencing death due to treatment-related AEs including high rates of Grade 3–4 events of hypotension (45%), vomiting (37%), diarrhea (32%), fever/chills (19%), confusion (13%), and dyspnea (10%). Side effects typically abate after treatment discontinuation and are thought to be due to capillary leak syndrome and lymphoid infiltration. 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 [18]. For patients who experienced a favorable response to treatment, median OS had not been reached, and for patients

Table 1 Selected key trials in BRAF mutant metastatic melanoma

Trial name (Ref)	Phase	#Patients enrolled	Studied combination	Control	ORR (combi vs. single)	Median PFS in months	Median OS in months
COMBI-d [11]	III	423	Dabrafenib + Trametinib	Dabrafenib + placebo	69% vs 53%	11.0 vs 8.8	25.1 vs 18.7
coBRIM [12, 13]	III	495	Vemurafenib + Cobimetinib	Vemurafenib	70% vs 50%	12.6 vs 7.2	22.5 vs 17.4
COLUMBUS [14, 15]	III	577	Encorafenib + Binimetinib	Vemurafenib	63.5% vs 40.8%	14.9 vs 7.3	33.6 vs 16.9
			Encorafenib + Binimetinib	Encorafenib	63.5% vs 51.5%	14.9 vs 9.6	33.6 vs 23.5

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

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.

Patients who are considered for HD IL-2 therapy typically have an appropriate performance status and cardiac and pulmonary function, and the significant toxicities observed with HD IL-2 require intensive monitoring and limit its use to specialized centers [19]. While previously considered a frontline therapy, it is now being taken to subsequent lines of therapy for refractory patients. A retrospective cohort was found to have a response rate of 12% for those who received HD-IL-2 without prior ipilimumab; however, in those who received ipilimumab, ORR was 21% for HD-IL-2 used after ipilimumab [20]. A similar retrospective cohort found 22.5% (n = 9/40) ORR for HD-IL-2 following progression on PD-1 (4 CR and 5 PR) with toxicity similar to expected with HD-IL-2 without prior CPI [21]. While its use has significantly decreased, HD IL-2 is still being used in adoptive cell protocols or considered for refractory patients, and novel recombinant IL-2 agents are being examined (see next section).

2.2 Chemotherapy

While chemotherapy rarely ever led to durable responses, it was the only option available for many patients until 2011. Various agents have been tested in melanoma in phase II and phase III trials, with an overview of the clinical data provided in Table 2.

Biochemotherapy (BCT) consists of the chemotherapy triplet cisplatin, vinblastine, and dacarbazine (CVD), as well as HD IL-2 and interferon. The efficacy of this regimen compared to CVD was evaluated in a phase III trial [36]. Response rates were only numerically higher for BCT (BCT, n = 200; CVD, n = 195; 19.5% versus 13.8%, p = 0.140), and median PFS was significantly longer for BCT than for CVD (4.8 versus 2.9 months; p = 0.015), but did not translate into longer OS (9.0 versus 8.7 months; p = 0.64). In addition, grade 3 and 4 toxicities were more commonly observed with the BCT regimen (95% versus 73%; p = 0.001).

While chemotherapy is now rarely used in front line, trials have explored the efficacy of combination chemotherapy agents with immunotherapy that have had only response rates no better than historical trials of immunotherapy alone. A phase II study of nab-paclitaxel combined ipilimumab in the first-line setting for advanced or metastatic melanoma (n = 21) achieving an ORR of 28% and 24-month OS of 60.6% (NCT0182711) [37]. In a phase II study of first-line ipilimumab combined with carboplatin and paclitaxel (n = 30), ORR response rate was 27% with median OS of 16.2 months (NCT01676649) [38]. Additionally, a phase II trial of pembrolizumab with carboplatin and paclitaxel has yet to publish results (NCT02617849), and the overall benefit of combining chemotherapy with immunotherapy remains to be defined.

Finally, while melphalan use has significantly diminished in era of CPI, it has been used for decades as part of isolated limb infusion (ILP) protocol for patients with localized in-transit metastases [39]. While ILP use has significantly diminished, long-term outcomes from a series of 687 first-time melphalan-based ILP (M-ILP) for stage IIIB or IIIC melanoma (AJCC seventh edition) had high ORR of 64.1% with CR of 28.9% and PR of 35.2% and a median OS of 38.2 months at a median follow-up of 47 months [40].

3 Adoptive Cell Therapy (ACT)

Adoptive cell therapy represents a patient-tailored therapeutic approach, using autologous-derived T cells, and while this approach has been used for decades, there currently still is no regulatory approval in melanoma or solid tumors. Furthermore, its use has been limited by the need for specialized laboratories and treatment centers able to manage the toxicities from HD IL-2, which continues regularly to be administered in conjunction with the T-cell products [41]. The

Table 2 Selected key trials using systemic chemotherapy

Agent (Ref)	Comparator	Phase	Line of therapy	Patients (n)	ORR	Median PFS	Median OS	Main Toxicity
Dacarbazine (DTIC) [22, 23]	None	III	First line	62	8–20%	4–6 months	NR	Myelosuppression, mild nausea, vomiting, minimal alopecia, and fatigue [24, 25]
Temozolomide (TMZ) [26]	DTIC	III	First line	305	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 [27, 28]
DHA-paclitaxel (DHA-P) [29]	DTIC	III	First line	393	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
Nab-paclitaxel [30]	DTIC	III	First line	529	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%) for nab- paclitaxel
Paclitaxel plus carboplatin [31, 32]	n/a	II	Treated once but no previous platinum or taxane	17	20%	NR	9 months	Grade ≥ 3: Granulocytopenia (17%), thrombocytopenia (9%)
Nab-paclitaxel and carboplatin (AUC 2) [33]	None	II	Chemotherapy naïve (CN) and previously treated (PT)	76	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%)
Carboplatin plus paclitaxel with sorafenib (CPS) [34]	Carboplatin plus paclitaxel (CP)	III	First line	823	20% vs. 18% (NS)	4.9 vs. 4.2 months	11.3 vs. 11.1 months	Grade ≥ 3: 84% v 78%; P = 0.027. Increased rash, hand-foot syndrome, and thrombocytopenia accounted for most of the difference
Cisplatin, vinblastine, and dacarbazine (CVD) [35]	None	II	Prior immunotherapy allowed	52	40%	3 months	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

NS difference was not statistically significant, *NR* not reported, *n/a* not applicable

cellular products may be derived directly from the tumor (TIL, tumor-infiltrating lymphocytes), via pheresis, or through off-the-shelf products, and most treatment protocols use lymphodepletion with fludarabine and cyclophosphamide prior to ACT [42].

Early trials utilized TIL-derived T-cell product combined with HD bolus IL-2 following prior surgical or systemic treatments (93% surgery, 50% immunotherapy, 23% chemotherapy). The ORR was 34% for all patients (N = 86) with majority of side effects attributed to HD IL-2 [42]. Later, another clinical trial reported an ORR of 51% (9% CR) in 35 pretreated metastatic melanoma patients (100% with prior immunotherapy, 51% with prior chemotherapy) and a mean duration of response of 11.5 ± 2.2 months [43]. A meta-analysis of 13 trials published between 1988 and 2016 analyzed pooled estimates of trials combining TIL-ACT with IL-2 for melanoma among 410 patients [44]. Pooled estimate for overall response was 41% (95% CI 35–48); complete response rate was 12% (95% CI 6–16). Of those who experienced complete response, almost all (n = 27/28) remained in remission at a median follow-up of 40 months. Since then, different approaches have been developed and tested to improve efficacy and toxicity profile of adoptive cell therapy. Novel approaches also investigate CD20-targeted CAR T-cell therapy (NCT03893019) as well as modified/transduced T cells (NCT01955460) [45, 46].

4 Immune Checkpoint Inhibitors

The development of checkpoint inhibitors (CPI) revolutionized the treatment of metastatic melanoma which has translated successfully to other cancer types. However, research to understand of the mechanisms of T-cell signal transduction and regulation and its relation to cancer therapy began decades ago [47]. 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) [48]. By binding, CTLA-4 downregulates pathways of T-cell activation by competitively binding to B7 proteins which is required for stimulation of T cells. Anti-CTLA-4 has also been shown to induce the expansion of an $ICOS^+$ Th1-like CD4 effector population engaging a cellular pathway distinct from 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 [49]. Similar to CTLA-4, PD-1 negatively regulates the anti-tumor response.

4.1 Anti-CTLA-4: Ipilimumab

Ipilimumab is a fully human, monoclonal IgG1 antibody that inhibits CTLA-4. Ipilimumab was initially approved in 2011 by the Food and Drug Administration (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 [50]. The OS in the combination arm and single-agent ipilimumab were significantly longer 10.0 and 10.1 months, respectively, versus 6.4 months. Ipilimumab as single agent resulted in a 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) [51]. The response rate (CR + PR) was 15.2% in patients who received ipilimumab/DTIC combination vs. 10.3% in 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 3 and 4 toxicities (56.3% versus 27.5%), with the most common grade 4 toxicity being elevation in liver enzymes.

While single-agent ipilimumab is rarely used in the first-line setting, unless given in combination with nivolumab, it has potential use in PD-1

refractory patients. In a phase II trial of patients progressing following first-line PD-1, the combination of pembrolizumab with low-dose ipilimumab (1 mg/kg) resulted in immune response rate of 27% (n = 19/70) including 5 complete responses [52]. A multicenter retrospective reviewed the response of 330 PD1-resistant melanoma patients' response to subsequent ipilimumab. Objective response rate for second-line ipilimumab with PD1 was 32% (n = 61/193) versus 13% for ipilimumab alone (n = 21/132) p = 0.0021 [53].

Ipilimumab continues to be tested in various combination, with radiotherapy, vaccines, cytokines, small molecules, and other CPIs (NCT02259231, NCT02307149, NCT02203604, NCT02073123, NCT03297463) [20].

4.2 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, also known as CD274, is frequently expressed throughout cancerous cells and TILs [54]. Its other ligand, PD-L2, is expressed mainly by antigen-presenting cells (APCs). Both ligands are members of B7 protein family [55]. PD-L1 expression in clinical trials of metastatic melanoma has yielded conflicting results of utility to predict response and presently is not recommended to select for giving CPI [56].

4.2.1 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 [57]. The ORR was 42.9% (95% CI, 33.3–47.0) in nivolumab-treated patients, with over 19.0% achieving a CR versus 14.4% overall response (95% CI, 9.5 to 19.4) and only 1.4% CR in the DTIC group. Nivolumab also compared favorable to dacarbazine with regard to grade 3 and 4 adverse events (15.0% versus 17.6%). Long-term 3-year follow-up has continued to show favorable comparison with 3-year OS of 51.2% for nivolumab versus 21.6% for DTIC with median OS of 37.5 versus 11.2 months, respectively [58].

Pharmacokinetic modeling in population of nivolumab recipients found a flat-exposure-response relationship with 24 mg q weekly dose comparable to 3 mg/kg dose [59]. Subsequently, simulation data for nivolumab pK exposure was utilized to compare to the 3 mg/kg every 2 week and 240 mg flat dose every 2 weeks which found the time-averaged steady-state exposure, and safety of nivolumab 480 mg every 4 weeks was found to be consistent with 3 mg/kg every 2 weeks across multiple tumor types [60].

4.2.2 Pembrolizumab

Pembrolizumab is another fully humanized IgG4 antibody directed against PD-1 receptor that received regulatory approval 2014. In KEYNOTE-002, a multicenter phase II study, 540 previously treated patients were randomly assigned (in a ratio 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) [61]. Progression-free survival was improved in patients assigned to pembrolizumab at both dose levels compared with those assigned to chemotherapy.

In KEYNOTE-006, a phase III study, 834 advanced or 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) [62]. The majority of patients were treatment naïve. Both pembrolizumab arms yielded higher response rates (33.7% for every 2 weeks, 32.9% for every 3 weeks ($p < 0.001$ vs. ipilimumab) and 11.9% for ipilimumab). 6 months PFS was nearly 47% for pembrolizumab in both groups versus 26.5% for ipilimumab. In

addition to improving PFS, 12 months OS was 74.1% for pembrolizumab every 2 weeks and 68.4% for pembrolizumab every 3 weeks compared to 58.2% for ipilimumab. Long-term follow-up shows median OS of 32.7 months in combined pembrolizumab group versus 15.9 months in the ipilimumab group [63]. Endocrine events related to thyroid were more frequently observed in the pembrolizumab groups, whereas colitis and hypophysitis were more frequent in the ipilimumab group. Following these landmark clinical trials, the 200 mg flat dose of pembrolizumab was established based on analysis of dose distributions for both 2 mg/kg and 200 mg doses [64]. Subsequently, a 400 mg every 6-week dose was added to the label following modeling and simulation data supporting its use [65]. 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.

Two different weight-based dosing regimens for Pembrolizumab were found to have similar toxicity rates and efficacy in melanoma [61]. Modeling of expected exposures were found to be similar at different intervals and are currently FDA approved at flat dosing of 200 mg every 3 weeks and 400 mg every 6 weeks [65].

4.2.3 Novel PD-1 Agents

Novel agents targeting PD-1/PD-L1 agents are being utilized in immunotherapeutic trials. Spartalizumab (also known as PDR-001) was tested in a diverse patient population in phase I study and was subsequently used in combination [66, 67].

A second novel PD-1 agent, toripalimab, was the first anti-PD1 monoclonal antibody approved for marketing in China and recently was granted fast track determination by the US Food and Drug Administration [68]. Recently, it has been utilized in a phase Ib trial in metastatic mucosal melanoma where the combination of toripalimab 1 or 3 m/kg every 2 weeks was given along with 5 mg axitinib BID. Overall response rate was 48.3% with 14PR and 1 CR occurring leading to a median OS of 20.7 months [69].

4.3 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 mechanism involved in activation of T cells, the combination of ipilimumab and nivolumab was evaluated. Checkmate-069 was a double-blinded phase II study, of 142 previously untreated patients with metastatic melanoma randomly assigned (in a 2:1 ratio) 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 [70]. The ORR for the combination therapy was 56%, with 22% of patients achieving a CR compared to 11% with ipilimumab ($p < 0.0001$). 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; $p < 0.0001$).

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 4 doses, followed by 3 mg/kg of nivolumab every 2 weeks; or ipilimumab (3 mg/kg) alone in patients with advanced or metastatic melanoma [71]. 945 previously untreated patients were assigned to the treatment arms in a 1:1:1 ratio. Overall response rates were 19% (2.2% CR) in the ipilimumab group, 43.7% (8.9% CR) in the nivolumab group, and 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 patients with high baseline lactate dehydrogenase, low baseline tumor PD-L1 expression, or BRAF mutation might benefit from the combination over monotherapy PD-1. As expected, more treatment-related grade 3 and 4 adverse events were observed in the combination group (55.0%) compared to either single-agent groups

[nivolumab group (16.3%) or ipilimumab group (27.3%)].

In attempts to retain the demonstrated efficacy while reducing treatment-related AEs, a reduced ipilimumab dosing schedule akin to that used for trials of RCC, TMB-high NSCLC, and metastatic gastric for nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (NIVO3 + IPI1) was studied [72–74]. 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). A lower discontinuation rate for NIVO3 + IPI1 23.9% versus 33.1% for NIVO1 + IPI3 In descriptive analyses, objective response rate was 45.6% (95% CI 38.1–53.1) in the NIVO3 + IPI1 group and 50.6% (95%CI 43.0–58.1) in the NIVO1 + IPI3 group (p = 0.35). Median PFS was 9.9 months in the NIVO3 + IPI1 group and 8.9 months in the NIVO1 + IPI3 group. Median OS has not been reached in either group. It should be emphasized that this study was not designed to formally demonstrate non-inferiority of NIVO3 + IPI1 to NIVO1 + IPI3 for efficacy end points [75].

4.4 Ipilimumab and Pembrolizumab in Combination

KEYNOTE-029 tested the combination of pembrolizumab with dose-reduced ipilimumab in a phase Ib trial [76]. Prior targeted therapy or chemotherapy was allowed, but 87% of patients were treatment-naïve. Patients (n = 153) were treated with the combination of pembrolizumab (2 mg/kg) and ipilimumab (1 mg/kg), followed by pembrolizumab (2 mg/kg) maintenance therapy. Objective response rate was 61%, with 15% of patients achieving a CR. Estimated 1-year PFS was 69% and estimated 1 year OS of 89%; grade 3 and 4 adverse events occurred in 45% of patients. A separate trial was conducted in patients who experienced progression on first-line PD1 therapy or within 6 months of completing adjuvant PD1 therapy [52]. The reduced-dose ipilimumab 1 mg/kg every 3 weeks for four doses with pembrolizumab 200 mg every 3 weeks schedule was utilized. Response by irRECIST was 27% (19/70) with five complete responses. Median overall survival in the population was 24.7 months with a PFS of 5.0 months. However, this regimen has currently no regulatory approval.

4.5 Immunotherapy in Patients with Brain Metastases

Clinical and autopsy data show that a significant number of patients with metastatic melanoma will develop brain metastases (MBM) during their course of disease [77]. Initial trials with single-agent ipilimumab and pembrolizumab both showed encouraging responses in melanoma patients with brain metastases [78, 79]. Importantly, all responses observed were durable [80].

Two recent studies evaluated the combination of ipilimumab and nivolumab for patients with MBM [71]. Checkmate-204 enrolled 101 melanoma patients with untreated yet asymptomatic brain metastases, 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 [81]. At median follow-up of 20.6 months, the intracranial clinical benefit rate was 58.4% (CR 29% and PR 26%), with a similar extracranial clinical benefit rate of 54% (95% CI, 44 to 64). Both progression and overall survival had not been reached. Treatment-related grade 3 or 4 adverse events were reported in 55% of patients, with the overall safety profile similar to CheckMate-067 [82]. Importantly, central nervous system specific grade 3 or grade 4 was seen in only 7%. Importantly, a second cohort (n = 18) assessed the efficacy of this regimen in symptomatic patients, treated with dexamethasone doses of <4 mg/day. Response rate was only 22%, and intracranial PFS was 1.2 months. 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 and 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) [83]. Patients that were symptomatic or had leptomeningeal disease (LMD) were treated in nonrandomized fashion with single-agent nivolumab (3 mg/kg) (cohort C, n = 16). At a median follow-up of 17 months, intracranial responses were achieved by 16 (46%) of 35 patients in cohort A, 5 (20%) of 25 in cohort B, and 1 (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. Compared to Checkmate-204, patients enrolled had a higher number of brain metastases and were allowed to have LMD.

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).

Patients with involvement of the leptomeninges have the worst prognosis of all patients with melanoma that is often just weeks [84]. NCT03025256 is an ongoing phase I/II first-in-human study of intrathecal nivolumab administered along with IV nivolumab in those with leptomeningeal disease. Of 15 treated patients at 3 dose levels, overall survival is 46.1 weeks (95%CI 0.1–83.3). The treatment was well tolerated, and no grade 3–5 AEs were attributed to IT or IV nivolumab [85]. Approaches using either intrathecal or intravenous CPI as well as with or without the addition of radiation or BRAF/MEK targeted therapies are currently under investigation (NCT02939300, NCT03719768, NCT03719768, NCT02910700).

4.6 Anti-PD-L1

Antibodies specific for PD-L1 have been developed which in preclinical data may more potently block PD-1 function, therefore hindering PD-L1 from binding its receptors PD-1 and B7.1 [86, 87]. While these agents have shown efficacy in the treatment of metastatic melanoma, none of the currently three available PD-L1 agents (atezolizumab [88], avelumab [89], and durvalumab [90]) have been approved for the treatment of metastatic melanoma, with multiple combination trials with PD-L1 inhibitors still ongoing (NCT02535078, NCT02639026, NCT03178851).

4.7 Anti-PD-1 or PD-L1 in Combination with BRAF and MEK Inhibitors

In BRAF WT unresectable or metastatic melanoma, the combination of atezolizumab with cobimetinib in comparison to pembrolizumab was examined in IMSPIRE 170 [91]. The primary endpoint PFS was not met as no improvement in PFS was seen for combination with a PFS of 5.5 versus 5.7 months for pembrolizumab alone (HR:1.15, p = 0.295).

A similar approach in BRAF mutant melanoma patients has been utilized to combine PD-1 or PD-L1 agents with BRAF/MEK inhibitors to examine the potential additive benefit of checkpoint inhibition to BRAF/MEK inhibitors. Each study was undertaken in untreated BRAF-mutant metastatic or unresectable melanoma with primary endpoint of PFS. COMBI-I (spartalizumab, dabrafenib, and trametinib) [92] as well as KEYNOTE-022 (pembrolizumab, dabrafenib, and trametinib) [93] triplets did not reach their prespecified PFS endpoints. However, IMspire 150 used the triplet combination of atezolizumab, cobimetinib, and vemurafenib in a randomized, blinded, placebo-controlled, phase III study [94]. Atezolizumab 840 mg on days 1 and 15, vemurafenib 720 mg BID days 1–21, and cobimetinib 60 mg daily days 1–21 in 28-day cycles after a

cycle lead in without atezolizumab was compared to vemurafenib plus cobimetinib plus placebo. Progression-free survival was 15.1 months (95% CI 11.4–18.4) versus 10.6 (9.3–12.7) in favor of triplet combination (HR: 0.78, p = 0.0249). Treatment-related adverse events were similar between groups with grade 3–4 AEs occurring in 79% of patients in triplet and 73% in the doublet. Interim survival analysis showed survival benefit of 28.8 months with triplet versus 25.1 months, but final OS data are yet to be reported. The FDA approved the triplet in 2020.

5 Immune-Related Adverse Events and Outcome

Given their different mode of action, CPIs can lead to a different types of side effects than previously observed with cytotoxic chemotherapy or targeted therapy, commonly referred to as immune-related adverse events (irAEs). The disrupted immune homeostasis is mediated by unchecked T-cell activation [95]. Importantly, early recognition and management are essential to expedite resolution of symptoms as irAEs may affect any organ at any time [75]. CPIs can also lead to delayed toxicities occurring weeks or months after discontinuation of therapy [96]. Additionally, combination regimen of two CPIs (typically nivolumab and ipilimumab) results in greater risk of and earlier onset for clinically significant irAEs [97].

Interestingly, the presence of irAE may portend improved outcome. The presence of irAE has been associated with improved survival. A retrospective analysis of 346 melanoma patients found any grade GI-irAE to be associated with improved survival (HR 0.53, 95% CI 0.36–0.78; p < 0.01) [98]. Additionally, a secondary analysis of double-blind EORTC1325/KEYNOTE-054 for adjuvant pembrolizumab following surgical resection found those who received adjuvant pembrolizumab and experienced irAE (n = 190/509) to have improved recurrence-free survival compared to those who experienced irAE in the placebo arm (HR, 0.61; 95% CI, 0.39–0.95; P = 0.03) [99]. A separate single-center analysis of patients of diverse tumor types enrolled in immunotherapy-based early-phase clinical trials of 290 patients found 5.2% of patients (n = 15) experienced grade 3 or greater irAE. However, compared to those without grade $\geq$ 3 irAE, those experiencing grade $\geq$ 3 irAE had improved ORR (25% vs 6%; p = 0.039) and longer time to progression (median 30 weeks vs 10.0 weeks, p = 0.0040) [100].

Following irAE to checkpoint inhibitor, side effect grade and patient clinical characteristics affect choice for rechallenge. In a study of the WHO pharmacovigilance cohort of 24,079 cases of irAE, recurrence of the same irAE occurred in 28.8% with hepatitis, colitis, and pneumonitis experiencing the highest rate of recurrence [101]. In a separate analysis of the French pharmacovigilance database of 180 patients with at least one grade 2 or greater irAE and subsequent CPI rechallenge, 38.9% experienced irAE with 70% of those being the same irAE [102].

6 Vaccination and Intratumoral Approaches

Multiple intratumoral and vaccine approaches have been tested in 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 identified such as melanoma antigen A1 (MAGE-A1), gp100, or melanoma antigen recognized by T cells (MART-1/Melan-A) [103]. However, as single-agent results have been underwhelming, combinatorial approaches may 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 [104]. 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 HD IL-2 only group. Recently, a phase Ib combined personal neoantigen-based therapy, NEO-PV-01, with nivolumab. This approach induced T cells with a cytotoxic phenotype found to traffic to tumors. Among the ITT population with melanoma (n = 34), ORR was 59% (95% CI 39–78%) with a PFS of 23.5 months [105].

6.1 T-VEC

Talimogene laherparepvec (T-VEC), a genetically modified herpes simplex virus (HSV) type 1, is currently the only intratumoral oncolytic virotherapy with regulatory approval 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 [106]. The approval was based on a randomized phase III trial in 436 patients with unresectable stage III or IV melanoma [107]. Patients were randomly assigned at a 2:1 ratio to intratumoral T-VEC or subcutaneous GM-CSF. The overall response rates for T-VEC were higher (26.4% vs. 5.7%), and a higher number of 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 statistically significance (p = 0.051). T-VEC injections were well tolerated, and the most common adverse events included 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% versus 5% for GM-CSF).

TVEC also has 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) [108]. 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 were received T-VEC (in day 1, day 22 then every 2 weeks, and pembrolizumab (200 mg) on day 36, and then every 2 weeks) [109]. 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 TVEC in combination with other CPIs, targeted therapy, as well as radiation (NCT02263508, NCT03088176, NCT02819843, NCT02965716).

6.2 PV-10 (Rose Bengal Disodium)

Rose bengal disodium (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 [110]. 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 a best ORR of 51% (CR in 26%), and 8% of patients still had no evidence of recurrence after 52 weeks [111]. Importantly, non-injected 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 [112]. Complete responses were associated with having less than 15 metastases at time of PV-10 injection. PV-10 administered intratumorally in combination with IV was carried out in a phase Ib/II trial. In the phase Ib results released, partial response was achieved in 57% of patients with complete response in 9% [113]. Expansion for the combination is ongoing (NCT02557321).

6.3 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 [114]. Many tumor types express functional TLRs, leading to tumor proliferation, formation of metastases, and resistance to apoptosis. Studies are now underway to evaluate TLR-based therapeutic approaches (esp. intratumoral) will increase the efficacy of anticancer immunotherapies (NCT00960752, NCT04401995, NCT04364230, NCT04570332, NCT04126876).

Tilsotolimod (also known as IMO-2125) is an oligonucleotide that binds to TLR-9 and rapidly upregulates IFN type 1 to induce innate and adaptive tumor response along with dendritic cell activation, was previously shown to have clinical activity in a phase I study [115]. A Phase I/II study was undertaken utilizing intratumoral injection of tilsotolimod along with IV infusion of ipilimumab in unresectable or metastatic melanoma after progression on PD-1 therapy (ILLUMINATE-204). Among 49 patients evaluable for response, 22.4% (n = 11/49) had objective response with two complete responses observed. Tumor regression was seen in injected and non-injected masses with treatment-related adverse events occurring in 26% [116]. A subsequent phase III study randomizing to ipilimumab versus ipilimumab + tilsotolimod is being undertaken (ILLUMINATE-301; NCT03445533).

Intratumoral TLR9 agonist, CMP-001, plus pembrolizumab demonstrated a best ORR of 23.5% (n = 23/98) in a phase Ib/II trial for anti-PD-1 refractory disease with a median duration of response greater than 1 year [117, 118]. Furthermore, another TLR9 agonist (SD-101) combined with pembrolizumab in a multicenter phase Ib study demonstrated an ORR of 78% in treatment-naive unresectable or metastatic disease (n = 7/9) while only 15% (n = 2/13) in PD-1 treated [119].

7 Adjuvant Therapies

The goal of systemic adjuvant therapy is to decrease recurrence for high-risk melanomas after surgery. Traditionally, this approach has focused on patients with stage III disease, which is defined as the presence of lymph node and/or in-transit metastasis. An increasing number of involved lymph nodes, but also an increase in primary tumor depth and mitotic rate, and the presence of ulceration in the primary tumor are all associated with worse outcomes [120]. Stage III disease outcomes are heterogeneous, and it was been redefined into 4 substages stratified by survival outcomes in the eighth edition of the American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma. The five-year melanoma-specific survival rates range from 93% for stage IIIA disease to 32% for stage IIID disease (thickness > 4 mm with ulceration and ≥ 4 involved lymph nodes) [121]. Adjuvant therapy remains an important focus of research as immediate complete lymph-node dissection is frequently omitted due to lack of improved melanoma-specific survival [122]. Furthermore, anti-PD-1 agents are now being tested in the adjuvant setting for patients with high-risk stage II disease (NCT03553836).

7.1 Previously Used Adjuvant Approaches

Interferon was the first agent tested in the adjuvant setting. While initial trials showed and improved recurrence free and overall survival benefit for treatment of high-dose interferon alpha-2 (HD INF-α) compared to observation

[123–125], longer follow-up and pooled analysis were not able to confirm the improved OS, apart from patients with ulcerated primary tumor [123, 126].

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 [127]. 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 deference was found between the two groups, but a trend toward favoring biochemical group was reported. Each treatment group experienced different toxicities, as expected. However, neither are currently used in the adjuvant setting.

7.2 CPIs in the Adjuvant Setting

The drastic improvements in survival and durable responses seen with CPI in unresectable advanced melanoma patients led to study its efficacy in the adjuvant setting. The first adjuvant CPI trial, EORTC 18071, was a phase III double-blind randomized study comparing high-dose ipilimumab (10 mg/kg every 3 weeks for 4 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) [128]. 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 6.9 years, the 5 year OS was 65.2% in the ipilimumab group, as compared with 54.1% in the placebo group with continued 7-year OS of 60.0% versus 51.3% (HR 0.73; 95%CI: 0.60–0.89) [129].

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 [130]. The 12-month RFS was remarkably higher in nivolumab group (70.5%) vs (60.8%) in the ipilimumab group (p < 0.001). An updated 4-year analysis showed RFS of 51.7% in nivolumab group compared to 41.2% in the ipilimumab group (HR 0.71 [95% CI 0·60–0·86]; p = 0·0003) [131]. 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 48-month RFS benefit (64.0% for nivolumab vs 52.3% for ipilimumab). 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 long-term follow-up 3-year RFS in pembrolizumab group was 63.7% vs. 44.1% in placebo (HR 0.56; 95% CI, 0.47 to 0.68). Grade 3–5 toxicities were reported in 14.5% of the patients in the pembrolizumab group and in 3.4% of patients in the placebo group. Of note, cross-trial comparison with CheckMate-238 which included stages IIIB–IV is difficult, as KEYNOTE-054 included patients with stage IIIA disease and excluded stage IV [132, 133].

Despite the successes of adjuvant CPI, 25–30% of those with high-risk melanoma recur within 1 year. In a multicenter retrospective cohort of patients receiving adjuvant CPI, 17% (n = 147/850) of patients recurred within 1 year at a median time of 4.6 months of which 76% were on PD1 therapy. Location of recurrence was locoregional in 43% (n = 49/126) and distant in 57% (n = 77/126). Of patients receiving systemic therapy following PD1, 24% (n = 8/33) responded to ipilimumab alone or in combination and 78% (n = 18/23) responded to BRAF/MEK inhibitors [134].

In addition to adjuvant immunotherapy, adjuvant targeted therapy with BRAF/MEK inhibitors following surgical resection has been used successfully to reduce recurrence [135–137]. A retrospective multicenter cohort of those receiving adjuvant targeted therapy found 85 patients with recurrence of which 22% during adjuvant treatment (n = 19/85). For subsequent treatment after recurrence, response rates were 63% (n = 12/19) for anti-PD-1, 62% (n = 8/13) for nivolumab + Ipilimumab, 25% (n = 4/16) for targeted therapy rechallenge, and 10% (n = 1/10) for ipilimumab single-agent [138].

Given the superior results of combination nivolumab and ipilimumab in the metastatic setting, studies have looked into testing it in the adjuvant setting. A small trial (NCT01176474) is assessing two treatment schedules of NIVO1 + IPI3 (cohort 1) vs 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 [139]. The phase 3 trial Checkmate 915 (NCT03068455) in resected stage IIIB/C/D or stage IV utilized adjuvant ipilimumab and nivolumab vs nivolumab alone, did not meet its endpoint for superior RFS of the combination therapy over monotherapy [140].

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

8 The Future of Melanoma Treatment

As our understanding of the tumor microenvironment and T-cell homeostasis deepens, new targets will be identified and undergo testing in clinical trials. We will highlight a selection of current targets under development in the section below.

8.1 Indoleamine-2,3-Dioxygenase (IDO) Inhibitors

Accumulation of tryptophan exerts an inhibitory effect on T cells, of which the intracellular enzyme, IDO, is the rate-limiting step. IDO inhibitors may alter the tumor microenvironment to allow for immunotherapy response [141, 142].

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) [143, 144]. However, reported result from phase III ECHO-301/KEYNOTE-252 (NCT02752074) did not show a clinical benefit of the combination epacadostat and pembrolizumab over pembrolizumab alone. PFS was 4.7 vs 4.9 months, and OS rate at 12 months was 74% in both groups [145].

A phase II study of epacadostat in combination with nivolumab in select cancer types showed an ORR of 62% (n = 31/50); however, this combination is not currently planned for phase III studies as enthusiasm fades for IDO inhibitors in melanoma [146].

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 [147]. Future successes for agents in this pathway may be dependent upon pharmacokinetic and pharmacodynamic studies to determine the proper dose to properly sustaining suppression of plasma and intratumoral kyneurenine. A phase I study of M4112, a dual inhibitor of indoleamine 2,3-dioxygenase 1 and tryptophan 2,3-dioxygenase 2, in advanced solid tumors found that while initial changes in kyneurenine were observed, there was no significant reduction of plasma kyneurenine when steady state was reached [148]. Additional trials studying IDO inhibitors are ongoing in advanced cancer types (NCT02658890, NCT03695250, NCT03854032).

8.2 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 [149]. 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. The development of LAG-3 blockade has now moved into clinical testing. In a phase I/IIa clinical trial, 68 melanoma patients who progressed on prior PD1/PD L1 exposure were treated with relatlimab 80 mg (previously known as BMS-986016) in combination with nivolumab 240 mg every 2 weeks [150, 151]. Disease control rate was 49%, and ORR was 11.5%. Importantly, relatlimab did not appear to add toxicity, as grade 3 or 4 toxicities were only observed in 10% of the treated patients. Multiple clinical trials are currently evaluating the efficacy of anti-LAG-3 in combination with other immunotherapies for patients in the neoadjuvant setting (NCT02519322) and for treatment naïve (NCT03470922) and progression on anti-PD-1 therapy (NCT03978611) [152].

8.3 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 [153]. It was shown that a subset of T cells (PD-1^{+} NY-ESO specific CD8+ T cells) in patients with advanced melanoma upregulate TIM-3 expression which appear to be dysfunctional producing less immunoregulatory cytokines compared to their TIM-3-expressing counterparts [154]. It was also shown that this is a severely exhausted phenotype of T cells, and concurrent blockade with anti-PD1 may act synergistic in reversing tumor-induced T-cell dysfunction [155].

In a phase I/II trial, the anti-TIM-3 mAb MBG453 was given alone and in combination with spartalizumab (anti-PD-L1 mAb) in patients with advanced cancer. In dose escalation of the single-agent MBG453, stable disease was seen in 25/87 (29%) of patients and 34 of 86 patients (40%) who received combination including five melanoma patients. Partial responses were seen in 5% (4/86), none of which were in melanoma. A dose expansion cohort of melanoma resistant to antiPD-1/PD-L1 is ongoing [67]. Several other TIM-3 antagonists are in early-phase clinical development as single agent or in combination with anti-PD-1/PD-L1 (NCT04370704, NCT03744468, NCT04641871NCT03099109, NCT03489343, NCT02817633) or as a bispecific anti-PD-1/TIM3 antibody (NCT03708328).

8.3.1 T-Cell Agonists

Distinct from immune checkpoint inhibitors, alternate strategies to improve T-cell response to cancer include T-cell agonists such as 4-1BB and OX40, which exert their effect through costimulatory molecules [156].

8.3.2 1 OX40

OX40 (or CD134) is a member of tumor necrosis factor (TNF) receptor superfamily (TNFRSF). Increased OX40 expression has been seen in TIL CD8+ T cells upon encountering tumors [157]. 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 [158]. In an initial phase I trial using an OX40 agonistic murine monoclonal antibody, 9B12 (later known as MEDI6469) 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 7 patients, and other grade 1 and 2 toxicities included fatigue, nausea, vomiting, rash, and flu-like symptoms. [159] MEDI0562, an agonistic humanized mAb which binds to OX40, has completed phase I; however, few melanoma patients were included [160]. Clinical trials are currently ongoing with OX40 agonists in combination with

checkpoint inhibitors, including MOX40916 with atezolizumab (NCT02410512) and MEDI-0562 with durvalumab (NCT02705482) or with 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) [161].

8.3.3 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 [162]. 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 [163]. The 4-1BB agonist development programs were placed on a hold due to hepatotoxicity, and analysis revealed statistics-related adverse event and trails were restarted [164]. However, due to preclinical synergism with nivolumab, synergistic activity of and the combination of nivolumab and urelumab were evaluated in a phase I/II study. Of 46 evaluable patients, 23 had objective response (18 confirmed, 5 unconfirmed) for an ORR of 50% with a side effect profile similar to single-agent nivolumab [165]. In addition, PF-05082566, another 4-1BB agonist mAb, has also been evaluated in combination of pembrolizumab in patients with solid tumors (NCT02253992, NCT02179918) [166] as well as in combination with avelumab in advanced melanoma patients (NCT02554812).

8.3.4 Glucocorticoid-Induced Tumor Necrosis Factor Receptor-Related Protein (GITR)

Glucocorticoid-induced tumor necrosis factor receptor-related protein is a type 1 transmembrane protein of the tumor necrosis factor receptor superfamily also known as TNFRSF18. GITR is expressed on natural killer (NK) cells and human T lymphocytes (predominantly on regulatory T cells). GITR expression increases as T cells are activated and its ligation positively modulates antigen-specific T-cell responses as costimulatory signal [167]. An open-label phase I study of the anti-GIR antibody MK-4166 was carried out as monotherapy or in combination with pembrolizumab. In the expansion cohort for the combination in metastatic melanoma, checkpoint inhibitor-naïve patients had overall response rate of 69% (n = 9/13) with 4 of 9 responses being complete response. However, in the checkpoint inhibitor-treated cohort, 7 of 7 patients had progressive disease with anti-GIR and anti-PD-1 combination. Incidence of severe adverse events were similar between monotherapy and combination, 4.2% and 7.7%, respectively [168]. A similar study was conducted with anti-GITR IgG4 monoclonal antibody MK-1248. 20 patients were treated with monotherapy, while 17 patients were treated in combination with pembrolizumab. 0 of the 20 patients in monotherapy had objective response; however, 3 of 17 patients in combination had objective response (1 partial response in a melanoma) [169]. Further studies with anti-GITR agents are ongoing (NCT03799003 and NCT04021043).

8.4 Novel Recombinant IL-2 Agents

Due to the pivotal role IL-2 plays in immune homeostasis and recruitment of multiple lymphocyte subsets, it remains an attractive target. Low concentrations of IL-2 induce signaling through high-affinity IL-2R (composed of IL-2R alpha, beta, and gamma subunits), which is mainly expressed on regulatory T cells, whereas high concentrations of IL-2 are necessary to activate the intermediate-affinity IL-2R (composed of IL-2Rbeta and gamma subunits) expressed on memory CD8+ T cells and NK cells [170]. Designing agents to target the efficacy of intermediate affinity IL-2R is desirable.

Bempegaldesleukin (NKTR-214/BEMPEG) has a preferential activation of the 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 [171]. Phase I study with NKTR-214 enrolled 28 patients (melanoma n = 7) and overall favorable tolerance with only 21.4% rate of grade ≥ 3 treatment-related adverse events [172]. The PIVOT-2 phase II trial evaluating NKTR-214 in combination with nivolumab in treatment-naive metastatic melanoma found ORR of 53% (n = 20/38) with 34% (n = 13/38) complete response rate. Median PFS was 30.9 months with median OS not yet reached [173]. Phase III trials with BEMPEG are ongoing including BEMPEG plus nivolumab in first-line setting (PIVOT IO 001; NCT03635983) and adjuvant setting (PIVOT-12; NCT04410445) as well as a phase 1/2 trial of BEMPEG combined with pembrolizumab PROPEL (NCT03138889) in advanced or metastatic solid tumors.

In a phase I/II study (ARTISTRY-1) utilizing combination ALKS4230 as monotherapy and with pembrolizumab, the single-agent dose-escalation cohort of checkpoint inhibitor pre-treated melanoma yielded partial response (n = 1/6) and stable disease (n = 2/6) as best observed response. In the rollover with pembrolizumab, a durable partial response was seen in a patient with mucosal melanoma. The treatment was well-tolerated with most common treatment related adverse event grade < 2 fever or hypotension [174]. Further studies are ongoing (NCT04592653, NCT03861793).

8.5 Bispecific Antibodies

Bispecific antibodies that simultaneously block multiple checkpoints are being developed for clinical use. The benefits of bispecific modalities allow the targeting of two receptors in a single agent that may be utilized to increase activity or target a mechanism of resistance. The combination of PD-L1-Fc-OX40 has shown synergistic preclinical activity [175]. Bispecific PD-1/4-1BB (NCT03809624) is undergoing testing in advanced solid tumors including melanoma.

9 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 [176]. In addition, a recently published phase I trial assessed the safety and feasibility of fecal microbiota transplantation (FMT) and re-induction of anti-PD-1 immunotherapy in ten patients with anti-PD-1-refractory metastatic melanoma. Clinical responses were observed in three patients, including two partial responses and one complete response [177]. 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).

10 Conclusion

The numerous breakthrough treatment discoveries utilizing immunotherapy for melanoma over the last decade have ushered in an immunotherapy revolution in oncology. While there is great cause for optimism, much remains unknown, and we eagerly await the results of ongoing trial to help guide oncologists to choose the best therapy for each patient.

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Immunotherapy in Lung Cancer: Are the Promises of Long-Term Benefit Finally Met?

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Abstract

Over the last few years, agents targeting immune checkpoints have shown potential to improve therapeutic outcomes in patients with lung cancer in multiple clinical settings. Inhibitors of PD-1/PD-L1 have been approved for the treatment of different types of lung cancer by the FDA either alone or in combination with chemotherapy or other immune checkpoint inhibitors, such as anti-CTLA-4 agents. The introduction of these agents in clinical practice has revolutionized the therapeutic approach to lung cancer, keeping the promises of long-term benefit in selected patient populations. The therapeutic indications of immunotherapy in lung cancer are rapidly growing, and multiple combinations entered clinical practice or are under active development. Furthermore, the quest for a reliable predictive biomarker is still ongoing to overcome the limits of currently approved tests for patients' selection. In this review, we summarized the current status and progress of anti-PD-1/PD-L1 agents in lung cancer treatment.

Keywords

PD-1 · PD-L1 · CTLA-4 · Immune checkpoint inhibitors · NSCLC · SCLC · Immunotherapy · TMB · bTMB

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1 Introduction

Clearly, we are seeing that lung cancer improves its survival year by year; NSCLC 2-year relative survival increased from 34% for persons diagnosed during 2009 through 2010 to 42% during 2015 through 2016, including absolute increases of 5% to 6% for every stage of diagnosis; survival for small cell lung cancer remained at 14% to 15%. This is due to the improvement in treatments, where immunotherapy plays a fundamental role [1].

Immunotherapy treatment is now a reality in clinical practice, and knowledge mechanism of

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action is key in understanding the benefit of the improved survival of the lung [2]. The development of immune checkpoint inhibitor (ICI) agents targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death protein 1 (PD-1), or programmed cell death protein ligand 1 (PD-L1) has garnered tremendous interests in the field of immuno-oncology because of the recent successful applications in multiple advanced cancers. Although CTLA-4 is the first immune checkpoint molecule identified, the PD-1/PD-L1 axis has been widely investigated due to the role in the exhaustion of CD8+ T cells. Physiologically, PD-1/PD-L1 has the task of limiting the activity of T cells in peripheral tissues at the time of an inflammatory response to infection, thereby limiting autoimmunity. 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. 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 [3, 4].

Since the first demonstration of activity of PD(L)-1 agents in lung cancer in early clinical trials in 2012, 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. 1) [5]. 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 nonresponders, and PD-L1 immunohistochemical expression still remains the only applicable marker in clinical practice to date [6].

To date, the primary biomarker used for lung cancer has been PD-L1 [10]. Different immunohistochemical assays have been developed, using different antibodies and scoring systems. However, multiple harmonization studies have consistently reported high concordance between most of these assays (22C3, SP263, 28-8, 73-10, and E1L3N) in terms of PD-L1 expression on tumor cells (TC) [11–14]. PD-L1 tumor proportion score (TPS) evaluated using the Dako 22C3 assay was developed and validated as the companion diagnostic for single-agent pembrolizumab in pretreated NSCLC in the randomized phase II/III KEYNOTE-010 trial [15]. Based on the positive results in pretreated patients, the use of ICIs was then moved to treatment-naïve patients, and PD-L1 expression represented the most extensively used biomarker for treatment selection of single-agent PD-1/PD-L1 inhibitors versus platinum-based chemotherapy [16]. PD-L1 TPS $\geq$50% identifies a subgroup of patients that accounts for approximately 30% of the patients with NSCLC that derives greater benefit from single-agent ICIs than platinum-based chemotherapy, and to date, three different agents (pembrolizumab, atezolizumab, and cemiplimab) [17–19] have been approved in this setting. Single-agent ICI in PD-L1 low expressors (TPS 1–49%) for first-line therapy is controversial, as the benefit seen in the KEYNOTE-042 trial with pembrolizumab [20] is likely driven by PD-L1 strong expressors [21].

Absence of PD-L1 expression does not conclusively identify patients who will not benefit from immunotherapy, leading to investigation of many other biomarkers [22–24].

In this review, we summarized the current status and progress of anti-PD-1/PD-L1 agents in lung cancer treatment.

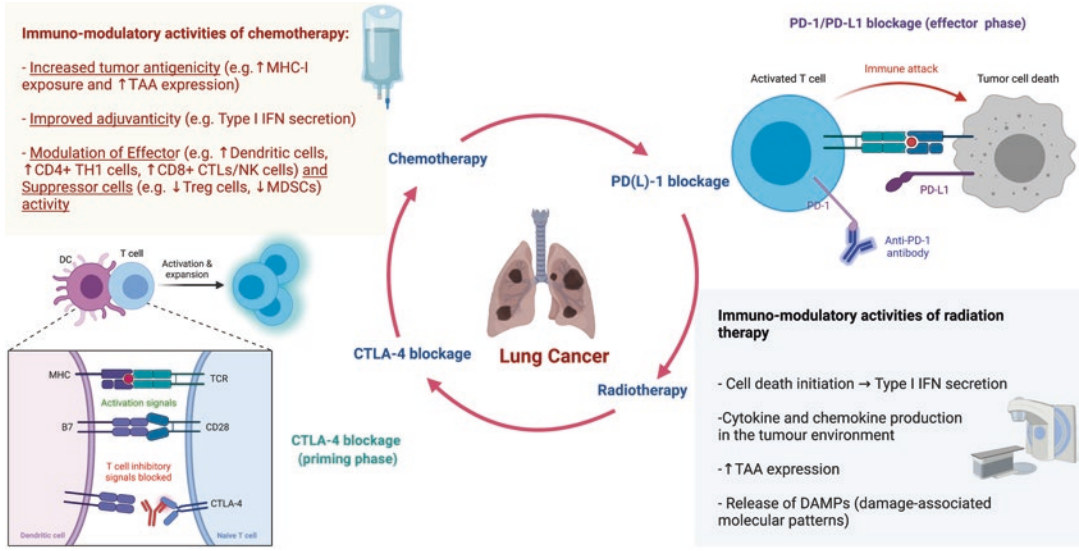

Fig. 1 Role of immune checkpoint inhibitors in lung cancer and immune-modulatory activities of conventional treatment strategies [3, 7–9]. Abbreviations: *MHC* major histocompatibility complex, *TAA* tumor-associated antigens, *IFN* interferon, *TH1* T-helper 1, *CTL* cytotoxic T-cell lymphocyte, *NK* natural killer, *Tregs* regulatory T cells, *MDSCs* myeloid-derived suppressor cells, *TCR* T-cell receptor, *DC* dendritic cell (Credit: Created with BioRender.com)

2 Early-Stage NSCLC and Locally Advanced NSCLC

Approximately 40% of NSCLC patients are diagnosed with locoregional disease that is potentially resectable [25]. Adjuvant platinum-based chemotherapy has been shown to improve survival in patients with stages II–III disease and can be considered high-risk stage IB disease (4 cm, poorly differentiated carcinoma, post-wedge resection, lymphovascular invasion, visceral pleural involvement, unknown lymph node status) [26]. Meta-analyses of randomized phase III trials conducted in the 1990s and early 2000s reported an absolute survival benefit at 5 years of 5% from adjuvant/neoadjuvant approaches in stage IB–IIIA NSCLC compared with surgery alone [27, 28].

Since these trials, the therapeutic landscape of early-stage NSCLC has little improved over the last two decades, and only recently, a randomized phase III trial has reported a survival advantage in a selected patient population (activating *EGFR* mutations) using osimertinib after platinum-based chemotherapy [29]. ICIs might potentially revolutionize the adjuvant setting, given the well-known ability of immunotherapy of inducing long-term responses, and multiple clinical trials are ongoing (Table 1).

Recently, the preliminary results of a single-arm phase II study (NCT03053856) evaluated the postoperative role of pembrolizumab in stage IIIA-N2 NSCLC who has undergone neoadjuvant concurrent chemoradiotherapy (weekly carboplatin/paclitaxel and radiation therapy to 44 Gy in 22 fractions) with curative resection for up to 2 years or until disease recurrence. The primary endpoint is disease-free survival (DFS), with a statistical goal of more than 20 months. Thus far, of 37 patients treated in this trial, 14 patients have discontinued treatment owing to disease progression (n = 9), adverse events (n = 4), or consent withdrawal (n = 1). Adverse events have included grade 4 pneumonitis (n = 1) and grade 3 autoimmune hepatitis (n = 1), which have led to discontinuation, as well as grade 1 or 2 hypothyroidism (n = 6), pneumonitis (n = 5), and skin rash (n = 3) [30].

On March 2021, Roche announced that phase III IMpower010 trial met the primary endpoint,

Table 1 Ongoing clinical trials evaluating immune checkpoint inhibitors as adjuvant therapy in radically resected NSCLC

Study	Population	Arm(s)	Phase	Endpoint(s)
IMpower010	Stage IB–stage IIIA NSCLC following resection and adjuvant chemotherapy	Atezolizumab vs. BSC	3	DFS
CANOPY-A	Completely resected stages II–III	Canakinumab vs. placebo	3	DFS
PEARLS	Completely resected stages IB–IIIA NSCLC, after standard adjuvant chemotherapy	Pembrolizumab vs. placebo	3	DFS
BR31	Completely resected stages IB–IIIA NSCLC, after standard adjuvant chemotherapy	Durvalumab vs. placebo	3	DFS in PD-L1 ≥ 25% EGFR/ALK WT
ANVIL	Stages IB–IIIA after surgery and adjuvant chemotherapy	Nivolumab vs. observation	3	DFS, OS
ALCHEMIST Chemo-IO	Completely resected stages IB–IIIA NSCLC	Chemotherapy +/− pembrolizumab	3	DFS, OS
LungMate-008	Completely resected *EGFR/ALK* WT stages II–IIIB(N2) NSCLC	Platinum-based chemotherapy +/− toripalimab	3	DFS
MERMAID-1	Completely resected stages II–III NSCLC who are MRD+ postsurgery	Durvalumab +/− chemotherapy	3	DFS
MERMAID-2	Stages II–III NSCLC MRD+ after curative intent therapy*	Durvalumab vs. placebo	3	DFS in PD-L1 ≥ 1%
NCT04585477	Stages I–III NSCLC who had positive ctDNA following definitive treatment with surgery or radiation and completion of adjuvant SoC chemotherapy	Durvalumab	2	ctDNA changes after two cycles
CATHAYA	Completely resected stages I–III NSCLC who had positive ctDNA results postsurgery	Adjuvant chemotherapy +/− atezolizumab	2	ctDNA clearance rate at 6 months
BTCRC-LUN19–396	Completely resected stages I (≥4 cm)–IIIA and ctDNA clearance	Adjuvant chemotherapy + atezolizumab	2	Percentage of patients with undetectable ctDNA after four cycles
NCT03053856	Completely resected stage IIIA N2 after neoadjuvant chemo-radiotherapy	Pembrolizumab	2	DFS
NCT04317534	Stage I (1–4 cm) NSCLC	Pembrolizumab vs. observation	2	DFS

Abbreviations: *NAC* neoadjuvant chemotherapy, *EFS* event-free survival, *OS* overall survival, *DFS* disease-free survival, *MRD+* minimal residual disease-positive, *MPR* major pathological response, *SoC* standard of care, *SBRT* stereotactic body radiotherapy, *pCR* pathologic complete response, *BSC* best supportive care, *SABR* stereotactic ablative radiotherapy, *WT* wild type

[a]Complete resection ± neoadjuvant and/or adjuvant therapy

demonstrating a statistically significant improvement in terms of DFS with the use of the PD-L1 inhibitor atezolizumab as compared with best supportive care (BSC) in patients with PD-L1-positive, stages II–IIIA NSCLC who have undergone surgical resection and received up to four cycles of adjuvant cisplatin-based chemotherapy. The presentation of the full results of the study is eagerly awaited.

In addition to the adjuvant setting, immunotherapy might have a role also in the neoadjuvant setting either as monotherapy or in combination with platinum-based chemotherapy. Preliminary data of these studies are encouraging, especially when considering chemo-immunotherapy combinations (Table 2).

Collectively, chemo-immunotherapy seems associated with higher ORR and increased probability of major pathological response (MPR)/pathologic complete response (pCR). This therapeutic strategy seems more promising than single-agent ICB and moved quickly to phase III. Several randomized trials evaluating the addition of a PD-1/PD-L1 inhibitor to a platinum-based doublet as neoadjuvant therapy in resectable NSCLC (stages I–IIIA) are underway, including KEYNOTE-671 (pembrolizumab), AEGEAN (durvalumab), NCT04316364 (adebrelimab/SHR-1316), NCT04379635 (tislelizumab), JS001 028 III (toripalimab), CheckMate-77 T (nivolumab), and CheckMate-816 (nivolumab/chemotherapy vs. nivolumab-ipilimumab).

In October 2020, Bristol Myers Squibb announced that the CheckMate-816 met its primary endpoint of improved pCR in patients who received nivolumab plus chemotherapy before surgery. The presentation of the full results of the study is expected in the next few months.

In patients with inoperable stage III disease, the use of chemoradiotherapy has been shown to increase survival as compared with radiotherapy alone [44], and concurrent chemoradiation (cCRT) increases 5-year overall survival by 4.5% as compared with a sequential approach [45].

Several studies have shown promise for immunotherapy following cCRT in patients with unresectable stage III LA-NSCLC. The PACIFIC trial (A Global Study to Assess the Effects of MEDI4736 Following Concurrent Chemoradiation in Patients with Stage III Unresectable Non-Small Cell Lung Cancer) reported encouraging phase III data on the use of the anti-PD-L1 antibody durvalumab in this context. PACIFIC was the first study to demonstrate improved outcomes in patients with LA-NSCLC who received an immune checkpoint inhibitor. In this phase III trial, patients with stage III unresectable NSCLC were randomly assigned in a 2:1 ratio to receive either durvalumab (a PD-L1 inhibitor) or placebo as consolidation therapy every 2 weeks for as long as 1 year [46]. The study population consisted of 713 patients who had received cisplatin-based chemotherapy with concurrent radiation to 66 Gy and had no disease progression following treatment. Progression-free survival (PFS), the primary endpoint, was significantly longer in the durvalumab group than in the placebo group (median PFS, 16.8 vs. 5.6 months; $P < 0.001$). In addition, the co-primary OS remained consistent with that previously reported (stratified HR = 0.69 [95% CI: 0.55–0.86]); the median OS was not reached with durvalumab but was 29.1 months with placebo. The 12-, 24-, and 36-month OS rates with durvalumab and placebo were 83.1% versus 74.6%, 66.3% versus 55.3%, and 57.0% versus 43.5%, respectively [47]. Improved OS with durvalumab was broadly observed irrespective of PD-L1 expression, which is consistent with findings from prespecified and post hoc analyses carried out at the time of the primary OS analysis [46]. Remember that PD-L1 data were based on pre-cCRT samples, which may not reflect changes in expression potentially incurred by cCRT, and should also be taken into consideration when drawing definitive conclusions. PACIFIC was not designed to evaluate the efficacy of durvalumab based on PD-L1 status. Overall, the findings of this analysis underscore the long-term survival benefit with durvalumab after cCRT and further establish the PACIFIC regimen as the standard of care in patients with unresectable stage III NSCLC who do not progress while undergoing cCRT. An exploratory analysis showed that

Table 2 Selected clinical trials with neoadjuvant ICIs in resectable NSCLC

Study	Patients (n)	Stage	Drug(s)	Cycles	MPR/pCR(%)	ORR (%)
Forde et al. [31]	21	IB–IIIA	Nivolumab	2	45%, 15%	10%
LCM3 [32]	144	IB–IIIA, selected IIIB	Atezolizumab	2	21%, 7%	–
NEOSTAR [33]	23	I–IIIA	Nivolumab	3	22%, 9%	23%
	21		Nivolumab-ipilimumab	1	38%, 29%	20%
NADIM [34]	46	IA–IIIA	Nivolumab/CP	3	83%, 63%	76%
Shu et al. [35]	30	IB–IIIA	Atezolizumab + nab-P/C	2	57%, 33%	63%
Ready et al. [36]	30	I–IIIA	Pembrolizumab	2	28%, 8%	–
MSK3475–223 [37]	15	I–IIIA	Pembrolizumab	2	40%, 20%	15%
Gao et al. [38]	22	IB–IIIA	Sintilimab	2	46%, 18%	13%
IoNESCO [39]	50	IB–IIIA	Durvalumab	3	18%, 0%	9%
PRINCEPS [40]	30	I (>2 cm)–IIIA	Atezolizumab	1	14%, 0%	7%
Zinner et al. [41]	13	I–IIIA	Nivolumab + chemotherapy	3	84%, 38%	–
TOP12 01 [42]	24	IB–IIIA	CP x2 → CP/ipilimumab	2	NR, 15%	58%
SAKK 16/14 [43]	67	IIIA	Cisplatin/docetaxel x3 → durvalumab	2	60%, 18%	59%

Abbreviations: *MPR* major pathological response, *pCR* pathologic complete response, *ORR* overall response rate, *CP* carboplatin/paclitaxel, *nab-P/C* nab-paclitaxel/carboplatin

patients who started treatment with durvalumab <14 days from completion of radiation therapy had improved efficacy outcomes compared with those who started treatment ≥14 days from completion of radiation therapy [48].

Besides consolidation after cCRT, other therapeutic strategies under active investigation include the concomitant use of ICIs during chemoradiotherapy (PACIFIC-2, CheckMate73L, EA5181, DETERRED-PART II, NICOLAS, KEYNOTE-799) and the use after sequential chemoradiotherapy (PACIFIC-6) or radiotherapy alone (DUART). The results of these trials will provide additional insights on the role of PD-1/PD-L1 inhibitors in inoperable stage III NSCLC.

3 Pretreated NSCLC

After few years since early clinical sights of activity of PD-1/PD-L1 inhibitors in lung cancer [49, 50], three PD-1/PD-L1 therapies have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in the second-line setting (nivolumab, pembrolizumab, and atezolizumab), on the basis of phase III studies demonstrating improved overall survival (OS) in comparison with the former standard-of-care therapy docetaxel.

In two phase III trials (CheckMate-017 and CheckMate-057), nivolumab showed an improvement in OS and favorable safety versus docetaxel in patients with previously treated, advanced squamous, and non-squamous NSCLC [51, 52]. After follow-up of 64.2 and 64.5 months for CheckMate-017 and CheckMate-057 [53], respectively, 50 nivolumab-treated patients and 9 docetaxel-treated patients were alive. Five-year pooled OS rates were 13.4% versus 2.6%, respectively; 5-year PFS rates were 8.0% versus 0%, respectively. Nivolumab-treated patients without disease progression at 2 and 3 years had an 82.0% and 93.0% chance of survival, respectively, and a 59.6% and 78.3% chance of remaining progression-free at 5 years, respectively. Treatment-related adverse events (TRAEs) were reported in 8 of 31 (25.8%) nivolumab-treated patients between 3 and 5 years of follow-up, 7 of whom experienced new events; one (3.2%) TRAE was grade 3, and there were no grade 4 TRAEs. Clearly, nivolumab compared to docetaxel exhibited a fivefold increase in OS rate, with no new safety signals. 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 PD-L1-negative patients [54]. Atezolizumab was compared with docetaxel in pretreated 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 [55, 56]. A longer OS was observed in patients receiving atezolizumab vs. docetaxel in POPLAR (median OS, 12.6 months vs. 9.7 months; HR, 0.76 [95% CI: 0.58–1.00]) and OAK (median OS, 13.3 vs. 9.8 months; HR, 0.78 [95% CI: 0.68–0.89]. Four-year OS rates in POPLAR were 14.8% (8.7–20.8) and 8.1% (3.2–13.0) for atezolizumab and docetaxel, respectively, and 15.5% (12.4–18.7) and 8.7% (6.2–11.3) in OAK. Most 4-year survivors in the docetaxel arms received subsequent immunotherapy (POPLAR, 50%; OAK, 65%). Of 4-year survivors, most had ECOG PS 0 and non-squamous histology; approximately half were responders (POPLAR, atezolizumab, 7/15; docetaxel, 3/4; OAK, atezolizumab, 24/43; docetaxel, 11/26). Treatment-related grade 3/4 adverse events occurred in 27% and 16% of atezolizumab 4-year survivors in POPLAR and OAK, respectively [57].

The development of pembrolizumab in NSCLC started with the phase I 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. Patients with squamous and non-squamous tumors were enrolled; however, PD-L1 expression had to be 1% or greater. All patients had progressed on first-line platinum-doublet therapy, and those with driver mutations had also progressed on appropriate TKI therapy [58]. The updated analysis with a 42.6 months follow-up [59] showed that the risk of death was reduced with pembrolizumab versus docetaxel in both the PD-L1 TPS ≥50% group (HR 0.53; $P < 0.00001$) and the TPS ≥1% group (HR 0.69; $P < 0.00001$). Median OS was 16.9 months (95% CI, 12.3 to 21.4 months) versus 8.2 months (95%CI, 6.4 to 9.8 months) in the TPS ≥50% group and 11.8 months (95% CI, 10.4 to 13.1 months) versus 8.4 months (95% CI, 7.6 to 9.5 months) in the TPS ≥1% group. Kaplan-Meier estimates of OS at 36 months were higher with pembrolizumab versus docetaxel in both TPS groups, with OS rates of 34.5% versus 12.7% in the TPS ≥50% group and 22.9% versus 11.0% in the TPS ≥1% group. The risk of disease progression or death (per RECIST v1.1 by BICR rather than per investigator) was reduced with pembrolizumab versus docetaxel in the PD-L1 TPS ≥50% (HR 0.57; $P = 0.00001$) and TPS ≥1% groups (HR, 0.83; $P = 0.005$). Kaplan-Meier estimates of PFS at 36 months were higher with pembrolizumab versus docetaxel in both TPS groups, with PFS rates of 21.9% versus 1.2% in the TPS ≥50% group and 12.7% versus 1.0% in the TPS ≥1% group.

Not all trials using PD-1 and PD-L1 checkpoint inhibitors for the second-line treatment of advanced NSCLC have yielded positive results. Avelumab, an anti-PD-L1 monoclonal antibody, was compared with docetaxel in the JAVELIN Lung 200 trial [60]. As a result, the OS was not significantly different between the avelumab and docetaxel groups, even in the subgroup with positive tumor PD-L1 expression. High post-study use of ICIs and the non-blinded design of the trial might have affected the results.

Currently, ICIs are now well established as the standard of care for second-line treatment of advanced NSCLC, but there is no data to suggest that one agent is superior to another in that setting. No head-to-head comparison has been conducted. Indeed, the meta-analyses of published studies with ICIs in pretreated NSCLC did not demonstrate significant evidence of survival differences between these agents [61, 62]. Therefore, in clinical practice, factors that could influence ICI selection might include drug access, dosing schedule, costs, and PD-L1 expression.

4 First-Line Metastatic NSCLC

The introduction of the anti-PD-1 nivolumab for metastatic lung cancer in second-line setting was just the beginning in the development of checkpoint inhibitors in different clinical scenarios [16], including first-line treatment either as monotherapy in selected patient populations or in combination with chemotherapy +/− antiangiogenic drugs or in combination with CTLA-4 inhibitors with or without chemotherapy.

Within the revolutionary first-line setting for metastatic lung cancer patients, two pivotal randomized phase III clinical trials have compared pembrolizumab vs. platinum-doublet regardless histology and without driver mutations (*EGFR/ALK* wild type) in patients with PD-L1 TPS ≥50% (KEYNOTE-024) and in those with PD-L1 TPS ≥1% (KEYNOTE-042).

KEYNOTE-024 met its primary endpoint, reaching its goal of demonstrating the superiority of pembrolizumab vs. chemotherapy for patients with strong PD-L1 expression (TPS ≥50%) regardless of tumor histology [17]. By reaching a median OS of 30 months [63], pembrolizumab is positioned as a less toxic and more effective treatment than platinum-doublet-based chemotherapy in this selected patient population, demonstrating for the first time a survival advantage over platinum-based chemotherapy as first-line treatment in non-oncogene addicted NSCLCs. Based on these results, PD-L1 ≥ 50% in absence of concomitant driver mutations identified a novel subgroup of patients that accounts for approximately 30% of all NSCLCs that benefits from a chemotherapy-free regimen in first line.

Similar results were more recently reported with two ICIs, atezolizumab and cemiplimab, in

patients with strong PD-L1 expression without *EGFR* mutations and/or *ALK* rearrangements and have been recently approved by the US FDA as first-line options.

Atezolizumab was compared with platinum-based chemotherapy in the randomized phase III trial IMpower110 in PD-L1 selected NSCLCs (PD-L1 expression on at least 1% of tumor cells or at least 1% of tumor-infiltrating immune cells as assessed by the SP142 immunohistochemical assay) [18]. The study demonstrated a statistically significant improvement in OS in the intention-to-treat (ITT) population (patients whose tumors were wild-type with respect to EGFR mutations or ALK translocations) within the subgroup of patients with strong PD-L1 expression (20.2 months vs. 13.1 months; hazard ratio for death, 0.59; P = 0.01). Furthermore, OS and PFS favored atezolizumab in the subgroups with a high blood-based tumor mutational burden (bTMB), assessed through the plasma 394-gene NGS panel FoundationOne CDx Liquid, suggesting a potential utility of this biomarker for patient selection [18].

Cemiplimab was compared with platinum-based chemotherapy in the randomized phase III trial EMPOWER-Lung 1 as first-line treatment in advanced NSCLC with PD-L1 tumor expression ≥50% and no *EGFR* mutations, *ALK* translocations, or *ROS1* fusions. Patients were ineligible if they had never smoked (defined as ≤100 cigarettes in a lifetime). This is the largest study in this setting (563 patients in the PD-L1 ≥ 50% population) and showed that cemiplimab was superior to chemotherapy in improving PFS (8.2 months vs. 5.7 months, HR 0.54; p < 0.0001) and OS (not reached vs. 14.2 months, HR 0.57; p = 0.0002) in PD-L1-strong positive NSCLC patients [19].

Recently, a multicenter retrospective study analyzed the impact of different PD-L1 expression levels on pembrolizumab outcome in the subgroup of patients with NSCLC PD-L1 TPS ≥50% without *EGFR/ALK* aberrations. Compared with patients with PD-L1 expression of 50%–89% (N = 107), patients with an expression level of 90%–100% (N = 80) had a significantly higher ORR (60.0% versus 32.7%), a significantly longer PFS (14.5 versus 4.1 months), and a significantly longer OS (not reached versus 15.9 months). These results suggest that in patients with NSCLC and PD-L1 expression ≥50% treated with first-line pembrolizumab, clinical outcomes are significantly improved in NSCLC with a very high PD-L1 expression of ≥90% [64].

Other studies have sought to expand the potential number of patients that might benefit from upfront PD-1/PD-L1 blockage as monotherapy, evaluating these agents in patients with PD-L1 expression ≥1%. The CheckMate-026 failed to demonstrate a survival benefit with nivolumab versus platinum-based chemotherapy. Nivolumab was not associated with significantly longer PFS than chemotherapy among patients with previously untreated stage IV or recurrent NSCLC with a PD-L1 expression level of 5% or more, the primary endpoint of the trial. Furthermore, no differences were observed in OS between groups, and no advantage was seen in the PD-L1 ≥ 50% subgroup (HR for PFS 1.07 and 0.90 for OS) [64]. However, an exploratory analysis evaluating the tumor mutation burden (TMB) with whole exome sequencing (WES), performed in a subgroup of patients (58% of the randomized patients), showed that nivolumab was associated with higher ORR (47% vs. 28%) and longer PFS (9.7 vs. 5.8 months; HR 0.62) in patients with high TMB (≥243 mutations). No correlation between TMB and PD-L1 expression level was observed. Interestingly, the subgroup of patients with both high TMB and strong PD-L1 expression identified the subgroup of patients with higher response rate (75%) than those with only one of these factors (32% among patients with a high TMB only and 34% among those with a PD-L1 ≥ 50% only) or neither factor (16%) [65].

In contrast, the KEYNOTE-042 met its primary endpoints, demonstrating a statistically significant OS benefit in patients with a TPS ≥50% (HR 0.69, p = 0.0003), ≥20% (HR 0.77; p = 0.0020), and ≥ 1% (HR 0.81; p = 0.0018) leading to the FDA approval of pembrolizumab in treatment-naïve *EGFR/ALK* wild-type NSCLC patients with a TPS ≥ 1% [20]. However, this decision raised some concerns as pembrolizumab

monotherapy may not represent the best treatment strategy for patients with tumor PD-L1 expression of 1–49%, as survival curves cross approximately 7 months after treatment initiation, with chemotherapy performing better than pembrolizumab during the first 6 months from randomization. These results suggest that a substantial number of patients progress rapidly and die within the first 6 months of treatment without obtaining any meaningful benefit from immunotherapy, and therefore other therapeutic strategies might be preferable in this subgroup of patients [21], especially in light of the positive results of chemo-immunotherapy trials in the first line.

Multiple randomized phase III trials have investigated the efficacy and safety (Table 3) of different chemo-immunotherapy trials.

Phase III KEYNOTE-189 trial evaluated the use of pembrolizumab in association with platinum-pemetrexed chemotherapy in patients of non-squamous *EGFR/ALK* wild-type NSCLC, regardless of PD-L1 expression [66]. The trial met the two primary endpoints, demonstrating a statistically significant improvement in terms of both OS and PFS with the combination, as assessed by blinded, independent central radiologic review. First-line pembrolizumab plus chemotherapy demonstrate substantially improved OS and PFS in metastatic non-squamous NSCLC, regardless of PD-L1 expression or liver/brain metastases, with acceptable safety profile [66, 67]. Pembrolizumab plus platinum-pemetrexed was associated with a median OS of 22.0 months vs. 10.6 months with chemotherapy alone (HR 0.60) with a 3-year OS almost doubled (31.3% vs. 17.4%). Median PFS was longer for the experimental arm (9.0 vs. 4.9 months; HR 0.50), with a 3-year PFS rate of 11.8% vs.1.3% [68]. The PFS/OS benefit was seen across all the PD-L1 subgroups, with the strong PD-L1-positive subgroup benefitting more from the addition of pembrolizumab. Patients who completed the planned 35 cycles of treatment (2 years) were associated with durable responses and were most still alive at the 4-year follow-up (79.6% OS rate after 2 years from treatment completion) [68].

