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Aung Naing
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Aung Naing • Joud Hajjar
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Editors
Aung Naing
MD Anderson Cancer Center
University of Texas
Houston, TX, USA

Joud Hajjar
Baylor College of Medicine-Texas
Children's Hospital
Houston, TX, USA

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Chapter 1
Overview of Basic Immunology for Clinical Investigators

Bettzy Stephen and Joud Hajjar

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

Keywords Adaptive • CTLA-4 • Immune checkpoints • Tumor immunotherapy • Innate • PD-1 • T cells

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

B. Stephen
The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
e-mail: BAStephen@mdanderson.org

J. Hajjar (✉)
Baylor College of Medicine, Department of Pediatrics, Houston, TX 77030, USA
e-mail: joud.hajjar@bcm.edu

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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 systems are distinct but interactive components of the human immune system that collectively contribute to the defense operations against foreign proteins [4]. In this chapter, we 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.

1.1 Innate Immune System

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

1.1.1 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 give rise to the cells of the innate immune system, leukocytes (neutrophils, monocytes, basophils, and eosinophils), mast cells, DCs, erythrocytes, and the megakaryocytes.

1.1.1.1 Leukocytes

The primary function of the leukocytes is to protect the body against invading microorganisms. However, microenvironmental factors at the site of inflammation produces 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, upregulates 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) versus a protumorigenic (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 towards N2 phenotype [13]. These neutrophils locally produce neutrophil elastase (ELA2) [22], oncostatin M [23], and alarmins S100A8/9 [24] that promotes 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]. Neutropohils thus assume multiple roles in development and

progression of tumor cells [26]. However, under certain conditions such as TGF-β blockade, TANs assume an N1 phenotype, which are more cytotoxic due to enhanced expression of immune-activating cytokines and chemokines, and lower levels of arginase [13]. N1 TANs also communicate with DCs to trigger an adaptive immune response [27]. 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) [28]. This phenotype has the potential to inhibit progression of the tumor, indicating the possibility of immunostimulation 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 [29]. 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 [30–33], where they differentiate into tissue-resident macrophages [34]. 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 [35]. The cytokine profile of the TME plays a central role in the phenotype orientation of the differentiating macrophages [36]. In general, M-CSF, TGF-β, and IL-10, the principal cytokines present in the TME strongly inhibits IL-12 production and NF-κB activation in TAMs [37]. This skews the differentiation of monocytes to macrophages M2 phenotype, characterized by IL-12^{low} IL-10^{high} [30, 38]. 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 by suppressing adaptive immunity through production of prostaglandins, IL-4, IL-6, IL-10, TGF-β, indoleamine dioxygenase (IDO) metabolites, and induction of T regulatory (Treg) cells [33, 38]. 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-γ (IFN-γ), where TAMs are educated to assume the more cytotoxic, antigen presenting, IL-12^{high} IL-10^{low} M1 phenotype [33]. They kill microbes and tumor cells by producing copious amounts of pro-inflammatory cytokines such as IL-12 and IL-23, toxic intermediates—nitric oxide, reactive oxygen intermediates (ROI), and TNF [30, 33]. The cytokines also initiate T-helper 1 (Th1) adaptive immunity. Though high macrophage content is often correlated with poor patient prognosis in breast [39, 40], bladder [41], endometrial [42], and cervical cancers [43], TAMs in tumor tissue confer survival advantage to patients with prostate cancer [44] and colon cancer [45]. Pharmacological skewing of macrophage polarization from M2 to M1 phenotype is likely to provide therapeutic benefit to cancer patients.

Eosinophils

Eosinophils are derived from the CMP cells, and they constitute less than 5% of circulating leukocytes [2, 46]. Traditionally, eosinophils are associated with host defense against large, multicellular parasitic helminths and fungi with allergic conditions [47]. Eosinophils express a number of receptors such as chemokine receptors, cytokine receptors, immunoglobulin (Ig) receptors, Toll-like pattern recognition receptors, and histamine receptors [48]. 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), CXCL5, and lipid mediators (platelet-activating factor and leukotriene C4) from the large, highly cytotoxic, secretory cytoplasmic granules at the sites of allergic inflammation [48, 49].

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 [50], oral squamous cell carcinoma (SCC) [51], laryngeal, and bladder carcinoma [52]. 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)] [53, 54], whereby they function as APCs and initiate antigen-specific immune responses by the T cells [55]. Kinetic studies have demonstrated that chemotactic factors such as eotaxins and damage-associated molecular patterns (DAMPs), high mobility group box 1 (HMGB1) released by necrotic tumor cells, preferentially induces eosinophilic migration to tumors [56, 57] prior to infiltration by CD8+ T cells [58]. Tumor-associated tissue eosinophils in its active form release chemokines such as CCL5, CXCL9, and CXCL10 that attracts CD8+ T cells to the tumor [59]. 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 [58].

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 leukocytes and were therefore considered redundant to mast cells functionally till about 15 years ago [60]. Basophils travel to the sites of allergic inflammation and microbial assault in response to cytokines and chemokines released locally [60]. 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 [61]. IL-4 and IL-13, released within an hour of stimulation, serve as chemoattractants for other immune cells and direct the differentiation of naïve T cells towards Th2 phenotype resulting in Th2 (allergic)-type immune responses in an IgE-dependent and IgE-independent manner [62, 63]. 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 [63].

Though the role of basophils in tumorigenesis has not been clearly understood, it is believed that basophils promote neoplastic angiogenesis [64]. Basophils express Angiopoietin-1 and Angiopoietin-2 messenger RNAs in the cytoplasmic vacuoles, and VEGFR-2 and Tie-1 receptors on the cell surface. And, activation of basophils releases pro-angiogenic factors VEGF-A and VEGF-B through a cross talk between the basophils and the mast cells, contributing to neoplastic angiogenesis. Further, the correlation between basophils in the tumor-draining lymph node with Th2 inflammation in patients with pancreatic ductal adenocarcinomas and the emergence of basophils as an independent prognostic factor of poor survival after surgery suggests a role for basophils in tumor development and disease recurrence [65].

1.1.1.2 Mast Cells

Mast cells are tissue-based inflammatory cells of hematopoietic origin [66]. 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 still retaining a limited capacity to differentiate into myeloid cells [67]. The pre-BMPs circulate in the blood and reach the peripheral tissue, where they get differentiated into basophils and mast cells in the presence of mutually exclusive transcription factors, C/EBPα and MITF, respectively [67]. 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 [68, 69]. 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” [70]. Mast cells express several surface receptors including KIT IgG receptor and Toll-like receptors (TLRs) [70]. 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 [71]. 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 [71], 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 [68, 70], and their ability to store TNF-α in a preformed state allows mast cells to orchestrate the first response to invading pathogens [66]. 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 [66].

Increased number 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 [72], lip cancer, [73], and diffuse large B cell lymphoma [74]. 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 [73, 75, 76]. On the contrary, mast cell infiltration has been associated with good prognosis in breast [77], ovarian [78], lung [79], and colorectal cancers [80]. This is due to the 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 [78, 81, 82]. It is therefore evident that the density and location of mast cells within the tumor samples and the cross talk between mast cells and stromal cells are better predictors of patient survival as they modulate the immune response [1].

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 [83]. In the steady state, they originate from the monocyte and dendritic cell progenitor (MDP) derived from the CMP cells in the bone marrow [84]. The MDPs give rise to monocytes and common DC progenitors (CDPs) in the bone marrow [85]. 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 [86]. Plasmacytoid DCs is an example of pre-DCs found in blood, thymus, bone marrow, and secondary lymphoid tissue, which produce type I IFN-α in response to viral exposure. The cDCs are broadly classified into migratory DCs and lymphoid tissue-resident DCs. The migratory DCs (Langerhans cells and dermal DCs) are immature DCs present in the peripheral tissue, which are very effective in capturing antigens. They sample the environment using several receptors including the TLRs and (NOD)-like receptors (NLRs). On encountering a pathogen, endocytosis is upregulated transiently to facilitate 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, 87]. Shortly thereafter, functional maturation of DCs ensues triggering the antigen-presenting machinery, which is the critical link between innate and adaptive immunity [88]. Endocytosis by the DCs decreases and expression of MHC-I, MHC-II, and co-stimulatory molecules increases at the surface possibly due to cessation of ubiquitination of MHC class II molecules [87]. 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 [87]. 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 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 [87]. They present the antigens in the lymphoid organ to the T cells [86]. 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) [86].

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 [83]. Therefore in the absence of infections, though DCs continuously present self-antigens and nonpathogenic 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, TGF-β, and IDO inhibit DC and T-cell function 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 [83].

1.1.1.4 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 [89]. 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 [90]. 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 [89].

The effector function of NK cells is triggered by the engagement of cell surface-activating receptors including the potent NKG2D receptor, killer cell Ig-like receptors (KIR-S), TLR, and NLR that identifies non-self-infected cells and self-cells under stress by recognizing pathogen-associated molecular patterns (PAMPs) [91]. However, activation of the NK cells is dependent on cellular cross talk 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 [92, 93]. 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 [94]. Activated NK cells induce cytotoxicity and/or promote cytokine production [94]. 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 [95]. Tumor cells however evolve and evade destruction by NK cells [95]. A common escape mechanism used by tumor cells is the proteolytic shedding of NKG2D ligands [96]. 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 [97, 98]. 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 [99]. NK cells express other activating receptors such as CD16, Fc-γ receptor IIIa (FCGR3A), which binds to the Fc region of Ig [100]. 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 [101]. 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 [102]. The immune memory function of NK cells lasts for several months after the initial exposure, is antigen specific, and transferable to naïve animals [102]. 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 [103]

1.2 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 germ line-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 [29]. There are two classes of adaptive immune response: the humoral and cell mediated. The humoral immune response is mediated by the B lymphocytes against antigens present outside the cells, in the blood and body fluids. On the other hand, the cell-mediated immune response is mediated by the T lymphocytes against intracellular pathogens presented as small antigenic determinants on MHC molecules.

1.2.1 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].

1.2.1.1 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 fail to recognize antigen-MHC complexes or distinguish self-antigens do not mature [104]. As the lymphoid progenitor cells migrate through the cortex, they undergo an education program based on the constant interaction with the thymic epithelial cells [105]. The lymphoid progenitor cells does not express TCR, or CD4 or CD8 co-receptors and are therefore called CD4/CD8 double-negative (DN) lymphocytes (DN1) [106]. As they move through the cortex from the corticomedullary junction to the capsule, the lymphoid progenitor cells lose their ability to form B cells or NK cells and become committed T-cell precursors (DN2) [107]. 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) [104]. 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 [104, 108]. 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 [105, 109].

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 [29]. 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, while those that express TCRs that bind with MHC Class II molecule mature into a single positive CD4 mature T cell. The 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 and 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 [110]. This results in clonal expansion and differentiation of naïve T cells in the lymph nodes into helper T cells (CD4 T cells) and cytotoxic effector T cells (CD8 T cells), which then migrate back to the tumor and destroys the tumor cell. Depending on the cytokine milieu and the transcription factors in the tumor environment, CD4 T cells differentiate into several subtypes that includes Th1 [111], T-helper 2 (Th2) [112], T-helper 17 (Th17) [113], induced Tregs (iTregs) [114], follicular helper T cell (Tfh) [115], and T-helper 9 (Th9) [116]. The helper T cells secrete cytokines and chemokines that regulate the immune response. Th1 cells favor cellular 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 T cells activated by antigen presentation on the MHC class I molecule or through CD4 helper T cells are directly cytotoxic. Besides, some of the activated T cells and B cells differentiate into memory cells that are responsible for the long-lasting immunological memory [117]. Subsequent exposure to the same antigen results in more rapid and robust immune response.

Regulation of T-cell response is a delicate balance between co-stimulatory and inhibitory signals that serve as immune checkpoints. Co-stimulatory receptors include CD28, ICOS, 41BB, and OX40, while CTLA-4, Tim-3, and programmed cell death 1 (PD-1) are co-inhibitory [118]. CD28 is 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 [119]. Activated T cells simultaneously express CTLA-4 and PD-1 on their surface as immune checkpoints [120, 121]. CROITLA-4 is a CD28 homologue with a higher affinity to bind with B7 molecules. On engagement, CTLA-4 blocks CD28 co-stimulation and abrogates T-cell activity and cytokine production. PD-1 is a CD28 family member and has two ligands, PD-L1 and PD-L2. PD-L1 is expressed on many cells including the tumor cells, activated B and T cells in response to IFN-γ produced by the activated T cells, while PD-L2 is expressed on macrophages and DCs [122]. 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 [123]. Under normal conditions, immune checkpoints play an important role in maintenance of peripheral tolerance and regulation of the amplitude and duration of T-cell responses [124]. There are other co-signaling receptors of the TNF receptor superfamily including 4-1BB [125], OX40 [126], and GITR [127] that synergize with TCR signaling to promote cytokine production and T-cell survival. The stimulatory effect of T cells is counterbalanced by a suppressive mechanism in order to maintain immune homeostasis. A chief contributor to this effect are the regulatory

T cells (Tregs), which are specialized T cells that suppress the function of other T cells [128]. They are classified as Natural Tregs and Inducible Tregs. Natural Tregs characterized by the expression of forkhead box P3 (FOXP3) are positively selected thymocytes with relatively high affinity for self-antigens presented on MHC class II molecules. Inducible Tregs differentiate from naïve T cells in the periphery and are characterized by the expression of immunosuppressive cytokines such as IL10 and TGF-β [114]. Decreasing the activity of Treg cells enhances both innate and adaptive immune response, which can be utilized to treat cancer [129].

1.2.1.2 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 [130]. 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) [131]. 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. Whereas, immature B cells that express a non-auto-reactive BCR with low basal BCR signaling insufficient to downregulate RAG expression and immature B cells that are strongly self-reactive are negatively selected for elimination by apoptosis (clonal deletion). Alternatively, these cells may be inactivated (anergy) or may undergo receptor editing, a process by which secondary rearrangement of L leads to formation of new BCRs that are not self-reactive, which allows for subsequent positive selection of these cells for further development [132].

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 reactivitity to self-antigens that are expressed only in the peripheral tissue [133]. And, 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 differentiate into either follicular (FO) B cell 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 [133, 134]. 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 lymphoid organs and are activated by foreign proteins in a T-cell-dependent manner [135]. 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 [136]. 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 pairs 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 [137].

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 [136]. 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 are 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 [137]. 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 [138]. 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, created by fusing murine variable region with human constant region [139]. Later, humanized mAbs that are 85–90% human, where only the CDRs are murine, were developed [140]. Currently, fully human mAbs produced by phage display are available [141]. 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 [142]. 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).

1.3 The Immune System in Action!

1.3.1 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 complex and the immune cells present in the TME include macrophages, DCs, NK cells,

mast cells, naïve lymphocytes, B cells, cytotoxic T cells, helper T cells, memory cells, and Tregs [143].

The human immune system exhibits a dual role in cancer. Though the primary function of the immune system is to suppress tumor growth, they also shape immunogenicity and promote tumor progression through a dynamic process called cancer immunoediting [144]. 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 [145]. 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 IFN-γ, 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. Innate and adaptive immune cells have the capacity to completely eliminate the tumor cells and halt the immunoediting process.

During the equilibrium phase, continuous interaction between immune cells and tumor cells that have escaped elimination phase prevents expansion of the tumor cells. This continuous immune pressure however selects or promotes the formation of new variants of tumor cells with reduced immunogenicity that escapes recognition by immune system [145]. This is the longest phase in the immunoediting process, when the tumor cell variants reside in a latent form before escaping eventually [146].

During the escape phase, tumor cells adopt several mechanisms to evade immunosurveillance [147]. 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 [148]. 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 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 [149]. In addition, myeloid-derived suppressor cells (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 [150, 151]. 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.

1.4 Cancer Immunotherapy

Immunotherapy has revolutionized cancer treatment due to its ability to produce durable responses in patients with advanced cancer. Though several immunotherapeutics including IL-2, IFN-α, and Sipuleucel-T vaccine were investigated, only small improvements in efficacy were observed. Several mAbs have also been used in the treatment of cancer [152] 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-TGF-β antibody) [153].