In a similar study design, pembrolizumab in combination with carboplatin plus paclitaxel/nab-paclitaxel demonstrated a PFS/OS as compared with chemotherapy in squamous NSCLC (KEYNOTE-407). The study met its two primary endpoints, demonstrating a statistically significant advantage for chemo-immunotherapy in terms of both OS (15.9 vs. 11.3 months, HR 0.64; $P < 0.001$) and PFS (6.4 vs. 4.8 months, HR 0.56; $P < 0.001$) [69]. Similar to the KEYNOTE-189 trial, the addition of pembrolizumab to chemotherapy was associated with survival benefit across all the PD-L1 subgroups, including among PD-L1-negative (TPS <1%) tumors [70]. At a 3-year follow-up, pembrolizumab plus carboplatin and paclitaxel/nab-paclitaxel continued to provide OS and PFS benefit vs. placebo plus chemotherapy (median OS 17.2 vs. 11.6 months with a 3-year OS rate of 29.7% vs. 18.2%; median PFS 8.0 vs. 5.1 months with a 3-year PFS rate of 16.1% vs. 6.5%). Among patients who completed the planned 35 cycles of treatment, durable responses were seen with a 1-year OS rate from completion of pembrolizumab of 96% [71].

In the IMpower150 was tested the addition of atezolizumab to bevacizumab plus chemotherapy as first-line treatment for metastatic non-squamous NSCLC, regardless of PD-L1 expression. In contrast with other chemo-immunotherapy trials, patients with known EGFR or ALK aberrations were included in the study but were excluded from the ITT population. Patients were randomly assigned, in a 1:1:1 ratio, to receive atezolizumab plus carboplatin plus paclitaxel (ACP group), atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP group), or bevacizumab plus carboplatin plus paclitaxel (BCP group) [72].

The two primary endpoints were PFS both among patients in the ITT WT population and among patients in the 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. ABCP was associated with longer PFS (8.3 versus 6.8 months, HR 0.62; $P < 0.001$) and longer OS

Table 3 Safety of chemo-immunotherapy regimens in first-line NSCLC

Name	n	Arms	Duration of IO	Median FU	AEs	AEs G3–4	Treatment-related death	Discontinuation rate
KEYNOTE-407 [69]	559	Carbo + pacli or nab-P ± pembro	Up to 35 cycles	7.8 mos	98.2% vs. 97.9%	69.8% vs. 68.2%	3.6% vs. 2.1%	23.4% vs. 11.8%
KEYNOTE-189 [66]	616	Cis/ carbo-pem ± pembro	Up to 35 cycles	10.5 mos	99.8% vs. 99.0%	67.2% vs. 65.8%	6.7% vs. 5.9%	13.8% vs. 7.9%
IMpower-150 (ARM B vs. C) [72, 73]	400 vs. 400	ABCP vs. BCP	Until PD	~20 mos	94% vs. 96%	57% vs. 49%	2.8% vs. 2.3%	34% vs. 25%
IMpower-150 (ARM A vs. C) [73]	402 vs. 400	ACP vs. BCP	Until PD	~20 mos	94% vs. 96%	43% vs. 49%	1% vs. 2.3%	13% vs. 25%
IMpower 130 [74]	451 vs. 228	Atezo + Carbo + nab-P vs. Carbo + nab-P	Until PD	19 mos	99.6% vs. 99.1%	73.3% vs. 60.3%	1.7% vs. 0.4%	26.4% vs. 22.0%
IMpower 131 (ARM B vs. C) [75]	343 vs. 340	Atezo + Carbo + nab-P vs. Carbo + nab-P	Until PD	17.1 mos	99% vs. 97%	68% vs. 57%	1% vs. 1%	30% vs. 17%
IMpower 132 [76]	292 vs. 286	Atezo + Cis/ Carbo + pem vs. Cis/Carbo + pem	Until PD	14.8 mos	98% vs. 97%	62% vs. 54%	4% vs. 3%	24% vs. 18%

(19.2 versus 14.7 months, HR 0.78; $P = 0.02$) as compared with BCP in the ITT population [72]. Interestingly, an exploratory analysis of the study showed that ABCP was associated with improved OS compared with BCP in patients with sensitizing *EGFR* mutations (HR 0.31) and in those with baseline liver metastases (HR 0.52). In contrast, no OS benefit was seen with ACP versus BCP in patients with sensitizing *EGFR* mutations (HR 0.90), in the ITT population (HR 0.85), or in patients with baseline liver metastases (HR 0.87) [73]. These data should be interpreted with cautions, given the low number of patients included in this analysis, but suggest a potential synergistic effect between bevacizumab and atezolizumab.

Another therapeutic strategy explored in treatment-naïve advanced NSCLC is the dual immune checkpoint blockage with PD-1 plus CTLA-4 inhibitors. Checkmate-227 (Part 1) trial was a randomized phase III study evaluating the role of nivolumab plus ipilimumab in either PD-L1-positive (≥1%) versus chemotherapy or nivolumab (Part 1a) or PD-L1-negative (<1%) NSCLC patients versus chemotherapy +/− nivolumab (Part 1b). In Part 1a, nivolumab-ipilimumab was significantly associated with a longer median duration of OS as compared with chemotherapy alone (17.1 vs. 14.9 months; $P = 0.007$). The OS benefit was also observed in the Part 1b of the study (PD-L1 < 1%) with a median duration of 17.2 months with nivolumab plus ipilimumab and 12.2 months with chemotherapy. This combination was associated with similar serious adverse event (G3–4 AEs) rates compared with chemotherapy (32.8% with nivolumab plus ipilimumab and 36.0% with chemotherapy) [77]. At a 3-year follow-up, nivolumab-ipilimumab continues to provide a survival benefit as compared with chemotherapy in both PD-L1 ≥ 1% and PD-L1 < 1% with a similar 3-year OS rate (33% and 34%, respectively) [78]. This chemotherapy-free regimen was recently FDA-approved in PD-L1 ≥ 1%.

In contrast with nivolumab-ipilimumab, the dual blockage with durvalumab plus tremelimumab was not associated with significant survival benefit in the randomized phase III MYSTIC trial. The primary endpoints, assessed in patients with PD-L1 ≥ 25%, were OS for durvalumab vs. chemotherapy and OS and PFS for durvalumab plus tremelimumab vs. chemotherapy. The study did not meet its primary endpoints with no statistically significant improvement in terms of OS with durvalumab vs. chemotherapy (HR 0.76, p = 0.04) or OS/PFS with durvalumab plus tremelimumab vs. chemotherapy in patients PD-L1-positive tumors (HR 0.85 and 1.05, respectively) [79]. However, this combination was associated with OS improvement in patients with high blood TMB (≥20 mutations per megabase), assessed with the 500-gene plasma NGS platform GuardantOMNI [80].

Whether dual PD-1/CLTA-4 blockage is superior to PD-1 inhibition alone in patients with PD-L1 TPS ≥50% is still debated. The randomized, double-blind, phase III trial, KEYNOTE-598, addressed this issue and compared pembrolizumab plus ipilimumab vs. pembrolizumab alone. The primary endpoints were OS and PFS. The trial failed to demonstrate a survival benefit in terms of both OS (21.4 months for pembrolizumab-ipilimumab vs. 21.9 months for pembrolizumab-placebo; HR 1.08, $p = 0.74$) and PFS (8.2 months for pembrolizumab-ipilimumab vs. 8.4 months for pembrolizumab-placebo; HR1.06, $p = 0.72$). Differences in grades 3–5 AEs occurred in 62.4% vs. 50.2% and resulted in death in 13.1% versus 7.5%. Despite the study being early stopped due to futility by the external data and safety monitoring committee, it provides evidence that the addition of an anti-CTLA-4 inhibitor to pembrolizumab in PD-L1-strong positive NSCLC patients does not improve the efficacy but also worsens the toxicity profile [81].

To increase the disease control during the first few weeks of immunotherapy, another therapeutic strategy recently investigated is the addition of a limited course (two cycles) of a platinum-based chemotherapy to the dual checkpoint blockage. In the CheckMate-9LA, patients were randomly assigned (1:1) to nivolumab (360 mg intravenously every 3 weeks) plus ipilimumab (1 mg/kg intravenously every 6 weeks) combined with histology-based, platinum-doublet chemotherapy

(intravenously every 3 weeks for two cycles, experimental group) or chemotherapy alone (every 3 weeks for four cycles, control group). Randomization was stratified by tumor histology, sex, and PD-L1 expression. The primary endpoint was OS in all randomly assigned patients [82]. The experimental group was associated with a significantly longer OS than control group (14.1 vs. 10.7 months, HR 0.69; $p = 0.00065$) at the preplanned interim analysis. In contrast with the CheckMate-227 study, the two OS curves early separated, suggesting that the addition of a short course of chemotherapy might overcome the limits of chemotherapy-free regimens that might be associated with a lower disease control in the first 3 months of treatment. No differences were observed across all PD-L1 TPS subgroup. This regimen was associated with an increased incidence of serious AEs as compared with chemotherapy alone (30% vs. 18%), although treatment-related deaths were similar in both groups (2%) [82]. Recently, the results of an exploratory analysis of the study, analyzing the role of tissue and blood TMB (tTMB and bTMB), were presented. Collectively, 64% and 73% of all randomized patients had tTMB (FoundationOne CDx assay) and bTMB (GuardantOMNI) evaluable samples, respectively. Similar to the CheckMate-227, the OS benefit with nivolumab-ipilimumab plus chemotherapy was observed regardless of TMB status with higher tTMB and bTMB associated with greater ORR and PFS benefit but similar OS outcomes. Collectively, these results support the use of nivolumab-ipilimumab plus two cycles of chemotherapy as first-line treatment option for patients with advanced NSCLC regardless of PD-L1 expression, TMB status, or their combination [83].

In summary, we have an arsenal of options when choosing first-line treatment for patients with metastatic lung cancer. The choice of the scheme will depend on a series of factors to take into account, considering that patients in everyday practice often do not always resemble the group selected and suitable for clinical trials.

5 ICIs and SCLC

Small cell lung cancer (SCLC) accounts for ~15% of all lung cancers and ~ 30,000 deaths in the USA annually, owing to the elusive pathophysiology of the disease, the poor prognosis of patients, and the minimal improvement in the effectiveness of therapies over the past decades. By the time that small cell lung cancer (SCLC) is diagnosed, nearly two-thirds of patients already have extensive stage disease (ES-SCLC) [84, 85].

ES-SCLC has a poor prognosis and a 5-year survival rate of <7% [84, 86]. For more than 20 years, the standard of care for ES-SCLC was platinum chemotherapy, which is associated with high initial response rates but a median survival of only 10 months. These findings highlight an unmet need for first-line (1 L) treatment of ES-SCLC [84, 86, 87]. In recent years, PD-L1/PD-1 inhibitors have demonstrated improved outcomes in patients with ES-SCLC.

Recent studies have shown that the efficacy of immunotherapy is related to a high tumor mutation burden (TMB), high genomic instability, and high immunogenicity in tumor cells. Some studies have shown that SCLC may have some advantages in immunotherapy.

PD-L1 expression in >1% of tumor cells is present in only a minority (~20%) of SCLC specimens [87, 88]. High counts of tumor-infiltrating lymphocytes (TILs) have been associated with better prognosis in SCLC in the pre-immunotherapy era [89]. Indeed, the presence of suppressive FOXP3+ regulatory T cells has been associated with a better prognosis in patients with LS-SCLC (HR 0.37; $P = 0.013$), and the presence of CD45RO+ memory T cells in brain metastases from ED-SCLC has been correlated with prolonged OS (11 vs. 5 months; $P = 0.007$) [90, 91]. However, data from studies designed to investigate the presence or absence of alternative, potentially clinically important immune checkpoints in SCLC, such as LAG3, TIM3, TIGIT, OX40, and ICOS, are currently unavailable. A better understanding of the immune microenvironment is an important area of unmet need in the

immunobiology of SCLC. To better understand the ES-SCLC treatment, it can be divided into first line, maintenance, and second or more lines.

6 First-Line Therapy

The first immune checkpoint inhibitor evaluated in SCLC was the CTLA-4 inhibitor ipilimumab, following the promising results of a randomized phase II study [92]. In a phase III, placebo-controlled randomized trial, ipilimumab was evaluated in combination with platinum-etoposide with a phased schedule (two cycles of chemotherapy followed by two cycles of ipilimumab plus chemotherapy and then two additional cycles of ipilimumab) vs. chemotherapy alone in patients with ES-SCLC. Addition of ipilimumab to chemotherapy did not prolong OS versus chemotherapy alone in patients with newly diagnosed ES-SCLC (13.4 vs. 12.4 months, HR 0.91; $p = 0.25$) and was associated with higher serious AEs and discontinuation rates due to treatment-related AEs [93].

In the IMpower 133 trial, the efficacy of atezolizumab in combination with carboplatin-etoposide was assessed in patients with ES-SCLC. Patients were randomized to receive four 21-day cycles of carboplatin-etoposide plus atezolizumab or placebo and then maintenance atezolizumab or placebo until unacceptable toxicity, disease progression, or loss of clinical benefit. The study met its two primary endpoints (investigator-assessed PFS and OS) [94]. The addition of atezolizumab was associated with a significantly longer median OS (12.3 vs. 10.3 months, HR 0.76; $p = 0.154$) compared with chemotherapy alone with an 18-month OS of 34% vs. 21%. The survival benefit was seen regardless of PD-L1 expression or bTMB status [95]. Atezolizumab was the first ICI approved in first-line ES-SCLC, and this trial was the first randomized phase III study reporting a survival benefit in this setting as compared with platinum/etoposide after three decades of inconsistent results.

A second PD-L1 inhibitor that demonstrated a survival benefit in first-line ES-SCLC was durvalumab in combination with cisplatin/carboplatin plus etoposide. The randomized phase III trial CASPIAN randomized 805 ES-SCLC patients to receive durvalumab/tremelimumab plus platinum/etoposide or durvalumab plus platinum/etoposide or platinum/etoposide alone. Primary endpoint was OS [96]. Durvalumab/tremelimumab plus platinum/etoposide failed to demonstrate a significant improvement in OS versus platinum/etoposide (10.4 vs. 10.5 months, HR 0.82; $p = 0.045$). In contrast, durvalumab plus platinum/etoposide showed a sustained improvement in OS versus platinum-etoposide (12.9 vs. 10.5 months, HR 0.75, $p = 0.0032$). The survival benefit observed with durvalumab plus platinum/etoposide versus platinum/etoposide consistently favored the combination across all prespecified patient subgroups, as well as post hoc subgroups defined by liver metastases at baseline [97]. The overall survival benefit observed with durvalumab plus platinum-etoposide in CASPIAN aligns with findings from the IMpower133 trial, adding a novel therapeutic option in the therapeutic armamentarium of ES-SCLC.

In contrast with the positive results of IMpower133 and CASPIAN, the randomized phase III trial KEYNOTE-604 failed to demonstrate a statistically significant OS benefit with the addition of pembrolizumab to platinum/etoposide. The study randomized 453 ES-SCLC patients to receive pembrolizumab plus platinum/etoposide for 4 cycles followed by pembrolizumab for up to 35 cycles vs. platinum/etoposide for 4 cycles. Primary endpoints were PFS (by blinded central review) and OS with prespecified efficacy boundaries where one-sided $P = 0.0048$ for PFS and $P = 0.0128$ for OS. The addition of pembrolizumab significantly improved PFS (HR 0.75; $P = 0.0023$) and was associated with durable responses (12-month PFS rates: 13.6% vs. 3.1%). Albeit median OS was longer in the experimental arm, the significance threshold was not met (HR, 0.80; $P = 0.0164$). Twenty-four-month OS estimates were 22.5% and 11.2%, respectively. The PFS and OS HRs were similar between PD-L1-positive and PD-L1-negative tumors and regardless of the choice of platinum [98]. Albeit formally negative, the results of this trial along with those of IMpower133 and

CASPIAN consolidate the use of platinum/etoposide plus an ICI as the novel standard of care for first-line ES-SCLC, which is associated with long-term clinical benefit in a small subgroup of patients. Inherited differences in the three trials and the enrollment of a poorer prognosis population in the KEYNOTE-604 trial might account for the survival differences seen in these studies. The identification of predictive biomarkers and the correlation with SCLC molecular subtypes might provide novel insights on patients benefitting most from this strategy.

7 Second-Line or Later Monotherapy

ICI monotherapy with nivolumab or pembrolizumab is FDA-approved for patients with ES-SCLC, independent of PD-L1 status, as a third or subsequent line of therapy.

The approval of nivolumab was based on the preliminary results of the phase II study CheckMate-032. Nivolumab monotherapy provided durable responses (median duration of response 17.9 months with 12-month and 18-month OS rates of 28.3% and 20.0%, respectively) and was well tolerated as a third- or later-line treatment for recurrent SCLC [99]. The randomized study compares nivolumab plus ipilimumab with nivolumab alone in pretreated ES-SCLC with ORR by blinded independent central review as primary endpoint. Although nivolumab plus ipilimumab was associated with higher ORR compared with nivolumab monotherapy (21.9% vs. 11.6%; $p = 0.03$), addition of ipilimumab did not prolong OS (median OS 4.7 vs. 5.7 months; 24-month OS rates 16.9% vs. 17.9%, respectively), at cost of higher-grade 3/4 treatment-related AEs (37.5% vs. 12.9%) [100].

The randomized phase III trial CheckMate-331 evaluated the efficacy of nivolumab in second line versus an active comparator (topotecan or amrubicin). The primary endpoint was OS. The trial failed to demonstrate a significant OS benefit with nivolumab compared with chemotherapy (7.5 vs. 8.4 months, HR 0.86; $P = 0.11$). No differences were noted between PD-L1-positive and PD-L1-negative tumors. Patients with baseline lactate dehydrogenase (LDH) under the upper limit of normal and those without baseline liver metastases seemed to benefit from nivolumab. A delayed separation in the survival curves at 12 months was observed, suggesting long-term benefit with nivolumab [101].

The FDA approval of pembrolizumab as third or subsequent line of therapy for ES-SCLC was based on the results of KEYNOTE-028 and KEYNOTE-158 trials. In the phase Ib KEYNOTE-028 trial, pembrolizumab was evaluated in PD-L1 selected patients with a tumor cell, immune infiltrate, and stromal summative PD-L1 combined positive score (CPS) ≥1%. The study included 24 patients (31.7% of all samples evaluated for PD-L1) with relapsed SCLC (12.5% receiving pembrolizumab as second line and 50% as third line). Pembrolizumab showed encouraging signals of activity in this setting with an ORR of 33%, a median PFS of 1.9 months (1-year PFS 23.8%), and a median OS of 9.7 months (1-year OS 37.7%) [102]. The KEYNOTE-158 was a phase II basket trial that enrolled 107 patients with relapsed SCLC (79% received pembrolizumab in the second-line or third-line setting), regardless of PD-L1 status (47% of patients had PD-L1-negative tumors). This study confirmed that promising antitumor activity (ORR 18.7%, median PFS 2.0 months, and OS 9.1 months) and durable responses (77% of the patients had a duration of response ≥9 months) were seen with pembrolizumab in pretreated SCLC, especially in patients with PD-L1-positive tumors (ORR 35.7% vs. 6.0% for PD-L1-positive and PD-L1-negative subgroups, respectively) [103]. A pooled analysis of these two trials, including 83 patients with recurrent SCLC, confirmed these findings. Pembrolizumab was associated with an ORR of 19.3% (2 complete responses and 14 partial responses) and a median duration of response not reached (61% of responders had responses lasting ≥18 months) [104].

In a phase II randomized clinical trial, the efficacy of atezolizumab monotherapy was compared with that of chemotherapy (with either topotecan or platinum rechallenge) in second-line SCLC, independent of PD-L1 expression.

The study included 73 patients (49 in the atezolizumab arm and 24 in the chemotherapy arm), and 64% had platinum-sensitive disease (defined as disease progression ≥90 days after completion of induction chemotherapy). No significant differences were observed in median OS (9.5 vs. 8.7 months, HR 0.84; $P = 0.60$), and the median PFS was statistically inferior in patients who received atezolizumab (1.4 vs. 4.3 months; $P = 0.004$). ORRs were low in both groups (2.3% in the atezolizumab arm and 10% the chemotherapy arm) [105].

In summary, both nivolumab and atezolizumab have failed to improve OS compared with standard chemotherapy in RCTs involving patients with relapsed SCLC requiring second-line therapy. FDA approval of ICI monotherapy, with either nivolumab or pembrolizumab, has been granted only in the third-line or later setting based on ORRs of 10–30% in single-arm studies.

8 Activity of ICIs in Special Populations

Poor Performance Status (PS) Patients with disease burden-determined poor performance status (PS) have generally poor prognosis. Evidence on first-line ICIs in PS ≥2 NSCLC with PD-L1 ≥ 50% expression is relatively scant, as this population is usually excluded from clinical trials. A recent retrospective multicenter study in a real-world setting addressed this issue. Among 153 patients included, the median PFS and OS were 2.4 (95% CI, 1.6–2.5) and 3.0 months (95% CI: 2.4–3.5), respectively. The 6-month PFS rate was 27% (95% CI, 21–35%). Patients with a PS 2 determined by comorbidities had significantly better results compared with PS 2 induced by disease burden (6-month PFS rate, 49% vs. 19%; median OS 11.8 vs. 2.8 months, respectively) [106]. Additional data are required to determine the best therapeutic approach for this poor prognosis subgroup of patients.

HIV/AIDS Anti-PD-1/PD-L1 checkpoint inhibitors have been approved for a variety of cancers that occur with higher incidence in people with HIV, including lung cancer. However, HIV-infected patients were excluded from all registrative trials with ICIs in solid tumors, and therefore the evidence on safety and activity of these agents in this population are relatively poor and mostly derived from small case series or case reports [107].

A recent prospective study explored the safety of pembrolizumab immunotherapy in solid tumor patients with HIV infection (CD4 count greater than or equal to 100 cells/μL, antiretroviral therapy for 4 or more weeks, and an HIV viral load of less than 200 copies/mL were eligible) and showed that pembrolizumab has a similar irAE profile for people with HIV and advanced cancer who have suppressed antiretroviral treatment for HIV as seen in HIV-negative participants in published studies [108]. The proportion of serious events was similar to that previously described in patients receiving anti-PD-1 therapy for FDA-approved indications. Hypothyroidism was the most frequent immune-mediated event in 20% of the participants and was adequately controlled with standard treatment [108].

Evidence available to date in HIV-infected NSCLC suggests that single-agent PD-1/PD-L1 inhibitors can be used safely in this subgroup of patients with similar efficacy results observed in the overall NSCLC population. The results of ongoing clinical trials evaluating ICIs in HIV-infected patients with NSCLC (CHIVA-2/NCT03304093) and/or different solid tumors (NCT03094286, NCT02408861) will provide definitive conclusions in this setting [109].

Preexisting Autoimmune Disorders The vast majority of clinical trials have excluded patients with significant preexisting autoimmune disorders (AID). However, AIDs are relatively common in clinical practice. Safety and efficacy of ICIs in patients with preexisting AIDs are largely unknown, and evidence available to date are mostly based on retrospective analyses.

In a large retrospective study including 751 patients, of whom 65.5% had an advanced NSCLC, 11.3% had preexisting AID, including

both clinically active (17.6%) and inactive (82.4%) diseases. Patients with preexisting AID experienced higher incidence of immune-related adverse events (irAEs) of any grade compared with patients without AIDs (65.9% vs. 39.9%). However, no significant differences were observed regarding grade 3/4 irAEs. Interestingly, preexisting AIDs were not significantly associated with ICI efficacy [110]. Similarly, another retrospective multicenter study evaluating the safety of PD-1/PD-L1 inhibitors in NSCLC patients with preexisting AID showed that exacerbation of AID occurred in a minority of patients (23%). Thirty-eight percent of the patients experienced an irAE (74% G1/2, 26% G3/4), and 14% discontinued treatment because of irAEs [111].

Given the paucity of data, treatment with ICIs in this patient population should be evaluated with caution and after an accurate evaluation of the risk-benefit ratio within a multidisciplinary team [112].

Solid Organ Transplant A scenario of the daily clinic surrounds the aspect that carries safety problems, solid organ transplant recipients (SOTR) who are routinely excluded from immunotherapy trials; therefore, there is limited data for these agents in this population. A first approximation to the information in relation to cancer patients and solid organ transplants was published in 2018 evaluating 26 solid organ recipients treated with ICIs. 3/7 had graft rejection with ipilimumab, 6/15 with PD-1 inhibitor, and 2/4 patients treated with sequential ipilimumab and PD-1 inhibitors. Graft rejection was observed in 7/10 patients who received prednisolone with or without cyclosporine and 4/16 patients treated with different immunosuppressive regimens (tacrolimus, everolimus, sirolimus, or mycophenolate mofetil), suggesting that solid organ transplant recipients considered for ICIs might need more intensive immunosuppressive therapy than prednisolone monotherapy. Tumor response was reported in 27% of patients treated with ipilimumab and in 32% in those treated with PD-1 inhibitors. Of the nine patients who obtained a CR/PR to ICIs, four patients were immunosuppressed with tacrolimus or sirolimus, while the other five were treated with prednisolone. This could suggest that immunosuppressive regimens containing tacrolimus or sirolimus can be continued when ICIs are administered to organ transplant recipients [113].

A recent meta-analysis of published data reported that 37% of the patients experienced organ rejection and 14% died as a result of graft rejection. Nivolumab was associated with the highest rejection rate (52.2%), followed by pembrolizumab (26.7%) and ipilimumab (25%). When analyzing transplant rejection by organ, the highest rejection rate was observed in patients with kidney transplants (40.1%), followed by liver (35%) and heart (20%) transplants, and 64% presented disease progression. In terms of efficacy, the response rate was highest for pembrolizumab (40%), followed by nivolumab (30%) and ipilimumab (25%) [114].

Integrating Special Populations An example of an inclusive clinical trial seeking to shed light on a daily problem in healthcare practice was given by a prospective cohort investigation with ICIs in special populations with stage IV or recurrent NSCLC, and no known sensitizing *EGFR* or *ALK* alterations, regardless of PD-L1 expression. CheckMate-817 was a phase IIIb/IV trial initiated due to limited available data on safety and efficacy of immunotherapy in patients with advanced NSCLC with poor performance status (ECOG PS 2) or other comorbidities, such as kidney and renal disease, and HIV-infected. First-line flat-dose nivolumab plus weight-based ipilimumab showed a consistent safety profile in special populations with advanced NSCLC, including those with ECOG performance score 2. Patients with either high TMB or higher PD-L1 1 expression exhibited improved outcomes. The safety profile was similar between the special population and a reference cohort. The mean time to the appearance of adverse events was similar between the cohorts [115]. Similarly, the TAIL study evaluated the safety and activity of atezolizumab in pretreated NSCLC with ECOG PS 2, renal failure, or preexisting autoimmune disease [116]**.** 615 patients received atezoli-

zumab. Serious AEs occurred in 7.8% of patients and irAEs in 8.3%. The median OS was 11.1 months (95% CI: 8.9, 12.9), the ORR was 11.1% (95% CI: 8.7, 13.8), and the median of DOR was 14.6 months (95% CI: 8.4, 15.4) [116]. Medium- and long-term safety profile data are awaited for this population.

Clinical trials in lung cancer with anti-PD-1/PD-L1 treatment have generally excluded patients with ECOG PS ≥ 2, organ transplantation, AIDS, chronic viral infection, or organ dysfunction. This group of patients does not have scientific support that the use of immunotherapy in these special populations is scarce and is derived mainly from case series or real-world experience. Therefore, cautions should be used in clinical practice when considering these agents in special patient populations, as the evidence available to date are low. The results of ongoing clinical trials in these peculiar clinical scenarios will provide definitive conclusions on the safety and efficacy of ICIs in these subgroups of patients.

9 Impact of Molecular Characterization in the era of Immunotherapy and Future Directions

In the era of personalized medicine, the increasing use of next-generation sequencing (NGS) in both tissue and plasma is rapidly expanding our knowledge on the molecular characteristics of lung tumors. The multitude of information that can be obtained with these techniques has considerably improved the therapeutic landscape of advanced NSCLC trough the identification of oncogene drivers exploitable with targeted therapies [117]. In the context of non-oncogene addicted tumors, the molecular characterization of the tumor might provide useful prognostic and predictive information, overcoming the limits of PD-L1 tumor expression.

There are reports regarding the increase in the acquisition of somatic mutations during tumorigenesis which is associated with the formation of neoantigens and the subsequent development of immunogenicity; therefore, it has been postulated that tumors with a higher number of somatic mutations could be more sensitive to blocking immune checkpoints. According to international consensus, tumor mutational burden (TMB) is defined as the total number of non-synonymous mutations per coding area of a tumor genome and is calculated as mutations per DNA megabase (Mb). This emerging biomarker has been variably associated with ICI efficacy, although its clinical utility in clinical practice is unclear. On the one hand, TMB on either tissue (tTMB) or plasma (bTMB) has been clearly associated with improved efficacy with single-agent PD-1/PD-L1 inhibitors in exploratory analyses of large randomized studies in advanced NSCLC [65; 118, 119] and is a tumor-agnostic FDA-approved biomarker for pembrolizumab [120]. On the other hand, the predictive role of tTMB and bTMB is questioned when using chemo-immunotherapy combinations [121, 122].

Recently, the presence of concomitant mutations has been associated with inferior outcomes in NSCLC patients treated with single-agent ICIs.

Retrospective studies have shown that the presence of *TP53* mutations without co-occurring *STK11* or *EGFR* alterations (TP53-mut/STK11-EGFR-WT), independent of *KRAS* mutations, identifies a group of tumors with the highest CD8 T-cell density and PD-L1 expression that is associated with a prolonged PFS with single-agent ICIs. In contrast, *STK11/LKB1* alterations are the most prevalent genomic driver of primary resistance to PD-1 axis inhibitors in KRAS-mutant lung adenocarcinoma [123, 124]. One of the possible explanations for these findings is that *STK11*, *EGFR*, or *SMARC4* mutations are usually enriched among PD-L1-negative tumors, whereas TP53 mutations are more often seen in PD-L1-strong positive patients, as recently reported [125]. Collectively, these data suggest that concomitant mutations might influence ICI activity due to the presence of a tumor microenvironment less immunogenic (“cold tumors”), despite a higher TMB than wild-type tumors

[126]. However, the debate on the prognostic/predictive role of these mutations is still open, and recent studies have shown that these mutations represent a poor prognostic factor rather a predictive factor that is independent of treatment received. Interestingly, the blood biomarker analysis of the MYSTIC trial showed that OS was shorter for patients with *KEAP1*-mutated or *STK11*-mutated NSCLC compared to wild-type patients irrespective of treatment received (durvalumab, durvalumab-tremelimumab, or chemotherapy) [127], suggesting that these mutations are likely a poor prognostic factor rather than a predictive factor to ICIs. Similar conclusions were recently reported in a large pan-cancer analysis evaluating the prognostic and predictive role of *STK11* mutations. Across multiple solid tumors, *STK11* alterations correlated with a poor prognosis regardless of therapy and were not associated with inferior immunotherapy outcome in the pan-cancer setting or in NSCLC. Furthermore, pan-cancer patients with co-altered *STK11/KRAS* did worse, regardless of treatment type [126]. In addition, the impact of these concomitant mutations among patients treated with chemo-immunotherapy combinations is still unclear, as initial retrospective studies reported that *STK11* and *KEAP1* genomic alterations are associated with shorter PFS with both platinum-pemetrexed-pembrolizumab chemo-immunotherapy and platinum-pemetrexed chemotherapy in non-squamous NSCLC and therefore represent adverse prognostic biomarkers, but the addition of pembrolizumab to platinum-pemetrexed does not result in prolonged PFS in PD-L1-positive *STK11* and/or *KEAP1*-mutant non-squamous NSCLC [127], suggesting a potential negative predictive role [128, 129]. However, an exploratory analysis of the KEYNOTE-189 study did not confirm these findings, as pembrolizumab plus platinum/pemetrexed is associated with better outcomes than chemotherapy regardless of *STK11* or *KEAP1* mutational status [130].

The scenario of permanent evolution in the search for the best therapeutic strategy for a first line entails the need for robust predictive biomarkers to advanced NSCLC [131], which can potentially allow counseling of these patients who do not benefit from the use of ICI alone or in combination with chemotherapy or a combination of different checkpoint inhibitors.

The cornerstone of treatment for advanced NSCLC is focused on the search for biomarkers capable of predicting the response with an adequate safety profile. The biomarkers reported so far showed limitations in the capacity to effectively predict therapeutic efficacy of ICIs either alone or in different combinations. The use of ICIs is rapidly revolutionizing the therapeutic landscape of lung tumors, providing a significant improvement in the overall survival of these patients in multiple clinical settings. Long-term follow-up of registrative trials of these agents is constantly demonstrating long-term survivals in an unprecedented percentage of the patients, transforming an incurable disease into a chronic disease. The next step will be to extend the survival benefit to a higher percentage of patients, through the identification of novel predicting biomarkers and the introduction of more effective therapeutic strategies in tumors with less immunogenic microenvironment.

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Landscape of Immunotherapy in Genitourinary Malignancies

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Abstract

The past decade has witnessed a revolution in the development of immune checkpoint inhibitors for the treatment of multiple tumor types, including genitourinary cancers. Immune checkpoint inhibitors have notably 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 except for microsatellite instability *high* (*MSI*-H) tumors. 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 great interest in biomarker development to predict response to immunotherapy and risk of toxicity. This book chapter is a comprehensive review of 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

1 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

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newly diagnosed with RCC have metastatic or advanced disease [2, 3]. Risk stratification of patients with newly diagnosed metastatic RCC is essential to determine both prognosis and treatment options. 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 independent prognostic value 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 one to two 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, since 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 1).

The treatment of ccRCC has witnessed tremendous evolution over the past decade both with the introduction of targeted therapies as well as 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 was approved in 2018 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. Combinations of immune CPIs with TKIs have now also been approved for metastatic RCC. 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. 1).

1.1 Rationale for Immunotherapy in RCC

RCC is known to be particularly resistant to chemotherapy, and this characteristic can 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 compared 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].

Table 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

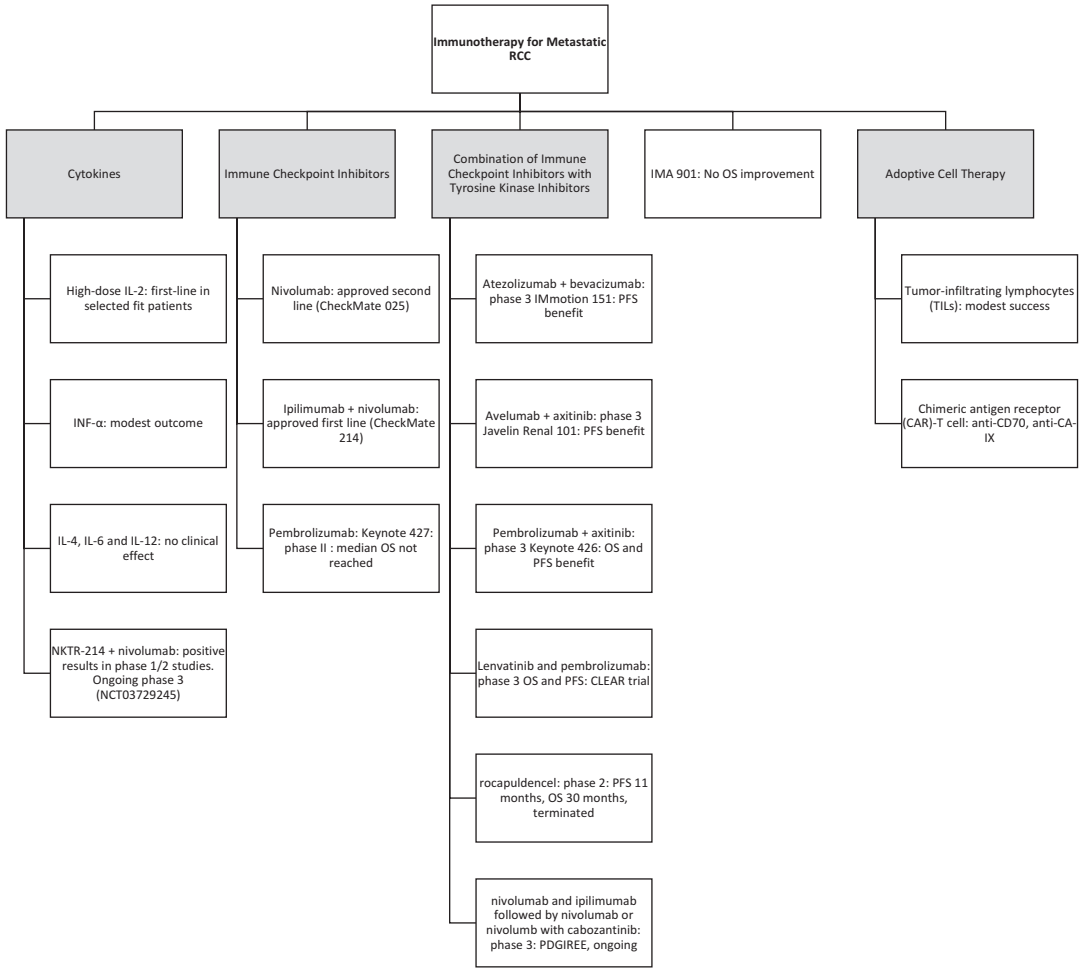

Fig. 1 Immunotherapy for the treatment of metastatic renal cell carcinoma. *RCC* renal cell carcinoma, *IL* interleukin, *INF* interferon, *Prelim* preliminary, *PFS* progression-free survival, *OS* overall survival

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 [14]. 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, $P = 0.048$) [17]. A recent prospective study of 352 patients [18] and another retrospective study of 391 patients [19] 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 [18, 19]. 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 [20].

Until 2005, IL-2 and INF-α were the only two approved therapies for RCC, and the median survival was approximately 1 year [21]. Since then, a number of new therapies have been approved that lead 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 [22, 23]. 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 of 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 [24]. The immune-editing mechanism comprises three phases: elimination, equilibrium, and escape [25]. 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 constrained environment in the presence of immune cells in the equilibrium phase. Finally, evasion of immune surveillance by cancer cells comprises the escape phase [25–27]. Under constant pressure from the immune system, tumor cells thrive through mechanisms that allow them to resist immune cells [28] 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) [29–33]. Ongoing efforts to counteract these immune escape mechanisms are driving scientific research and clinical trials in the exploration of the best treatment modalities for RCC.

Immune-related adverse effects (irAEs) are seen in these patients treated with these agents. Interestingly, in a retrospective study of about 500 patients with advanced RCC who received immunotherapy, 80 of those patients developed irAEs that required treatment interruption. 36/80 (45%) patients were rechallenged, and fewer of the patients who were retreated required corticosteroids and hospitalizations for treatment of IrAE compared to the patients who had immunotherapy permanently discontinued [34].

1.2 Immune Checkpoint Blockade in Locally Advanced or Metastatic RCC (Fig. 2)

1.2.1 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, thus interfering 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 indications of the clinical benefit of immune checkpoint blockade in the treatment of RCC.

The promising activity of the phase 1 trials led to the launch of a phase 2 randomized blinded multicenter clinical trial of nivolumab in metastatic ccRCC [38]. Three arms were included in the study with 1:1:1 randomization to 3 different doses of nivolumab, 0.3, 2, and 10 mg/kg. The randomization was stratified based on the number of prior therapies (1 versus >1 (70%)) and MSKCC risk group (favorable/intermediate versus poor (25%)). The primary endpoint was evaluation of the dose-response relationship as measured by progression-free survival (PFS); secondary endpoints included ORR, OS, and safety. One hundred and 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 the 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 3 arms. The most common treatment-related AE was fatigue (24%, 22%, and 35%, respectively). Nineteen patients (11%) experienced grade three to four treatment-related AEs (nausea, arthralgia, and elevation of alanine and arginine transaminases), of which 4 were in the 0.3 mg/kg group, 14 were in the 1 mg/kg group, and 1 was in the 10 mg/kg group [38].

The successful phase 2 trial again led to the investigation of nivolumab in metastatic ccRCC in a phase 3, multicenter, international, open-label randomized study, CheckMate 025 [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].

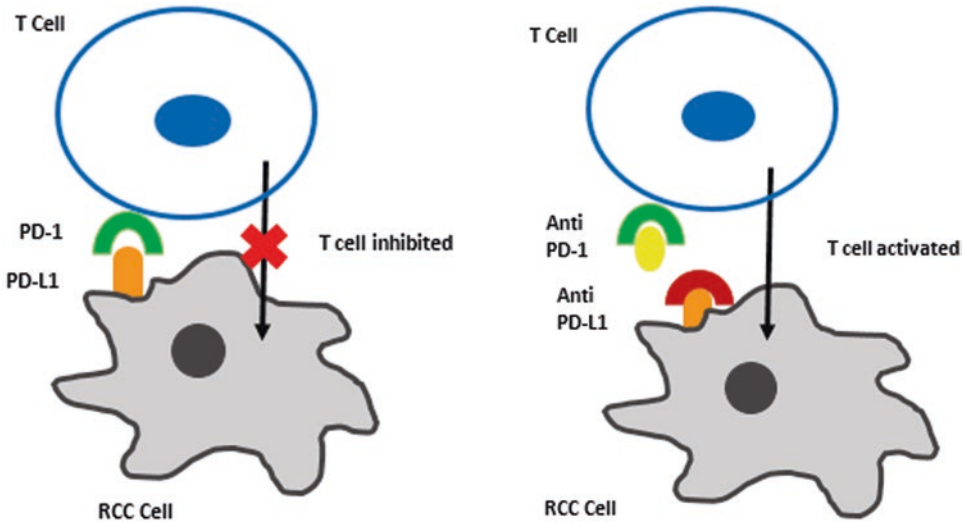

Fig. 2 Principle of immune checkpoint inhibition. *RCC* renal cell carcinoma, *PD-1* programmed death 1, *PD-L1* programmed death-ligand 1

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% versus 5%, odds ratio, 5.98 [95% CI: 3.68 to 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 two points from baseline) HRQoL improvement with nivolumab (200 [55%] of 361 patients) versus everolimus (126 [37%] of 343 patients; $p < 0{\cdot}0001$). 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 exists on the role of nivolumab monotherapy in the frontline treatment of advanced RCC. Biomarkers have been investigated to prognosticate patients with advanced RCC receiving immunotherapy. Bilen et al. reported a retrospective study where 38 patients were treated with nivolumab and a pretreatment NLR < 5.5 was found to be associated with superior PFS and OS. Low NLR was associated with prolonged OS (95% CI: 0.01–0.55; $P = 0.012$) [44].

1.2.2 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 ccRCC [8]. Previously untreated patients with advanced or metastatic ccRCC 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 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 versus 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% versus 32%), but there was no difference in PFS (median 12.4 versus 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 versus 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% versus 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 versus 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% versus 25%, median PFS 22.8 versus 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 versus 28% for sunitinib) or median PFS (11 months for the combination versus 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% versus 52%), and PFS was shorter (median 15.3 versus 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 suggests that the nivolumab-ipilimumab combination has better outcomes in the favorable risk group than initially presented [45].

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 in 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, and among those, 35% received high-dose corticosteroids. It is important to note, however, that 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 [46]. 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 to 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 to 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 results from the CheckMate 214 clinical trial, the combination of ipilimumab and nivolumab was approved by the US FDA for the treatment of previously untreated patients with intermediate- to poor-risk advanced or metastatic RCC, on April 16, 2018.

1.2.3 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 [47]. Preliminary results from cohort A of this trial were presented at the 2018 American Society of Clinical Oncology

(ASCO) annual meeting. One hundred and 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 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 lead to discontinuation of treatment in 12 patients, and treatment-related death due to pneumonitis occurred in one patient.

1.3 Combined Antiangiogenic Plus CPI Immunotherapy in Locally Advanced or Metastatic RCC

1.3.1 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 [47, 48]. This was confirmed in a phase 1b trial of the combination in the frontline setting of metastatic RCC with ORR of 73% (95% CT 59–84) [49].

The phase 3 Keynote-426 trial demonstrated an OS and PFS benefit of the combination of pembrolizumab and axitinib in the frontline treatment of advanced or metastatic RCC [50]. 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 or 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 were 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 FDA approved as a first-line treatment in advanced RCC on April 19, 2019, regardless of IMDC risk score or PD-L1 status.

1.3.2 Avelumab with Axitinib

Another combination of antiangiogenesis with immunotherapy composed of avelumab and axitinib showed promising results in a phase 3 study. The Javelin Renal 101 phase 3 trial involved 886 treatment-naïve patients with advanced ccRCC, randomly assigned to the combination of avelumab and axitinib versus sunitinib [51]. 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 in 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 to 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 matures. 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.

1.3.3 Atezolizumab with Bevacizumab

Positive results of the phase 2 trial of bevacizumab and atezolizumab [52] led to a phase 3 trial of this combination in 915 untreated patients with metastatic RCC (IMmotion 151). Patients were randomized to receive either atezolizumab with bevacizumab or sunitinib [53]. Median PFS was longer in the combination arm as opposed to the sunitinib arm (11.2 versus 8.4 months, HR 0.83, 95% CI 0.70–0.97), ORR were 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 versus 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. A subgroup analysis of patients with tumors with sarcomatoid features who were treated with atezolizumab plus bevacizumab had longer PFS (8.3 vs 5.3 months; HR, 0.52) and higher ORR (49% vs 14%) compared with patients treated with sunitinib [54].

1.3.4 Nivolumab and Cabozantinib

In September of 2020, the results of the CheckMate 9ER study, which evaluated nivolumab plus cabozantinib compared with sunitinib as first-line treatment of advanced RCC, were reported at the European Society for Medical Oncology (ESMO) Virtual Congress 2020. In this clinical trial, 651 patients were stratified by IMDC risk score, tumor PD-L1 expression, and region and randomized to receive 1:1 nivolumab plus cabozantinib or sunitinib until disease progression or unacceptable toxicity for a total duration of 2 years. Of the 651 patients enrolled in the trial, 22.6% were in the favorable risk category, 57.6% were intermediate risk, and 19.7% were of poor risk. Median follow-up was 18.1 months. The study met all of the efficacy endpoints; the combination of nivolumab and cabozantinib doubled PFS compared to sunitinib (16.6 months versus 8.3 months; HR 0.51, 95% CI 0.41–0.4). OS was also improved with nivolumab plus cabozantinib versus sunitinib though the median OS was not reached (HR 0.60, 98.89%, CI 0.40–0.89, $p = 0.0010$). ORR was higher with the combination (55.7% versus 27.1%, 95% CI 22.4–32.3) compared to sunitinib. Approximately 8.0% of patients achieved CR with the combination compared to 4.6% of the patients taking sunitinib. Any-grade treatment-related adverse events (TRAE) were noted in 96.5% of patients who received the combination compared with 93.1% of patients receiving sunitinib. Grade 3 or higher TRAEs were reported in 60.6% in patients receiving the combination versus 50.9% of patients receiving sunitinib. Based on this study, it was concluded that the combination of nivolumab plus cabozantinib demonstrated superior PFS, OS, and ORR to

sunitinib in the first-line treatment of advanced ccRCC [55]. The US FDA approved this combination on October 19, 2020.

1.3.5 Lenvatinib and Pembrolizumab

The phase 3 CLEAR trial (NCT02811861) investigated the combination of lenvatinib with pembrolizumab, which resulted in improved OS, PFS, and ORR over sunitinib in the frontline treatment of patients with advanced RCC. These findings were recently published. The trial comprised 1069 patients who were randomized in a 1:1:1 fashion to receive lenvatinib plus pembrolizumab, lenvatinib plus everolimus, or sunitinib.

Objective response was 71.0% with lenvatinib plus pembrolizumab, 53.5% with lenvatinib plus everolimus, and 36.1% with sunitinib. CR was seen in 16.1% of patients receiving lenvatinib plus pembrolizumab, 35% receiving lenvatinib plus everolimus, and 15% who received sunitinib. The median PFS with lenvatinib and pembrolizumab was 23.9 months (95% CI, 20.8–27.7) and was 9.2 months (95% CI, 6–11) with sunitinib (HR, 0.39; 95% CI, 0.32–0.49; $P < 0.001$). Patients who received lenvatinib plus everolimus achieved a median PFS of 14.7 months (95% CI, 11.1–16.7) compared to the 9.2 months in the sunitinib arm (HR, 0.65; 95% CI, 0.53–0.8; $P < 0.001$). The PFS benefit extended across both the lenvatinib and pembrolizumab and lenvatinib and everolimus treatment arms. Median OS was longer in the lenvatinib and pembrolizumab arm compared to the sunitinib arm (HR, 0.66; 95% CI, 0.49–0.88; $P = 0.005$). No observed OS benefit in the lenvatinib and everolimus treatment arm was seen over sunitinib alone (HR, 1.15; 95% CI, 0.88–1.5; $P = 0.3$). Over 90% of patients in each treatment arm, lenvatinib plus pembrolizumab (96.9%), lenvatinib plus everolimus (97.7%), and sunitinib (92.1%), experienced any treatment-related AE. Patients receiving lenvatinib plus pembrolizumab (67.3%) and lenvatinib plus everolimus (69.3%) treatment arms were more likely to experience TRAEs compared to sunitinib arm leading to dose reductions. These findings support potentially adding lenvatinib plus pembrolizumab as potential first-line treatment for patients with advanced RCC [56].

1.3.6 Other Combinations

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 [57, 58]. A current phase 2 study is assessing whether cabozantinib, nivolumab, and ipilimumab in combination is safe and effective in treated advanced clear cell RCC (NCT04413123). A phase 3 trial (NCT 03793166) compares treatment with ipilimumab and nivolumab followed by nivolumab alone to treatment with ipilimumab and nivolumab, followed by nivolumab with cabozantinib in patients with advanced RCC. COSMIC-313 is a controlled phase 3 trial evaluating the effect of cabozantinib in combination with nivolumab and ipilimumab in two arms: (1) nivolumab and ipilimumab with cabozantinib versus (2) nivolumab and ipilimumab in combination with matched placebo. This study will report the effect of cabozantinib on the duration of PFS versus nivolumab and ipilimumab, and the secondary objective is to evaluate the effect of combination on the duration of OS.

The hypoxia-inducible factor-2α (HIF-2α) inhibitor belzutifan has been reported to have clinical activity in patients with advanced clear cell RCC in a recent phase 2 study (NCT02974738). Currently, a phase 3 trial is investigating the efficacy and safety of pembrolizumab plus belzutifan plus lenvatinib or pembrolizumab/quavonlimab, an anti-CTLA-4 antibody, plus lenvatinib versus pembrolizumab plus lenvatinib as first-line treatment (NCT04736706).

Table 2 summarizes phase 3 combination trials.

1.4 Other Immunotherapy Approaches in Locally Advanced or Metastatic RCC

1.4.1 Vaccines

The use of vaccines to enhance the immune recognition of tumor has been investigated in RCC. Rocapuldencel-T is an autologous immunotherapy prepared from fully matured and opti-

Table 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					
				PFS (months)	OS (months)	CR	ORR	DCR	Grade 3–4 adverse events
Clear/ NCT02811861	Lenvatinib/ pembrolizumab vs everolimus/ lenvatinib	Sunitinib	PFS	23.9 vs 9.2; 14.7 vs 9.2	Not reached in any arm	57 vs 35 v 15	71.0 vs 53.5 vs 36.1	90.2 vs 87.1 vs 74.2	Grade 3–4: 82.4 any event vs 83.1 vs 71.8
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%	Grade 3–4: LFT abnormalities (3%) in treatment arm vs LFT abnormalities (4%) in control arm 16% of pts. 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 Grade 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%	Grade 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	16.6 vs 8.3	Not reached in either arm	8.0% vs 4.6%	55.7% vs 27.1%		All grade TRAEs occurred in 96.6% vs 93.1% Grade 3–4: TRAEs in 60.6% vs 50.9%

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, *Pts* patients, *HTN* hypertension, *HFS* hand-foot-syndrome, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

mized monocyte-derived DCs, which are co-electroporated with amplified tumor RNA from nephrectomy specimens plus synthetic CD40L RNA. Rocapuldencel-T was evaluated in combination with sunitinib in an open-label phase 2 study of 21 patients with intermediate and poor-risk, treatment-naïve metastatic RCC [59]. 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 led to the phase 3 ADAPT study (NCT01582672) in which patients with metastatic RCC undergoing debulking nephrectomy are randomly assigned to either sunitinib with rocapuldencel-T or sunitinib alone. However, due to lack of clinical efficacy, the trial was terminated [60].

Another cancer vaccine, IMA901, that is based on tumor-associated peptides was administered in the frontline setting to patients with metastatic RCC who were positive for HLA-A*02 antigen, leading to positive results in a phase 2 study [61]. A phase 3 study, IMPRINT, investigated its addition to sunitinib [62]. Three-hundred and 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 versus not reached, HR 1.34, 95% CI 0.96–1.86, $P = 0.08$).

1.4.2 Other Cytokines

Multiple interleukins have been studied for the use in RCC, including IL-4 [63], IL-6 [64], and IL-12 [65, 66], but their antitumor activities were modest or toxicities of some were concerning. 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 [67].

A novel prodrug of peglyated 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 [68]. 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 [69] 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-naïve RCC, pre-specified efficacy criteria were met for ORR in stage 1 with 7/11 (64%) of 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 frontline treatment of metastatic RCC (NCT03729245). A phase 1 study evaluated bempegaldesleukin (NKTR-214/BEMPEG) plus nivolumab in solid tumors which included RCC. Total objective response rate across tumor type and dose cohorts was 59.5% (22/37) with 7 CR (18.9%). For RCC, ORR was 10/14 or 71.4%. IL-10 receptor agonist, pegilodecakin, was studied in an open-label, phase 1b trial where it was given in combination with pembrolizumab or nivolumab to patients with advanced solid tumors refractory to prior lines of therapies. 111 patients were enrolled in the study, and 38/111 (34%) had renal cell carcinoma and 1/111 (1%) had bladder

cancer. Out of the 38 patients with advanced RCC, 29 patients received study drug with nivolumab, and 9 patients received study drug with pembrolizumab. Primary endpoints were safety and tolerability, while objective response was a secondary endpoint. Objective response, overall, was seen in 14/35 (40%) patients with advanced RCC [70, 71].

1.4.3 Adoptive Cell Therapy

The generation and adoptive transfer of tumor-infiltrating lymphocytes (TILs) has demonstrated durable CR in metastatic melanoma [72], but the success rates of this strategy are much lower in other cancers [73]. A number of studies have shown that the tumor microenvironment in RCC harbors tumor-reactive T cells [73, 74], 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 [75]. 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 tumor-associated antigens (TAAs) [76]. Second- and third-generation CAR are engineered to express a costimulatory molecule, such as CD28, 4-1BB, CD27, ICOS, or OX40, to increase the antitumor effect, proliferation, and survival of CAR T cells [77]. The greatest challenge in solid tumors is the identification of antigen targets. Many TAAs are also expressed at low levels 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 [78]. CA-IX is a metalloprotease that is considered a 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 [79, 80]. Preclinical studies of first-generation CA-IX-directed T cells in RCC demonstrated a robust cytokine production and cytotoxic activity [81]. 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 [82]. Two of these patients developed grade 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 pre-administered unmodified antibody from which scFv was derived to saturate the liver before CAR-T-cell administration and abrogate liver toxicity [78]. 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 [83]. A clinical trial of anti-CD70 CAR in CD70-expressing solid tumors including RCC is now suspended.

Multiple mechanisms are involved in T-cell suppression and are mediated via myeloid-derived suppressor cells (MDSCs) [61, 84], through arginase-mediated downregulation of the T-cell receptor ζ chain [85] as well as circulatory regulatory T cells (Tregs) [86, 87]. Sunitinib is a multikinase inhibitor for the treatment of metastatic RCC, and it has been shown to decrease MDSCs [88], enhance type-I INF responses, and

decrease Treg function [89]. It would be intriguing to investigate the role of VEGFR-TKI in preconditioning and maintenance after CAR-T cell therapy in RCC [90].

1.5 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 was 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 [91]. 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 versus 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 receive either 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 [92]. 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 receive either the tumor vaccine or observation postoperatively if the disease was high risk (pT2-T3b, pN0-3) [93]. The vaccine was administered every 4 weeks for a total of six doses. There was a modest 5-year PFS improvement in the vaccine arm (77.4% versus 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 [94]. Its use in the adjuvant setting was investigated in a multicenter phase 3 randomized trial of patients with cT1b-T4N0M0 or TanyN1-2 M0 RCC who planned to undergo curative nephrectomy [95]. The vaccine was administered weekly for 4 weeks 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 takes 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 are 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.

1.6 Biomarkers for Response

Research into 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 the 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 ≥1% PD-L1 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 (≥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 [96] 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 expression.

Another biomarker used in other diseases to predict response to immunotherapy is tumor mutational burden (TMB) and non-synonymous expression where higher tumor expression of neo-antigens was linked to a favorable response to immunotherapy [97, 98]. 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 [99]. 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 neo-antigens 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 [99]. Moreover, TMB did not differ between clear cell and sarcomatoid components of different tumor samples, suggesting that TMB is not associated with worse clinical features, although this hypothesis needs to be further investigated [100]. Another study carried out whole exome and transcriptome sequencing of nine patients with metastatic RCC receiving nivolumab [101] and determined that RCC had relatively few nonsynonymous mutations and neoantigens. Interestingly, among nivolumab-treated patients, the neoantigen load was significantly higher in non-responders than 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 the role of TMB in predicting response to immunotherapy in 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 [102]. These findings were presented by Rini et al. at the ESMO 2018 Congress. 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 versus 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 versus 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 versus 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 versus 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.

Analyses of baseline tumor samples from the phase 3 JAVELIN Renal 101 trial (NCT02684006) which compared avelumab + axitinib versus sunitinib in advanced RCC found that neither expression of PD-L1 nor TMB differentiated PFS in either study arm. The presence of FcγR single nucleotide polymorphisms did not have any impact. The authors reported new immunomodulatory and angiogenesis gene expression signatures (GESs) to provide insight into determining combined PD-1/PD-L1 and angiogenic pathway inhibition in advanced RCC [103].

1.7 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. Recently, the combination of nivolumab and cabozantinib was approved in the frontline setting as well. 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 to potentiate T-cell and immune cell responses in the presence of immune CPIs or other immunotherapies will lead to an increase in the scope of benefit from these breakthrough treatments.

2 Immunotherapy for Urothelial Carcinoma

Bladder cancer is the sixth most common cancer with an estimate of 80,470 new cases diagnosed in the USA in 2019 and 17,760 deaths during the same year [104]. Urothelial carcinoma (UC) is the most common subtype in the USA and Europe [105, 106]. Bladder cancer is most frequently diagnosed among people aged 65–74 [107]; therefore, it is important to factor other medical comorbidities into treatment choices. Approximately 75% of new cases are non-muscle-invasive and characterized by a tendency to recur [108, 109]. 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 [110]. Despite the effectiveness of platinum-based therapies, metastatic UC still has a modest median OS of around 15 months [108, 111]. Similarly, second-line chemotherapies provide a suboptimal OS [112, 113]. CPIs have flipped the equation for both platinum-refractory and platinum-ineligible patients [114–121]. Actionable genetic alterations, which are found in >50% of high-grade UCs, are gaining interest, especially fibroblast growth factor receptor (FGFR) alterations [122]. Additionally, several TAAs in UC are attractive targets for antibody drug conjugate (ADC) development, which are being studied alone and in combinations with CPIs [123, 124]. Here, we describe the FDA-approved immune-oncology (I-O) modalities and the prominent investigational strategies for early or advanced stage UC.

2.1 Rationale for Immunotherapy in UC

In 1976, immune modulation was found to be helpful in the management of non-muscle-invasive bladder cancer (NMIBC) with the use of Bacillus Calmette-Guerin (BCG) [125]. 40 years later, genomic studies showed that bladder cancer ranks third after melanoma and non-small cell lung cancer in terms of somatic mutation rate [126, 127]. This high mutational burden and genomic instability seem to determine sensitivity to immunotherapy [128, 129]. Genomic alterations are translated into foreign proteins that could be recognized by cytotoxic T cells, and potentiate cancer cells response to CPI [130]. However, infiltrating CD4+ and CD8+ T cells express high levels of PD-1 in UC [131], rendering them ineffective at eradicating tumors. Furthermore, expression of PD-L1 on UC cells is associated with higher grade, stage, rate of post-operative recurrence, and risk of death after cystectomy [131–133]. These findings provide the rationale for using anti-PD1 and anti-PD-L1 immunotherapies to treat patients with UC.

Currently, there is not accepted risk score in metastatic UC. The modified Glasgow Prognostic Score (mGPS) is a system that incorporates albumin and C-reactive protein. Brown et al. studied the effect of mGPS on survival outcomes of patients with metastatic UC receiving ICI. From 53 patients, increased mGPS at the time of when patients first received ICI was associated with shorter OS and PFS in univariate and multivariate and Kaplan-Meier analyses [134]. Shabto et al. reported a novel risk stratification system for UC patients treated with ICI using platelet-to-lymphocyte risk as an inflammatory marker in addition to Eastern Cooperative Oncology performance status, presence of liver metastasis, and albumin [135].

2.2 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 [136].

2.2.1 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 [137]. This was followed by FDA approval for this indication in 1990 [138]. In terms of reducing recur-

rences, BCG post-resection of high-grade NMIBC is superior to observation and superior to intravesicular chemotherapy [139–141]. 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) [142]. BCG unresponsiveness 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 [138]. Combination intravesicular pembrolizumab + intravesicular BCG is being investigated in BCG-naïve high-risk NIMBC and BCG-relapsing NIMBC (NCT02808143).

2.2.2 BCG-Unresponsive 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. In January 2020, the FDA approved pembrolizumab for the treatment of patients with BCG-unresponsive, high-risk, non-muscle invasive bladder cancer based on results from KEYNOTE-057. This was a multicenter, single-arm trial that enrolled 148 patients with high-risk NMIBC, 96 of whom had BCG-unresponsive CIS with or without papillary tumors. The CR rate in 96 patients with high-risk BCG-unresponsive NMIBC with CIS was 41% (95% CI: 31, 51), and median response duration was 16.2 months. Forty-six percent (46%) of responding patients experienced a CR lasting at least 12 months. 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 [143]. 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 [144].

2.3 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 [145, 146]. 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 [147]. Therefore, neoadjuvant chemotherapy followed by radical cystectomy is a category 1 recommendation for MIBC.

2.3.1 Neoadjuvant Immunotherapy in Cisplatin Ineligible Patients

Patients with hearing loss, neuropathy, poor performance status, or cardiac or renal insufficiency are typically deemed cisplatin-ineligible. It is estimated that 50% of patients are cisplatin-ineligible [148, 149]. Neoadjuvant therapy with anti-CTLA-4 showed a measurable immunologic effect, consisting of an increased frequency of CD4 + $ICOS^{hi}$ T cells in tumor tissues and the systemic circulation [150]. 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. 50 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 [151]. 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 [152].

Gao et al. reported findings from a trial that evaluated the combination of durvalumab and tremelimumab in cisplatin-ineligible patient with high-risk urothelial cancer. Primary endpoint was safe, and 6 out of 28 patients had grade 3 or higher immune-related AEs. In fact, 37.5% of patients had pathological CR and 58% had downstaging to pT1 or less at time of surgery [153]. The DUTRENEO study (NCT03472274) compared the durvalumab plus tremelimumab combination to cisplatin in the neoadjuvant setting for cisplatin eligible patients. In this study, tumors were categorized as "hot" or "cold" according to a tumor TIS determined by Nanostring technology. Patients randomized in the "hot" arms received standard neoadjuvant chemotherapy (n = 22) or combination of durvalumab plus tremelimumab (n = 23) and had a pCR rate of 8/22 pts. (36.4%) vs 8/23 pts. (34.8%), respectively. In the "cold" arm, 16 patients who received conventional chemotherapy obtained a pCR rate of 68.8% (11/16 pts). Grade 3–4 toxicities were more frequent in the CT arms. From this trial, it was concluded that the combination of durvalumab and tremelimumab is safe and active in MIBC patients in the neoadjuvant setting [154]. CPI plus cisplatin chemotherapy is also being investigated (NCT02690558).

2.3.2 Immunotherapy in Combination with Radiotherapy for Localized Bladder Cancer

Studies have assessed combined radiotherapy with CPIs alone for cisplatin ineligible MIBC. The DUART study is a phase 1b study of concurrent durvalumab and radiation therapy (DurvaRT) followed by adjuvant durvalumab. Six patients were enrolled and five patients completed the DurvaRT phase. Three out of four patients had ORR and one patient had progression. Overall, the combination of this immunotherapy agent and radiation therapy seems to be tolerated. Radiotherapy with CPIs plus chemotherapy for MIBC cisplatin eligible patients has also been explored. The ongoing ANZUP 1502 trial NCT02662062) reported interim results that indicate the combination of pembrolizumab and cisplatin with concurrent radiation seemed to be well-tolerated, and 9/10 patients achieved a complete cystoscopic response to treatment post CRT and were free of distant metastatic disease. The study 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 [155].

2.3.3 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. In this trial, median DFS was significantly longer for patients who received nivolumab after resection (21 months) compared to patients who received placebo (10.9 months). More treatment-related side effects (grade 3–4) were seen among patients who received nivolumab (17.9%) than in the placebo group (7.2%). Longer follow-up is needed for this trial [156]. The IMvigor010 (NCT02450331) trial did not meet the primary endpoint of DFS [157]. It is unclear why there is difference in between IMvigor010 and CheckMate274 results; this could be due to difference in the trial design vs the different mechanism of action given nivolumab is a PD-1 inhibitor and atezolizumab is a PDL-1 inhibitor. The AMBASSADOR trial (NCT03244384) is studying pembrolizumab in the adjuvant setting. Table 3 lists completed and ongoing phase 3 trials studying adjuvant checkpoint therapy for invasive UC. NIAGARA (NCT03732677) is a phase 3 study of neoadjuvant durvalumab + cisplatin-based chemotherapy followed by durvalumab adjuvant therapy.

2.3.4 Immunotherapy for Advanced Stage UC

To date, the US FDA has approved five CPI agents as a frontline or second-line treatment for

Table 3 Completed and ongoing phase 3 trials studying adjuvant checkpoint therapy for invasive UC

NCT identifier (trial)	Intervention	Phase	Population	Sample size	Results
NCT02632409 (CheckMate 274)	Nivolumab	3	Adjuvant therapy high-risk MIBC	709	NR
NCT02450331 (IMvigor010)	Atezolizumab	3	Adjuvant therapy high-risk MIBC	809	NR
NCT03244384 (AMBASSADOR)	Pembrolizumab	3	Adjuvant therapy high-risk MIBC and locally advanced UC	739	NR

MIBC muscle invasive bladder cancer, *NR* not reported

patients with advanced bladder cancer who are either ineligible or progressed after cisplatin [114–121].

2.3.5 Platinum Ineligible

Pembrolizumab

KEYNOTE-052 is the phase 2 trial that studied pembrolizumab as first-line treatment for cisplatin-ineligible patients with metastatic UC [120]. 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 of frontline pembrolizumab in metastatic UC. Arms of treatment are pembrolizumab monotherapy, pembrolizumab plus cisplatin-based chemotherapy, or chemotherapy alone [158, 159]. Cisplatin was replaced by carboplatin in cisplatin-ineligible patients. Based on KEYNOTE-052 results, the US FDA approved the use of pembrolizumab for the cisplatin-ineligible population in 2017. However, in June 2018, the FDA announced that treatment-naïve 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. 3).

Atezolizumab

The phase 2 IMvigor210 trial included two cohorts (treatment-naïve and previously treated patients). Cohort 1 studied atezolizumab in treatment-naïve cisplatin-ineligible metastatic UC patients [160]. 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-naïve patients to three arms: atezolizumab plus platinum-based chemotherapy, atezolizumab alone, and chemotherapy alone [161]. Stratification is similar to the IMvigor210 study. Similar to pembrolizumab, in June 2018, the FDA announced that treatment-naïve 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. 3).

2.3.6 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% [114–119].

Pembrolizumab

Pembrolizumab for UC was first studied in the phase 1b KEYNOTE-12 trial [162] which required ≥1% PD-L1 expression. ORR was 26% in unselected patients with good tolerance, i.e., only 15% with grade ≥ 3 AEs. The phase 3 KEYNOTE-45 compared pembrolizumab to second-line chemotherapy in platinum-refractory UC [121]. The 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 (in 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,

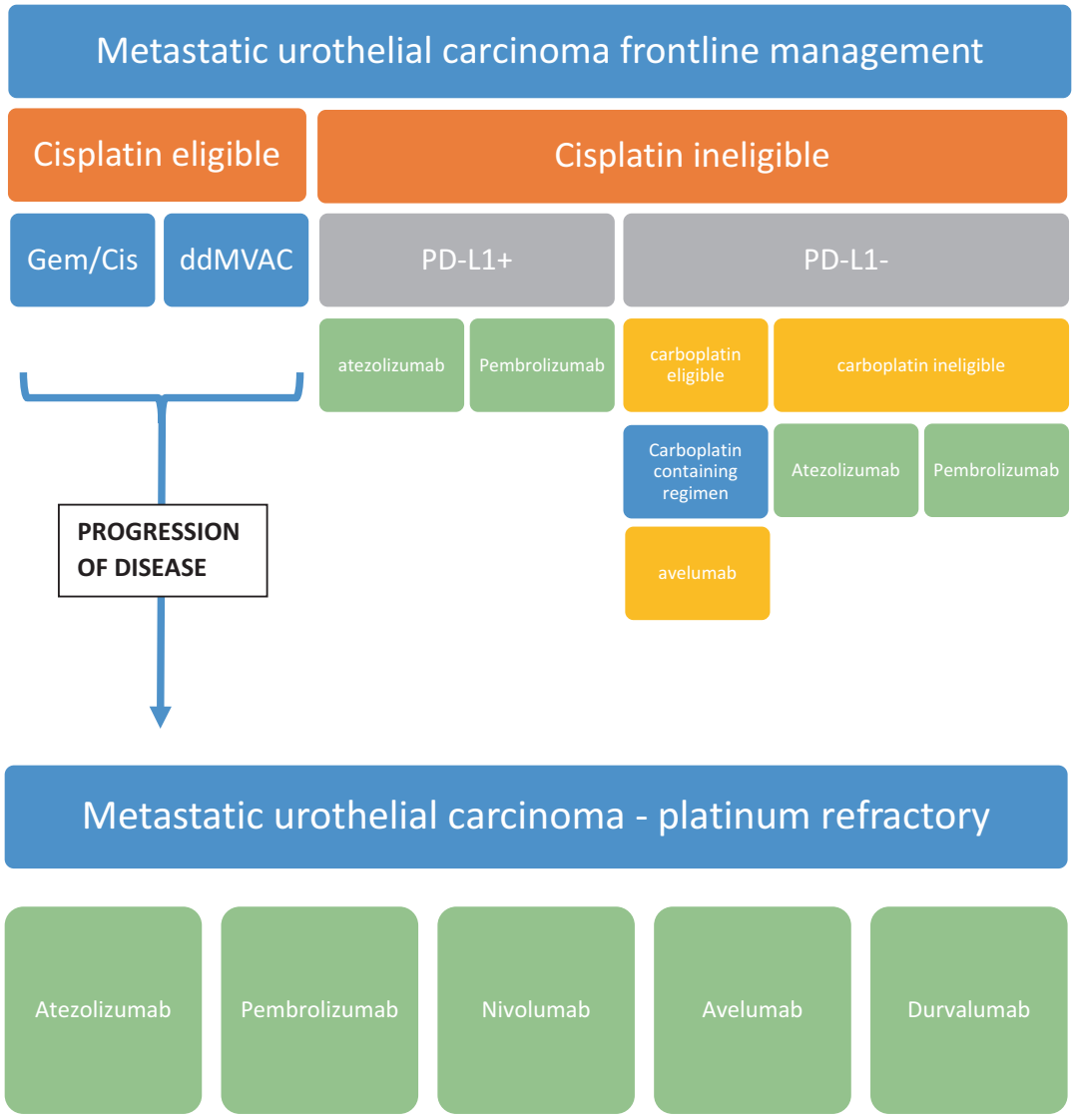

Fig. 3 Current treatment algorithm for metastatic urothelial carcinoma

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 that enrolled 68 patients with previously treated metastatic UC, atezolizumab had an ORR ranging from 11% to 43% [118]. 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 above-mentioned IMvigor210 study had an ORR in all-comers of 15% versus historical control of ORR with second-line cytotoxic chemotherapy of 10%. However, ORR was 27% in patients with IC2/3 and 18% for IC1/2/3 [116]. This provided the basis for the FDA to approve atezolizumab as second-line therapy 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 [152]. Nonetheless, atezolizumab is approved by the FDA for post-platinum therapy of metastatic UC based on improvement of ORR in comparison to historical rates for second-line chemotherapy.