But, a deeper understanding of the mechanism of immune responses in TME is what led to major breakthrough in cancer immunotherapy, the discovery of immune checkpoint CTLA4, and strategies to unleash the immune harnessing power of T cells to combat cancer [154]. On activation, T cells express CTLA-4, which on binding with B7 molecules blocks co-stimulation of T cells resulting in immune suppression. Tumor cells frequently hijack these immune checkpoints to promote immune suppression and immune evasion. This observation led to the development of ipilimumab, a CTLA-4 inhibitor, which produced durable responses in about 20% of patients and considerable improvement in the overall survival (OS) of patients with metastatic melanoma, resulting in FDA-approval of the drug in 2011 [155]. The dramatic response with ipilimumab laid the foundation for exploration of other T-cell inhibitory pathways. PD-1 is another immune checkpoint, which on ligation binding with PD-L1/PD-L2 produces immune suppression. In response to immune attack, tumor cells overexpress PD-L1 and PD-L2 resulting in immune suppression. This favors immune evasion and tumor progression. Based on strong preclinical evidence, blockade of PD-1/PD-L1 pathway by mAbs produced durable responses in several tumor types [156–160]. As a result, FDA granted accelerated approval of the following checkpoint inhibitors besides ipilimumab: nivolumab for the treatment of patients with unresectable or metastatic melanoma, metastatic non-small cell lung cancer, RCC, classical Hodgkin's lymphoma, and recurrent or metastatic squamous cell carcinoma of the head and neck (HNSCC); pembrolizumab (PD-1 inhibitor) for metastatic non-small cell lung cancer, unresectable or metastatic melanoma, recurrent or metastatic HNSCC; and atezolizumab (PD-L1 inhibitor) for urothelial carcinoma bladder cancer and metastatic non-small cell lung cancer [161]. This offers proof of concept that checkpoint inhibition provides durable and meaningful response in a subset of patients with responsive tumors. Despite the success with checkpoint inhibitors (CTLA-4, PD-1/PD-L1 blockade), many patients are primarily resistant or develop resistance to treatment after an initial period of response [162]. Among several mechanistic approaches being investigated

in the clinic to overcome primary and secondary resistance to the immune checkpoint inhibitors, there is growing evidence that combination therapies are potentially synergistic and are far more effective than monotherapies to combat resistance mechanisms as tumors use multiple pathways to evade immune elimination [163]. Recently, FDA-approved nivolumab in combination with ipilimumab for the treatment of patients with *BRAF V600* wild-type, unresectable, or metastatic melanoma [161] IDO is another such immunosuppressive pathway exploited by tumor cells to evade immune surveillance [164]. Currently, four IDO inhibitors are under clinical development INCB024360 [165, 166], indoximod [167], IDO peptide vaccine [168], and NLG919 [169] .

Further, generating a robust therapeutic immune response requires not only the release of "brakes" on T cells, but also stepping on the "gas." T-cell co-stimulation through receptors, like OX40 or 4-1BB, provides a potent "go" signal that actively promotes the optimal "killer" CD8 T-cell responses [170]. Several ongoing clinical trials are investigating immune checkpoint therapies as single-agent or in combination with other immunotherapies, chemotherapy, targeted therapy, vaccines, or radiotherapy.

1.5 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, spare the patients who are less likely to benefit from avoidable toxicities and cost of treatment [171]. These biomarkers are applicable across tumor types that are responsive to the therapy. Some of the important predictive biomarkers are:

1.5.1 PD-L1 Expression

Early phase I trials suggests that cell surface expression of PD-L1 on tumor cells in pretreatment tissue samples could serve as biomarker of response to treatment with anti-PD-1/PD-L1 therapies. In a phase I study of MDX-1106, an anti-PD-1 inhibitor, in 39 patients with advanced cancers, tumor biopsies from 9 patients were analyzed for PD-L1 expression by immunohistochemistry (IHC) [156]. Objective response was observed in 3 of 4 patients (75%) with PD-L1-positive tumors, while none of the 5 patients with PD-L1-negative tumors had a response. Similar results were observed in another phase I study of BMS-936558 (nivolumab), an anti-PD1 therapy, in which pretreatment tumor tissue from 42 patients with advanced cancer was analyzed for PD-L1 expression by IHC [172]. Nine of 25 patients (36%) with

PD-L1-positive tumors had objective response, while none of the 17 patients with PD-L1-negative tumors had a response indicating the possibility of an association between PD-L1 expression on pretreatment samples and objective response. Recently, FDA-approved expression of PD-L1 by IHC using 22C3 pharmDx as a diagnostic test for selecting NSCLC patients for treatment with pembrolizumab [173]. 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 [174]. Surprisingly, response to treatment was observed even in patients with PD-L1-negative disease. In addition, the association between PD-L1 expression and response to therapy was discordant between tumor cells and 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 ($P = 0.079$). There is also marked heterogeneity in PD-L1 expression between samples from the primary and metastatic sites in the same individual [175]. Further, multiple immune assays use different PD-L1 antibody clones for IHC staining with different staining procedures and scoring patterns. As a result, there is lack of defined criteria to determine PD-L1-positive tumor. 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 [174, 176]. Constitutively expressed PD-L1 in the tumor tissue, for example, in the context of aberrant signaling in the PI3K pathway or molecular alterations as in Hodgkin's lymphoma is associated with poor prognosis [177].

1.5.2 Intratumoral T-Cell Infiltration

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, renal cell, colorectal and bladder carcinoma among other solid tumors [178, 179]. On the contrary, infiltration of the tumor tissue by Tregs is associated with poor survival in ovarian, breast cancer, and hepatocellular carcinoma [180–182]. Interestingly, mismatch repair-deficient tumors are not eliminated despite strong intratumoral infiltration by CD8+ T cells and Th1 cells due to strong expression of several immune checkpoint ligands such as PD-1, PD-L1, CTLA-4, LAG-3, and IDO by the TME, which made them responsive to checkpoint blockade [183]. As a result, mismatch repair status may be predictive of response to checkpoint inhibition. Further,

multiple immune markers including total T lymphocytes (CD3), T-cell effectors (CD8), their associated cytotoxic molecule (GZMB), 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 [184]. The type, density, and location of immune cells (collectively known as immune contexture) had prognostic value. 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. Further, presence of markers for Th1 polarization, cytotoxic, and memory cells were predictive of low recurrence rate. Similarly, flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) and tumor-infiltrating lymphocytes is used to evaluate the effect of therapy on low-frequency immune subsets such as Tregs, and MDSCs [185].

1.5.3 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 [184]. 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 tumor TME in TNM classification of tumors. On the contrary, immune contexture discussed above has better prognostic value. Therefore, immunoscore, a ratio of two lymphocyte populations, CD3/CD45RO, CD3/CD8, or CD8/CD45RO, in the CT and IM also has a strong prognostic value for DFS and OS [186]. 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.

1.5.4 Mutation Load and Molecular Alterations

Tumors with high mutational load such as melanoma, NSCLC, and HNSCC are more likely to respond to treatment with checkpoint inhibitors [187]. However, Snyder and colleagues described that in melanoma patients, high mutational load correlated to sustained response to CTLA-4 blockade, but not all patients with high mutational load responded to therapy [188]. Nevertheless, the presence of neoepitope signature peptides correlated strongly with OS in these patients. On the contrary, response to treatment with checkpoint inhibitors may not be seen in patients whose tumors have low mutational loads, e.g., pancreatic and prostate cancer. Also, molecular alterations in the PI3K pathway may promote tumor immune evasion

through constitutive expression of PD-L1 [189]. 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 [179].

1.5.5 Absolute Lymphocyte Count

In a compassionate use trial with ipilimumab in patients with advanced refractory melanoma, ALC $\geq$1000 μL^{-1} after two treatments with ipilimumab was significantly associated with clinical benefit and OS [190, 191]. Though absolute lymphocyte count (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^{-1} may be required for adequate activation of the immune system for patients to derive meaningful antitumor response with therapy.

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 [176]. 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 [156, 172]. Therefore, identification of a single immunologic biomarker may not be predictive of response [176]. This indicates a need to identify multifactorial biomarker panels that would help to determine the immunogenic nature of the tumor and predict response or resistance to treatment. For example, presence of intratumoral CD8+ T cells, expression of PD-L1 on tumor cells, and increased mutational load has been associated with greater likelihood of response to PD-1/PD-L1 checkpoint inhibition [171].

1.6 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 potential of the antitumor immune response by T cells has now emerged as a powerful new 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 molecular profiling will guide the future development of new immunotherapeutic strategies as well as the identification of potential biomarkers of clinical response.

References

1. Benito-Martin A, Di Giannatale A, Ceder S, Peinado H. The new deal: a potential role for secreted vesicles in innate immunity and tumor progression. Front Immunol. 2015;6:66.
2. Murphy K, Weaver C. Janeway's immunobiology. 9th ed. New York: Garland Science, Taylor & Francis Group, LLC; 2016.
3. Mellman I. Dendritic cells: master regulators of the immune response. Cancer Immunol Res. 2013;1:145–9.
4. Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol. 2010;125:S24–32.
5. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539–45.
6. Janeway Jr CA, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002;20:197–216.
7. Finn OJ. Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. Ann Oncol. 2012;23 Suppl 8:viii6–9.
8. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 2007;117:1175–83.
9. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883–99.
10. Fedeles BI, Freudenthal BD, Yau E, Singh V, Chang SC, Li DY, Delaney JC, Wilson SH, Essigmann JM. Intrinsic mutagenic properties of 5-chlorocytosine: a mechanistic connection between chronic inflammation and cancer. Proc Natl Acad Sci U S A. 2015;112:E4571–80.
11. Dvorak HF, Flier J, Frank H. Tumors—wounds that do not heal—similarities between tumor stroma generation and wound-healing. N Engl J Med. 1986;315:1650–9.
12. Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Nat Immunol. 2011;12:1035–44.
13. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. 2009;16:183–94.
14. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annu Rev Pathol. 2014;9:181–218.
15. Borregaard N. Neutrophils, from marrow to microbes. Immunity. 2010;33:657–70.
16. Kobayashi Y. Neutrophil infiltration and chemokines. Crit Rev Immunol. 2006;26:307–15.
17. Scapini P, Carletto A, Nardelli B, Calzetti F, Roschke V, Merigo F, Tamassia N, Pieropan S, Biasi D, Sbarbati A, Sozzani S, Bambara L, Cassatella MA. Proinflammatory mediators elicit secretion of the intracellular B-lymphocyte stimulator pool (BLyS) that is stored in activated neutrophils: implications for inflammatory diseases. Blood. 2005;105:830–7.
18. Theilgaard-Monch K, Knudsen S, Follin P, Borregaard N. The transcriptional activation program of human neutrophils in skin lesions supports their important role in wound healing. J Immunol. 2004;172:7684–93.
19. Fridlender ZG, Albelda SM. Tumor-associated neutrophils: friend or foe? Carcinogenesis. 2012;33:949–55.
20. Piccard H, Muschel RJ, Opdenakker G. On the dual roles and polarized phenotypes of neutrophils in tumor development and progression. Crit Rev Oncol Hematol. 2012;82:296–309.
21. Gregory AD, Houghton AM. Tumor-associated neutrophils: new targets for cancer therapy. Cancer Res. 2011;71:2411–6.

22. Houghton AM, Rzymkiewicz DM, Ji H, Gregory AD, Egea EE, Metz HE, Stolz DB, Land SR, Marconcini LA, Kliment CR, Jenkins KM, Beaulieu KA, Mouded M, Frank SJ, Wong KK, Shapiro SD. Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth. Nat Med. 2010;16:219–23.
23. Queen MM, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. Cancer Res. 2005;65:8896–904.
24. Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, Manova-Todorova K, Leversha M, Hogg N, Seshan VE, Norton L, Brogi E, Massague J. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 2012;150:165–78.
25. Shojaei F, Singh M, Thompson JD, Ferrara N. Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. Proc Natl Acad Sci U S A. 2008;105:2640–5.
26. Liang W, Ferrara N. The complex role of neutrophils in tumor angiogenesis and metastasis. Cancer Immunol Res. 2016;4:83–91.
27. van Gisbergen KPJM, Geijtenbeek TBH, van Kooyk Y. Close encounters of neutrophils and DCs. Trends Immunol. 2005;26:626–31.
28. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, Cassatella MA. The neutrophil as a cellular source of chemokines. Immunol Rev. 2000;177:195–203.
29. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol. 2010;125:S3–23.
30. Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev. 2006;25:315–22.
31. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med. 2001;193:727–40.
32. Duyndam MC, Hilhorst MC, Schluper HM, Verheul HM, van Diest PJ, Kraal G, Pinedo HM, Boven E. Vascular endothelial growth factor-165 overexpression stimulates angiogenesis and induces cyst formation and macrophage infiltration in human ovarian cancer xenografts. Am J Pathol. 2002;160:537–48.
33. Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur J Cancer. 2006;42:717–27.
34. Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. Cancer Lett. 2008;267:204–15.
35. Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol. 2009;86:1065–73.
36. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer. 2004;4:71–8.
37. Sica A, Saccani A, Bottazzi B, Polentarutti N, Vecchi A, van Damme J, Mantovani A. Autocrine production of IL-10 mediates defective IL-12 production and NF-kappa B activation in tumor-associated macrophages. J Immunol. 2000;164:762–7.
38. Mantovani A, Allavena P, Sica A. Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. Eur J Cancer. 2004;40:1660–7.
39. Tsutsui S, Yasuda K, Suzuki K, Tahara K, Higashi H, Era S. Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. Oncol Rep. 2005;14:425–31.
40. Zhang J, Yan Y, Yang Y, Wang L, Li M, Wang J, Liu X, Duan X, Wang J. High infiltration of tumor-associated macrophages influences poor prognosis in human gastric cancer patients, associates with the phenomenon of EMT. Medicine (Baltimore). 2016;95:e2636.
41. Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. Int J Urol. 2000;7:263–9.
42. Salvesen HB, Akslen LA. Significance of tumour-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumour angiogenesis and prognosis in endometrial carcinomas. Int J Cancer. 1999;84:538–43.

43. Fujimoto J, Sakaguchi H, Aoki I, Tamaya T. Clinical implications of expression of interleukin 8 related to angiogenesis in uterine cervical cancers. Cancer Res. 2000;60:2632–5.
44. Shimura S, Yang G, Ebara S, Wheeler TM, Frolov A, Thompson TC. Reduced infiltration of tumor-associated macrophages in human prostate cancer: association with cancer progression. Cancer Res. 2000;60:5857–61.
45. Forssell J, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. Clin Cancer Res. 2007;13:1472–9.
46. Fulkerson PC, Rothenberg ME. Targeting eosinophils in allergy, inflammation and beyond. Nat Rev Drug Discov. 2013;12:117–29.
47. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006;24:147–74.
48. Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. Immunol Rev. 2011;242:161–77.
49. Muniz VS, Weller PF, Neves JS. Eosinophil crystalloid granules: structure, function, and beyond. J Leukoc Biol. 2012;92:281–8.
50. Fernandez-Acenero MJ, Galindo-Gallego M, Sanz J, Aljama A. Prognostic influence of tumor-associated eosinophilic infiltrate in colorectal carcinoma. Cancer. 2000;88:1544–8.
51. Dorta RG, Landman G, Kowalski LP, Lauris JRP, Latorre MRDO, Oliveira DT. Tumour-associated tissue eosinophilia as a prognostic factor in oral squamous cell carcinomas. Histopathology. 2002;41:152–7.
52. Costello R, O'Callaghan T, Sebahoun G. [Eosinophils and antitumour response]. Rev Med Interne. 2005;26:479–84.
53. Ohkawara Y, Lim KG, Xing Z, Glibetic M, Nakano K, Dolovich J, Croitoru K, Weller PF, Jordana M. CD40 expression by human peripheral blood eosinophils. J Clin Invest. 1996;97:1761–6.
54. Woerly G, Roger N, Loiseau S, Dombrowicz D, Capron A, Capron M. Expression of CD28 and CD86 by human eosinophils and role in the secretion of type 1 cytokines (interleukin 2 and interferon gamma): inhibition by immunoglobulin a complexes. J Exp Med. 1999;190:487–95.
55. Shi HZ, Humbles A, Gerard C, Jin Z, Weller PF. Lymph node trafficking and antigen presentation by endobronchial eosinophils. J Clin Invest. 2000;105:945–53.
56. Lotfi R, Herzog GI, DeMarco RA, Beer-Stolz D, Lee JJ, Rubartelli A, Schrezenmeier H, Lotze MT. Eosinophils oxidize damage-associated molecular pattern molecules derived from stressed cells. J Immunol. 2009;183:5023–31.
57. Cormier SA, Taranova AG, Bedient C, Nguyen T, Protheroe C, Pero R, Dimina D, Ochkur SI, O'Neill K, Colbert D, Lombari TR, Constant S, McGarry MP, Lee JJ, Lee NA. Pivotal advance: eosinophil infiltration of solid tumors is an early and persistent inflammatory host response. J Leukoc Biol. 2006;79:1131–9.
58. Minton K. Granulocytes: eosinophils enable the antitumour T cell response. Nat Rev Immunol. 2015;15:333.
59. Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, Hammerling GJ. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. Nat Immunol. 2015;16:609–17.
60. Falcone FH, Zillikens D, Gibbs BF. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. Exp Dermatol. 2006;15:855–64.
61. Schroeder JT, MacGlashan Jr DW, Lichtenstein LM. Human basophils: mediator release and cytokine production. Adv Immunol. 2001;77:93–122.
62. Haas H, Falcone FH, Holland MJ, Schramm G, Haisch K, Gibbs BF, Bufe A, Schlaak M. Early interleukin-4: its role in the switch towards a Th2 response and IgE-mediated allergy. Int Arch Allergy Immunol. 1999;119:86–94.
63. Schroeder JT. Basophils beyond effector cells of allergic inflammation. Adv Immunol. 2009;101:123–61.