Nivolumab

Nivolumab was first studied in 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) groups had similar responses (24% vs. 26%). However, the median OS was longer in patients with PD-L1 high vs. low tumor (16.2 months vs 9.9 months) [117]. CheckMate 275 was the phase 2 study to verify these findings [163]. 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, besides checkpoint inhibition, to lyse PD-L1-expressing tumor cells by an antibody-dependent cell-mediated cytotoxicity [164]. 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 was the focus of the JAVELIN Bladder 100 phase 3 trial (NCT02603432). Results from this trial were recently published and showed that maintenance avelumab compared to supportive care prolonged OS significantly. The median OS was 21.4 months in all patients who received avelumab and 14.3 months in the supportive care alone arm (HR: 0.69; 95%CI: 0.56, 0.86; $p = 0.001$). Among patients with PD-L1-positive tumors (51%), the HR for OS was 0.56 (95% CI: 0.40, 0.79; $p < 0.001$). Given this, the FDA granted accelerated approval for maintenance avelumab in June of 2020. 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 subgroup (defined as ≥25% of tumor cells or tumor-infiltrating immune cells) and 0% in the PD-L1-negative subgroup [165]. A phase 1/2 trial for metastatic UC patients followed, and 95.3% of enrolled patients had failed platinum therapy [115]. 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. As of February 2021, durvalumab was voluntarily withdrawn as an option for bladder cancer.

2.4 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 [131], worse clinical outcomes, and less postoperative survival [132]. 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 [116]. In contrast, the CheckMate 275 showed nivolumab responses irrespective of tumor PD-L1 expression [163]. Using PD-L1 as a predictive marker faces several challenges. 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 [116, 163, 165]. 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 [166]. In the CheckMate 275 trial, a 25-gene interferon-γ (IFN-γ) signature was associated with response PD-L1 expression [163]. Genomic defects in IFN-γ pathway genes are linked to anti-PD-1 and anti-CTLA-4 resistance [167–171]. An exploratory subgroup analysis of IMvigor210 Cohort II showed a significant increase in TMB in responding patients relative to non-responding patients (12.4 mutation/megabase vs. 6.4 mutation/megabase) [116]. 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 [127]. 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. The importance of finding biomarkers for CPIs is recognized as it will help with identifying the optimal patient who will respond the best with minimal toxicity to treatment. Currently, the patient's serum, tumor tissue, circulating DNA, and gut microbiome are sources of biomarkers. However, no single biomarker can help predict a patient's response to treatment or the toxicity that he or she may encounter and that combining multiple biomarkers may help develop a model [172].

2.5 Future Directions and Ongoing Trials

Although CPI offers an effective alternative option in a disease that has historically 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 elevated PD-L1 expression [173], and many patients in the front-line are also cisplatin ineligible [148]. Thus, additional therapies are necessary, and research is ongoing to investigate combinations of CPIs along with other agents that target the immune microenvironment [158].

2.5.1 Combination Anti-PD-L1 + Anti-CTLA4

DANUBE (NCT02516241) was a 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 was the primary endpoint for this three-arm trial. Unfortunately, this trial was negative for its co-primary endpoints of OS in comparing durvalumab monotherapy versus chemotherapy in the PD-L1 high patient population and the combination of durvalumab and tremelimumab versus chemotherapy in the

intention-to-treat population. However, secondary analyses suggested that the combination had improved antitumor activity, particularly in the group of patients with high PD-L1 expression [174]. As mentioned before, durvalumab was voluntarily withdrawn in February 2021. CheckMate 901 (NCT03036098) is a similar phase 3 trial evaluating nivolumab + ipilimumab and nivolumab + SOC chemotherapy vs SOC chemotherapy in treatment-naive patients with metastatic UC [175].

2.5.2 Combination CPI + Chemotherapy

Recently, several trials (summarized in Table 4) have attempted to address whether combination immunotherapy-chemotherapy will be more effective than immunotherapy alone in metastatic UC. The IMvigor130 study (NCT02807636) enrolled 451 patients with metastatic UC to be randomized into three groups: (A) atezolizumab in addition to platinum-based chemotherapy, (B) atezolizumab alone, and (C) placebo plus platinum-based chemotherapy. Median follow-up time for survival was 11.8 months for all patients. Co-primary efficacy endpoints were PFS and OS (group A vs group C) and OS (group B vs group C). The median PFS was statistically significantly longer in group A than group B (8.2 vs 6.3 months). Median OS did not differ significantly between group A and group C (16 vs 13.4 months). Also, median OS did not differ significantly between group B and group C (15.7 months vs 13.1 months). AEs leading to withdrawal of any agent occurred in 156 patients (34%) in group A, 22 (6%) in group B, and 132 (34%) in group C. In addition, 50 patients (11%) in group A, 21 (6%) in group B, and 27 (7%) in group C experienced AEs that led to discontinuation of atezolizumab or placebo.

KEYNOTE-361 looked at utilizing pembrolizumab +/− chemotherapy in advanced UC. Unfortunately, the addition of pembrolizumab to platinum-chemotherapy did not provide a statistically significant benefit for PFS or OS [176]. CheckMate 901 (NCT03036098) results are pending at this time [158, 159, 161, 175]. 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 [116]. 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 post-neoadjuvant chemotherapy specimens than in matched pre-neoadjuvant specimens, supporting this hypothesis [177].

2.5.3 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 (EV), an antibody-drug conjugate against nectin-4, which is highly expression on surface of UC cells, combined with pembrolizumab for cisplatin ineligible patients with locally advanced or metastatic UC was launched (NCT03288545). In this trial, 45 patients with metastatic urothelial cancer received EV + pembrolizumab. Most common treatment-emergent AEs were fatigue (58%, 11% ≥ G3), alopecia (53%), and peripheral sensory neuropathy (53%, 4% ≥ G3). With a median follow-up of 11.5 months, ORR was 73.3% (95% CI, 58.1, 85.4) including 15.6% CRs. The ORR in patients with available PD-L1 status was 78.6% in PD-L1 high (11/14) and 63.2% in PD-L1 low (12/19). The median PFS was 12.3 months (95% CI, 7.98, −). Recently, results from the phase 3 trial EV-301 were published; 608 patients were enrolled, one arm received EV, and another arm received investigator's choice of chemotherapy. OS was longer in the EV group than in the chemotherapy group (median OS, 12.88 vs. 8.97 months; HR for death, 0.70; 95% CI, 0.56–0.89; $P = 0.001$). PFS was also longer in the EV group than in the chemotherapy group (median PFS, 5.55 vs. 3.71 months; HR for progression or death, 0.62; 95% CI, 0.51–0.75; $P < 0.001$). The EV302 trial (NCT04223856) is a phase 3 trial evaluating the

Table 4 Completed and ongoing phase 3 studies assessing CPIs with chemotherapy combinations for treatment-naïve metastatic or unresectable UC

NCT identifier (trial)	Intervention	Comparator	Phase	Primary outcome	Results
NCT02853305 (KEYNOTE-361)	Pembrolizumab (P) plus cisplatin-based (C) chemotherapy or Pembrolizumab alone	Platinum-based chemotherapy	3	PFS, OS	Median PFS for P + C, P, and C for total patients was 8.3 months, 3.9 months, and 7.1 months, respectively; median OS was 17.0 months, 15.6 months, and 14.3 months, respectively. HR (95% CI) for P + C vs C was 0.78 (0.65–0.93, P = 0.0033) for PFS and 0.86 (0.72–1.02, P = 0.0407) for OS. ORR was 54.7% for P + C, 30.3% for P, and 44.9% for C
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

combination of EV + pembrolizumab versus standard of care gemcitabine + platinum-containing chemotherapy, in subjects with previously untreated locally advanced or metastatic urothelial cancer [178].

On April 12, 2019, the FDA granted erdafitinib approval for metastatic platinum-refractory UC with susceptible 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 of the combination of FGFR3 inhibitor vofatamab plus pembrolizumab in platinum refractory UC. Bintrafush alfa, or 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 3 TGFβ isoforms, covalently linked to the C-terminus of the heavy chain of the anti-PD-L1 antibody derived from avelumab [179]. 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 [180]. 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 [181].

2.5.4 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, the 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 study of T-cell receptor-transduced T cells targeting NY-ESO-1 for treatment of patients with NY-ESO-1-expressing malignancies.

2.6 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.

3 Immunotherapy for Prostate Cancer

Prostate cancer (PC) was expected to be the most common cancer diagnosed in men in 2019, accounting for nearly one in five new diagnoses. In the USA, it was estimated that PC would still be the second leading cause of death from cancer in men in 2019 [104]. PC deaths have been increasing from an estimated 26,739 in 2017 and 29,430 in 2018 to 31,620 in 2019 [182, 183]. Perhaps, this could be explained by the recommendations against screening and as a result an increased rate of distant metastases at diagnosis [184, 185]. Androgen deprivation therapy (ADT), commonly using medical castration, remains the current standard of care for the initial treatment of patients with metastatic PC [186]. In February 2018, the FDA approved abiraterone with prednisone to be added to ADT for newly diagnosed castration-sensitive PC (CSPC) per the LATITUDE trial [187, 188]. Additionally, chemotherapy (docetaxel) added to ADT (chemohormonal therapy) is also an option for metastatic CSPC based on the CHAARTED and

STAMPEDE phase 3 trials [189, 190]. The ENZAMET trial investigated the efficacy of enzalutamide with ADT in metastatic castrate-sensitive prostate cancer mCSPC [191], and the TITAN trial [192] demonstrated that the addition of apalutamide to lifelong ADT also improved OS in mCSPC; both phase 3 trials led to the approval of enzalutamide and apalutamide in the hormone-sensitive settings. Despite the effectiveness of the previously mentioned therapies, eventually, all CSPC patients will progress to castrate-resistant PC (CRPC) [187–190]. 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 [193]. 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. Currently approved therapies for metastatic CRPC (mCRPC) include abiraterone, enzalutamide, radium-223, sipuleucel-T, and chemotherapy including docetaxel and cabazitaxel (Fig. 4) [194–201]. For men with mCRPC, the median survival in recent phase 3 studies has ranged from 12.2 to 21.7 months [194–200]. The inevitable resistance to hormonal and chemotherapy indicates the need to develop novel therapeutic approaches [202] 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.

3.1 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) [203–205]. These proteins unique 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 allows the immune system time to mount a response [206, 207]. In fact, effector T cells responsive to PC TAAs have been identified in the peripheral blood of patients with PC, especially those with CRPC [208, 209]. Preclinical data showed that anti-prostate immune responses can exclusively target normal as well as cancerous prostate tissues without affecting other tissues that lack PC TAAs [210–212]. Additionally, histological evaluation of PC tissue has identified infiltrating CD4+ and CD8+ lymphocytes (TILs) that are oligoclonally expanded, suggesting that their presence is due to specific antigenic stimulation [213]. Treatment with ADT modulates the immune microenvironment by inducing infiltration of CD8+ TILs as well as CD68+ macrophages into prostate tumors [214, 215]. CD68+ macrophages seem to be associated with increased risk of biochemical recurrence [215] 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 [213]. 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 [216]. Based on these findings, effective immunotherapy strategies against PC, especially CRPC, have focused on training the immune system against PC TAAs (via therapeutic vaccines) [217] and antagonizing immune checkpoints.

3.2 Vaccines

"Vaccine" 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) [218]. Ex vivo processed vaccines are usually personalized (i.e., generated from the

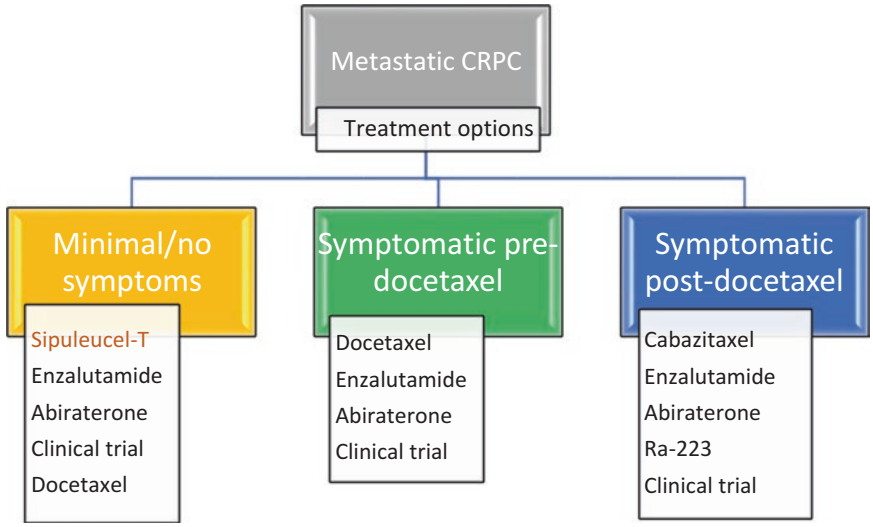

Fig. 4 Current treatment options for metastatic CRPC including the only approved immunotherapy sipuleucel-T

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) [219]. Several vaccines were developed to target PC, but failed to show clinical efficacy [220]. We will be discussing agents that have reached FDA approval or a late-stage clinical trial.

3.2.1 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. 5). D9901, 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 [221, 222]. 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 [221]. D9902B or the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial was a larger phase 3 trial that made OS its primary outcome. 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 [198]. Based on these findings, sipluleucel-T was the first anticancer immunotherapy to be approved by the FDA. Despite sipuleucel-T approval, the IMPACT study has been 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 [223]. However, these concerns were not credited during the FDA review due to the careful consideration given to the leukapheresis procedures in the placebo arm [224].

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) [225] 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 vs sequential enzalutamide with sipuleucel-T in metastatic CRPC, but is not sufficiently powered to assess differences in OS or PFS [226].

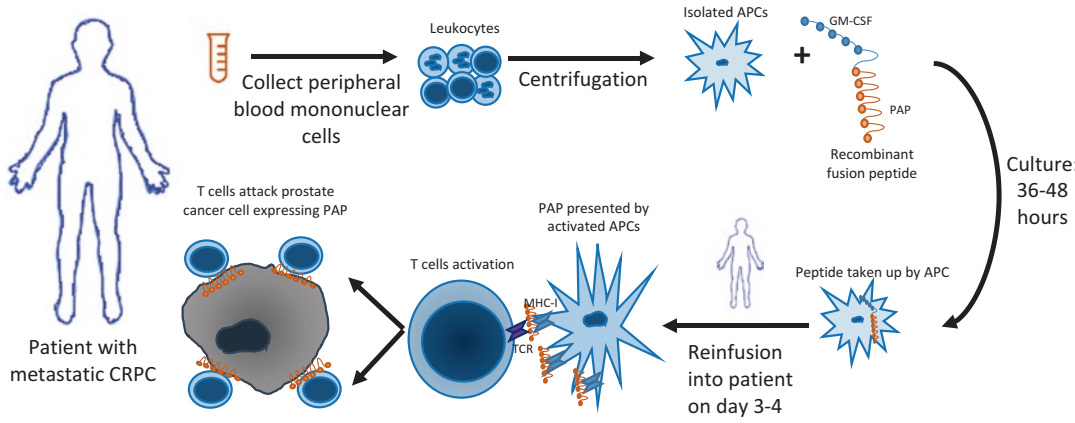

Fig. 5 The manufacturing process and proposed mechanism of action for sipuleucel-T

STAMP (NCT01487863) is a similar study to STRIDE using abiraterone instead of enzalutamide and is also not powered to report differences in clinical outcomes [227]. 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 vs. delayed addition of ipilimumab to sipuleucel-T and was 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 [228]. The absence of objective parameters to judge whether or not sipuleucel-T is benefitting patients poses a major difficulty in determining when to consider sipuleucel-T ineffective and switch treatment.

3.2.2 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 [229, 230]. However, phase 2 and phase 3 trials are so far not promising. NCT00771017, a phase 2 combination with ADT trial for non-metastatic 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 [220].

3.2.3 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 was not effective in inducing sufficient PSA-specific T-cell population [231, 232]. 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 costimulatory molecules (CD80, CD54, and CD58), hence the name PROSTVAC-VF/TRICOM. Despite showing 8.5 months OS benefit, the phase 2 trial with this vaccine failed to show PFS benefit in metastatic CRPC which was its primary endpoint [233]. Consequently, the phase 3 trial, PROSPECT, was conducted to further investigate these findings but failed to show a benefit in OS. In fact, the trial was stopped early after meeting criteria for futility [234, 235]. Nonetheless, combination trials with PROSTVAC-VF are underway. For example, in

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

3.3 CPIs

CPIs have revolutionized the management of solid tumors in the past few years [236, 237]. Unfortunately, CPIs have not been as successful in PC, perhaps due to its multifaceted and pleotropic immune tumor microenvironment [238]. 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 to improve outcomes [239] (Tables 5 and 6). AZD4635 has been shown to inhibit adenosine 2a receptor signaling and lead to improved immune activation and anti-tumor activity; given this, a phase 1 trial (NCT02740985) was conducted using AZD4635 as monotherapy and in combination with durvalumab in patients with solid tumors refractory to multiple lines of therapy. Thirty-eight adult subjects with advanced malignancies were treated with AZD4635 monotherapy (n = 15) or in combination with durvalumab (n = 23). Interestingly, three responses were seen in eight RECIST evaluable subjects with mCRPC; one had partial response treated with monotherapy AZD4635 and one had complete response and another had partial response treated in combination with durvalumab. A PSA decrease greater than 99% was observed on AZD4635 monotherapy in one of four RECIST non-evaluable mCRPC patients [240].

3.3.1 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 [241]. 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 [242]. NCT01057810 was the second phase 3 trial that randomized patients with chemotherapy-naive metastatic CRPC without visceral metastases to ipilimumab alone vs placebo [243]. In both studies, ipilimumab did not improve OS and when given alone 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 [244]. Another phase 2 trial evaluated ipilimumab combined with ADT early on for CSPC and established the safety of the combination [245]. A phase 1 trial combined ipilimumab with sipuleucel-T in metastatic castrate-resistant prostate cancer (mCRPC) and found the combination was well-tolerated [246]. Combination trials of ipilimumab with abiraterone (NCT01688492) and ADT (NCT01194271, NCT01377389, NCT00170157) are ongoing.

3.3.2 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) [247]. 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). 131 patients had measurable PD-L1+ disease (C1), 67 patients had measurable PD-L1- disease (C2), and 60 patients had nonmeasurable, bone-predominant

Table 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-naïve 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 pts. DCR ≥ 6 mos: 29%. ORR: 7% (28 evaluable pts) [275]
			Cohort C: Pembrolizumab + Enzalutamide	Post abiraterone acetate but pre-chemotherapy mCRPC	70	Enrolled 69 pts. DCR ≥ 6 mos: 33%. ORR: 20% (25 evaluable patient) [256]
			Cohort D: Pembrolizumab + Abiraterone + prednisone	Post enzalutamide acetate but pre-chemotherapy mCRPC	70	NR
NCT02787005 (KEYNOTE-199: cohort 4 and 5)	2	Primary: ORR Secondary: DCR, PSA RR, AEs, discontinuation rate	Pembrolizumab + enzalutamide	Chemotherapy-naïve mCRPC	370 (for all cohorts)	Results for single-agent pembrolizumab in post-docetaxel cohorts 1, 2, and3 show ORR 3–5% [248]
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 pre-chemotherapy 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	Enrolled 759 pts. median OS 15.2 months in atezo+enza group and 16.6 months in enza group
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 pts. enrolled. 47% had PSA responses >50% [265]

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, *pts.* patients, *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 Selected combination trials of vaccines with checkpoint inhibitors for prostate cancer

NCT identifier (trial)	Phase	Status	Outcome measures	Intervention	Population	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-naïve mCRPC	29	NR
NCT03315871	2	Recruiting	Primary: PSA RR Secondary: AEs, PSA slope over time	PROSTVAC-VF +Avelumab linked with TGFbeta-Trap molecule	Biochemically recurrent prostate cancer	34	NR
NCT03532217	1	Active, not 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-naïve mCRPC	Actual enrollment of 9	Increased PAP/PA2024-specific immune response [246]
NCT01804465	2	Completed	Primary: PAP/PA2024-specific immune response. Secondary: PSA, radiographic, clinical response, T-cell activity	Sipuleucel-T + immediate or delayed ipilimumab	Chemotherapy-naïve 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, *pts.* patients

disease (C3). Chemotherapy-naïve 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 mo 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) [248]. 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, three experienced a biochemical response and 2 a radiological response. Genetic analysis revealed markers of MSI in one patient [249]. MSI has been shown to be a predictive factor for response to pembrolizumab [250].

Pembrolizumab in High MSI

The prevalence of MMR deficiency in metastatic CRPC is estimated at 2–5% [251, 252]. In one series from MSKCC, 20/839 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 13/20 MSI-H patients who consented to germline analysis, 3/13 (23%) had a germline MMR gene mutation. In total, 10 patients with MSI-H tumors received a PD-1/PDL-1 targeting agent. 5/10 had radiographic PR or PSA decline of >60%. 1/10 had SD for 6 months. 4/10 had no response or were inevaluable [253]. 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.

3.3.3 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 study [254]. Asymptomatic/minimally symptomatic patients with mCRPC were divided into pre-taxane therapy (cohort 1) and after taxane (cohort 2). Treatment was nivolumab 1 mg/kg + ipilimumab 3 mg/kg Q3W for 4 doses and then nivolumab 480 mg every 4 weeks. Co-primary endpoints were ORR and radiographic PFS per PC working group 2 [255]. 62 patients were enrolled, and ORR was 26% and 10% in cohorts 1 and 2, respectively. Higher activity in the chemotherapy-naïve cohort is consistent with data from other immunotherapy modalities such as sipuleucel-T. In both cohorts, ORR was higher in pts. 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.

3.3.4 CPIs Plus Enzalutamide

KEYNOTE-365 is a phase 1b/2 umbrella trial [256] that is based on the activity seen with pembrolizumab in KEYNOTE-199 and following reports of adding enzalutamide [248, 249]. 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). Updates on cohorts A, B, and C have been released and report clinical activity with each combination with pembrolizumab. PSA response was observed in 9% of cohort A, 28% of cohort B, and 22% of patients in cohort C [256–258]. CheckMate 9KD (NCT03338790) is another phase 2 umbrella trial evaluating nivolumab in combination with either rucaparib (PARP inhibitor), docetaxel, or enzalutamide [239]. So far, the combination of nivolumab + docetaxel exhibited clinical activity with confirmed ORR of 36.8% and confirmed PSA response rate of 46.3% as per the initial analysis [259]. With regard to chemotherapy, KEYNOTE 921 (NCT03834506) will be investigating pembrolizumab (MK-3475) and docetaxel in the treatment of men with metastatic CRPC

who have not received chemotherapy for mCRPC but have progressed on or are intolerant to next-generation hormonal agent (NHA). Otherwise, there are a few ongoing trials also combining pembrolizumab with enzalutamide. KEYNOTE-991 (NCT04191096) will be studying the safety and efficacy of pembrolizumab and enzalutamide with ADT in metastatic CSPC compared to placebo and enzalutamide with ADT and is currently ongoing. KEYNOTE-641 (NCT03834493) is a phase 3 trial evaluating the efficacy and safety of pembrolizumab and enzalutamide versus placebo and enzalutamide in patients with castrate-resistant disease, on the other hand. IMbassador 250 (NCT03016312) is a phase 3 multicenter trial evaluating atezolizumab with enzalutamide vs enzalutamide alone for CRPC [260]. This combination did not show an OS improvement vs enzalutamide alone, and the study was terminated [261].

3.4 Other Ongoing Immunotherapeutic Trials in PC

3.4.1 CPIs Plus PARP Inhibitors

Data suggests 25–30% of sporadic mCRPC patients have somatic or germline defects in DNA repair pathways that may confer sensitivity to PARP inhibition (PARPi) [193]. Data from the above-mentioned CheckMate 650, KEYNOTE-199 and other reports suggest there may be improved activity in CRPC with DDR mutations when treated with CPIs [248, 254, 262]. NCT02484404 is phase 1/2 trial based on the hypothesis that increased DNA damage by olaparib will complement anti-tumor 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 [263]. Among 17 treated CRPC patients, 8 (47%) had PSA responses >50%. Six of the eight responders had mutations in the DDR pathways [264, 265]. This was the first study to demonstrate activity for the PARPi+CPI combination in PC patients without having 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 studies are aiming to further address this question.

3.4.2 PSMA Radioligand Therapy and Combinations with Immunotherapy

PSMA is upregulated in dedifferentiated and CRPC making it an attractive target for therapy [266]. ^{177}Lu-PSMA-617 is composed of the therapeutic radionuclide Lutetium-177 attached to a 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 [267]. 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 USA.

A phase 2 trial, TheraP, in Australia (NCT03392428) aimed to compare ^{177}Lu-PSMA-617 versus cabazitaxel in patients with metastatic CRPC. 65% PSA responses were seen in men in the ^{177}Lu-PSMA-617 group compared to the cabazitaxel group, which was 37% by intention to treat (95% CI 16–42; $p < 0{\cdot}0001$). With regard to AEs, 32 (33%) of 98 men in the ^{177}Lu-PSMA-617 group versus 45 (53%) of 85 men in the cabazitaxel group experienced grade 3–4 AEs. Given this, ^{177}Lu-PSMA-617 could be a potential alternative to cabazitaxel in a heavily pretreated population. The secondary findings from this trial were recently reported. The 1-year PFS rate was significantly better in the LuPSMA arm than in the cabazitaxel arm, at 19% and 3%, respectively, and an HR of 0.63. Interestingly, the median PFS was 5.1 months in both arms [268].

Also conducted in Australia, the UpFrontP SMA trial is a phase 2 trial determined to evaluate the activity and safety of ^{177}Lu-PSMA-617 followed by docetaxel in newly diagnosed metastatic mCSPC.

3.4.3 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 pre-metastatic niche and, as a result, reduce the chance of developing cancer metastasis [269].

3.5 Chimeric Antigen Receptor and Bispecific T-Cell Engager

In CRPC, two groups reported developing a CAR construct targeting PSMA [270, 271]. 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 human PC mouse models [272]. 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 [273].

T-cell redirection can be achieved via CAR-T as well as by a bispecific T-cell engager, or BiTE®. A phase 1 trial was designed to study AMG 160, a half-life extended (HLE) bispecific T-cell engager (BiTE®) antibody construct, alone and in combination with pembrolizumab for patients with metastatic CRPC that is heavily pretreated. Results were recently reported, and as of July 20, 2020, 43 patients had received at least 1 dose of AMG 160 at 6 dose levels, and 19 patients (44.2%) were still on treatment for more than 6 months. As far as safety profile, cytokine release syndrome was the most common AE ($n = 30$, 90.7% all grade; $n = 11$, 25.6% grade 3) but was reversible and manageable and most severe in cycle 1. There were no grade 5 TRAEs or discontinuation of treatment.

68.6% of patients showed any PSA decline across all monotherapy dose cohorts, 34.3% had a PSA reduction >50%, and among 15 patients with measurable disease, there were 3 partial responses and 8 patients with stable disease. The maximum tolerated dose has yet to be established. AMG 160 in combination with pembrolizumab is also being studied (NCT03792841) [274].

3.6 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 [198]. 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). 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.

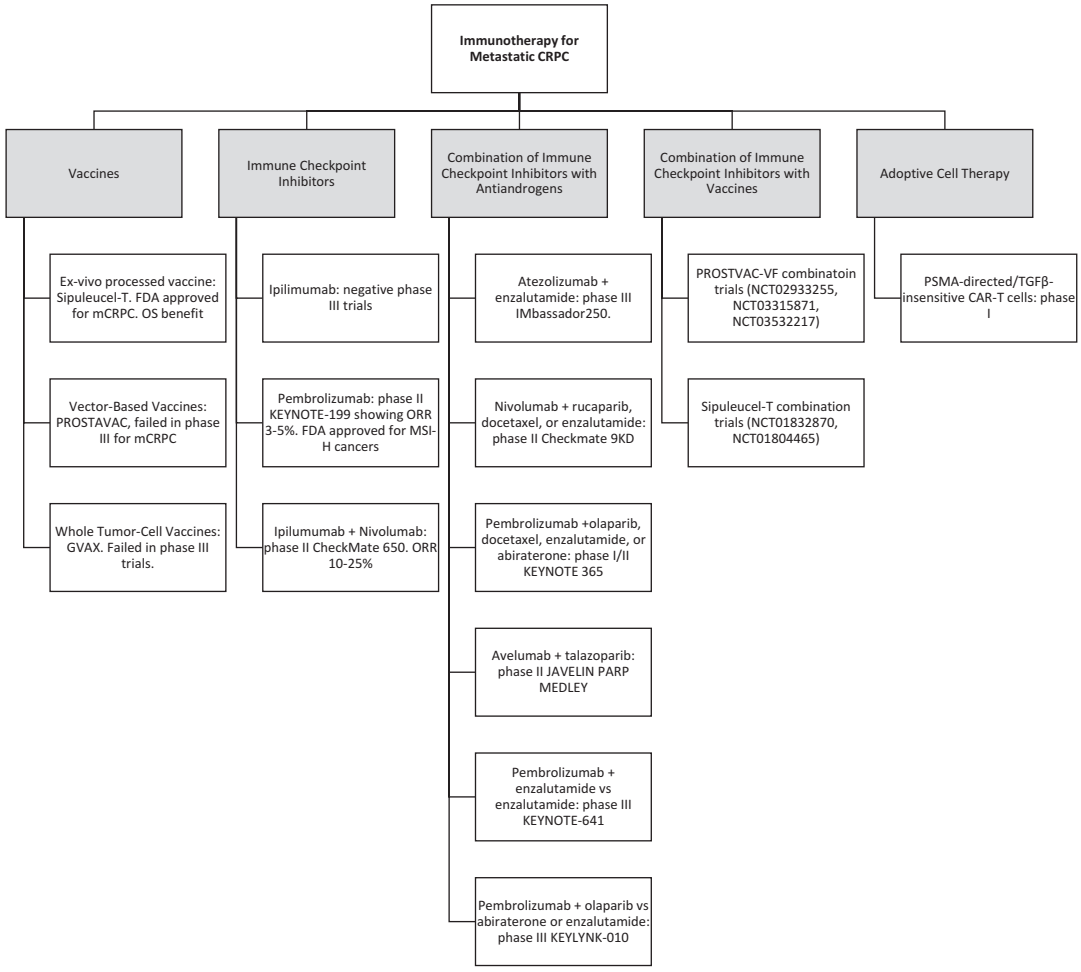

Fig. 6 Selected categories of current immunotherapy landscape for prostate cancer. Sipuleucel-T remains the only FDA-approved agent

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Immuno-Oncology for Gynecologic Malignancies

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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 instability-high cancers, cancers with high tumor mutational burden, 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 will review 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

1 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.

1.1 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, etc.), anti-PD-1 antibodies (e.g., pembrolizumab, nivolumab, dostarlimab, etc.), and/or anti-PD-L1 antibodies (e.g., durvalumab, avelumab, atezolizumab, etc.) serves as potential therapeutic options to augment the antitumor activity of adaptive immunity.

1.2 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 premalignant 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 lessen 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 vaccine 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].

1.3 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 toward tumor associated antigens), genetically engineered TCR and CAR T cells are T-lymphocyte populations modified with the same high-affinity tumor recognition moiety that is 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, and 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 of TCR T cells is their utility is limited to patients with common HLA types (typically HLA-A*0201) used in engineering the TCR. Another limitation is the possibility of tumors downregulating 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.

2 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), and 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. Defect of MMR function results 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 non-polyposis 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].

2.1 Immune Checkpoint Inhibitors in Endometrial Cancer

2.1.1 MSI-H Tumors

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 2 study of MMR-deficient (dMMR) colorectal cancers and non-colorectal solid tumors and MMR-proficient (pMMR) 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 pMMR tumors demonstrated no responses [24]. Although this cohort predominantly consisted of colorectal cancer patients, there were two dMMR 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 dMMR cancer types who had progressive disease on at least one prior treatment (Table 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, 26]. In a phase II basket trial of MSI-H/dMMR tumors, KEYNOTE-158 reported a 57.1% ORR (28 of 49; 8 complete and 20 partial responses) in advanced MSI-H endometrial cancer patients who failed prior systemic therapy. Additionally, the median duration of response that was not reached (NR) (95% CI 2.9 to 27.0+ months) [27]. Pembrolizumab had an impressive, favorable impact on survival outcomes. The median PFS was 25.7 months (95% CI 4.9 to NR), and the median overall survival (OS) was NR (95% CI 27.2 to NR). Given its clinical efficacy, pembrolizumab was awarded United States Food and Drug Association (FDA) – accelerated approval for the use in treatment of MSI-H/dMMR 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) [28]. Tamura and colleagues found an overall ORR of 23% in 23 uterine cancer patients with acceptable drug safety profile. ORR was similar regardless of the presence or absence of PD-L1 expression (25% vs. 21.4%, respectively) [28]. MSI testing was performed in 8 patients, and the ORRs for MSI-H and MSI-L tumors were 100% (2 of 2 had partial responses) and 0% (0 of 6), respectively. In the NCI-MATCH trial, patients with relapsed or refractory non-colorectal tumors were screened for MMR-deficiency by immunohistochemistry and administered IV nivolumab for the primary endpoint of ORR [29]. For the evaluable patients in the endometrial tumor cohort (n = 14), the ORR was 42.9% (4 partial and 2 complete responses) with a disease control rate of 64.3% (9 of 14) [29].

Dostarlimab is another PD-1 inhibitor that has been investigated in endometrial cancer and was evaluated in the GARNET study. In this phase 1b/II trial, the investigators administered dostarlimab at 500 mg IV every 3 weeks for the first 4 cycles and then 1000 mg IV every 6 weeks across multiple tumor types, including dMMR endometrial cancer (n = 104) [30]. Among the evaluable dMMR recurrent/advanced endometrial cancer patients, the ORR was 42.3% (30 of 71) with 21 partial (29.6%) and 9 (12.7%) complete responses; the median duration of response (DOR) was NR [30]. The most frequent treatment-related adverse events (TRAEs) were asthenia, diarrhea, fatigue, and nausea. The TRAE rate for grade 3 or higher was 11.5% with anemia being most commonly reported (2.9%) [30].

PD-L1 inhibitors have also demonstrated favorable activity in dMMR/MSI-H endometrial

Table 1 Reported immune checkpoint inhibitors trials in endometrial cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
PD-1 inhibitors						
Ott et al. 2017 [36]	Phase 1B	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)
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[a]: 74% (mainly rash/pruritus, fatigue, diarrhea/colitis). Grade 3–4: 20% (diarrhea/colitis, pancreatitis, hyperamylasemia)
Marabelle et al. 2019 [27]	Phase II	49	MSI-H endometrial cancer with progression on prior systemic therapy	Pembrolizumab 200 mg IV q3 weeks	ORR 57.1% (20 PR/8 CR) mPFS 25.7 months (95% CI 4.9 to NR) mOS NR (95% CI 27.2 to NR)	Overall[a]: 64.8% (mainly fatigue, pruritus, diarrhea, and asthenia) with 15% grade 3–5 TRAE (there was 1 grade 5 TRAE and was a treatment-related pneumonia)
Tamura et al. 2019 [28]	Phase II	23	Advanced/recurrent EC	Nivolumab 240 mg IV q2 weeks	Overall: ORR 23% (similar regardless of PD-L1 status), mPFS 3.4 mo, mOS 8.7 mo MSI-H: ORR 100% (2 of 2), mPFS NR MSS: ORR 0% (0 of 6), mPFS 2.2 mo	Overall[a]: 56.3%, grade 3–4 toxicities: 12.5% (mainly pruritus, increased lipase, diarrhea)
Azad et al. 2020 [29]	Phase II	14	Recurrent or refractory MMR-deficient endometrial cancer	Nivolumab 3 mg/kg IV q2 weeks (28 day cycles) followed by 480 mg IV q4 weeks after cycle 4	ORR 42.9% (4 PR/1 CR) DCR 64.3%	Mainly mild toxicities (most commonly fatigue, anemia, rash, and hypoalbuminemia). Grade 4 toxicities in 2 patients (sepsis and pneumonitis)
Marabelle et al. 2020 [33]	Phase II	82	Advanced EC with progression on prior systemic treatment	Pembrolizumab 200 mg IV q3 weeks	ORR 46.7% (7 of 15) in TMB-H tumors and 6% (4 of 67) in TMB-L tumors	Overall[a]: 15% grade 3–5 TRAE (colitis was the only AE that occurred in more than 1 patient; there was 1 grade 5 TRAE and was a treatment-related pneumonia). Any irAE = 16% (most commonly hypothyroidism)

(continued)

Table 1 (continued)

Study	Design	N	Patient population	Therapy	Results	TRAE
Oaknin et al. 2020 [30]	Phase Ib/II	104	dMMR recurrent or advanced endometrial cancer	Dostarlimab 500 mg IV q3 weeks for 4 cycles then 1000 mg IV q6 weeks	ORR 42.3% (21 PR/ 9 CR) DCR 57.7% mDOR NR mPFS 8.1 months (95% CI 3–18 months) mOS NR	Most TRAEs were grade 1 (most commonly asthenia, diarrhea, fatigue, and nausea). Grade 3+ TRAE was 11.5% (most commonly anemia)
Oaknin et al. 2020 [38]	Phase I/II	142	Recurrent or persistent EC (MMR proficient cohort)	Dostarlimab 500 mg IV q3 weeks for 4 cycles then 1000 mg IV q6 weeks	ORR 13.4% (16 PR / 3 CR) in MSS). DCR 50%, 18 month DOR 61.3%	Most common grade 3+ TRAEs were anemia (12.2%), abdominal pain (4.8%), and dyspnea (4.1%)
PD-L1 inhibitors						
Antill et al. 2019 [31]	Phase II	71	Advanced EC that progressed on prior systemic therapy	Durvalumab 1500 mg IV every 4 weeks	*dMMR EC (n = 35)* ORR 40% (10 PR/4 CR) DCR 60% *pMMR EC (n = 36)* ORR 3% (1 PR/0 CR) DCR 19%	IrAEs occurred in 14 patients (most commonly hyper−/hypothyroidism)
Liu et al. 2019 [38]	Phase I	15	Recurrent uterine cancer	Atezolizumab 15 mg/kg or 1200 mg every 3 weeks	ORR 13.3% (2 PR/0 CR) DCR 26.7%	Overall: TRAE in 46.7% (mainly grade 1–2 with the most common TRAEs being diarrhea and fatigue)
Konstantinopoulos et al. 2019 [32]	Phase II	31	Recurrent or persistent EC stratified by mutational status Hypermutated: dMMR/ polymerase ε (POLE) mutant Hypomutated: Non-dMMR	Avelumab 10 mg/kg IV every 2 weeks	*Hypermutated (n = 12)* ORR:33.3% (3 PR, 1 CR), DCR: 66.7%, 6-month PFS rate: 40%, mPFS 4.4 months, mOS NR *Hypomutated (n = 14)* ORR: 7.1% (1 PR), DCR: 35.7%, 6-month PFS rate: 6.3%, mPFS 1.9 months, mOS 6.6 months	Overall: TRAE occurred in 71% with grade 3 TRAEs in 19.4%

Combination therapy						
Makker et al. 2020 [40]	Phase II	124	Metastatic endometrial cancer	Pembrolizumab 200 mg IV q3 weeks and Lenvatinib 20 mg po qday	ORR 38.9% (overall previously treated; 34 PR/8 CR), 37.2% (MSS), 63.6% (MSI-H) DCR 84.3% (overall), 84% (MSS), 90.9% (MSI-H) mPFS 7.4 months and mOS 16.7 months	Overall: 96.8% (common: Hypertension, diarrhea, fatigue, hypothyroidism), grade 3: 68%, with 2 treatment-related deaths due to sepsis and intracranial hemorrhage. Majority required lenvatinib dose reduction (62.9%) and interruption (70.2%)
Rubinstein et al. 2019 [41]	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, *DOR* duration of response, *irAE* immune-related adverse event, *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, *NR* not reached, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *PO* oral, *q* every, *PR* partial response, *TMB-H* high tumor mutational burden, *TMB-L* low tumor mutational burden, *TRAE* treatment related adverse events. 95% CI = 95% confidence interval

[a]includes other non-endometrial cancers

tumors. In the preliminary results of a phase II PHAEDRA trial, the investigators administered durvalumab 1500 mg IV every 4 weeks to advanced endometrial cancer patients that progressed on prior systemic therapy (n = 71) [31]. Among the dMMR endometrial cancer patients, the ORR was 40% (14/35) with 10 partial and 4 complete responses with favorable safety profile [31]. In another PD-L1 inhibitor trial, Konstantinopoulos et al. administered avelumab 10 mg/kg IV every 2 weeks to two cohort endometrial cancers stratified by mutational profile: 1) hypermutated (dMMR/polymerase ε (POLE) mutant (n = 15)) and 2) hypomutated (non-dMMR (n = 16)) [32]. In the 12 evaluable patients in the hypermutated cohort, there were no POLE mutations, and the ORR was 33.3% (3 partial and 1 complete response). Furthermore, the 6-month PFS rate was 40% with responders having negative PD-L1 expression and ongoing response by the data cutoff date [32]. In contrast, avelumab was observed to have poor activity in the non-dMMR cohort with an ORR of 7.1% and a 6-month PFS rate of 6.3% [32].

2.1.2 TMB-H Tumors

Similar to MSI status, tumor mutational burden (TMB) demonstrates potential as a biomarker of response for PD-1 inhibitors [33–35]. TMB is defined as the total number of somatic mutations per coding area in the tumor genome with high TMB (TMB-H) generally defined as tumors with ≥10 mutations/megabase [33, 35]. Compared to tumors with low TMB (TMB-L), tumors with TMB-H are postulated to produce greater numbers of neoantigens and thereby generate a stronger response to immune checkpoint inhibitors across a diverse number of tumor types [33–35]. In a prospective exploratory analysis of the KEYNOTE-158 trial, endometrial cancer patients with TMB-H (n = 15) had improved response to pembrolizumab compared to those with TMB-L (n = 67) (ORR 46.7% vs. 6%, respectively) [33]. It should be noted that 10 of the 15 endometrial tumors with TMB-H also were MSI-H, while there were no MSI-H tumors in the TMB-L cohort [33]. In a retrospective study performed at the Memorial Sloan Kettering Cancer Center, Valero et al. correlated TMB with response to immune checkpoint inhibitors in patients with MSS solid tumors and who received treatment with PD-1/PD-L1 monotherapy or combination therapy [34]. In the endometrial cohort, the TMB-H and TMB-L tumors had an ORR of 66.7% (2 of 3) and 20.5% (9 of 44), respectively [34]. In June 2020, the FDA granted accelerated approval to pembrolizumab in the treatment of unresectable or metastatic TMB-H solid tumors that have progressed on prior therapy.

2.1.3 MSS Tumors

For MSS tumors, monotherapy immune checkpoint has shown more limited benefit. 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 [36]. 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 [36]. The ORR was 12.5% (n = 3; all partial responses) with a DCR of 25% (n = 6) [36]. 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, and they were predominantly MSS; the sole patient with an MSI-H tumor had progressive disease [36]. One of the three patients with a partial response was found to have a POLE mutant tumor [36]. 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, 37]. 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-H and MSS endometrioid tumors [18, 22, 23, 37]. Other PD-1/PD-L1 trials have demonstrated similar ORRs among MSS tumors. In a trial by Tamura et al., nivolumab had an ORR of 0% (0 of 6) among MSS tumors [28]. Avelumab, durvalumab (PD-L1 inhibitor), and dostarlimab have shown ORRs of 7.1%, 3%, and 13.4%, respectively,

among pMMR tumors [31, 32, 38]. In a phase I study by Liu et al., atezolizumab (PD-L1 inhibitor) demonstrated an overall ORR of 13.3% in a predominantly MSS uterine cancer population 2 partial responses, each in a MSI-H and MSS patient [39].

Although immune checkpoint inhibitor monotherapy has been limited in MSS tumors, the combination of immune checkpoint inhibitors and multi-tyrosine kinase inhibitors has been reported to result in substantially higher response rates. In a phase Ib/II study (KEYNOTE-146/Study 111), lenvatinib (20 mg po daily) (inhibitor of vascular endothelial growth factor 1–3, fibroblast growth factor receptor 1–4, and other kinases) and pembrolizumab (200 mg IV every 3 weeks) were administered in advanced endometrial cancer patients with predominantly MSS tumors (85%) [40]. Among 108 evaluable patients, the overall ORR was 38.9% (8 complete responses and 34 partial responses) and DCR was 84.3% [40]. Remarkably, this regimen had efficacy in serous histologies as well with an ORR of 42.4% [40]. Based on efficacy results, pembrolizumab and lenvatinib therapy was given accelerated FDA approval for use in non-MSI-H/dMMR advanced endometrial cancer that failed at least 1 prior line of systemic therapy in September 2019. Although impressive tumor responses were seen, toxicity was significant with a grade 3–4 TRAE rate of 66.9% (most common being hypertension, fatigue, and diarrhea) [40]. There were two deaths related to TRAE (sepsis and intracranial hemorrhage) [40]. There were 17.7% of patients who discontinued treatment due to toxicity (mainly related to lenvatinib), and the majority of patients had lenvatinib-dose interruptions (70.2%) [40]. Despite the combination regimen receiving accelerated FDA approval with lenvatinib dosing at 20 mg/daily, the majority of patients had lenvatinib dose reductions (62.9%), and the mean lenvatinib dose intensity was 14.4 mg/daily [40]. The combination of pembrolizumab and lenvatinib provides a promising alternative for treatment of recurrent endometrial cancer, but it remains to be seen the tolerability and feasibility of this regimen in clinical practice. Currently, a phase 3 trial investigating lenvatinib/pembrolizumab vs. physician's choice is underway (NCT03517449).

At the 2019 American Society of Clinical Oncologists Meeting, the preliminary results of a phase II trial of durvalumab with or without tremelimumab (CTLA-4 inhibitor) in persistent/recurrent endometrial cancer were presented (NCT03015129) [41]. 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 [41]. 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 [41]. Grade 3 and 4 TRAE were 7% and 4% in the monotherapy group and 32% and 11% in the combination group, respectively [41].

There are numerous ongoing clinical trials of combination therapy with immune checkpoint inhibitors, and these include but are not limited to the following:

- KEYNOTE-775 (NCT03517449): phase III trial of pembrolizumab and lenvatinib vs. physician's choice
- LEAP-001 (NCT03884101): phase III trial of pembrolizumab and lenvatinib vs. carboplatin and paclitaxel
- RUBY (NCT03981796): phase III trial of carboplatin, paclitaxel, and dostarlimab vs. carboplatin, paclitaxel, and placebo
- AtTEnd (NCT03603184): phase III trial of carboplatin, paclitaxel, and atezolizumab vs. carboplatin, paclitaxel, and placebo
- NRG-GY018 (NCT02549209): phase II trial of carboplatin/paclitaxel plus pembrolizumab
- DOMEC (NCT03951415): phase II trial of durvalumab plus olaparib
- EndoBARR (NCT03694262): phase II trial of rucaparib, bevacizumab, and atezolizumab

2.2 Vaccines in Endometrial Cancer

One of the identified tumor-associated antigens that has been utilized, as a target for therapeutic

vaccinations, is a product of the Wilm's tumor gene: WT1 [42, 43]. 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 [43]. 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 2) [43]. 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 [43]. In another phase I/II study, a mixed cohort of end-stage serous endometrial carcinoma (n = 3) and leiomyosarcoma (n = 3) patients received 4 weekly vaccines of autologous dendritic cells electroporated with WT1 mRNA [44]. Although all three serous endometrial carcinoma patients (two HLA-A2 positive and one HLA-A2 negative) 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 [44]. 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) [44].

Another targeted epitope is associated with NY-ESO-1, which is classified as a "cancer-germ line 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 [45]. 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 [45].

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 [46]. 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 [46]. Of the 24 patients enrolled, two endometrial cancer patients had received the vaccines after 2 failed chemotherapy treatments with one of the patients demonstrating high antibody production and partial response [46].

Folate binding protein (FBP) is another immunogenic protein overexpressed in endometrial (as well as ovarian) cancer [47]. In a phase I/IIa trial by Brown and colleagues, a very heterogeneous cohort of 51 patients with endometrial or ovarian cancer who all had no evidence of disease in either the frontline or recurrent setting who received an HLA-A2-restricted, FBP-derived E39 peptide vaccine +/− booster inoculations to prevent recurrence [48]. Overall, the vaccine was well tolerated, and the disease-free survival (DFS) was improved in the higher dosage vaccine group (1000 mcg) compared to the lower dosage vaccine (<1000 mcg) or control group (77.9% vs 31.2% vs 40%; p = 0.013) [48]. Other factors associated with decreased risk of recurrence included use of booster inoculations, vaccination in frontline setting, and low FBP expression in tumors [48].

2.3 ACT in Endometrial Cancer

There are few reported studies discussing TIL, TCR-T, or CAR-T therapy in endometrial cancer. In a phase I trial by Qiao et al., the investigators administered several therapeutic options (hyperthermia + ACT +/− pembrolizumab +/− chemotherapy) to a heterogeneous group of solid tumors that failed prior therapy [49]. With the ACT, mononuclear cells were collected from the peripheral blood, and the cultured cytokine-induced mix of T and natural killer immune effector cells was infused back into the patient [49]. In the endometrial cohort (n = 5), there was 1 patient with a partial response and 2 patients with stable disease [49]. Overall, the majority of toxicities were associated with grade 1 or 2 and chemotherapy [49]. Another ACT therapeutic option involves lymphokine-activated killer

Table 2 Reported vaccine therapy trials in endometrial cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
Jager et al. 2006 [44]	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 two 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 [45]	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 x 3, additional vaccinations given later based on if there were toxicity	ORR 50% (1 PR / 0 CR), DCR = 50%	Grade 3[a]: 12.5%, (diarrhea, pain, hyperglycemia)
Ohno et al. 2009 [42]	Phase II	2	HLA-A[a]2402-positive endometrioid adenocarcinoma and carcinosarcoma resistant to standard therapy	Intradermal injections of 3.0 mg of HLA-A[a]2402-restricted adjuvant modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant administered qweek for 12 weeks	ORR 0%, DCR 0%	Mild erythema at injection site with no grade 3–4 toxicities
Coosemans et al. 2013 [43]	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

(continued)

Table 2 (continued)

Study	Design	N	Patient population	Therapy	Results	TRAE
Brown et al. 2019 [47]	Phase I / IIa	Treatment group (n = 29) Controls (n = 22)	Endometrial and ovarian cancer patients at risk of recurrence in the frontline or recurrent setting	HLA-A2 restricted, FBP-E39 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 monthly for 6 doses. Within the treatment group, patients were randomized to receive E39 or E39' booster inoculations	2-year DFS rate 55.5% (vaccine group) vs. 40% (control group) (p = 0.339); by dosage, 1000 mcg had improved DFS rate compared to <1000 mg or control group: 77.9% vs 31.2% vs 40% (p = 0.013). DFS rate was improved in those with vaccine boosters (77.2% vs 45.5%, p = 0.023). DFS rate was improved among those treated with vaccination in the primary setting with 1000 mcg compared to <1000 mcg or control (90% vs 33.6% vs 42.9%, p = 0.007)	Most common: Induration at injection site, erythema, and pruritus; 1 grade 3 toxicity (chest pain/dyspnea) 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

[A]includes other non-endometrial cancers

(LAK) cells. This process involves collection of peripheral blood containing mononuclear cells that are stimulated in vitro with IL-2 to become LAK cells [50]. These LAK cells are re-infused into the patient and are capable of lysing tumor cells without MHC restriction while sparing normal tissue [50]. In study by Steis et al., they selected patients with various cancers that had metastatic disease restricted to the peritoneal cavity [51]. These patients received IL-2 (100,000 U/kg IV every 8 h) for 3 days, followed by leukapheresis for 5 days [51]. LAK cells were expanded in vitro by incubating the peripheral blood mononuclear cells in IL-2 for 7 days and then administered IP for 5 days with IL-2 (25,000 U/kg IP every 8 h) [51]. 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 [51]. In another study, Santin et al. observed stable disease in a patient with endometrial cancer with unresectable, chemoresistant liver metastases who was treated with infusion of peripheral T cells stimulated with tumor lysate-pulsed autologous dendritic cells [52].

3 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 result 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.

3.1 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 [53–55]. 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 [56]. 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 3) [56]. In an interim analysis in the KEYNOTE-158 phase 2, open-label trial, 98 cervical cancer patients received pembrolizumab (200 mg every 3 weeks), including 83.7% of patients who had PD-L1-expression (defined as combined positive score (CPS) ≥1) in their tumors and 78.6% who had prior lines of chemotherapy for recurrent or advanced disease (NCT02628067) [57]. Among these patients, the ORR was 12.2% (nine had a partial response and three 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 [57]. Furthermore, the median duration of response was NR, and 91% of patients had a response duration of at least 6 months [57]. Since June 2018, the FDA has approved pembrolizumab in advanced cervical cancer expressing PD-L1 (CPS ≥1) 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 stereotactic body radiation [58, 59]. In a larger study, nivolumab (240 mg every 2 weeks) was tested in five HPV-associated malignancies including cervical, vulvar, and vaginal cancers that previously had up to two failed prior systemic therapies (CheckMate358; NCT02488759) [60]. 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 [60]. 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 [60]. In the phase II results of another trial with nivolumab (NRG-GY002), the agent was demonstrated to have poor response rate (despite PD-L1 positivity in 77.3% of tumors) with an ORR of 4% (1 partial response) with a DCR 36% in a cohort of 25 cervical cancer patients with persistent or recurrent disease who failed at least 1 prior line of systemic therapy [61]. 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) [62]. There were 10 evaluable patients with no confirmed responses and a DCR of 50% [62]. The median PFS was 2.9 months,

Table 3 Reported immune checkpoint inhibitors trials in cervical cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
Monotherapy						
Frenel et al. 2017 [56]	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
Hollebecque et al. 2017 [60]	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%[a], mPFS 5.5 months	Overall 70.8% Grade 3–4 12.5%
Lheureux et al. 2018 [65]	Phase I/II	42	Metastatic or recurrent cervical cancer	Ipilimumab 3 mg/kg q3 weeks x 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)
Chung et al. 2019 [57]	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%
Rischin et al. 2020 [63]	Phase I	Arm#1 (n = 10) Arm#2 (n = 10)	Recurrent or metastatic cervical cancer	ARM#1: Cemiplimab 3 mg/kg IV every 2 weeks for 48 weeks ARM#2: Cemiplimab with hypofractionated radiotherapy	ORR 10% (both ARMs and each had 1 PR) DCR 40% (ARM#1) and 60% (ARM#2) DOR 11.2 months (ARM#1) and 6.4 months (ARM#2)	Any grade TRAE: 90% (ARM#1) and 100% (ARM#2): Most common TRAEs were diarrhea, fatigue, and hypokalemia. Grade 3+ TRAE: 40% in both ARMs
Santin et al. 2020 [61]	Phase II	26	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 36%, 6 month PFS rate 16%, 6 month OS rate 78.4%	Overall: 84% Grade 3: 24% Grade 4: 8%
Combination therapy						
Mayadev et al. 2017 [63]	Phase I	19	Stage IB2 – IVA cervical cancer with node positive disease undergoing chemoradiation	Cisplatin (40 mg/M^2) qweek x 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 [62]	Phase II	10	Recurrent, persistent, or metastatic cervical cancer	Atezolizumab 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% (arachnoditis, sensorineural hearing loss, lower extremity weakness, thrombosis, rectal bleed)
Naumann et al. 2019 [66]	Phase II	Arm#1 (n = 45) Arm#2 (n = 46)	Recurrent or metastatic cervical cancer with or without prior systemic therapy (PST)	*ARM#1 low dose ipi* Nivolumab 3 mg/kg q2 weeks and ipilimumab 1 mg/kg q6 weeks *ARM#2 high dose ipi* Nivolumab 1 mg/kg and ipilimumab 3 mg/kg q3 weeks x 4 doses then nivolumab 240 mg IV q2 weeks	*Arm#1* ORR 31.6% (no PST) and 23.1% (PST) DCR 63.2% (no PST) and 53.8% (PST) mPFS 13.8 months (no PST) and 3.6 months (PST) mOS NR (no PST) and 10.3 months (PST) *Arm#2* ORR 45.8% (no PST) and 36.4% (PST) DCR 60.8% (no PST) and 72.8% (PST) mPFS 8.5 months (no PST) and 5.8 months (PST) mOS NR (no PST) and 25.4 months (PST)	Any grade: 80% (ARM#1) and 28.9% (ARM#2) Grade 3–4 TRAE: 28.9% (ARM#1) and 37% (ARM#2)

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, *NR* not reached, *OS* overall survival, *PFS* progression-free survival, *PR* partial response, *PST* prior systemic therapy, *q* every. *TRAE* treatment related adverse events

[a]includes 5 vaginal and vulvar cancers

and overall survival was 9 months with 23% of patients having grade 3 TRAE [62]. In a phase I study, Rischin et al. reported the safety and antitumor activity results of cemiplimab (PD-L1 inhibitor) with or without hypofractionated radiation therapy evaluated in recurrent or metastatic cervical cancer patients [63]. The ORR was 10% in both the monotherapy and combination therapy group with a duration of response of 11.2 and 6.4 months, respectively [63].

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 [64]. In another phase I/II clinical trial, 42 patients with metastatic cervical cancer (squamous cell or adenocarcinoma) with progression on at least one line of platinum chemotherapy received ipilimumab [65]. 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 [65]. Expression of CD3, CD4, CD8, FoxP3, indoleamin 2,3-dioxygenase, and PD-L1 did not predict benefit [65]. More recently, at the 2019 European Society of Medical Oncology Congress, the investigators of CheckMate-358 (NCT02488759) presented their preliminary results of an ongoing phase I/II study evaluating two dosing regimens of ipilimumab and nivolumab in patients with advanced/recurrent cervical cancer [66]. Of note, this study was stratified based on whether patients had received prior systemic chemotherapy [66]. Both regimens [low-dose ipilimumab (1 mg/kg) and high-dose nivolumab (3 mg/kg) vs. high-dose ipilimumab (3 mg/kg) and low-dose nivolumab (1 mg/kg) followed by maintenance nivolumab (1 mg/kg)] demonstrated impressive objective response rates that were higher in subjects who had received no prior systemic therapy (31.6% and 45.8%, respectively) [66]. The clinical benefit rate of mirroring responses was also impressive for both regimens and higher in subjects who had received no prior systemic therapy (63.2% versus 70.8%, respectively). Furthermore, responses were noted regardless of PD-L1 status [66]. Although there were no safety concerns, 28.9% and 37% of patients in the low-dose ipilimumab and high-dose ipilimumab regimens, respectively, had grade 3–4 treatment-related adverse events [66].

There are numerous ongoing clinical trials of combination therapy with immune checkpoint inhibitors, and these include but are not limited to the following:

- KEYNOTE-826 (NCT03635567): phase III trial of pembrolizumab and investigator's choice of chemotherapy vs. placebo and investigator's choice chemotherapy
- BEATcc (NCT03556839): phase III trial of platinum chemotherapy, paclitaxel, bevacizumab, and atezolizumab vs. platinum chemotherapy, paclitaxel, and bevacizumab
- NCT03614949: phase II trial of stereotactic body radiation therapy and atezolizumab
- NCT03508570: phase Ib trial of intraperitoneal nivolumab +/− ipilimumab
- KEYNOTE-A18/ENGOT-cx11 (NCT04221945): phase III trial of chemoradiation with or without pembrolizumab in locally advanced cervical cancer
- CALLA (NCT03830866): phase III trial of chemoradiation with or without durvalumab in locally advanced cervical cancer
- NCT03894215: phase II study of balstilimab with or without zalifrelimab in second-line treatment of cervix cancer

3.2 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 phase I vaccine trial, Hasan et al. administered MEDI0457, DNA-based vaccine targeting E6 and E7 of HPV-16/18 that is coinjected with an

IL-12 plasmid followed by electroporation with the CELLECTRA 5P device in cervical cancer patients following chemoradiation in the primary and recurrent setting [67]. In this small 10-patient study, they observed detectable cellular or humoral immune responses in 8 of 10 patients with 6 of 10 generating anti-HPV antibody and IFN-gamma producing T-cell responses [67]. The vaccine demonstrated tolerable safety profile [67]. 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 [68]. ADXS11–001 was also examined in the GOG/NRG0265 phase II study (NCT01266460) (Table 4) [69]. 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 [69]. The 12-month OS was 38% with an ORR of 2% (1 complete response) and DCR of 32% [69]. 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 [69]. 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 cell carcinomas of the head and neck (NCT02291055) [70]. In the phase I portion of the trial, combination therapy was examined with eight cervical cancer patients treated [70]. Among the five evaluable patients, the ORR and DCR were 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.

In the interim results of another trial combining immune checkpoint inhibitors and vaccine therapy, Youn et al. administer pembrolizumab and GX-18E (therapeutic HPV DNA vaccine that encodes for HPV-16 and HPV-18 E6 and E7) to inoperable recurrent or advanced HPV-16 or 18 positive cervical cancer (n = 36) [71]. In the 26 evaluable patients, the ORR was 42% (7 partial and 4 complete responses) and tolerable safety profile [71]. Of note, the responses were mainly seen among those with PD-L1-positive tumors: ORR 50% (10 of 20) in PD-L1-positive tumors and 17% (1 of 6) in PD-L1-negative tumors [71].

3.3 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 [72, 73]. In the cervical cancer subcohort, the ORR and DCR were 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 4) [72, 73]. The proportion of HPV-reactive T cells in peripheral blood post-infusion was positively correlated with improved clinical response [72]. 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 [74]. 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) [75].

Table 4 Reported vaccine therapy and adoptive cell therapy trials in cervical cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
Slomovitz et al. 2016 [70]	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)	[a]Overall: 91%. The most frequent was chills/rigors, fever, nausea, hypotension, diarrhea, fatigue, tachycardia, and headache. Grade 3 and 4: 27% and 9%, respectively
Huh et al. 2017 [69]	Phase II	50	Persistent or recurrent metastatic cervical cancer	ADXS11–001 (1×10^9 CFU) q3 weeks x 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 and 4: 39% and 4%, respectively
Lu et al. 2017 [76]	Phase I	3	Metastatic or locally advanced/recurrent cancer. HLA-DPB1[a]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
Basu et al. 2018 [68]	Phase II	Mono (n = 35) combo (n = 34)	Recurrent or treatment-refractory	ADXS11–001 1 cycle (3 infusions) (1 x 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 x 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
Stevanovic et al. 2019 [73]	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)	[a]Grade 3–4: Conditioning agent (myelosuppression and infection)
Jazaeri et al. 2019 [75]	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

Study	Design	N	Patient population	Therapy	Results	TRAE
Hasan et al. 2020 [67]	Phase I	10	Newly diagnosed stage IB1-IVA or persistent/ recurrent cervical cancer undergoing chemoradiation	MEDI0457 monthly x 4 doses following chemoradiation (2–4 weeks following treatment)	Responses: Cellular/humoral: 8/10 Anti-HPV antibodies: 6/10 Interferon-gamma producing T-cells: 6/10	TRAE were all grade 1, related to primary injection site.
Youn et al. 2020 [71]	Phase II	36	Inoperable recurrent or advanced HPV-16/18 positive cervical cancer	Pembrolizumab 200 mg IV q3 weeks and GX-188E 2 mg IM at week 1, 2,4, 7, 13, and 19 with one optional dose at week 46.	ORR 42% (7 PR/ 4 CR); 50% in PD-L1+ tumors and 17% in PD-L1- tumors. DCR 58%	Any grade TRAE: 44% Grade 3–4 TRAE: 11%

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

[a]includes other cancers

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 [76]. 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 [76].

4 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 [77]. 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 [78, 79].

4.1 Immune Checkpoint Inhibitors in Epithelial Ovarian Cancer

In a multicenter phase 1 trial, Brahmer et al. administered an anti-PD-L1 antibody, a heterogeneous cohort of advanced cancers, including 17 ovarian cancer patients [80]. In the ovarian cancer cohort, the ORR was 6% (1 partial response) with a DCR of 23.5% (Table 5) [80]. 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 [81]. 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 [81]. In KEYNOTE-028, 26 patients with PD-L1-positive advanced, metastatic ovarian cancer received pembrolizumab with the majority of patients having at least three prior lines of systemic therapy [82]. The ORR was 11.5% (2 partial and 1 response) with a DCR of 38.5% and acceptable side effect profile [82]. 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 [83]. 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) $\geq$ 10) appeared to be correlated with higher clinical response (ORR 17.1% vs. 5.2% vs. 5.0% for CPS $\geq$ 10, 1–10, <1, respectively) [83].

The JAVELIN trials have investigated the use of avelumab in epithelial ovarian cancer. In the phase 1B JAVELIN Solid Tumor study, avelumab was administered to 125 patients with advanced, recurrent, or refractory ovarian cancer [84]. The ORR was 9.6% (including 1 complete and 11 partial responses) and DCR of 52% [84]. The 1-year PFS rate was 10.2% with a median OS of 11.2 months and acceptable side effect profile [84]. The study authors did not find an association between PD-L1 nor BRCA status and treatment response [84]. In JAVELIN Ovarian 200, 566 platinum-resistant/refractory ovarian cancer patients were randomized to one of three treatment arms: avelumab alone, pegylated liposomal doxorubicin alone, or both (NCT02580058) [85]. 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) [85]. 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 toward improved OS (17.7 vs. 13.1 months; HR 0.72, 95% CI 0.48–1.08) [85]. Grade 3

Table 5 Reported immune checkpoint inhibitors trials in epithelial ovarian cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
Monotherapy						
Brahmer et al. 2012 [80]	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)	[a]Overall: 61% (fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache). Grade 3 or 4 TRAE in 9%
Hamanishi et al. 2015 [81]	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%
Infante et al. 2016 [86]	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)
Disis et al. 2019 [84]	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 5 (continued)

Study	Design	N	Patient population	Therapy	Results	TRAE
Matulonis et al. 2019 [83]	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
Varga et al. 2019 [82]	Phase 1B	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
Marabelle et al. 2019 [27]	Phase II	15	Recurrent MSI-H ovarian cancer	Pembrolizumab 200 mg IV q3 weeks	ORR 33.3% (2 PR/3 CR) mPFS 2.3 months (95% CI 1.9–6.2) mOS NR (95% CI 3.8 – NR)	Overall[a]: 64.8% (mainly fatigue, pruritus, diarrhea, and asthenia) with 15% grade 3–5 TRAE (there was 1 grade 5 TRAE and was a treatment-related pneumonia)
Combination therapy: IO-chemotherapy						
Wenham et al. 2018 [87]	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
Pujade-Lauraine et al. 2019 [85]	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%)

Ledermann et al. 2020 [89]	Phase III	998	Stage III–IV (post debulking surgery or candidates for neoadjuvant chemotherapy)	Randomized 1:1:1 ARM#1: Carboplatin (AUC 5–6) q3 weeks and paclitaxel 175 mg/m^2 q3 weeks or paclitaxel 80 mg/m^2 qweekly with avelumab maintenance (10 mg/kg IV q2 weeks) IV ARM#2: Chemotherapy with avelumab 10 mg/kg IV q3 weeks followed by avelumab maintenance ARM#3: Chemotherapy followed by observation	HR (95% CI) for PFS in avelumab arms versus control: 1.43 ((95% CI 1.051–1.946) for ARM#1 vs ARM#2; 1.14 ((95% CI 0.832–1.565) Median PFS was 16.8 months (ARM#1), 18.1 months (ARM#2), NR (ARM#3). ORR 30.4% (ARM#1), 36.0% (ARM#2), and 30.4% (ARM#3)	Grade ≥ 3 treatment-emergent adverse events of any causality occurred in 66.5% (ARM#1), 70.8% (ARM#2), and 62.6% (ARM#3)
Moore et al. 2020 [90]	Phase III	1301	Stage III/IV who underwent debulking surgery with gross residual disease or candidates for neoadjuvant chemotherapy	Randomized 1:1 ARM#1: Carboplatin AUC 6, paclitaxel 175 mg/m^2, and atezolizumab 1200 mg IV q3 weeks x 6 cycles followed by atezolizumab maintenance 1200 mg IV q3 weeks ARM#2: Chemotherapy + placebo followed by placebo maintenance	No statistically significant PFS improvement with chemotherapy and atezolizumab (HR 0.92 [95% CI 0.79–1.07]. mPFS 19.5 months (ARM#1) vs.18.4 months (ARM#2)	Any grade TRAE: 100% in both ARMs Grade 3+ TRAE: 79% (ARM#1) and 73% (ARM#2)
Zsiros et al. 2020 [88]	Phase II	40	Recurrent EOC patients	Pembrolizumab 200 mg IV and bevacizumab 15 mg/kg IV q3 weeks with cyclophosphamide 50 mg po daily	*Platinum-resistant (n = 30)* ORR 43.3%, DCR 93.3%, DOR 5.5 months *Platinum-sensitive (n = 10)* ORR 60%, DCR 100%, DOR 11.5 months	Most common grade 3–4 TRAE: Hypertension and lymphopenia. Most common TRAE: Fatigue, diarrhea, and hypertension
Combination therapy: IO-targeted therapy						

(continued)

Table 5 (continued)

Study	Design	N	Patient population	Therapy	Results	TRAE
Lee et al. 2017 [91]	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
Drew et al. 2018 [94]	Phase II	32	gBRCAm platinum-sensitive relapsed ovarian cancer	Olaparib 300 mg po BID x 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
Lee et al. 2018 [93]	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
Liu et al. 2018 [96]	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
Konstantinopoulos et al. 2019 [95]	Phase I/II	60	Recurrent ovarian cancer	Pembrolizumab 200 mg q3 weeks + niraparib 200 mg qday	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 bevacizumab, somatic BRCA mutations or HRD biomarker status	Most common: Fatigue, nausea, anemia, constipation. Grade 3: Myelosuppression

Combination therapy: IO combinations						
Zamarin et al. 2020 [97]	Phase II	Arm#1 (n = 49) Arm#2 (n = 51)	Recurrent ovarian cancer with platinum-free interval < 12 months	ARM#1: Nivolumab 3 mg/kg IV then q2 weeks x 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 x 4 then maintenance nivolumab 3 mg/kg IV q2 weeks	ORR 31.4% vs. 12.2% (p = 0.034) mPFS 3.9 months vs. 2 months (p = 0.004) mOS 28.1 months vs. 21.8 months (p = 0.43)	AE: More frequently from ARM#2 vs. ARM#1 Grade 3 or more: 49% (ARM#2) vs. 33% (ARM#1)

AE adverse events, *CPS* combined positivity score, *CR* complete response, *DCR* disease control rate = stable disease + partial response + complete response rates, *DOR* duration of response, *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

TRAEvwere highest in the combination arm (42.9%) followed by PLD alone (31.6%) and avelumab alone (16.0%) [85].

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 [86]. In preliminary results of the nine patients with an evaluable response, there was a 22% ORR and DCR (two patients with partial response) [86].

4.1.1 Combination Therapy: IO + Chemotherapy

Given the strength immunosuppressive tumor microenvironment 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) [87]. 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 [87]. In another phase II nonrandomized clinical trial, Zsiros et al. evaluated pembrolizumab, bevacizumab, and oral cyclophosphamide in a mixed cohort of platinum-sensitive and platinum-resistant ovarian cancer (n = 40) [88]. In the platinum-resistant cohort (n = 30), there were 10 partial and 3 complete responses, making an ORR of 43.3% [88]. The median duration of response was 5.5 months, median PFS 7.6 months, and the disease control rate 93.3% [88]. This regimen was well tolerated and represents a potential therapeutic option for platinum-resistant ovarian cancer that should be investigated in larger studies.