64. Prevete N, Staiano RI, Granata F, Detoraki A, Necchi V, Ricci V, Triggiani M, De Paulis A, Marone G, Genovese A. Expression and function of angiopoietins and their tie receptors in human basophils and mast cells. J Biol Regul Homeost Agents. 2013;27:827–39.
65. De Monte L, Wormann S, Brunetto E, Heltai S, Magliacane G, Reni M, Paganoni AM, Recalde H, Mondino A, Falconi M, Aleotti F, Balzano G, Ul HA, Doglioni C, Protti MP. Basophil recruitment into tumor-draining lymph nodes correlates with Th2 inflammation and reduced survival in pancreatic cancer patients. Cancer Res. 2016;76:1792–803.
66. Frossi B, De Carli M, Pucillo C. The mast cell: an antenna of the microenvironment that directs the immune response. J Leukoc Biol. 2004;75:579–85.
67. Qi X, Hong J, Chaves L, Zhuang Y, Chen Y, Wang D, Chabon J, Graham B, Ohmori K, Li Y, Huang H. Antagonistic regulation by the transcription factors C/EBPalpha and MITF specifies basophil and mast cell fates. Immunity. 2013;39:97–110.
68. Marone G, Galli SJ, Kitamura Y. Probing the roles of mast cells and basophils in natural and acquired immunity, physiology and disease. Trends Immunol. 2002;23:425–7.
69. Galli SJ, Franco CB. Basophils are back! Immunity. 2008;28:495–7.
70. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol. 2010;125:S73–80.
71. Metcalfe DD. Mast cells and mastocytosis. Blood. 2008;112:946–56.
72. Nonomura N, Takayama H, Nishimura K, Oka D, Nakai Y, Shiba M, Tsujimura A, Nakayama M, Aozasa K, Okuyama A. Decreased number of mast cells infiltrating into needle biopsy specimens leads to a better prognosis of prostate cancer. Br J Cancer. 2007;97:952–6.
73. Rojas IG, Spencer ML, Martinez A, Maurelia MA, Rudolph MI. Characterization of mast cell subpopulations in lip cancer. J Oral Pathol Med. 2005;34:268–73.
74. Fukushima H, Ohsawa M, Ikura Y, Naruko T, Sugama Y, Suekane T, Kitabayashi C, Inoue T, Hino M, Ueda M. Mast cells in diffuse large B-cell lymphoma; their role in fibrosis. Histopathology. 2006;49:498–505.
75. Kormelink TG, Abudukelimu A, Redegeld FA. Mast cells as target in cancer therapy. Curr Pharm Des. 2009;15:1868–78.
76. Ribatti D, Vacca A, Nico B, Crivellato E, Roncali L, Dammacco F. The role of mast cells in tumour angiogenesis. Br J Haematol. 2001;115:514–21.
77. Rajput AB, Turbin DA, Cheang MC, Voduc DK, Leung S, Gelmon KA, Gilks CB, Huntsman DG. Stromal mast cells in invasive breast cancer are a marker of favourable prognosis: a study of 4,444 cases. Breast Cancer Res Treat. 2008;107:249–57.
78. Chan JK, Magistris A, Loizzi V, Lin F, Rutgers J, Osann K, DiSaia PJ, Samoszuk M. Mast cell density, angiogenesis, blood clotting, and prognosis in women with advanced ovarian cancer. Gynecol Oncol. 2005;99:20–5.
79. Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. J Clin Oncol. 2005;23:8959–67.
80. Tan SY, Fan Y, Luo HS, Shen ZX, Guo Y, Zhao LJ. Prognostic significance of cell infiltrations of immunosurveillance in colorectal cancer. World J Gastroenterol. 2005;11:1210–4.
81. Latti S, Leskinen M, Shiota N, Wang YF, Kovanen PT, Lindstedt KA. Mast cell-mediated apoptosis of endothelial cells in vitro: a paracrine mechanism involving TNF-alpha-mediated down-regulation of bcl-2 expression. J Cell Physiol. 2003;195:130–8.
82. Leskinen MJ, Lindstedt KA, Wang YF, Kovanen PT. Mast cell chymase induces smooth muscle cell apoptosis by a mechanism involving fibronectin degradation and disruption of focal adhesions. Arterioscl Throm Vas. 2003;23:238–43.
83. Hammer GE, Ma A. Molecular control of steady-state dendritic cell maturation and immune homeostasis. Annu Rev Immunol. 2013;31:743–91.
84. Liu K, Nussenzweig MC. Origin and development of dendritic cells. Immunol Rev. 2010;234:45–54.
85. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, Chu FF, Randolph GJ, Rudensky AY, Nussenzweig M. In vivo analysis of dendritic cell development and homeostasis. Science. 2009;324:392–7.

86. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. Nat Rev Immunol. 2007;7:19–30.
87. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. Annu Rev Immunol. 2005;23:975–1028.
88. Steinman RM. Decisions about dendritic cells: past, present, and future. Annu Rev Immunol. 2012;30:1–22.
89. Ljunggren HG, Karre K. In search of the missing self—Mhc molecules and Nk cell recognition. Immunol Today. 1990;11:237–44.
90. Vivier E, Nunes JA, Vely F. Natural killer cell signaling pathways. Science. 2004;306:1517–9.
91. Tomasello E, Blery M, Vely F, Vivier E. Signaling pathways engaged by NK cell receptors: double concerto for activating receptors, inhibitory receptors and NK cells. Semin Immunol. 2000;12:139–47.
92. Strengell M, Matikainen S, Siren J, Lehtonen A, Foster D, Julkunen I, Sareneva T. IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells. J Immunol. 2003;170:5464–9.
93. Brady J, Carotta S, Thong RP, Chan CJ, Hayakawa Y, Smyth MJ, Nutt SL. The interactions of multiple cytokines control NK cell maturation. J Immunol. 2010;185:6679–88.
94. Lunemann A, Lunemann JD, Munz C. Regulatory NK-cell functions in inflammation and autoimmunity. Mol Med. 2009;15:352–8.
95. Becknell B, Caligiuri MA. Natural killer cells in innate immunity and cancer. J Immunother. 2008;31:685–92.
96. Kaiser BK, Yim D, Chow IT, Gonzalez S, Dai Z, Mann HH, Strong RK, Groh V, Spies T. Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. Nature. 2007;447:482–6.
97. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature. 2002;419:734–8.
98. Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, Biassoni R, Bottino C, Moretta L, Moretta A. Transforming growth factor beta 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. Proc Natl Acad Sci U S A. 2003;100:4120–5.
99. Sconocchia G, Titus JA, Segal DM. Signaling pathways regulating CD44-dependent cytolysis in natural killer cells. Blood. 1997;90:716–25.
100. Wang W, Erbe AK, Hank JA, Morris ZS, Sondel PM. NK cell-mediated antibody-dependent cellular cytotoxicity in cancer immunotherapy. Front Immunol. 2015;6:368.
101. Ferlazzo G, Thomas D, Lin SL, Goodman K, Morandi B, Muller WA, Moretta A, Munz C. The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. J Immunol. 2004;172:1455–62.
102. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature. 2009;457:557–61.
103. Albertsson PA, Basse PH, Hokland M, Goldfarb RH, Nagelkerke JF, Nannmark U, Kuppen PJ. NK cells and the tumour microenvironment: implications for NK-cell function and antitumour activity. Trends Immunol. 2003;24:603–9.
104. Robey E, Fowlkes BJ. Selective events in T cell development. Annu Rev Immunol. 1994;12:675–705.
105. Germain RN. T-cell development and the CD4-CD8 lineage decision. Nat Rev Immunol. 2002;2:309–22.
106. Scollay R, Wilson A, D'Amico A, Kelly K, Egerton M, Pearse M, Wu L, Shortman K. Developmental status and reconstitution potential of subpopulations of murine thymocytes. Immunol Rev. 1988;104:81–120.
107. Blackburn CC, Manley NR. Developing a new paradigm for thymus organogenesis. Nat Rev Immunol. 2004;4:278–89.
108. Vonboehmer H, Teh HS, Kisielow P. The thymus selects the useful, neglects the useless and destroys the harmful. Immunol Today. 1989;10:57–61.

109. Leung RK, Thomson K, Gallimore A, Jones E, Van den Broek M, Sierro S, Alsheikhly AR, McMichael A, Rahemtulla A. Deletion of the CD4 silencer element supports a stochastic mechanism of thymocyte lineage commitment. Nat Immunol. 2001;2:1167–73.
110. Sharma P, Wagner K, Wolchok JD, Allison JP. Novel cancer immunotherapy agents with survival benefit: recent successes and next steps. Nat Rev Cancer. 2011;11:805–12.
111. Lugo-Villarino G, Maldonado-Lopez R, Possemato R, Penaranda C, Glimcher LH. T-bet is required for optimal production of IFN-gamma and antigen-specific T cell activation by dendritic cells. Proc Natl Acad Sci U S A. 2003;100:7749–54.
112. Zhu JF, Guo LY, Watson CJ, Hu-Li J, Paul WE. Stat6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. J Immunol. 2001;166:7276–81.
113. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008;453:236–40.
114. Chen WJ, Jin WW, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4(+)CD25(-) naive T cells to CD4(+)CD25(+) regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med. 2003;198:1875–86.
115. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, Wang YH, Watowich SS, Jetten AM, Tian Q, Dong C. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity. 2008;29:138–49.
116. Staudt V, Bothur E, Klein M, Lingnau K, Reuter S, Grebe N, Gerlitzki B, Hoffmann M, Ulges A, Taube C, Dehzad N, Becker M, Stassen M, Steinborn A, Lohoff M, Schild H, Schmitt E, Bopp T. Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells. Immunity. 2010;33:192–202.
117. Saule P, Trauet J, Dutriez V, Lekeux W, Dessaint JP, Labalette M. Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4(+) versus effector memory and terminally differentiated memory cells in CD8(+) compartment. Mech Ageing Dev. 2006;127:274–81.
118. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13:227–42.
119. Linsley PS, Clark EA, Ledbetter JA. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. Proc Natl Acad Sci U S A. 1990;87:5031–5.
120. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991;174:561–9.
121. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027–34.
122. Liang SC, Latchman YE, Buhlmann JE, Tomczak MF, Horwitz BH, Freeman GJ, Sharpe AH. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol. 2003;33:2706–16.
123. Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci U S A. 2001;98:13866–71.
124. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.
125. Nam KO, Kang H, Shin SM, Cho KH, Kwon B, Kwon BS, Kim SJ, Lee HW. Cross-linking of 4-1BB activates TCR-signaling pathways in CD8+ T lymphocytes. J Immunol. 2005;174:1898–905.
126. Godfrey WR, Fagnoni FF, Harara MA, Buck D, Engleman EG. Identification of a human OX-40 ligand, a costimulator of CD4+ T cells with homology to tumor necrosis factor. J Exp Med. 1994;180:757–62.
127. Nocentini G, Riccardi C. GITR: a modulator of immune response and inflammation. Adv Exp Med Biol. 2009;647:156–73.

128. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Holenbeck AE, Lerman MA, Naji A, Caton AJ. Thymic selection of CD4(+)CD25(+) regulatory T cells induced by an agonist self-peptide. Nat Immunol. 2001;2:301–6.
129. Croft M. The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol. 2009;9:271–85.
130. Nagasawa T. Microenvironmental niches in the bone marrow required for B-cell development. Nat Rev Immunol. 2006;6:107–16.
131. Hardy RR, Carmack CE, Shinton SA, Kemp JD, Hayakawa K. Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J Exp Med. 1991;173:1213–25.
132. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. J Exp Med. 1993;177:1009–20.
133. Carsetti R, Kohler G, Lamers MC. Transitional B cells are the target of negative selection in the B cell compartment. J Exp Med. 1995;181:2129–40.
134. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. J Allergy Clin Immunol. 2013;131:959–71.
135. Shlomchik MJ, Weisel F. Germinal center selection and the development of memory B and plasma cells. Immunol Rev. 2012;247:52–63.
136. Schroeder Jr HW, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010;125:S41–52.
137. Lipman NS, Jackson LR, Trudel LJ, Weis-Garcia F. Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. ILAR J. 2005;46:258–68.
138. Kung P, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T cell surface antigens. Science. 1979;206:347–9.
139. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc Natl Acad Sci U S A. 1984;81:6851–5.
140. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature. 1988;332:323–7.
141. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. mAbs. 2010;2:256–65.
142. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer. 2012;12:278–87.
143. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12:298–306.
144. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22:329–60.
145. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
146. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
147. Teng MW, Galon J, Fridman WH, Smyth MJ. From mice to humans: developments in cancer immunoediting. J Clin Invest. 2015;125:3338–46.
148. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480:480–9.
149. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253–68.
150. Huang B, Pan PY, Li QS, Sato AI, Levy DE, Bromberg J, Divino CM, Chen SH. Gr-1(+) CD115(+) immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res. 2006;66:1123–31.
151. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology. 2013;138:105–15.

152. Tangri S, LiCalsi C, Sidney J, Sette A. Rationally engineered proteins or antibodies with absent or reduced immunogenicity. Curr Med Chem. 2002;9:2191–9.
153. Weiner LM, Surana R, Wang SZ. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol. 2010;10:317–27.
154. Krummel MF, Allison JP. Cd28 and Ctla-4 have opposing effects on the response of T-cells to stimulation. J Exp Med. 1995;182:459–65.
155. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJM, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.
156. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I, Topalian SL. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28:3167–75.
157. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhaufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crino L, Blumenschein Jr GR, Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373:1627–39.
158. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Aren Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373:123–35.
159. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro Jr G, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540–50.
160. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y, Srinivas S, Retz MM, Grivas P, Joseph RW, Galsky MD, Fleming MT, Petrylak DP, Perez-Gracia JL, Burris HA, Castellano D, Canil C, Bellmunt J, Bajorin D, Nickles D, Bourgon R, Frampton GM, Cui N, Mariathasan S, Abidoye O, Fine GD, Dreicer R. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016;387:1909–20.
161. U.S. Food and Drug Administration: Hematology/Oncology (Cancer) Approvals & Safety Notifications. 2016. http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm
162. Aspeslagh S, Postel-Vinay S, Rusakiewicz S, Soria JC, Zitvogel L, Marabelle A. Rationale for anti-OX40 cancer immunotherapy. Eur J Cancer. 2016;52:50–66.
163. Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. J Clin Oncol. 2011;29:4828–36.
164. Moon YW, Hajjar J, Hwu P, Naing A. Targeting the indoleamine 2,3-dioxygenase pathway in cancer. J Immunother Cancer. 2015;3:51.
165. Koblish HK, Hansbury MJ, Bowman KJ, Yang G, Neilan CL, Haley PJ, Burn TC, Waeltz P, Sparks RB, Yue EW, Combs AP, Scherle PA, Vaddi K, Fridman JS. [INCB preclin] Hydroxyamidine inhibitors of indoleamine-2,3-dioxygenase potently suppress systemic tryptophan catabolism and the growth of IDO-expressing tumors. Mol Cancer Ther. 2010;9:489–98.

166. Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, Leffet L, Hansbury MJ, Thomas B, Rupar M, Waeltz P, Bowman KJ, Polam P, Sparks RB, Yue EW, Li Y, Wynn R, Fridman JS, Burn TC, Combs AP, Newton RC, Scherle PA. [INCB preclin] Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood. 2010;115:3520–30.
167. Metz R, Rust S, Duhadaway JB, Mautino MR, Munn DH, Vahanian NN, Link CJ, Prendergast GC. [Indoximod preclin] IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: a novel IDO effector pathway targeted by D-1-methyl-tryptophan. Oncoimmunology. 2012;1:1460–8.
168. Iversen TZ, Engell-Noerregaard L, Ellebaek E, Andersen R, Larsen SK, Bjoern J, Zeyher C, Gouttefangeas C, Thomsen BM, Holm B, Straten PT, Mellemgaard A, Andersen MH, Svane IM. [IDO pep vac] Long-lasting disease stabilization in the absence of toxicity in metastatic lung cancer patients vaccinated with an epitope derived from indoleamine 2,3 dioxygenase. Clin Cancer Res. 2014;20(1):221–32.
169. Mautino MR, Jaipuri FA, Waldo J, Kumar S, Adams J, Allen CV, Marcinowicz-Flick A, Munn D, Vahanian N, CJJ L. NLG919, a novel indoleamine-2,3-dioxygenase (IDO)-pathway inhibitor drug candidate for cancer therapy. AACR. 2013:491.
170. Linch SN, McNamara MJ, Redmond WL. OX40 agonists and combination immunotherapy: putting the pedal to the metal. Front Oncol. 2015;5:34.
171. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer. 2016;16:275–87.
172. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.
173. U.S. Food and Drug Administration: FDA approves Keytruda for advanced non-small cell lung cancer. 2015. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm465444.htm
174. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563–7.
175. Madore J, Vilain RE, Menzies AM, Kakavand H, Wilmott JS, Hyman J, Yearley JH, Kefford RF, Thompson JF, Long GV, Hersey P, Scolyer RA. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res. 2015;28(3):245–53.
176. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348:56–61.
177. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, Rodig SJ, Chapuy B, Ligon AH, Zhu L, Grosso JF, Kim SY, Timmerman JM, Shipp MA, Armand P. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372:311–9.
178. Hadrup S, Donia M, Thor Straten P. Effector CD4 and CD8 T cells and their role in the tumor microenvironment. Cancer Microenviron. 2013;6:123–33.
179. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348:203–13.
180. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10:942–9.