The interim results of two phase III trials combining chemotherapy and immune checkpoint inhibitors in the frontline setting have not demonstrated improved efficacy over chemotherapy alone. In JAVELIN 100, stage III–IV advanced ovarian cancer patients (n = 998) undergoing frontline therapy (with primary cytoreduction or candidates for neoadjuvant chemotherapy and interval cytoreduction) were randomized 1:1:1 to (1) 6 cycles of chemotherapy (carboplatin/paclitaxel) followed by avelumab maintenance, (2) chemotherapy with avelumab followed by avelumab maintenance, and (3) chemotherapy followed by observation [89]. The response rates were similar across all cohorts (ORR 30.4% vs 36% vs 30.4%, respectively) [89]. The hazards ratios for PFS were not statistically significant when comparing the avelumab arms versus the control arm: 1.43 (95% CI 1.1–1.9) for arm 1 versus 3 and 1.14 (95% CI 0.8–1.6) for arm 2 versus 3 [89]. In the IMagyn050/GOG 3015/ENGOT-OV39 trial, stage III/V ovarian cancer patients (n = 1301) undergoing frontline therapy (primary cytoreductive surgery with gross residual disease or candidates for neoadjuvant chemotherapy and interval cytoreduction) were randomized 1:1 to receive 6 cycles of chemotherapy (carboplatin/paclitaxel/bevacizumab) with atezolizumab followed by atezolizumab maintenance or chemotherapy with placebo followed by placebo maintenance [90]. There was no statistically significant improvement in PFS with the atezolizumab arm (median PFS 19.5 months vs. 18.4 months, respectively; HR 0.92 (95% CI 0.70–1.07)) [90]. Furthermore, PD-L1 status did not significantly improve PFS [90].

4.1.2 Combination Therapy: IO + Targeted Therapy

In a phase 1 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%) [91]. 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% [91]. 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 who had somatic BRCA mutations had stable disease). For the six evaluable patients who received durvalumab and intermittent cedi-

ranib and were assessed for response, the ORR was 50% (all partial responses) and had a DCR of 83% [91]. 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 [91]. A biomarker analysis of a subset of the tumors demonstrated some clinical benefit correlated with tumoral PD-L1 expression [92]. 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% (five partial responses, two with germline BRCA mutated, and three with BRCA wild type) and DCR of 52.9% (NCT02484404) [93]. 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) [94]. 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 [94]. 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 [95]. In this phase I/II study, niraparib and pembrolizumab were given to a cohort of 67 patients with ovarian or triple-negative breast cancer [95]. In the 60 evaluable ovarian cancer patients, the ORR was 18% (eight partial and three complete responses), and the DCR was 65% (three with acceptable treatment side effect profile) [95]. 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 [95].

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 [96]. 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) [96].

4.1.3 Combination Therapy: IO Combinations

In the phase II NRG-GY003 trial, 100 recurrent ovarian cancer patients with a platinum-free interval < 12 months were randomized to either nivolumab alone (n = 49) or nivolumab/ipilimumab (n = 51) followed by maintenance nivolumab [97]. The 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) [97]. Furthermore, median PFS was slightly better in the combination group (3.9 vs 2 months, respectively; HR 0.53 (95% CI 0.34–0.82). Interestingly, clear cell histologies had fivefold odds of response with combination therapy compared to other histologies (p = 0.0498) [97]. Grade ≥ 3 adverse events were higher in the combination group than the monotherapy group (49% vs 33%, respectively) but were overall well tolerated [97].

There is a plethora of ongoing clinical trials of combination therapy with immune checkpoint inhibitors, and these include but are not limited to the following:

- NCT02839707: phase II/III trial of atezolizumab and pegylated liposomal doxorubicin vs atezolizumab, pegylated liposomal doxorubicin, and bevacizumab vs pegylated liposomal doxorubicin and bevacizumab
- ATLANTE (NCT02891824): phase III trial of physician's choice platinum chemotherapy, bevacizumab, and atezolizumab vs platinum chemotherapy, bevacizumab, and placebo
- FIRST (NCT03602859): standard of care chemotherapy with niraparib vs standard of care chemotherapy with dostarlimab vs standard of care chemo with placebo
- MK-7339-001/KEYLYNK-001/ENGOT-ov43/GOG-3036 (NCT03740165): phase III trial of standard of care chemotherapy with niraparib and pembrolizumab vs standard of care chemotherapy with niraparib-placebo and pembrolizumab vs standard of

care chemotherapy with niraparib and pembrolizumab-placebo
- DUO-O (NCT03737643): phase III trial of standard of care chemotherapy with olaparib, durvalumab, and bevacizumab vs standard of care chemotherapy with olaparib-placebo, durvalumab, and bevacizumab vs standard of care chemotherapy with olaparib, durvalumab-placebo, and bevacizumab
- ATHENA (NCT03522246): phase III trial of maintenance rucaparib with or without nivolumab following frontline chemotherapy
- NCT02608684: phase II trial of pembrolizumab, gemcitabine, and cisplatin

4.2 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 [98] (Table 6). In a study by Diefenbach et al., high-risk ovarian cancer patients with HLA-A*0201 positivity had the administration of NY-ESO-1b peptide and Montanide vaccination series following primary debulking and chemotherapy [99]. In the nine 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 and three patients with NY-ESO-1-negative tumors having clinical remission at 25, 38, and 52 months [99]. 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% [100]. 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 [101].

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 [102]. Overall 3-year survival was 90% with a trend toward survival in those who received cyclophosphamide therapy prior to vaccination [102]. 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 [103]. 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 [103]. Increased immune response and reduced immune-suppressive factor secretion were also evident [103]. Another study compared autologous dendritic cell vaccine with chemotherapy to chemotherapy alone for recurrent platinum-sensitive ovarian cancers and demonstrated a trend toward improved ORR (87.5% vs. 62.5%, respectively) for the vaccine cohort (NCT02107950) [104]. 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) [105].

Kuwano et al. investigated the use of personalized vaccination based on HLA-type and preexisting host immunity (by IGG response levels to tumor-associated antigens) and have demonstrated some disease stabilization with good tolerability [106]. Personalized vaccine generated by autologous dendritic cells pulsed with oxidized autologous whole-tumor cell lysate also demonstrated broad antitumor immune response activity [107].

Table 6 Reported vaccine therapy trials in epithelial ovarian cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
Diefenbach et al. 2008 [99]	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 x 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
Chu et al. 2011 [102]	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
Sabbatini et al. 2012 [101]	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
Odunsi et al. 2014 [100]	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) x 4 cycles	ORR 10% (1 PR/0 CR) DCR 60%	Mainly injection side reactions. Grade 3 or 4: Neutropenia, injection site reactions

(continued)

Table 6 (continued)

Study	Design	N	Patient population	Therapy	Results	TRAE
Baek et al. 2015 [103]	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 [104]	Phase II	32 per ARM	Platinum-sensitive recurrent advanced stage EOC	ARM#1: Chemo + DCVAC. DCVAC (1 x 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
Dorigo et al. 2018 [108]	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
Rob et al. 2018 [105]	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
Tanyi et al. 2018 [107]	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
O'Cearbhaill et al. 2019 [110]	Phase I	ARM#1 (n = 86) ARM#2 (n = 85)	Ovarian cancer patients in their 2nd or 3rd clinical remission	Randomized 1:1 ARM#1: Polyvalent vaccine conjugate with adjuvant OPT-821 at weeks 1,2,3, 7, 11, and every 12 weeks ARM#2: Adjuvant OPT-821 only at weeks 1,2,3, 7, 11, and every 12 weeks	HR for PFS 0.98 (95% CI: 0.7–1.36). mOS 47 months (ARM#1) and 46 months (ARM#2)	Grade 4 myeloid dysplastic syndrome (n = 1) and grade 4 depression (n = 1) and grade 3 gastrointestinal disorders (n = 4)

Rocconi et al. 2020 [109]	Phase II	ARM#1 (n = 47) ARM#2 (n = 44)	Stage III/IV high grade serous, endometrioid, or clear cell histologies with complete response to treatment	ARM#1: Gemogenovatucel-T ARM#2: Placebo	*Overall analysis* PFS was similar between the ARMs (11.5 months vs. 8.4 months, respectively; p = 0.078). *Post-hoc analysis among patients with BRCA wild-type tumors* PFS was improved in ARM#1: HR 0.50, 90% CI 0.30–0.88; p = 0.02. The 1-year PFS rate improved in ARM#1: 51% vs 28%, respectively, p = 0.036 The 2-year PFS rate improved in ARM#1: 33% vs. 14%, respectively, p = 0.048	Well-tolerated No grade 3–4 TRAEs
Kawano et al. 2014 [106]	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

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 , *Type of HLA haplotype

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) [108]. Preliminary results in the 10 evaluable patients demonstrated an ORR of 30% (3 partial responses) and DCR of 60% with good treatment tolerability [108].

In the VITAL study, the investigators utilized an autologous tumor cell vaccine (gemogenovatucel-T) in stage III/IV high-grade serous, endometrioid, or clear cell ovarian cancer that had a complete response to carboplatin/paclitaxel frontline therapy [109]. Gemogenovatucel-T is an autologous tumor cell vaccine generated from harvested tumor and transfected ex vivo with a plasmid encoding the *GMCSF* gene and a bifunctional short-hairpin RNA that ultimately reduces expression of immunosuppressive TGF-β1 and TGF-β2 [109]. In this phase IIb trial, 91patients were randomized to gemogenovatucel-T (n = 47) or placebo (n = 44) [109]. The recurrence-free survival (RFS) was similar between the treatment and placebo groups (11.5 months vs. 8.4 months, respectively; p = 0.078) [109]. However, in a post-hoc analysis among patients with BRCA wild-type tumors, RFS was improved in the treatment group, compared to the placebo group (HR 0.50, 90% CI 0.30–0.88; p = 0.02) [109]. The 1-year and 2-year RFS rates were improved in the treatment group compared to the placebo group (51% vs 28%, respectively, p = 0.036; 33% vs. 14%, respectively, p = 0.048) [109]. The vaccine was observed to safe with no grade 3–4 TRAEs [109].

A trial by O'Cearbhaill et al. sought to investigate the safety and efficacy of a polyvalent vaccine-Keyhole limpet hemocyanin (KLH) conjugate with the adjuvant OPT-821 compared to OPT-821 alone [110]. The investigators randomized, in 1:1 fashion, 171 ovarian cancer patients in their second or third clinical remission to vaccine and adjuvant (n = 86) vs. adjuvant alone (n = 85). Despite being tolerable, the combination therapy was modestly immunogenic and did not improve PFS or OS [110].

4.3 ACT in Epithelial Ovarian Cancer

Multiple trials have examined ACT in ovarian cancer. The first trial that was by a 1991 study by Aoki et al. examined TIL therapy without IL-2 infusion in advanced or recurrent ovarian cancer with or without cisplatin-containing combination chemotherapy [111]. In the TIL group without chemotherapy, there was an ORR of 71.4% (one complete and four partial responses), while the group with both TIL and chemotherapy had a 90% ORR (seven with complete response and two with partial responses) which 4 of the 7 patients with complete responses did not have recurrence for >15 months of follow-up (Table 7) [111]. Another study by Ikarashi et al. demonstrated that TIL therapy may also induce increased cytotoxic T-cell and natural killer cell activity [112]. 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 [113]. In contrast to the above previous three studies, Pedersen et al. utilized an IL-2 infusion following TIL therapy in six patients with progressive platinum-resistant disease [114]. The DCR was 100% with five 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 [114]. 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 [114]. In another study by the previous group, Kverneland et al. treated six patients with advanced-stage metastatic high-grade serous ovarian cancer with ipilimumab followed by TIL extraction and re-infusion with expanded TILs with low-dose IL-2 and nivolumab [115]. In their results, there was one partial response with the remaining five hav-

Table 7 Reported trials in adoptive cell therapy in epithelial ovarian cancer

Study	Design	N	Patient population	Therapy	Results	TRAE
Aoki et a. 1991 [111]	Phase I	TIL only (n = 7) TIL + chemo (n = 10)	Advanced or recurrent EOC	ARM#1: TIL (at least $1x10^{10}$ 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%
Freedman et al. 1994 [116]	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
Ikarashi et al. 1994 [112]	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 ($5x10^8$ 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 [113]	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 ($5x10^8$ 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 [114]	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

(continued)

Table 7 (continued)

Study	Design	N	Patient population	Therapy	Results	TRAE
Kverneland et al. 2019 [115]	Phase I	6	Platinum-resistant advanced high grade serous ovarian cancer	Ipilimumab (3 mg/kg) IV x 1 dose 2 weeks prior to TIL extraction for ex vivo expansion. Following lymphodepleting chemotherapy, reinfusion of TIL was performed with nivolumab (3 mg/kg q2 weeks x 4 doses) and low-dose IL-2 for 2 weeks	1 partial response and 5 patients with stable disease (84–342 days)	Most grade 3+ TRAE were secondary to lymphodepleting chemotherapy, followed by IL-2 (including performance status 3–4 in 3 patients, fever in 3 patients, fatigue in 2 patients)

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 response, *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

ing stable disease for up to 12 months [115]. Most of the grade 3–4 toxicity was related to the conditioning chemotherapy prior to TIL infusion [115]. 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 [116].

5 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 [117]. In the six patients that received ipilimumab, there were four patients with progressive disease, one stable response, and one patient who had a partial response but 89% reduction in tumor volume and a survival of 31 months [117]. In the eight patients that were treated with nivolumab, there were partial responses in four patients [117]. One vaginal melanoma patient had received both ipilimumab and nivolumab and had a partial response [117]. In CheckMate 358, the vulvovaginal cohort (two vaginal and three vulvar squamous cell carcinomas) was treated with nivolumab with one partial response observed in the vulvar cancer patient [118]. In a phase II basket trial of advanced rare tumors (including cohorts of squamous cell carcinoma of the vagina or vulva (two vaginal and one vulva), granulosa cell tumor of the ovary (four adult type and one juvenile type), and gynecologic extrapulmonary small cell carcinoma), patients were treated with pembrolizumab [119–121]. Although there were no confirmed responses, one vaginal cancer had an 81% reduction in her target tumor lesions, and one vulvar cancer patient had 30% reduction in her target tumor lesions but discontinued treatment due to a grade 3 mucositis before a confirmatory scan was performed for the partial response [119]. In the patients with granulosa cell tumor of the ovary, there were no responses, but the disease control duration was 565 and 453 days for 2 adult-type granulosa cell tumors [120]. In the cohort with gynecologic extrapulmonary small cell carcinoma, there were six cervical and one vulvar carcinomas [121]. However, pembrolizumab demonstrated minimal activity in these patients (no responses: one patient with stable disease and six with progressive disease) [121]. Future studies should evaluate the use of combinational immunotherapeutic regimens in these rare tumors.

6 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 populations (e.g., pembrolizumab for MSI-H/dMMR/TMB-H tumors and PD-L1 positive cervical cancers). Combination immune checkpoint inhibitor therapy demonstrates promise in the treatment of advanced/recurrent cervical cancer. 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. As demonstrated with the impressive response rates of pembrolizumab and lenvatinib in MSS endometrial cancer, combination therapy can overcome immune checkpoint inhibitor resistance. However, with a wealth of new immune-modulatory 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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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, 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, GBM, and rare CNS cancers.

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

1 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

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lymph nodes [3]. CNS antigens are presented to 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 to CNS lymphatics, immune cells are able to infiltrate 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.

2 Immunotherapy for Brain Metastasis

BM is the most common form of intracranial malignancy, and its incidence is on the rise as systemic treatment options have improved 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 the location of a non-infiltrative CNS tumor does not preclude 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.

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]. Kluger and colleagues reported the final results and long-term follow-up for the melanoma cohort and showed that 26% had a brain metastasis response [20]. The study had median progression-free survival (PFS) and overall survival (OS) times of 2 and 17 months, respectively, with 48% alive at 24 months. An updated analysis of the phase II trial of pembrolizumab in patients with NSCLC brain metastases was published looking at a cohort of patients with greater than or equal to 1% of PD-L1 expression and a cohort with less than 1% of PD-L1 expression [21]. A brain metastasis response was seen in 29.7% [95% CI, 15·9–47·0%] of the cohort with greater than or equal to 1% PD-L1 expression, while no response was seen in the cohort with less than 1% PD-L1 expression.

Combinations of CPIs have also been shown to be effective in treating BMs from solid tumors. Recently, a post hoc analysis of the phase III CheckMate 227 trial showed that the combination of nivolumab plus ipilimumab was at least as effective as chemotherapy in front-line therapy for patients with advanced NSCLC and brain metastases at baseline [22]. In patients with baseline brain metastases and PD-L1 expression of 1% or higher, the mOS was 20.6 months for the nivolumab/ipilimumab group vs. 13.7 months for the chemotherapy group and 12.0 months with nivolumab alone. However, in patients without baseline brain metastases and PD-L1 expression of 1% or higher, the mOS was 16.7 months for the nivolumab/ipilimumab group vs. 15.0 months for the chemotherapy group and 16.1 months with nivolumab alone. Based on these findings, the nivolumab/ipilimumab combination was approved by the US Food and Drug Administration in May 2020 as a first-line therapy in metastatic NSCLC without EGFR or ALK aberrations and PD-L1 expression of 1% or higher. The success of CPI in BM 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 1.

3 Glioblastoma

GBM is the most common malignant brain tumor in adults with mOS of 14.6 months with the current standard of care [23]. The standard of care includes maximal safe resection when possible [24] 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-day cycles for 6–12 cycles. Despite this multimodality treatment, GBM invariably recurs leading to death with a 2-year survival rate of 26.5% [23].

Table 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	65	Melanoma BM: RR, 26%; mPFS, 2 mo; mOS, 17 mo NSCLC BM (≥1% PD-L1 expression): RR: 29.7%	NCT02085070	[20, 21]
CheckMate 227	Nivolumab/ ipilimumab	III	2220	mOS 20.6 mo nivolumab/ipilimumab group vs. 13.7 mo chemo vs. 12.0 mo nivolumab	NCT02477826	[22]

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

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 [25, 26]. 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) [27]. In addition, there was no survival benefit when nivolumab was added to radiation and temozolomide in newly diagnosed GBM in two large phase III study (CheckMate 498 and CheckMate 548) [28, 29]. 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 [30] 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.

3.1 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 [31] in HLA class I is frequent in adult GBM and is associated with shorter overall survival [32]. In addition, GBM cells overexpress the T cell inhibitory ligand, PD-L1 [33], 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) [34, 35]. 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) [36–38]. 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 [39, 40]. 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 [41]. Similarly, direct interactions between GBM cells and NK cells via atypical HLA molecules suppress NK cell activity [42, 43]. Immunosuppressive soluble factors such as TGF-β [44] and IL-10 [45] 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 [45–47]. 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 [47]. 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) [48] 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 [49]. The systemic immunosuppression in GBM is further exacerbated by lymphotoxic effects of radiation, TMZ, and corticosteroids [46, 50]. Overall, profound local and systemic immunosuppressive mechanisms in GBM should be targeted for the successful implementation of immunotherapy in GBM.

3.2 Checkpoint Inhibitors for the Treatment of GBM

3.2.1 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 [51] and successful use of pembrolizumab in a patient with germline POLE deficiency and GBM metastatic to the spine [52]. High mutational load and mismatch repair deficiency are known markers of response to CPI in a number of solid tumors [53], but these molecular characteristics are only found in a minority of GBM patients [54], and their associations with clinical response to CPI are 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 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) [27]. At a median follow-up of 9.5 months, mOS was comparable between groups: nivolumab, 9.8 months (95% CI, 8.2–11.8); bevacizumab, 10.0 months (95% CI, 9.0–11.8); HR, 1.04 (95% CI, 0.83–1.30); and $P = 0.76$.

An exploratory phase I cohort within CheckMate 143 assessed nivolumab monotherapy ($n = 10$) versus nivolumab plus ipilimumab ($n = 30$). Adverse events leading to discontinuation occurred more commonly in patients receiving dual immunotherapy [55]. 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 [56]. However, a phase III trial of nivolumab plus radiation versus temozolomide plus radiation in MGMT-unmethylated and MGMT-methylated GBM demonstrated no survival benefit [28, 29].

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%) [57]. 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 [60, 61] and respectable lung cancer [62] 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 [58]. 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

Table 2 Completed checkpoint inhibitor 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	[27]
CheckMate 143 recurrent GBM	Cohort 1B NIVO +/− IPI	I	40	Adverse event profile superior in NIVO monotherapy than in combination arms	NCT02017717	[55]
CheckMate 143 ND GBM	Cohort 1C: NIVO+TMZ+RT-> TMZ (MGMT methylated and unmethylated)	I	55	Neurological adverse events were similar to other trials without immunotherapy	NCT02017717	[56]
	Cohort 1D: NIVO+RT-> TMZ (MGMT unmethylated)	I	58			
Recurrent GBM	Pembro versus pembro + BEV	II	80	PFS-6 6.7% versus 26%	NCT02337491	[57]
Recurrent GBM	Neoadjuvant pembro versus adjuvant pembro	II	35	mOS 13.7 mo versus 7.5 mo mPFS 3.3 versus 2.4	NCT02852655	[58]
Recurrent GBM	Neoadjuvant nivo	II	30	mOS 7.3 mo mPFS 4.1 mo	NCT02550249	[59]
Recurrent GBM	Neoadjuvant pembro	II	15	mPFS: 4.5 mo mOS: 20 mo OS-12 rate: 63%	NCT02337686	[37]

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

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 [59]. In another single-arm neoadjuvant study by de Groot and colleagues, neoadjuvant pembrolizumab was tested in 15 patients with recurrent GBM where mPFS was 4.5 months and mOS was 20 months with an estimated 1-year OS rate of 63% [37]. 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.

Two PD-L1 inhibitors, atezolizumab and durvalumab, were tested in newly diagnosed GBM patients (NCT03174197 and NCT02336165, respectively), and final results are pending.

3.2.2 CTLA-4 Axis Inhibitors

Dual immunotherapy targeting both PD-1/PD-L1 and CTLA-4 pathways has been more successful than monotherapy in melanoma [63]. However, higher rates of adverse events were seen when dual therapy was used in CheckMate 143 GBM trial [57]. Several combinatorial therapies with CPI and other forms of immunotherapy are ongoing.

3.2.3 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 most importantly 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 [64], GBM has a higher expression of the T cell inhibitory ligand, PD-L1, than BM [65]; 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 [66]. In fact, GBM utilizes a variety of immunosuppressive mechanisms to prevent its immune detection and eradication [67]. These immunosuppressive mechanisms include infiltration of GBM microenvironment by immunosuppressive T cells (regulatory T cells) and macrophages [68] and release of immunosuppressive soluble factors such as TGF-ß and IL-10 [67]. In addition to local immune suppression, systemic immune suppression has been described in GBM patients even prior to the start of radiation and chemotherapy [47]. Local and systemic immunosuppressive mechanisms in GBM are described in detail in "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 [69]. 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 [70]. 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 successful [71, 72].

3.3 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 allogeneic immune cells that trigger antitumor immune responses.

3.3.1 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 [73]. 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 [74]. 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 minimal 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 ($P = 0.029$) [74]. 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 [75]. The most common of such mutations is the R123H mutation in IDH1 which accounts for approximately 70% of all IDH mutations [75]. 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 [76]. 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 [77]. 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 [78]. 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 [79]. 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 [80]. 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 [80]. 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 [81].

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 [82]. 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 [82]. 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 who 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 [83]. 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 [84]. 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 [84]. 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 [85]. 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 [86]. 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 [87]. Completed peptide and cell vaccine trials are summarized in Table 3.

Combinations of peptide vaccine with standard therapy have shown some promise. Ahluwalia and colleagues have recently published the results of combination therapy with the immunotherapy vaccine SurVaxM (SVN53-67/M57-KLH) plus standard of care in newly diagnosed glioblastoma. This particular peptide vaccine targets survivin which is an anti-apoptotic protein that is highly expressed in many different cancer types including malignant gliomas. The vaccine demonstrated minimal toxicity and generated an immune response that consists of a survivin-specific antibody and CD8-positive T cells. SurVaxM produced an increase in survivin-specific IgG titer from pre-vaccine baseline to ≥1:10,000 in 67% of pts. and ≥1:100,000 in 27% that was correlated with OS [88].

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.

3.3.2 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 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 [91].

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 [89].

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 [90]. 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.

3.4 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 the development of cell therapy in GBM are the intracranial

Table 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	[74]
NOA-16 ND GBM and AA (IDH1R132H-mutated)	IDH1 peptide vaccine	I	32	Demonstrated safety and immunogenicity	NCT02454634	[79]
IMA950 ND GBM	GBM multipeptide vaccine IMA950	I	40	Well tolerated with multi- TUMAP responses in at least 30%	NCT01222221	[80]
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	[81]
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	[84]
GP96 heat-shock protein- peptide complex vaccine recurrent GBM	HSPPC-96	I/II	41	mPFS 19.1 weeks mOS 42.6 weeks	NCT00293423	[87]
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	[89]
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	[90]
SurVaxM ND GBM	SVN53–67/M57-KLH peptide vaccine	II	64	93.5% were alive in 1 year vs. 65% with standard therapy	NCT02455557	[88]

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

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.

3.4.1 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 [92]. CAR T cell therapies are at the forefront of immunotherapy approaches for the treatment of highly clonal neoplasms such as lymphoma and leukemia [93]. Aside from ubiquitously expressing monoclonal antigens, the location of the tumor cells (peripheral blood) makes hematological malignancies perfect candidates for CAR T cell therapies.

CAR T cell therapies have not been as successful in solid tumors [94]; 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 [95]. Local tumor control was maintained, 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 was 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 localized in the CSF space: 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 [96, 97]. 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. Limited numbers of CART-EGFRvIII cells were identified in tumor. However, despite the promising correlative outcome, mOS of the patients was not improved [96]. 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 [97]. A phase I trial of HER2-CAR T cells in progressive HER2-positive GBM was conducted [98]. Infusions were well tolerated, and HER2-CAR T cells were detected in the peripheral blood for up to 12 months after the infusion. Of 16 evaluable patients, 1 had a partial response for more than 9 months, 7 had stable disease for 8 weeks to 29 months, and 8 progressed after T cell infusion.

Recently, Weathers and colleagues reported a clinical trial of autologous polyclonal CMV pp65-specific T cells expanded ex vivo and administered to patients after temozolomide-induced lymphodepletion [99]. Repeated intravenous infusions of CMV-T cells paralleled significant increases in circulating CMV^+ $CD8^+$ T cells, but cytokine production showing effector activity was suppressed, especially from T cells obtained directly from glioblastomas.

Several factors contribute to the lack of response to CAR T cells or autologous antigen-specific T cells in GBM including lack of stably expressed antigens, intratumoral heterogeneity, effective T cell trafficking to the tumor, and an immunosuppressive microenvironment. Determining the most effective route of cell delivery of cell therapy (intravenous vs. intracavitary vs. intrathecal vs. intratumoral routes) remains one of the most important steps in improving the effectiveness of cell therapy in GBM. Efforts in altering the tumor microenvi-

ronment 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 [100], and combination of EGFRvIII CAR T cells with pembrolizumab is currently being evaluated in newly diagnosed GBM (NCT03726515).

3.4.2 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 [101].

NK cells for the treatment of solid tumors have shown promise [102]. 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 [103]; 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. A phase I trial of human placental hematopoietic stem cell-derived NK cells (CYNK-001) in adults with recurrent GBM is currently enrolling patients at MD Anderson Cancer Center (NCT04489420) [104]. This study evaluates safety and efficacy of intravenous and intratumoral routes of delivery of CYNK-001 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).

3.5 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 [105]. 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 [106]. 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 poliovirus, retrovirus, adenovirus, 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 4. The recombinant oncolytic poliovirus, PVSRIPO, is a genetically engineered form of poliovirus Sabin type 1 with attenuated neurovirulence. PVSRIPO received breakthrough therapy designation from the 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 [107]. 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

Table 4 Select virus therapy clinical trials for GBM

Title/setting	Route of delivery	Phase	*N*	Outcome	Clinical trial identifier number	Reference
Poliovirus (PVSRIPO) Recurrent GBM	Convection-enhanced delivery	I	61	OS rate: 21% at 24 and 36 months	NCT01491893	[107]
Retrovirus Toca 511 (vocimagene amiretrorepvec) Recurrent GBM	Injection of virus into the resection cavity	III	403	mOS: 11 mo Toca 511/FC groups vs. 12.22 months for the control group ($p = 0.62$)	NCT02414165	[108]
Adenovirus DNX-2401 Recurrent GBM	Injection of virus into the tumor	I	37	OS rate: 20% at 72 months	NCT00805376	[109]

that converts the prodrug 5-fluorocytosine (5-FC) to the potent anticancer drug, 5-fluorouracil (5-FU), in an infected tumor cell [110]. 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. In a phase I open-label study, out of 53 efficacy-evaluable recurrent or progressive high-grade glioma patients receiving ascending dose escalation of Toca 511, 6 (11.3%) had complete response [111]. In the 23 patient phase III eligible subgroup, the percentage of patients with objective response was 21.7% (5 complete responders), and the percentage of patients with clinic benefit rate was 43.5%. A multicenter, randomized, open-label phase II/III trial (TOCA 5) comparing posttumor resection treatment with Toca 511 followed by Toca FC vs. a defined single choice of approved (SOC) therapies was conducted [108]. The trial did not meet its primary endpoint. The median OS was 11.10 months for the Toca 511/FC group and 12.22 months for the control group (HR, 1.06; 95% CI 0.83, 1.35; $P = 0.62$).

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 [109]. 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 [112]. Retrospective analysis and literature review have shown similar survival rates in patients enrolled in other nonviral therapy trials [112, 113]. The patients with longer survival seem to possess favorable biological and/or demographic features [114]. 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.

3.6 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 and IL13Ra1 and nivolumab are currently ongoing (NCT03726515 and NCT04003649). Another approach to increase intratumoral T cells is vaccination with DCs [115–117]. Similarly, 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 immunomodulating 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 kynurenine and its by-products [118]. Elevated IDO1 expression is thought to down-regulate T cell activity via depletion of tryptophan and induces T cell apoptosis via increased levels of kynurenine and its by-products [119]. 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 [120]. 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 antisense oligonucleotides targeting soluble extracellular TGF-βII [121], TGF-β receptor sequestering soluble TGF-β (GC1008) [122], and TGF-βI receptor kinase inhibitor (galunisertib/LY2157299) [123]. These agents have not been shown to be efficacious in treatment of recurrent GBM as monotherapy when compared with chemotherapy [121, 122]. Their lack of effectiveness may be 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 [124]. 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 (NCT02423343) 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 [125]. 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 [126]. 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 [127] and was shown to be present in perivascular niche of the tumor in six of nine of human GBM samples tested [128]. 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 [128]. A phase I/II study of nivolumab with anti-LAG3 antibody or urelumab (anti-CD37) in recurrent GBM is currently ongoing [129] (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 [130].

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. Phase I and II trials of pembrolizumab in combination with MRI-guided laser ablation (MLA) in recurrent GBM are currently enrolling patients (NCT02311582).

In addition, therapeutic interventions that increase tumor mutational burden may overcome CPI resistance in GBM. It has been hypothesized that DNA damage response (DDR) and poly(ADP-ribose) polymerase (PARP) inhibitors can enhance the tumor mutational burden and increase the neoantigen load by inducing S-phase DNA damage [131]. Several clinical trials of PARP inhibitors in gliomas have been conducted [132], and clinical trials of DDR inhibitors in GBM are on the rise [133]. It remains to be determined whether PARP and DDR inhibitors aug-

ment a response to CPI in GBM clinical trials. We advocate for window-of-opportunity studies to determine if the inhibition of DNA repair pathways elevates the tumor neoantigen load and increases alterations in its immune cell composition to lay the foundation for future rationale combinatorial studies of PARP and DDR inhibitors plus CPIs.

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

4 Immunotherapy in Rare Primary CNS Tumors

Nearly 150 entities of primary CNS tumors have been identified by the World Health Organization (WHO) together with its updates by the Consortium to Inform Molecular and Practical Approaches to CNS tumor Taxonomy (c-IMPACT NOW) [134, 135]. Most rare CNS tumors affect less than 1000 patients in the United States per year [136]. These entities include tumors such as pituitary carcinoma, ependymoma, atypical meningiomas, and embryonal tumors which have limited treatment options. Conducting clinical trials in rare aggressive primary CNS tumors is exceedingly difficult due to the rarity of these tumors, lack of funding and pharmaceutical interest to run clinical studies, and limited access to centers with expertise for most patients. Growing evidence supports the role of CPI in solid cancers with the first tumor-agnostic approval for pembrolizumab for microsatellite instability high or mismatch repair-deficient solid tumors [137]. There have been efforts to evaluate the efficacy of CPI in patients with advanced rare cancers in multiarm basket trials. These efforts include a phase II study of pembrolizumab in rare, advanced cancers [138] and a phase II study of nivolumab in adult patients with select rare CNS tumors (NCT 03173950). Here, we briefly review published clinical reports of CPI results in select rare CNS tumors.

Pituitary carcinoma (PC) is defined anatomically, not histologically, as a pituitary adenoma that has metastasized outside of the sellar region [139]. Similar to pituitary adenomas, PCs originate from the various cell types with the anterior pituitary gland. However, unlike benign pituitary adenomas which are indolent, PCs are aggressive tumors that account for only estimated 200–300 cases annually in the United States [140]. Four cases of PCs with response to CPI have been reported. Interestingly all responses were seen in ACTH-secreting tumors. Lin and colleagues reported a marked response of an ACTH-secreting PC to ipilimumab and nivolumab [141]. Similarly, Duhamel and colleagues treated two PC patients with ipilimumab and nivolumab and observed biochemical and radiographic partial responses in the patient with ACHT-secreting PC but not in the patient with prolactin-secreting PC [142]. Majd and colleagues reported four patients with PC treated with pembrolizumab as part of the phase II trial of pembrolizumab in rare cancers (NCT02721732) in whom two partial responses were seen in patients with ACTH-secreting PC [143], but not in patients with nonsecreting corticotroph tumor and prolactin-secreting carcinoma. The case reported by Lin and colleagues and the case with durable response reported by Majd and colleagues demonstrated hypermutator phenotypes including MMR mutations attributed to prior exposure to temozolomide. Elevated tumor PD-L1 expression was not seen in any of the four responders. The role of CPIs in treating patients with PC and the relationship between tumor subtype, hypermutation, and response to immunotherapy in PC warrant further investigation.

Ependymomas are gliomas which generally have low cell density and a low mitotic index [134]. Ependymomas have a variable clinical outcome primarily dependent upon molecular subgroups [144] and are more common in children than in adults. Myxopapillary ependymoma (MPE) is a slow-growing variant almost exclusively found in the region of the conus medullaris. With en bloc resection, MPEs in general have a favorable prognosis and seldom disseminate within the CNS or to extra-neural sites.

There are currently no FDA-approved systemic therapies in adults. Tapia Rico and colleagues reported a patient with metastatic MPE who had stable disease after treatment with anti-PD-1 therapy (tislelizumab (BGB-A317)) for more than 18 months [145], which is a much longer progression-free survival than previously reported with systemic treatment options in ependymomas. Detailed next-generation sequencing was not performed on pre-treated tissue in this study. PD-L1 expression tumor-infiltrating cells were 5% and 0% on tumor-infiltrating immune cells and tumor cells, respectively.

Meningiomas are the most common primary tumor of the CNS and compromise a heterogeneous group of tumors driven by a wide number of mutations [146]. There are no FDA-approved systemic treatment options available for high-grade meningiomas at the time of recurrence. PD-L1 is expressed in a subset of meningiomas and is associated with higher-grade tumors [147] suggesting a potential for treating meningioma with CPIs. A phase II study of nivolumab in patients with recurrent high-grade meningioma (NCT02648997) is ongoing, and a durable therapeutic response in a patient with atypical meningioma enrolled in this trial has been reported [148]. Here, the tumor at initial diagnosis was MMR-deficient, and there was a progressive increase in TMB. High TMB and mutations in MMR-related genes are rare events based on two large cohorts of meningioma sampled [149]. However, CPIs may provide benefit in molecularly selected patients with aggressive rare CNS tumors including atypical meningiomas.

Embryonal tumors of the CNS, such as medulloblastoma, atypical teratoid/rhabdoid tumors (ATRT), and a category of tumors formerly called primitive neuroectodermal tumors (PNETs), are exceedingly rare malignant tumors in adults. Medulloblastoma, a malignant tumor of the posterior fossa, represents the most common malignant brain tumor in children and is therefore the most studied CNS embryonal tumor. Treatment options for recurrent medulloblastoma are limited, and efforts in clinical development of immunotherapy are ongoing. Various immunotherapy trials in medulloblastoma and other rare embryonal tumors include CPIs, CAR T cells, NK cells, oncolytic viral therapies, and peptide vaccines [150]. Similar to GBM, successful clinical development of immunotherapy in these tumors will require mechanisms to overcome tumor heterogeneity, immunosuppressive tumor microenvironment, and the BBB. In addition, as our knowledge of molecularly defined classification of rare CNS tumors continues to grow, immune profiling of these tumors and understanding of the tumor microenvironment with attention to molecular subgroups are needed to define biomarkers of response and immunotherapy modalities that are most effective in rare CNS tumors.

5 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. Neoadjuvant trials of CPIs in recurrent GBM have been instrumental in improving our understanding of the GBM microenvironment and potential mechanisms of resistance. 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 antigen selection in GBM (GAPVAC vaccine and NK cells), T cell activation (antibodies against T cell stimulatory ligands and pro-inflammatory cytokines), and maintenance of T cell activation (CPI and TGF-β inhibition). Given the success of immunotherapy for the treatment of BM from melanoma and NSCLC and select cases in GBM and rare CNS tumors, 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 and neoadjuvant 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 and other CNS tumors.

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Immunotherapy in Gastrointestinal Malignancies

Rishi Surana and Shubham Pant

Abstract

Gastrointestinal (GI) cancers represent a heterogeneous group of malignancies, each with a unique tumor biology that in turn affects response to treatment and subsequent prognosis. The interplay between tumor cells and the local immune microenvironment also varies within each GI malignancy and can portend prognosis and response to therapy. Treatment with immune checkpoint inhibitors has changed the treatment landscape of various solid tumors including (but not limited to) renal cell carcinoma, melanoma, and lung cancer. Advances in the understanding between the interplay between the immune system and tumors cells have led to the integration of immunotherapy as standard of care in various GI malignancies. For example, immunotherapy is now a mainstay of treatment for tumors harboring defects in DNA mismatch repair proteins and tumors harboring a high mutational load, regardless of primary site of origin. Data from recent clinical trials have led to the integration of immunotherapy as standard of care for a subset of gastroesophageal cancers and hepatocellular carcinoma. Here, we outline the current landscape of immunotherapy in GI malignancies and highlight ongoing clinical trials that will likely help to further our understanding of how and when to integrate immunotherapy into the treatment of various GI malignancies.

Keywords

Immunotherapy · Gastric · Colon · Liver · Cancer

1 Introduction

In 2020, over 330,000 individuals in the United States are expected to be diagnosed with a gastrointestinal (GI) cancer, and roughly 50% of these patients 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 immunother-

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apy 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 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. There are numerous hypotheses as to why certain GI malignancies tend to have higher response rates to immunotherapy compared to other GI malignancies, including differences in tumor mutational burden and variations in the quantity and phenotype of tumor-infiltrating lymphocytes [2–4].

Below, we will assess the current role of immunotherapy in the treatment of GI malignancy and discuss various promising strategies of integrating immunotherapy into the standard of care of various GI malignancies.

2 Gastroesophageal Cancer

2.1 Current Evidence

Expression of programmed death ligand 1 (PD-L1) in gastric cancer was first reported well before the widespread clinical use of immune checkpoint inhibitors [5, 6]. In 2007, Sun et al. described the association between PD-L1 expression by immunohistochemistry (IHC) and clinical outcomes in gastric cancer with PD-L1-expressing tumors exhibiting higher rates of lymph node metastasis, larger tumor size, greater depth of invasion, and decreased overall survival [5]. Results from KEYNOTE-012, a phase Ib study evaluating the use of pembrolizumab in patients with advanced solid tumors, demonstrated an overall response rate (ORR) of 22% and a median overall survival (OS) of 11.4 months in a cohort of 39 patients with recurrent or metastatic PD-L1 positive gastric or gastroesophageal junction (GEJ) cancers [7]. The phase II KEYNOTE-059 study enrolled 259 patients with previously treated gastric and GEJ cancers, including both PD-L1-positive and PD-L1-negative tumors, and demonstrated an ORR of 11.6% in all patients [8]. Notably, the ORR was 15.5% in patients with PD-L1 positive tumors and only 6.4% in PD-L1 negative tumors. Complete responses were seen in both the PD-L1-positive and PD-1 L-negative cohorts. 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 III 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 [9]. The initial 489 patients were enrolled regardless of PD-L1 status, but following a protocol amendment, the remaining patients were required to have a PD-L1 combined positive score (CPS) of at least 1. 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 in patients with gastric of GEJ cancers that have progressed on first-line chemotherapy. They also noted that protocol-specific and post-hoc subgroup analyses did suggest improved 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 and CheckMate-032 trials [10]. The ATTRACTION-2 trial was a phase III study performed in East Asia that randomized 493 patients with gastric or GEJ cancers who had received at least two prior lines of systemic therapy in a 2:1 ratio to the anti-PD-1 monoclonal antibody, nivolumab, or placebo. The median OS in the nivolumab group was 5.26 months, compared to 4.14 months in the placebo group (HR 0.63, *P*: 0.0001), with 10% of patients in the nivolumab group experiencing a grade 3 or 4 adverse event compared with 4% in the placebo group [11]. Similarly, the phase III ATTRACTION-3 study evaluated the role of nivolumab in patients with advanced squamous (or adenosquamous) esophageal or GEJ cancer who had progressed on first-line fluoropyrimidine and platinum-based therapy and demon-

strated a median OS of 10.9 months in the nivolumab group vs. 8.4 months in the chemotherapy group (HR 0.77, 95% CI 0.62–0.96, p = 0.019) indicating that nivolumab has activity in the second-line setting in this patient population [12]. CheckMate-032 randomized patients in the United States and Europe with chemotherapy refractory gastric, esophageal, or GEJ cancer to receive nivolumab or combination nivolumab with the anti-CTLA-4 antibody ipilimumab. Patients treated with nivolumab monotherapy had an ORR of 12% and a 12-month progression-free survival (PFS) of 8% [10]. The clinical activity of pembrolizumab in patients with metastatic esophageal cancer (adenocarcinoma or squamous cell carcinoma) who had progressed on one line of therapy was evaluated in the KEYNOTE-181 trial. Patients with a CPS of >10 treated with pembrolizumab had a median OS of 9.3 months compared to 6.7 months in the chemotherapy arm (HR 0.69; 95% CI: 0.52–0.93, p = 0.0074) further solidifying the role of immunotherapy in the second-line and beyond settings in patients with advanced esophageal and gastric cancers [13].

The anti-PD-L1 antibody, avelumab, has also been evaluated in patients with advanced gastric or GEJ cancers. A group of 150 patients with gastric or GEJ cancers were enrolled in the phase Ib JAVELIN Solid Tumor trial, 90 in the first-line maintenance setting and 60 in the second-line setting [14]. In both groups, the RR was modest 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 evaluated the utility of maintenance avelumab vs. continuation of chemotherapy in patients with advanced gastric or GEJ cancers with at least stable disease after 12 weeks of first-line fluoropyrimidine and platinum-based chemotherapy. This study failed to demonstrate an OS benefit with avelumab maintenance versus continuation of chemotherapy [15]. 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 [16]. No difference was observed between the avelumab and chemotherapy arms, with a median OS of 4.6 and 5.0 in the avelumab and chemotherapy arms, respectively [16]. Although technically negative trials, both JAVELIN Gastric 100 and JAVELIN Gastric 300 studies suggested a safety profile favoring avelumab over chemotherapy.

Patients harboring a high tumor mutational burden (TMB) have also demonstrated clinical benefit to treatment with checkpoint inhibitors, regardless of the primary site of cancer. KEYNOTE-158 enrolled patients with MSI-H/dMMR solid tumors (non-colorectal primary) who had progressed on prior treatment and had no standard of care treatment options remaining and treated them with single-agent nivolumab. Patients that had a high TMB (>10 mutations/megabase) had an ORR of 29% with a 4% CR rate, and 50% of patients have a duration of response ≥24 months [17]. It was on the basis of this study that the FDA approved pembrolizumab for patients with TMB-high tumors without alternative treatment options.

2.2 Future Strategies

A major focus of ongoing clinical trials of gastric and esophageal cancer is to evaluate the efficacy of T-cell checkpoint inhibitors in localized disease and in earlier lines of therapy for advanced disease. Results from the phase III Checkmate-577 study evaluated the utility of adjuvant nivolumab in patients with esophageal or GEJ cancer who had residual disease on surgical specimen following neoadjuvant chemotherapy and radiation. The results of this study were recently presented at the European Society for Medical Oncology (ESMO) 2020 Virtual Congress and demonstrated that patients receiving adjuvant nivolumab had a median disease-free survival (DFS) of 22.4 months compared to 11.0 months in patients receiving placebo (HR: 0.69; 95% CI: 0.56–0.86); p = 0.0003) [18]. It is interesting to note that most patients in this study had tumors that were PD-L1 negative. OS data are not yet mature, but the improvement seen with the primary endpoint of DFS in patients receiving adjuvant nivolumab will likely change the standard of care of patients with localized esophageal or GEJ with

residual disease after neoadjuvant chemotherapy and radiation. Checkmate-649 evaluated the role of nivolumab plus chemotherapy vs. chemotherapy alone vs. combination ipilimumab+nivolumab in patients with previously untreated advanced gastric cancer, esophageal adenocarcinoma, or GEJ cancer. The primary endpoint was OS and PFS in patients with a PD-L1 CPS score of ≥5. Results from this trial were also presented at the ESMO 2020 Virtual Congress and showed that patients with a PD-L1 CPS score of ≥5 receiving nivolumab+chemotherapy had a median OS of 14.4 months vs. 11.1 in the chemotherapy arm (HR: 0.71; 95% CI: 0.59–0.86; p < 0.0001) [19]. Median PFS also favored the nivolumab+chemotherapy group, with a PFS of 7.7 months vs. 6.1 months in the chemotherapy group. Interestingly, patients with a PD-L1 CPS score of ≥1 and all randomized patients had a prolonged OS with nivolumab+chemotherapy vs. chemotherapy alone. Results from the ipilimumab+nivolumab arm are not yet available. ATTRACTION-4 is a phase II/III study conducted in Asia evaluating the efficacy of first-line nivolumab+chemotherapy (oxaliplatin+S-1 or capecitabine) vs. chemotherapy alone in patients with advanced gastric or GEJ cancer regardless of PD-L1 status. Patients receiving nivolumab+chemotherapy had a median PFS of 10.5 months vs. 8.3 months in patients receiving chemotherapy alone (HR:0.68; 95% CI: 0.51–0.90; p = 0.007). There was no difference in OS between the two groups [20]. It is unknown as of yet whether regulatory agencies will grant approval for nivolumab+chemotherapy only in patients with a CPS score of ≥5 or will extend approval more broadly.

Keynote-590 is a phase III study evaluating the utility of adding pembrolizumab to chemotherapy in patients with previously untreated advanced esophageal or Siewert type 1 GEJ cancer. In patients with a PD-L1 CPS score of ≥10, median OS was 13.5 months in patients receiving pembrolizumab+chemotherapy vs. 9.4 months in patients receiving chemotherapy alone (HR: 0.62; 95% CI: 0.49–0.78; p < 0.0001). In the intention to treat analysis, median OS regardless of PD-L1 status was 12.4 months vs. 9.4 months (HR: 0.73, 95% CI: 0.62–0.86; p < 0.0001) [21].

There is also interest in incorporating immunotherapy into earlier lines of therapy in patients with HER-2 amplified GI malignancies. 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 ORR of 83% with three complete responses and a median PFS of 11.4 months [22]. This combination is currently being evaluated in the phase III KEYNOTE 811 trial [23]. Another study in Japan is evaluating the combination of nivolumab and trastuzumab combined with chemotherapy in patients with HER-2 amplified gastric cancers [24].

3 Colorectal Cancer: MSI-H

3.1 Current Evidence

The subset of patients with colorectal cancer (CRC) who have benefited most from advances in immunotherapy have been those whose tumors are microsatellite instability-high (MSI-H) or harbor defects in the mismatch repair apparatus (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 [25, 26]. Only 4–5% of patients with metastatic CRC are MSI-H, and the majority of these cases result from sporadic mutations in mismatch repair proteins rather than being associated with Lynch syndrome [27]. The immunogenicity of MSI-H tumors has been well-described, with the primary hypothesis being that high mutational load leads to a higher density of tumor-infiltrating lymphocytes (TIL) and increased expression of checkpoint inhibitors [28–30].

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 a landmark phase II study, which included a cohort

of patients with pretreated metastatic dMMR and mismatch repair-proficient CRC [31]. The ORR of patients with dMMR CRC was 40% vs. 0% in patients with mismatch repair-proficient CRC. 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% [32, 33]. 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.

CheckMate-142 was a phase II study evaluating the use of nivolumab monotherapy or nivolumab in combination with ipilimumab in patients with MSI-H and MSS metastatic CRC who had progressed on at least one line of therapy [34]. The results from the initial 74 patients with MSI-H metastatic CRC treated with nivolumab monotherapy were published in 2017. The ORR in patients with MSI-H metastatic CRC 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 [35]. 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 grades 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 who have progressed on at least one line of prior therapy.

While immune checkpoint inhibitors have demonstrated clinical benefit in patients with pretreated MSI-H CRC, it was unknown until recently whether there was any clinical benefit to moving immunotherapy to first-line therapy in these patients. KEYNOTE-177 was a phase III open-label trial evaluating the efficacy of pembrolizumab vs. chemotherapy in 307 treatment-naïve patients with metastatic MSI-H-dMMR CRC [36]. Treatment with pembrolizumab resulted in a media PFS 16.5 months vs. 8.2 months in the chemotherapy arm. ORR was 43.8% in the pembrolizumab and 33.1% in the chemotherapy group. Notably, of those patients responding to treatment, ongoing responses were observed at 24 months in 83% of patients in the pembrolizumab arm vs. 35% in the chemotherapy arm. Pembrolizumab was better tolerated than chemotherapy, with 22% of patients receiving pembrolizumab experiencing a grade 3 or greater adverse event vs. 66% in the chemotherapy arm [36]. It was on the basis of this study that the FDA approved the use of pembrolizumab as first-line treatment for patients with MSI-H/dMMR metastatic CRC.

3.2 Future Strategies

Results from KEYNOTE-177 solidified the role of pembrolizumab as first-line therapy for metastatic MSI-H/dMMR CRC, but there are several ongoing trials evaluating other checkpoint inhibitors in this setting. 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 [37].

Another avenue of exploration is the utility of immune checkpoint inhibitors in patients with stage III MSI-H CRC. The ATOMIC trial is evaluating adjuvant FOLFOX with or without atezolizumab, and the POLEM trial is evaluating maintenance avelumab for 24 weeks after com-

pletion of adjuvant chemotherapy and includes patients with POLE exonuclease domain mutations [38, 39]. Both of these trials are ongoing and results are eagerly awaited.

4 Colorectal Cancer: MSS

4.1 Current Evidence

Despite successes of several immune checkpoint inhibitors in the treatment of patients with MSI-H metastatic CRC, the vast majority of patients with metastatic CRC do not have MSI-H/dMMR disease and have not yet realized a benefit from immunotherapy. The phase II Canadian Cancer Trials Group (CCTG) CO.26 study 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 toward improved 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, $P = 0.07$) [40]. IMblaze370 was a phase III study that evaluated third-line combination therapy with atezolizumab and cobimetinib (MEK inhibitor) vs. atezolizumab monotherapy vs. regorafenib in patients with metastatic CRC. Approximately 90% of enrolled patients in this study had MSS CRC. The study did not meet its primary endpoint of improved OS [41]. To date, there are no approved indications for immunotherapy in patients with MSS CRC.

4.2 Future Strategies

Strategies aimed at converting immunologically "cold" tumors, such as MSS CRC, into inflamed tumors are an area of active investigation. There are several ongoing clinical trials combining radiation therapy with immunotherapy in patients with MSS CRC with the goal of harnessing the "abscopal effect." In this hypothesis, radiation therapy would induce local cell death (and release of neoantigens) and stimulate a productive immune response that extends to distant sites beyond the radiation field. 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 [42, 43]. 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 group of patients [44, 45]. There continues to be great interest in understanding the role of immunotherapy in patients with MSS CRC with many ongoing clinical trials evaluating either combination immunotherapies (e.g., dual immune checkpoint blockade), chemoimmunotherapy, or immunotherapy + a tyrosine-kinase inhibitor (TKI) in this patient population (Table 5.2).

5 Anal Cancer

5.1 Current Evidence

Squamous cell carcinoma (SCC) of the anus is a human papillomavirus (HPV)-associated malignancy with a pathophysiology which resembles that of other HPV-associated malignancies [46–48]. The safety and efficacy of pembrolizumab was evaluated in KEYNOTE-028, a phase Ib multicohort study [49]. The ORR in a cohort of 24 anal SCC patients was 17% and disease control rate was 58%. Sixty-four percent of patients experienced treatment-related adverse events. KEYNOTE-158 is another phase Ib multicohort study that evaluated pembrolizumab in patients with heavily pretreated solid tumors. One hundred twelve patients with anal SCC were enrolled with an ORR of 11.6% and five complete responses in these patients and no differences in response observed in those with PD-L1-positive or PD-L1-negative tumors [50]. The multicenter phase II trial, NCI9673, evaluated the clinical benefit of single-agent nivolumab in 37 patients

with pretreated metastatic anal SCC [51]. The ORR was 24%, including two complete responses. Immunohistochemistry analysis of tumor samples from patients in this study demonstrated a significantly higher expression of PD-1 and PD-L1 within tumors of those who responded to nivolumab compared with those who 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.

5.2 Future Strategies

Similar to other tumor types, investigations are ongoing to evaluate combination immunotherapy with anti-CTLA-4 and ant-PD-1 in patients with anal SCC. 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. Data from this arm are still pending.

Pembrolizumab is also being studied as monotherapy in a phase II study in patients with refractory metastatic anal SCC. A phase II study in France will be assessing the efficacy of the combination of atezolizumab and a HPV-directed vaccine, UCPVax, in patients with HPV-positive cancers (NCT03946358). 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 (NCT03233711). The SCARCE trial will evaluate the addition of atezolizumab with chemotherapy vs. chemotherapy alone in patients with chemotherapy-naïve metastatic anal SCC [52].

Novel immunotherapeutic approaches such as autologous T-cell therapy utilizing transgenic T cells or tumor-infiltrating T cells and vaccine-based approaches are also being investigated in anal SCC [53] (NCT02858310, NCT02399813). These approaches exploit the natural immunogenicity of HPV-related proteins and are a promising strategy to improve clinical outcomes in this disease.

6 Hepatocellular Carcinoma

6.1 Current Evidence

Immune checkpoint inhibitors have changed the landscape of treatment for hepatocellular carcinoma (HCC). Tremelimumab was the first immune checkpoint inhibitor studied in HCC [54]. 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 treatment with tremelimumab was fairly well tolerated. In 2017, single-agent nivolumab was granted accelerated approval by the FDA as a second-line agent without any biomarker requirement. This approval was based on the CheckMate-040 study, a phase I/II trial which included 262 total patients, some with treatment-naïve disease and some having previously been treated with sorafenib [55]. The safety profile was manageable in this study, and the objective RR was 20% (95% CI: 15–26%) with nivolumab 3 mg/kg in the dose-expansion phase. This trial also had an arm evaluating the toxicity and activity of nivolumab 1 mg/kg + ipilimumab 3 mg/kg with and ORR of 32%, CR rate of 8%, and a median OS of 22.8 months [56]. KEYNOTE-240 was a phase III study evaluating pembrolizumab as a second-line therapy in patients with advanced HCC. The co-primary endpoints of OS and PFS did not reach statistical significance per prespecified criteria [57]. KEYNOTE-224 was a phase II study that evaluated pembrolizumab in patients with HCC previously treated with sorafenib. Of the 104 patients treated, 18 (17%) experienced a response, with one complete response and an OS of 54% at 12 months [58]. Based on the results of CheckMate-040 and KEYNOTE-224, both nivolumab and pembrolizumab and combination nivolumab 1 mg/kg and ipilimumab 3 mg/kg are approved by the FDA for the treatment of

advanced HCC that has progressed on one line of therapy.

Results from the IMbrave-150 study were recently published and have since changed the standard of care for first-line therapy of patients with unresectable, treatment-naïve HCC. IMbrave-150 is a phase III, open label trial of patients with systemic treatment-naïve, unresectable HCC who were randomized to receive atezolizumab+bevacizumab vs. sorafenib. Overall survival at 12 months was 67.2% in the atezolizumab+bevacizumab group vs. 54.6% with sorafenib with a PFS of benefit of approximately 2.5 months in favor of the atezolizumab+bevacizumab group [59]. It was on the basis of this study that the FDA granted approval of atezolizumab+bevacizumab in patients with treatment-naïve, unresectable HCC.

6.2 Future Strategies

There are ongoing efforts to combine immunotherapy with TKIs or local therapies to help improve both response rates and durability of responses. IMMULAB is a phase II study of pembrolizumab in combination with local ablation in patients with HCC (NCT03753659). The EMERALD-1 trial is phase III study evaluating the efficacy of transarterial chemoembolization (TACE) in combination with durvalumab and bevacizumab in patients with locoregional HCC [60]. These trials are currently active and recruiting patients. The combination of pembrolizumab+lenvatinib was evaluated in a phase Ib trial in patients with treatment-naïve advanced HCC with an ORR of 46% and a median duration of response of 8.6 and one CR [61]. Based on available data, it is likely that the addition of immune checkpoint inhibitors to local therapies and TKIs will improve response rates, but it is unclear as to how these treatments should be sequenced. Similarly, more work is needed to determine the optimal subsequent therapies once patients have experienced disease progression on immunotherapy.

7 Biliary Tract Cancers

7.1 Current Evidence

Biliary tract cancers (BTCs) are a rare subset of GI malignancies, comprising cholangiocarcinoma and gallbladder 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 (as low as 1% and as high as 10%) of BTC patients who have tumors with MSI-H or dMMR disease [62]. The phase II KEYNOTE-158 basket trial had a total of 104 patients with BTC, none of whom had MSI-H tumors. The ORRwas a dismal 5.8%, with a PFS of 2.0 months and an OS of 7.4 months [63].

7.2 Future Strategies

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 of patients 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 [64]. In addition to immune checkpoint inhibitors, other immunotherapy strategies such as adoptive T-cell therapy and vaccines are being evaluated in BTC. Mucin protein 1 (MUC1) and Wilms' tumor protein 1 (WT1) are two tumor-associated antigens that are expressed on >80% of BTCs [64]. In a phase I study of eight BTC patients treated with gemcitabine and a WT1 vaccine, half of the patients achieved stable disease at 2 months [65]. Another phase I study with a MUC1 vaccine in eight BTC and pancreatic cancer patients yielded an even lower disease control rate [66]. A clinical trial

assessing the utility adjuvant adoptive T-cell therapy combined with a postoperative dendritic cell vaccine in resectable intrahepatic cholangiocarcinoma patients demonstrated an increased PFS in OS in favor of the patients receiving adjuvant vaccine-based therapy vs. surgery alone [67]. These results are encouraging and warrant further investigation into the role of immunotherapy in patients with BTC.

8 Pancreatic Cancer

8.1 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 [68, 69]. Several studies have concluded that PD-L1 expression in PDAC is associated with a poor prognosis [70]. Despite these formative barriers, 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 evaluating impact of nivolumab in patients with advanced solid tumors [71]. Unfortunately, none of the PDAC patients achieved an objective response. Ipilimumab as a monotherapy administered at a dose of 3 mg/kg dose was evaluated in a phase II trial of patients with advanced PDAC [72]. 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. A Johns Hopkins study combined ipilimumab with GVAX, a GM-CSF cell-based vaccine, in patients with advanced PDAC. Compared to ipilimumab alone, the combination of ipilimumab and GVAX showed a trend toward increased median OS (3.6 vs. 5.7 months, HR: 0.51, P = 0.07) and 1-year OS (7% vs. 27%) [73]. 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 [74]. 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. Similarly, a phase I study of nivolumab in combination with gemcitabine+nab-paclitaxel in patients with advanced PDAC demonstrated a modest ORR of 18% [75].

Currently, the role of immunotherapy with immune checkpoint inhibitors is relegated to patients with MSI-H/dMMR PDAC, a population that may represent as little as <1% of all PDAC patients [76, 77].

8.2 Future Strategies

There continues to be interest in combining immunotherapy with other treatment modalities, including radiation, in early stages of disease in patients with PDAC. One study evaluated 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. Similarly, a phase I/II study of durvalumab with SBRT in locally advanced pancreatic cancer showed a 54% partial response rate with a 6% CR rate and a high rate of margin-negative resections [78]. A number of other studies evaluating the combination of radiation and immunotherapy in patients with PDAC are currently ongoing (NCT02648282, NCT03915678, NCT03563248).

CD40 has proved to be an emerging target for the treatment of PDAC. CD40 agonist antibodies have been shown to change the phenotype of tumor-infiltrating macrophages from an immunosuppressive phenotype to an antitumor phenotype. Combination therapy with a CD40 agonist

antibody and gemcitabine resulted in tumor regression in preclinical models as well as in a subset of patients with PDAC [79]. A phase Ib study evaluated the CD40 agonist antibody, APX005M, in combination with gemcitabine+nab-paclitaxel with or without nivolumab in patients with advanced PDAC. The regimen with or without nivolumab was tolerable and resulted in response rate of 58% [75]. While this response rate is impressive, caution must be exercised in interpreting the results from this phase Ib study as larger, phase III studies are needed to truly elucidate any clinical benefit from the addition of APX005M to standard chemotherapy in these patients.

Therapeutic approaches utilizing adoptive cell therapy such as chimeric antigen receptor T cell (CAR-T) are also being evaluated in patients with PDAC. CAR-T cells have significantly advanced the treatment of patients with certain relapsed and refractory hematologic malignancies, but attempts to carry these benefits over to patients with solid tumors are still in early stages. For patients with PDAC in particular, various CAR-T cells have been engineered to recognize MUC1, carcinoembryonic antigen (CEA), and mesothelin (MSLN) [80–82]. There is cautious optimism that CAR-T cell therapy for PDAC may represent a novel immunotherapeutic strategy for this devastating malignancy. It is likely that an effective immunotherapeutic treatment package for patients with PDAC will involve strategies targeting T cells (e.g., immune checkpoint inhibitors, adoptive T-cell therapy) in combination with strategies to reprogram the suppressive tumor immune microenvironment (e.g., CD40-agonist antibodies).

9 Conclusion

Immunotherapy has changed the treatment landscape for a variety of solid tumors, including a subset of GI malignancies. Patients with MSI-H/dMMR tumors and tumors with a high TMB experience durable responses to immune checkpoint inhibitors. Combination therapy with immune checkpoint inhibitors and chemotherapy will likely become the new, front-line standard of care in patients with gastric and GEJ tumors. The combination of immune checkpoint inhibitor and bevacizumab has already supplanted single-agent TKI as front-line therapy of patients with advanced HCC. Despite these recent advances, response rates to immunotherapy in GI malignancies continue to be low. Malignancies such as PDAC and BTC seem particularly refractory to efforts to harness the immune system for therapeutic benefit. A more thorough understanding as to why some patients benefit from immunotherapy while others do not is needed in order to improve response rates and overall clinical outcomes. Emerging data suggest that the immunosuppressive tumor microenvironment is a key player in limiting the efficacy of traditional forms of immunotherapy, and effort to reprogram this microenvironment in GI malignancies is an area of active research. Novel combination strategies with chemotherapy, radiotherapy, and/or targeted therapy are currently being studied and may provide a critical immunologic boost to overcome resistance to immunotherapy. The next generation of cancer vaccines and adoptive cell therapy offer promise in the treatment of GI malignancies. It is likely that the future of immunotherapy of GI malignancies will involve a combinatorial approach utilizing various forms of immunotherapy, including immune checkpoint inhibitors, costimulatory engagers, adoptive cell therapy, and efforts to target the tumor immune microenvironment in conjunction with traditional chemotherapy and radiation in order to maximize clinical benefit.

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An Update on Immune Based Therapies in Acute Myeloid Leukemia: 2021 and Beyond!

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Abstract

Despite advances in the treatment of acute myeloid leukemia (AML), relapse is still widely observed and represents the major cause of death among patients with AML. Treatment options in the relapse setting are limited, still relying predominantly on allogeneic hematopoietic stem cell transplantation (allo-HSCT) and cytotoxic chemotherapy, with poor outcomes. Novel targeted and venetoclax-based combinations are being investigated and have shown encouraging results. Immune checkpoint inhibitors in combination with low-intensity chemotherapy demonstrated encouraging response rates and survival among patients with relapsed and/or refractory (R/R) AML, especially in the pre- and post-allo-HSCT setting. Blocking the CD47/SIRPα pathway is another strategy that showed robust anti-leukemic activity, with a response rate of around 70% and an encouraging median overall survival in patients with newly diagnosed, higher-risk myelodysplastic syndrome and patients with AML with a *TP53* mutation. One approach that was proven to be very effective in the relapsed setting of lymphoid malignancies is chimeric antigen receptor (CAR) T cells. It relies on the infusion of genetically engineered T cells capable of recognizing specific epitopes on the surface of leukemia cells. In AML, different CAR constructs with different target antigens have been evaluated and demonstrated safety and feasibility in the R/R setting. However, the difficulty of potently targeting leukemic blasts in AML while sparing normal cells represents a major limitation to their use, and strategies are being tested to overcome this obstacle. A different approach is based on endogenously redirecting the patient's system cells to target and destroy leukemic cells via bispecific T-cell engagers (BiTEs) or dual antigen receptor targeting (DARTs). Early results have demonstrated the safety and feasibility of these agents, and research is ongoing to develop BiTEs with longer half-life, allowing for less frequent administration schedules and developing them in earlier and lower disease burden settings.

Keywords

Immunotherapy · Checkpoint inhibitors · AML · Nivolumab · Pembrolizumab · Azacitidine · Magrolimab · BiTE · DART · TriKE

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1 Background

Acute myeloid leukemia (AML) is a malignant clonal hematopoietic disorder arising from genetic and/or epigenetic alterations affecting the hematopoietic progenitor cells in the bone marrow and resulting in detrimental effects to critical cellular pathways such as self-renewal, differentiation, and proliferation [1]. AML is diagnosed at a median age of 67 years, with around one-third of patients aged ≥75 years at diagnosis [2]. It can occur de novo in around 80% of cases or can occur secondary to a previous hematologic disorder or myelotoxic therapies.

Acute myeloid leukemia is a heterogeneous disease, stratified into three risk-groups based on cytogenetic and molecular characteristics, according to the European LeukemiaNet (ELN 2017) criteria, with implications on remission and survival [3]. The cure rates of AML are relatively low and decrease with age, with a 5-year overall survival (OS) rate of around 35% and 11% in patients aged <60 years and ≥ 60 years, respectively [4]. Cytotoxic chemotherapy has traditionally been the backbone of AML therapy, including high-intensity cytarabine-based regimens and/or allogeneic hematopoietic stem cell transplantation (allo-HSCT) for intermediate-/high-risk patients [5]. Intensive therapies, however, are associated with a high mortality rate in older patients and individuals with comorbidities and/or poor performance status [6, 7]. Alternative lower-intensity therapies with hypomethylating agents (HMAs) have been used in frail patients over the past 15–20 years. These regimens were associated with lower rates of remission, less early mortality, and a median OS of around 7–8 months [8]. Recently, the combination of the hypomethylating agent azacitidine with venetoclax showed improvement over azacitidine alone, with higher remission rates around 70% and improved median OS of 15–18 months.

Nevertheless, disease relapse following conventional therapy represents the major cause of death in patients with AML. Currently, allo-HSCT is considered the only therapeutic modality that is potentially curative in relapsed and/or refractory (R/R) AML. The efficacy of this approach is mainly driven by the graft-versus-leukemia effect that occurs when donor T cells recognize foreign antigens on the host's hematopoietic tissues and eliminate tumor (graft-versus-leukemia effect) [9, 10]. However, relapse following allo-HSCT is still a major challenge with a dismal prognosis [11]. Therefore, there remains significant unmet need for patients with R/R AML, especially those with no actionable mutations (such as FLT3 and IDH) and for patients who have exhausted standard treatment options.

Attractive strategies have been recently investigated and focused on redirecting the patient's own immune system to target leukemic cells. These approaches include T-cell or macrophage immune checkpoint inhibitors, chimeric antigen receptor (CAR) T cells, and bispecific T-cell engaging antibodies (BiTEs) and will be discussed in this review [12, 13].

2 Immune-Based Therapies in Acute Myeloid Leukemia

Over the past few years, different immunotherapy-based strategies have been evaluated in both the pre-clinical and the clinical settings among patients with hematologic malignancies, with significant efficacy and cure rates, even in the relapsed/refractory setting [14]. Progress has been most remarkable in B-lymphoid malignancies, such as Hodgkin's and non-Hodgkin's lymphomas and acute lymphoblastic leukemia (ALL), where CAR T cells, BiTEs, and immune checkpoint inhibitors have demonstrated robust clinical responses [15–18]. Efforts have been made to apply these modalities to try and duplicate these positive results in the field of myeloid malignancies.

2.1 Immune Checkpoint Inhibitors

CTLA-4 (or CD152) is a B7/CD28 family member that inhibits T-cell functions through its interaction with B7 present on the surface of

antigen-presenting cells such as dendritic cells, macrophages, and monocytes, thereby diminishing signaling through the costimulatory receptor CD28 [19, 20]. The anti-CTLA-4 antibody ipilimumab was the first immune checkpoint inhibitor (ICI) to be US FDA approved for the treatment of metastatic melanoma [21, 22]. PD-1 is another important checkpoint molecule expressed on T cells, homologous to CD28, and is primarily involved in inhibitory immune signaling [23]. PD-L1 (CD274) and PD-L2 (CD273) are the two ligands of PD-1 and can be found on the surface of antigen-presenting cells (Fig. 1) [24, 25]. PD-1 interferes with TCR/CD28 signaling and results in reduced cytokine production (such as interleukin-2, interferon gamma, and tumor necrosis factor alpha), cell cycle progression, and pro-survival Bcl-xL gene expression [26–28]. The inhibition of the PD-1/PD-L1 and CTLA-4 pathways leads to the stimulation of an antitumor response [29]. This strategy was tested and showed significant clinical efficacy in the treatment of multiple cancer types, which led to a number of US FDA approvals for multiple tumor types over the past several years, in both the advanced and early disease settings [30, 31].

The remarkable efficacy with ICI therapies in solid malignancies has not been reproducible to date in myeloid malignancies. One possible explanation is the low mutational burden described in AML that is one of the lowest among human cancers, meaning that fewer neoantigens are recognized by T cells [32, 33].