181. Gobert M, Treilleux I, Bendriss-Vermare N, Bachelot T, Goddard-Leon S, Arfi V, Biota C, Doffin AC, Durand I, Olive D, Perez S, Pasqual N, Faure C, Coquard IR, Puisieux A, Caux C, Blay JY, Menetrier-Caux C. Regulatory T cells recruited through ccl22/ccr4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res 2009;69:2000–9.
182. Fu JL, Xu DP, Liu ZW, Shi M, Zhao P, Fu BY, Zhang Z, Yang HY, Zhang H, Zhou CB, Ya JX, Jin L, Wang HF, Yang YP, Fu YX, Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology. 2007;132:2328–39.
183. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan HN, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM, Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015;5:43–51.
184. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pages F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960–4.
185. Ma WJ, Gilligan BM, Yuan JD, Li TH. Current status and perspectives in translational biomarker research for PD-1/PD-L1 immune checkpoint blockade therapy. J Hematol Oncol. 2016;9(1):47.
186. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, Lugli A, Zlobec I, Hartmann A, Bifulco C, Nagtegaal ID, Palmqvist R, Masucci GV, Botti G, Tatangelo F, Delrio P, Maio M, Laghi L, Grizzi F, Asslaber M, D'Arrigo C, Vidal-Vanaclocha F, Zavadova E, Chouchane L, Ohashi PS, Hafezi-Bakhtiari S, Wouters BG, Roehrl M, Nguyen L, Kawakami Y, Hazama S, Okuno K, Ogino S, Gibbs P, Waring P, Sato N, Torigoe T, Itoh K, Patel PS, Shukla SN, Wang YL, Kopetz S, Sinicrope FA, Scripcariu V, Ascierto PA, Marincola FM, Fox BA, Pages F. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J Pathol. 2014;232:199–209.
187. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan JD, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124–8.
188. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371:2189–99.
189. Lastwika KJ, Wilson W, Li QK, Norris J, Xu HY, Ghazarian SR, Kitagawa H, Kawabata S, Taube JM, Yao S, Liu LN, Gills JJ, Dennis PA. Control of PD-L1 expression by oncogenic activation of the AKT-mTOR pathway in non-small cell lung cancer. Cancer Res. 2016;76:227–38.
190. Ku GY, Yuan JD, Page DB, Schroeder SEA, Panageas KS, Carvajal RD, Chapman PB, Schwartz GK, Allison JP, Wolchok JD. Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting lymphocyte count after 2 doses correlates with survival. Cancer. 2010;116:1767–75.
191. Delyon J, Mateus C, Lefeuvre D, Lanoy E, Zitvogel L, Chaput N, Roy S, Eggermont AMM, Routier E, Robert C. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol. 2013;24:1697–703.

Chapter 2
Interleukin-2: Old and New Approaches to Enhance Immune-Therapeutic Efficacy

Pooja Dhupkar and Nancy Gordon

Abstract Interleukin-2 (IL-2) is a very well-known cytokine that has been studied for the past 35 years. It plays a major role in the growth and proliferation of many immune cells such NK and T cells. It is an important immunotherapy cytokine for the treatment of various diseases including cancer. Systemic delivery of IL-2 has shown clinical benefit in renal cell carcinoma and melanoma patients. However, its use has been limited by the numerous toxicities encountered with the systemic delivery. Intravenous IL-2 causes the well-known "capillary leak syndrome," or the leakage of fluid from the circulatory system to the interstitial space resulting in hypotension (low blood pressure), edema, and dyspnea that can lead to circulatory shock and eventually cardiopulmonary collapse and multiple organ failure. Due to the toxicities associated with systemic IL-2, an aerosolized delivery approach has been developed, which enables localized delivery and a higher local immune cell activation. Since proteins are absorbed via pulmonary lymphatics, after aerosol deposition in the lung, aerosol delivery provides a means to more specifically target IL-2 to the local immune system in the lungs with less systemic effects. Its benefits have extended to diseases other than cancer. Delivery of IL-2 via aerosol or as nebulized IL-2 liposomes has been previously shown to have less toxicity and higher efficacy against sarcoma lung metastases. Dogs with cancer provided a highly relevant means to determine biodistribution of aerosolized IL-2 and IL-2 liposomes. However, efficacy of single-agent IL-2 is limited. As in general, for most immunetherapies, its effect is more beneficial in the face of minimal residual disease. To overcome this limitation, combination therapies using aerosol IL-2 with adoptive transfer of T cells or NK cells have emerged.

P. Dhupkar
Department of Pediatrics-Research, , The Children's Cancer Hospital, University of Texas M.D. Anderson Cancer Center, 7777 Knight Road, Houston, TX 77030, USA

Experimental Therapeutics Academic Program, The University of Texas Health Science Center at Houston, Houston, TX, USA
e-mail: pmdhupkar@mdanderson.org

N. Gordon (✉)
Department of Pediatrics-Research, , The Children's Cancer Hospital, University of Texas M.D. Anderson Cancer Center, 7777 Knight Road, Houston, TX 77030, USA
e-mail: ngordon@mdanderson.org

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Using a human osteosarcoma (OS) mouse model, we have demonstrated the efficacy of single-agent aerosol IL-2 and combination therapy aerosol IL-2 and NK cells or aerosol IL-2 and interleukin 11 receptor alpha-directed chimeric antigen receptor-T cells (IL-11 receptor α CAR-T cells) against OS pulmonary metastases. Combination therapy resulted in a better therapeutic effect. A Phase-I trial of aerosol IL-2 was done in Europe and proved to be safe. Others and our preclinical studies provided the basis for the development of a Phase-I aerosol IL-2 trial in our institution to include younger patients with lung metastases. OS, our disease of interest, has a peak incidence in the adolescent and young adult years. Our goal is to complete this trial in the next 2 years.

In this chapter, we summarize the different effects of IL-2 and cover the advantages of the aerosol delivery route for diseases of the lung with an emphasis on some of our most recent work using combination therapy aerosol IL-2 and NK cells for the treatment of OS lung metastases.

Keywords Aerosol IL-2 • Osteosarcoma • Immunotherapy • Lung metastasis • NK cell therapy • IL-2 clinical trial

2.1 Introduction

Interleukin-2 (IL-2) is primarily one of the first cytokines that has been characterized to stimulate the growth of T cells. IL-2 was discovered in 1976, as a T-cell growth factor (TCGF), that was capable of growing and differentiating T-lymphocytes in vitro from undifferentiated bone marrow extracts [1]. Mier et al. later characterized the molecule and named it as "IL-2". The introduction of IL-2 as an antitumor agent represented the first successful immunotherapy cancer treatment approach for humans [1]. Purified natural IL-2 formulations were produced by DuPont from the Jurkat T tumor cell line following phytohemagglutinin (PHA) stimulation and used for the treatment of patients with advanced cancer [2, 3]. The IL-2 gene was cloned in 1983, followed by the production of recombinant IL-2 (rIL-2) by *E. coli*. This stimulated preclinical research in animals and led to various IL-2 clinical trials for the treatment of metastatic disease. Thereafter, several strategies are being utilized to develop novel and more effective IL-2 formulations for cancer immunotherapy [4].

2.2 IL-2 Receptor and Signaling

IL-2 is a 15.5 kDa cytokine, which exerts its activity by binding with the IL-2 receptor. The IL-2 receptor consists of three components: the α chain (IL-2Rα), the β chain (IL-2Rβ), and the γ chain (IL-2Rγ). Different combinations of these three components have different affinities to IL-2, and their heterodimerization may vary according to the cell type and activation. The αβγ heterotrimer, βγ dimer, and α chain monomer

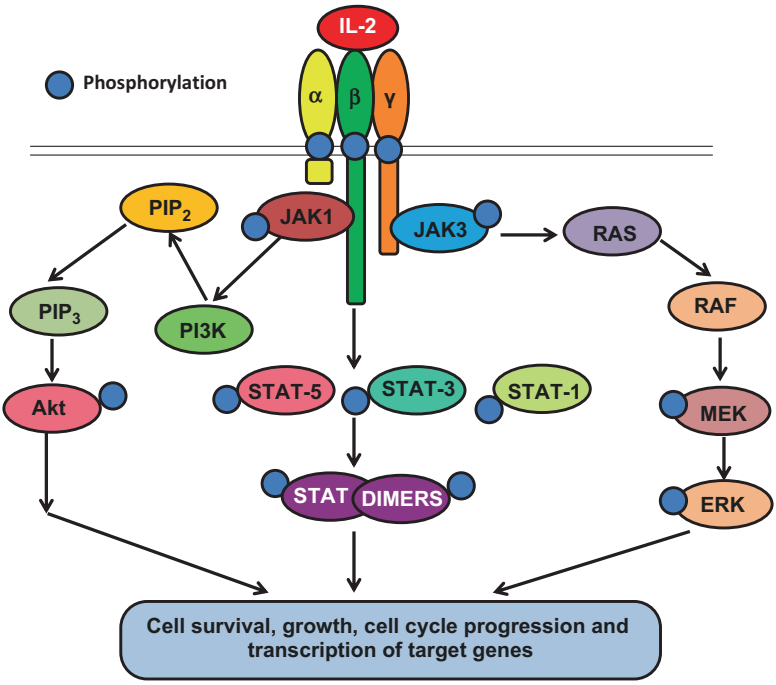

Fig. 2.1 IL-2 and IL-2R signaling pathway. Interaction of IL-2 with β and γ receptors causes JAK1 and JAK3 phosphorylation, which in turn activates and dimerizes STAT proteins with subsequent nuclear translocation and transcription of various genes. IL-2 activation may also lead to the activation of PI3K-Akt or MEK-Erk pathway and lead to cell survival and cell cycle progression

have "high," "intermediate," and "low" affinities to IL-2, respectively. As depicted in Fig. 2.1, binding of IL-2 with its receptor induces tyrosine phosphorylation of numerous proteins, as kinases bind to the cytoplasmic domains of the receptor subunits. IL-2Rβ and IL-2Rγ heterodimerize to activate Janus kinases (JAK) 1 and 3, which are also associated with β and γ chain, respectively. Either of the Signal Transducers and Activators of Transcription family of Transcription factors 1 (STAT1), 3 (STAT3), and 5 (STAT5) in T cells, or 4 (STAT4) in NK cells, are recruited and bind to the phosphorylated IL-2 receptor, resulting in their dissociation and subsequent dimerization. STAT dimers are then translocated to the nucleus, resulting in the transcription of target genes. The proto-oncogene tyrosine-protein kinase Src may also bind to the phosphorylated receptor leading to activation of the extracellular signal-regulated kinases 1 (Erk-1) and 2 (Erk-2) and cell cycle progression. In addition, IL-2 can activate the phosphatidylinositol-3 (PI3K)—protein kinase B (Akt)-p70S6 kinase pathway, thus promoting cell survival and growth.

IL-2 has been known to be involved in the growth and expansion of immune cells such as T cells, NK cells, and B cells. IL-2 can also stimulate the differentiation of CD4+ cells and CD8+ T cells into memory cells and terminally differentiated lymphocytes. Moreover, IL-2 plays an important role in augmenting the cytolytic activity of NK cells and T-lymphocytes.

2.3 Systemic IL-2 as an Immunotherapeutic Tool to Treat Malignant Diseases

Preliminary studies in animal models demonstrated that rIL-2 caused tumor regression of established pulmonary metastases and subcutaneous sarcoma tumors [5]. The preclinical findings from mice models were translated into clinical trials, where for the first time, 23 patients with various tumors, were treated with different regimens and doses of rIL-2 [2, 3]. Immunological changes such as cytokines and IFN-γ release were found in the serum; however, tumor regression was not seen when IL-2 was given alone perhaps because one of the major limitations of this therapy includes treatment-associated toxicity [6].

However, the ability of IL-2 to sustain the growth of T-lymphocytes encouraged the idea of the high-dose administration of IL-2 as a better immunotherapeutic strategy against cancer. IL-2 was shown to activate a population of lymphocytes, the lymphokine-activated killer cells (LAK), which have the unique property of killing the tumor cells irrespective of their histocompatibility expression status [7]. This phenomenon was further addressed by other investigators where exposure of peripheral blood mononuclear cells (PBMCs) or mouse splenocytes to supernatants containing IL-2 generated by LAK cells enhanced killing of various human cancer cell lines in vitro and primary human tumors in vivo [8–11]. Furthermore, preclinical studies using adoptive transfer of LAK cells expanded in vitro and given to mice with hepatic and pulmonary tumors caused significant antitumor in vivo activity [12–16]. These studies also showed that IL-2 augmented the in vivo activity of LAK cells in murine models of sarcomas and in one adenocarcinoma model. Therapeutic efficacy of IL-2 was shown to be dose dependent [15, 16].

Preclinical studies in animals prompted the use of high-dose IL-2 delivery in the clinical setting. The first study to discover the efficacy of high-dose IL-2 administration in mediating tumor regression was published in 1985 by Rosenberg et al. in patients with advanced cancer [17]. Twenty five patients with metastatic cancer were treated with escalating doses of IL-2 until toxicity was observed. The initial dose was 60,000 IU/kg followed by 180,000 or 600,000 IU/kg. Objective regression was observed in 11 of 25 patients. Four of seven patients with metastatic melanoma and 3 of 3 patients with renal cancer showed regression of metastatic disease. As the best durable responses were seen in melanoma and renal cell carcinoma, follow-up studies to investigate ways to improve IL-2 efficacy were only focused on these diseases. These initial studies uncovered the unique concept of immune modulation as a way to stimulate the immune system against tumor progression.

2.4 Efficacy of High-Dose IL-2 in Melanoma and Renal Cancer

A significant breakthrough in the IL-2 immunotherapy was highlighted by the Rosenberg et al. study at the National Cancer Institute, where 283 patients with metastatic melanoma or renal cancer were treated with high-dose IL2. High-dose

IL-2 was delivered either as a bolus or as a continuous infusion [18]. IL-2 exerted significant antitumor effects, with 7% complete response and 10% partial responses in metastatic melanoma, and 7% complete response and 13% partial responses in metastatic renal carcinoma.

Similar efficacy was observed in further clinical trials using various high-dose IL-2 infusion schedules either alone or in combination with other cytokines or adoptive cell therapy [19–23]. Seven Phase-2 clinical trials of high-dose IL-2 for metastatic renal cancer patients resulted in an overall response of 14% and a complete response of 5% [23]. Due to the durability of responses from multicenter trials, United States Food and Drug administration (US-FDA) approved high-dose bolus IL-2 for the treatment of patients with metastatic renal cancer in 1992. IL-2 was the second immunotherapeutic agent approved for patients with cancer.

Similar multicenter trials were conducted by 22 institutions for the high-dose IL-2 delivery in patients with metastatic melanoma. From 272 patients, 6% had a complete response and 10% had a partial response [19]. Because high-dose IL-2 was shown to induce durable responses associated with disease-free survival in a small percentage of patients, in 1998, high-dose IL-2 was approved by the US-FDA for the treatment of metastatic melanoma.

However, because of the significant toxicities associated with high-dose IL-2, its application was limited to highly selected patients. Further combination regimens containing lower doses of IL-2 were investigated.

2.5 Systemic IL-2 as an Immunotherapeutic Approach for Sarcomas

No effective immunotherapies have thus far been identified for the treatment of sarcomas. However, building on additional immunotherapeutic advances made in other tumors has provided encouraging alternatives. The role of the immune system as a therapeutic approach to target cancer was first described in sarcomas where tumor regression was observed in a patient who developed postoperative infections [6]. This suggested that the ability of the body to respond to infections may also play a role in the antitumor response. As a consequence, few immunotherapy approaches have been investigated which included cytokines such as IL-2 and IFN-γ.

Initial studies using combination of IL-2 and LAK cells by Rosenberg et al. in patients with multiple malignancies demonstrated responses in metastatic renal cell cancer, melanoma, colorectal cancer, and non-Hodgkin's lymphoma. However, six sarcoma patients in the study showed no response [24]. Another study included ten heavily pretreated pediatric patients with multiple malignancies including four osteosarcoma and two Ewing's sarcoma patients. Variable responses were noted. Two osteosarcoma patients had durable complete responses with a median follow-up of 28 months and the two other had progressive disease suggesting that IL-2 efficacy was limited to only a subset of patients [25]. Further studies have been developed using combination therapy with IL-2. Nevertheless, no specific benefit of this cytokine alone has been described in sarcomas.

2.6 Limitations and Alternatives to Systemic IL-2 Administration

Systemic IL-2 administration provided high regression rates in patients with various advanced cancers. However, the high doses exerted a variety of serious dose-dependent systemic toxicities. One of the most predominant toxicity of IL-2 is the "capillary leak syndrome," caused by extravasation of fluid into the organs, causing multi-organ damage. This translates into pulmonary congestion, hypotension, pre-renal azotemia, adult respiratory distress syndrome, and myocardial infarction [26]. These latter biologic effects are the result of IL-2 activation of NK and other immune cells, which in turn stimulate the release of inflammatory cytokines, which eventually leads to the so-called cytokine storm [27]. The cytokine storm is a major driver of the IL-2-mediated side effects, some of which include fever, chills, malaise, diarrhea, nausea, anemia, thrombocytopenia, eosinophilia, elevation of hepatic enzymes, and confusion [28]. An increase in the vascular permeability may be caused by IL-2 and CD-25-dependent endothelial cell damage and indirectly by the release of NK cell-mediated tumor necrosis factor-α (TNF-α) [29, 30].