2.1.1 Efficacy in the Pre-allogeneic Hematopoietic Stem Cell Transplantation Setting

In hematologic malignancies, blocking PD-1 with single-agent nivolumab resulted in substantial clinical efficacy in patients with relapsed or refractory Hodgkin's lymphoma [34]. In non-Hodgkin's lymphoma, ICIs were not successful as monotherapy [35] but rather when used in specific combinations [36]. In AML, given the limited efficacy of ICIs as single agents, combination strategies have been the focus of clinical investigation. Hypomethylating agents, azacitidine and decitabine, are used for the treatment of patients with myelodysplastic syndromes (MDS) and for older patients with newly diagnosed AML [37, 38]. Both agents were found to upregulate interferon-gamma pathway genes, increase the

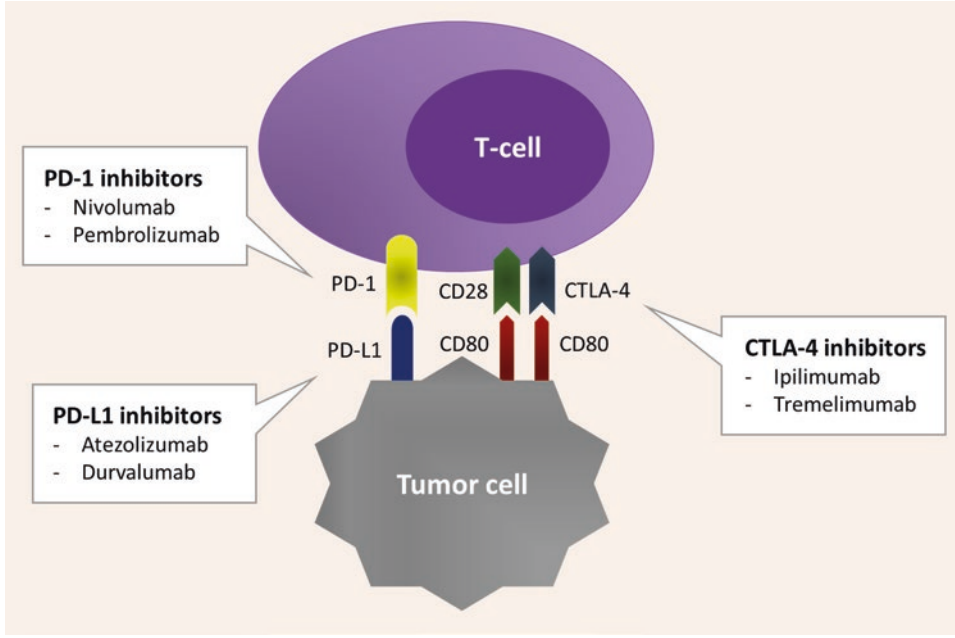

Fig. 1 Mechanism of action of immune checkpoint inhibitors

expression of human leukocyte antigen (HLA) class I antigens, and activate viral defense pathways, thereby promoting antitumor immune signaling [39]. The use of HMAs in MDS/AML, as well as in solid tumors, also leads to an upregulation of PD-1 and PD-L1 expression by a direct hypomethylation of the PD-1 and PD-L1 promoters resulting in increased transcription [40, 41]. Therefore, combining ICIs with HMAs represented a potential synergistic approach. A phase 2 study was conducted at the University of Texas MD Anderson Cancer Center (MDACC) wherein patients with R/R AML were treated with azacitidine 75 mg/m^2 on days 1–7 in combination with nivolumab 3 mg/kg on days 1 and 14, every 4–6 weeks [42]. Seventy patients were treated, with a median age of 70 years (range: 22–90) and a median of 2 (range: 1–7) prior therapies. Fifteen patients (22%) achieved a complete remission (CR)/CR with incomplete count recovery (CRi), one partial response (PR), and seven hematologic improvement maintained >6 months, for an overall response rate (ORR) of 33%. The ORR was higher in the HMA-naïve group compared to the HMA-prior exposed group (58% versus [vs.] 22%). The median OS with this combination compared favorably to the historical cohort treated with azacitidine alone, both in the "all salvage" population (6.3 vs. 4.6 months, P = 0.013) and especially so in the "first salvage" population (10.6 vs. 5.3 months, P = 0.011). Grade 3–4 immune-related adverse events (AEs) occurred in eight (11%) patients and were manageable with early identification and use of steroids. The presence of a higher proportion of CD3- and CD8-positive T cells in the pretherapy bone marrow or peripheral blood was associated with an increased CR/CRi rate suggesting that biomarker-driven strategies may be more successful, allowing for enrichment of patients more likely to respond to such immune-based strategies. The results of this trial suggest that the combination of nivolumab and azacitidine appeared to be safe and effective for the treatment of patients with R/R AML, particularly in HMA-naïve patients and in first salvage patients (median OS in salvage 1 was 10.6 months) [42].

Low-intensity chemotherapy in combination with pembrolizumab was also examined in a multicenter, phase 2 trial of patients with R/R AML. Thirty-seven patients with a median age of 65 years (range: 19–83) were enrolled to receive azacitidine 75 mg/m^2 on days 1–7 every 4–6 weeks, with pembrolizumab 200 mg started on cycle 1 day 8 and continued every 3 weeks thereafter. Twenty-one patients (57%) had poor risk cytogenetics, and eight (22%) had *TP53* mutation. Twenty-nine patients were evaluable for response: four achieved CR/CRi (14%), one PR (4%), four hematologic improvement (14%), and seven stable disease for ≥6 cycles (24%). With a median follow-up of 14.9 months, the median OS was 10.8 months for the whole cohort and 17.2 months for patients in CR/CRi/PR. The median DFS for patients in CR/CRi was 8.5 months. Grade 2 immune-related AEs were observed in five patients (11%) and grades 3–4 in nine patients (24%), managed with steroids and supportive care [43]. This trial showed that the combination of azacitidine and pembrolizumab was also safe, feasible, and well tolerated for patients with R/R AML.

The combination of checkpoint inhibitors with more intensive chemotherapy was also evaluated. In addition to directly inducing the death of tumor cells, chemotherapy may also reinstate immune surveillance [44, 45]. Multiple mechanisms have been postulated to enhance the immune response, including an improved antigen uptake and chemotactic response by antigen-presenting cells, better recognition of neo-epitopes by the major histocompatibility complex (MHC) I and T-cell receptors, and a higher susceptibility of tumor cells to immune-mediated cytotoxicity, resulting in rapid antigen dispersion and immune priming [46, 47]. Chemotherapy is capable of inducing an immunogenic cell death [44], a phenomenon characterized by the secretion of interferon-gamma, which leads to the proliferation of cytotoxic T cells and also causes an increased expression of PD-L1 on leukemic blasts [48, 49]. The upregulation of PD-L1 on leukemic cells is considered to limit the ability of cytotoxic T cells to eradicate malignant cells, which may lead to AML relapse [50]. Notably, anthracyclines,

which represent the backbone of the "3 + 7" regimen in AML, have been shown to be potent inducers of immunogenic cell death [51]. Based on this rationale, the combination of chemotherapy with immune checkpoint inhibition was tested.

Nivolumab was combined with high-intensity IA chemotherapy in a phase 2 trial conducted at MDACC. Patients aged 18–60 years (or > 60 years if fit for intensive chemotherapy) with newly diagnosed AML or high-risk MDS received induction chemotherapy with cytarabine 1.5 g/m^2 by continuous infusion over 24 hours daily on days 1–4 (3 days in patients >60 years) plus idarubicin 12 mg/m^2 daily on days 1–3. Nivolumab was administered at the dose of 3 mg/kg every 2 weeks, starting at day 24. Consolidation therapy consisted of attenuated doses of idarubicin (8 mg/m^2 daily for 2 days) and cytarabine (0.75 g/m^2 over 24 hours daily for 3 days) every 4 to 6 weeks for up to five cycles or allo-HSCT [52]. Forty-four patients were enrolled, with a median age of 54 years (20% aged >60 years) with ELN adverse-risk cytogenetics in 50%. After a median follow-up of 17.25 months, the median OS was 18.5 months, and the relapse-free survival (RFS) among responders was 18.5 months. Mortality during induction was seen in two patients (5%). Six patients (14%) had grades 3–4 immune-related AEs consisting of rash (n = 2), colitis (n = 2), transaminitis, pancreatitis, and cholecystitis (n = 1, each). Nineteen patients (43%) proceeded to allo-HSCT, with grades 3–4 graft-versus-host disease (GVHD) seen in five patients (26%). The examination of baseline bone marrow samples revealed a higher percentage of CD4-positive T-effectors expressing PD-1/TIM-3 (P = 0.01) and PD-1/LAG-3 (P = 0.04) in nonresponders compared to responders [52].

Another phase 2 trial examined the role of the combination of the anti-PD-1 agent pembrolizumab with high-dose cytarabine (HiDAC) in patients with R/R AML [53]. Thirty-seven patients were enrolled, 16 (43%) with refractory disease and 21 (57%) with relapsed AML. Patients received induction therapy with age-adjusted HiDAC (<60 years, 2 g/m^2 every 12 hours on days 1–5; ≥60 years, 1.5 g/m^2 every 12 hours on days 1–5) followed by pembrolizumab 200 mg on day 14. Responders received maintenance therapy with pembrolizumab 200 mg every 3 weeks for up to 2 years or until progression of the disease. The median age was 54 years (range: 24–70) and the ORR was 46% (CR/CRi 38%, PR 5%, MLFS 3%). After a median follow-up of 7.8 months, the median OS among survivors was 8.9 months, EFS 6.9 months, and DFS 5.7 months. Nine patients (24%) received allo-HSCT, with no grades 4–5 GVHD or veno-occlusive disease post-transplant. Among nine patients (24%) who received maintenance therapy with pembrolizumab, seven relapsed after the maintenance phase. The most common AEs were febrile neutropenia (57%), ALT and AST elevation (43% and 32%, respectively), fatigue (27%), alkaline phosphatase elevation (24%), and maculopapular rash (19%). Immune-related AEs of grade > 3 were uncommon, maculopapular rash (5%), transaminitis (5%), and hepatitis (3%) [53]. These results suggest the safety and efficacy of pembrolizumab administration following HiDAC in the salvage setting, with an encouraging ORR of 46% in patients with R/R AML. Future studies are needed to better assess the role of pembrolizumab in AML and to identify immunogenomic biomarkers of response to treatment.

2.1.2 Efficacy in the Post-allogeneic Hematopoietic Stem Cell Transplantation Setting

The blockade of CTLA-4 in an autologous culture system led to an increase in the activity and proliferation of AML-reactive T cells [54]. An early phase 1 clinical trial demonstrated the potential role of CTLA-4 inhibition in AML. In this study, 28 patients with hematologic malignancies relapsing following allo-HSCT, including 12 patients with AML and 2 patients with MDS, were treated with ipilimumab at the dose of 3 or 10 mg/kg every 3 weeks. Among the 14 patients with AML/MDS, five (36%) had a complete response (four out of five patients had extramedullary disease), and all responders were treated with ipilimumab at the higher dose of 10 mg/kg. Six patients in the total cohort (21%)

experienced grades 3–4 immune-related AEs, including one death from possible immune-related AEs, and four (14%) had GVHD that precluded further administration of ipilimumab [55]. These single-agent findings supported the use of ICIs following allo-HSCT failure in order to restore antitumor activity and achieve responses through a graft-versus-leukemia effect.

In AML patients relapsing after bone marrow transplant, PD-1 expression was found to be significantly upregulated on T cells compared to diagnosis, with no impact on proliferation or cytokine production [56]. On the other hand, a significant increase in the expression of PD-L1 on the leukemia cells was not consistently detected [57]. Nevertheless, the expression profile of PD-1 and its ligands impacted prognosis, with $PD1^{-/-}$ mice inoculated with AML having slower disease progression compared to PD-1-sufficient mice [58], and the increased expression of PD-L1 and/or PD-L2 by AML blasts was probably associated with poor prognosis [59]. Therefore, blocking the interaction between PD-1 and PD-L1/PD-L2 may be exploited in AML patients relapsing following allo-HSCT.

One theoretical concern associated with the use of ICIs before or after allo-HSCT is the induction of GVHD. Therefore, the first trial of ipilimumab in relapsing hematologic malignancies was initiated with very low doses of the drug. Among 29 patients with recurrent or progressive disease after allo-HSCT treated on this study, including two patients with AML, no dose-limiting toxicity and no evidence of GVHD were reported [60]. On the other hand, reports of anti-PD-1 agents used for lymphoma therapy prior to allo-HSCT resulted in higher than predicted grades 2–4 and grades 3–4 acute GVHD in 44% and 23%, respectively [61]. Despite the increased risk of GVHD associated with PD-1 blockade, ongoing trials with nivolumab and pembrolizumab following allo-HSCT are ongoing.

The risk of acute GVHD following allo-HSCT in patients with prior ICIs therapy was analyzed in a retrospective study of 43 patients with AML and/or MDS treated with anti-PD-1 and/or anti-CTLA-4 agents prior to allo-HSCT. Patients were retrospectively stratified by GVHD prophylaxis with or without post-HSCT cyclophosphamide (PTCy). A higher incidence of grades 3–4 acute GVHD was observed among patients treated with ICIs in the pre-transplant setting compared to a matched cohort, as well as in patients who received >4 immunotherapy cycles prior to allo-HSCT if they were not given prophylactic PTCy post-allo-HSCT (43% vs. 12%). However, that increased risk was limited to patients who did not receive PTCy. Patients who received PTCy prophylaxis had a trend toward lower grades 3–4 acute GVHD compared with patients who did not (5% vs. 22%), with no detrimental effect on survival with a 1-year PFS rate of 55% in the PTCy group, compared to 22% in the non-PTCy group. These results indicate that ICIs therapy prior to allo-HSCT was feasible and safe in patients with AML, and the use of PTCy as GVHD prophylaxis improves outcomes [62]. Additional strategies, such as platforms with low GVHD potential with T-cell depleted allo-HSCT, can lower the incidence of acute and chronic GVHD [63].

2.1.3 Efficacy in the Frontline Setting of Elderly and Unfit Patients

In a multicenter phase 2 study, 22 newly diagnosed older AML patients with a median age of 75 years (range: 67–83) were treated with azacitidine 75 mg/m^2 on days 1–7 every 4 weeks and pembrolizumab 200 mg started at cycle 1 day 8 and continued every 3 weeks thereafter. Fourteen patients (64%) had poor risk cytogenetics, and five patients (23%) had *TP53* mutation. Among 17 evaluable patients, 8 achieved CR/CRi (47%), 2 PR (12%), 2 hematologic improvement (12%), and 4 stable disease for at least 6 cycles (24%). With a median follow-up of 19 months, the median OS was 13.1 months for the whole cohort and not reached for patients in CR/CRi/PR (1-year OS rate, 79%). The median DFS for patients in CR/CRi was 16.6 months. Grade 2 immune-related AEs were observed in four patients (18%) and grades 3–4 in three patients (14%), managed with steroids and supportive care in the majority of cases [43]. These findings showed encouraging activity of this combination in newly diagnosed elderly AML patients.

The combination of HMA plus ICI among older patients was also evaluated in a large randomized, multicenter study that enrolled 129 AML patients aged ≥65 years who were ineligible for intensive chemotherapy. Patients were randomized (1:1) to receive azacitidine 75 mg/m^2 on days 1–7 plus the anti-PD-L1 agent durvalumab 1500 mg on day 1 every 4 weeks (Arm A, 64 patients) or azacitidine alone (Arm B, 65 patients). No statistically significant differences in ORR were noted between the two treatment arms (Arm A, 31.3% vs. Arm B, 35.4%). Median OS and PFS were similar between Arm A and Arm B, 13.0 vs. 14.4 months and 8.1 vs. 7.2 months, respectively. The most frequent treatment-related AEs (≥15%) were hematologic and GI toxicity. Seventeen immune-mediated AEs were observed, all treated and resolved [64]. This first large randomized trial of HMA plus ICI (albeit with PD-L1 and not PD-1 inhibitors) compared to HMA alone in older AML patients showed no significant difference in efficacy between both cohorts, with no new safety signals identified with the combination.

2.1.4 Future Perspectives and Biomarkers of Response

Although early results are encouraging, ongoing and future studies are awaited to clarify the role of checkpoint inhibitors, the use of biomarkers, and combinations with ICIs in AML. In addition to PD-1, PD-L1, and CTLA-4, other immune checkpoint molecules have been described and are in early clinic development, such as lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), and the leukocyte immunoglobulin-like receptor B4 (LILRB4). These represent promising targets in AML as shown in preclinical studies [65–68].

Costimulatory signals are also known to be involved in the activation of T cells. Different costimulatory molecules are expressed on the surface of T cells and are essential for their activation, such as OX40, 4-1BB, inducible T-cell costimulator, and glucocorticoid-induced tumor necrosis factor receptor. It has been suggested that agonists of these costimulatory molecules may improve T-cell activation and, subsequently, tumor control, either individually or in combination with co-inhibitory checkpoint blockade. Some agents have been evaluated in preclinical studies and have demonstrated signs of clinical activity in early clinical trials, particularly when combined with ICIs, chemotherapy, or radiotherapy, which underlines a potential synergistic effect of the combination therapy by leveraging different aspects of the immune system against tumors. Although the majority of these studies are currently being conducted in solid tumors and non-Hodgkin's lymphoma, the strategy to combine immune agonists with standard therapy or with inhibitory checkpoints may also be of interest in myeloid malignancies and is currently being investigated in an ongoing multi-arm immunotherapy combination platform study in AML (NCT 03390296) [69]. Clinical trials are needed to evaluate the most effective synergistic combinations of checkpoint inhibitors with other forms of therapy and the best safety profile in patients with R/R AML.

Various biomarkers have been proposed as predictive of response to treatment with immune checkpoint inhibitors, with an early focus on PD-L1. Significant responses have been observed among patients with various cancer subtypes having an increased PD-L1 expression. Nevertheless, responses have also been reported in several studies among patients with negative PD-L1 staining, suggesting the indefinite role of PD-L1 expression as a predictive biomarker. The presence of tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment represents an immune response and has also been examined as a potential biomarker of response to ICI therapy. While multiple studies have demonstrated a favorable outcome in tumors with baseline TIL infiltration, others failed to show any correlation between the presence of TILs and the clinical activity or response. Regulatory T cells (Tregs) are an important element of the tumor microenvironment where they exhibit a dual function by activating T cells and maintaining immune homeostasis and by suppressing the cytotoxic activity of CD8-positive T cells which promotes tumor proliferation. Different other molecules

have been also analyzed with conflicting evidence. This highlights the unmet need to identify predictive biomarkers of response to ICIs which can help personalize treatment by selecting patients with a high likelihood of response to ICIs [70].

With the expansion of ICIs used in cancer treatment, immune-related AEs (irAEs) are more frequently observed among patients. These irAEs have a distinct pattern compared to the traditional side effects produced by conventional chemotherapies, resulting from the uncontrolled activation of the immune system which affects multiple organ systems. To date, no validated biomarkers have been established to predict patients at risk of developing irAEs. The effective management of irAEs relies on the early recognition of symptoms and the immediate treatment with corticosteroids and other immunosuppressive agents. One first step among others to help improve outcomes is to provide physicians and patients with proper education regarding the potential side effects which allows early recognition, reporting, and intervention; standardize the reporting of irAEs; and harmonize irAE management guidelines. A second more advanced step constitutes applying novel diagnostic tools to try to predict a given patients' baseline risk for developing irAEs and the likely organ to be impacted with such irAE, such as radiomics for pneumonitis and T-cell receptor beta variable region sequencing for severe other irAEs. This latter approach requires proper validation in larger prospective clinical trials for reliability and generalizability but then may be useful tools for irAE prediction in clinical practice (blood samples and CT images) [71].

One particular side effect of interest with ICIs use is pneumonitis, with a clinical picture similar to that of infectious pneumonia and which can lead to severe consequences if misdiagnosed and/or mistreated. Patients with AML are at significant risk of infectious complications, notably pneumonia, making distinction from immune-related pneumonitis often challenging. In order to better characterize the cellular and molecular mechanisms underlying the development of pulmonary complications in AML/MDS patients treated with ICIs and potentially predictive biomarkers to distinguish infectious pneumonia from immune pneumonitis, a multi-departmental study was conducted at MDACC to analyze the lymphocytes from bronchoalveolar lavage (BAL) fluid and peripheral blood from AML/MDS patients with pulmonary symptoms after ICI-based therapy. Seven patients with AML/MDS and pulmonary symptoms after being exposed to ICI-based therapy (ICI group) and four ICI-naïve AML/MDS patients with extracellular bacterial or fungal pneumonias (controls) were enrolled and analyzed. This study showed that BAL T cells in the ICI group were clonally expanded and BAL samples was enriched with IFNγ+ IL-17− CD8+ T and CXCR3+ CCR6+ Th17/Th1 cells, compared to controls. These pilot findings suggest that a distinct T-cell profile may be identifiable in patients with ICI-related pulmonary complications. A better understanding of the pathophysiology may also provide predictive biomarkers of ICI-related pulmonary complications and may allow differentiation of pneumonitis from infectious pneumonia in AML/MDS patients receiving ICI-based therapies, but such studies need to be done prospectively and on larger number of patients prior to considering them for routine clinical application [72].

2.2 CD47/SIRPα Blockade

CD47 is a widely expressed transmembrane protein and represents the ligand for the signal regulatory protein alpha (SIRPα), which is identified on macrophages and dendritic cells [73]. The activation of SIRPα triggers a signal transduction cascade that leads to the inhibition of phagocytosis [74–76]. CD47 is overexpressed on myeloid leukemia cells and mediates cancer cell evasion of phagocytosis by the innate immune system [77]. Thus, through its interaction with SIRPα, CD47 on the surface of leukemic cells appears to play an important role in potentially allowing cancer stem cells to overcome intrinsic expression of their pro-phagocytic "eat me" signals and thereby avoid phagocytosis.

As a macrophage inhibitory checkpoint, CD47/SIRPα interaction may be amenable to targeted strategies. The potential specificity of the CD47/SIRPα signaling pathway blockade is a major benefit of anti-CD47 therapies, causing the elimination of cancer cells while sparing most normal cell counterparts [78]. This robust rationale led to the development of an anti-CD47 antibody that showed promising efficacy both in vitro and in vivo [79]. A first-in-class humanized IgG4 anti-CD47 antibody, magrolimab, was developed with potent efficacy and favorable pharmacokinetic properties and toxicity profile [80]. However, while preclinical studies demonstrated robust anti-leukemic effects of anti-CD47 monotherapy, the single-agent activity was modest in humans with R/R AML [79]. In a phase 1 trial, magrolimab was evaluated as a single agent in patients with R/R AML. Anemia was the most common drug-related AE. Fifty-eight percent of patients had blast count reduction, but no objective responses were observed [81].

Therefore, combination strategies aiming to enhance the efficacy of CD47 blockade were initiated, relying on the synergy between enhancing pro-phagocytic and blocking anti-phagocytic signals. Pro-phagocytic signals on cancer cell (such as calreticulin) can be induced by cell damage following treatment with cytotoxic chemotherapies or by epigenetic therapies such as HMAs [82]. This approach was investigated preclinically with the combination of the hypomethylating agent, azacitidine, and the anti-CD47 antibody, magrolimab [83]. The combination was more effective than single agent alone and significantly enhanced the phagocytic elimination of AML cells, with dramatically improved survival in AML xenograft models [84].

Magrolimab was then investigated in a phase 1b trial in combination with azacitidine in 52 untreated AML patients, ineligible for induction chemotherapy, with a median age of 73 years. Sixty-four percent of participants had poor-risk cytogenetics, and 65% had *TP53* mutation. The *TP53* enrichment was by design as the trial was amended early on to focus on the high-risk unmet need population of *TP53*-mutated AML. The safety profile of the combination was similar to azacitidine monotherapy, with the most frequent AEs being anemia (31%), fatigue (19%), increase in bilirubin (19%), neutropenia (19%), thrombocytopenia (17%), and nausea (15%). Among the 34 evaluable patients, 65% had an objective response (44% CR, 12% CRi, 3% PR, 6% morphologic leukemia-free state [MLFS]), 32% stable disease, and 3% progressive disease. Among the group harboring *TP53* mutation, 15 out of 21 patients (71%) achieved an objective response (48% CR, 19% CRi, 5% MLFS), 24% stable disease, and 5% progressive disease, suggesting the efficacy of magrolimab in poor-risk disease. The median duration of response was 9.9 months, with 89% of patients maintaining response at 6 months. After a median follow-up of 12 months, the median OS for *TP53* wild-type patients was 18.9 months. The median OS for *TP53*-mutant patients was 12.9 months, but this was with a short median follow-up of 4 months at the time of presentation [85].

Following these encouraging results, a triplet combination approach with azacitidine plus venetoclax and magrolimab is currently being evaluated in a phase 1b/2 investigator-initiated trial at MDACC among older AML patients, ineligible for intensive chemotherapy (NCT04435691). It is hoped that this approach will increase the duration of CR/CRi, median OS, and MRD-negativity rate, with a particular interest in high-risk patients such as patients with secondary AML, or those with adverse cytogenetics, or *TP53* mutations with a high variant allele frequency.

Other investigational approaches including the combinations of magrolimab with monoclonal antibodies, such as with rituximab in follicular lymphoma, were shown to have a synergistic effect [86]. For AML or MDS, combinations of magrolimab with anti-CD33 or anti-CD123 antibodies may be efficacious approaches to consider. The association of magrolimab with ICIs may lead to improved responses and further enhancement of T-cell responses and efficacy by cross-presentation of antigens by activated macrophages [87–90]. A phase 1b trial was recently initiated to evaluate the combination of magrolimab with the anti-PD-L1 agent, atezolizumab,

in patients with R/R AML (NCT03922477). Similarly, combining magrolimab or other CD47 antibodies with cytotoxic chemotherapy may be synergistic, as cytotoxic therapy will also likely increase the stress response pro-phagocytic signals, and such strategies are planned to be investigated clinically for AML.

2.3 Chimeric Antigen Receptor (CAR) T Cells

CAR T cells are tumor-reactive and genetically engineered T cells, composed of three different domains. The extracellular, or antibody-like surface domain, consists of a single-chain variable fragment (formed by antibody-derived heavy and light chains) that recognizes tumor antigens independently of major histocompatibility complex proteins. The intracellular domain consists of the CD3ζ chain along with a costimulatory domain, responsible of T-cell activation. The extra- and intracellular parts are connected together via a transmembrane domain, typically constructed from CD8 or IgG4 molecules [91]. This structure allows CAR T cells to express the binding site of specific antibodies and induce targeted tumor killing [92].

First-generation CARs were constructed with only the CD3ζ domain, with a subsequent limited signaling ability and incapacity to prime resting T cells and lasting T-cell responses or sustained cytokine release [93, 94]. In order to improve the efficacy of CAR T cells, second-generation CARs were developed with an additional costimulatory signaling domain (e.g., CD28 or 4-1BB) coupled with the intracellular domain, resulting in enhanced activation, survival, and effective expansion of T cells [95, 96]. Third-generation CARs were then constructed with the simultaneous combination of both costimulatory domains (e.g., both CD28 and 4-1BB). In order to further improve the antitumor activity of CAR T cells, additional costimulatory ligands or transgenes for cytokine secretion (e.g., interleukin-12) were incorporated to produce fourth-generation CARs, known as TRUCKs (T cells redirected for universal cytokine-mediated killing) [97–99] (Fig. 2).

Anti-CD19 CAR T cells have already been evaluated among patients with B-cell malignancies, particularly ALL [100]. In a phase 2 trial, remarkable efficacy was observed with a single infusion of the CD19-directed CAR T-cell tisagenlecleucel that resulted in durable remission, with long-term persistence in pediatric and young adult patients with R/R B-cell ALL resulting in US FDA approval of this construct for R/R pediatric and young adults relapsed ALL [101].

2.3.1 CAR T Cells in Acute Myeloid Leukemia

Nevertheless, in the AML setting, CAR T cells' clinical trials are still in a much earlier phase of development. The biological activity of CAR T cells in AML was first evaluated in 2013 during a phase 1 clinical trial, using second-generation CD28-ζ CAR directed against the Lewis Y antigen, which is expressed on a wide range of malignancies including AML, but with only limited expression on normal tissue [102–104]. Five older patients, aged between 64 and 78 years with AML in first salvage, were treated with a single infusion of freshly transduced autologous T cells. Two patients achieved stable disease following CAR T-cell infusion, of whom one patient maintained response at 23 months. Additional two patients had a transient response defined by a cytogenetic remission and reduction in blasts, in one patient each [105]. No serious AEs were observed in this study, notably the cytokine release syndrome (CRS) that may occur after CAR T-cell infusion as a result of increases in interferon gamma and interleukin-6 production [106, 107]. This study demonstrated the safety and feasibility of CAR T-cell therapy, as well as the durable persistence (for up to 10 months) of infused cells among patients with R/R AML.

Additional preclinical studies have shown the activity of CAR T cells directed against the CD123 antigen, which is overexpressed on AML blasts and leukemic stem cells [108]. CD123 is the low-affinity binding segment of the interleukin-3 receptor expressed on the surface of monocytes, basophils, and plasmacytoid dendritic cells, where it plays a major role in proliferation

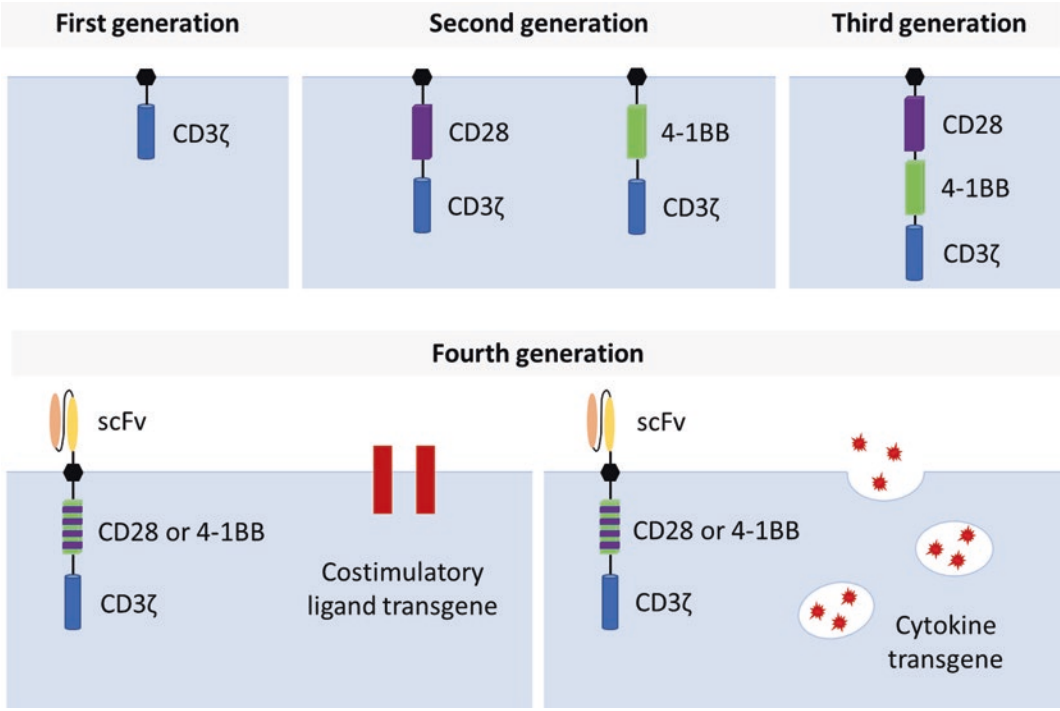

Fig. 2 Evolution of chimeric antigen receptor generations [97]
scFv single-chain variable fragment

and differentiation of hematopoietic progenitor cells [109–111]. The overexpression of CD123 on AML cells is associated with a poor prognosis and lower responses rates [112]. A phase 1 clinical trial was conducted to evaluate the safety and efficacy of a CD123-CAR constructed with an anti-CD123 single-chain variable fragment, an optimized IgG4 CH2CH3 linker, a CD28 costimulatory domain, and a CD3-ζ signaling domain. Six patients (five AML patients and one patient with blastic plasmacytoid dendritic cell neoplasm) with R/R disease following allo-HSCT, with a median of four prior lines of therapy, were treated with one to two doses of the CD123 CAR T-cell construct. Two patients achieved complete remission with successful bridge to a second allo-HSCT, and two additional patients achieved reduction in the percentage of blasts not classified as CR. The majority of AEs were grades 1–2, with only one grade 3 AE (rash) that were all reversible and manageable, with no treatment-limiting AE observed [113].

2.3.2 Limitations to the Development of CAR T Cells in Acute Myeloid Leukemia

Circulating CAR T cells are unable to differentiate between normal and malignant cells. For example, CD19 CAR T cells infused during the treatment of hematologic malignancies target the CD19 surface antigen present on both malignant and normal cells derived from B-cell lineages. This often induces on-target off-tumor side effects such as B-cell aplasia that can be treated by monthly intravenous immunoglobulin infusions [114, 115]. This represents a major obstacle for the development of CAR T-cell therapy in AML, where leukemic cells share many surface antigens with healthy hematopoietic stem or progenitor cells (HSPCs) and myeloid and/or lymphoid precursors, such as CD33, CD34, CD123, and others [116]. CAR T cells targeting CD33 and CD123 were developed and showed highly potent antitumor activity in preclinical models [117–119]. However, the shared expression of surface antigens on both healthy cells and malig-

nant blasts may result in the complete elimination of both normal and cancerous myeloid-derived cells following CAR T-cell therapy. This leads to prolonged bone marrow failure with an ultimately fatal risk of neutropenic infections and bleeding complications [120]. Several strategies and solutions are being developed and tested in order to mitigate the toxicities and the risk of myeloablation associated with AML CAR T-cell therapy.

One potential strategy is to limit the long-term persistence of CAR T cells in the body after infusion, via the implementation of a suicide switch, such as with inducible caspase 9. The inducible caspase 9 system, which is a fusion protein, allows the rapid destruction of T cells upon the administration of a synthetic drug (AP1903) [121, 122]. This strategy has been explored in the preclinical setting of CAR T-cell therapy but has never been tested in the clinical setting [123]. Another approach is to engineer CAR T-cells for co-expression of a specific truncated surface antigen that is targetable by monoclonal antibodies (e.g., truncated CD20 or EGFR, targetable by rituximab and cetuximab, respectively). This would allow the elimination of the infused CAR T cells by a mechanism of complement-dependent cytotoxicity or antibody-dependent cellular cytotoxicity [124]. Other nonselective drugs, such as the anti-CD52 monoclonal antibody alemtuzumab, or the anti-thymocyte globulin, are able to eliminate both the infused CAR T cells and the endogenous T cells [125]. One different strategy is to incorporate mRNA into the manufactured CAR T cells, whose function will be inherently limited due to the degradation of the mRNA [126]. On the other hand, based on the experience from CD19 CAR T cells in B-cell leukemia, CAR T-cell expansion and persistence for 3–6 months are associated with durable clinical response and decreased risk of relapse [101, 127, 128]. Thus, limiting CAR T-cells' persistence could negatively affect their therapeutic impact and increase the risk of relapse.

Antigen editing represents a novel approach allowing CAR T cells to persist in vivo while sparing the myeloablative side effects. One strategy is to transplant the patient with CD33-negative HSPCs from a modified donor allograft and allow for the engraftment and subsequent normal hematopoiesis of CD33-negative cells. After engraftment, the patient is infused with CD33-specific CAR T cells that are able to target AML blasts while sparing the edited CD33-negative precursors [118]. This approach has been tested preclinically and is being evaluated in a clinical trial of patients with R/R AML (NCT03971799). A similar strategy currently under investigation is the editing of the CD123 antigen, which serves as the alpha subunit of the interleukin-3 receptor (NCT04014881). However, given that interleukin-3 is a pleiotropic cytokine involved in hematopoietic development, the complete knockout of CD123 can result in a wide range of detrimental side effects [129]. Therefore, an alternative solution consists in the targeted removal of the epitope on the CD123 molecule present on the surface of donor HSCPs, which allows a selective recognition by CAR T cells while preserving normal CD123 signaling and hematopoiesis.

2.4 T-Cell Engagers in Acute Myeloid Leukemia

2.4.1 BiTEs and TriKEs

Bispecific T-cell engagers are stable, single-chain variable fragment antibody constructs, formed by the fusion of the minimal binding domains of two different antibodies, tandemly arranged on a single-polypeptide linker [130]. The first domain consists of a binding site for the invariant epsilon subunit of CD3 on T cells, while the second domain binds the tumor antigen. The low molecular weight of these compounds, around 55 kDa, results in rapid clearance by the kidneys and therefore a short half-life or around 1–4 hours, which necessitate continuous intravenous infusion [131].

BiTE antibodies induce the formation of an immunological lytic synapse between CD3-positive T cells and target tumor cell membranes causing the recruitment of polyclonal cytotoxic T cells, without the dependency on T-cell receptor specificity or MHC class I presentation [132,

133]. This triggers the activation and proliferation of the lymphocyte, with cytotoxic granule fusion, cytokine release, and liberation of perforin and granzymes which ultimately result in the destruction of the target tumor cell [134, 135] (Fig. 3).

The efficacy of BiTE antibodies in the treatment of acute leukemias was first demonstrated in B-cell ALL, with the CD3/CD19 bispecific molecule, blinatumomab. In a phase 3 trial, blinatumomab was compared to standard chemotherapy in the treatment of adult patients with R/R B-cell ALL and was shown to improve median OS (7.7 vs. 4 months, P = 0.01), induce higher remission rates within 12 weeks after treatment initiation (34% vs. 16%, P < 0.001), as well as observe higher rates of event-free survival (6-month estimates, 31% vs. 12%, P < 0.001) and longer median duration of remission (7.3 vs. 4.6 months). Among the patients who achieved remission, a higher rate of MRD negativity was observed in the blinatumomab arm (76% vs. 48%) [136]. These data showed the therapeutic activity of CD19-directed BiTE antibodies in patients with relapsed or refractory disease.

In AML, the potential role of antibodies was first highlighted with the introduction of the CD33 antibody-drug conjugate, gemtuzumab ozogamicin, which improved the survival of selected patients with AML (especially patients with core binding factor AML and ELN favorable group) [137]. CD33 is a transmembrane cell surface receptor expressed on myeloid-derived hematopoietic cells during advanced stages of maturation and is not expressed or expressed at low levels on normal primitive stem cells [138]. CD33 is expressed on AML blasts in more than 90% of cases and on leukemic stem cells [139].

AMG330 was the first BiTE targeting CD33 in AML and was shown to activate and expand T cells, leading to tumor lysis ex vivo [140]. Preclinical studies showed that AMG330 was able to suppress the growth of AML cell line xenograft in mice, thereby improving survival [141]. In vitro findings also suggested that, despite similar CD33 expression on the surface of myeloblasts from patients with newly diagnosed and relapsed/refractory AML, the cytotoxic effect induced by AMG330 therapy was significantly more pronounced in specimens of patients with newly diagnosed disease [142].

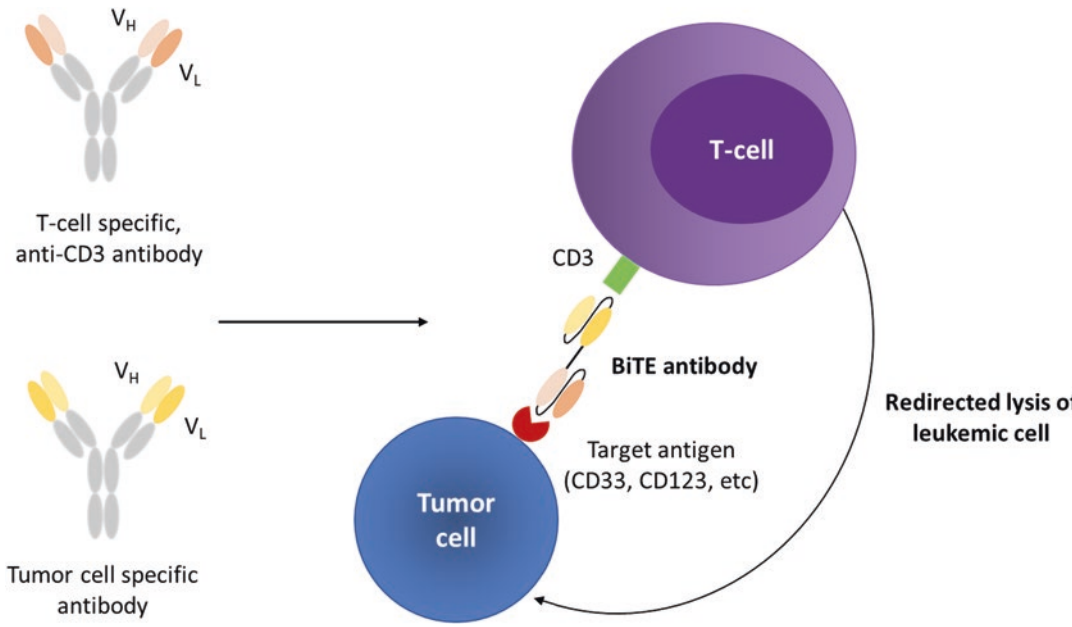

Fig. 3 Mechanism of action of bispecific T-cell engager antibody constructs

Treatment with AMG330 had an acceptable safety profile, drug tolerability, and anti-leukemic activity, as supported by early data from a phase 1 trial of 40 heavily pretreated patients with R/R AML and a median of four prior therapies (range: 1–15) [143]. In a recent update of this study with 55 patients enrolled, 49 (89%) reported AMG330-related AEs, with the most frequent being CRS in 67% (grades 3–4 in 13%) that was reversible and dose-dependent, occurring within the first 24 hours of the drug administration. Among 42 evaluable patients receiving a target dose ≥120 μg, 8 achieved a response (19%): 3 CR, 4 CRi, and 1 MLFS. Among responders, 67% had adverse cytogenetics and 50% had ≥4 prior lines of therapy. Notably, higher response rates were observed among patients with a lower leukemic burden (<25%) at the beginning of treatment [144].

Additional in vitro studies showed that the exposure of AML blasts to AMG330 resulted in the activation of T cells, the overexpression of PD-L1 on T cells, and the release of pro- and anti-inflammatory cytokines [141, 145]. Based on this rationale, an ongoing clinical trial is currently investigating the safety and efficacy of the combination of AMG330 with pembrolizumab in adults with R/R AML (NCT04478695). This approach has further evolved into a single bifunctional checkpoint inhibitory T-cell-engaging (CiTE) construct, combining immune checkpoint blockade with T-cell redirection to CD33 on AML cells. In a preclinical model, the CiTE induced complete elimination of AML cells in a murine xenograft model with no infusion-related AEs [146].

A novel CD33-directed BiTE construct was engineered with the addition of a single-chain IgG Fc region, thereby extending the half-life of the molecule to 1 week, allowing weekly dosing. In a phase 1 trial, AMG673 dosed up to 72 μg per dose was evaluated in 30 patients with R/R AML, with a median age of 67.5 years and having received ≥4 prior lines of therapy, including allo-HSCT in 23%. The most common treatment-related AE was CRS in 50% of the patients, with the majority being grades 1–2 (73% of cases). The most common treatment-related AEs of grade ≥ 3 were abnormal hepatic enzymes (17%), CRS (13%), leukopenia (13%), thrombocytopenia (7%), and febrile neutropenia (7%). Bone marrow evaluation showed a decrease in blasts in 44% of evaluable patients, with one patient achieving CRi [147]. These preliminary clinical data showed an acceptable toxicity profile and anti-leukemic activity of AMG673 in patients with R/R AML.

Similarly to BiTE, tri-specific killer engager (TriKE) construct consists of two single-chain variable fragments (scFvs) that bind CD33 and CD16, on leukemic cells and natural killer (NK) cells, respectively (Fig. 4). An interleukin-15 linker bridging the CD33 and CD16 scFvs is added in order to maintain cell activation. GTB 3550 is a first-in-class TriKE that was evaluated in a phase 1 dose-escalation trial among patients with R/R AML and led to NK cell proliferation in all patients at initial dose levels with no clinically significant AEs [148]. The study is still ongoing to evaluate its efficacy in the clinical setting (NCT03214666).

2.5 Tandem Diabodies

Tandem antibodies consist of two single-chain variable fragments for each target, linked together via a single polypeptide. This configuration allows them to have a higher molecular weight exceeding the threshold of renal clearance while maintaining the avidity of a bivalent antibody [149].

AMV564 is a tetravalent anti-CD3/CD33 tandem antibody with an increased molecular weight (106 kDa) designed to minimize renal clearance and allow for a 14-day continuous intravenous infusion over a 28-day cycle. Preliminary results from a phase 1 trial of 16 patients with R/R AML showed initial evidence of activity, with a 13–38% reduction of bone marrow blast in ten patients. The treatment had a favorable safety profile with no drug-related grades 3–4 toxicities and with manageable grade 2 CRS that occurred in one patient. This early finding showed that AMV564 is safe and tolerable, with objective clinical responses in some patients [150].

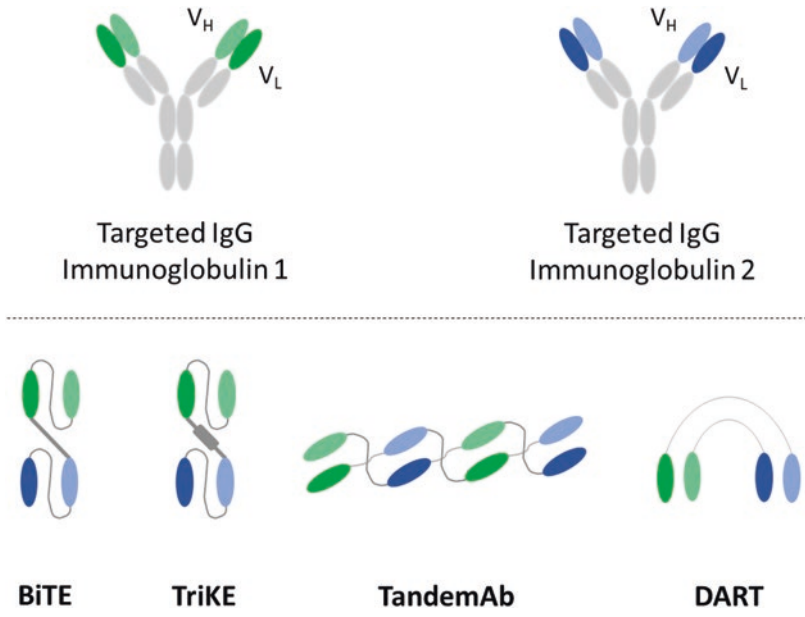

Fig. 4 Mechanism of action of BiTE antibody constructs *BiTE* **bispecific T-cell engager,** *DART* **dual affinity retargeting antibody,** *TandemAb* **tandem diabodies,** *TriKE* **tri-specific killer engager,** *VH* **heavy chain,** *VL* **light chain**

G333 is an anti-CD3 x CD33 antibody construct arranged in a single-chain bispecific tandem format and capable of redirecting T cells to AML blasts without affecting normal progenitor or stem cells [151, 152]. It is currently being investigated in the ongoing clinical trial NCTT03516760, and results are awaited.

2.5.1 Dual Affinity Retargeting Antibodies

Dual affinity retargeting antibodies (DARTs) are composed of two independent polypeptide chains, each one consisting of the VL potion of one antibody in tandem with the VH portion of the other antibody, linked together via a disulfide bond [153]. One of the earliest antibody constructs developed in patients with AML is the anti-CD123 and CD3 bispecific DART, flotetuzumab (previously MGD006 or S80880). Preclinical models demonstrated that this first-in-class DART mediated T-cell activation, proliferation, and blast/T-cell engagement [154]. The safety and efficacy of flotetuzumab as a continuous infusion were evaluated in a phase 1/2 trial of 88 patients with R/R AML, 42 in a dose-finding segment and 46 at the recommended phase 2 dose (RP2D) of 500 ng/kg/day [155]. Among 30 patients with primary induction failure or early relapse (within the first 6 months) treated at the RP2D, ORR was 30%, with 26.7% of patients achieving CR or CR with partial hematological recovery (CRh). The median OS for patients in CR/CRh was 10.2 months, with a 6- and 12-month survival rates of 75% and 50%, respectively [155]. Interestingly, around half of the enrolled patients with *TP53* abnormalities achieved CR/CRi following flotetuzumab treatment, with an associated median OS of 10.3 months (range: 3.3–21.3), suggesting that flotetuzumab therapy may alleviate the negative prognostic impact of *TP53* mutations. Responders had a significantly higher baseline tumor inflammation signature and PD-1 gene expression compared to nonresponders [156].

In an update of this study presented at ASH 2020, 24 patients with primary refractory AML and 14 patients with early relapse were treated at the RP2D. The median age was 63 years, and the vast majority (94.7%) had nonfavorable risk by ELN 2017 criteria [157]. The overall response rate (CR/CRh/CRi) was 42.1%, with 68.8% of responders subsequently able to undergo allo-HSCT. After a median follow-up of 10.8 months, the median OS was 4.5 and 7.7 months in the entire population and in responding patients, respectively. The most common side effect

observed was CRS, most commonly of grade ≤ 2, that progressively decreased during dosing at RP2D [157]. These findings show promising activity in AML patients with poor prognosis and high unmet medical need.

Vibecotamab (XmAb14045) is a novel CD123/CD3 BiTE with an extended half-life, which allows intermittent dosing [158]. This drug was examined in a phase 1 trial among 104 heavily pretreated AML patients, at dosages from 0.003 to 12.0 μg/kg with a recommended initial priming dose of 0.75 μg/kg. The most common side effect was CRS occurring in 62 patients (58%), mostly mild to moderate in severity (85% grades 1–2, 15% grade ≥ 3). Responses were observed in 7 out of 51 patients treated at higher dose levels (0.75 μg/kg), with an ORR of 14% (2 CR, 3 CRi, 2 MLFS), and stable disease in 36 patients (71%). Responding patients were characterized by a lower pretherapy burden of disease and specific T-cell subtypes compared to nonresponders [159].

APVO436 is another CD123/CD3 BiTE with prolonged half-life that was shown to be associated with a lower risk of CRS in preclinical studies [160]. In a phase 1 trial of patients with R/R AML, secondary AML, and MDS, APVO436 was shown to be well tolerated with a manageable safety profile [161]. The study is ongoing to evaluate its clinical efficacy (NCT03647800).

CLEC12A (also referred to as CLL-1 or CD371) is a myeloid differentiation antigen selectively expressed on 90–95% of leukemic stem cells but not on hematopoietic stem cells and represents a potential target in AML [162]. MCLA-117 is a full-length IgG1 bispecific antibody that binds CD3 on T cells from one side, and CLEC12A on granulocyte-macrophage progenitors from the other side, while sparing CD34+/CD38- hematopoietic stem cells. Preclinical studies demonstrated that treatment with MCLA-117 induced T-cell activation and T-cell-mediated lysis of AML cells [163, 164]. MCLA-117 is currently under clinical investigation for the treatment of patients with R/R AML (NCT03038230).

Currently, a novel therapeutic approach is being investigated consisting of a triple antibody agent (SPM-2) engaging NK effector cells via CD16 with leukemic cells via CD33 and CD123, with encouraging clinical activity. In vitro studies from 29 AML patients, including patients with poor molecular subtypes, showed that SPM-2 resulted in blast elimination at nanomolar concentrations. A positive association was observed between the sensitivity to treatment and the density of CD33 and CD123, with a maximum susceptibility for cells with a combined density above 10,000 copies/cell. These early results suggest that SPM-2 may be capable of eliminating AML leukemic stem cells and thus prolong survival [165].

2.6 Summary

Despite the improvements in AML treatments, the majority of patients with standard or high-risk AML will die of relapsed and/or progressive disease. Novel and powerful therapies potentially capable of eradicating leukemic cells, including putative leukemic stem cells, have been recently investigated. These strategies include T-cell or macrophage immune checkpoint inhibitors, chimeric antigen receptor (CAR) T cells, and bispecific T-cell engaging antibodies, alone or in combination. These agents demonstrated particular efficacy in an area of unmet need, in patients with high-risk genetic features such as *TP53* mutations and those relapsing following bone marrow transplant. Further studies are warranted to combine these drugs with chemotherapy or other targeted agents capable of increasing immunogenicity and therefore enhancing response. Nevertheless, this enhancement of the immune system can result in immune-mediated adverse events and cytokine-related toxicities. Timely suspicion and diagnosis of these side effects can allow proper management and avoidance of more severe complications.

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Authorship Contributions FH and ND analyzed the data, wrote the paper, reviewed and approved the manuscript, and shared final responsibility for the decision to submit.

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CAR T Cells

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Keywords

Non-Hodgkin lymphoma · Immunotherapy · Adoptive cell therapy · Chimeric antigen receptor (CAR) T cell · Cytokine release syndrome (CRS) · Toxicity · Cellular therapy · CAR-T cell related encephalopathy syndrome · Neurotoxicity · Axicabtagene ciloleucel · Tisagenlecleucel · Lisocabtagene maraleucel · Brexucabtagene autoleucel

1 Introduction

In 1891, Dr. William B. Coley, an American surgeon, made a compelling observation that 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 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 two decades, due to our deeper understanding of the immune system and the cellular and molecular pathways leading to tumor development. Two groundbreaking therapies which have shown great promise in this field involve "taking the breaks off" and "pushing the pedal" of the immune system. These therapies, namely, immune checkpoint inhibitors and adoptive cell therapy, respectively, 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 TCR (T-cell receptor) therapy and CAR (chimeric antigen receptor) T-cell therapy. The former requires antigen presentation by innate T cells, while the latter has receptors transduced in T cells which offers 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. Although an immune therapy, they use T cells in the crudest of forms, with varying degree of success, and have become the treatment of choice for many relapsed refractory hemato-

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logical cancers due to lack of more effective or less toxic options. However due to its nonselective nature (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 and bypass immune tolerance and enhanced 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 moeity 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 [26]. 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 led 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. 1. In this chapter, we will review the various aspects of CAR T-cell and their efficacy, toxicity, and management in different tumors presented in recent clinical trials and its future potential.

2 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. 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's cell 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 1). 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. 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) and IgD or CD8 domains and connects the antigen recognition part, scFV, with the transmembrane domain.

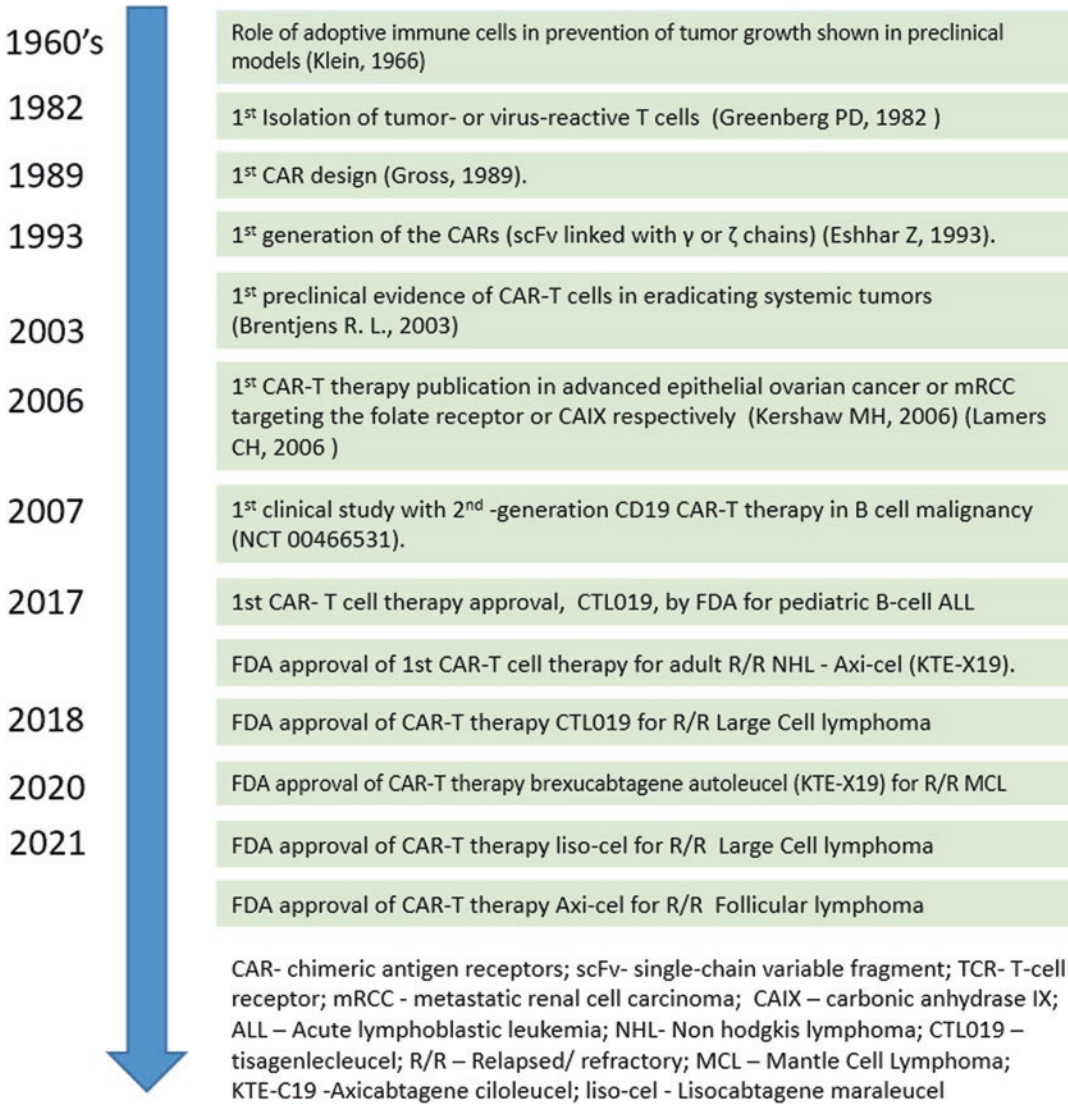

Fig. 1 Timeline of progress in the development of CAR T-cell therapies

The hinge, though inconspicuous in the overall structure, has a significant impact on the overall function and cytokine signature during T-cell expansion [3]. 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 [31].

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 signal gateway to the intracellular compartment. This is usually derived from CD3-ζ, CD4, CD8, or CD28 molecules.

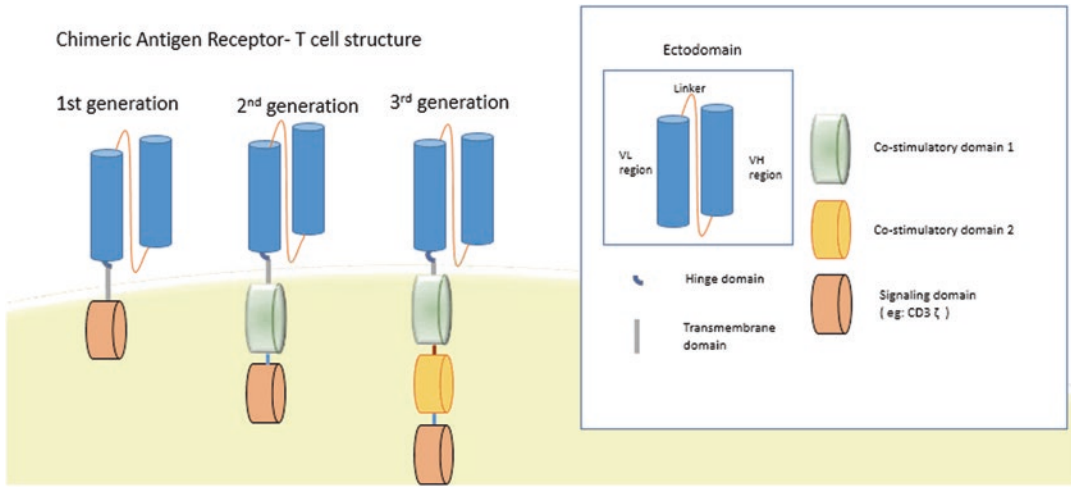

Fig. 2 Structure of first-, second, and third-generation chimeric antigen receptor

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 preclinical model, the effect could not be translated in vivo due to poor T-cell expansion, less stability, and anti- tumor 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 variable. Tandem CARs (Tan CARs) generated through co-transduction, 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 [28, 60]. The fourth-generation CARs which have functional modification in addition to its structural change, the so-called TRUCKs (T-cells redirected for universal cytokine-mediated killing), use T cells as vehicles to produce and release a tumoricidal cytokines inside the targeted tumor tissue. This causes direct killing and also a second wave of immune recruitment [14]. To deliver the pleotropic 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 the different technologies including a presence of suicide gene, switchable dual-antigen receptors, or synthetic control devices (using inducible caspase 9 (iCasp9), Synthetic Notch (synNotch) receptors.) [79].

3 Manufacturing and Treatment

Building autologous CAR T cells requires a series of well-organized steps (Fig. 3). The process starts with the collection and enrichment of CD3+ lymphocytes through the process of leukapharesis. The principle of leukapharesis is same as that for peripheral blood stem cell (PBSC) collection in hematopoietic stem cell

Table 1 TAA 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's 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; GPC3, glypican 3; GD2, gangliocide; 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; MUC1, mucin 1, cell surface associated; FBP, folate-binding protein; FAP, fibroblast activation protein; BCMA, B-cell maturation antigen; NY-ESO-1, New York esophageal squamous cell carcinoma 1; NKG2D, natural killer group 2 member D; SLAM7, self-ligand receptor of the signaling lymphocytic activation molecule

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 [5, 6, 72]. 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) [23, 33]. A minimum absolute peripheral blood lymphocyte count greater than 100–200 cells/mL is expected to result in successful T-cell collection [52, 65].

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 the placing a dependable venous access (central or peripheral), removing blood and filtering the peripheral blood mononuclear cells [70]. 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 [5, 6, 11].

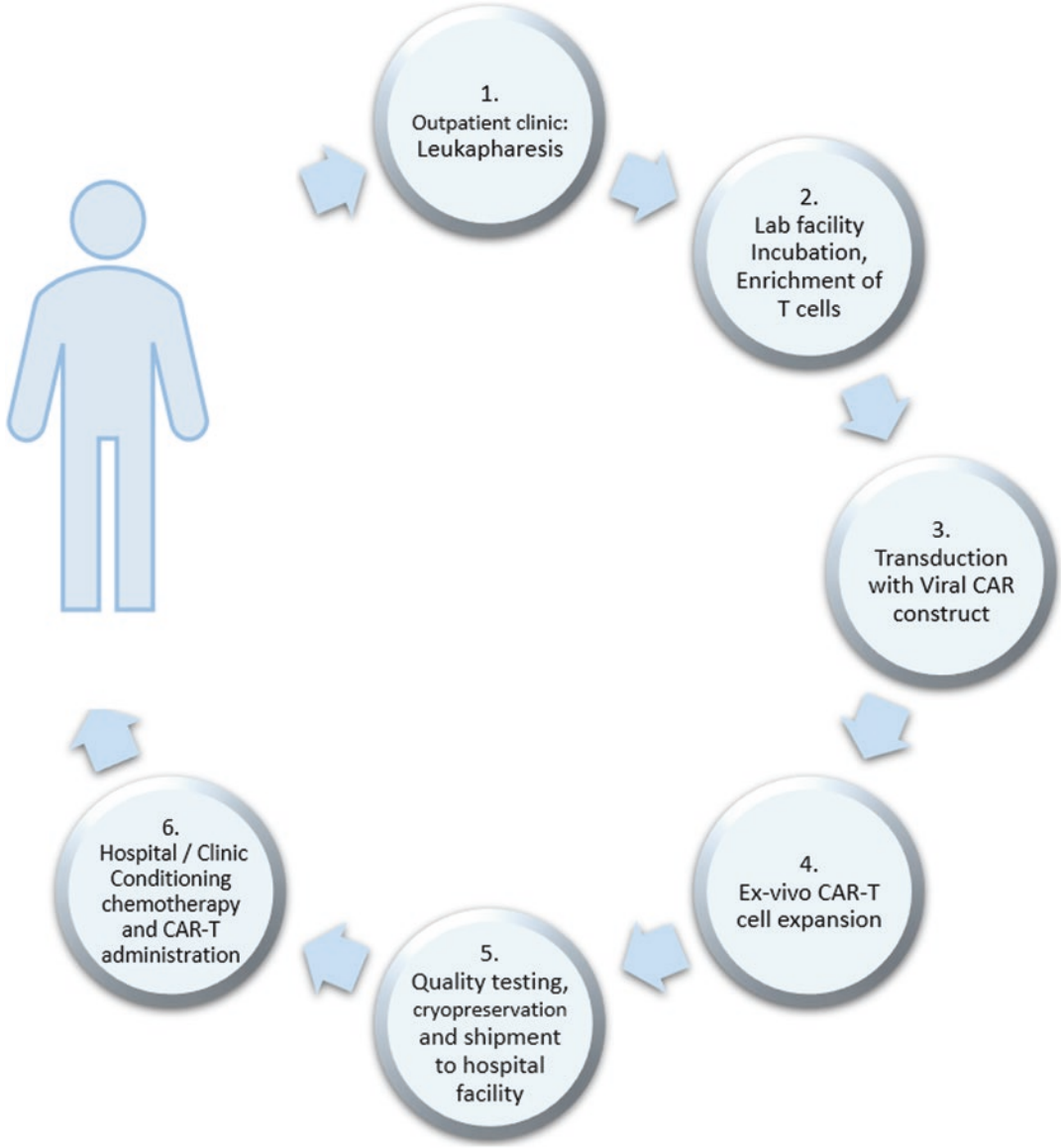

Fig. 3 Simplified version of manufacturing process of autologous CAR T cell therapy

FDA-approved instruments are available to perform extraction of T cells from the blood that is withdrawn, which involves elutriation, a technique which 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. The is followed by purification of the T cell from other blood cells by a complex process of washing and antibody-bead conjugate selection [64]. 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 expres-

sion vectors used for clinical applications: gamma retroviral vectors, lentivirus vectors, and the transposon/transposase system. Lentivirus vectors have a safer integration site profile than gamma retroviral vectors and hence commonly used in clinical practice for 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 utilized regimen is fludarabine and cyclophosphamide, but other regimes such as bendamustine have also been utlilized. 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 as majority of patients enrolled in these studies have highly refractory and heavily pretreated disease [8, 16, 34, 55, 73, 75]. The conditioning helps to create a less competitive environment for the adoptive transferred T cells by promoting host lymphocyte depletion, more supportive cytokine milieu, decreased immunosuppressive cells such as regulatory T cells, and myeloid-derived suppressor cells [32, 78].

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) which 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. 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).

The side effect profile of the currently approved CAR T precludes wide-scale application in the outpatient setting. In ZUMA-1 trial, patients could be discharged at day 7 post treatment in the absence of any sign of CRS or ICANS, while in ELIANA and JULIET trial, patients could be discharged same day [48, 52, 69]. 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. 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. In TRANSCEND NHL 001 study, out of the 269 patients who received at least 1dose of liso-cel, 25 patients were treated in the outpatient setting, and approximately a third of these patients did not require any further hospitalization. For patients who required hospitalization, the median time from liso-cel infusion to hospitalization was 5 days (range 3–22) [2].

4 CAR T-Cell Therapy in Different Cancer Types

4.1 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 [15, 19]. 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's 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 three CAR T-cell products that are FDA approved as of 2021, tisagenlecleucel (CTL019, Kymriah), axicabtagene ciloleucel (axi-cel, KTE-19, Yescarta), and lisocabtagene maraleucel (Lis-cel, Breyanzi). Tisagenlecleucel was approved for the treatment of pediatric relapsed and/or refractory B-cell precursor acute lymphoblastic leukemia, and 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, in October 18, 2017 [52]. Liso-cel is the most recent CAR T to receive approval for DLBCL. On February 5, 2021, the FDA approved this treatment for adult patients with non-Hodgkin's lymphoma after two or more lines of systemic therapy, including DLBCL not otherwise specified (NOS) (including transformed DLBCL), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B.

Axicabtagene Ciloleucel 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 [45]. 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 center B cell and activated B-cell DLBCL subgroups, respectively [38, 52]. Median PFS for the whole group was 5.9 months. In a recent real-world analysis of axi-cel in the standard of care setting (n = 295), the safety and efficacy in patients with relapsed/refractory LBCL was comparable to the registrational ZUMA-1 trial [51].

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 pilot study of 28 patients with refractory B-cell lymphomas treated with this construct (CTL019). Among refractory DLBCL, 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 [68, 69].

Lisocabtagene Maraleucel TRANSCEND NHL 001, a large multicenter trial, which started

as a phase I first-in-human study of JCAR017, used a defined composition of CD19-directed CAR T cell (equal ratio of CD4+ and CD8+ CAR T cells) and used 4-1BB costimulatory domain. In this trial, which has the largest cohort of patients for any CAR T study to date in large-cell lymphoma, 344 patients underwent leukapheresis for manufacture of liso-cel, of whom 269 patients received at least 1 dose of liso-cel. The trial reported an ORR of 74% for the entire patient population, with CR rate of 53%. The estimated duration of response rate at 1 year was 55% for the total population and 65% among those who achieved a complete response. Median progression-free survival was 6.8 months.

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, best ORR was 81%, and 3-month CR rate was 60%. In those who relapsed within 12 months of a stem cell transplant, the ORR was 85% [1, 2]. (Table 2)

Mantle cell lymphoma: Eight patients with mantle cell lymphoma (MCL) (four of them receiving Cy/Flu conditioning) were included in the study at Fred Hutchinson Cancer Research Center, with no CRs reported and only two PRs in the cohort of MCL [73]. The phase I TRANSCEND study included patients with MCL; however results reported were primarily for patients with relapsed large B-cell lymphomas. In the NCI trial (NCT00924326), which included 22 patients with relapsed/refractory advanced-stage lymphoma, there was 1 one patient with MCL who experienced a CR and had ongoing response +17 months [38]. Given the promising results from NCI trial, the CD19-targeted CAR T-cell product KTE-X19 (Tecartus; brexucabtagene autoleucel) was investigated in patients with relapsed/refractory MCL in the ZUMA-2 trial (NCT02601313). In an intention-to-treat analysis of the 74 patients, the responses were unprecedented in this highly aggressive disease cohort, which included MCL with Ki67 > 30% (82%), Tp53 mutated (17%), blastoid/pleomorphic (31%), with an ORR of 85% (CR 59%). At a median follow-up of 12.3 months, 57% of the 60 patients in the primary efficacy analysis were in remission. At 12 months, the estimated progression-free survival and overall survival were 61% and 83%, respectively. This study led to the first and only CAR T-cell therapy approval in MCL to date.

Indolent Lymphoma An indolent B-cell lymphoma can have ominous clinical features, either manifesting as early relapse after therapy or by transformation histologically to DLBCL or high FLIPI scores (as in high-risk follicular lymphoma). These features have been consistently associated with poor outcomes. Relapse of follicular lymphoma (FL) after first-line treatment with R-CHOP within 2 years defines a unique category of patients at substantially high risk of death from lymphoma.

The first patient report of CAR T therapy in lymphoma was on a phase I trial at the NCI with a second-generation CD19-targeted CAR T (CD28 costimulatory domain) where a patient with advanced relapsed/refractory FL received lymphocyte-depleting regimen with cyclophosphamide and fludarabine. The day after the last fludarabine dose, the patient received $1x10^8$ anti-CD19 CAR Ts intravenously, followed by $3x10^8$ 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 $0.3x10^7$ to $3.0x10^7$ CAR Ts/kg bodyweight. Results from this trial showed that three of four patients with FL

Table 2 Summary of the three anti-CD19 CAR T-cell therapy in aggressive B-cell NHLs
Abbreviations: *DLBCL-NOS* **Diffuse Large B cell Lymphoma – not otherwise specified,** *FL* **Follicular Lymphoma,** *SCT* **Stem cell transplant,** *HGBCL* **High grade B cell lymphoma**

Study	ZUMA-1	JULIET	TRANSCEND
Co-stimulatory domain	CD-28	4-1BB	4-1BB
Median Age (Range), years	58 (23-76)	56 (22-76)	60 (20-82)
Evaluable Patients	101	93	269
Response	ORR: 83% CR: 58%	ORR: 52% CR: 40% PR: 12%	ORR: 74% in FULL dataset CR: 52% in FULL dataset
Lymphoma Subtype and FDA approval	DLBCL- NOS Transformed FL HGBCL PMBCL	DLBCL- NOS Transformed FL HGBCL	DLBCL- NOS Transformed FL HGBCL PMBCL FL grade 3B
Double Hit Lymphoma	5 patients	27%	13%
Relapse Post Auto SCT	21%	49%	33%
Bridging Therapy	Not Permitted	92% received	59% received.

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 [34].

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 11+ months at the time of report [38].

Building up on the success in aggressive B-cell lymphoma, ZUMA-5 trial enrolled patient in a phase II, multicenter, single-arm study of axi-cel for R/R indolent advance stage NHL, including FL and MZL. 146 patients (124 FL; 22 MZL) received axi-cel. With a median follow-up of 17.5 months, the ORR was 92% among efficacy-evaluable patients with 76% CR rate. In patients with FL (n = 84), the ORR was 94% (80% CR rate); in those with MZL (n = 20), the ORR was 85% (60% CR rate). This study led to FDA's first CAR T therapy approval in FL, and current indication includes treatment of adult patients with relapsed or refractory FL after two or more lines of systemic therapy.

Refractory FL (14 patients) who relapsed within 24 months of initial diagnosis and/or remained refractory to least 2 lines of therapy were treated in the University of Pennsylvania trial using CTL019 [68]. At the time of the most updated report, 3-month ORR and CR rates 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) and relapsed post-autologous/ allogeneic, with a median progression-free survival (PFS) that was not reached. 70% of patients were disease-free after a median follow-up of 29 months. It remains unclear if responding patients will have sustained durable responses, and/or potential cure, or if the disease will eventually relapse as happens with many indolent lymphoma therapies. ELARA is a phase II study evaluating the efficacy and safety of tisagenlecleucel in patients with heavily pre-treated relapsed/refractory FL. In the early interim analysis of the 52 evaluable patients who received tisagenlecleucel (median follow-up, 6.5 months), the ORR was 83% and with CRR was 65%. The treatment was overall very tolerable, and in patients with best response of CR, the responses appear durable [20]. Turtle et al. published their experience with the use of 1:1 ratio CD4/CD8 CAR T in 8 patients with FL even of 8 patients with FL achieving complete remission

(CR; 88%) after CAR T cells. The median time to CR was 29 days (range, 27–42), and all who achieved CR remained in remission (median follow-up, 24 months; range, 5–37). One patient received additional therapy (allogeneic HCT) while still in CR. One patient with stable disease at first restaging received radiation 2.3 months after CAR T cells and has not progressed 36 months after CAR T-cell infusion. The study demonstrated a high rate of durable CR in high-risk FL patients treated with CD19 CAR T cells, comparable to that reported in another study where CRs were only seen in the cohort that received fludarabine/cyclophosphamide conditioning chemotherapy with none in the cyclophosphamide alone conditioning arm (0/2 at 0%) [29, 73].

In CLL, CAR T cells have produced responses ranging from 57 to 74%, with CRs lower in comparison to DLBCLs and range from 21 to 29% [55]. 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 [37, 57, 58]. 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 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 [9]. 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) negative marrow CR was 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 [25]. Several studies are currently ongoing to prove this concept on a wider population cohort [21, 22].

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% to 15% of patients with localized Hodgkin's disease and approximately a third of those with advanced-stage disease. Around 10% to 15% of patients will have refractory disease to first-line therapy. With the advent of ASCT, 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 universal 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 x 10^7 CAR+ cells/m2 (DL1), two patients received 1 x 10^8 CAR+ cells/m2 (DL2), and five patients received 2 x 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 was likely due to two main reasons, one due to the heavy microenvironmental T-cell suppressive infiltrate in Hodgkin's lymphoma and second, which was common to these trials, was the absence of conditioning therapy. Currently two parallel phase I/II trials (NCT02690545 and NCT02917083/RELY30) at two independent centers involving patients with relapsed or refractory HL are ongoing. In the study, a total of 41 patients with R/R HL received autologous CD30 CAR Ts after lymphodepletion with either bendamustine alone, bendamustine, and fludarabine or cyclophosphamide and fludarabine. All patients had received at least 2 prior lines of therapy (and as many as 23) and a median of 7 prior therapies. Of the 37 evaluable patients, 34 received fludarabine conditioning. Two of those patients had attained complete remission prior to CAR T-cell infusion and were not included in the efficacy analysis. The treatment led to objective responses in 23 of 32 (72%) patients, consisting of 19 complete responses and 4 partial responses. Three additional patients had stable disease. 1-year PFS and OS for all evaluable patients were 36% and 94%, respectively [63].

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 [77], with 10-year overall survival reaching almost 80% [77]. Achieving a CR in relapsed patients occurs in about a third of patients [18, 54]. The prognosis is grim for patients with primary refractory disease, and relapse post allogeneic hematopoietic stem cell transplantation (HSCT) results in a median overall survival of 3–6 months.

CAR T cells have shown to be very promising in these groups of patient with induction of remission rates as high as 70–90% seen across multiple trials with different CAR T-cell constructs (scFv and costimulatory domains) and in heavily pretreated with prior CD19 targeted therapies (e.g., blinatumomab) or SCT. Remission is also seen in Philadelphia chromosome-positive (Ph+) disease and in down syndrome-associated ALL [40, 43].

Tisagenlecleucel is the only FDA-approved autologous CD19-targeted CAR T-cell product for treatment of R/R B-cell ALL in patients under 25 years old. The multicenter international ELIANA trial that led to its approval reported an ORR rate of 81%. Majority of patients in the study were not bridged to transplant. 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. Relapse rate was significantly higher in subjects who did not have a HSCT after CAR therapy (6/7; 85.7%) compared to those who did (2/21; 9.5%) ($p = 0.0001$) [43].

In the ZUMA-3 phase I study, KTE-X19 (same construct of axi-cel) is being evaluated in adult patients with R/R ALL. The interim analysis reported showed encouraging efficacy with manageable safety. The CR rate was noted to be 68%, and all patients were MRD negative. The phase II portion of the study is ongoing (NCT02614066).

It is challenging to draw definitive conclusions from these studies and many open question currently remain; once MRD negative status is achieved, whether to consolidate with HSCT, especially for transplant-naïve patients, or is CD19-CAR T a better bridging therapy than other novel therapies (e.g., blinatumomab) if an MRD negative status can be achieved prior to HSCT.

Multiple Myeloma Patients with relapsed and refractory multiple myeloma (RRMM) who progress on immunomodulatory agents, proteasome inhibitors, and anti-CD38 antibodies have dismal outcomes and have a high unmet need for novel therapies including CAR T. 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. Though there are no FDA approvals, there are a few strong contenders in the race. 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 minimal residual disease (MRD) undetectable disease in bone marrow [4, 10]. Bluebird Bio's bb2121 cell therapy product (4-1BB costimulatory domain), currently marketed as idecabtagene vicleucel (ide-cel), has further set the benchmark in multiple myeloma in the large phase II study (KarMMa) study. The study included patients with triple-class-exposed relapsed/refractory myeloma. The median number of prior therapies was 6 (range, 3–16), and 94% had previously undergone at least one autologous hematopoietic stem cell transplant. The phase II results of this study showed an ORR, CR, and median duration of response of 73%, 33%, and 10.7 months, respectively, across the target dose levels of 150–450 × 10^6 CAR+ T cells and 82%, 39%, and 11.3 months at the highest target dose of 450 × 10^6 CAR+ T cells, a response independent of the degree of BCMA expression. A multicenter, randomized, open-label, phase III study, KarMMa-3 is currently open to evaluate the role of ide-cel as an earlier line of treatment [62, 50].

Nanjing Legend Biotech in China recently reported long-term follow-up results from LCAR-B38M CAR T-cell trial, LEGEND-2 study (NCT03090659) (using 4-1BB costimulatory domain), a clinical trial featuring a CAR T therapy with 2 BCMA-targeting single-domain antibodies designed to confer avidity. Patients on this trial had fewer lines of prior therapy and achieved an ORR of 88% with CR in 74% of patients, with overall favorable safety profile. At 18 months, the PFS rate was 50% for all pts. and 71% for MRD negative-negative patients with CR [76, 80]. The same construct under the name ciltacabtagene autoleucel (cilta-cel; JNJ-4528) is currently undergoing a phase I/II study in patients with RRMM who have received at least three prior lines of therapy or are double class refractory to a proteasome inhibitor and an immunomodulatory drug.

Overall response rate per independent review committee (primary endpoint) was 95% with a stringent CR rate of 56%. Of 52 MRD-evaluable patients, 94% were MRD-negative at 10^5. The 6-month PFS and OS rates were 87% and 94%, respectively.

Other BCMA CAR T trials with different products are currently ongoing with data preliminary at this point [56]. 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 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 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 to 12 days) in axi-cel and 3 days (range, 1–51) in tisagenlecleucel. 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 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 trigger 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 (tisagenlecleucel), and 37% of patients (grade ≥ 3 in 2%) 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) [2, 52, 69].

There is considerable difference and overlap in the management of these toxicities across grades, clinical trials, and different institutions. The American Society for 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 [44]. Organ toxicity associated with CRS is graded according to CTCAE v5.0. Most patients have a compromised immune system or have ongoing neutropenia, and the symptoms mimic sepsis syndrome; clinical management needs a concerted 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 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 CRS. Shifting patient for more intensive care in critical care unit should be considered in these scenarios. Dexamethasone is reserved if hypotension persists despite IL6 blockade or fluid boluses or if there is 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, nonrebreather mask, or Venturi mask not attributable to any other cause [44]. 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 of refractory to IL-6 blockade. Dexamethasone is dosed 10 to 20 mg every 6 hours for grade 3 and up to methylprednisolone 1000 mg/day for grade 4. If clinical improvement is 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 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, nonrebreather mask, or venturi mask	Requiring positive pressure (e.g., CPAP, BiPAP, intubation, and mechanical ventilation)
Management	• Antipyretics • Antiemetics • IV fluid • Sepsis work-up • 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

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.

ASTCT CRS consensus grading and management

ASBMT guidelines for ICANS

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 in August, 2017 for the management of severe CRS	Off label use
Dose and frequency	Minimum interval of 8 hours to a maximum total of 4 tocilizumab doses 4–8 mg/kg (max 800 mg)	One dose in 3 weeks 11 mg/kg IV

ICANS, a unique neurotoxicity syndrome, is the second most-common adverse event that can occur concurrently with or after 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(axicel), 39% (grade ≥ 3 in 12%) of patients in JULIET trial (Tisagenlecleucel), and 19% (grade ≥ 3 in 12%) of patients in TRANSCEND NHL 001 (liso-cel) trial. Though there is 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 [27, 67]. An analysis showed higher levels of cytokines, which are usually associated with a 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 routine assessment. This includes diminished attention, impaired handwriting which can deteriorate quickly to language disturbance, confusion, disorientation, agitation, aphasia, somnolence, and tremors. More severe cases of ICANS are associated with motor weakness, seizures, incontinence, mental obtundation, increased

intracranial pressure, papilledema, and cerebral edema.

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 [53]. Though IL-6 blockade can reverse CRES during the first phase, it is found to be suboptimal by itself during 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 3). A 10-point immune effector cell-associated encephalopathy (ICE) score is assessed across this five domains, which includes elements for assessing orientation, naming, command-following, writing, and attention. Other neurological domains assessed for ICANS grading include level of consciousness, seizures, motor weakness, and raised intracranial pressure/cerebral edema.

ASBMT guidelines for ICANS

ICE:
• Orientation: Orientation to year, month, city, 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 2 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 backwards 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

ASBMT ICANS grade	Defining features of grade	Management
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

Hemophagocytic lymphohistiocytosis (HLH)/ macrophage activation syndrome (MAS) is an uncommon event (1% incidence with CAR- T therapies) characterized by 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 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 majority of cases, HLH/MAS is managed in same way as for CRS and resolves with CRS resolution [44]

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 [47, 61, 66]. 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 [34, 35, 47, 57]. 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 [9, 12, 36]. Other toxicities rarely associated with CAR 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 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.

5 Resistance Pathways

Prognosis of patients after failure of CAR T is poor. The resistance of the tumor and the cause of T-cell failure is an area of active research; some potential mechanisms include loss of target, genetic reprogramming, and T-cell exhaustion. In the international trial which included young adults and pediatric patients with acute lymphoblastic leukemia, around third of the relapses were with CD19-negative variants [39, 47]. 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 [42]. 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 receptor 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 selection of target-negative tumor cells. It could also be the presence of a sub-detection level presence of a CD19-negative clone [71, 74]. Selection pressure, with genetic reprogramming 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 [24, 30]. 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 [7, 49]. This would incapacitate T-cell 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) [13]. 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. 4-1BB costimulation has shown to abrogate the persistent exhaustion induced by CAR signaling [17, 46]. Trials are underway using different combinatorial approach 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 clear understanding of these pathways is critical to build up on the early success of CAR T.

6 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 (including multispecific CAR T cells; tandem CARs or Tan CARs) and reduce the toxicity of treatment by using suicide switch technology (caspase 9 (iCasp9) and Synthetic Notch (synNotch) receptors. Allogeneic off-the-shelf CAR T-cell therapy is underway with minimal GVHD and 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 [14, 41, 59]. As we learn more on 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

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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 60% for cutaneous AEs among the seven FDA-approved drugs used as monotherapy or combination therapy. Although only 2% are reported as grade 3 or 4 events with monotherapy, the incidence can be as high as 6–9% for combination therapy and the impact on quality of life can be significant for these patients. 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

With the increased use of immune checkpoints inhibitors (ICPs), different types of side effects than previously observed with cytotoxic chemotherapy or targeted therapy, commonly referred to as immune-related adverse events (irAEs), are increasingly seen. The disrupted immune homeostasis is mediated by unchecked T-cell activation [1]. 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 60% for cutaneous AEs among FDA-approved drugs. Although only 2% are reported as grade 3 or 4 events with monotherapy, the incidence can be as high as 6–9% for combination therapy, and the impact on quality of life can be significant for these patients [2–4]. 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 poten-

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tially 9% of patients have dose interruption of ipilimumab because of their cutaneous AEs [2]. 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 bind the programmed 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) [2]. 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 [2]. Programmed death-ligand 1 (PD-L1) inhibitors and a second-generation CTLA-4 inhibitor are now being used in clinical trials, increasingly as combination therapies, and it appears that they in combination tend to have more common and severe CAEs. At our institution 52.3% of patients on combination therapy experienced CAEs. 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, if well managed, can be an indicator of a good prognosis [5–7].

1 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 syndrome (SJS)/toxic epidermal necrolysis (TEN) on the severe end [8, 9].

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 spreading 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 vesicles appear, 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, intravenous immunoglobulin therapy, or TNFα inhibition.

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.

2 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

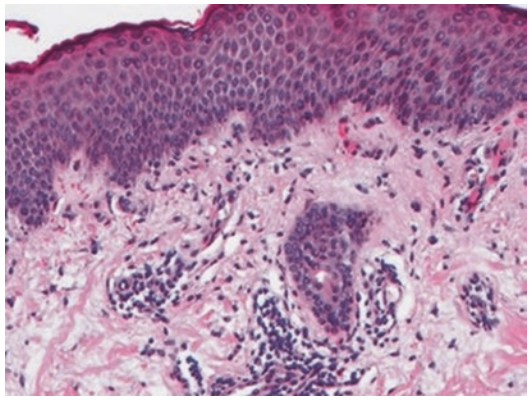

Fig. 2 Eczema, spongiotic dermatitis with dermal eosinophils

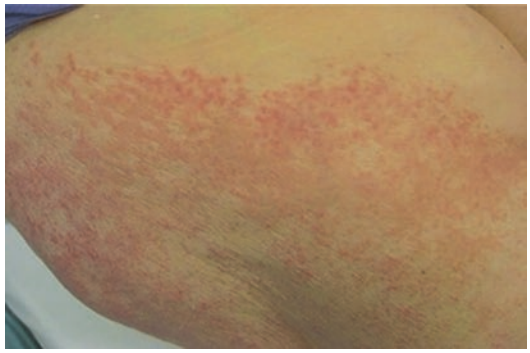

Fig. 1 Eczema, erythematous papules coalescing into plaques that are rough and have minimal scale

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 [10–16]. 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 require systemic treatments, some of which are specific to the type of inflammatory reaction (Figs. 1 and 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, are 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 [17]. Treatment consists of topical steroids, usually mid-strength creams, such as triamcinolone 0.1%, to begin with and graduating to superpotent 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 diphenhydramine 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 systemic therapies can be helpful. Typically oral steroids, such as prednisone at 1 mg/kg, has been 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 [18, 19].

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) has been used successfully for severe refractory eczema in patients requiring continuing therapy with immune checkpoint inhibitors [20].

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 often times 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 originally reported exclusively in melanoma patients, but now seen with a variety of primary malignancies (Fig. 3). Incidence is about 2% for anti-CTLA-4 and anti-PD-1 therapies [7]. Histology shows loss of melanocytes at the dermal–epidermal junction (Fig. 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 [21]; 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.

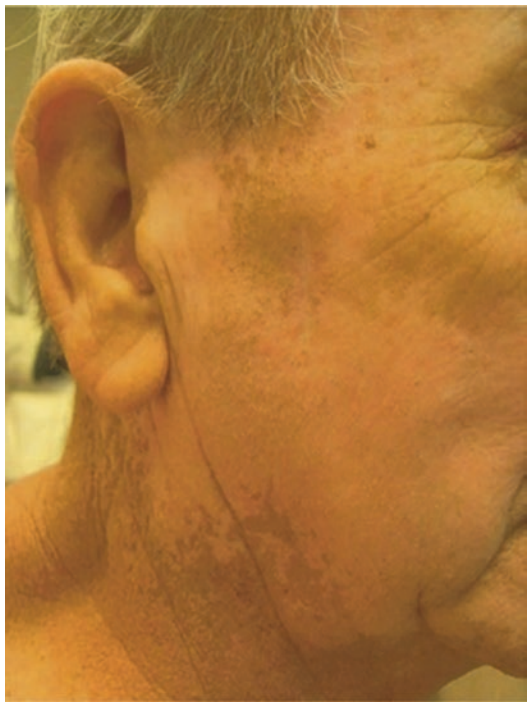

Fig. 3 Vitiligo, depigmented patches of head and neck

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 [22, 23]. 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 [24].

3 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 pruritus from a quarter to one-third [25]. The type of rash varied from mild eczema to epidermal necrolysis [26], with the majority experiencing a more traditional morbilliform drug eruption or an eczematous atopic dermatitis-like eruption [25]. The onset of rash has been reported to appear at about 3 weeks and then usually resolves around 2.5 months [25]. 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 [27], granulomatous dermatitis [28], and pyoderma gangrenosum [29].

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

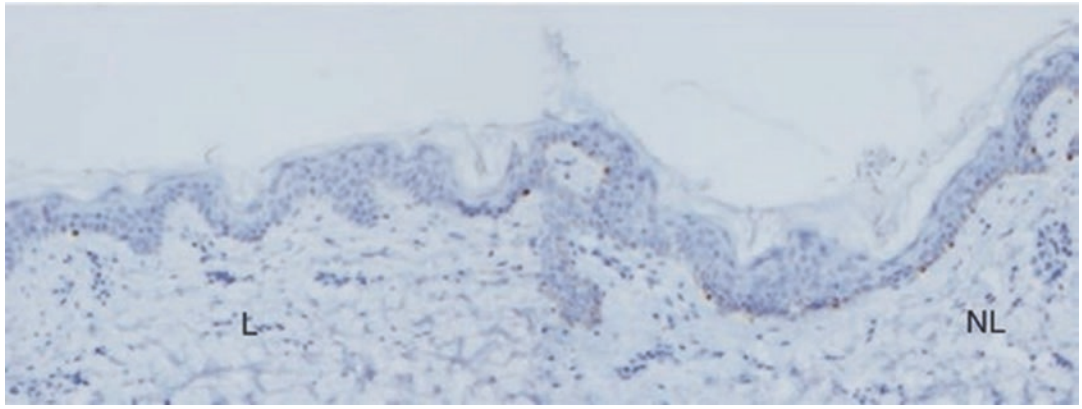

Fig. 4 Vitiligo-MART1 immunostain in lesional skin (L) showing decreased melanocytes at the dermal–epidermal junction compared to MART1 immunostain of nonlesional (NL) skin

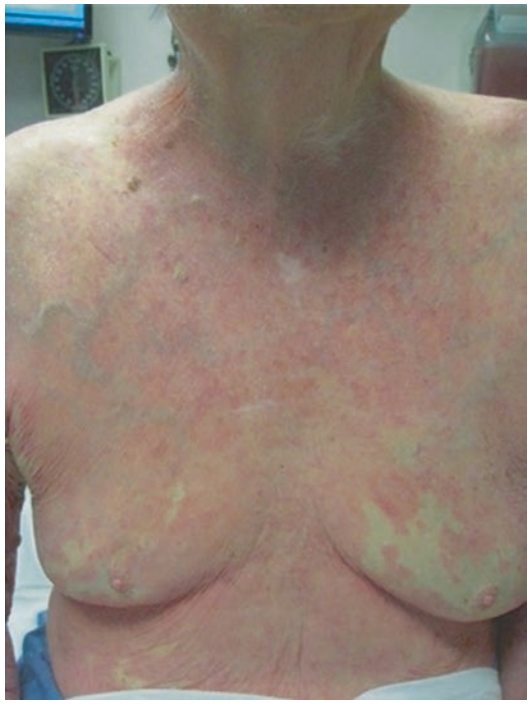

Fig. 5 Psoriasiform dermatitis, erythematous well-demarcated plaques with fine adherent scale

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% [30]

4 CAE in Anti-PD-1

In addition to the shared inflammatory skin reactions discussed earlier, psoriasis [31, 32], lichenoid dermatitis [33], and bullous pemphigoid have been induced by anti-PD-1 antibodies [34, 35]. More recently, eruptive keratoacanthomas has been reported in patients receiving anti-PD-1 therapy [36] (Figs. 5 and 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 [32] or in the pustular variant [36]. 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), IL-12/23, and IL-23

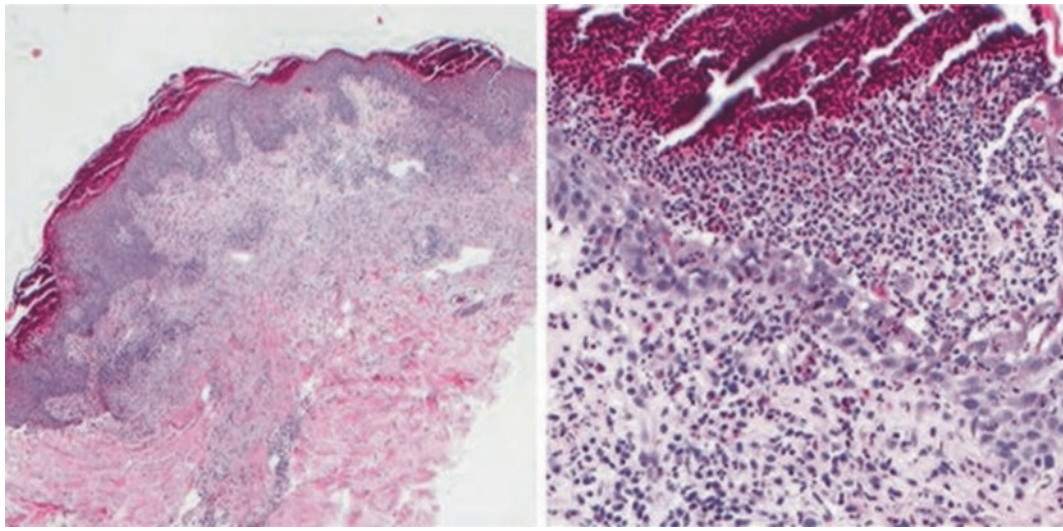

Fig. 6 Spongiotic psoriasiform dermatitis with subcorneal pustules, irregular acanthosis, and numerous eosinophils

inhibitors are potential therapies for refractory cases and have been used anecdotally with success [37].

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 bullae. The dermal–epidermal split is cleaved and the epidermal roof is intact. Dyskeratosis is not a feature. Direct immunofluorescence highlights IgG deposition at the dermal–epidermal junction. Topical and oral steroids, rituximab [38], anti-IgE monoclonal antibodies (omalizumab) [39], and anti-IL4,13 antibodies (dupilimumab) [40] have been used successfully in this slow-to-appear cutaneous AE. Similarly pemphigus has been reported [41].

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 [36].

5 Combination Therapies

Combination checkpoint inhibitor therapies are being used more frequently with loading doses of anti-CTLA4 and anti-PD-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.

6 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 1 and Fig. 7).

Table 1 Common terminology criteria for adverse events [42]

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. 7 Management of skin irAEs in patients treated with ICPIs [43]

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 had previously only been reported in patients being treated with melanoma [5, 6, 44, 45], but has since been seen in other cancer types [21]. Incidence of rash was also associated with increased survival and tumor response [5].

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. 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. 7 (continued)

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Immunotherapy-Mediated Luminal Gastrointestinal Toxicities

Anusha S. Thomas and Yinghong Wang

Abstract

The advent of immune checkpoint blockade and its application in the management of advanced malignancies has revolutionized cancer therapies, outcomes, and survival. As beneficial as these class of drugs have been proven to be, their use is not devoid of complications, viz., immune-related adverse events (irAEs). The gastrointestinal (GI) tract is the second most frequently affected organ system, and toxicities may vary in severity from mild disease to aggressive life-threatening clinical presentations. Timely diagnosis that incorporates clinical, biochemical, imaging, endoscopic, and histologic evaluation is imperative for efficacious management of this disease process to ensure good outcomes. Management varies depending on severity and can comprise supportive care in milder disease patterns as well as vigorous immunosuppression in aggressive cases.

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Keywords

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

1 Epidemiology and Risk Factors

The overall incidence of immune checkpoint inhibitor (ICI) enterocolitis (IMC) has been reported to range from 10 to 30% [1–5]. This wide range rests significantly on several risk factors as pertains to the type of ICI, the cancer type, and the patient. Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) blockade therapy is notorious for a higher incidence and grade of toxicity as opposed to Programmed cell death protein 1 (PD-1)/Programmed death-ligand 1 (PD-L1) blockade as is combination therapy compared to single agent therapy [6]. Furthermore, higher doses of ICI therapy appear to pose a greater risk of developing IMC [7, 8]. Toxicity secondary to CTLA4 blockade often presents earlier (1 month) than that due to PD1/L1 blockade (2–3 months) which is possibly reflective of a longer half-life of the latter class.

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However, cases may occur up to 2 years after the first infusion which is highly suggestive of a persistence of the biological impact of the drug long after its clearance [9]. In terms of cancer types, it has been suggested that patients with advanced stage cancers, in particular malignant melanoma, may predispose to an increased risk of developing IMC [10]. Patient characteristics may play a crucial role in determining the risk of developing IMC, viz., gender and baseline microbiome. One might speculate that given the significantly varied immune response pattern and tumor biology between men and women [11], this might translate similarly in terms of irAEs; however conclusive data is still lacking. Furthermore, while the literature favors the role of the baseline gut microbiome unique to the patient to predict both therapeutic response to ICI as well as the risk of developing IMC [12], controlled clinical trials are warranted to confirm the same. Lastly, preexisting IBD with active disease may confer a higher risk of IMC [13].

2 Evaluation of a Patient with Immune-Mediated Enterocolitis

Clinically, it is important to grade the presentation of IMC using the Common Terminology Criteria for Adverse Events *version 5.0* [14]. Despite data suggesting a poor correlation between the grading of diarrhea and colitis on this scale that relies heavily on clinical signs and symptoms alone, the utility of the same in triaging patients based on severity of disease has been employed in numerous clinical trials [15].

Infectious workup is imperative to rule out bacterial (e.g., *Clostridium difficile*), viral (e.g., CMV), parasitic, or fungal infections in an immunocompromised patient population which may present in a similar fashion [16]. 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. Fecal lactoferrin and calprotectin may serve as useful biomarkers of inflammation. While data suggests that the former can be highly sensitive in detecting endoscopic and histologic inflammation, stool calprotectin testing can be applied as an alternative to endoscopic surveillance to assess treatment response [17, 18].

Contrasted imaging is routinely reserved to rule out acute intra-abdominal processes other than IMC or complications related to the same in those with grade $\geq$ 2 diarrhea. This stems from the poor negative predictive value and correlation between imaging and endoscopic findings. However, three imaging signs have been established for this process, namely, diffuse colitis pattern, segmental colitis with diverticulosis, and isolated rectosigmoid colitis without diverticulosis with a good positive predictive value [19].

Early endoscopic evaluation is key to identifying patients with high-risk features of colitis and has been shown to facilitate prompt and efficacious management thereby decreasing steroid dependency and improving overall outcomes particularly in terms of prolonged hospitalization and recurrence in a critically ill patient population [15, 17]. Endoscopic manifestations may range from normal appearing mucosa (up to one third of patients) to non-ulcerative inflammation and mucosal ulcerations [15]. There is no established validated tool to grade endoscopic severity in IMC currently; the Mayo Clinic scoring system (Table 1) for ulcerative colitis is beneficial in triaging those with high-risk features (Fig. 1c) (large ulcers >1 cm, deep ulcers >2 mm and extensive colitis involvement) and moderate-risk features(Fig. 1b) (small ulcers <1 cm, shallow ulcers<2 mm in depth, non-ulcerative inflammation, normal colonic mucosa with abnormal histology and isolated left sided colitis) from those with low-risk features(Fig. 1a) (normal endoscopy with histology).

Three distinct histologic patterns of IMC have been identified, namely, acute colitis, chronic colitis, and microscopic colitis. It is the third type, albeit rare, that demonstrates an aggressive disease course with a significantly increased need for systemic immunosuppression [20]. Acute colitis pattern (Fig. 2a) is most frequently encountered and is notable for neutrophil and/or eosinophil infiltration, epithelium apoptosis,

Table 1 Mayo Endoscopic score

Score	Disease activity	Endoscopic features
0	Normal/inactive	None
1	Mild	Erythema, decreased vascular pattern, mild friability
2	Moderate	Marked erythema, absent vascular pattern, friability, erosions
3	Severe	Spontaneous bleeding, ulceration

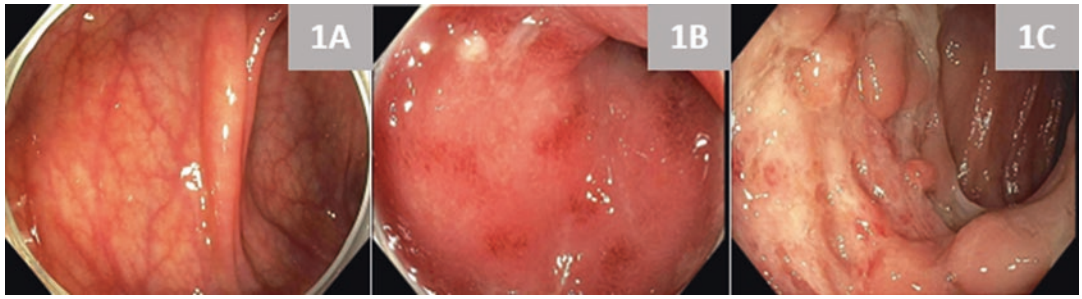


Fig. 1 (**a**) Normal-appearing colonic mucosa; (**b**) moderate-risk endoscopic features characterized by edema, erythema, and non-ulcerative inflammation; (**c**) high-risk endoscopic features characterized by deep ulcerations

cryptitis, and crypt micro-abcesses. Chronic colitis pattern (Fig. 2b) demonstrates features very similar to inflammatory bowel disorders such as crypt architectural distortion, basal lymphoplasmocytosis, granulomas, and Paneth cell metaplasia [15]. The microscopic colitis pattern may resemble lymphocytic or collagenous colitis. (Fig. 2c, d) It is important to emphasize that there is no correlation between clinical symptoms of this disease process and histologic inflammation, a phenomenon observed in IBD [21, 22]. Interestingly, the onset of histologic inflammation likely occurs before clinical symptomatology [15].

3 Treatment of IMC

Efficacious management of IMC involves prompt initiation of appropriate therapy to ensure avoidance of complications, recurrence, and delay in cancer care. Grade 1 IMC manifested as a mild and self-limiting diarrhea may be managed with supportive care, i.e., hydration, correction of electrolyte imbalances, bland diet, anti-diarrheals (once infection has been ruled out), or 5-ASA-based therapies. In most cases, ICI therapy may be resumed after resolution of the acute episode [23].

Grade ≥2 IMC is routinely managed with prompt immunosuppression. Importantly, ICI therapy should be halted temporarily for grades 2 and 3 and permanently for grade 4 [24, 25]. Patients with low-risk endoscopic features on evaluation can be treated with weight-based systemic corticosteroids (prednisone or equivalent with a dose of 1–2 mg/kg) with a taper over a duration of 4 weeks after symptoms resolution to ensure fewer complications secondary to infections [15]. In the rare absence of improvement in 3 days from steroid initiation, patients may be administered selective immunosuppressive therapy (SIT) with either infliximab or vedolizumab to reach clinical remission.

Early introduction of SIT is associated with favorable clinical outcomes in patients with IMC regardless of steroid response, especially in patients with severe disease presentation. Infliximab is a chimeric human mouse IgG monoclonal antibody that targets the TNF-α receptor thereby suppressing inflammation. While the evidence shows a significant decrease time to symptom resolution and steroid titration with this drug [26], it does have a plethora

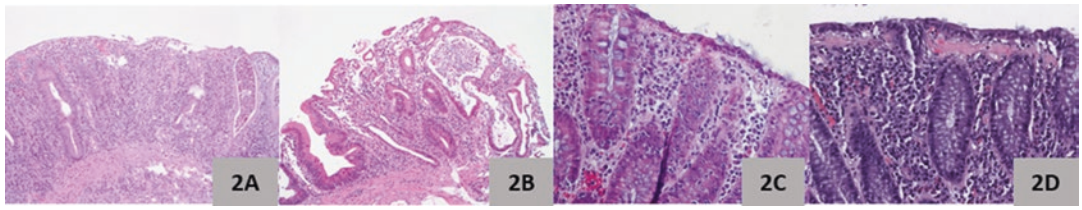


Fig. 2 (**a**) Acute active colitis, (**b**) chronic active colitis, (**c**, **d**) microscopic colitis patterns

of side effects and is contraindicated in the setting of congestive heart failure, hepatotoxicity, and demyelinating disease. It has also been implicated in an increased risk of malignancy/lymphoma [27] with long-term usage. Vedolizumab is a gut selective fully humanized monoclonal antibody that targets the α4β7 integrin that has shown encouraging clinical outcomes, comparable efficacy, and favorable safety profile [28].

Patients with high-grade endoscopic features have a significantly higher risk of prolonged hospitalization and recurrence [17] and therefore benefit from early initiation of at least three doses of SIT [29] in conjunction with weight-based systemic corticosteroid taper. Once clinical remission is attained, it is highly recommended that SIT therapy continue if ICI is resumed. Presently, endoscopic surveillance is recommended to ensure adequate treatment response. Partial endoscopic improvement and/or residual histologic inflammation should prompt continuation of SIT and PD1/PDL1 blockade may be reinstituted with caution. We also note that up to a third of patients who resume ICI after IMC experience recurrence. Factors that predispose to IMC recurrence with resuming ICI therapy such as CTLA 4 blockade, long duration of the initial IMC episode, and its requirement for SIT should be strongly considered [30].

Fecal microbiota transplantation (FMT) has been proposed to be effective in patients with ICI-induced enterocolitis refractory to above-mentioned immunosuppression [31]. The utility of mycophenolate mofetil, tofacitinib, and ustekinumab in the management of refractory cases is also being explored [32–35].

Lastly, the evidence suggests that IMC, particularly when the disease course exceeds 3 months in duration with features of chronicity on colon histology [36], is associated with improved survival outcomes in terms of cancer and may in fact reflect persistent antitumor activity of the ICI therapy. Diarrhea is an independent predictor of an improved survival regardless of treatment requirement. As we learn more about this disease process, it appears that striking a fine balance between ICI therapy and toxicity is key to ensure maximum benefit of this revolutionary class of drugs in advanced malignancies.

4 Conclusion

IMC is the second most frequently encountered irAE. Early recognition with clinical, biochemical, imaging, and prompt endoscopic evaluation bears favorable outcomes. Early introduction of SIT with a minimum of three doses is associated with faster symptom resolution and decreased steroid exposure. IMC is associated with better cancer outcomes.

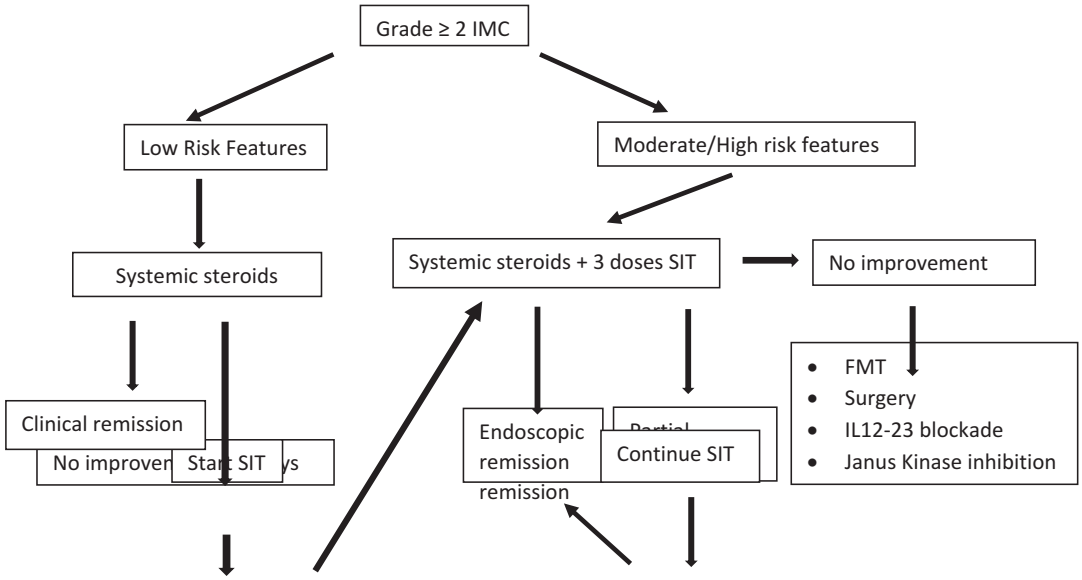
5 Immune-Mediated Upper GI Toxicity (From the Mouth to the Ligament of Trietz)

Upper GI (mouth to ligament of Trietz) toxicity secondary to ICI use is rare, and therefore the body of evidence is limited. Upper GI symptoms occur far more commonly in conjunction with IMC, and isolated upper GI involvement is rare. PD-1/PD-L1 blockade has been more frequently implicated in toxicity involving the upper GI tract compared to CTLA4 blockade [37–39], which may have attributed to variable expression of targets in different tissues [39, 40]. However,

the distribution of CTLA-4 and PD-1/PD-L1 expression along the GI tract has not been well described.

Reports suggest clinical symptomatology might include dysphagia, odynophagia, intractable nausea, and emesis [37, 42, 43]. This entity is typically a diagnosis of exclusion by upper endoscopy with biopsy. Endoscopic features include erythema, edema, friability, erosions, and ulcerations. On histology, commonly described features in the gastric mucosa are lamina propria expansion and intraepithelial neutrophilic infiltration. In duodenal biopsies, villous blunting, lymphoplasmacytic lamina propria expansion, and plasma cells and eosinophilic infiltrates, neutrophilic cryptitis, and/or villitis have been reported [44, 45].

Most patients have mild symptoms and can be effectively managed with non-immunosuppressive treatments such as proton pump inhibitors or H_2 receptor blockers. Anecdotal reports favor use of systemic steroids or vedolizumab in patients with aggressive disease refractory to supportive management [46]. Larger prospective studies are needed to help further characterize this disease process longitudinally and determine optimal management of the same.



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Hepatobiliary and Pancreatic Adverse Events

Hao Chi Zhang, Lan Sun Wang, and Ethan Miller

Abstract

The expanded approval of immune checkpoint inhibitors (ICIs) for the treatment of multiple cancer types has offered patients more opportunities in treatment selection and survival.

Hepatotoxicity is a well-recognized immune-related adverse event (irAE) associated with treatment with ICI. It is considered a type of drug-induced liver injury (DILI). Depending on the specific ICI and whether the patient receives single- or dual-drug therapy, the incidence of hepatotoxicity in general could be as high as 30%. As more patients receive treatment with ICI, more cases of hepatotoxicity are expected to occur. Clinicians must exercise close pharmacovigilance to recognize liver-related irAEs early.

ICI-mediated hepatobiliary toxicity (or "IMH") generally presents as asymptomatic elevations of alanine transaminase and aspartate transaminase, with or without alkaline phosphatase elevation. Some patients may present with jaundice, fever, or malaise. Rarely, it may cause liver failure and death. The diagnosis of IMH is made after careful exclusion of other causes of acute hepatitis based on medical history, laboratory evaluation, imaging, and liver histological findings. In clinically significant cases of IMH, the management involves discontinuation of ICI followed by close monitoring and the initiation of immunosuppression. Current society guidelines, which are not based on robust evidence, specify treatment recommendations depending on the grade of liver injury, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. However, our clinical experience suggests possible alternatives, including lower corticosteroid dosing with adjunct therapies. Whereas current guidelines endorse permanent cessation of future ICI treatment in patients diagnosed with grades 3–4 IMH, published clinical experience suggests potential for flexibility when assessing for candidacy of resuming ICI.

Because histologic bile duct injury has been observed in cases ascribed to IMH, ICI-mediated cholangiopathic disease probably exists on a spectrum within IMH. Even extrahepatic bile duct involvement has been observed. This phenotype warrants special considerations in treatment and surveillance.

ICI-related cholecystitis has been rarely reported in the literature. Management follows current standards of care for typical cases of cholecystitis. No relationship with ICI-

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mediated cholangiopathic disease has been observed.

Assessing for and managing ICI-associated pancreatic injury remain challenging to the clinician. Many cases of asymptomatic serum lipase elevation are detected on routine labs without clinical signs or symptoms of typical acute pancreatitis. However, symptomatic patients should be initially managed like traditional cases of acute pancreatitis requiring hospitalization for evaluation and inpatient management.

Keywords

Immune checkpoint inhibitors · Immunotherapy · Hepatitis · Hepatobiliary toxicity · Cholangiopathy · Pancreatitis · Cholecystitis

1 Hepatobiliary Toxicity

1.1 Nomenclature

The nomenclature used to describe hepatotoxicity associated with immune checkpoint inhibitors (ICIs) is variable. Examples of terms that have been used include "hepatic irAE" and "immune-mediated hepatitis." The most recent review on this topic in *Hepatology* by Peeraphatdit et al. refers to this entity as "IMH" for "immune-mediated hepatotoxicity" [1]. Because bile ducts derive from the liver and with the knowledge that bile duct injury can occur simultaneously (or overlap) with hepatocellular injury, "immune-mediated hepatobiliary toxicity" or "ICI-mediated hepatobiliary toxicity" captures a broader spectrum of its heterogeneous presentations while maintaining the abbreviation of IMH for consistency and brevity. We will maintain this nomenclature henceforth.

1.2 Incidence

IMH is a well-recognized irAE [2]. The incidence of IMH varies depending on the ICI agent and whether monotherapy or dual ICI therapy is being employed. The overall incidence of hepatotoxicity associated with anti-PD-1/PD-L1 inhibitors is reported to be up to 12%, where the incidence of IMH associated with anti-PD-1 inhibitors (specifically pembrolizumab and cemiplimab) is relatively lower at 0.7–2.1% [1]. Anti-CTLA-4 inhibitors (most commonly ipilimumab) are associated with a hepatotoxic risk as low as 1–7% but as high as 16% [1, 3–5].

The risk of IMH increases up to 30% in patients receiving ICI combination therapy [3, 4, 6]. Grades 3–4 IMH were reported in 1–3% of patients receiving ICI monotherapy and in 8–14% of patients treated with a combination of anti-PD-1 and anti-CTLA-4 therapy [5–13]. Overall, the incidence of at least grade 3 IMH occurred in 1.1%, 1.7%, and 9.2%, associated with anti-PD-1/L1, anti-CTLA-4, and combination of ICI treatment, respectively [14]. Therefore, IMH is not an uncommon irAE. The diagnosis of grades 3–4 IMH has important implications for the patient's future cancer treatment course and prospects for future candidacy for ICI treatment.

1.3 Pathophysiology

Aside from T-cell activation pathways that affect hepatocytes, the specific mechanism by which IMH develops is not understood. Hypotheses for mechanisms include the notion of a possible dose-dependent risk and permissive hepatotoxicity in those with preexisting autoimmune disease, although no studies have included those with idiopathic autoimmune hepatobiliary disease [1]. Currently, IMH is regarded as a form of "indirect" hepatotoxicity, which is not expected to be dose-related, but instead occuring due to the drug mechanism, which is immune-mediated [1]. IMH is not considered a form of idiosyncratic liver injury. Patient-specific risk factors and predictive models have not been identified. Interleukin-6 (IL-6) and its associated pathways have a well-described role in general liver biology, but the specific immunobiologic pathways to the development of IMH in relation to downstream signaling mechanisms

have yet to be delineated. Because cholangiocytes express PD-L1, which would interact with PD-1 on activated T-cells, this may provide insight into possible mechanisms associated with cholangiopathic phenotypes of IMH.

1.4 Clinical Presentation

IMH manifests along a spectrum of hepatocellular and/or cholestatic injury [11, 15–17]. The presentation of IMH remains highly heterogeneous, ranging from an asymptomatic state with the rise in liver enzymes to, rarely, death as a consequence of acute liver failure [18–20]. Although hepatotoxicity is commonly an incidental finding on routine laboratory screening during the course of ICI treatment, clinical signs and symptoms of IMH can include jaundice, acholic stool, malaise, abdominal pain, and fever [15, 21, 22]. Increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin are the commonly used biomarkers of IMH as suggested by the Common Terminology Criteria for Adverse Events (CTCAE)-based guidelines, regardless of the class of ICI [2, 5, 15, 19]. In general, IMH is often identified at about 5–13 weeks after initiation of ICI therapy, but its onset can be as early as after the first week [1, 13, 16, 19, 23]. IMH can also emerge after cessation of ICI therapy, which may depend on the half-life of the agent that was administered.

1.5 Diagnosis

1.5.1 Initial Diagnostic Evaluation

The diagnosis of IMH is approached in nearly the same manner as with other cases of suspected DILI. Like assessment for DILI in general, IMH is a diagnosis of exclusion. The clinician should perform a careful evaluation of the patient's medical history including competing medications, the use of supplements (including herbal medications), and alcohol history. Other differential diagnoses should be explored and excluded [1, 5, 24]. These include viral etiologies (viral hepatitis A, B, C, or E; cytomegalovirus; Epstein-Barr virus; herpes simplex virus), idiopathic autoimmune liver disorders, and metabolic liver disorders (Wilson disease, hemochromatosis, and alpha-1 antitrypsin deficiency). Importantly, IMH is an entity that is distinct from idiopathic autoimmune hepatitis (AIH) and drug-induced AIH. AIH may be excluded when histologic features on liver biopsy are not compelling for AIH in conjunction with a normal total serum IgG level. In cases with cholestasis or jaundice, etiologies to be considered include acute liver dysfunction, choledocholithiasis, tumor causing obstructive jaundice, hemolytic disorder, or, rarely, IgG4-related cholangiopathy. In a patient of metastatic burden to the liver organ should be carefully considered.

Calculating the R factor will characterize the pattern of liver injury:

$$\text{R factor} = \frac{\left(\text{ALT} / \text{ALT}_{\text{ULN}}\right)}{\left(\text{ALP} / \text{ALP}_{\text{ULN}}\right)}$$

ALT, alanine aminotranferase; ALP, alkaline phosphatase; ULN, upper limit of normal. Hepatocellular-predominant injury corresponds to an R factor greater than 5.0. Cholestatic-predominant injury corresponds to an R factor less than 2.0, when the ALP is at least 2× ULN. Mixed hepatocellular and cholestatic injury corresponds to an R factor from 2.0 to less than 5.0, when the ALT and ALP are both at least 2× ULN.

Based on both the magnitude of liver biochemical tests and the clinical presentation, the CTCAE version 5.0 grading is used to determine the specific management and/or treatment of IMH [25]. Liver enzymes include two transaminases (ALT and AST) and alkaline phosphatase. Liver function tests (LFTs) are the INR, total bilirubin, and albumin. From here forth, we will use the term "liver biochemical tests" (LBT) to refer to the combination of liver enzymes and liver function tests. In current society guidelines, the CTCAE grading of IMH is based on the ALT, AST, and total bilirubin. The standard criteria for CTCAE grading are summarized in Table 1.

Table 1 CTCAE (version 5.0) grading schema for liver biochemical laboratory tests

Lab parameter	Grade 1	Grade 2	Grade 3	Grade 4
ALT	>ULN to 3.0× ULN	>3.0–5.0× ULN	>5.0–20.0× ULN	>20.0× ULN
AST	>ULN to 3.0× ULN	>3.0–5.0× ULN	>5.0–20.0× ULN	>20.0× ULN
ALP	>ULN to 2.5× ULN	>2.5–5.0× ULN	>5.0–20.0× ULN	>20.0× ULN
Total bilirubin	>ULN to 1.5× ULN	>1.5–3.0× ULN	>3.0–10.0× ULN	>10.0× ULN

Abbreviations: *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *ULN* upper limit of normal

The correct interpretation of the liver biochemical profile is crucial in the interpretation of the patient's clinical status and planning the appropriate follow-up strategy [22]. ALT is more specific than AST as an indicator of hepatocellular injury, although, in general, the magnitudes of their levels are similar and track together over time. In DILI, the ALT level is generally similar or higher than the AST level. Because there is otherwise no universal standard ULN for the AST level, we recommend using the ULN of AST reported by the interpreting laboratory.

The alkaline phosphatase (ALP) level can be directly influenced by age, ethnicity, and the presence of metastatic disease to the liver or bone [26]. Society guidelines do not feature ALP in the overall CTCAE grading. However, significant ALP elevations should prompt further characterization to determine, for instance, whether the elevation is predominantly from a cholestatic condition or from bone turnover, which could be differentiated by first checking the GGT value. Alkaline phosphatase isoenzyme evaluation may also be helpful in some circumstances.

Any case of elevated bilirubin must be carefully characterized to determine whether it is associated with liver dysfunction, biliary obstruction, or another cause, because unconjugated hyperbilirubinemia may point toward a hematologic process (such as hemolysis) instead of impaired liver synthetic function. Therefore, this CTCAE grading should only be used when the direct bilirubin proportion is at least 50% of the total bilirubin, and after focal biliary obstruction is excluded. Serum albumin and INR levels should also contribute to the interpretation of liver synthetic function.

IMH is an entity that is distinct from idiopathic autoimmune hepatitis and drug-induced autoimmune hepatitis. There is no relationship between autoimmune serologic markers, such as ANA, with the diagnosis of IMH [1, 13]. AIH may be excluded when histologic features on liver biopsy are not compelling for AIH in conjunction with a normal total serum IgG level. The AIH scoring systems can be used to gauge this further [27]. The expectation would be that those patients with IMH should yield low-probability AIH scores. In the absence of positive AMA M2 type, normal total serum IgM level, and lack of typical histologic features such as florid duct lesions and ductopenia, primary biliary cholangitis can be excluded. The correct diagnosis of the observed laboratory derangements is crucial as it affects prognosis, indications for steroid treatment and its duration, clinical outcomes, and candidacy for ICI rechallenge.

1.5.2 Imaging

Abdominal imaging, such as computerized tomography (CT), magnetic resonance imaging (MRI), and abdominal ultrasound (US), must be part of the initial evaluation although findings in IMH are usually nonspecific [28]. Imaging can help detect alternative diagnoses such as liver metastasis, intrahepatic and extrahepatic biliary tree abnormalities, or vascular disease such as hepatic or portal vein thrombosis [15, 29, 30]. In patients who present with cholestasis suspicious for biliary tract disease, high-quality imaging targeting the biliary tree such as magnetic resonance cholangiopancreatography (MRCP) must be performed to exclude entities such as choledocholithiasis or other causes of obstructive jaundice. MRCP is also the preferred initial diagnostic imaging test for evaluating primary sclerosing cholangitis.

In general, IMH alone is associated with normal appearance of the liver or no new interval changes compared to prior liver imaging [15, 31]. However, reported radiological features in IMH that could manifest include periportal edema, hepatomegaly, periportal MRI T2 hyperintensity, attenuated liver parenchyma, and enlarged periportal lymph nodes on CT or MRI in severe IMH [15, 32, 33].

1.5.3 Role of Liver Biopsy and Interpretation of Histologic Features

The role of routine liver biopsy for diagnosing IMH is controversial since the liver biopsy is an invasive procedure [1, 34, 35]. Across published guidelines, liver biopsy has not been endorsed as an initial diagnostic test for confirmation of the diagnosis of IMH before treatment (i.e., steroids) is initiated. In clinical practice, if liver biopsy is not initially performed during the diagnostic phase, it may be considered later in those patients whose LBT fail to improve, either spontaneously or in response to systemic corticosteroid treatment.

There are currently no known pathognomonic histologic features of IMH. The most common histologic descriptions attributed to patients with IMH include nonspecific features of lobular or pan-lobular hepatitis, necroinflammatory findings that are either spotty or confluent, fibrin ring granulomas (particularly in those with anti-CTLA-4 exposure), central vein endotheliitis, prominent sinusoidal lymphohistiocytic infiltrates, and bile duct injury [10, 19, 21, 36, 37]. Despite their limitations, the histologic findings serve to exclude other causes of liver injury when the serologic data may not be revealing or may suggest a distinct, competing disease process under consideration. In a study of melanoma patients to gauge the utility of liver biopsy for suspected IMH, 58 patients with grades 3–4 liver injury underwent liver biopsy, three of whom were actually diagnosed with a condition other than IMH [38]. Whether or not the patient has positive autoimmune antibodies, the pattern of histologic inflammation *could* differentiate IMH from AIH. The finding of interface hepatitis with inflammatory cells that are plasma cell-predominant in AIH are distinct from the findings of lymphocyte and histiocyte predominance with typically lobular inflammation in IMH. In cases with cholestatic injury LBT patterns, the biopsy can confirm whether there is cholestasis and whether bile duct injury is seen in conjunction with hepatitis. If a patient's diagnostic work-up does reveal a positive autoimmune marker such as ANA, anti-smooth muscle antibody, or anti-mitochondrial antibody, then such a case would carry a very compelling indication to pursue a liver biopsy.

Although the diagnosis of IMH traditionally addresses hepatocellular injury, ICI-mediated bile duct injury is probably severely underrecognized as a distinct entity. In a case series of patients regarded as having immune checkpoint inhibitor-mediated hepatotoxicity, 56% of the cases had histologic evidence of bile duct injury [13]. The majority of such cases are reported in association with anti-PD-1 inhibitors, especially nivolumab and pembrolizumab [39–45]. Concomitant alkaline phosphatase elevations, jaundice, and histologic bile duct injury have been acknowledged in cases attributed traditionally to ICI-mediated "hepatotoxicity" [13, 46, 47]. Therefore, ICI-mediated cholangiopathy or cholangiohepatitis may exist within the spectrum of IMH. Its diagnosis can be corroborated by liver biopsy.

1.6 Management and Treatment Options

1.6.1 General Diagnostic Approach

The grading schema offered by the CTCAE version 5.0 influences the treatment approach to IMH [22, 48]. A primary limitation in the current treatment guidelines offered across multiple societies is that the recommendations are based on expert consensus without robust data. Given the dearth of evidence, monitoring and treatment strategies must be tailored to each patient's specific scenario. Here, we offer our diagnostic algorithm and treatment recommendations based on existing guidelines, appraisal of published evidence, and our own clinical experience (Fig. 1).

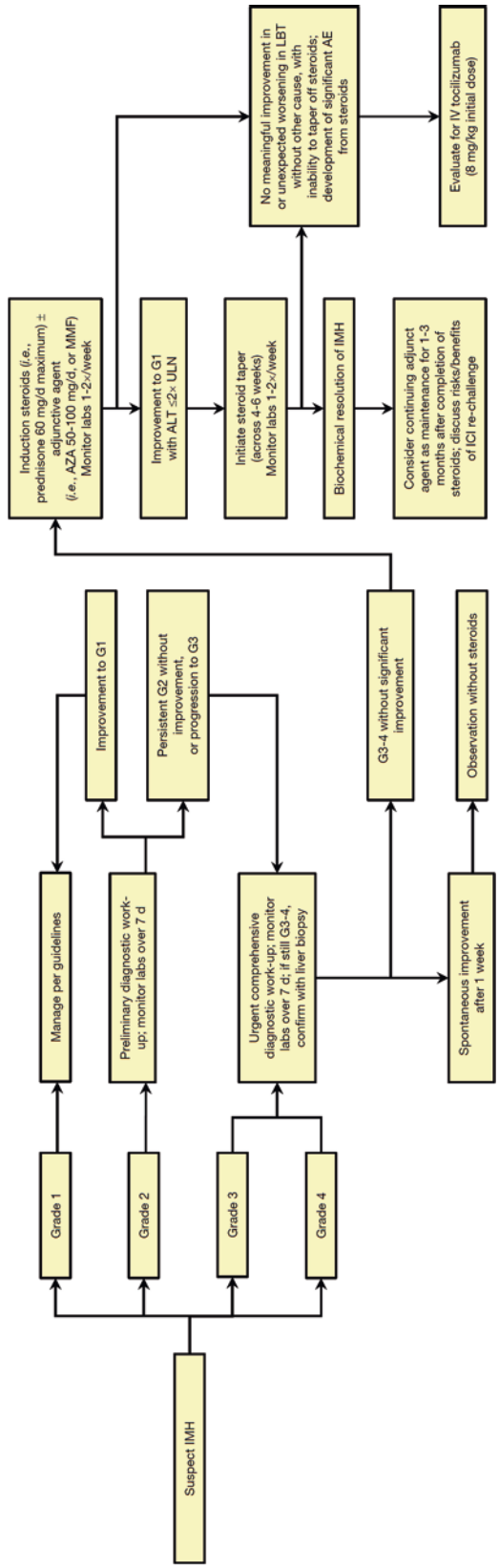

Fig. 1 Schema delineating the approach to the diagnosis of IMH and its treatment. The proposed steps reconcile current guidelines with the latest understanding from clinical experience. *G* grade (by CTCAE definitions), *AZA* azathioprine, *MMF* mycophenolate mofetil, *LBT* liver biochemical tests, *AE* adverse events or effects, *IV* intravenous

Grade 1

Patients may continue ICI treatment with close monitoring of the LBT. Liver biopsy is not necessary to make the diagnosis.

Grade 2

ICI should be temporarily withheld with close monitor of the trends in LBT. Like in many cases of DILI, because spontaneous improvement could be observed in the short term, the first week may be used to initiate a more comprehensive liver disease workup, including the need to exclude acute infectious hepatitis, before deciding on steroid initiation. If LBT do not improve or worsen while remaining within grade 2 parameters, oral prednisone dosed at 0.5–1 mg/kg/day with maximum of oral prednisone 60 mg/day can be considered with a subsequent taper. Weekly lab monitoring is recommended.

Grade 3

ICI must be withheld. As part of the initial liver disease workup, liver biopsy should be considered to increase confidence for the diagnosis of IMH. Because grade 3 IMH has the potential to demonstrate spontaneous improvement, it is reasonable if not encouraged to monitor for signs of improvement in LBT for 1–2 weeks after recognition of LBT, during the diagnostic testing phase, before deciding to initiate steroids [14]. However, initiation of steroids should not be delayed once infection is confidently excluded and if LBT are not already improving.

Traditionally, a steroid dose range equivalent to either IV methylprednisolone or PO prednisone 1–2 mg/kg is suggested. The necessity of this dosing paradigm has been challenged [1, 49, 50]. We recommend a steroid range equivalent to prednisone of 0.5–1 mg/kg/day with maximum of oral prednisone 60 mg/day (regardless of patient weight) for induction [49–51]. If IV methylprednisolone is selected, then a dose of 60 mg/day can be administered. Weekly lab monitoring should be considered to track the evolution of changes in LBT. Once transaminases approach either complete biochemical remission or near biochemical remission (i.e., ALT of 2× ULN or less), steroids may then be tapered over 4–6 weeks, or longer, closely based on individual LBT trends [5, 52, 53].

Grade 4

ICI must be withheld. Thorough liver disease workup should be immediately pursued, including liver biopsy. Since grade 4 IMH also has the potential to demonstrate spontaneous improvement, barring evidence of liver failure, the patient's LBT may be monitored for about 1 week while waiting for results from diagnostic testing before deciding to initiate steroids [14]. However, as with grade 3 IMH, if the LBT trends are not favorable by the end of the first week, initiation of corticosteroids should not be delayed if infection is not suspected or has been excluded. We generally recommend steroid dosing of the equivalent of prednisone 1 mg/kg/day, with maximum dose of oral prednisone 60 mg/day for induction. Steroids are eventually to be tapered once biochemical remission is achieved. It may take longer than 4–6 weeks depending on the starting dose of steroids and evolution of LBT. Close follow-up of laboratory values and careful examination for evidence of liver failure are key. If a patient demonstrates features of acute hepatic synthetic dysfunction (such as jaundice and significant elevation in the INR level), or if clinical features of acute liver failure are present (including presence of asterixis or an abrupt abnormal change in mental status), then hospitalization would be warranted to avoid delays in diagnosis and management.

In all patients initiated on the path of steroid treatment for IMH, we prefer prophylaxis against PJP pneumonia using atovaquone. Dapsone is an alternative. We avoid the use of trimethoprim-sulfamethoxazole, if possible, to lower the risk of hepatotoxicity. If the patient is not already on a proton pump inhibitor (PPI), a low dose of PPI should be prescribed for gastric protection while on steroids. Prolonged steroid use should prompt evaluation for glycemic control, particu-

larly in patients who have an established diagnosis of prediabetes or diabetes.

1.6.2 Adjunctive Treatments

In patients initiated on steroids who do not respond satisfactorily after 3 days of treatment, clinicians should consider addition of adjunctive agent(s) to control IMH [1, 22, 23, 35, 54–57]. Many adjunctive therapies have been selected in real-world clinical use based on knowledge of an agent's theoretical effects on targeting T-cell subpopulations [54]. Early or simultaneous adjunct treatment may also confer the benefit of a shorter time to ALT improvement in those with grade 3 IMH, thereby potentially reducing overall steroid exposure [58]. We prefer azathioprine (50–100 mg/day), which is established as a first-line adjunct treatment in idiopathic AIH, to be prescribed during the initial steroid induction phase [50, 58–61]. Ursodeoxycholic acid (UDCA or ursodiol), based on its mechanism and low risk of AE, may be prescribed in cases with cholestatic features, histologic bile duct injury, with or without jaundice [62–64]. The optimal dose of UDCA is not defined, although 13–15 mg/kg/day in divided doses can be adopted from the management of PBC.

The following agents and treatments have been published:

- Mycophenolate mofetil [13, 14, 20, 34, 49, 54, 58, 62, 65–70]
- Tocilizumab [44, 45, 71]
- 6-mercaptopurine [19]
- Tacrolimus [49, 54, 68]
- Antithymocyte globulin (ATG) [20, 66, 72]
- Plasma exchange [73]
- Cyclosporine [59]
- Budesonide [63]
- Intravenous immunoglobulin (IVIG) [69, 70]
- N-acetylcysteine [63]
- Infliximab [49, 68]

To date, there are no studies comparing the relative efficacies of these agents. The potential adverse effects from the adjunctive agent utilized should be carefully weighed. Importantly, infliximab is *not* recommended to treat IMH due to its own potential hepatotoxicity [21, 22, 74, 100, 101]. Tocilizumab, an IL-6 receptor antagonist, has current applications in treating cytokine release syndrome and immunotherapy-mediated rheumatoid disease [35]. Along with emerging case reports to treat immunotherapy-mediated hepatobiliary disease, the use of tocilizumab may represent a favorable steroid-sparing strategy in cases of steroid-resistance or steroid-dependence, or in cases where serious adverse events develop from steroid use while treating IMH (Fig. 1). Additional focused studies for the use of tocilizumab in the context of IMH treatment are needed to demonstrate efficacy and safety. In summary, close and open communication amongst the hepatologist, the patient, and the oncologist is paramount in managing and monitoring IMH.

1.7 Outcomes

Treatment with corticosteroids will achieve improvement or normalization of liver enzymes in most patients [19, 37, 75]. Particularly in those with CTCAE grades 3–4 IMH, favorability of response to steroids is assessed over the first 3 days before reassessing the need to escalate treatment with higher steroid dose, immunomodulators, or other adjunctive agents, as suggested in current society guidelines. The median time from corticosteroid initiation to resolution is approximately 8 weeks [76].

In clinical practice, spontaneous resolution of IMH without corticosteroid therapy, including those with grades 3–4 liver injury, has been observed [13, 14, 77]. However, the factors that predict this favorable outcome are not yet defined. Therefore, most patients will ultimately go on to receive corticosteroids.

For patients undergoing steroid treatment, the first week after recognition of abnormal LBT offers a reasonable window to gauge whether the liver enzymes have or will soon reach its peak. LBT should be monitored at least once a week depending on the trends since AST and ALT may rebound even after completion of corticosteroid therapy and biochemical resolution. The utility of

defining histologic remission in IMH has not been studied.

The timing and manner of steroid taper are important. Premature taper of steroids, particularly when the LBT have not adequately improved, could lead to rebound and uncontrolled IMH. This may lead to prolonging the current steroid dose or increasing the steroid dose to regain immunosuppression effects. As such, we generally recommend that the ALT and AST have both approached CTCAE grade 1 levels, but specifically less than 2× ULN (so that it is not immediately at the threshold with CTCAE grade 2 levels), before starting the taper from the initial induction dose (Fig. 1). In general, we recommend weekly tapering of each dose. The clinician should continue to monitor the LBT 1-2× per week during the taper through the date of completion of steroids (Fig. 1).

Liver failure and death related to IMH are rare [78–82]. The role of underlying liver disease such as metastatic tumor burden or cirrhosis (especially in patients with liver cancer) in the risk of IMH fatality has yet to be examined [30].

1.8 Rechallenging with ICI After Recovery From Grades 3–4 IMH

Society guidelines from NCCN, ASCO, SITC, ESMO, and AGA recommend permanent discontinuation of ICI for those who are diagnosed with grade 3 and grade 4 IMH [22, 35, 55–57, 100, 101]. This recommendation is based on expert consensus, but real-world experience and clinical practice challenge this paradigm. Available studies imply the opportunity for flexibility in patients where ICI was deemed effective but caused high grades IMH [14, 65, 83]. The risks, benefits, and alternative treatment options should be discussed with the patient and oncologist with expectation for very close pharmacovigilance. In efforts to attenuate the theoretical risk of ICI recurrence, the clinician may opt for monotherapy ICI rather than dual therapy with ICI rechallenge or resume ICI at a modified dose. Prophylactic use of adjunctive agents such as immunomodulators may also be considered. In our experience, careful ICI rechallenge guided by a multidisciplinary care team can often be successful.

1.9 Conclusions

IMH is increasingly encountered as ICI use becomes more expansive. It can occur as early as 1 week and as late as 13 weeks from initiation of ICI treatment. Delayed presentation of IMH is also possible depending on the half-life of the specific ICI. In most cases, IMH is asymptomatic and identified via routine lab surveillance. Potential symptoms, including abdominal pain, fever, jaundice, and malaise, are rare. Pharmacovigilance is paramount to permit early diagnosis. Mortality associated directly with IMH is rare.

As IMH remains a diagnosis of exclusion, other etiologies for new abnormal liver tests must be thoroughly explored. IMH is distinct from both idiopathic autoimmune hepatitis and drug-induced autoimmune hepatitis. No relationship to autoimmune markers is observed. Liver biopsy could be beneficial in select cases to corroborate a suspected case of IMH. Although no pathognomonic findings are defined in the histopathology of IMH, commonly described histologic findings can help distinguish IMH from autoimmune hepatitis or primary cholestatic liver diseases. Cholangiopathic phenotypes have been observed in the spectrum of IMH, which can be detected by first utilizing the R factor.

Once the diagnosis of IMH is made, management and treatment will depend on the overall CTCAE grade. Some patients, even those with grades 3–4 IMH, can exhibit spontaneous improvement without steroids upon ICI withdrawal. The goal of steroid treatment is biochemical remission with return of liver enzymes to baseline or normal values. Clinical observations suggest that lower doses of steroids compared to what is delineated in the current guidelines are effective while minimizing the risk of steroid-induced AE. The duration of corticosteroids is based on the trends of the liver enzymes, comorbidities, and prospects of being rechallenged with

ICI while minimizing the risk of adverse events from steroids. Additional research is needed to establish the efficacy, the timing of initiation, and the selection of adjunctive treatments in IMH. Published clinical experience shows that not all patients who recover from grades 3–4 IMH experience recurrent IMH during ICI rechallenge. Therefore, the recommendation for permanent discontinuation of ICI in those categories should be revisited, particularly in cases where the patient's cancer had responded well to ICI therapy and no good alternative treatment is available.

2 Gallbladder Injury

Very limited data are available regarding ICI-related cholecystitis. However, recognition and management of rare adverse events of ICI therapy are essential to maintain effective cancer treatment. Acute cholecystitis with or without cholangitis has been reported in case studies and case series [42, 84–86]. One study showed an incidence of 0.6% of acute acalculous cholecystitis, higher than the incidence (0.2%) among cancer patients without ICI exposure. The incidence is relatively higher in cases related to anti-CTLA-4 inhibitors [84]. The median time to cholecystitis was about 6 months after initiation of ICI therapy or after a median of four infusions of ICI. Traditional diagnostic tests and treatment strategies may be adopted from typical non-ICI-related cholecystitis. Management may include surgical cholecystectomy and percutaneous drainage, but the role of steroids is not yet defined [84]. Gallbladder wall perforation and sepsis have been reported with ICI-related cholecystitis [84]. To date, there is no study to document an association between patients who develop ICI-related gallbladder injury and those who develop ICI-mediated bile duct injury.

3 Pancreatic Toxicity

3.1 Incidence and Diagnosis

Among different ICI classes, the reported incidence of ICI-induced pancreatic injury is 0.6–4% [21, 87–89]. ICI-related clinical pancreatitis is therefore considered rare [21, 56]. A common scenario is asymptomatic elevations in serum lipase and amylase without clinically apparent pancreatitis. It is important to distinguish between asymptomatic ICI-related effects causing elevated serum lipase and amylase levels from those with clinically significant pancreatic injury in the form of true pancreatitis. Elevated lipase and amylase levels are generally an incidental finding detected during routine monitoring through expectations in the treatment protocol. Lipase and amylase elevations are usually recorded after a median of 3 months from ICI therapy initiation [90]. In cases of acute epigastric abdominal pain and nausea consistent with typical acute pancreatitis, toxicities involving other parts of the gastrointestinal tract, which could even coexist with pancreatic injury, must be ruled out. For example, choledocholithiasis must be excluded.

CTCAE version 5.0 offers a grading schema accounting for laboratory values, clinical signs, and symptoms (Table 2) [25].

Table 2 CTCAE (version 5.0) grading schema for lipase and amylase levels

Lab parameter	Grade 1	Grade 2	Grade 3	Grade 4
Lipase	>ULN to 1.5× ULN	>1.5–2.0× ULN or >2.0–5.0× ULN and asymptomatic	>2.0–5.0× ULN with signs or symptoms or >5.0× ULN and asymptomatic	>5.0× ULN with signs or symptoms
Amylase	>ULN to 1.5× ULN	>1.5–2.0× ULN or >2.0–5.0× ULN and asymptomatic	>2.0–5.0× ULN with signs or symptoms or >5.0× ULN and asymptomatic	>5.0× ULN with signs or symptoms

Abbreviations: *ULN* upper limit of normal

A retrospective study by Freeman-Keller et al. showed that 7 of 148 patients (4.7%) treated with nivolumab developed elevations in lipase/amylase of grades 1–3, of which 2 patients had grade 3 elevation [91]. The time to onset of these enzyme abnormalities was a median of 2 weeks after starting ICI therapy. A retrospective study at a cancer hospital of melanoma showed that 2 of 119 patients (1.7%) in the study were diagnosed with clinical pancreatitis but 52 patients (43.7%) had elevations in serum lipase and/or amylase (grades 3–4) without meeting diagnostic criteria for pancreatitis [92]. The pathophysiologic mechanism for asymptomatic elevations in these enzymes is currently unknown [92].