High-dose IL-2 has also been linked to the development of eosinophilic myocarditis [31]. It can also enhance neutrophil chemotaxis, which makes patients who receive IL-2, more prone to Gram-positive and Gram-negative organism infections [32]. Due to the complexity of IL-2-induced toxicities, appropriate care and management of treated patients is crucial. Patients are initially screened to rule out any cardiac pathology, and those found to have a cardiac abnormality are not favored for IL-2 therapy. Early termination of treatment or interruption is followed when toxicity is noted.

Some other limitations are related to the fact that systemic IL-2 delivery has a very short half-life (15–30 min) and is rapidly excreted through the kidneys upon intravenous administration [30, 33–36]. Moreover, the route of administration also affects the absorption, biodistribution, and the half-life of IL-2. Intramuscular and subcutaneous IL-2 delivery results in higher systemic absorption as compared to intravenous administration [36].

Other methods of administration such as intraperitoneal, intrapleural, intrathecal, intraventricular, and inhalational delivery routes have emerged as they are more advantageous in providing a better local sustained effect in addition to a greater therapeutic efficacy [36]. Low-dose bolus, continuous infusions or subcutaneous delivery regimens have also been developed as an alternative approach to decrease the serious side effects of IL-2 [37]. However, low-dose IL-2 therapy was shown not to be effective as antitumor therapy. By contrary, it results in the expansion of immunosuppressive T regulatory (T-reg) cells, which can be detrimental to the patients. Lastly, methods to incorporate cytokine proteins into multi-lamellar liposomes have been developed. This methodology has provided an alternative way to maintain more stable and sustained cytokine levels locally within the tumor bed, which has translated in a better local therapeutic benefit [38–40].

2.7 Strategies to Enhance the Efficacy of IL-2 Therapy

Several strategies are being studied to enhance the therapeutic efficacy and decrease the systemic side effects elicited by IL-2.

2.7.1 IL-2/Anti-IL-2 Monoclonal Antibody (mAb) Complexes

In this approach, target tumor cell killing is enhanced as the anti-IL-2 mAb is fused with the IL-2 cytokine, which in turn recognizes and binds to the IL-2 receptors on immune cells. The increase in the IL-2/anti-IL-2 mAb conjugate formation allows for a better biological response [41, 42]. IL-2 antibody fusions, has also been used in several mouse models to direct IL-2 to cells expressing CD122 (IL-2-c-x), such as CD8+ T and NK cells [43–47]. Treatment with antibody fusion caused a robust T and NK cell expansion, which resulted in more effective antitumor immune responses [30, 45]. The advantages of antibody fusions compared to high-dose IL-2 administration are prolonged in vivo half-life, preferential stimulation of CD8+ T cells over T-regs and reduction in side effects [30, 45, 48, 49]. Currently, humanized and anti-human IL-2 antibodies (IL-2-c-x) are being developed for clinical testing.

2.7.2 IL-2 Muteins

Another approach was to design mutants of IL-2 to preferentially create binding to the IL-2 receptor chains responsible for cytotoxic T cells proliferation. An IL-2 "superkine" was generated by amino acid substitutions between positions 80 and 92. These modifications caused an enhanced binding to the IL-2Rβ and spare IL-2Rα binding [47]. In addition, the IL-2 "superkine" resulted in a more stable and flexible helix of the IL-2Rβ binding site, which allowed for a sustained phosphorylation and signaling. The end result translated in a greater cytotoxic T-cell production as opposed to T-regs, and a reduction in pulmonary edema in mice treated with the superkine.

An additional mutein, the no-α mutein, was designed to reduce the affinity of IL-2 to CD25, the IL-2 receptor responsible for T-reg proliferation, and maintain normal binding with IL-2Rβγ [50]. No-α mutein inhibited the metastasis of B16 melanoma variant and 3LL-D122 Lewis lung carcinoma in mice. It also increased NK cell activation and exerted less toxicities compared to wild-type IL-2.

As compared to IL-2/Anti-IL-2 mAb complexes, IL-2 muteins have a shorter half-life in vivo and hence require frequent administration. Also, IL-2 muteins contain epitopes that may be immunogenic. Patients could potentially develop antibodies that could inhibit IL-2 muteins biological activity [51].

2.7.3 IL-2 Fusion Proteins

An additional novel alternative to reduce the side effects associated with systemic IL-2 delivery is a fusion protein in which IL-2 is covalently linked to a specific inhibitory binding component separated by a protease cleavage site. The proteases overexpressed in the tumor microenvironment such as matrix metalloproteinases (MMPs), cleave the protease cleavage site of the fusion protein, which in turn allows for a local increase in IL-2 concentration and as a consequence immune cell activation. Immune cells can further produce more cytokines, thus accounting for a better antitumor effect. One example is Selectikine I or NHS-IL2LT, a fully humanized IL-2 fusion protein used for the treatment of solid tumors and B-cell non-Hodgkin's lymphoma. It caused only mild grade 1 hypotension and vascular leak syndrome compared to wild-type IL-2 [52–54]. It is composed of a fully human de-immunized monoclonal antibody, NHS76, which binds free DNA from the dying tumor cells usually released after tumors are exposed to either radiation or chemotherapy. In addition, to decrease the toxicity effects of IL-2, a D20T mutation was introduced to the IL-2 motif. This mutation has little to no effect on the activity of free IL-2, is highly specific for activating the high-affinity IL-2 receptor, and has reduced binding to endothelial cells. In a Phase-I clinical trial in humans, the investigators were able to demonstrate that Selectikine has a favorable safety profile and induced the biological effects typical of IL-2 [54].

Two additional IL-2 fusion proteins, GA504 and GA501, comprise a fusion of the IL-2 mutein with a disrupted binding to CD25 and a humanized antibody targeting either carcinoembryonic antigen (CEA-GA504) or the familial adenomatous polyposis antigen (FAP-GA501). Both GA504 and GA501 were shown to cause strong activation and expansion (about 100-fold) of NK and CD8+ T cells and shift the CD4+:CD8+ ratio towards CD8+ T cells systemically in the peripheral blood and at the tumor site. These fusion proteins showed therapeutic efficacy in the murine colon adenocarcinoma model, MC38-CEA, and in the murine pancreatic carcinoma model, PancO2-CEA, as compared to the wild-type IL-2 [55]. Lastly, another IL-2 fusion protein, hu14.18-IL-2, was created in an effort to improve the antitumor effects of IL-2 or monoclonal antibody, ch14.18. It contains IL-2 molecularly linked to the carboxy terminus of the IgG heavy chains of the humanized hu14.18 monoclonal Ab, which recognizes disialoganglioside (GD2) on neuroblastoma and melanoma tumors [56, 57]. Intra-tumoral injection of hu14.18-IL2 led to increased NK and CD8+ T-cell infiltration and improved antitumor efficacy [57].

In summary, different strategies have provided different alternative benefits. However, the limitations of IL-2 to be considered as single-agent therapy still remain.

2.8 The Aerosol Route: A Novel Approach to Deliver IL-2

Drug concentration in lung tumors is low after systemic administration. This might be one of the major contributors of treatment failure [58]. In humans, drug concentration in the tumors appears to be a key parameter for drug efficacy [59]. The

inhalation route discovered in the 1990s offers several advantages over systemic delivery. These include: (1) direct local high-dose concentrations in the lungs and airways with lower doses and fewer side effects and (2) the use of a noninvasive delivery system, which avoids first-pass metabolism of the drug through the liver. Moreover, the alveolar surface also provides a large surface area for fast systemic absorption [60]. Owing to the toxicities of the systemic delivery approach, aerosolized delivery of IL-2 has been studied as an alternative delivery route for the treatment of certain diseases limited to the lung.

A number of studies in renal cell carcinoma and melanoma have used aerosol IL-2 and confirmed that this route of administration can be safely administered and that it has modest efficacy against pulmonary metastases. Bronchoalveolar lavage recovered from patients treated by this approach demonstrated not only an increase in the number of lymphocytes but also an activated phenotype, indicating predominantly localized lymphocyte activation by the aerosol IL-2 treatment in the lung. Further studies demonstrated feasibility of this approach for the treatment of patients with various sarcoma types in addition to patients with immune deficiency diseases [61, 62]. These included patients with osteosarcoma (OS). Lung metastasis is the main cause of death in patients with OS. Aerosolized IL-2 offers an advantage as the target organ of treatment are the lungs. Biodistribution and pharmacokinetics of aerosolized IL-2 and IL-2 liposomes has been demonstrated in dogs with OS. Interestingly, nuclear medicine studies showed that lung retention times were significantly prolonged for both free IL-2 and IL-2 liposomes [38, 39]. Aerosol delivery of IL-2 was proved to be safe in dogs with spontaneous OS lung metastases [38, 39].

Our group has previously published work supporting the rationale and efficacy of aerosol IL-2 for the treatment of OS lung metastases. Using our LM7 OS mouse model, we demonstrated single-agent aerosol IL-2 efficacy against OS lung metastasis [63, 64]. Aerosolized IL-2 treatment given twice a week in the presence of micrometastatic disease, developed 5 weeks after i.v. injection of tumor cells, demonstrated therapeutic benefit. The number of macro- and micro-metastasis significantly decreased after 5 weeks of treatment with aerosol IL-2 at a dose of 2000 U/day twice a week. In addition, there was a significant increase in the number of apoptotic cells in the aerosol IL-2-treated lung metastases as compared to the PBS-treated groups as demonstrated by an increase in terminal deoxynucleotidyl transferase dUTP end labeling (TUNEL) staining (data not shown). Furthermore, aerosol IL2 caused an increase in the migration and activation of local immune cells in the lung. Fig. 2.2a, b shows that there was a significant increase in the number of mouse NK cells in the lung tumors of the aerosol IL-2-treated group compared to the aerosol PBS group as shown by an increase in the NKp46 immunohistochemistry (IHC) staining ($p = 0.05$). In addition, the number of macrophages was also significantly increased in the lung tumors from the aerosol IL-2-treated group as compared to the PBS control group as demonstrated by an increase in the F4/80 IHC staining ($p = 0.05$) (Fig. 2.2a, b).

The effect of aerosolized IL-2 on the local immune system in the lung was also demonstrated using immunocompetent mice. Mice received 2000 U/day of aerosol rhIL-2 or aerosol PBS for a total of 15 days. Single cell isolation from lungs of both

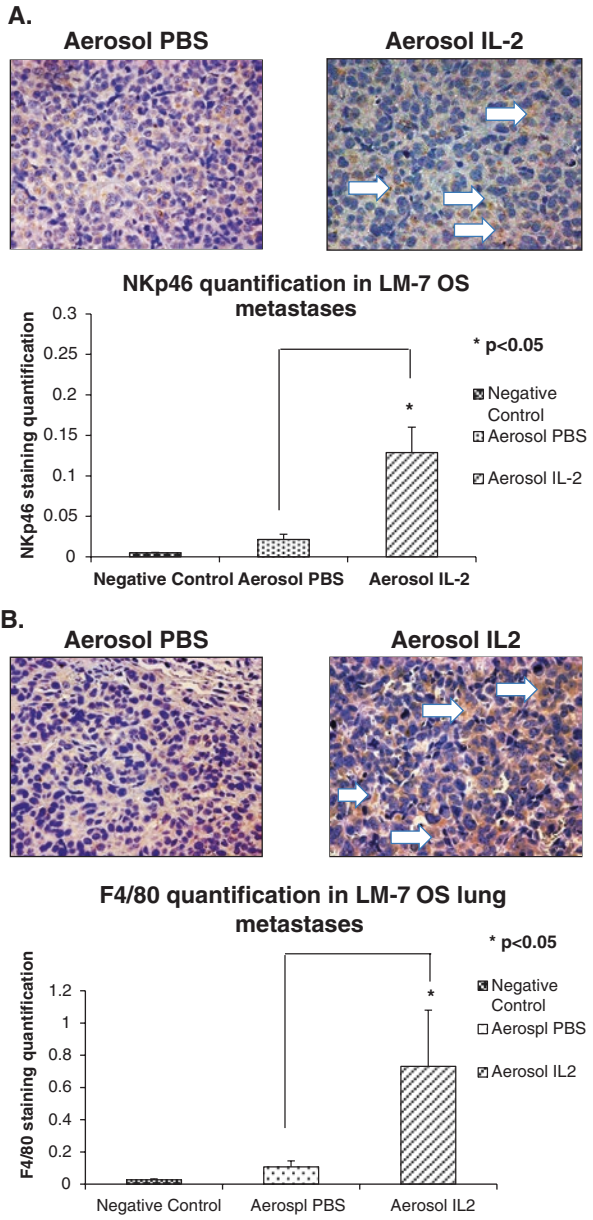

Fig. 2.2 Increase in the number of local NK cells and macrophages in osteosarcoma lung metastases from mice treated with aerosol IL-2. Representative images of (**a**) Significant increase of NK cells in LM7 OS lung metastases from mice treated with aerosol IL-2 as determined by NKp46 (*brown*) immunohistochemistry (IHC) staining (*left top*) and quantification (*right top*) $p < 0.05$ as compared to untreated control and; (**b**) Significant increase of macrophage infiltration in LM7 OS lung metastases from mice treated with aerosol IL-2 as demonstrated by F4/80 (*brown*) IHC staining (*left bottom*) and quantification (*right bottom*) $p < 0.05$ as compared to untreated control

treatment groups was performed and compared. Fluorescence-activated cell sorting (FACS) analysis depicted that the number CD3+, CD8+, and CD4+ cells significantly increased on day 13 following aerosol IL-2 treatment compared to aerosol PBS, and persisted for 3 days after terminating the treatment. IHC staining also showed a slight increase in T regulatory cells (T-regs), as demonstrated by forkhead

box P3 (Foxp3) staining. However, the increase in Foxp3+ cells was smaller compared to the increase in CD4+ cells, indicating that T-regs do not account for the majority of the CD4+ cells induced by aerosol IL-2. The number of CD4+ and Foxp3+ cells returned back to the original levels, 1 month after terminating aerosol IL-2 treatment (data not shown). CM-Dil-labeled T cells injected intravenously localized and proliferated in the lung after aerosol IL-2 administration [65].

Lastly, survival studies using the human LM7 OS mouse model revealed an improved median survival time of mice treated with aerosol IL-2 as compared with aerosol PBS (89.5 versus 71 days; $p = 0.03$) [63].

In summary, our preclinical studies using an OS mouse model constitute real evidence of the immunotherapeutic benefit of this approach for the treatment of OS lung metastases and provide a strong rationale for the design of future clinical trials.

2.9 Different Combination Therapy Approaches Using IL-2

2.9.1 IL-2 in Combination with Cytokines and/or Chemotherapy

Several strategies to augment the immunotherapeutic effect of IL-2 have been studied. Combination treatments of IL-2 with IFN-α showed a limited advantage over high-dose IL-2 treatment, but served as a good outpatient management strategy [66, 67]. Furthermore, combination therapy of IL-2 with Cisplatin and Dacarbazine resulted in efficacious outcomes for metastatic melanoma patients [68]. IFN-α and IL-2 along with 5-Fluorouracil therapy resulted in 48% response rate in metastatic renal cancer patients [69]. Patients with metastatic melanoma, who did not respond initially to chemotherapy, showed better responses when high-dose IL-2 was added to the regimen [70].

2.9.2 IL-2 in Combination with Antibody Therapy

Low doses of IL-2 in combination with the anti-vascular endothelial growth factor A (VEGF-A), Bevacizumab, resulted in significant antitumor activity in patients with metastatic renal cell carcinoma [71].

2.9.3 IL-2 in Combination with Vaccines

Phase-III clinical trials using a GP-100 peptide vaccine, based on the ability of melanoma-derived tumor-infiltrating lymphocytes to recognize melanocyte-specific antigen gp-100 in combination with high-dose IL-2 resulted in better clinical activity

than high-dose IL-2 alone in metastatic melanoma [72, 73]. In addition, Phase-II clinical trials using combination of modified vaccinia Ankara virus (MVA) expressing 5T4, a non-secreted membrane glycoprotein expressed on clear cell and papillary renal cell carcinoma, and IL-2 resulted in disease stabilization in patients with metastatic renal cell carcinoma [74]. In some instances, studies evaluating the combination of IL-2 as an immune adjuvant for a peptide vaccine against melanoma demonstrated IL-2 to likely be immunosuppressive in this setting, suggesting no additional benefit [75].

2.9.4 IL-2 in Combination with Adoptive T-Cell Therapy

Adoptive T-cell therapy has been described as an effective strategy to target metastases. However, a major barrier in adoptive transfer is the survival and persistence of the T cells in vivo. Combination with cytokines such as IL-2 has helped overcome this barrier. Hence, studies showed that combination of autologous T-cell adoptive transfer with high-dose IL-2 therapy with previous lymphodepletion using cyclophosphamide and fludarabine or total body irradiation, resulted in objective responses from 50 to 70% in metastatic melanoma patients [76, 77]. In these studies, the CD8+ lymphocytes obtained from tumors were clonally expanded using high-dose IL-2 initially for 5–6 weeks. Further expansion was performed using feeder cells and anti-CD3, in addition to high-dose IL-2, for an additional 2 weeks before the cells were reinfused to the patients [76]. Additional Phase-II clinical trials from the NCI showed that combination treatment of adoptive T-cell therapy with high-dose IL-2 resulted in a 50% clinical response rate and 13% durable responses with complete regression lasting more than 5 years in metastatic melanoma patients [78]. Similar clinical responses have also been shown in adoptive T-cell transfer trials in metastatic melanoma conducted at M.D. Anderson Cancer Center and Sheba Medical Center in Tel Hashomer, Israel [79–81]. Nevertheless, the widespread application of this treatment is limited by the complex procedure of T-cell expansion and the toxicities elicited by high-dose IL-2. Recently, a pilot trial was conducted by researchers at Danish Translational Research Center, using low-dose subcutaneous IL-2 injection along with adoptive T-cell transfer in melanoma patients. The results showed durable, complete responses with reduced toxicity, suggesting that low-dose IL-2 may be sufficient to prolong the survival of T cells [82].

Our laboratory, previously demonstrated that intravenous delivery of genetically modified T cells expressing chimeric antigen receptor (CAR) targeting IL-11Rα, expressed in OS tumors, resulted in regression of OS lung metastasis. IL-11Rα-CAR-T cells were generated by ex vivo expansion in the presence of IL-2 and IL-15 and a feeder system of antigen-presenting cells (APCs) [83]. This therapy was further enhanced when aerosol IL-2 was added. Others' and our findings suggest that the addition of IL-2 to T-cell therapy offers additional therapeutic benefit for the treatment of many tumors.

2.9.5 IL-2 in Combination with NK Cell Therapy

Although we and others have demonstrated that aerosolized IL-2 alone has therapeutic effect against some tumors, complete control of metastatic spread has not been achieved. In our preclinical studies using a human OS mouse model, complete eradication of OS lung metastasis was not attained with aerosol IL-2 alone as there was evidence of relapsed disease. In order to augment the effect of IL-2, we combined aerosolized IL-2 with adoptive NK cell immunotherapy.

NK cells possess an advantage over T cells as they can target tumor cells without the requirement of tumor-antigen recognition or major histocompatibility complex (MHC) restriction. However, infusion of NK cells also requires culture and ex vivo expansion, as the number of cells that are usually harvested is very small and not enough to lead to a good therapeutic outcome. Several platforms have been used to expand NK cells ex vivo using the feeder cells or APCs. Genetically modified K562 cells, like K562-membrane bound 15-41BBL, have had successful applications to expand allogeneic NK cells from patients treated for acute myelogenous leukemia, gastric cancer, and multiple myeloma [84, 85]. Denman and colleagues showed that membrane bound IL-21-expressing K562 APCs caused high rate of proliferation of NK cells and constitutes an additional successful approach [86]. Either approach is feasible and provides the advantage of not only expanding the NK cells but also enhancing their activity.

The addition of aerosolized IL-2 to the adoptive transfer of NK cells showed additional therapeutic advantage against OS lung metastasis in our human OS mouse model [64]. Aerosolized IL-2 increased the number of human NK cells in the lung. The combination therapy resulted in a greater decrease in the number and size of lung metastases and greater tumor cell apoptosis as compared to aerosol IL-2 or the administration of NK cells alone (Fig. 2.3a, b). Lastly, aerosol IL-2 and NK cells combination therapy led to an improved overall survival as the median survival of mice treated with the combination was 130 days as compared to 71 days for untreated mice. The improved survival demonstrated by the combination therapy was also superior to either IL-2 or NK cell treatment alone confirming the benefit of this approach [63]. Figure 2.4 summarizes our proposed model and expected outcomes.

Additionally, clinical trials of adoptive transfer of in vitro-activated autologous NK cells have not demonstrated any significant clinical benefit in any of the tumors studied perhaps due to the self-tolerance of autologous NK cells [87, 88]. As a consequence, allogeneic NK cell infusions have been generally administered after immunosuppressive chemotherapy or after an HLA-mismatched transplantation.

Adoptively transferred human-mismatched (haploidentical) allogeneic NK cells have been shown to be more effective against AML and solid cancers such as metastatic melanoma, renal cell carcinoma, and Hodgkin's disease [89–91]. A Phase-I trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer has demonstrated some benefit [92]. However, there are so far, no studies to address the benefit of combining IL-2 with the adoptive transfer of NK cells. We have an ongoing Phase-I study at M.D. Anderson Cancer Center, of

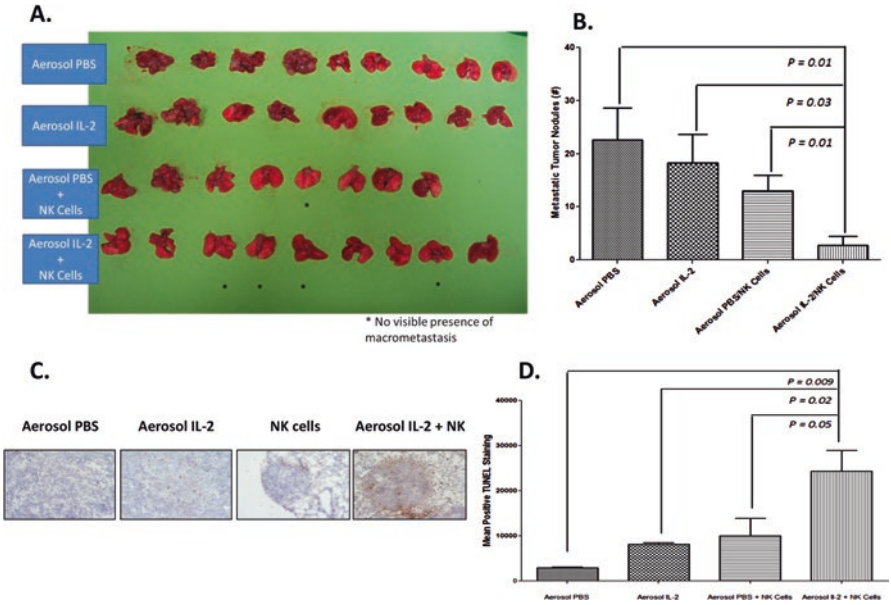

Fig. 2.3 Therapeutic benefit of the combination therapy aerosol IL-2 and NK cells for the treatment of OS lung metastases. (**a**, **b**) Aerosol IL-2 and NK cell combination therapy resulted in a significant decrease in the number of metastatic lung tumor nodules as compared to PBS control ($p = 0.01$), aerosol IL-2 ($p = 0.03$), or NK cell ($p = 0.01$) treatments alone in a human LM7 OS mouse model, (**c**, **d**). Aerosol IL-2 and NK cell combination therapy led to a significant increase in tumor cell apoptosis as determined by TUNEL staining (*brown*) as compared to either treatment alone, ($p = 0.009$-PBS, $p = 0.02$-aerosol IL-2 and $p = 0.05$-NK cells)

aerosol IL-2 for the treatment of lung metastases to address the feasibility of this approach in the pediatric population and facilitate a future combination therapy trial using adoptive transfer of NK cells in addition to aerosolized IL-2.

2.10 Summary

IL-2 has been one of the most widely studied cytokines and immunotherapeutic agents for the treatment of various cancers and other diseases. IL-2 has been approved by the US-FDA for the treatment of melanoma and renal cell carcinoma. Due to the serious toxicities and limited efficacy of systemic IL-2, alternative unique approaches have been studied. In this chapter, we summarized all these approaches with a greater emphasis on aerosol IL-2 as a potential safe and feasible method capable of enhancing local and adoptive transfer of immune cells for the treatment of lung metastases. Successful completion of the Phase-I/II clinical trial of aerosol IL-2 will allow for further combination therapy trials using this less invasive and less toxic approach.

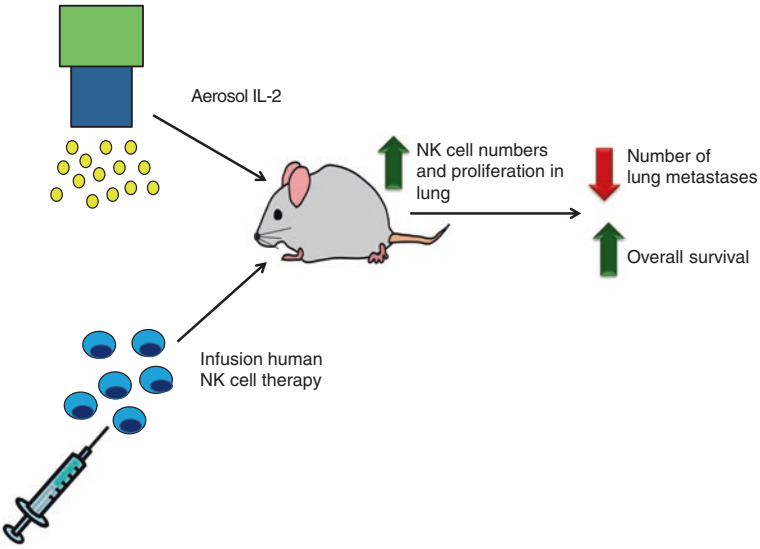

Fig. 2.4 Schematic proposed model of the combination therapy aerosol IL-2 and NK cells with expected outcomes. Mice with micrometastatic disease were treated with aerosol IL-2 twice a week, along with infusion of human NK cell therapy (50 million cells). Aerosol IL-2 increased the number of infused NK cells proliferating in the lung leading to enhanced tumor cell apoptosis and improved survival

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References

1. Taniguchi T, Minami Y. The IL-2/IL-2 receptor system: a current overview. Cell. 1993;73(1):5–8.
2. Lotze MT, et al. In vivo administration of purified human interleukin 2. I. Half-life and immunologic effects of the Jurkat cell line-derived interleukin 2. J Immunol. 1985;134(1):157–66.
3. Lotze MT, et al. In vivo administration of purified human interleukin 2. II. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2. J Immunol. 1985;135(4):2865–75.
4. Parkinson DR. Interleukin-2 in cancer therapy. Semin Oncol. 1988;15(6 Suppl 6):10–26.
5. Rosenberg SA, et al. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. J Exp Med. 1985;161(5):1169–88.
6. D'Angelo SP, et al. Sarcoma immunotherapy: past approaches and future directions. Sarcoma. 2014;2014:391967.
7. Fagan EA, Eddleston AL. Immunotherapy for cancer: the use of lymphokine activated killer (LAK) cells. Gut. 1987;28(2):113–6.
8. Grimm EA, et al. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982;155(6):1823–41.

9. Yang SC, et al. Clinical and immunomodulatory effects of combination immunotherapy with low-dose interleukin 2 and tumor necrosis factor alpha in patients with advanced non-small cell lung cancer: a phase I trial. Cancer Res. 1991;51(14):3669–76.
10. Yang SC, et al. Induction of lymphokine-activated killer cytotoxicity with interleukin-2 and tumor necrosis factor-alpha against primary lung cancer targets. Cancer Immunol Immunother. 1989;29(3):193–8.
11. Rosenberg SA, et al. Prospective randomized trial of high-dose interleukin-2 alone or in conjunction with lymphokine-activated killer cells for the treatment of patients with advanced cancer. J Natl Cancer Inst. 1993;85(8):622–32.
12. Mazumder A, Rosenberg SA. Successful immunotherapy of natural killer-resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin 2. J Exp Med. 1984;159(2):495–507.
13. Mule JJ, et al. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science. 1984;225(4669):1487–9.
14. Mule JJ, Shu S, Rosenberg SA. The anti-tumor efficacy of lymphokine-activated killer cells and recombinant interleukin 2 in vivo. J Immunol. 1985;135(1):646–52.
15. Lafreniere R, Rosenberg SA. Successful immunotherapy of murine experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin 2. Cancer Res. 1985;45(8):3735–41.
16. Lafreniere R, Rosenberg SA. Adoptive immunotherapy of murine hepatic metastases with lymphokine activated killer (LAK) cells and recombinant interleukin 2 (RIL 2) can mediate the regression of both immunogenic and nonimmunogenic sarcomas and an adenocarcinoma. J Immunol. 1985;135(6):4273–80.
17. Rosenberg SA, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med. 1985;313(23):1485–92.
18. Rosenberg SA, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. JAMA. 1994;271(12):907–13.
19. Atkins MB, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol. 1999;17(7):2105–16.
20. Kruit WH, et al. Dose efficacy study of two schedules of high-dose bolus administration of interleukin 2 and interferon alpha in metastatic melanoma. Br J Cancer. 1996;74(6):951–5.
21. Keilholz U, et al. Interferon-alpha and interleukin-2 in the treatment of metastatic melanoma. Comparison of two phase II trials. Cancer. 1993;72(2):607–14.
22. de Gast GC, et al. Phase I trial of combined immunotherapy with subcutaneous granulocyte macrophage colony-stimulating factor, low-dose interleukin 2, and interferon alpha in progressive metastatic melanoma and renal cell carcinoma. Clin Cancer Res. 2000;6(4):1267–72.
23. Dutcher JP, et al. Interleukin-2-based therapy for metastatic renal cell cancer: the Cytokine Working Group experience, 1989-1997. Cancer J Sci Am. 1997;3(Suppl 1):S73–8.
24. Rosenberg SA, et al. Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Ann Surg. 1989; 210(4):474–84; discussion 484–5.
25. Schwinger W, et al. Feasibility of high-dose interleukin-2 in heavily pretreated pediatric cancer patients. Ann Oncol. 2005;16(7):1199–206.
26. Rosenstein M, Ettinghausen SE, Rosenberg SA. Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin-2. J Immunol. 1986;137(5):1735–42.
27. Panelli MC, et al. Forecasting the cytokine storm following systemic interleukin (IL)-2 administration. J Transl Med. 2004;2(1):17.
28. Lotze MT, et al. Clinical effects and toxicity of interleukin-2 in patients with cancer. Cancer. 1986;58(12):2764–72.
29. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012;12(3):180–90.
30. Boyman O, Surh CD, Sprent J. Potential use of IL-2/anti-IL-2 antibody immune complexes for the treatment of cancer and autoimmune disease. Expert Opin Biol Ther. 2006;6(12):1323–31.

31. Schuchter LM, et al. Eosinophilic myocarditis associated with high-dose interleukin-2 therapy. Am J Med. 1990;88(4):439–40.
32. Klempner MS, et al. An acquired chemotactic defect in neutrophils from patients receiving interleukin-2 immunotherapy. N Engl J Med. 1990;322(14):959–65.
33. Donohue JH, Rosenberg SA. The fate of interleukin-2 after in vivo administration. J Immunol. 1983;130(5):2203–8.
34. Shaker MA, Younes HM. Interleukin-2: evaluation of routes of administration and current delivery systems in cancer therapy. J Pharm Sci. 2009;98(7):2268–98.
35. Konrad MW, et al. Pharmacokinetics of recombinant interleukin 2 in humans. Cancer Res. 1990;50(7):2009–17.
36. Anderson PM, Sorenson MA. Effects of route and formulation on clinical pharmacokinetics of interleukin-2. Clin Pharmacokinet. 1994;27(1):19–31.
37. Kammula US, White DE, Rosenberg SA. Trends in the safety of high dose bolus interleukin-2 administration in patients with metastatic cancer. Cancer. 1998;83(4):797–805.
38. Khanna C, et al. Interleukin-2 liposome inhalation therapy is safe and effective for dogs with spontaneous pulmonary metastases. Cancer. 1997;79(7):1409–21.
39. Khanna C, et al. Nebulized interleukin 2 liposomes: aerosol characteristics and biodistribution. J Pharm Pharmacol. 1997;49(10):960–71.
40. Dow S, et al. Phase I study of liposome-DNA complexes encoding the interleukin-2 gene in dogs with osteosarcoma lung metastases. Hum Gene Ther. 2005;16(8):937–46.
41. Rabinovitch A, et al. Combination therapy with sirolimus and interleukin-2 prevents spontaneous and recurrent autoimmune diabetes in NOD mice. Diabetes. 2002;51(3):638–45.
42. Shin JI, Park SJ, Kim JH. A possible role of leptin-associated increase in soluble interleukin-2 receptor diminishing a clinical response to infliximab in rheumatoid arthritis: comment on the article by Klaasen et al. Arthritis Rheum. 2011;63(9):2833–4; author reply 2834.
43. Tomala J, et al. In vivo expansion of activated naive CD8+ T cells and NK cells driven by complexes of IL-2 and anti-IL-2 monoclonal antibody as novel approach of cancer immunotherapy. J Immunol. 2009;183(8):4904–12.
44. Votavova P, Tomala J, Kovar M. Increasing the biological activity of IL-2 and IL-15 through complexing with anti-IL-2 mAbs and IL-15Ralpha-Fc chimera. Immunol Lett. 2014;159(1–2):1–10.
45. Krieg C, et al. Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. Proc Natl Acad Sci U S A. 2010;107(26):11906–11.
46. Letourneau S, et al. IL-2/anti-IL-2 antibody complexes show strong biological activity by avoiding interaction with IL-2 receptor alpha subunit CD25. Proc Natl Acad Sci U S A. 2010;107(5):2171–6.
47. Levin AM, et al. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. Nature. 2012;484(7395):529–33.
48. Rojas G, et al. Deciphering the molecular bases of the biological effects of antibodies against interleukin-2: a versatile platform for fine epitope mapping. Immunobiology. 2013;218(1):105–13.
49. Rojas G, et al. Fine epitope specificity of antibodies against interleukin-2 explains their paradoxical immunomodulatory effects. MAbs. 2014;6(1):273–85.
50. Carmenate T, et al. Human IL-2 mutein with higher antitumor efficacy than wild type IL-2. J Immunol. 2013;190(12):6230–8.
51. Rotte A, et al. Immunotherapy of melanoma: present options and future promises. Cancer Metastasis Rev. 2015;34(1):115–28.
52. Gillies SD, et al. A low-toxicity IL-2-based immunocytokine retains antitumor activity despite its high degree of IL-2 receptor selectivity. Clin Cancer Res. 2011;17(11):3673–85.
53. Gubbels JA, et al. Ab-IL2 fusion proteins mediate NK cell immune synapse formation by polarizing CD25 to the target cell-effector cell interface. Cancer Immunol Immunother. 2011;60(12):1789–800.
54. Gillessen S, et al. A phase I dose-escalation study of the immunocytokine EMD 521873 (Selectikine) in patients with advanced solid tumours. Eur J Cancer. 2013;49(1):35–44.