Initial investigation for both asymptomatic and symptomatic cases should include a close review of alcohol consumption behaviors, medication reconciliation to identify any pharmacologic agents that can predispose to pancreatitis, exclusion of hypertriglyceridemia, and cross-sectional imaging to evaluate for pancreatic lesions including metastasis of primary cancer. Traditional criteria for the diagnosis of acute interstitial pancreatitis include satisfying two of the following three features: compatible abdominal/gastrointestinal symptoms, serum lipase >3× ULN, and findings on cross-sectional imaging consistent with interstitial pancreatitis. 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. Commonly observed features of ICI-induced pancreatitis are segmental hypoenhancement, peripancreatic fat stranding, and pancreatic enlargement with heterogeneous enhancement (Fig. 2). Because typical imaging features of interstitial pancreatitis could be observed in patients who are asymptomatic, it is reasonable to offer CT or MRI for additional workup. Some findings, such as pancreatic ductal dilation, may prompt further revaluation by endoscopic ultrasound. Autoimmune pancreatitis and IgG4-related pancreaticobiliary disease may manifest similarly and must be excluded.

Significant adverse consequences can occur in up to 10% of patients with ICI-induced pancreatitis. A case of severe pancreatitis with progressive morbid sequelae has also been reported [93]. One case report described a patient treated with nivolumab and ipilimumab who developed abdominal pain later diagnosed as pancreatic disease ascertained by PET-CT and endoscopic ultrasound, showing a diffusely lobular pancreas. This patient had concomitant distal common bile duct stricture associated with abnormal liver enzymes but no ANA or IgG4 elevation [94].

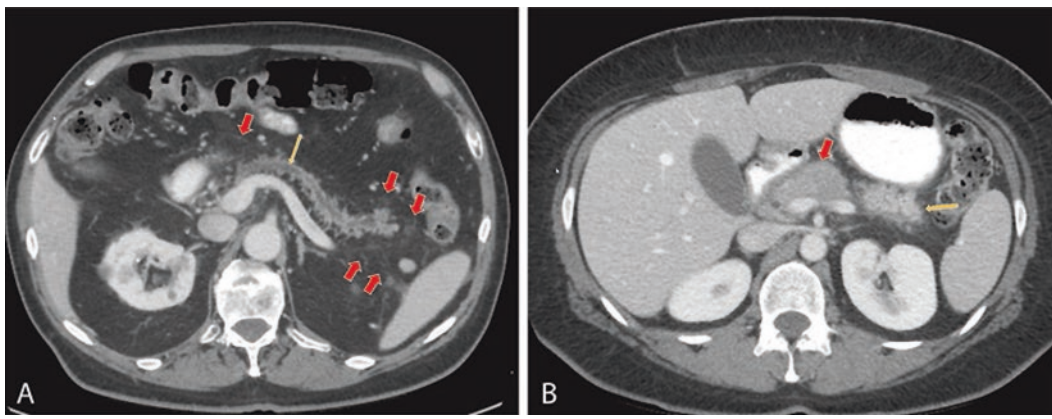

Fig. 2 (**a**) The peripancreatic fat stranding (short block arrows) are suggestive of pancreatitis. Pancreatic duct dilatation (long arrows) is due to metastasis in the pancreatic head. (**b**) The segmental hypoenhancement of pancreatic head and proximal pancreatic body (short block arrow) versus normal 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)

Exocrine pancreatic insufficiency (EPI) has also been observed as a sequela [95, 96]. In the context of ICI exposure, a retrospective study at a single-center cancer hospital revealed nine patients diagnosed with EPI (after a median time of 589 days after initiation of ICI) and received prescription for pancreatic enzyme replacement therapy [95]. Therefore, in patient presenting with new-onset diarrhea, the differential diagnosis may include EPI in addition to IMDC. A history of steatorrhea, combined with subsequent 24-h fecal fat testing and fecal elastase testing, would be informative.

The NCCN guidelines offer guidance for categorizing patients with asymptomatic pancreatic disease based on the levels of serum lipase and amylase and also for categorizing those with clinical symptomatic pancreatitis into mild (grade 1), moderate (grade 2), and severe disease (grades 3–4) [35]. Limited guidance is offered in the ASCO and SITC guidelines [22, 56, 101]. No recommendations are offered in the ESMO guidelines [55, 57].

3.2 Management and Treatment Options

Given the similarities between symptomatic ICI-induced pancreatitis and "classic" acute pancreatitis, ICI-induced pancreatitis with clinical symptoms should be managed in a similar fashion to classic acute pancreatitis [97].

The unusual part of the diagnosis is related to the majority of patients who do not present with clinical symptoms. These patients may only have isolated elevations in serum lipase/amylase, and some patients, despite the lack of symptoms, could manifest in findings of pancreatitis on abdominal imaging. The optimal management of the asymptomatic case has not been systematically studied. Surveillance of pancreatic enzyme levels and the decision to continue ICI treatment is at the discretion of the clinician.

In patients who do present with typical symptoms of acute pancreatitis, ICI must be withheld, and the patient should be immediately evaluated in the emergency department with anticipation to be treated as a case of acute interstitial pancreatitis, which includes implementing initial *nil* per os status, administration of aggressive intravenous fluids such as lactated ringers within the first 24 h, and analgesic medication. Standard of care for acute pancreatitis should be followed.

The role of corticosteroids and other immunosuppressive agents in such patients is not well-established [22, 88]. The ASCO guidelines briefly suggest that asymptomatic disease may not warrant corticosteroid treatment [22]. In patients diagnosed with moderate or severe (grades 2–4) pancreatitis, NCCN guidelines recommend initiating oral prednisone or intravenous methylprednisolone at a dose of 0.5–1 mg/kg/day (for grade 2) or 1–2 mg/kg/day (for grades 3–4), with eventual taper of steroids over 4–6 weeks [35]. In the retrospective with 119 patient, ICI was withheld in 12.6% of patients, and 7.6% of patients were treated with oral steroids for lipase/amylase elevations without development of clinical pancreatitis [92].

Because serum lipase and amylase have no compelling clinical value in the management trajectory for acute pancreatitis, surveillance of these labs is not recommended. The patient should be assessed for signs of clinical improvement. Nonetheless, as with typical cases of acute pancreatitis, it remains important to monitor for development of morbid sequelae of clinical pancreatitis, especially in patients who are diagnosed with early-onset pancreatitis, as well as those with a history of smoking and hyperlipidemia, since these patients bear an increased risk of pancreatic injury [88, 98, 99]. Imaging abnormalities at the pancreas or pancreatic duct in some cases may warrant further characterization by endoscopic ultrasound. In patients who suffer from grades 1–2 symptomatic pancreatitis, the patient should discuss with the clinician about the prospects of resuming ICI treatment; ICI treatment should be permanently discontinued for those who suffered from grades 3–4 (severe) symptomatic pancreatitis [35].

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Pulmonary Toxicities of Immunotherapy

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Abstract

Immune checkpoint inhibitors are a form of immunotherapy that are increasingly being used in a wide variety of cancers. Immune-related adverse events (irAEs) pose a major challenge in the treatment of cancer patients. Pneumonitis, the most common lung irAE, can cause significant disruptions in the treatment of cancer and may be life-threatening. 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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1 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 in which the immune system is weaponized against cancers to induce a potentially durable reduction in tumor burden [3–5]. Immune checkpoint inhibitors (ICIs), particularly inhibitors of the programmed cell death protein-1(PD-1)/its ligand (PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), have transformed the treatment of cancer in the recent years [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), or can benefit from the intrinsic negative regulatory pathways [7].

Inhibitors of the PD-1 and CTLA-4 pathways boost antitumor immune responses by preventing homeostatic downregulation of T-lymphocyte activity that normally occurs during chronic

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infection to prevent excessive tissue injury [8, 9]. However, a reinvigorated immune system may lead to disturbances in 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 toxicities of immunotherapies.

2 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, T- and B-lymphocytes [10–12]. PD-1 is primarily expressed in mature T-cells and appears within 24 hours 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. The expression of PD-L1 and PD-L2 is regulated by the inflammatory milieu. Several inflammatory cytokines can induce PD-L1 expression on the surface of lymphocytes and on non-immune cells [10, 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 co-stimulatory 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].

3 Immune Checkpoint Inhibition as a Therapeutic Strategy in Cancer

Cancer cells harness checkpoint activation through the PD-1 and CTLA-4 pathways to induce anergy 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, pembrolizumab, and cemiplimab; 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, 37]. 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, pembrolizumab, and cemiplimab bind competitively to PD-1 to

form PD-1:monoclonal antibody complexes [38]. These 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 PD-1 complexes, without inhibiting the PD-L2/PD-1 pathway [39]. The FDA has approved several PD-1 and PD-L1 inhibitors to treat many tumor types, and several more trials of therapy are underway. Further details about current FDA-approved immune checkpoint inhibitors and their indications can be found in Chap. 1.

Pneumonitis is the most common pulmonary toxicity of ICI therapy and is associated with one of the highest rates of therapy-related mortality among all immune-related adverse events (irAEs) [40]. While pneumonitis can be seen as the only immune therapy-related toxicity, concomitant or sequential irAEs involving other organ systems are seen in 58% of patients with pneumonitis [41]. Additionally, in a review of toxicities that led to fatality, colitis, hepatitis, and cardiac and neuromuscular toxicities were also reported to co-occur in up to 16% of patients who developed pneumonitis [40].

Rarely, other non-pneumonitis lung irAEs are seen after ICI therapy, such as de novo sarcoid-like reactions or exacerbations of preexisting lung diseases [42–47]. However, most of this chapter will focus on pneumonitis.

4 Clinical and Radiologic Patterns of Pneumonitis

In the following section, we discuss presentations of pneumonitis after ICI therapy. Pneumonitis after ICI therapy presents as an interstitial lung disease [48]. Pneumonitis usually 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 consider NSIP and HP as subtypes of a single category (interstitial pneumonitis [IP]), due to similarities in presentation and in therapeutic approaches. Table 1 summarizes the clinical, radiological, and pathological features associated with each pattern of pneumonitis, and Fig. 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 [49, 50].

Pneumonitis after ICI therapy generally presents as OP or NSIP, but may rarely present as DAD and can have a fulminant course. 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 [51] or CTLA-4 inhibitor therapy [52].

OP OP is a common manifestation of pneumonitis after ICI therapies [51]. OP primarily affects distal bronchioles, respiratory bronchioles, alveolar ducts, and alveolar walls [53]. Symptoms of OP may include low-grade fever, malaise, and cough, and the onset of symptoms in idiopathic cases is often subacute [54–57]. Respiratory infections are often associated with the development of OP though the mechanism remains unclear [58]. 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 [59]. 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 [60]. 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. 2) in distal airspaces with infiltration by lymphocytes and plasma cells [59]. 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 [59]. The treatment of OP depends upon the severity of the disease. We recommend use of the Common Terminology Criteria for Adverse Events (CTCAE, Table 2) to grade the severity of pneumonitis [61]. Mild

Table 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	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
		Multiple alveolar opacities, solitary opacities, or infiltrative opacities can be seen		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
Interstitial pneumonia (IP)	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

cases (Grade 1) of OP may resolve spontaneously, but close monitoring for early signs of pulmonary impairment is imperative [62]. 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 [63].

Non-corticosteroid 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 [64–67]. 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 [68–71]. Infliximab has been reported to be effective in severe pneumonitis, but this requires validation in a prospective study [51, 72]. Tocilizumab, a humanized monoclonal anti-

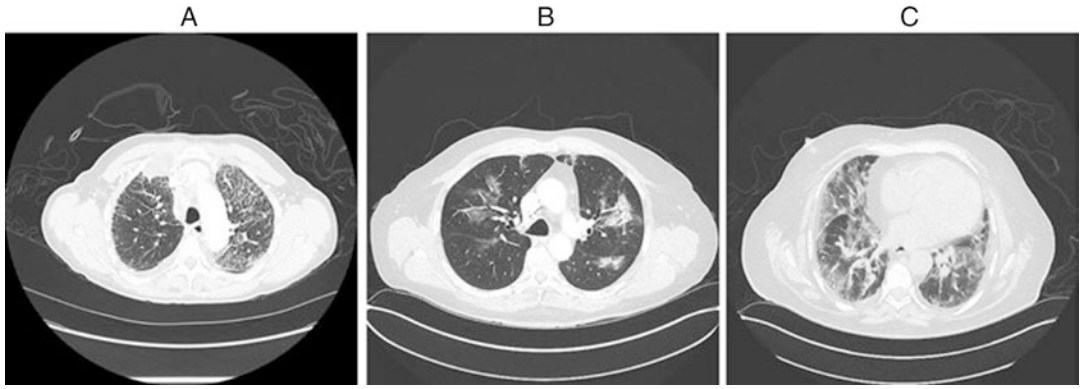

Fig. 1 Representative images of (**a**) interstitial pneumonitis, (**b**) organizing pneumonia, and (**c**) diffuse alveolar damage in patients receiving precision oncology therapies

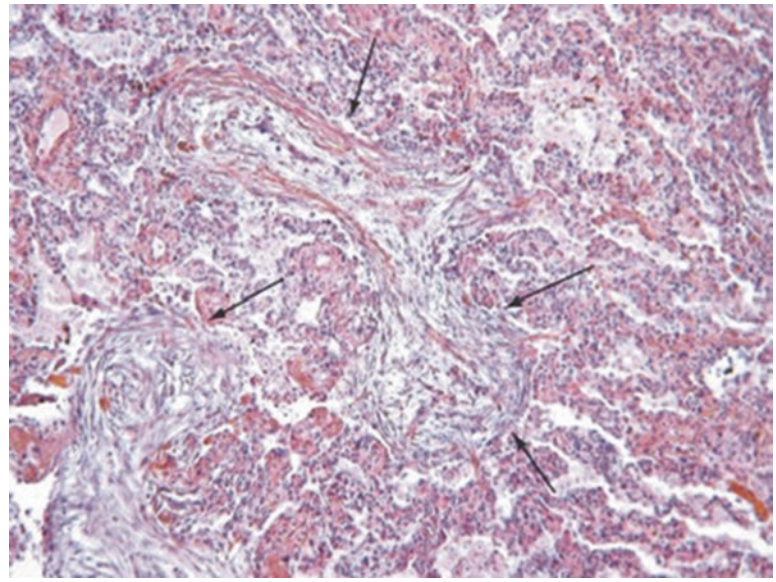

Fig. 2 Buds of granulation tissue (*arrows*) in the lumen of alveoli. (Reproduced with permission from *Clinical Respiratory Medicine*, Cottin V. and Cordier J., 2012, Elsevier Publishing)

body against the interleukin-6 receptor, 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 included pneumonitis and were refractory to corticosteroid therapy. Of those, 27 patients (around 80%) showed clinical improvement, and the median time to discharge was 4 days [73]. Anakinra is an interleukin-1 receptor antagonist protein used for the treatment of inflammatory disorders such as rheumatoid arthritis, and experience of its use in pulmonary involvement of rheumatologic disease is very limited [74]. 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, yet no real-life experience is available for this utilization. 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.

Table 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)	

IP IP is a rare ILD that is often associated with autoimmune diseases or human immunodeficiency virus infection and, along with OP, is a common manifestation of pneumonitis after ICI therapy [75]. IP typically presents with nonspecific symptoms of cough and dyspnea, though the duration of symptoms may vary from case to case. Thoracic CT imaging of IP typically reveals ground-glass opacities, reticular infiltrates, and traction bronchiectasis [76–78]. Subpleural sparing of lung infiltrates may help distinguish IP from idiopathic pulmonary fibrosis [79]. The HP variant of ICI-related pneumonitis may be characterized by air trapping on expiratory chest CT imaging [80]. However, unlike HP that occurs in the general population, there is no clear link to pulmonary exposures such as aerosolized molds [81] or toxic chemicals [82]. Histologically, IP 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 [83]. Fibroblastic foci may be present, but are less common in cases of IP [84]. The HP variant of pneumonitis may be characterized by poorly formed non-caseating granulomas [80]. In general, patients who develop IP 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 [62]. For ICI-related NSIP, interruption of ICI therapy is generally recommended [85].

DAD DAD is a severe form of pneumonitis caused by widespread alveolar injury that results in severe capillary leak and non-cardiogenic pulmonary edema [85, 86]. 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 IP, 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 [87–91]. 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. 3) [92, 93]. 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 [87]. Thoracic CT images of DAD show widespread airspace opacities, which may be more prominent in the dependent areas of the lung [94–96]. 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

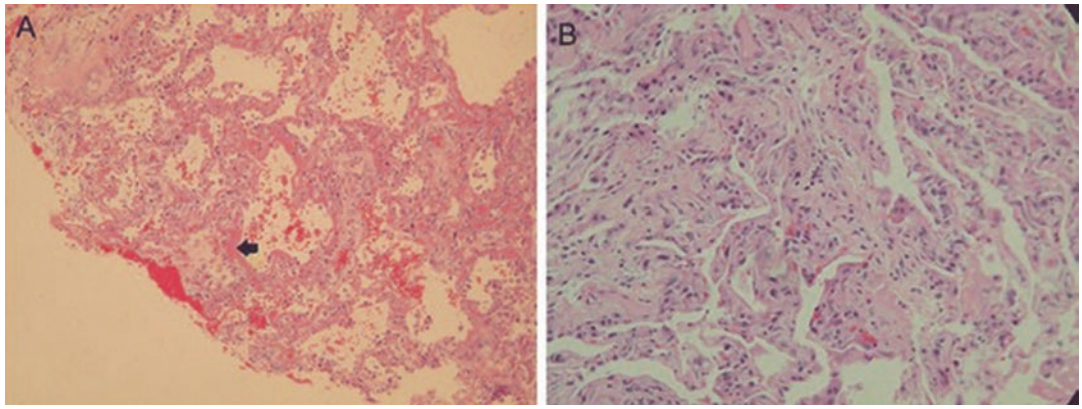

Fig. 3 Pathological findings of diffuse alveolar damage. (**a**) Diffuse alveolar damage in the acute phase. The interstitium is edematous. Hyaline membrane (arrow) is seen lining the alveolar ducts (hematoxylin and eosin stain, ×100). (**b**) Diffuse alveolar damage in the organizing phase. The interstitium is thickened with organizing connective tissue. Prominent type 2 pneumocyte hyperplasia is seen (hematoxylin and eosin stain, ×200) [72, 87]

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 [97].

5 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 [98, 99]. 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 [100]. 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 [101]. Pulmonary function testing should be performed at the time of evaluation, as early impairment in pulmonary function may herald the onset of pneumonitis [102]. 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. An elevated lymphocyte count may be indicative of pneumonitis in the absence of infections that may also elevate BAL lymphocytes (e.g., respiratory viruses) [103]. 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 [104]. The use of cryobiopsy is becoming more prevalent for the diagnosis of ILD, due to the better acquisition of tissue specimens for histology, without the need for a thoracotomy as in surgical lung biopsies [105, 106].

6 Incidence and Clinical Characteristics of Pulmonary Toxicities in Patients Receiving Immune Checkpoint Therapies

The incidence of pneumonitis with immune checkpoint inhibitor varies by agent class (PD-1/PD-L1 blockade versus CTLA-4), tumor type, and disease setting, as well as the complexity of the ICI regimen (monotherapy versus combination therapy). Although many irAEs, including pneumonitis, were identified during preapproval clinical development phase of ICI, our knowledge on clinical characterization of this toxicity in terms of real-world incidence, timing, and outcomes have been more recently complemented by large-scale retrospective studies, pharmacovigilance analyses, unplanned analysis of pooled data from clinical trials, as well as clinical trials particularly focusing on irAEs [41, 107–112].

In earlier 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% [113–118]. 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% [41, 109, 119–121]. In general, the median onset of pneumonitis is about 3 months, but disease onset can significantly vary from days to years [51, 109, 111, 112, 122]. In this section, we discuss incidence rates and specific forms of pneumonitis that occur with each FDA-approved ICI therapy.

6.1 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 [123]. 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 [52]. While some irAEs are more common with CTLA-4 inhibitors than PD-1 or PD-L1 inhibitors [124, 125], pneumonitis is less common, though the mechanism for this difference is unclear [126]. Pneumonitis occurs at about one-third the rate in patients treated with ipilimumab for melanoma as compared to those being treated for renal cell cancer or non-small cell lung cancer [126]. One possibility for this may be the presence of lung disease from cigarette smoking, as has been described in other ILDs [127].

6.2 PD-1 and PD-L1 Inhibitors

In this section, we will discuss the PD-1 inhibitors, nivolumab, pembrolizumab, and cemiplimab, and the PD-L1 inhibitors, atezolizumab, avelumab, and durvalumab. Pneumonitis after PD-1 inhibition occurs as much as three times more frequently as compared to conventional chemotherapy regimens across several types of cancers [128].

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% [41, 109, 128]. 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% [41, 109, 128, 129]. 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%) [128].

Preexisting fibrotic ILD appears to be a predictive risk factor in the development of immune checkpoint-related pneumonitis [129]. In one study, the incidence of PD-1-related pneumonitis among patients with NSCLC and mild baseline pulmonary fibrosis was 28.6% compared to 5.8% among patients with no preexisting fibrotic ILD, suggesting that even mild ILD at baseline may confer higher rates of pneumonitis [47]. ICI-related pneumonitis in this setting may exacer-

bate preexisting ILD or promote de novo disease [47, 129, 130].

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 [131]. 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 [128]. 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 treated outside of 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% [132]. 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 [133]. A review of fatal immune checkpoint inhibitor toxicities from a WHO pharmacovigilance database reported that pneumonitis was the most common cause of therapy-related mortality, with a case fatality rate exceeding 10% [134].

The lung is one of the most sensitive organs to ionizing radiation, and due to overlapping risk for lung inflammation, concurrent treatment with ICI and radiation therapy 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 (G $\geq$ 1), 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) [110]. Concurrent use of ICI with chemotherapy and radiation had reported grade $\geq$3 pneumonitis in 8% of patients in an ongoing study [135]. 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% [136]. 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%) [119, 137]. 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.

6.3 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 that may increase antitumor responses in certain cancers [138]. 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 [41]. 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 [41]. 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% [128]. 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 [139]. 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 [140]. 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.

6.4 ICIs in Combination with Other Antineoplastic Therapies

Increasingly, ICIs are used in combination with other chemotherapies, particularly in metastatic solid tumors, due to improved rates of response compared to conventional strategies or sequential treatment [141–143]. Many combinations did not raise concern for increased rates of pneumonitis [142–144]; it is possible that immunosuppressive effects of chemotherapy or the frequent utilization of steroids as a part of symptom management strategies for chemotherapy may be playing a role. However, increased pneumonitis had been reported for a subset of targeted therapies when given with combination of ICI. For example, combination of atezolizumab, vemurafenib, and cobimetinib for BRAF mutant melanoma had increased risk of pneumonitis compared with placebo, vemurafenib, and cobimetinib combination, and in this study, any grade pneumonitis was reported 10% vs 5%, respectively, but grade ≥3 or above pneumonitis rate was low and 1% in the triple therapy arm [145]. Interstitial lung disease was reported as an adverse event 5/23 (22%) of the patients who received durvalumab in combination with osimertinib, a third-generation kinase inhibitor of epidermal growth factor (EGFR) [146]. The prolonged half-lives of anti-PD-(L)1 therapies raise concerns for increased pulmonary toxicities when osimertinib is used subsequently used after ICI in a short interval. In a retrospective study, a cohort of 41 patients, 6/41 of all patients treated with sequential anti PD-(L)1 therapy followed later by osimertinib developed a severe irAE. Severe irAEs were most common among those who began osimertinib within 3 months of prior anti-PD-(L)1 therapy (5 of 21), as compared with an interval of greater than 3 months. By contrast, no severe irAEs were identified among patients treated with osimertinib followed by anti-PD-(L)1 or anti-PD-(L)1 therapy followed by other kinase inhibitors of EGFR (afatinib or erlotinib) [147]. Toxicity after combined therapy with ICIs and other antineoplastic agents is an emerging area of research that may become increasingly important with greater use.

6.5 Rare Patterns of Pulmonary Toxicity After ICI Therapy

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 [148]. Rapidly recurrent pleural and pericardial effusions were reported in two patients within 8 weeks of initiating nivolumab therapy [149]. 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 [114]. 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 [44, 52, 150] and with PD-1 inhibition [45, 151]. 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 that occurs in the general population [44, 152]. However, inhibition of immune checkpoint pathways may increase the population of Th17 cells, which are thought to be involved in non-ICI-related sarcoidosis [153, 154]. 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.

7 Areas of Uncertainty

7.1 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 [111, 155], while others have not [156]. Therefore, rechallenge with ICI therapies after the occurrence ICI-related pneumonitis may be desirable. Several groups have reported the safety of resuming ICI therapy after irAEs [157, 158]. Additionally, the overall incidence of irAEs is higher upon drug rechallenge, with about half of patients experience any-grade irAEs. Furthermore, about 20% of patients experience irAEs which are different from the initial irAE [158]. In other words, patients who develop pneumonitis after ICI therapies may experience a non-pneumonitis irAE upon drug rechallenge. Generally, these events are treatable with corticosteroids and are not fatal [111] though rare fatalities have been reported [158]. 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 that has resolved completely, as well as in select patients with grade 3 pneumonitis that have resolved completely and in whom the benefits of ICI therapies outweigh the risks of recurrent irAEs [68]. Patients with grade 4 pneumonitis should not undergo rechallenge with ICI therapies. Further work in this area is necessary to guide practice algorithms.

7.2 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 [159]. A similar approach led to the development of a radiomic-based algorithm which predicted the onset of pneumonitis from pre-treatment thoracic CT scans of patients who underwent ICI therapies [160]. These findings need to be externally validated but highlight the power of imaging as a biomarker of disease risk.

Since cancer symptoms are subjective reports, patients are the best source of information. Patient-reported outcomes (PROs) provide patients the opportunity to describe what he or she is experiencing during and after treatment. The potential use on PROs, in recognition of irAEs including pneumonitis, is intriguing, and studies are ongoing to further utilize this platform for immunotherapy toxicities [161].

Interleukin-17 is an inflammatory cytokine that is upregulated in many autoimmune diseases, including inflammatory bowel disease [162]. Elevated serum IL-17 levels were predictive of colitis in patients with melanoma treated with ipilimumab [163]. Similarly, in patients with leukemia, Th1/Th17 cells are expanded in bronchoalveolar lavage (BAL) fluid from patients with leukemia who developed pneumonitis after ICI therapy as compared to control patients with leukemia who had not received ICI therapy [164, 165]. Similarly, elevations in BAL total lymphocyte counts are often present in patients with pneumonitis. Bronchoalveolar lavage samples from patients with pneumonitis secondary to immunotherapy showed increased lymphocytosis, mainly composed of CD4+ T-cells, increased central memory T-cell numbers, and decreased CTLA-4 and PD-1 protein expressions within the Treg population [103]. This decrease in checkpoint inhibitor expression may lead to an increase in T-cell activation and impaired regulatory T-cell function [166]. Biomarkers that may distinguish pneumonitis from mimicking conditions, such as pneumonia, disease progression, or atelectasis, represent an important unmet need. Further work is necessary to identify inflammatory biomarkers in the blood or in bronchoalveolar lavage fluid that can help predict the onset of pneumonitis after ICI therapy. Early engagement of a multidisciplinary team to rule out other etiologies is critical on recognition and therapy of pneumonitis, which can also complement biomarker studies by accurate toxicity attribution [167].

8 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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Immune Checkpoint Inhibitor (ICI)-Related Cardiotoxicity

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Abstract

The growing success of immune checkpoint inhibitors (ICIs) has led to improved outcomes in several types of cancers with studies looking for expanding their indications and use. However immune-related adverse events have been recognized of which myocarditis is associated with a high mortality. Other cardiac events such as arrhythmias, pericardial disease, and coronary atherosclerosis have been observed in patients on ICI therapy. These cardiac toxicities are thought to be the result of increased inflammatory responses after inhibition of specific checkpoint proteins on T cells. 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 · CTLA4 · PD1 · PDL1 · Cardiotoxicity · Myocarditis

1 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]. There are currently seven Food and Drug Administration-approved checkpoint inhibitors with activity on three different checkpoint proteins. Ipilimumab was the first approved checkpoint inhibitor and works on the cytotoxic T lymphocyte-associated protein 4 (CTLA-4). There are three programmed death 1 (PD-1) checkpoint inhibitors including pembrolizumab, nivolumab, and cemiplimab. There are three programmed death ligand 1 (PD-L1) inhibitors including durvalumab, atezolizumab, and avelumab. Many other checkpoint inhibitors are in development among these same targets and more. Checkpoint inhibitors can lead to different immune-related adverse events (irAEs) which are caused by disrupted immune homeostasis which is mediated by unchecked T cell activation [48]. With the increase use of ICIs, potential short- and long-term cardiac toxicities are

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emerging. The following chapter will focus on ICI-related myocarditis which is the most well-described and well-established cardiac toxicity due to ICI therapy. This will be followed by descriptions of other cardiac toxicities increasingly recognized after starting therapy with ICI which include pericardial disease, arrhythmias, hypertension, and atherosclerosis.

2 Myocarditis

2.1 Suggested Mechanism

Myocarditis induced by ICI is most likely caused by inhibition of the CD-28 family regulatory molecules, CTLA-4 and PD-1, which are important in suppressing T cell responses [3]. Normally, these molecules prevent inflammation in the tissue and protect against cardiac muscle injury associated with the inflammatory response [4]. 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 [5]. 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 an antigen-specific autoimmune response [6]. In CTLA-4-deficient mice, multi-organ lymphoproliferative diseases develop within few weeks of life, including T cell-mediated myocarditis [7]. Induction of tolerance and upregulation of regulatory T cells (Treg) could be a pharmacologic approach to preventing autoimmune myocarditis [8]. 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 [9]. In addition, there is some data to suggest the expression of PD-1 and PD-L1 receptors in cardiac tissue, which could lead to inflammation from ICI therapy [33] (Fig. 1).

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 [10]. 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 [10]. Myasthenia gravis and myositis have been found to be concurrent in a high percentage of patients who develop ICI-related myocarditis [49]. The overlap in these syndromes warrants investigation when one of these manifestations are detected.

2.2 Incidence and Mortality

Myocarditis was rarely observed in early clinical trials; however given the increasing use of immune checkpoint inhibitors, there have 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 [11]. 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 one patient after the first dose requiring glucocorticoids as treatment [12]. 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 mel-

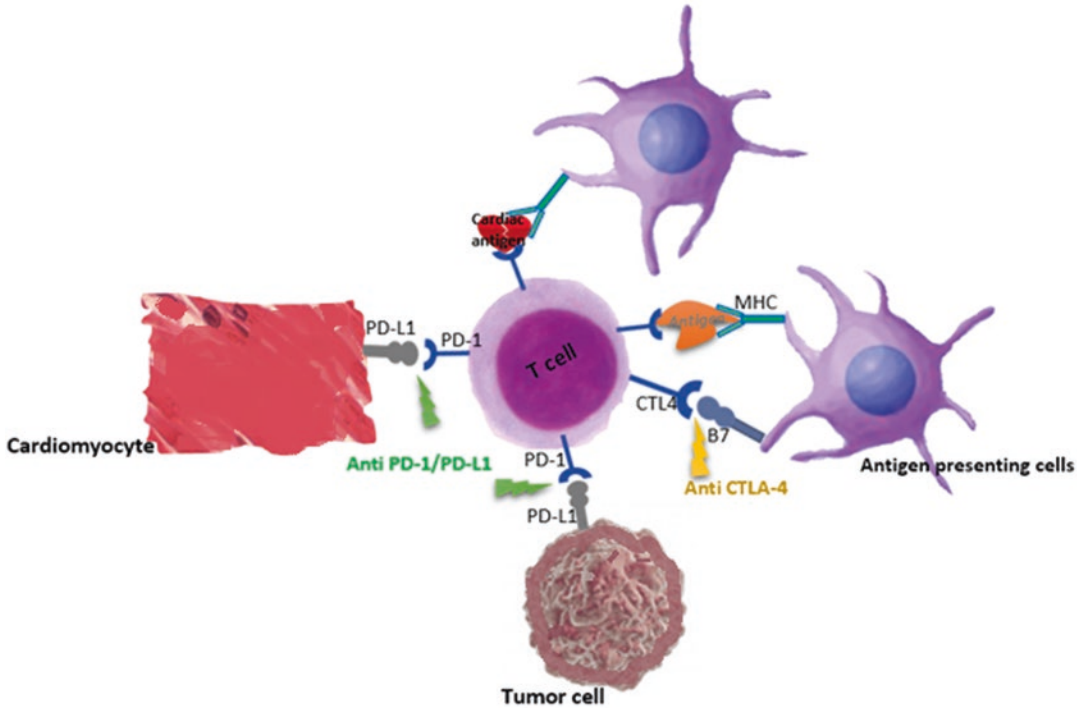

Fig. 1 Mechanism of cardiotoxicity of immunotherapy (**a**) Immune checkpoint inhibitors mechanism of action. *MHC* major histocompatibility complex, *TCR* T-cell receptor, *CTLA-4* cytotoxic T lymphocyte-associated protein 4, *PD-1* programmed cell death 1, *PD-L1* programmed cell death ligand 1 (**b**) PD-L1 expression on injured cardiomyocytes likely representing a protective mechanism for cardiac tissue during inflammation

anoma of the uvea due to autoimmune myocarditis after institution of pembrolizumab [13]. In a more extensive case series among six clinical cancer centers with substantial experience in the administration of ICI, 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 [9]. In this case series, severe myocarditis was 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 vs the nivolumab arm, five (0.17%) versus one (<0.01%), respectively [10]. Since recognition and awareness of myocarditis has improved, the incidence has increased with Mahmood et al. reporting an incidence of 1% [14].

Improved awareness of ICI-related myocarditis is necessary despite it being an uncommon toxicity because of its high mortality. When compared among other fatal irAEs, myocarditis is the most fatal with a reasonable estimate of mortality between 25 and 50% [15]. Unfortunately, the only established risk factor for myocarditis is use of combination therapy [9, 10]. Traditional cardiovascular risk factors such as history of heart

failure, coronary artery disease, and hypertension have not increased the risk of toxicity as seen with traditional cancer therapeutics like anthracyclines.

2.3 Diagnosis

The diagnosis of myocarditis is dependent on a combination of the clinical presentation, laboratory studies, noninvasive cardiac imaging, and endomyocardial biopsy. Each of these factors has strengths and weaknesses for making the diagnosis of myocarditis and only when used in combination with the knowledge of each test's limitations is an accurate diagnosis possible. There are considerable implications to the patient when starting therapy for ICI myocarditis and for future cancer therapeutic options for their cancer, and thus appropriate diagnosis is needed. Proposed diagnostic criteria for myocarditis due to cancer therapeutics were presented in a white paper that includes all of the following factors [16].

1. Clinical presentation – There is a clinical spectrum of patients presenting with myocarditis that ranges from asymptomatic elevation in cardiac biomarkers to fulminant myocarditis requiring mechanical circulatory support and mechanical ventilation [17]. Patients can present with dyspnea, fatigue, chest pain, and clinical heart failure. Most patients present within the first 2 months of initiating ICI therapy and typically after the second dose [14]. While rare reports of late cases of myocarditis up to 454 days after initiation have been reported, this is not common [14].
2. Laboratory studies – The most well-established cardiac biomarker for the diagnosis of myocarditis is troponin. It is preferred to check troponin I rather than troponin T due to cross-reactivity of troponin T with skeletal muscle injury; however regardless it has been shown that higher troponin I or T values are associated with increased risk of major adverse cardiovascular events [16]. Mahmood et al. demonstrated a fourfold increased risk of major adverse events when troponin T was greater than 1.5 ng/ml [14]. There is not an established cutoff troponin elevation for when to consider myocarditis, and this laboratory test needs to be used in combination with the clinical presentation and other factors presented below. Natriuretic peptides are not specific for myocarditis as not all patients present with heart failure.
3. Noninvasive cardiovascular imaging – Electrocardiographic (EKG) changes are observed with myocarditis and in fulminant cases include advanced atrioventricular blocks, profoundly prolonged PR intervals, and ventricular tachycardia. Other nonspecific EKG changes and arrhythmias are discussed below. Diagnostic echocardiographic changes are not observed with ICI-related myocarditis, and this differs from viral myocarditis in which EF declines are expected. For ICI-related myocarditis, the majority of patients maintain normal EF, and despite this the patients with normal EF have the same risk of major adverse cardiovascular events [14]. New pericardial effusions can be observed with myocarditis and are supportive criteria when myocarditis is suspected. Cardiac magnetic resonance imaging provides myocardial tissue characterization superior to echocardiography by using a combination of T1, T2, and late gadolinium enhancement features (Fig. 2). There are established criteria, Lake Louise criteria, for the diagnosis of myocarditis by CMR, and for viral myocarditis, CMR has robust correlation with endomyocardial biopsy [18]. However with ICI-related myocarditis, CMR shows poor correlation with biopsy results. In addition, the findings on CMR such as the presence of late gadolinium enhancement and fibrosis did not predict major adverse cardiovascular events [19]. For this reason, it is recommended to consider endomyocardial biopsy as below in combination with CMR in all cases of suspected ICI-related myocarditis.
4. Endomyocardial biopsy – Two important aspects of using endomyocardial biopsy are that the cardiologist performing the procedure is experienced with endomyocardial biopsy

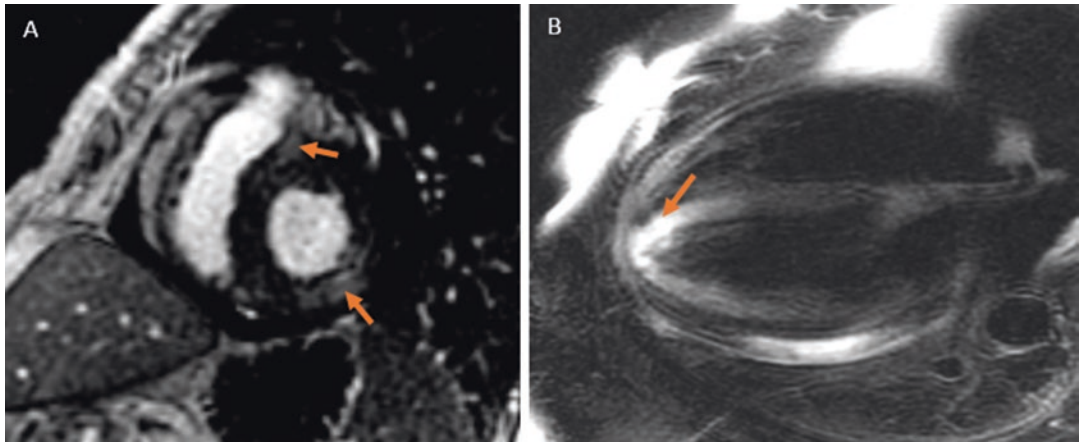

Fig. 2 Cardiac MRI findings showing myocarditis (**a**) Image showing late gadolinium enhancement within the myocardium (**b**) Increased T2 signal showing edema

and that the pathologist evaluating the biopsy specimens is an experienced cardiac pathologist familiar with heart transplant rejection pathology. The typical features of ICI-related myocarditis on endomyocardial biopsy are the presence of inflammatory infiltrate and myocyte necrosis which resembles that of acute cellular rejection in transplanted hearts [10, 20]. On immunohistochemical staining the inflammatory infiltrate is typically lymphohistiocytic with a predominance of CD8(+) T cells [10, 17]. At the same time as endomyocardial biopsy, a left and right heart catheterization is also typically performed to evaluate for coronary artery disease which can mimic many of the findings of myocarditis and for right heart filling pressures.

2.4 Monitoring Strategies

Since immune-mediated myocarditis has an early onset after receiving immunotherapy and a fulminant progression, a monitoring strategy has been suggested especially when receiving combination therapy [10]. A baseline ECG and weekly testing of troponin levels during weeks 1–3 for patients receiving combination immunotherapy can be considered [10]. However, the utility of troponin surveillance strategies is not established, and the low incidence of myocarditis brings into question the benefit of this strategy. For example, when a troponin monitoring strategy was studied in 76 patients prospectively, none of the patients developed clinical or subclinical myocarditis [21]. Nonetheless, upon the development of symptoms, a more extensive workup is necessary and should be directed by a consultant cardiologist [22]. Initial workup would include EKG, troponin, brain natriuretic peptide (BNP), echocardiogram, and a chest X-ray. Additional testing to be guided by cardiology may include cardiac catheterization with endomyocardial biopsy and CMR [22].

2.5 Treatment

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 [22, 23]. Contrary to the general notion with majority of ICI-related adverse events where toxicity can be closely monitored if it is grade 1, the recommendation is to hold ICI for grade 1 cardiac adverse events 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 setting, and some centers advocate for pulse dose steroids (500–1000 mg IV methylprednisolone daily for 3 days). This is likely secondary to the majority of reported myocarditis cases in the literature receiving corticosteroids [14]. In the limited data available, it is observed that patients receiving high-dose steroids have improved outcomes compared to low-dose steroids [14]. Most centers advocate for a rapid taper of steroids over 1–2 months using troponin monitoring to guide steroid taper; however no systematic studies have evaluated this strategy.

The addition of further immunomodulation per available guidelines is dependent on failure of response clinically or in troponin decline to steroid therapy. However many centers are evaluating upfront immunomodulatory therapy in addition to steroids to improve outcomes. Many other therapies have been described to be effective in treating ICI-related myocarditis; however, they are mostly limited to small case series and case reports. These therapies include mycophenolate, infliximab, antithymocyte globulin, intravenous immunoglobulin, tocilizumab, tacrolimus, plasmapheresis, alemtuzumab, and abatacept [24–30]. Their use is dependent on the availability of each of these agents and the experience of the treating physician with their use, administration, and safety profile.

Once treated, rechallenging such patients with ICI is not recommended given the high risk for recurrence [22] (Table 1). Cardiac symptoms should be managed according to American College of Cardiology/American Heart Association guidelines and with individualized guidance from a cardiologist familiar with immune-related cardiac side effects [31, 32]. 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 mechanical circulatory support.

3 Clinical Spectrum of Immune-Mediated Cardiotoxicity

1. Pericarditis/Pericardial Effusion

Pericardial disease has been reported to be an associated immune-related adverse effect. Nesfeder et al. [33] 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 [34].

There have been multiple case reports, involving the use of ipilimumab (anti-CTLA-4), showing a late-onset pericardial effusion 3–4 months after completing therapy. Dasanu et al. describe a

Table 1 Cardiac toxicities of immune checkpoint inhibitors

Cardiac toxicity	Time to onset	Management
Myocarditis	2–32 weeks	High-dose corticosteroids (1 to 2 mg/kg of prednisone) initiated rapidly Mycophenolate, infliximab or antithymocyte globulin
Pericarditis/pericardial effusion	6–15 weeks	
Arrhythmia	2–8 weeks	Standard treatment can be followed per AHA/ACC guidelines
Hypertension	17–22 weeks	
Vascular disease	Within 26 weeks	

65-year-old women, 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 [35]. Another case of late-onset pericardial disease was reported 12 weeks following treatment with ipilimumab. The patient presented with hypotension, and a metabolic workup 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 [36]. Another more recent combination phase Ib 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 [37].

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) [38]. While uncommon, when compared to those requiring pericardiocentesis who were not on ICI, the relative risk was 3.1 which suggests that the ICI was contributing the development to the effusion [38]. Nivolumab had the highest prevalence of 0.61% followed by pembrolizumab (0.19%) and atezolizumab (0.32%) [38].

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.

2. 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 [39].

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 II trial of patients receiving nivolumab for advanced clear cell renal cell carcinoma. Electrocardiograms were obtained at baseline, pre-dose, end of infusion, and 3 h 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 [40]. However, in a small phase I 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 [41]. 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 [42]. In another phase II 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 that occurred near the end of treatment [43]. It is unclear if the occurrence of atrial fibrillation was secondary to myocarditis or occurs through a different mechanism. Cardiac rhythm monitoring should be continued during ICI therapy to identify and treat for potential conduction abnormalities and dysrhythmias.

3. 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 [43]. 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 [44]. 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.

4. Vascular Disease

As the use of ICI has increased, many patients have been found to have concomitant coronary artery disease (CAD). Given the high prevalence of coronary artery disease, it was not suspected until recently that ICIs may have an effect on the arterial vasculature and development of atherosclerosis. One of the first reports that showed large vessel aortic inflammation after ICI use was reported in 20 patients with melanoma being treated with checkpoint inhibitors. Increased [^{18}F] fluorodeoxyglucose (FDG) uptake was observed in aortic calcifications after initiation of checkpoint inhibitors suggesting increased inflammatory activity [45]. Then D'Souza et al. evaluated melanoma and lung cancer patients in a nationwide Danish population-based cohort which found that within 6 months of ICI initiation, the risk of cardiac events were two- to four-fold that of patients not receiving ICI therapy [46]. More research is needed to understand the vascular effects of ICI therapy.

4 Future Directions

Due to the success of ICI therapies in treating refractory malignancies, new checkpoint inhibitor pathways beyond CTLA-4, PD-1, and PD-L1 are currently under investigation. These include LAG-3, TIM-3, TIGIT, VISTA, and B7/H3. In addition, agonists of stimulatory checkpoints including OX40, ICOS, GITR, 4-1BB, and CD40 are also being studied to improve the immune

reaction against tumor cells [47, 50]. With the rapid development of immune-mediated therapies, further research will be needed to identify potential cardiotoxic effects. The need for standardization in the monitoring, diagnosis, and treatment of ICI-related cardiotoxicity is essential for the continued safe administration of these medications. Providers must tailor the best possible treatment for their individual patients accounting for their expectations, risks, and quality of life [51]. Although advanced diagnostic tools are available at certain institutions, further study into simpler methods of screening patients, including biomarkers, should be a priority to enable all physicians to quickly diagnose and intervene before patients experience worsening outcomes [48, 52].

5 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

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 review, we will discuss the renal toxicities associated with a checkpoint inhibitor (CPI) from the typical acute tubulointerstitial nephritis to glomerulonephritis and their proposed mechanisms and treatments. We also discuss the use of CPI and reactivation of preexisting autoimmune disease with a focus on renal cell cancer in setting of chronic kidney disease (CKD). Transplant rejection in setting of CPI use has been further evaluated with single-center and multicenter retrospective studies, and available data will be presented in this chapter.

Keywords

Acute interstitial nephritis · Autoimmune disease induction · Organ transplant rejection · Renal cell cancer · Immune-related adverse events

1 Introduction

With the advent of the era of immunotherapy, there has been marked increased 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 other targets such as the programmed cell death protein 1 (PD-1) and its ligand (PD-L1) has resulted in also unregulated organ-specific toxicities. The expansion in the use of the checkpoint inhibitors has gained great momentum to being used from solid tumors to hematological malignancies in clinical trial. The recognition of increasing adverse events associated with checkpoint inhibitors has created the terminology of immune-related adverse events (irAEs). The adverse events have been associ-

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ated with poorer survival outcomes at times, but there is increasing evidence indicating otherwise [1–4]. Autoimmune colitis, hepatitis, endocrinopathies, and cutaneous irAEs were the most frequently reported adverse irAEs with renal toxicity incidence being reported as 1.4 to 4.9%, with AKI evaluated in terms of doubling of serum creatinine in some reports and a 1.5-fold increase in creatinine from baseline in others [5, 6]. However, a recent study of 309 patients found incidence of AKI after ICI treatment to be as high as 16.5% using the definition of 50% increase in creatinine from baseline with a median time of AKI of 30 days [7]. Predictors of AKI have been reported as lower baseline estimated glomerular filtration rate (eGFR), combination of ICI therapy, other irAEs, and PPI use [5–7]. However, impaired renal function does not preclude patients from receiving ICI especially that the associated AKI is a rare occurrence. In addition, it has not been demonstrated that a specific type of malignancy is more associated with CPI-induced AKI than others.

The incidence of grade III or IV AKI or the need for dialysis has been reported to be 0.6% [5, 8]. AKI occurred more frequently in patients who received combination therapy with ipilimumab and nivolumab (4.9%) than in patients who received monotherapy with ipilimumab (2.0%), nivolumab (1.9%), or pembrolizumab (1.4%) [8]. The association of adverse events with survival has been evaluated, and in some reports, patients with tumor response with an anti-PD-1/anti-PD-L1 antibody were more likely to report a related adverse event [9]. In the setting of AKI induced by ICI, a multivariable model showed that the absence of renal recovery was an independent predictor of increased mortality [5]. In another study, AKI associated with the use of ICIs was not associated with increased risk of mortality [7].

In this chapter, we would like to address renal toxicity associated with checkpoint inhibitors and its implication on development of chronic kidney disease which will effect overall survival especially in renal cell carcinoma patients [10].

2 Renal Toxicity AIN

The most commonly associated renal toxicity with CPI has been acute interstitial nephritis (AIN) with some reports of granulomatous interstitial nephritis [8, 11–14]. AKI has been noted to occur from 1 to 8 months with a reported median time for development of AKI of 3 months from starting treatment [7] [8]. Patients often present pyuria, sub-nephrotic proteinuria with rare cases of eosinophilia, rash, or fevers as typical of AIN [15]. 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 multi-organ lymphocytic infiltration and tissue destruction [16, 17]. PD-1 regulates tolerance primarily at the level of target organs. In mice models, PD-1 and PD-L1 were important inhibitory regulators of CD8(+) T cells in tubulointerstitial inflammation and provide protection from ischemic reperfusion injury [18, 19]. 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 that 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 [20] 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 [11, 21]. 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 [22, 23]. There has been increasing evidence that nonsteroidal anti-inflammatory drugs (NSAIDs) and proton pump inhibitors (PPIs) have been shown to be associated with increased risk of acute interstitial nephritis [5, 8, 11, 14].

3 Autoimmune Induction and Preexisting Autoimmune Disease

Interestingly, irAE has included induction of autoimmune diseases after the use of CPI such as sarcoidosis, lupus, psoriasis, diabetes type I, and polymyalgia rheumatic/arteritis among others. Not all patients develop autoimmune disease 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 [24, 25]. 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 [26]. Rheumatologists have appreciated the autoimmune induction post-CPI and have advocated for questionnaires for patients on CPI and autoimmune serology screening [27]. Autoimmune diseases have not escaped the kidney: there have been case reports of lupus nephritis, minimal change disease, and thrombotic microangiopathy after CTLA-4 antibody treatment [28, 29]. Interestingly, there is evidence that PD-1 is involved in autoimmune diseases as demonstrated in PD-1 knockout mice models who develop lupus and severe arthritis [30]. Membranous nephropathy, ANCA vasculitis, IgA nephropathy, C3 glomerulopathy, AA-type amyloid, and typical AIN after CPI have also been reported. One of the cases in the series with AIN had aggressive T-cell infiltration with CD4+ and CD8+ and further demonstrated in another case in the literature [23, 31]. The glomerulonephritis (GN) noted in these biopsies presented with either CTLA-4 antibody or PD-1 inhibitor treatments [32]. 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 meta-analysis among 123 patients, 92 (75%) had irAEs, of which 50 patients (41%) had exacerbation of their current autoimmune symptoms, 31 (25%) had a new irAEs, and 11 (9%) had both. Interestingly, two cases had preexisting autoimmune nephritis (IgA nephropathy and IgM nephropathy) [33]. A prospective study of 45 patients, with cancer and preexisting autoimmune or inflammatory disease, treated 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 vs. the group without was no different [34]. Treating patients with ICI and autoimmune disease continues to be challenging; however, in a recent case report, a patient with mesothelioma who developed reactivation of membranous nephropathy was successfully treated with rituximab and continued maintenance of CPI treatment with both cancer and membranous nephropathy remissions [35]

4 Kidney Transplant and CPI

There is an increased incidence of melanoma of 2.4-fold higher in solid organ recipients compared to general population with renal or liver transplant recipients having a higher risk [36] . Treatment protocols and management of possible organ rejection are 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 ipilimumab; however, more recently, cases of acute rejection were published [37, 38]. 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 treatment of CPI with four patients developing rejection leading to the conclusion that patients treated with PD-1 inhibitors and combination therapy of ipilimumab and PD-inhibitors were more likely to develop rejection [39–41]. Since PD-1 and PD-L1 interactions might participate in the induction of allograft tolerance, PD-L1 can limit effector T-cell function and expansion and 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 [42, 43]. 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 [44].

A comprehensive review further supports that PD-1 antibodies may be more likely to lead to rejection. In a recent study by Abdelwahab et al., 39 patients with allograft transplant were identified from both institutional and literature reviews of case reports. 59% had prior renal transplantation with a median time to CPI initiation after SOT of 9 years (range 0.92–32 years). Allograft rejection occurred in 41%. There was no difference in rejection rates in anti-CTLA-4 and antiPD-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% and death was reported in 46% [45, 46]. A recent retrospective multicenter study of 69 kidney transplant patients who received ICI indicated similar rejection rates of 42%. Interestingly, factors associated with a lower risk of rejection were mTOR inhibitor use and triple-agent immunosuppression. The study was notable for improved overall survival of patients with squamous cell carcinoma and treated with CPI when compared to patients, 19.8 months vs. 10.6 months, respectively [47]. The high rates of kidney transplant rejection associated with CPI exposure emphasize the importance of a multidisciplinary approach to insure prioritization of the cancer care. With the emergence of transplant oncology as a new specialty, it has been well recognized as a great asset in this population where a more guided approach to future prospective studies to identify optimal anticancer therapies, dosing strategies, class and dose of immunosuppressant, and time since transplant is needed to help balance between graft and ultimately patient survival. Establishing a national registry of transplant cancer patients who are treated with ICI would be of importance since this is a very rare population [48].

5 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. Several observational studies have shown the high prevalence of CKD in patients with solid tumors [49–52]. RCC account for 2.4% of adult malignancies, the vast majority being clear cell histology: ccRCC [53]. Evaluating data from the Fox Chase Cancer Center, Canter et al. [54] 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 [54]. 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 [55]. These VEGR TKIs 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; $P < 0.001$) [56]. 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 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) [57]. 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 [57, 58].

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]) vs. 26 months for sunitinib (95% CI [22-NR]) (HR 0.63, 99.8%CI [0.44–0.89]) [59]. 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 [60, 61]. These combinations of VEGFR TKI and PD-1/PD-L1 inhibitor will require a great focus on renal toxicity. The first combination of VEGF inhibition plus PD-L1 inhibition to have reported in phase III to date is the IMmotion 151 trial of atezolizumab plus bevacizumab compared with sunitinib in first-line setting. Grades 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 with no renal irAEs [62].

6 Management of Renal Toxicity

The mainstay treatment for renal toxicity associated with CPI has been steroids as is typically done with other organ irAEs [63]. However, it has become evident that biomarkers for organ toxicity associated with CPI are much needed to understand novel treatments [64]. For example, interleukin-17 has been noted to be high in patients treated with ipilimumab [65], and therefore the 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 [66]. 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. It has become clear in the last few years that the guidelines at hand have variations in the approach of CPI-induced nephritis [67–70]. However, with increased experience, it has become evident that an early renal consult and a kidney biopsy would be of great importance to delineate if patient has AIN vs. a glomerular process that may require more than steroids. Therefore, the more recent guidelines and updates have changed to emphasize early nephrology and kidney biopsy [71]. Based on case reports and CKIN (Cancer and Kidney International Network Workgroup on Immune Checkpoint Inhibitors), steroids are the mainstay treatment with AIN starting at 1 mg/kg and taper over 1–2 months with a close follow-up [58]. 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. Based on a multicenter retrospective study, complete, partial, or no kidney recovery occurred in 40%, 45%, and 15% of patients, respectively. Relapse of ATIN is also a challenge and associated with worse kidney prognosis [5]. There has been increasing interest of oncologist and subspecialists toward the use of biologics targeting TNF-alpha inhibition (infliximab) [72, 73] and IL-6 inhibitors (tocilizumab) [74]. As far as renal toxicity is concerned, there is a recent case series which demonstrated the effectiveness and durability of infliximab in treating cases of relapsed AIN post-steroid taper where 80% had complete or partial renal recovery [75]. 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 a 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 of preventing organ rejection while on immunotherapy [76]. In addition, based on published data, increased immunosuppressive medication before CPI infusion may prevent increased risk of rejection [47].

Although there has been a concern of use of steroids and hampering the 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 had no effect on survival or time to treatment failure [77].

7 Conclusion

Given the wide use of CPI across tumor types, physicians should be trained to detect renal complications and effectively treat to prevent further renal compromise. The large majority of cases present with either creatinine level impairment or proteinuria, the most common being acute interstitial nephritis. Prompt identification and management are needed to prevent chronic kidney disease to improve both renal outcomes and overall survival.

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Immune-Related Oral, Otologic, and Ocular Adverse Events

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Keywords

Immunotherapy · Side effects · Dental · Eyes · Ears

Emerging immunotherapeutic agents, including immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated antigen-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 (ICI) ipilimumab, an anti-CTLA-4, was approved in 2011. Since then, the US 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.

1 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 chemo-

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therapy- or radiotherapy-induced mucosal injury [6]. Oral mucositis 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 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 or stomatitis, irrespective of severity, has been reported to range from 59.4 to 100% in head and neck cancer patients receiving radiation or chemotherapy, between 70 and 86.6% in HSCT patients, and from 14.4 to 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].

1.1 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 were carried out. A systematic search was conducted on February 2, 2019, and data was extracted from all completed trials (Phases 1, 2, and 3) with reported adverse event data. Oral toxicity data, irrespective of toxicity grading, primary tumor, or drug dosage, was 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 effect model in which each study was weighted equally. Detailed methodology and interpretation are published elsewhere [13, 14].

A total of 20 clinical trials (Table 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. 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. 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 ten clinical trials (Fig. 3).

Table 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 2 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 Pre-Surgery 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 gastroesophageal junction	Phase 2

(continued)

Table 1 (continued)

NCT number	Immunotherapy	Title	Malignancy	Trial phase
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 1|phase 2
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-PDL1 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

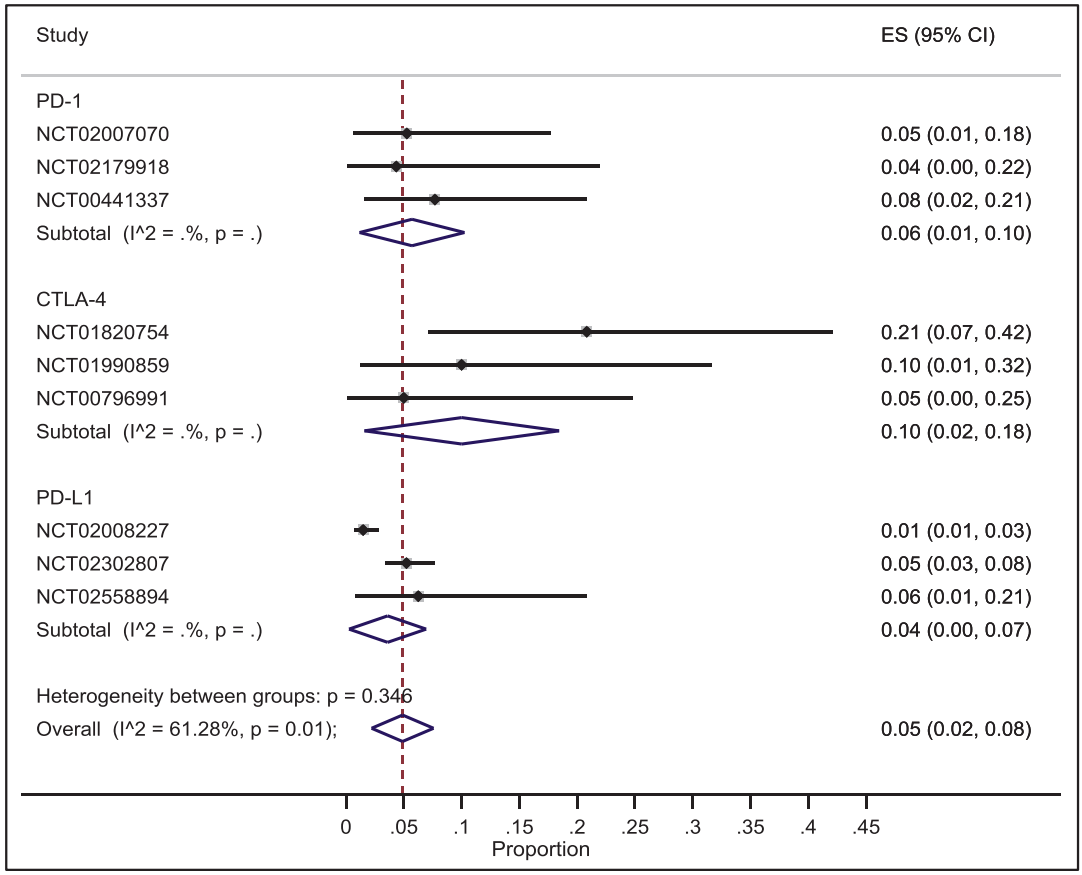

Fig. 1 Forrest plot for meta-analysis of prevalence of oral mucositis

1.2 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 (IV, 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 two 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

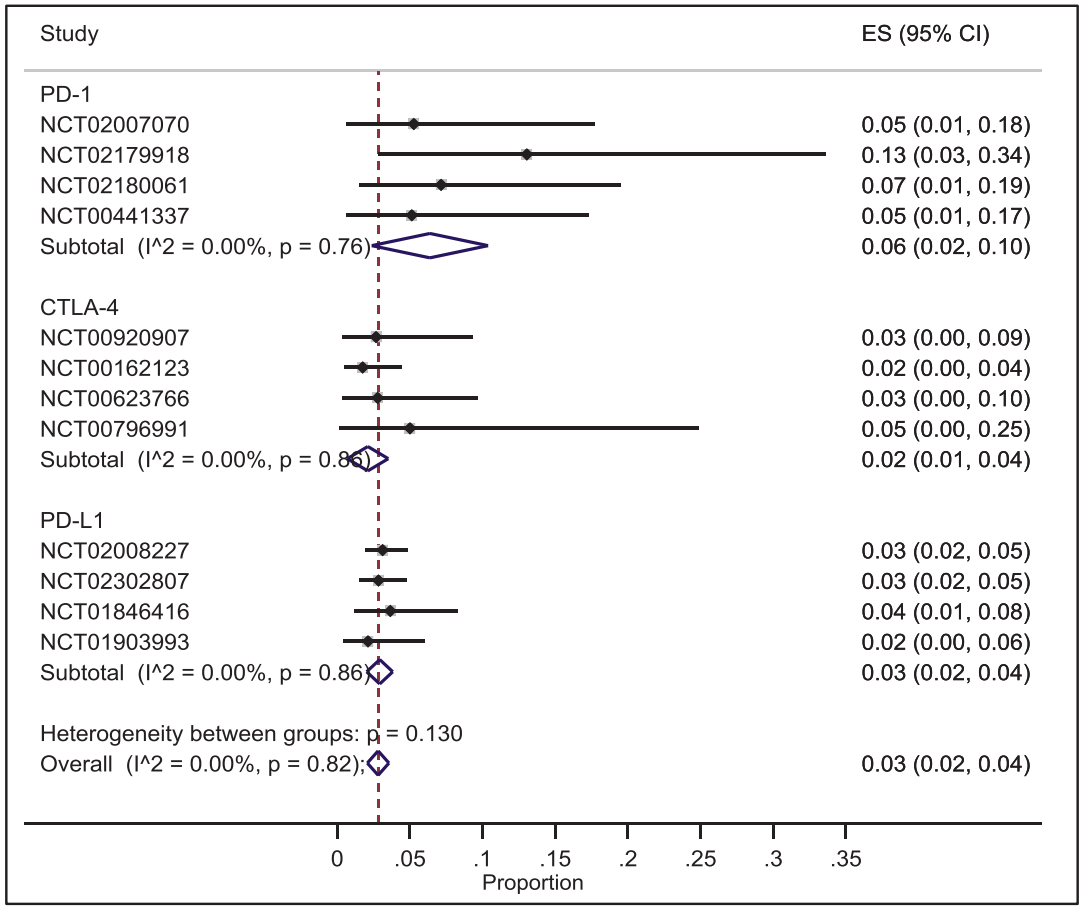

Fig. 2 Forrest plot for meta-analysis of prevalence of stomatitis

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 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.

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

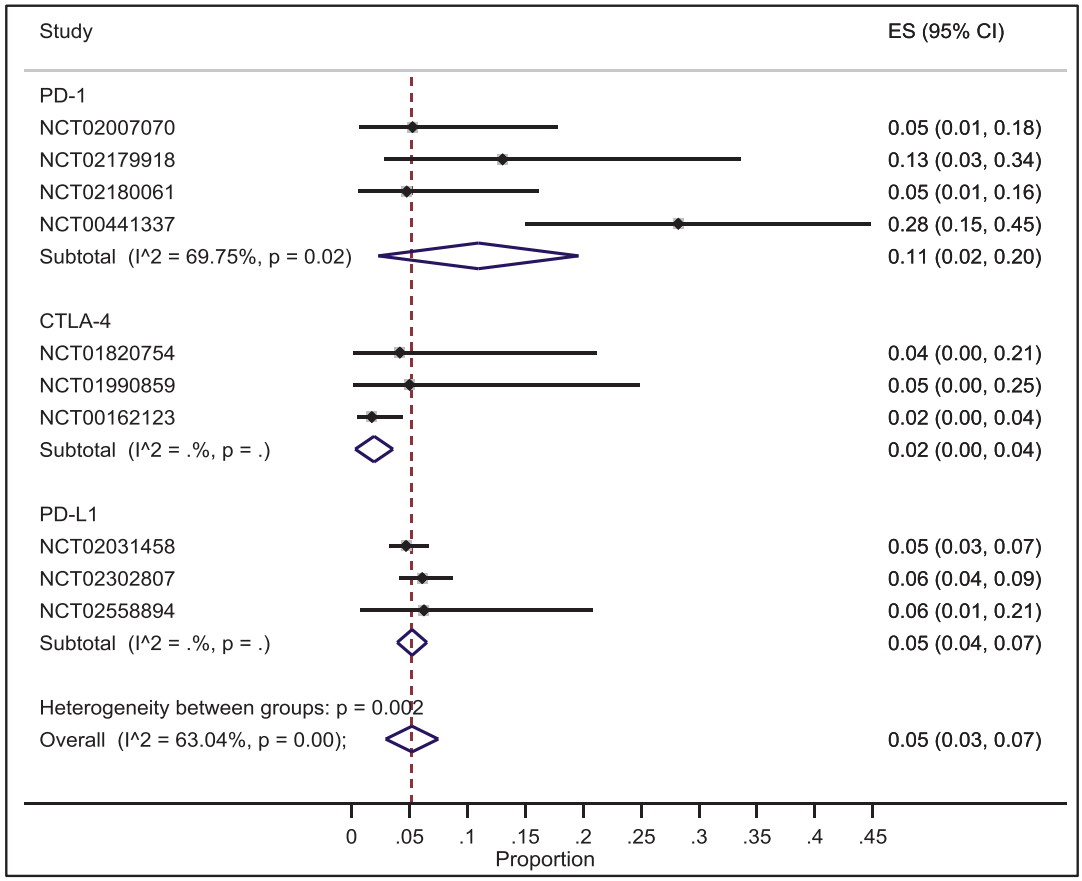

Fig. 3 Forrest plot for meta-analysis of prevalence of xerostomia

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.

2 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 (SNHL) and conductive hearing loss, respectively. Traditional chemotherapy modalities, such as carboplatin and cisplatin, also have well-known and well-studied ototoxicity profiles.

2.1 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 (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 SNHL. The most common form of non-syndromic, congenital SNHL involves genetic mutation 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 destruction is the underlying cause of hearing loss following ACI.

2.2 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 SNHL. 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 intravitreal 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.

2.3 Animal Models

Spielbauer et al. evaluated the impact of anti-PD-1 therapy on a murine animal model [31]. 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. Szepesy

et al. showed in their murine model treated with anti-PD-1 therapy similar threshold findings as well as preservation of the number and morphology of spiral ganglion neurons in all cochlear turns [32]. The apical-middle turns (<32 kHz) showed preservation of the inner and outer hair cells (OHCs), and surprisingly, the age-related loss of OHCs in the basal turn (>32 kHz) was mitigated. The latter finding, though not likely clinically significant, demonstrates the possibility of a protective effect. Kuzucu et al. found evidence of ototoxicity in their murine model evidenced by shifts in auditory brainstem responses (ABR) and mild OHC loss. Notably, the toxicity resolved following treatment cessation. Tonotopic details were not reported in the study [33].

2.4 Case Series and 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.

Rosner et al. recently published the largest case series in the literature to date [34]. They report six cases of metastatic melanoma treated with ipilimumab, nivolumab, pembrolizumab, and/or recombinant IL-21. Bilateral symptoms, including tinnitus and variable degrees of hearing loss, were most commonly seen with an average onset around 4 months after the initial dose. Half of the patients experienced only mild symptoms including no hearing loss, mild SNHL, and mild to moderate SNHL. Only one of the six received corticosteroids (prednisone) to manage the ototoxic effects. This patient was the only one who showed improvement from an otologic perspective. The authors do not advocate for but do introduce the idea of baseline audiograms in all patients treated with ICIs.

Though few reports are available in the literature, the general trend that emerges is cessation of the offending agent along with the application of steroids – either oral or intratympanically – which can have a positive impact on the return of hearing and vestibular function. Case reports to date are summarized in Table 2. Further case details are included in the text below.

2.4.1 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, and a PD-1 inhibitor (2 mg/kg every 3 weeks), due to disease progression [35]. Following his second dose of pembrolizumab, the patient noted bilateral hearing loss.

Audiometry confirmed a mild to moderately severe symmetric SNHL with word recognition scores (WRS) 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), six injections on the right and four 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 WRS improved to 88% and 84%. He continued his pembrolizumab therapy and had no further audiovestibular symptoms.

2.4.2 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 [36]. 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 3 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

Table 2 Summary of case reports

Author (citation)	Patient age (sex)	Tumor type (stage)	ICI	Degree of hearing loss	Associated symptoms	Treatment, dose (route)	Outcome of hearing loss	Tumor status
Zibelman [35]	82 (M)	Mucosal melanoma	Ipilimumab, pembrolizumab	AU: moderate-severe SNHL	None	Dexamethasone, 10 mg/mL (IT, 6 on right, 4 on left)	Persistent high Hz loss, full low Hz and WRS recovery	Significant response
Diamantopoulos [36]	81 (F)	Cutaneous melanoma (IIIb)	Binimetinib, encorafenib	AD, moderate SNHL; AS, mild SNHL	Bilateral panuveitis	Methylprednisolone, 64 mg daily for 7 days (PO)	Not reported	Complete resolution
Tampio [37]	67 (M)	Cutaneous melanoma	Nivolumab	AU: mild-severe SNHL	Vertigo, bilateral panuveitis	Prednisone, 60 mg daily, tapered over 5 weeks (PO)	Return to normal	Complete resolution
Hobelmann [38]	67 (M)	Cutaneous melanoma	Pembrolizumab	AU, moderate-severe SNHL	None	Prednisone, 60 mg daily for 5 days, tapered over 10 days (PO); dexamethasone, 10 mg/mL (IT; 1x each ear)	Improved to mild-moderate SNHL	Not reported
Rajapakse [39]	69 (M)	Non-small cell lung cancer	Nivolumab	AD: severe SNHL	None	Methylprednisolone for 3 days (IV, dosing not reported), prednisolone taper (PO, dosing not reported)	Improved to moderate SNHL	Complete resolution
Choi [40]	54 (M)	Cutaneous melanoma	Ipilimumab, nivolumab	AU: moderate-severe SNHL	Vertigo	Methylprednisolone (IV), prednisone (PO), dexamethasone (IT), infliximab[a]	Persistent high Hz loss, full low Hz and WRS recovery	Complete resolution
Gambichler [41]	63 (F)	Cutaneous melanoma	Nivolumab	AU: severe SNHL	Vertigo, bilateral uveitis, vitiligo	Methylprednisolone, 1000 mg for 3 days (IV), prednisolone, 40 mg taper (PO)	Return to normal	Complete resolution

M male, *F* female, *IT* intratympanic, *Hz* frequency, *WRS* word recognition score, *AU* both ears, *AD* right ear, *AS* left ear, *SNHL* sensorineural hearing loss, *PO* by mouth

[a]see text for treatment details for this case

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 is given regarding resolution of her hearing loss.