55. Klein C. Novel CEA-targeted IL-2 variant immunocytokine for immunotherapy of cancer. J ImmunoTherapy Cancer. 2014;2(Suppl 2):18.
56. Hank JA, et al. Immunogenicity of the hu14.18-IL2 immunocytokine molecule in adults with melanoma and children with neuroblastoma. Clin Cancer Res. 2009;15(18):5923–30.
57. Shusterman S, et al. Antitumor activity of hu14.18-IL2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study. J Clin Oncol. 2010;28(33):4969–75.
58. Sharma S, et al. Development of inhalational agents for oncologic use. J Clin Oncol. 2001;19(6):1839–47.
59. Minchinton AI, Tannock IF. Drug penetration in solid tumours. Nat Rev Cancer. 2006;6(8):583–92.
60. Gagnadoux F, et al. Aerosolized chemotherapy. J Aerosol Med Pulm Drug Deliv. 2008;21(1):61–70.
61. Merimsky O, et al. Targeting pulmonary metastases of renal cell carcinoma by inhalation of interleukin-2. Ann Oncol. 2004;15(4):610–2.
62. Lorenz J, et al. Phase I trial of inhaled natural interleukin 2 for treatment of pulmonary malignancy: toxicity, pharmacokinetics, and biological effects. Clin Cancer Res. 1996;2(7):1115–22.
63. Guma SR, et al. Aerosol interleukin-2 induces natural killer cell proliferation in the lung and combination therapy improves the survival of mice with osteosarcoma lung metastasis. Pediatr Blood Cancer. 2014;61(8):1362–8.
64. Guma SR, et al. Natural killer cell therapy and aerosol interleukin-2 for the treatment of osteosarcoma lung metastasis. Pediatr Blood Cancer. 2014;61(4):618–26.
65. Huang GX, et al. Genetically modified T cells targeting interleukin-11 receptor alpha-chain kill human osteosarcoma cells and induce the regression of established osteosarcoma lung metastases. Cancer Res. 2012;72(1):271–81.
66. Atkins MB, et al. Randomized phase II trial of high-dose interleukin-2 either alone or in combination with interferon alfa-2b in advanced renal cell carcinoma. J Clin Oncol. 1993;11(4):661–70.
67. Sparano JA, et al. Randomized phase III trial of treatment with high-dose interleukin-2 either alone or in combination with interferon alfa-2a in patients with advanced melanoma. J Clin Oncol. 1993;11(10):1969–77.
68. Keilholz U, et al. Results of interleukin-2-based treatment in advanced melanoma: a case record-based analysis of 631 patients. J Clin Oncol. 1998;16(9):2921–9.
69. van Herpen CM, et al. Immunochemotherapy with interleukin-2, interferon-alpha and 5-fluorouracil for progressive metastatic renal cell carcinoma: a multicenter phase II study. Dutch Immunotherapy Working Party. Br J Cancer. 2000;82(4):772–6.
70. Tarhini AA, et al. A phase 2 trial of sequential temozolomide chemotherapy followed by high-dose interleukin 2 immunotherapy for metastatic melanoma. Cancer. 2008;113(7):1632–40.
71. Panares RL, Garcia AA. Bevacizumab in the management of solid tumors. Expert Rev Anticancer Ther. 2007;7(4):433–45.
72. Bakker AB, et al. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med. 1994;179(3):1005–9.
73. Smith FO, et al. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res. 2008;14(17):5610–8.
74. Kaufman HL, et al. Phase II trial of Modified Vaccinia Ankara (MVA) virus expressing 5T4 and high dose interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma. J Transl Med. 2009;7:2.
75. Block MS, et al. Pilot study of granulocyte-macrophage colony-stimulating factor and interleukin-2 as immune adjuvants for a melanoma peptide vaccine. Melanoma Res. 2011;21(5):438–45.
76. Dudley ME, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008;26(32):5233–9.

77. Dudley ME, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002;298(5594):850–4.
78. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol. 2012;12(4):269–81.
79. Radvanyi LG, et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clin Cancer Res. 2012;18(24):6758–70.
80. Besser MJ, et al. Minimally cultured or selected autologous tumor-infiltrating lymphocytes after a lympho-depleting chemotherapy regimen in metastatic melanoma patients. J Immunother. 2009;32(4):415–23.
81. Besser MJ, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clin Cancer Res. 2010;16(9):2646–55.
82. Ellebaek E, et al. Adoptive cell therapy with autologous tumor infiltrating lymphocytes and low-dose interleukin-2 in metastatic melanoma patients. J Transl Med. 2012;10:169.
83. Huang G, et al. Genetically modified T cells targeting interleukin-11 receptor alpha-chain kill human osteosarcoma cells and induce the regression of established osteosarcoma lung metastases. Cancer Res. 2012;72(1):271–81.
84. Garg TK, et al. Highly activated and expanded natural killer cells for multiple myeloma immunotherapy. Haematologica. 2012;97(9):1348–56.
85. Fujisaki H, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Res. 2009;69(9):4010–7.
86. Denman CJ, et al. Membrane-bound IL-21 promotes sustained ex vivo proliferation of human natural killer cells. PLoS One. 2012;7(1):e30264.
87. Krause SW, et al. Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase i trial. Clin Cancer Res. 2004;10(11):3699–707.
88. Parkhurst MR, et al. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression. Clin Cancer Res. 2011;17(19):6287–97.
89. Ruggeri L, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097–100.
90. Miller JS, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. Blood. 2005;105(8):3051–7.
91. Curti A, et al. Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. Blood. 2011;118(12):3273–9.
92. Iliopoulou EG, et al. A phase I trial of adoptive transfer of allogeneic natural killer cells in patients with advanced non-small cell lung cancer. Cancer Immunol Immunother. 2010;59(12):1781–9.

Chapter 3
Optimizing Radiotherapy with Immunotherapeutic Approaches

Jonathan E. Schoenhals, Tijana Skrepnik, Ugur Selek, Maria A. Cortez, Ailin Li, and James W. Welsh

Abstract Several factors must be considered to successfully integrate immunotherapy with radiation into clinical practice. One such factor is that concepts arising from preclinical work must be tested in combination with radiation in preclinical models to better understand how combination therapy will work in patients; examples include checkpoint inhibitors, tumor growth factor-beta (TGF-β) inhibitors, and natural killer (NK) cell therapy. Also, many radiation fields and fractionation schedules typically used in radiation therapy had been standardized before the introduction of advanced techniques for radiation planning and delivery that account for changes in tumor size, location, and motion during treatment, as well as uncertainties introduced by variations in patient setup between treatment fractions. As a result, radiation therapy may involve the use of large treatment volumes, often encompassing nodal regions that may not be irradiated with more conformal techniques. Traditional forms of radiation in particular pose challenges

J.E. Schoenhals • M.A. Cortez, Ph.D.
Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

T. Skrepnik, M.D.
Department of Radiation Oncology, University of Arizona, Tucson, AZ, USA

U. Selek, M.D.
Department of Radiation Oncology, Koc University, Istanbul, Turkey

Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Anderson Central (Y2.5316), 1515 Holcombe Blvd., Unit 0097, Houston, TX 77030, USA

A. Li, M.D., Ph.D.
Department of Radiation Oncology, The First Hospital of China Medical University, Shenyang, China

Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

J.W. Welsh, M.D. (✉)
Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Anderson Central (Y2.5316), 1515 Holcombe Blvd., Unit 0097, Houston, TX 77030, USA
e-mail: jwelsh@mdanderson.org

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for combination trials with immunotherapy. This chapter explores these issues in more detail and provides insights as to how radiation therapy can be optimized to combine with immunotherapy.

Keywords Radiation • Immunotherapy • SABR • SBRT • CTLA4 • PD1 • TGF-β • CD8+ T-cell • Clinical trial

Radiation was first used to treat cancer by Emil H. Grubbe in 1896. Since that time, it has become a major component of cancer treatment. More than half of all patients with cancer will be treated with radiation at some point during the course of the disease. Many forms of radiation therapy involve daily doses of relatively small fractions (e.g., 1.8–2 Gy), but advances in the technology used to plan and deliver radiation therapy allows the delivery of high radiation doses to tumors while sparing the nearby normal tissue. One such technique, stereotactic ablative radiation therapy (SABR), can produce local control rates of 98% and overall survival rates of 55% for patients with inoperable stage I lung cancer [1]; however, neither SABR nor other forms of radiation on its own can control distant disease (metastases) in patients with stage IV disease. Radiation can induce antitumor effects outside the radiation field (i.e., abscopal effects), but such reactions are rare and require a functioning immune system [2]. That radiation affects aspects of immune function is clear; the potential for inducing abscopal (systemic) effects with an essentially local form of therapy underscores the importance of clarifying more precisely how radiation modulates the immune system to identify which forms of immunotherapy will provide synergistic effects with radiation.

3.1 Checkpoints and Radiation Therapy

3.1.1 Checkpoint Inhibitors

Checkpoint inhibitors are a group of immune system molecules that act as negative regulators to help maintain a balance between autoimmunity and immune response. The most clinically relevant examples of checkpoint inhibitors include cytotoxic T lymphocyte associated protein 4 (CTLA4) and programmed cell death protein-1/programmed cell death ligand-1 (PD1/PDL1).

CTLA4 is a receptor found on mature, activated CD4+ and CD8+ T-cells and interacts with the B7-1(2)/CD80(86) ligand on antigen-presenting cells. It shares this ligand with its co-stimulatory molecule, CD28, which is also found on CD4+ and CD8+ T-cells. Once tumor antigens are recognized by antigen-presenting cells, they are presented via major histocompatibility complexes (MHCs) to T cells, which recognize the antigen by means of T-cell receptors (TCRs). Subsequently,

CD28 binds its ligand B7-1(2)/CD80(86) and activates the T cells. Downstream effects include CD25 induction followed by activation of the interleukin-2 (IL-2) receptor, which ultimately induces differentiation and survival of $T_{effector}$ cells. However, CTLA4 has a much higher binding affinity for the shared ligand, and it can outcompete CD28 for this position, causing the opposite effect. When activated, CTLA4 dampens T-cell activation and response by suppressing IL-2 production and T-cell proliferation. CTLA4 functions early during T-cell activation and can also attenuate signaling by kinases such as the PI3K/AKT pathway, which is originally activated by TCRs and CD28 [3]. Further, the CTLA4 receptor is constitutively expressed on regulatory T-cells (T_{regs}), and its activation can enhance their proliferation. These immunosuppressive cells function to directly and negatively control dendritic cell (DC) maturation as well as downregulating B7 expression on DCs to block the immunostimulatory signal of CD28:B7 [4, 5]. Ultimately, a lopsided balance in favor of T_{reg} versus T_{helper}/$T_{effector}$ cells results in T-cell anergy and tolerance.

In both preclinical and clinical models, radiation and CTLA4 blockade mediate synergistic effects that culminate in systemic clearance of tumor outside the radiation field (the "abscopal" or "bystander" effect [6–8]). The term abscopal comes from the Latin *ab* meaning [to position] "away from" and *scopus* referring to the target. Abscopal effects are consistent with increased release of tumor-associated antigens, resulting in tumor antigen-specific T-cells that infiltrate tumors both locally and distantly. The addition of CTLA4 blockade to radiation releases the inhibition on the immunostimulatory interaction of CD28 and its ligand B7-1, an interaction that improves T-cell activation and effector-cell generation. The combination of the two therapies creates a larger pool of effectively primed T-cells and creates an environment where they can proliferate without CTLA4 inhibition. The primed T-cells subsequently migrate, recognize, and attack tumor cells at distant sites, occasionally leading to eradication of systemic disease. This combination does not produce abscopal effects in all patients; however, additional studies are needed to determine which combinations of immunotherapies with radiation will result in systemic immunity. Because radiation therapy is traditionally considered a local therapy, the combination of immunotherapy and radiation therapy is in effect converting a local therapy modality into a systemic therapy [9, 10].

The optimal radiation dose for T-cell priming via tumor-associated antigens is still under investigation; however, one group suggested that hypofractionation, specifically three fractions of 8 Gy each, was the most effective at secondary tumor control compared with other fractionation schemes, such as one fraction of 20 Gy or five fractions of 6 Gy [6]. Another group, evaluating the effects of fractionation on local tumor control, showed that treatment with two fractions of 7.5 Gy each maintained lower splenocyte T_{reg} levels than did other fractionation schemes such as five fractions of 3 Gy, three fractions of 5 Gy, and 1 fraction of 15 Gy. However, no significant differences in local control were found between any of the treatment conditions [11]. Another study supports the notion that single ablative doses may be better for local tumor control. In that study, a single fraction of 30 Gy was able to eradicate most of the tumors in the CT26 murine model. However, when the mice

were given an additional 30 Gy in 10 fractions (3 Gy each, 60 Gy total), most mice experienced tumor regrowth and death [12]. This group further showed that the 30 Gy doses induced high CD8+ infiltration, whereas the additional fractionated 30 Gy caused recruitment of myeloid-derived suppressor cells to the tumor. This discrepancy in results may have been related to differences in the tumor models used in the various studies; however, they all suggest that SABR-like doses may be effective at invoking antitumor immunity. Notably, however, none of these studies tested conventional 2-Gy-per-fraction schemes. At this time, the abscopal effect remains elusive, but the hope is that such responses will become more common as the search continues for the optimal dosage and timing in terms of patient response and toxicity, and as more data become available [13].

PD1 is another immune checkpoint receptor that is expressed by T-cells, B cells, NK cells, DCs, and macrophages to help negatively regulate the immune response [14]. The PD1/PDL1 pathway acts differently from CTLA4, as it inhibits T-cell activity in the effector phase within peripheral tissues and tumors [3, 15]. Once the TCR recognizes antigen, the activated T-cell ultimately upregulates PD1 to dampen its own activation. PD1 has a multitude of immunosuppressive effects, including inhibition of T-cell proliferation, survival, and effector functions; cytokine release; cytotoxicity; induction of apoptosis of tumor-specific T-cells; and promotion of differentiation of CD4+ cells into T_{regs} [16, 17]. Therefore, upregulation of PD1 on immune cells and expression of PDL1 on tumors tends to promote tumor immune evasion.

Although radiation can modify the local tumor microenvironment, as discussed below, tumors can also exploit regulatory mechanisms to undermine T-cell responses. For example, tumor cells that express PDL1 can successfully undergo immunoevasion when that ligand interacts with its receptor PD1 on effector T-cells, which then mediates apoptosis of the infiltrating T-cells [18]. Interestingly, a negative feedback loop has been discovered in which effector T-cells in the tumor's microenvironment produce interferon-gamma (IFN-y), which directly initiates PDL1 expression and then downregulates effector T-cells via the mechanism described above [19]. Several reports support this hypothesis, including a recent human melanoma study demonstrating strong correlations among intratumoral T-cell infiltration, PDL1, and IFN-γ [20]. Other studies have shown that fractionated radiation increases the expression of PDL1 by the tumor in response to effector CD8+ T-cell production of IFN-γ and that combining PD1/PDL1 inhibitors concurrently with radiation significantly improved durable systemic immune responses [21]. Another recent preclinical study examined the influence of PD1 expression on the abscopal effect after treatment with PD1/PDL1 blockade and radiation therapy [22]. That group found that the combination of conventional 2 Gy per fraction radiation and PD1/PDL1 blockade produced a curative rate of 66% in the CT26 model, and that concurrent administration of the drug with radiation produces the best effect. As the radiation reaches the tumor, type I IFNs (IFN-α and IFN-β) and IFN-ɣ modulate the microenvironment in ways that upregulate VCAM-1 (which allows T-cell trafficking and entry into the tumor), MHC-I, and chemokines to bring additional immune cells to local sites [23, 24].