2.4.3 Case #3

Tampio et al. reported a case of a 67-year-old man with a history of sarcoidosis with widely metastatic melanoma [37]. 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 WRS 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 four cycles of nivolumab, and repeat MRI and PET/CT at this time showed resolution of neoplastic disease. At 6-week follow-up, the patient noted completely resolved ocular symptoms and improved hearing. Repeat audiogram at the 4-month follow-up showed normalization of the speech reception thresholds.

2.4.4 Case #4

Hobelmann et al. reported a 67-year-old man with metastatic melanoma of the toe [38]. He underwent amputation and lymph node dissection and was started on pembrolizumab. After his first dose, he complained of bilateral ear fullness which was attributed to congestion, and the decision was made to continue treatment. Following his second dose, he continued to complain of hearing loss, and his audiogram revealed a new bilateral moderate-severe symmetric SNHL with WRS of 72% and 68% in the left and right ears, respectively. Treatment was discontinued, and he was given 60 mg prednisone for 5 days followed by a taper over 10 additional days.

On evaluation 2 weeks later, the patient reported subjectively improved hearing and had recovered low tones to the mild range and showed mild improvement in his WRS but demonstrated no improvement in the high frequencies. He subsequently underwent intratympanic injection of 0.4 ml of dexamethasone 10 mg/ml bilaterally. His final audiogram was performed 12 weeks after the initial hearing loss was noted and revealed stable symmetric mild SNHL in the low frequencies and moderate SNHL in the high frequencies. WRT was 84% on the left and 95% on the right. The patient did not have a pre-intervention audiogram for comparison.

2.4.5 Case #5

Rajapakse et al. reported a 69-year-old man with non-small cell lung cancer who underwent paclitaxel/carboplatin and concurrent radiation following disease recurrence after initial surgical management several years earlier [39]. Due to tumor progression within weeks of starting chemoradiation, nivolumab was started as second-line therapy. After his second dose, he presented with a right-sided sudden hearing loss. Audiogram revealed severe unilateral SNHL (WRS not reported). An MRI of the brain ruled out retrocochlear pathology. He commenced high-dose IV methylprednisolone for 3 days,

followed by an oral prednisolone taper (dosing not reported). Improvement in his hearing was noted within 10 days. Ultimately, his 3-month posttreatment audiogram showed a persistent moderate SNHL. Of note, nivolumab was discontinued following symptom onset. Fortunately, PET/CT performed at the initial hearing loss following the second cycle showed a complete metabolic response. The patient did not have a pre-intervention audiogram for comparison.

2.4.6 Case #6

Choi et al. reported a 54-year-old male with history of stage IA cutaneous melanoma initially treated with wide local excision and negative sentinel lymph node biopsy who presented to the emergency room 6 years after treatment with diplopia and lateral gaze palsy [40]. On workup, the patient was found to have a left lateral rectus lesion along with several other distant metastatic lesions. Radiation therapy was performed for the lateral rectus lesion followed by administration of four cycles of ipilimumab and nivolumab over 10 weeks. Four weeks after completion, the patient presented with complaint of imbalance, tinnitus, and rapidly progressive bilateral hearing loss. He was admitted for workup to rule out stroke and infectious etiologies. Repeat MRI showed new scattered T2 hyperintensities, which were considered reactive, as well as resolution of the lateral rectus mass. He was ultimately referred to the otology service.

An audiogram revealed bilateral moderate to severe sloping low- to high-frequency SNHL with speech recognition scores of 68% in the left ear and 60% in the right ear. He was started on IV methylprednisolone 1 mg/kg/day and noted immediate improvement in his hearing, vertigo, and gait. He was discharged 3 days later and transitioned to an oral prednisone 1 mg/kg/day for 1 week tapered over 30 days.

Subsequent MRI 1 month later revealed resolution of prior enhancement but new hyperintensities in the right globus pallidus and mild enhancement in the right internal auditory canal. He was thus started on a second tapering course of oral prednisone 1 mg/kg/day for 1 week tapered over 2 weeks. Repeat audiogram was stable. The patient was seen 4 weeks later and again complained of worsening hearing and tinnitus. Audiogram confirmed bilateral mild sloping to severe SNHL, and a third oral prednisone taper was given followed again by resolution of symptoms. He experienced a third and final episode of hearing loss bilaterally 2 weeks after receiving his third course of oral steroids. He was treated with one intratympanic injection of dexamethasone 24 mg/mL in each ear and oral steroids 1 mg/kg/day tapered over 4 weeks. He was also started on infliximab 5 mg/kg with plan to repeat dosing. Audiograms at 2 weeks and 8 months showed significant hearing recovery.

This case is the first to highlight that audiovestibular and neurological irAEs may occur in a recurrent manner despite discontinuation of immunotherapy and multiple courses of systemic steroid therapy.

2.4.7 Case #7

Gambichler et al. reported a 63-year-old female with stage IV melanoma with metastatic deposits in the lungs, liver, and paratracheal region [41]. Nivolumab monotherapy was initiated by administering 240 mg fixed-dose infusions every other week. After the third cycle of nivolumab, the patient developed bilateral blurry vision as well as hearing loss, vertigo, and ataxia, all within 3 days. Her audiogram revealed bilateral severe SNHL. Other workup included brain MRI, which was negative for metastatic disease, slit lamp exam, which showed uveitis anterior/intermedia and calorics which showed no response.

Nivolumab was stopped immediately, and the patient received 1000 mg IV methylprednisolone over 3 days, followed by a tapered-dose regimen starting with 40 mg prednisolone orally. Notably, the patient developed generalized vitiligo 3 weeks after the initiation of steroids. Importantly, interval CT imaging of the chest and abdomen showed an almost complete response of the metastatic lesions. Her cochleovestibular and ocular symptoms quickly improved with the steroids, and by 8 months, the only sequela remaining was vitiligo.

The authors of this report suggest that the occurrence of VKHD-type symptoms (i.e.,

hearing loss, vestibulopathy, uveitis, and vitiligo) following ICI initiation in melanoma patients may be a strong indicator for ICI efficacy. While no large prospective studies are available to confirm the association between otologic irAEs and oncologic response, the vast majority of patients reported to have sustained ototoxicity from ICI have had a response to treatment. Of the 13 cases of ototoxic irAEs that have been described in the case series and case reports previously discussed in this chapter, data on oncologic response is available for 12. Of these, only one patient had tumor progression, while five had partial response and six complete response. Of note, a growing number of retrospective reports have showed an association between the presence of irAEs and an improved oncologic outcome [42–44].

2.5 Management

Treatment guidelines have not been defined by the National Comprehensive Cancer Network as it has been done for other irAEs. Additionally, there are no prospective trials to date on the management algorithm for ototoxic irAEs. Thus, recommendations can only be based on the sparse case reports summarized above and the generalizable similarities in management for sudden audiovestibular symptoms frequently managed in a tertiary neurotology practice.

Generally, the decision to discontinue ICIs based on the development of audiovestibular symptoms will have to be weighed against the risks of stopping the therapy. This is a decision that the patient, oncologist, and surgeon should make together, leaving the ultimate choice with the patient.

As reported in the cases above, corticosteroids appear to have a positive impact on the recovery from ototoxic irAEs. This finding is consistent with the large body of literature supporting corticosteroid use for other causes of sudden SNHL and/or vestibular symptoms. Steroids are generally administered orally, but there are many contraindications to consider such as poorly controlled diabetes, psychiatric disorders, osteoporosis or other bone-related pathologies, gastritis, and more. If contraindications to oral therapy exist, IT injections can be considered as the systemic side effects are thought to be less. Intratympanic delivery may also be considered as salvage therapy if oral treatment fails. Therefore, while no prospective data is available specific to ototoxic irAEs, corticosteroids are recommended. The optimal dosing and delivery methods are unclear and should be the subject of future prospective trials.

If hearing deficits persist following corticosteroid treatment, hearing rehabilitation options can be useful. Specifically, for mild to moderate SNHL, hearing aids should provide benefit. Once hearing drops below this level, the patient may become a candidate for a cochlear implant (CI). Cochlear implants require intact spiral ganglion cells for proper functioning and therefore, theoretically, can be very effective in this patient population given irAEs appear to predominantly negatively impact hair cell function. The decision to proceed with CI evaluation will also be contingent on the patient's disease status as patients with active disease may not be candidates.

3 Immunotherapy and Ocular Toxicity

The majority of described ocular irAEs are mild- and low-grade, non-sight-threatening such as blurred vision, conjunctivitis, and ocular surface disease (dry eye). Serious and sight-threatening events such as corneal perforation, Vogt-Koyanagi-Harada syndrome, optic neuropathy, and retinal vascular occlusion can occur but are infrequent and may lead to treatment withdrawal and fulminant events. Knowledge and awareness of ocular side effects are imperative to guide the proper treatment plan. A multidisciplinary approach between oncologists, internists, ophthalmologists, and other specialists is essential in the identification and management of these events [45–47].

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 the 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-threatening 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 [48–54].

Abdel-Rahman et al. conducted a systematic review to assess the incidence of ocular irAEs. Eleven prospective trials were analyzed including 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 inhibitor 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 [55–57].

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 [58, 59].

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 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 1 with orbital myopathy; five (62.5%) developed exophthalmos [60].

Fang et al. looked at the association between immune checkpoint inhibitors and ophthalmic adverse effects using data from US 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 [47, 51, 61].

Alba-Linero et al. reviewed 35 articles of Phase 3 clinical trials of checkpoint inhibitors used in the treatment of kidney, and lung cancers, or melanoma. Of the 35 articles, 13 articles were on the treatment of melanoma, and 10 and 12 were on the treatment of renal and lung cancers, respectively. One Phase 2 clinical trial addressed the ocular toxicity after cemiplimab for cutaneous squamous-cell carcinoma. Unspecified ocular inflammation reported with tremelimumab in 13 patients (4%), anterior uveitis reported in 4 patients (1.5%) with pembrolizumab, and

increased tearing were reported in 3 patients (1%) with nivolumab. Other irAEs were reported with atezolizumab, e.g., exophthalmos uveitis, retinopathy, and ocular inflammation each in one patient (0.2%) and optic neuritis in two patients (0.3%). Optic neuritis and retinopathy were considered grade 3–4 toxicity, and all others were described as grade 1 toxicity. Conjunctivitis, dry eye, and ocular myasthenia reported with cemiplimab. The study concluded that ocular irAEs were underestimated in the clinical trials and recommended that the ophthalmological assessment should be part of the assessment for patients on checkpoint inhibitors [62].

3.1 Management

It is important for ophthalmologist and physician to be aware and have a high level of suspicion to recognize symptoms of the potential irAEs and be vigilant in detecting these ocular toxicities promptly to avoid irreversible damage. Ocular irAEs from treatments may seem indistinguishable from the direct effects of the cancer itself or its indirect complications.

Recognition and differentiation of these irAEs are imperative to the proper care and treatment of the patient. An ophthalmic baseline examination pretreatment may help detect any preexisting ocular conditions and lead to the reduction of ocular side effects when predisposed patients are screened and examined regularly during and after therapy. Recommendations regarding management are mostly based on case reports, case series, and expert consensus. Risk-to-benefit balance should be considered. 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 [63–65].

4 Summary

Immune-based cancer therapy has revolutionized the treatment of various malignancies. Clinicians should be familiar with likely adverse events associated with immunotherapies. Ocular toxicities are among the most common adverse events resulting from the use of these agents. The majority are mild, and not sight-threatening; 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 dysfunction has also been reported in patients treated with immunotherapy directed toward melanocytes. These irAEs tend to respond well to systemic and intratympanic corticosteroids. While the overwhelming majority of patients having sustained ototoxic irAEs have showed an oncologic response to treatment, the association between these irAEs and effectiveness of ICI needs to be confirmed in larger studies.

A multidisciplinary approach with good communication is crucial for prompt referral and management of such complications. Presently, there is a lack of standardized surveillance

guidelines and protocols. Establishing ophthalmic, otolaryngologic, audiologic, and oral surveillance protocols with baseline screening would be ideal. The specific frequency and exam parameters need to be elucidated and may be dependent on the agent and its toxicity profile. Other strategies may include emphasizing patient education, standardizing reporting systems, and optimizing the choice of immunosuppressive agents [66].

Further research is needed to establish the prevalence/incidence of immunotherapy-induced oral, ocular, and audiovestibular toxicities as well as their pathophysiology, risk factors, and management. Areas of future research may include investigating the oral microbiome and its association with ICI-induced toxicities [67]. Moreover, similar to studies on biomarkers and their association with tumor response, there is a need for biomarkers to help identify patients at risk for irAEs [68].

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Neurologic Toxicities of Immunotherapy

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Abstract

Immunotherapy has revolutionized treatment of cancer over the past two decades. The antitumor effects of immunotherapy approaches are at the expense of growing spectrum of immune-related adverse events (irAEs) due to cross-reactivity between the tumor and normal host tissue. These adverse events can happen in any organ and range from mild to severe and even life-threatening conditions. While neurological irAEs associated with immune checkpoint inhibitors (CPIs) are rare, they pose a significant challenge in management as the clinical phenotypes are heterogenous and frequently necessitate cessation of therapy and systemic immune suppression and lead to transient functional decline. On the other hand, immune effector cell-associated neurotoxicity (ICANS) is common, frequently occurs in conjunction with cytokine release syndrome (CRS), and poses a significant clinical challenge to the development and widespread use of these effective therapies. Early recognition of these neurological syndromes, timely diagnosis, and thoughtful management are key for further clinical development of these effective therapies in cancer patients. Here, we describe clinical phenotypes of CPI-induced neurological complications and ICANS and discuss steps in clinical monitoring, diagnosis, and effective management.

Keywords

Immunotherapy · Neurotoxicity · Myasthenia gravis · Myositis · Encephalitis · Checkpoint inihibitor · CAR T cell therapy · Immune effector cell-associated neurotoxicity

1 Introduction

Immunotherapy has changed the landscape of cancer therapy and is now established as the fourth pillar of cancer treatment in parallel with surgery, radiation, and chemotherapy. Immunotherapy takes advantage of intrinsic susceptibility of tumor cells to the immune system and achieves effective antitumor responses via active transfer of cytotoxic immune cells targeting specific antigens (CAR T cells) or immune cells without the need for antigen presentation (NK cells), T cell activation (dendritic cells, viral therapy, antibodies against inducible T cell antigens), and maintenance of T cell effector function (checkpoint inhibitors). Checkpoint inhibitors (CPIs) and CAR T cell therapies have been at the forefront of these advances for treat-

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ment of solid cancers and liquid malignancies, respectively.

CPI-induced immune-related adverse events (irAEs) can affect any organ and have variable presentations. Although most CPI-induced irAEs are mild, some impact quality of life significantly and are life-threatening. Development of irAEs can be considered as a biomarker of response to immunotherapy as improvements in survival have been reported in the patient population who experience irAEs [1] possibly due to a more responsive immune system. However, biomarkers of development of irAEs are not understood, and development of these adverse events remains unpredictable and poses challenges in management. Given the high incidence of CPI-induced irAEs, identifying patients who are likely to respond to treatment with CPIs is a priority, and significant efforts have been ongoing to determine the biomarkers of response to CPIs in order to limit toxicities to patients who are not likely to benefit from these therapies [2].

Neurological adverse events, although rare, are frequently associated with significant morbidity, require immunosuppressive therapies, and limit further cancer treatment. CPI-induced nervous system complications affect both the peripheral and central nervous system. Peripheral nervous system complications including myasthenia gravis, myositis, and polyneuropathies are more common than CNS manifestations and are a growing spectrum of complicated clinical phenotypes often affecting more than one segment of the peripheral nervous system [3]. CPI-induced CNS complications include encephalitis, meningitis, and myelitis. Multisystem involvement with combined CNS and PNS irAEs and co-occurring non-neurological irAES have been reported and are more common in combination with CPI treatments [3].

Unlike high-grade CPI-induced neurological complications which are relatively rare (1%) [4], rates of high-grade immune effector cell-associated neurotoxicity syndrome (ICANS) in pivotal CAR T cell therapy trials have ranged from 3 to 31% depending on the particular products' variability, dose, and peak CAR T cell expansion [5–10]. Management of patients with ICANS can be particularly challenging as neurological symptoms develop very rapidly in a severely immunosuppressed and frail patient population who has endured multiple lines of prior therapies.

Early identification and treatment irAEs are key in ameliorating the effects of these complications on neurological outcome and future cancer treatments. Strategies such as harmonizing irAE management guidelines, standardizing reporting in clinical trials, conducting translational research with the focus to identify predictive biomarkers, providing patient education, and implementing patient-reported outcomes for measuring symptomatic toxicity are crucial in our efforts to lessen the impact of irAEs on oncological care [11, 12]. In this chapter, we describe clinical phenotypes of CPI-induced PNS and CNS complications and ICAN and proposed biological mechanisms. We further discuss steps in clinical monitoring, diagnosis, and effective management.

2 Checkpoint Inhibitor Therapy

2.1 Therapeutic Rationale

Checkpoint inhibitors function by facilitating the recognition of cancer cells by the immune system. There are several points of regulation, or checkpoints, whereby the immune response against a foreign entity is hindered. The balance of these inhibitory signals, as well as stimulatory signals, keeps the immune system as it is in check. Many cancers are thought to protect themselves from immune recognition and attack by exploiting these mechanisms of inhibition. In order for a T cell to become active, it requires both a cognate antigen and a costimulatory signal. Checkpoint inhibitors can act at either to block their signal and dampen the immune response.

CPIs are antibodies to various epitopes on antigens and immune cells that interact to maintain antitumor immune responses. The antigen-epitope interactions they target are naturally occurring to inhibit immune activation; thus their

blockade facilitates immune activation. The checkpoint inhibitors are broadly classified based on the antigens that are targeted. Ipilimumab, a CTLA-4 antibody, was the first CPI to be approved by the FDA for treating patients with melanoma [13]. Since then, many more antibodies have been developed, targeting antigens such as PD-1 and PD-L1 on T cells and cancer cells, respectively. CPIs have significantly improved the survival of patients with various solid cancers, and their indications are on the rise. In addition to CPIs, other immunotherapy approaches promoting antitumor T cell function are now being explored. T cell agonists such as 4-1BB, OX40, inducible T cell co-stimulatory, and glucocorticoid-induced tumor necrosis factor receptors have demonstrated promising results in preclinical and early phase trials and now emerging in ongoing clinical trials [14]. These studies are still development, and more time is needed to fully understand the spectrum of irAEs related to T cell agonists.

CPI-induced immune-relate adverse effects (irAEs), including those affecting the nervous system, are proposed to occur through multiple different mechanisms, though these are not fully elucidated [15]. Blockade of the inhibitory checkpoints for T cell immune response may allow for aberrant recognition and activation to self-antigens. While pre-existing autoimmune antibodies have been considered a possible contributor to the development of irAEs, a prior history of autoimmune disease has not been found to be consistently predictive of their development or their severity [16, 17]. Molecular mimicry between antigens on both cancer cells and components of the nervous system has also been proposed as a mechanism. Supportive of this is the finding that there are shared antigens, such as gangliosides, found in both melanoma cancer cells and on Schwann cells [18, 19]. To what extent this mechanism contributes is unclear. There has been some association between tumor response to therapy and the development of irAEs, with increased adverse events in both NSCLC patients treated with the PD-1 inhibitor nivolumab [20] and in patients with hypermutated tumours associated with increased antitumor response of CPI [21]. CPI-induced nervous system complications result in clinical decline and impaired quality of life and can be life-threatening. Early recognition and treatment of these clinical syndromes is of outmost importance to the medical oncologist, general neurologists, and neuro-oncologists.

2.2 Clinical Syndromes

2.2.1 Background

Multiple syndromes affecting both central and peripheral nervous systems have been characterized after therapy with CPI. These generally take on a similar phenotype to previously characterized neurologic conditions that occur de novo. A notable distinction, however, is that most of those occurring associated with CPI treatment take on a monophasic course, while those occurring de novo frequently assume a relapsing-remitting or chronic trajectory.

2.2.2 Peripheral Nervous System

Myasthenia Gravis

Myasthenia gravis is a disorder of neuromuscular transmission, whereby pathologic antibodies target the neuromuscular junction or muscle-specific kinases. Most frequently, cases of myasthenia gravis after CPI therapy occur in patients with no known history of myasthenia gravis or thymic malignancy [22], with two-thirds of cases being such in one series [23]. There are exceptions where these are premorbid conditions, however [24, 25]. Serum acetylcholine receptor antibody positivity is variable [23, 26, 27]. A distinguishing feature of myasthenia from CPI treatment is the association with elevated serum creatine kinase and clinical myositis. This is highly atypical in non-iatrogenic myasthenia gravis but found in over three quarters of those with CPI-associated MG [28], commonly accompanied by electrodiagnostic findings of muscle membrane irritability and myositis. A concurrent myocarditis may also occur in this population [29], and as such, cardiac enzymes, cardiac MRI, and early cardiology consultation

should be considered when suspected. Another important feature of CPI-associated MG is its high morbidity and mortality. Nearly one-third of patients died of MG-specific causes in one series [23], and concurrent myocarditis elevated mortality to half of patients in another series [29].

With respect to treatment, early and aggressive identification and management is indicated. First, a thorough clinical evaluation for the extent and severity of MG is warranted. Clinical grading is important for assessment, progression, and grade-based management [30]. Early neurological consultation, close clinical monitoring, and consideration of ICU admission are generally warranted, given the potential for severe phenotype and rapid clinical decline. Diagnostic work-up includes serum acetylcholine receptor, anti-striated muscle antibodies, CK, ESR and CRP (to evaluate for concurrent myositis), and consideration of CNS imaging to rule out CNS involvement depending on symptoms [31]. Particular attention should be drawn to respiratory function, with repeat pulmonary function testing frequently indicated given the potential for rapid decline in this population. In cases of myasthenia gravis associated with myositis, close attention to extent of cardiac injury via performing a cardiopulmonary exam, obtaining cardiac specific enzymes (troponin I), electrocardiogram, telemetry, echocardiogram, cardiac MRI, and early cardiology consultation should be considered when suspected. Once a diagnosis is suspected, immunotherapy should be ceased. The diagnosis can be confirmed via electrodiagnostic studies including neuromuscular junction testing with repetitive nerve stimulation, nerve conduction studies, and needle EMG. Therapeutic intervention is generally based on clinical severity [31]. Low to medium dose (less than 1 mg/day prednisone) in combination with or without IVIG or plasma exchange is recommended in most patients. Higher dose steroids for MG with myositis along with IVIG or plasma exchange are recommended. Tandem simultaneous treatments are recommended rather than tiered escalating approach given the possibility of acute, subacute presentation and possibility of concurrent presentations of the three M's (myasthenia, myositis, and myocarditis). This would also facilitate faster taper of steroids. Few patients whose disease is limited to lower-grade myasthenia could be managed with prednisone and close clinical monitoring over the next few weeks for any progression into higher grades or development of other organ toxicity. Pyridostigmine is frequently used for symptomatic management, and medications known to exacerbate de novo myasthenia gravis [32] are to be avoided.

Myositis

Myositis, or inflammation of the muscle, may occur in isolation or as part of an overlap syndrome with other irAEs, such as AIDP or myasthenia gravis. This can take on various forms, including isolated hyperCKemia, dermatomyositis, or polymyositis [26, 33, 34]. As noted above, concurrent myocarditis may occur and has been noted in up to one-third of cases [34]. Troponin T elevation can be elevated in neuromuscular conditions, and troponin I is recommended for accurate myocarditis diagnosis. In 1 series of 19 patients with CPI-associated myositis, nearly half of patients had a severity classified as severe, and proximal myalgias and weakness were common, and pathologic review of muscle biopsy frequently revealed necrotic myositis [34]. The sequelae of CPI-associated myositis may be severe, and as such, early identification and management are key in successful management. Diagnostic work-up includes serum CK, aldolase, myositis panel, cardiac biomarkers,, ESR, CRP, electrocardiogram, echocardiogram, electromyography. Consideration of MRI of involved muscles and biopsy maybe warranted on an individual basis when diagnosis is uncertain. Referral to rheumatology, neurology, and cardiology in cases of cardiac involvement is warranted. Therapy is similar to that of myasthenia gravis and is based on clinical severity. It constitutes cessation of CPI therapy and early administration of steroids. Plasma exchange and IVIG should be considered in cases that are not responsive to steroids or initiated in tandem with steroids in higher grades. Patients with bulbar and diaphragm weakness should be considered as higher grades. Nonsteroidal anti-inflammatory drugs are typi-

cally needed for management of pain from myositis.

Neuropathy

The most common peripheral nerve disorders associated with CPI therapy are acute and chronic inflammatory demyelinating polyneuropathy (AIDP and CIDP, respectively). Less common reported phenotypes include cranial neuropathies, small fiber neuropathy, sensory ganglionopathy, and neuralgic amyotrophy [35–42]. Isolated root inflammation and plexopathies are rare entities as well. Concurrent inflammatory pathology affecting other organ systems is common, occurring in over half of all patients [39]. These neuropathies are often seen concurrently with other neurologic manifestations, with a review of 12 trials of ipilimumab or nivolumab revealing over 60% of patients with some form of neurologic toxicity which had a component of neuropathy as part of the irAE [41]. Diagnostic work-up includes MRI of spine to rule out compressive lesions, lumbar puncture to evaluate for evidence of intrathecal inflammation and malignancy, serum antiganglioside antibody tests as well as screening for reversible neuropathy causes, electrodiagnostic studies, and pulmonary function tests [31]. Neurological consultation and cessation of CPI therapy is indicated as in other cases of CPI-induced nervous system complications. Steroid treatment has been associated with improvements in modified Rankin Score and disability index [39]. Notably, steroids are also used in the treatment of CPI-associated AIDP, in contrast to how classical AIDP is clinically managed. IVIG and plasma exchange remain important therapies for those with significant clinical syndromes. Symptomatic management of concurrent neuropathic pain, autonomic dysfunction, constipation, and/or ileus is also warranted.

2.2.3 Central Nervous System

Central Demyelination

CPI therapy has been associated with de novo CNS demyelination [43]. There is also data to support these therapies leading to multiple sclerosis exacerbations in patients with prior diagnosis of MS. On review of cases reported to the FDA of newly diagnosed or relapsed multiple sclerosis in patients with CPI treatment, 57% of cases occurred in patients with pre-existing multiple sclerosis [44]. In these patients, symptoms tended to appear 29 days after treatment initiation and were associated with rapid progression. Two of 14 patients in this series died from their relapse. Severe relapse in a patient with pre-existing relapsing remitting MS has also been reported, supporting a worsening of pre-existing MS in patients with active disease [45]. Florid multifocal CNS demyelination consistent with ADEM (acute demyelinating encephalomyelitis) has been reported with nivolumab in a patient with no prior MS history [46], with subsequent improvement after steroids and IVIG. One case of de novo demyelination was associated with enhanced responses of myelin-reactive peripheral CD4+ T cells, similar to controls who had multiple sclerosis in the absence of pre-existent checkpoint inhibitor therapy [47].

Meningitis/Encephaliitis

Aseptic meningitis has been reported after multiple different CPI therapies [48–52]. The precise incidence of these entities is not known, though in one institutional series, 3 of 29 patients treated with atezolizumab developed aseptic meningitis [53]. These patients present in a typical fashion for meningitis, with headache, photophobia, and nausea. Cerebrospinal fluid testing often shows elevated opening pressure, lymphocytic pleocytosis, and negative infectious studies. Encephalitis is diagnosed with involvement of the brain parenchyma. Most reported cases are not associated with synaptic or paraneoplastic antibodies [51, 54], though cases of encephalitis associated with anti-Hu antibodies [55], GAD-65 encephalitis [56], and NMDA receptor antibodies [57] have been identified. Diagnostic work-up includes MRI of brain which may be normal or reveal T2/fluid-attenuated inversion recovery (FLAIR) abnormalities as is the case in autoimmune encephalitis. Lumbar puncture is necessary to rule out infection and malignancy. CSF should be evaluated for paraneoplastic antibody panels, oli-

goclonal bands, and IgG index and EEG to evaluate for seizures and serum inflammatory and rheumatological panels. Work-up for viral meningitis is also necessary (i.e., HSV, HHV6). Management includes neurological consultation, cessation of CPI, concurrent IV acyclovir for HSV coverage pending negative CSF PCR tests, steroids, and in severe cases plasma exchange or rituximab.

Myelitis

Longitudinally extensive transverse myelitis can rarely occur, with reported cases being seronegative [58, 59]. Diagnostic work-up includes MRI of spine and consideration of MRI brain to evaluate for concurrent encephalitis (encephalomyelitis), lumbar puncture for evaluation of infection, malignancy and onconeural antibodies, serum HIV, RPR, TSH, rheumatological panel, and aquaporin-4 IgG. Management involves permanent discontinuation of CPI, high-dose steroids, and IVIG. Symptomatic management of pain, autonomic dysfunction in higher cervical cord lesions, urinary retention, constipation, and spasticity is warranted.

Vasculitis

Rheumatological disorders including vasculitis and lupus-like syndromes have been documented in patients receiving CPIs [60] and should be considered as the underlying etiology of peripheral neuropathy and ischemic and hemorrhagic strokes. One systematic review identified 53 cases of suspected vasculitis associated with CPI therapy [61]. In these cases, the majority involved large or medium vessel involvement. Of those with CNS involvement, four were considered primary angiitis of the CNS, three were giant cell artieritis, one was isolated retinal vasculitis, and three had specified vasculitis polyneuropathy. No fatalities for vasculitis were observed. Diagnostic work-up of suspected CPI-induced CNS vasculitis includes MRI brain, noninvasive angiogram studies such as MR or CT angiogram, four-vessel cerebral angiogram in cases with high degree of suspicion and negative noninvasive angiograms, echocardiagram to evaluate for cardiogenic sources of stroke, and serum inflammatory markers. Initial management should follow guidelines for acute ischemic stroke or intracerebral hemorrhage [31, 60]. Once a diagnosis of CNS vaculitis is confirmed, rheumatology consultation for consideration of steroids and other stronger immunosuppresants such as cytoxan is warranted.

Table 1 summarizes the clinical syndromes discussed above and their diagnosis and management.

3 Adoptive Cell Therapy

Adoptive cell therapy involves the manipulation or engineering of tumor-infiltrating or peripheral immune cells, with reintroduction into the host with the goal of augmenting the antitumor immune response. The most common of these cellular therapies are chimeric antigen receptor T cell (CAR T) therapies. This is the first of these therapies to demonstrate efficacy and be incorporated into standard-of-care treatment. At this time, they are used in the treatment of several hematologic malignancies and are currently under investigation for the treatment of multiple solid tumors. At present, there are five products approved by the US Food and Drug Administration. Given their increasing use, a fundamental fluency in their associated toxicities is important for all clinicians that interface with these patients.

3.1 Neurotoxicity Syndrome

Immune effector cell-associated neurotoxicity syndrome (ICANS) is the current nomenclature used the encephalopathy syndrome associated with these therapies. The precise incidence of ICANS is difficult to define, as the diagnostic criteria have evolved over time [62], though two of the initial CAR T cell clinical trials noted 28–62% of patients developing central neurotoxicities [8, 63, 64]. The most commonly observed toxicity after CAR T cell therapy is cytokine release syndrome (CRS) [65], which can include fever, hypotension, hypoxia, and tachycardia. Distinctly, ICANS involves encephalopathy, sei-

Table 1 Summary of checkpoint inhibitor-induced immune-related neurological adverse events, clinical syndromes, diagnosis, and management

Peripheral nervous system			
Clinical syndromes	Diagnosis	Management	References
Myasthenia gravis[a]	Acetylcholine and antistriated muscle antibodies, CK, aldolase, ESR, CRP, troponin MRI brain and/or spine EMG/NCS If CK elevated, refer to "myositis"	Neurology consultation Hold CPIs for all grades Steroids IVIG or PLEX for G3 and higher PFTs Pyridostigmine Avoid medications that worsen MG	[31]
Myositis[a]	CK, aldolase, ESR, CRP, AST, ALT, LDH, troponin EKG, echocardiogram MRI muscle EMG Muscle biopsy	Rheumatology and/or neurology consultation Hold CPIs for G2 or higher IVIG or PLEX for G3 or higher NSAIDs Methotrexate, azathioprine, or mycophenolate mofetil for severe cases	
Peripheral neuropathies	Screen for reversible causes of neuropathy MRI spine and/or brain (if cranial neuropathies) EMG/NCS Paraneoplastic antibodies Orthostatic vital signs	Neurology consultation Hold CPIs for G2 or higher Steroids Neuropathic pain medications IVIG or PLEX for G3 or higher	
Acute demyelinating or axonal polyneuropathy	MRI spine Lumbar puncture EMG/NCS	Neurology consultation Hold CPIs for all grades MRI spine PFTs Steroids IVIG or PLEX for all grades	
Central nervous system			
Encephalitis[a]	MRI brain ESR, CRP, ANCA (if vasculitis suspected), TPO, thyroglobulin Lumbar puncture EEG Paraneoplastic panel	Neurology consultation Hold CPIs for all grades Steroids IVIG or PLEX Rituximab in severe cases	[31]
Meningitis[a]	MRI brain AM cortisol, ACTH Lumbar puncture EEG ESR, CRP	Neurology consultation Hold CPIs for all grades IV acyclovir and antimicrobials Steroids	
Myelitis[a]	MRI spine Lumbar puncture B12, HIV, RPR, ANA, Ro/La, TSH, AQP-4 IgG	Neurology consultation Hold CPIs for all grades Steroids IVIG	

[a]*Concurrent presentation possible*

Abbreviations: *CK* creatinine kinase, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *EMG* electromyogram, *NCS* nerve conduction studies, *CPIs* checkpoint inhibitors, *IVIG* intravenous immunoglobulin, *PLEX* plasma exchange, *PFT* pulmonary function test, *MG* myasthenia gravis, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *EKG* electrocardiogram, *NSADIs* nonsteroidal anti-inflammatory drugs, *ANCA* antineutrophil cytoplasmic antibodies, *TPO* thyroid peroxidase antibody, *EEG* electroencephalograph, *ACTH* adrenocorticotropic hormone, *RPR* rapid plasma reagin, *ANA* antinuclear antibody, *TSH* thyroid-stimulating hormone, *AQP-4 IgG* aquamarine-4 Immunoglobulin G

zure, and tremor and can occur with or without concurrent CRS. Significant clinical and academic effort has been made to characterize this syndrome and devise screening protocols and management algorithms given its frequency and potential severity. Of note, this syndrome was initially termed CRES (CAR T cell-related encephalopathy syndrome) but has now adopted the broader term of immune-effector cell neurotoxicity syndrome (ICANS) to include the other adoptive cell therapies and bispecific antibodies that may lead to this syndrome [62, 66, 67]. Risk factors for its development have been identified across studies and include younger patient age, pre-existing neurologic and medical conditions, high disease burden of the underlying malignancy, and early CRS with high cytokine levels [63, 68, 69].

The level of neurologic dysfunction observed with ICANS is variable. Most commonly, patients develop a delirium with preserved level of alertness [63]. Tremor and myoclonus may also occur and warrant dedicated evaluation on clinical examination. Notable in many cases is the distinct predilection for language involvement. Challenges with naming, comprehension, and repetition are frequently observed, despite no clear structural correlate for these changes on neuroimaging. It is unclear why language circuitry seems particularly vulnerable in this syndrome. Frontal-type cognitive changes and behaviors have also been noted, with verbal perseveration as well as hypokinesis being observed. Focal neurologic deficits, including ataxia, dysgraphia, and parkinsonism, have been noted. Seizures are an important element of this syndrome, and prophylactic antiseizure medications are routinely used at many centers for all patients [66] or those deemed at high risk for neurotoxicity [70]. Focal dyscognitive seizures need to be identified as potential contributors to a patient's encephalopathy or aphasia, and electroencephalogram is commonly indicated to aid in this distinction. The frequency of seizures in this population has been estimated to be less than 10% in patients as a whole, but in over two-thirds of patients with severe neurotoxicity [63]. Other potential confounders like encephalopathy related to neutropenic sepsis, cephalosporin toxicity, hepatic and renal dysfunction, and rarely steroid psychosis from higher-dose steroids can make a diagnosis of ICANS challenging. Rare reactivation of indolent viruses like HHV6,7 should be considered for late-onset ICANS. Rapid improvement or incremental improvements to few doses of steroids is reassuring.

In most patients that develop ICANS, it co-occurs with or appears after the development of CRS. The median time to onset of ICANS has been found to be 4–5 days post-infusion and usually reaches its greatest clinical severity within a day of onset [63]. Nearly all patients have at least some element of concurrent CRS, with the most common CRS element being fever [63, 71]. On average, neurotoxicity lasts 10–11 days and resolves by day 28 post-infusion [63]. This study found that the neurologic adverse events in patients without CRS were mild, subjective, and transient. Fever, elevated serum IL-6 concentration, and elevated monocyte chemoattractant protein-1 (MCP-1) in the first 36 h after infusion predicted grade 4 or higher neurotoxicity with 100% sensitivity and 97% specificity [71]. Higher-grade CRS seems to be the strongest predictor of higher-grade ICANS. While rates are variable, most studies document ongoing neurologic symptoms in up to 10% of patients that develop acute toxicities [6, 8, 71, 72]. The precise relationship between acute management and long-term outcomes is not understood.

3.2 Clinical Testing

The clinical identification of ICANS prompts several investigations to better define the syndrome and rule out other contributors to the clinical change. Because of the frequent co-occurrence of fever and encephalopathy typically in severely immunosuppressed patients, systemic and CNS infection must be ruled out with appropriate investigations to include lumbar puncture. In some cases, empiric antibiotic testing is clinically indicated. Non-contrast CT head is routinely obtained, as cerebral edema has been reported in rare cases [73]. With severe or pro-

longed ICANS, MRI brain imaging is frequently obtained. In one series, 30% of patients that underwent an MRI brain had some identifiable abnormality, ranging from cerebral edema, strokes, leptomeningeal enhancement, to cerebral microhemorrhages [71]. MRI may also be used to identify previously undiagnosed CNS involvement of disease which may be worsening neurologic symptoms or have contributed to the development of severe ICANS. Evaluation of optic disc for early signs of cerebral edema is recommended.

Electroencephalography (EEG) plays a critical role in the assessment of these patients. It is routinely performed in cases of suspected or defined ICANS, as seizures are common and may be challenging to identify clinically. While generalized slowing is the most commonly identified electrographic pattern in patients with encephalopathy [71], triphasic waves, frontal intermittent rhythmic delta activity (FIRDA), and focal or generalized epileptiform activity [63, 71] have been identified.

Frequent clinical assessment, clinical grading, intermittent neuroimaging, and spot EEGs in the absence of continuous EEG monitoring are useful in most scenarios and in facilities with limited resources. More advanced perfusion studies such as transcranial Doppler and nuclear medicine cerebral perfusion scans can provide additional insight if available.

Serum laboratory studies may show evidence of inflammation in patients with ICANS, attributable to concurrent CRS. Elevated levels of pro-inflammatory cytokines, such as IL-2, IL-6, and TNF-alpha, have been identified in patients with severe ICANS. Serum ferritin levels have been found to peak with onset of ICANS, and higher ferritin levels have been associated with greater neurotoxicity [68]. Evaluation of cerebrospinal fluid (CSF) is not routinely performed and, however, can be done to rule out infection or CNS involvement of the underlying cancer. ICANS has been associated with normal CSF, or elevations in protein and leukocytosis [69, 74].

Management involves administration of steroids and dose depends on severity of symptoms (i.e., ICANS grade). Tocilizumab is administered in cases of concurrent CRS and ICANS [75]. Tocilizumab should not be used for isolated ICANS given the displacement of IL-6 from receptor and increase in IL-6 levels. Patients with severe symptoms benefit from management in the intensive care unit as they may require mechanical ventilation and intubation for airway protection. Seizures and cerebral edema are treated per standard of care using seizure medications, high-dose steroids, hyperosmolar therapy, hyperventilation, and in rare circumstances neurosurgical consultation for CSF diversion [66] . Even though early studies showed that steroids may inhibit CAR T cell efficacy [76], more recent studies have evaluated the impact of steroids on CAR T cell therapy treatment response and indicated that steroids did not compromise the treatment effect of CAR T cells, proliferation, or duration [77, 78]. The differing results may be due to duration of steroid therapy in these studies, therefore emphasizing the judicious use of steroids and cessation of steroids as soon as clinically appropriate. There is ongoing research in early prophylactic use of biologics like IL-1 blocker, anakinra, and agents to affect macrophage-monocyte lineage to prevent CRS and ICANS. Table 2 summarizes immune effector cell-associated encephalopathy (ICE) assessment score and ICANS grades and corresponding steps in management.

3.3 Proposed Biologic Mechanisms

There are multiple biologic pathways proposed to contribute to ICANS, though the mechanisms have not been fully understood. As it has a distinct timeline from CRS, it is thought to have unique drivers, though given their common co-occurrence, a mechanistic link is likely. A central hypothesis is that diffuse systemic inflammation and elevated cytokine levels contribute to endothelial dysfunction, alterations in blood-brain barrier permeability, and thus aberrant inflammation in the CNS. Findings of high CSF protein and T cells [71], as well as increases in systemic inflammation with findings of elevated

Table 2 Summary of immune effector cell-associated neurotoxicity management

Grade	Management	References
1 ICE 7–9 No seizures, motor weakness or raised ICP	Aspiration precautions EEG Head imaging Tocilizumab if concurrent CRS	[62, 75]
2 ICE 3–6 No seizures, motor weakness or raised ICP	As in grade 1 plus steroids	
3 ICE 0–2 Any seizures that resolve with treatment No motor weakness Focal/local edema	As in grade 2 plus additional seizure medications as needed	
4 ICE 0 and patient unarousable or stuporous Status epilepticus Focal motor weakness Diffuse cerebral edema, decerebrate or decorticate posturing or CN VI palsies, or papilledema or Cushing's triad	As in grade 3 plus imaging of spine for focal motor weakness and ICP management (hyperventilation, hyperosmolar therapy, neurosurgery consult for CSF diversion)	

Abbreviations: *ICE* immune effector cell-associated Encephalopathy assessment score, *ICP* intracranial pressure, *EEG* electroencephalograph, *CRS* cytokine release syndrome, *CN* cranial nerve, *CSF* cerebrospinal fluid

C-reactive protein, ferritin, and pro-inflammatory cytokines, are supportive of this [63]. Direct penetrance of CAR T cells into the CNS has also been proposed to contribute to intrathecal cytokine production, contributing further to the syndrome [79].

4 Conclusion

Immunotherapies such as checkpoint inhibitors and immune effector cell therapies are receiving increasing indications in oncology, making the associated toxicities increasingly important to understand. The neurologic toxicities in particular have been noted with multiple agents and can be associated with morbidity and mortality in this population. Given that the treatment of these entities involves immunosuppression, a deeper understanding of the underlying biology will be important in elucidating treatment approaches that do not negate the anticancer impact of the treatment.

There are several areas of further understanding in the area of neurologic irAEs. At this time, we lack robust predictive strategies to identify those patients that are most vulnerable. If individual demographic, medical, or disease-related factors are found to be predictive, this may influence treatment selection or monitoring strategies for patients receiving immunotherapy. The long-term sequelae of treatments are also yet to be defined. Given the potential for these therapies to impact the central and peripheral nervous systems, longitudinal study of neurologic function is warranted to understand their long-term impact. These are two of the several potential areas that warrant further exploration with these therapies. Efforts to better characterize their clinical phenotype and their biologic underpinnings and to effectively treat their toxicities warrant continued attention. A clinical approach to neurologic irAEs is important for all clinician's interaction with this population.

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Cancer Imaging in Immunotherapy

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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. Response Evaluation Criteria in Solid Tumors (RECIST) or WHO criteria, developed to identify early effects of cytotoxic agents, may not provide a complete evaluation of new emerging treatment response pattern of immunotherapeutic agents. Therefore, new imaging response criteria, such as the immune-related Response Evaluation Criteria in Solid Tumors (irRECIST), immune Response Evaluation Criteria in Solid Tumors (iRECIST), and immune-related Response Criteria (irRC), are proposed. 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.

Keywords

Immunotherapy · Imaging · Responses criteria

1 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]. An approach to treating cancer by augmenting or generating an immune response against cancer cells, immunotherapy 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

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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 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, and patients receiving 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. 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 is lacking. 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 response criteria for the evaluation of tumor response in patients who are undergoing immunotherapy. We will also briefly discuss some of the immunotherapy-induced adverse events.

2 Conventional Imaging Response Criteria

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 [6, 7]. These criteria depend on reduction in tumor size and do not take in consideration appearance of new lesions when evaluating responses that may be related to treatment (Table 1) [6, 7].

2.1 WHO Criteria

In 1981, the WHO published the first tumor response criteria, thus establishing a standard assessment metric and nomenclature to evaluate treatment response [7]. The WHO criteria introduced the concept of assessing tumor burden using the sum of 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 [7]. 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 1). 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, 7].

2.2 RECIST 1.0 and 1.1

2.2.1 RECIST 1.0

In 2000, the RECIST criteria were established and addressed some of the pitfalls of the WHO

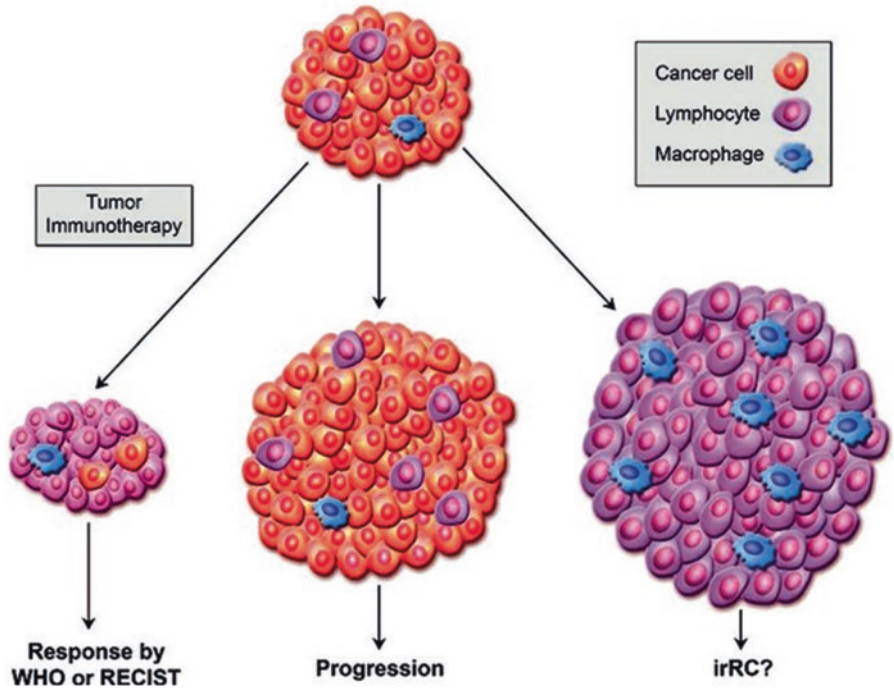

Fig. 1 Cancer imaging in immunotherapy

criteria [6]. Of these, the key features of RECIST 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 1) [6].

2.2.2 RECIST 1.1

In 2009, the RECIST 1.1 was developed to address multiple questions regarding the assessment of lymph nodes, number of lesions to be assessed, and use of new imaging modalities such as multidetector CT (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 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 (non-nodal lesions), its portions should be added together (as lesions coalesce) and measure its longest dimensions [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 it must be reassessed in follow-up examination to determine if it represents a new lesion (Table 2) [5]. Table 1 shows a brief comparison of WHO, RECIST 1.0, RECIST 1.1, irRC, and irRECIST criteria.

2.3 Modified RECIST (mRECIST)

Modified RECIST (mRECIST) was created to measure the response rate in hepatocellular carcinoma (HCC) [9]. 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

Table 1 Comparison between the basis of WHO, RECIST 1.0, RECIST 1.1, irRC, and irRECIST criteria

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 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 mm
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)
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
Complete response (CR)	Complete resolution of lesions at two consecutive scans >4 weeks apart	Disappearance of all nontarget lesions and normalization of tumor marker level	Complete resolution of all target lesions, nodes must regress to <10 mm in short axis	Complete resolution of all lesions including non- index lesions at two consecutive scans >4 weeks apart. No new measurable lesions. Referred to as irCR	Disappearance of all target and nontarget lesions, no new lesions
Partial response (PR)	≥50% decrease in SPD of all lesions (confirmed at 4 weeks)	≥ 30% decrease in tumor burden. No need to confirmation	≥30% decrease in tumor burden. Confirmation required	≥50% decrease in tumor burden. (Confirmed at 4 weeks). Referred to as irPR	Decrease of ≥30% in tumor burden relative to baseline Non-unequivocal progression of nontarget lesions No new lesions
Stable disease (SD)	Doesn't meet criteria of CR, PR, or PD	Doesn't meet criteria of CR, PR, or PD	Doesn't meet criteria of CR, PR, or PD	Doesn't meet criteria of irCR, irPR, or irPD Referred to as irSD	Doesn't meet criteria of CR, PR, or PD

(continued)

Table 1 (continued)

Criterion	WHO	RECIST 1.0	RECIST 1.1	irRC	irRECIST
Progressive disease (PD)	≥25% increase in SPD relative to nadir or appearance of new lesions	Appearance of one or more new lesions, increase in size of one or more nontarget lesions	≥20% +5 mm absolute increase in tumor burden compared with nadir, appearance of new lesions or progression of nontarget lesions	≥25% increase in tumor burden, at 4 weeks. Referred to as irPD	**iUPD**: – Increase ≥20% of the sum of longest diameters compared with nadir or progression of nontarget lesions or new lesions – Confirmation is required 4–8 weeks later the first iUPD assessment **iCPD**: – Increased size of target or nontarget lesions – Increase in the sum of new target lesions >5 mm – Appearance of another new lesion

irCR immune-related complete response, *irPR* immune-related partial response, *irSD* immune-related stable disease, *irPD* immune- related progressive disease, *iUPD* immune-unconfirmed progressive disease, *iCPD* immune-confirmed progressive disease

agent in the arterial phase on CT or MRI [10, 11]. 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 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.

2.4 Choi Response Criteria

The Choi criteria were initially proposed for assessment of GIST tumors on imatinib, a tyrosine kinase receptor inhibitor [12]. 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 [13]. The Choi criteria focus 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; these cannot be applied to MRI, and there is lack of sufficient validation in other tumors.

2.5 EORTC

The European Organisation for Research and Treatment of Cancer (EORTC) criteria have 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 def-

Table 2 Summary of immune-related RECIST 1.1

Method of assessment of lesion	The single longest diameter is measured except for nodal lesion where shortest diameter is considered for assessment
Total tumor burden evaluation	Sum of single longest diameters of all target lesions is measured and sum of shortest diameters of nodal lesions
New target lesions	If the new lesions fulfill the criteria of target lesion assessment, the single longest diameter is determined and incorporated into 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
Target lesion criteria	Target lesions should measure at least 10 × 10 mm, and nodal lesions must measure at least 15 mm in shortest diameter. A maximum of five target lesions could be selected. No more than two 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)
Complete response	irRECIST 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	irRECIST 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	irRECIST requires a total increase of tumor burden of at least 25% from the smallest reported tumor burden (nadir). However, irRECIST advice against 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	If percentage change shows an increase less than 25% from 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	Requires further testing to ensure reproducibility and accuracy of unidimensional assessment for capturing immune-related antitumor effect

inition of [18F]-FDG tumor response [14, 15]. The criteria follow 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 the 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, a 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 [14, 15].

2.6 Response Assessment in Neuro-Oncology (RANO) Criteria

The Response Assessment in Neuro-Oncology (RANO) criteria was proposed to overcome the significant limitations in the McDonald criteria for response assessment in high-grade gliomas. The McDonald criteria didn't take into account,

for example, pseudoprogression, pseudoresponse observed with antiangiogenic agents, and the inability to capture recurrence in the non-enhancing component of the lesion, due to using only the contrast-enhancing component of the tumor in it [15]. Similar to the McDonald criteria, the RANO criteria uses two-dimensional tumor measurements; however, the RANO criteria also accounts for changes in the non-enhancing T2/FLAIR signal abnormality. Measurable disease is defined 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. RANO criteria addressed pseudoprogression and pseudoresponse. The RANO criteria for high-grade glioma are summarized in Table 3 [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, and PR or the lesions became nonmeasurable; if the latter, the scan that showed "preliminary PD" is noted as "pseudoprogression" [17]. 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, while their non-enhancing component shows progression [17].

2.6.1 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 4) [16].

2.7 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 analyze the size and the metabolic activity of the tumor during the course

Table 3 RANO criteria for response assessment in high-grade gliomas

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

Table 4 RANO-BM criteria for response assessment in brain metastases

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 does 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

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 by Tirkes et al. (Table 5) [19].

2.8 PERCIST Criteria

While a range of factors have been linked with FDG uptake, there appears to be a considerably strong association between FDG uptake and quantity of cancer cells in a substantial number of studies [20, 21]. Additionally, 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 [22]. Thus, metabolic response may enhance the morphologic criteria. Therefore, the Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST 1.0) were proposed and are based mainly on FDG uptake to evaluate tumor response to refine and validate quantitative approaches to monitoring PET [23]. 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 [23]. 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 [23]. PERCIST defines four metabolic response categories [23]. In brief, according to these criteria, complete metabolic 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 [23]. Of note, the lesion at follow-up may be a different lesion than previously measured since the most active lesion needs to be followed. For classification as stable metabolic disease, an increase or decrease in SUL peak of less than 30% is required [23]. Progressive metabolic disease is defined as an increase (>30%) in SUL peak or the appearance of a new metabolically active lesion [23].

3 Immunotherapy Imaging Response Criteria

The emerging use of immunotherapeutic agents has led to the appearance of new treatment response patterns, and conventional response evaluation criteria might not be sufficient in evaluating immunotherapy response. One of the main differences in tumor response to immunotherapy in

Table 5 Cheson response criteria definitions

Table response definitions for clinical trials				
Response	Definition	Nodal masses	Spleen, liver	Bone marrow
CR	Disappearance of all evidence of 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 six 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 the 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

comparison to conventional therapies is a longer delay time for suitable response [24]. Another major response difference associated with immunotherapy is the enlargement of preexisting lesions and development of new lesions during the initial phase of treatment, which would necessitate classification as progressive disease (PD) with conventional criteria [24]. However, in patients on immunotherapy, therapeutic response can be observed in later follow-up scans after initial enlargement and emerging of new lesions. The initial increase in tumor burden or development of new lesions during the initial phase of treatment with immunotherapies could be due to transient flare-up and explained on a histological basis as either tumor growth until development of adequate immune response or transient immune cell infiltrate [24]. Thus, a well-tailored set of criteria to capture accurate and exact response to this new line of therapeutic agents is needed. To this end, immune-related Response Criteria (irRC), immune-related Response Evaluation Criteria in

Solid Tumors (irRECIST), immune RECIST (iRECIST), and immunotherapy Response Assessment in Neuro-Oncology (iRANO) were developed. Since their inception, immune-related evaluation criteria have been used in several clinical trials in patients receiving immunotherapies and have potentially representing improvement over conventional criteria for assessment of treatment response; however, they have their own challenges [2, 4, 25, 26]. While these criteria are the mainstay in the early-phase clinical trials, they have yet to be implemented for use in phase III trials; therefore, further prospective robust validation is warranted. Table 6 shows comparison of irRC, irRECIST, and iRECIST.

3.1 Immune-Related Response Criteria

Arising from the heightened awareness by the national and international community as to the unique radiographic response patterns seen with vaccines and immunotherapeutics, modifications were made to the WHO and RECIST criteria in 2004 and 200. In 2009, the immune-related Response Criteria (irRC) published by Wolchok et al. [4] were based on observed patterns in treatment response from phase II clinical trials in advanced melanoma patients who were receiving ipilimumab in 2009 [4]. In this study [4], four patterns of treatment responses were recognized, and two of them were captured with conventional response criteria: (1) a decrease in the size of the lesion and without new tumors and (2) stable disease after completion of treatment; the other two response patterns were new and involve (3) a delay in tumor response after an initial increase in total tumor burden and (4) a decrease in total tumor burden during or after the emerging of new lesion at time points later than week 12.

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 compared to the baseline scan [4]. The irRC was derived from WHO criteria, and therefore, the thresholds of response remain similar. However, the irRC response categories have been modified from those of 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 ten visceral lesions and five cutaneous index lesions). At every time point, the index lesions and any new measurable lesions are added together to accu-

Table 6 Features of immune response criteria

	irRC	irRECIST	iRECIST
Model based on	WHO criteria	IrRC and RECIST 1.1	RECIST 1.1
Method of measurement	Bidimensional	Unidimensional	Unidimensional
Definition of measurable disease	Selection of five lesions (≥5 × 5 mm) per organ (up to ten visceral and five cutaneous)	Selection of maximum five lesions (two per organ) (≥10 mm in diameter, ≥15 mm for nodal lesions)	Selection of maximum five lesions (two per organ) (≥10 mm in diameter, ≥15 mm for nodal lesions)
Progressive disease definition	25% increase from the nadir	20% increase from the nadir	20% increase from the nadir; results in iUPD; confirmation is necessary for iCPD
New lesion	New lesion does not define progression; the measurements of the new lesion are included in the sum of the measurements and added to total tumor burden at follow-up	New lesion are included in the sum of target lesions to define total tumor burden at follow-up	New lesion does not indicate progression; the measurements of the new lesion are not included in the tumor burden
Confirmation	≥4 weeks later	≥4 weeks later	≥4 weeks later no longer than 8 weeks

rately measure the total tumor burden (TTB) [(TTB = $SPD_{index\ lesions}$ + $SPD_{new,\ measurable\ lesions}$)]. This is a major difference from the WHO criteria which considers all new measurable lesions as progressive disease [5, 7]. 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 7.

The irRC does not mention the use of specific imaging modalities in 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 the cell proliferation or cell death is being investigated [16]. 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]. While potentially an advancement over traditional criteria for immunotherapy, the irRC may still not evaluate or completely characterize all relevant patterns of clinical activity. For example, one drawback for the irRC is that the term "irSD" represents both for cases of minimal change in tumor burden in time and for large increases in tumor burden followed by a reduction to baseline levels [4].

3.2 Immune-Related RECIST Criteria

The newly proposed irRECIST was developed based on irRC to evaluate tumor burden in patients receiving immunotherapy [2, 24]. The irRECIST adjusted the approach of unidimensional measurement and the number of lesions according to RECIST 1.1 while adding the important new features such as approval of PD and inclusion of new lesion measurements to assess immunotherapy treatment responses (Table 1) [2, 24]. In comparison to the bidimen-

Table 7 Summary of immune-related response criteria (irRC)

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 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 contributes to the evaluation of total tumor burden
New non-target 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. This includes CT, MRI, and PET-CT
Target lesions criteria	Target lesions should measure at least 5 × 5 mm. A maximum of five cutaneous lesions and ten 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

(continued)

Table 7 (continued)

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 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 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

sional method used by irRC, unidimensional measurement is more reproducible, demonstrates fewer variability, and results in lower misclassification rates for treatment response evaluation in clinical trials [2, 24]. The irRECIST is simple and practical and provides response evaluations that can be easily compared and implemented to the results from other studies applying RECIST [2, 24].

3.3 Immune RECIST Criteria

In 2017, immune RECIST (iRECIST) was proposed by the RECIST group to assess patients treated with immunotherapy [25]. iRECIST is based on RECIST 1.1, and the response categories (PD, SD, PR, CR) are assigned a prefix of "i" to indicate "immune" *(*i.e., immune complete response (iCR)) [25]. The continued use of RECIST 1.1. is suggested to approach tumor lesions and measurements; but new lesions are evaluated and subclassified as new target and new nontarget lesions [25]. The principles applied to determine tumor response are almost unchanged from RECIST 1.1 [25]. However, iRECIST defines immune unconfirmed progression (iUPD) which requires confirmation, and assessment of iUPD will be made if there is more than 20% increase in tumor burden or appearance of new target or nontarget lesions [24, 25]. Confirmation should be done by observing either a further increase of at least 5 mm of target tumor burden or new target lesion or any increase in nontarget disease [24, 25]. If no change is determined, the response is classified as iUPD. This method allows identification of atypical responses such as delayed treatment responses seen after pseudoprogression (Table 8) [24, 25].

3.4 Immunotherapy Response Assessment for Neuro-Oncology Criteria

Immunotherapy RANO (iRANO) criteria were presented as an update to RANO criteria to evaluate patients with neuro-oncological malignancies undergoing immunotherapy [26]. During the initial phase of immunotherapy treatment, the size of the tumor might increase, and/or new inflammatory lesions appear. These temporary changes typically stabilize or subside, but they are generally difficult to differentiate from PD [27]. This PD resembling event is called pseudoprogression (PsP) [27]. To overcome this challenge, iRANO was proposed (put table). In brief, the iRANO follows the same guidelines as the RANO criteria (Table 9). 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 are recommended (Table 10). 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. Promisingly, a recent study by our group demonstrated that radiomics can discriminate between patients who have PsP and true tumor progression with high sensitivity (97%), specificity (79%), and accuracy (95%) in patients with glioblastoma [28].

Table 8 iRECIST response criteria

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 one 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

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 are summarized in Table 11 [26].

4 Future Directions for Immune Therapy Imaging Assessment

Although irRECIST, irRC, and iRECIST represent an improvement over the conventional assessment criteria to evaluate tumor response in immunotherapy, there remain limitations and challenges, and further refinements are warranted. Therefore, RECIST is still a highly validated and reproducible tool, and majority of clinical trials continue to perform RECIST 1.1 for evaluation of treatment response. Plans for

Table 9 Summary of immune therapy Response Assessment in Neuro-Oncology (iRANO)

Method of assessment of lesion	Bidimensional assessment of the longest perpendicular diameters of all enhancing lesions
Total tumor burden evaluation	Sum of product of 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 three more months taking in reference the time point at which progression was initially reported
Target lesion criteria	Target lesions should measure at least 10 × 10 mm. A maximum of five target lesions could be selected
Complete response	Requires 100% decrease in tumor burden including total remission of all enhancing and non-enhancing 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 non-enhancing 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 product of bi-perpendicular diameters of T2FLAIR lesions. With no new lesions, no clinical decline and stable or decreased dose of steroids

(continued)

Table 9 (continued)

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

improving imaging response criteria include volumetric (3D) imaging, dynamic contrast imaging, and functional (molecular) imaging. Despite these aforementioned tremendous efforts to improve the radiological criteria and guidelines in tumor response evaluation, there still lie challenges to capture the precise volume of the tumor due to a variety of elements such as its shape irregularity. In addition to that, conventional imaging failed to describe local tumor heterogeneity, as well as molecular and biological com-

Table 10 iRANO criteria for high-grade glioma, low-grade glioma, and brain metastases

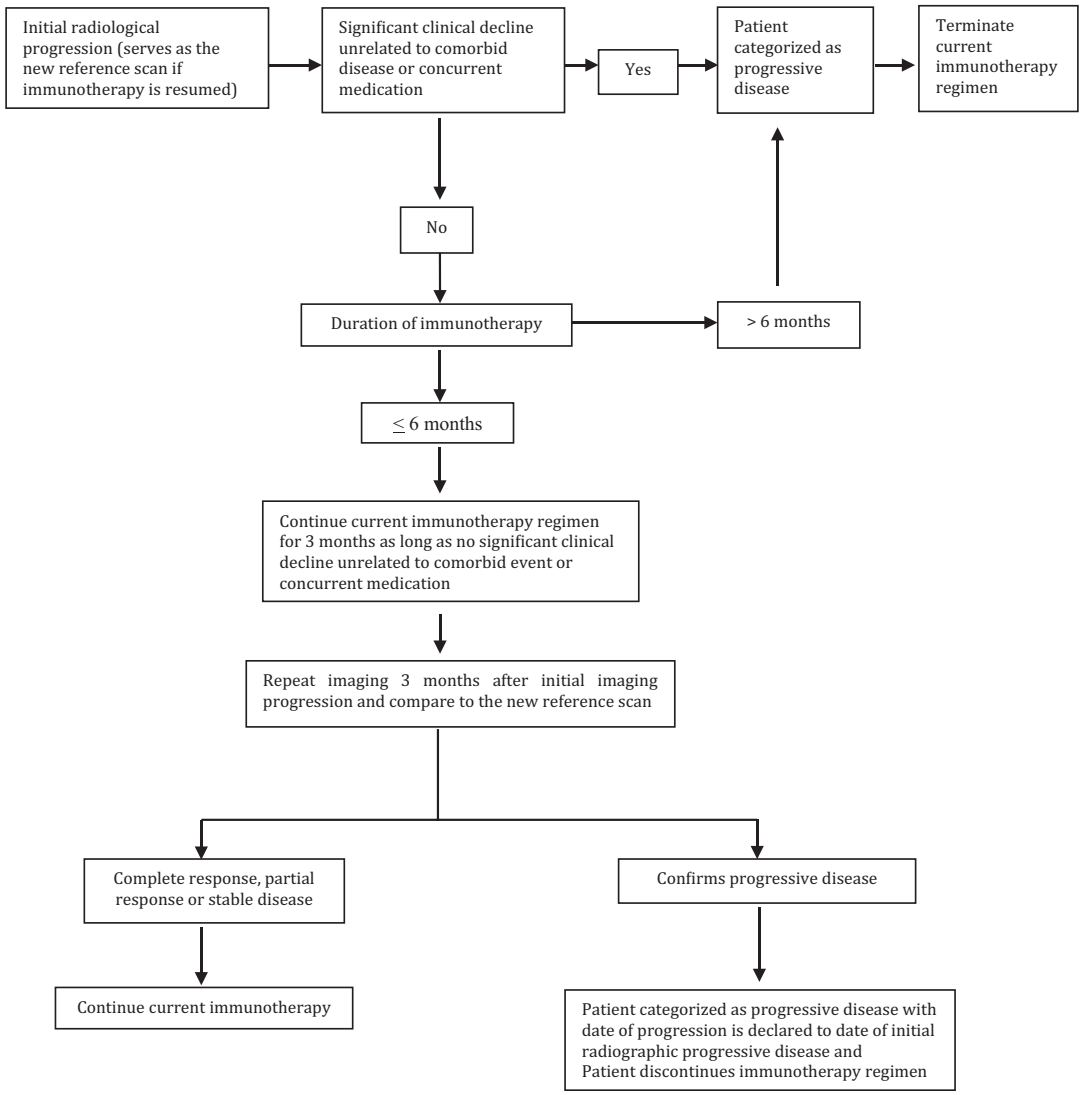

Table 11 Summary of immune therapy response assessment in brain metastases (iRANO-BM)

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

plexity of the tumor. Even with the obvious advancement in the quality of MR and CT imaging technologies, reporting is still subjective, descriptive, and nonquantitative. Additionally, despite immunotherapy have revolutionized the treatment of several malignancies, only a subset of patient derived clinical benefit as the absence of predictive biomarkers. As a promising rapidly evolving field, radiomics has potential to overcome these challenges [29]. Radiomics is a method that extracts large amount of imaging features from medical images [29]. As an extraordinary innovation in computational imaging, radiomics has led to providing significant information for personalized therapy such as tumor biology [30], genomics [31], spatial heterogeneity [31], and immune infiltration [32]. Also, radiomics has been demonstrated to predict immunotherapy response multiple cancers, including non-small cell lung cancer [33, 34], melanoma [34, 35], and advanced solid tumors [32]. These studies highlight that radiomics can potentially play a significant role in the clinical setting as an imaging biomarker to predict immunotherapy response a priori. Radiomics have many advantages; it is noninvasive, and features are extracted from standard medical images, making it ideal for clinical implementation. As a conclusion, radiology will continue to adjust the new tumor response patterns observed with the current and future immunotherapeutic agents. With the advent of molecular medicine and radiomics in the era of personalized medicine, the essential aim of research is to accommodate treatments to both the specific type of cancer and the patient.

5 Immune-Related Adverse Events

Immune-related adverse events (irAEs) are a unique spectrum of adverse effects of immunotherapy that resembles autoimmune responses. irAEs affect almost every organ system and are usually observed in the skin, gastrointestinal tract, lung, endocrine, and musculoskeletal system [36]. 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 [18]. Immunotherapy can generally continue in the presence of mild irAEs with close observation. However, moderate to severe irAEs may be related with severe declines in organ function and quality of life, and fatal outcomes have been reported; thus, these toxicities need early detection and proper management. 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 [36]. However, success in outcome lies heavily on correctly identifying and interpreting these complications.

In general, irAEs most experienced across the spectrum of the current immunotherapeutic agents may include but not limited to colitis, diarrhea, hepatitis, pneumonitis, thyroiditis, myocarditis, pericarditis, temporal arteritis, conjunctivitis, sarcoid-like reaction such as lymphocytic vasculitis, organizing pneumonia, fasciitis, hypophysitis, and thyroiditis [36]. A recent study by our group demonstrated that specific radiomic imaging features were able to predict those patients that will subsequently develop pneumonitis (Fig. 2) [37]. This study highlights

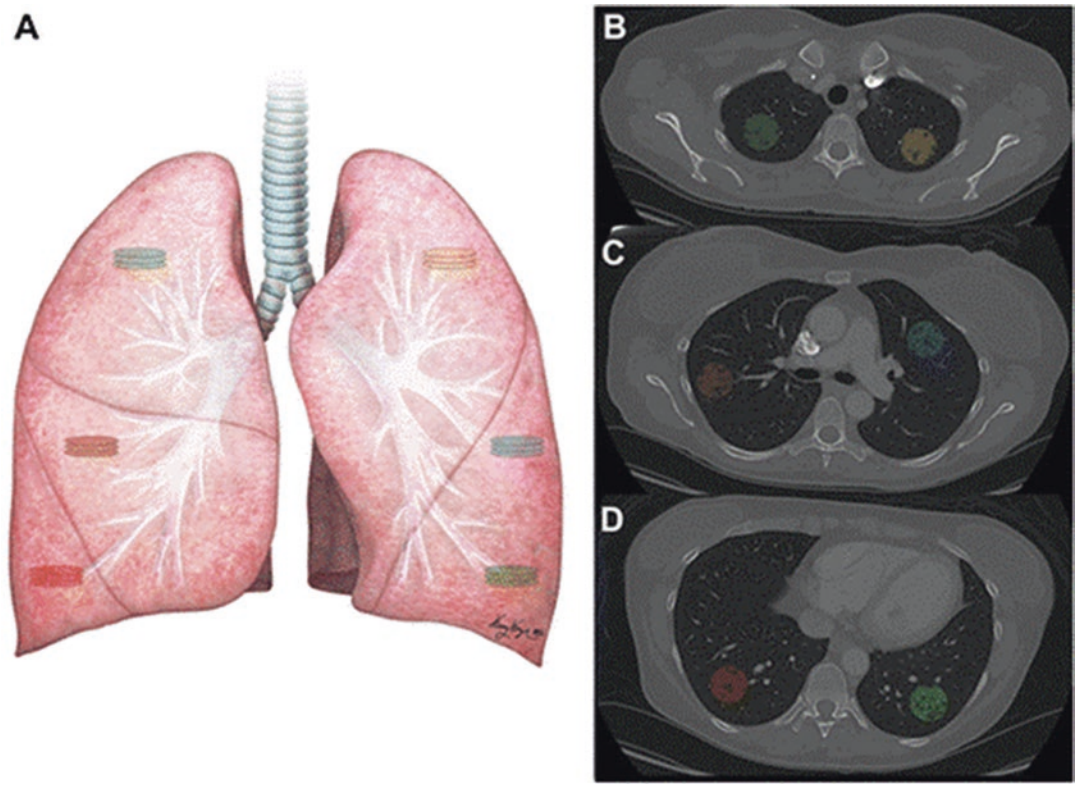

Fig. 2 (**a**) An illustration of the outlined regions of interest (ROIs) in the lungs. An ROI containing three consecutive slices, taken in each lobe in the right lung, and ROIs outlined in the left lung correspond to the same level as the right lung ROIs. Postcontrast lung CT images depicting the segmented 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

the ability of imaging to identify those patients that might be most susceptible to irAE before the irAE even occurs [38].

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