3.2 Radiation and Immune Function

Ionizing radiation is known to release "danger-associated molecular patterns" such as upregulation of high mobility group box 1 protein (HMGB1), ATP, and calreticulin, all of which increase immune activation at the tumor site [25]. Also, release and uptake of tumor-associated antigens by antigen-presenting cells in the tumor microenvironment increase the probability of successful T-cell priming (Table 3.1) [36]. Curiously, the immune system seems to be unable to exploit these mechanisms to consistently produce specific, durable immune responses. One possible reason why is that even when T-cells are specifically activated in appropriate lymph nodes, homing and retention of $T_{effector}$ cells into the tumor are limited because of the known ability of tumor cells to restrict the infiltration and activation of cytotoxic T-lymphocytes. This tumor-cell immunoevasion and ultimately immunosuppression is related to specific properties of tumor cells, some of which are highlighted below. General principles by which tumor cells evade the immune system include successful unchecked cell proliferation, de novo angiogenesis, suppression of T-cell penetration into tumor, increasing the ratio or concentration of immunosuppressive cells such as T_{regs}, and suppressing antigen presentation. Indeed, several of the ways by which tumor cells escape immune surveillance are identical to the immunosuppressive effects induced by radiation, particularly recruiting T_{regs} and producing TGF-β (Table 3.1) in addition to stimulating myeloid-derived suppressor cells and alternatively activated macrophages.

Table 3.1 Immunological effects of radiation on the tumor microenvironment

Immunostimulatory effects [26]
• *General*: T-cell priming, trafficking, and effector responses
• Inducing pro-inflammatory cytokines (TNF-α, IL-1B, IL-6) that recruit NK and CD8+ cells to local sites of cancer [27]
• Exposing novel tumor-associated antigens that lead to creation of tumor-specific T-cells [28, 29]
• Increased adhesion molecules such as VCAM-1 for lymphocyte trafficking, dependent on IFN-γ in irradiated tumors [23]
• Increased death receptors in tumor cells, stroma [30]
• Inducing expression of MHC-I molecules [31]
• Calreticulin translocation to surface [32]
• Release of HMGB1 by dying tumor cells → activate dendritic cells via toll-like receptor-4 [33]
Immunosuppressive effects
• Increasing the percentage/ratio of T_{regs}, a more radioresistant immune cell, by preferential killing of more radiosensitive and immunostimulatory cells [11, 34]
• Increasing TGF-β levels [35]

3.2.1 TGF-β

TGF-β is a multifunctional, polypeptide cytokine, and part of a cytokine superfamily that coordinates response to tissue injury and stress. It maintains immune self-tolerance while also assisting cancer cells in evading the host immune system. Of the three major isotypes of TGF-β (TGF-β1, TGF-β2, and TGF-β3), TGF-β1 is expressed mostly in the immune system. It is synthesized as an inactive pre-protein that is cleaved to form two homodimers joined by a disulfide bond. The homodimers interact with latency-associated protein and latent TGF-β-binding protein, which combine to form the so-called large latent complex. Release of free TGF-β requires first that the large latent complex be released, and then that the latency-associated protein be cleaved; this is accomplished in the extracellular matrix by metalloproteinases and thrombospondin. Changes or interactions with integrins can also activate latent TGF-β. Subsequently, free TGF-β binds to its transmembrane serine/threonine kinase heterodimeric receptor (TGFβRI/TGFβRII). Once bound, TGF-β has many downstream effects involved in tissue fibrosis, wound healing, carcinogenesis (e.g., proliferation, differentiation, migration, invasion, the epithelial–mesenchymal transition), and immune suppression [37, 38].

3.2.2 TGF-β and the Immune System

Notably, TGF-β activity differs substantially in early versus late immune responses although the molecular event prompting the "switch" between early and late effects is unclear. Paradoxically, during the early stages of immune response, TGF-β suppresses malignancy directly by stopping tumor cell cycle progression, and indirectly through stromal effects (Table 3.2). However, later during tumorigenesis (i.e., the late stages

Table 3.2 Role of TGF-β in cancer

Early tumor suppressor activity of TGF-β
• *Cell cycle arrest*: Inhibition of c-Myc, which promotes cell cycle entry into S phase (41–43). Inhibition of cyclin D-CDK4/6 and cyclin A/E-CDK2 activity (45, 46).
• *Tumor cell death*: Induction of apoptosis via induction of caspases, downregulation of Bcl-2 apoptotic proteins
Late tumorigenic effects of TGF-β
• *Cell proliferation*: Induction of transcription of CDK inhibitors such as p21 and p16
• *Angiogenesis and metastasis*: Assisting the phenotypic change in tumor-associated macrophages from the M1 phenotype to the alternatively activated M2 phenotype, an anti-inflammatory, pro-angiogenic and pro-metastasis promoting cell
• *Cytokine regulation*: Blocking of IL-2 suppresses NK cells and activated T-cells
• *Decreased antigen presentation*: Downregulating expression of MHC class II, thereby affecting the ability of dendritic cells to present antigens effectively
• *Upregulation of PD-L1* via interferon production
• *Stimulation of immunosuppressive cells*: Tregs that act on tumor antigen-specific T-cells and kill them
• *Induction of more aggressive phenotypes*: Support for the EMT phenotype leading to malignant progression, metastasis, and drug/chemo/radio resistance

of immune response), TGF-β contributes significantly to the immunosuppressive microenvironment in ways that encourage tumor progression, metastasis, resistance to therapy, and an overall poorer prognosis [39]. TGF-β is produced by both hematologic and solid tumor cells, such as breast, colon, liver, and lung and is also upregulated as a result of ionizing radiation. The latter mechanism directly implicates TGF-β in tissue fibrotic processes such as those that lead to radiation pneumonitis.

Another way in which TGF-β regulates a pro-invasive tumor microenvironment is by inducing the epithelial-to-mesenchymal transition. Cancer cells that have gone through this transition acquire stem-cell-like properties like self-renewal and resistance to chemotherapy or radiation [40]. Recent studies have linked suppression of CD8+ tumor-infiltrating lymphocytes with the epithelial-to-mesenchymal transition, which is modulated by a well-known transcription factor, zinc finger E-box binding homeobox 1 (ZEB1) [41]. That study further showed that microRNA-200 negatively targets PDL1 on tumor cells, but ZEB1 antagonizes this interaction by repressing miR-200, ultimately resulting in suppression of tumor-infiltrating lymphocytes and metastasis.

Radiation can also induce TGF-β production, which can have devastating consequences in delicate tissues such as the lung. TGF-β stimulates the formation of tissue collagen while reducing its degradation, which leads to tissue fibrosis. TGF-β has been linked with radiation pneumonitis, a potentially disabling condition that develops in up to 30% of patients who receive radiation therapy for thoracic malignancies. Usually appearing 1–6 months after radiation therapy, radiation pneumonitis manifests as shortness of breath, nonproductive cough, and fever [42], which can cause significant morbidity and, in severe cases, can be lethal. TGF-β may elevated in patients who develop RP; one recent study showed a seven-fold rise in TGF-β among patients who developed pneumonitis after 40 Gy of radiation, peaking at 3 months after therapy [43]. However, dosimetric variables did not predict who would develop pneumonitis in that study, prompting the authors to suggest stratifying patients at risk of developing pneumonitis based on their TGF-β levels before treatment. This topic is controversial, as other studies have shown weak or no correlation between serum TGF-β levels and RP after thoracic radiotherapy [44–46].

3.2.3 Anti-TGF-β

Preclinical efforts and some trials are now ongoing to evaluate whether blocking TGF-β expression or its downstream effects will promote immune surveillance and reverse the environment to a tumor-suppressing phenotype. Various methods of blocking this cytokine including TGF-β receptor kinase inhibitors (LY2109761, currently in preclinical testing for pancreatic cancer), antisense TGF-β oligonucleotides (Trabedersen/AP12009, currently in phase III trials for glioma, and in phase I trials for pancreatic, melanoma, and colorectal carcinoma), antibodies (fresolimumab/GC1008, now in preclinical and clinical evaluations), soluble receptors, and tumor cell vaccines (Lucanix/Belagen-pumatucel, being evaluated in a phase III for non-small cell lung cancer) [38].

3.3 NK Cells and the Tumor Microenvironment

Natural killer (NK) cells are key components of the innate immune system that specialize in eliminating targeT-cells via direct cytotoxicity and release of immunoregulatory cytokines. NK cells targeT-cells that express reduced numbers of MHC class I molecules, or, according to the "missing self" hypothesis, an incompatible or incomplete repertoire of MHC class I molecules [47, 48].

NK cells express numerous activating receptors that engage stress-induced ligands. NKG2D ligands have been found to be upregulated on murine tumor cells after stress-inducing events such as exposure to high doses of ionizing radiation, which can potentiate the antitumor cytotoxicity of both CD8+ T-cells and NK cells [49]. Interestingly, susceptibility to NK cell-mediated cytolysis seems to be enhanced after irradiation of KM12 and HeLa cells via upregulation of the NKG2D ligands MICB, ULBP1, and ULBP2 [50]. These findings suggest that combining radiation and NK cell-based therapy may have additive effects.

3.4 Radiation Dose: High or Low?

In certain settings, radiation can enhance the activity of the immune system against cancer. However, as mentioned previously, the optimal dose and fractionation of that radiation have yet to be clearly defined. Variations in both dose and fractionation have different biological effects that need to be understood to effectively use radiation to induce immune responses. A "one size fits all" solution is unlikely, but different radiation regimens may be needed according to the intrinsic radiosensitivity of a particular tumor, the composition of the tumor microenvironment, and the type of immunotherapy to be combined with the radiation.

Several lines of evidence indicate that low-dose radiation (10–50 cGy per fraction) is effective at enhancing an immune response. Epidemiologic findings have shown that individuals exposed to higher-than-normal levels of background radiation may actually have *lower* cancer mortality than those exposed to lower levels [51–53]. However, this remains controversial as other studies have shown either no effect or more incidence of cancer [54, 55]. One preclinical study showed that exposure of the whole body to low-dose radiation reduced lung metastasis and delayed tumor growth [56]. These findings were attributed to enhanced Th1-like cellular immunity [57–60], such as activated NK cells, DCs, macrophages, and T-cells and decreased numbers of T_{regs} [58, 59, 61]. On the contrary, low-dose radiation was also used as an immune suppressant to treat diseases like rheumatoid arthritis. Nevertheless, the applicability of low-dose radiation in clinical settings is limited.

High-dose radiation such as SABR has been shown to improve local disease control in addition to promoting abscopal regression when given with immunotherapeutic agents [7, 62, 63]. Preclinical studies have shown that ablative radiation can lead to the release of tumor-specific antigens, which then direct antigen-presenting cells to induce a T-cell-dependent response [2, 6, 62, 64, 65]. Moreover,

induction of MHC class I expression on tumor cells may be radiation dose-dependent, with higher doses inducing more MHC-I expression [66].

Some preclinical studies have found that hypofractionated radiotherapy (use of fractions >5 Gy) enhances the effectiveness of immunotherapy [66, 67] although more conventional fractionation can also have similar effects [21]. However, use of conventionally fractionated radiation over several weeks may continuously kill off infiltrating $T_{effector}$ cells, as suggested by a study indicating that single-dose radiation was more effective than fractionated radiation or chemotherapy given with radiation [68]. Indeed, as mentioned earlier, another preclinical study involving the CT26 colon cancer model showed that an initial 30-Gy radiation dose resulted in $CD8^+$ T-cell infiltration, but giving another 30 Gy in 10 fractions after initial treatment led to decreases in CD8+ T-cells and increases in myeloid-derived suppressor cells [12]. Although a single 30 Gy dose is not clinically relevant, these findings do suggest that higher radiation doses given in fewer fractions may result in better immune responses. On the other hand, another group found that fractionated and not single-dose radiation induced an immune-mediated abscopal effect in combination with an anti-CTLA4 antibody [6]. Notably, the fractionated doses in this study were ≥6 Gy, which is considered high-dose radiation; however, no low-dose fractions were tested.

In conclusion, radiation dose and fractionation are clearly important factors in activating an immune response, but the radiation regimens will necessarily differ depending on the tumor type. Additional investigations of which dose and fractionation schemes best enhance the immune system are needed if this type of therapy is to be effectively extended to patients.

3.5 Sequence of Radiation and Immunotherapy Combinations

Because radiation and immunotherapeutics have different mechanisms of action against cancer, their rational combination may well achieve excellent antitumor effects with few side effects. The question of which sequence is best is urgent, as it will dictate clinical practice when these therapies are extended to patients. However, because retrospective analyses of various combinations of radiation and ipilimumab have produced controversial and inadequate results (as noted below), prospective studies are needed to address this question.

In one retrospective analysis of 166 patients with metastatic melanoma given radiation therapy and ipilimumab, the median overall survival time was 9 months for patients who had concurrent radiotherapy and ipilimumab (radiation was given between first and fourth doses of ipilimumab) and 39 months in patients who received radiotherapy after the last dose of ipilimumab [69]. In a separate retrospective trial of 46 patients with melanoma brain metastases, patients who received stereotactic radiosurgery during or before ipilimumab had better overall survival and less regional recurrence than did patients who received stereotactic radiosurgery after ipilimumab [70]. Indeed, most of the trials investigating ipilimumab before, during,

or after radiation to date have not revealed significant differences among treatment groups [71–74]; however, these trials were not designed to address the issue of sequencing. Another preclinical study also did not reveal significant differences between concurrent vs. sequential combinations of radiation and ipilimumab [75].

In contrast to ipilimumab, most preclinical studies that combine radiation with anti-PD1/PDL1 antibodies have given the two modalities concurrently; this approach has generated strong systemic antitumor effects [64, 76]. One preclinical study that did consider differences between concurrent versus sequential anti-PDL1 therapy and radiation found that anti-PD1 seemed to work better when given before the radiation is completed [21]. Because radiation induces PDL1 expression in the tumor microenvironment [64, 76], anti-PD1/PDL1 drugs given during the radiation should achieve the best synergistic effect.

Thus, although the doses and fractionation schedules of radiation seem to be important when radiation is to be combined with immunotherapy, few preclinical studies have compared various sequences of radiation doses or fractions to determine which doses and fractions achieve optimal antitumor immunity. This should be a focus in future preclinical investigation, as it will provide guidance for clinical trial designs.

3.6 Clinical Integration

Radiation therapy as currently used in the clinic has largely been optimized for local therapy, and historically it has been hampered by older imaging and radiation-delivery techniques that cannot precisely locate tumors, especially tumors that move, and rely on low-energy photon beams. Indeed, many of the techniques in current use for local control may not be optimal in terms of producing systemic immunologic responses. This section focuses on the implications of using different radiation field sizes with immunotherapy and how to identify the optimal sequencing of immunotherapy and radiation. A great many other important clinical issues also remain to be considered (e.g., appropriate radiation doses, use of induction chemotherapy, the number of sites to be irradiated, differences in T-cell priming based on the location of metastatic lesions [e.g., bone versus liver]), but these considerations are beyond the scope of this chapter.

3.6.1 Radiation Field Size

The highly conformal techniques used to plan and deliver SABR have several advantages when SABR is to be used in combination with immunotherapy. First, SABR treatment volumes are usually quite small, often directed to relatively small metastatic lesions that are typically safe distances from critical structures such as lung or heart. Because immunotherapy has its own forms of toxicity (e.g., colitis and pneumonitis), the ability to treat small volumes has inherent advantages in terms of nonadditive toxicity. Smaller volumes may also spare the draining lymphatics,

which are important for T-cell education and priming. Also, the often-ablative doses used with SABR have unique immunologic effects on the tumor that may also be beneficial for eradicating the tumor stroma.

Despite these advantages, and the excellent local control possible with SABR, most patients with locally advanced disease require much larger treatment volumes that often encompass the tumor, involved lymphatics, and possibly other high-risk lymphatics. Although sterilizing draining lymphatics may be beneficial in terms of tumor control, it can also have detrimental effects on the host's immunologic response against the tumor, because newly exposed tumor neoantigens are delivered to the lymph nodes, where T-cell priming takes place. Moreover, treating larger fields often requires prolonged courses of fractionated radiation, for example, giving 2-Gy fractions daily for 30–35 days. Thus, the antigen-presenting cells and the T-cells in the lymph node and in the tumor are essentially eradicated daily for 6–7 weeks. Further improvements in local control for locally advanced disease often require that radiation be combined with radiosensitizing agents. This poses further immunologic challenges because chemotherapy can cause myelosuppression and deplete lymphocytes [77]. Moreover, chemotherapy is often given with steroids to minimize the unpleasant side effects of chemotherapy; steroids can prevent T-cell priming, but they may not affect previously activated T-cells [78]. Finally, the large fields needed to treat locally advanced disease often approach the dose-volume limits necessary to minimize radiation-induced toxicity to normal tissues, and adding concurrent immunotherapy agents has a higher potential for toxicity, especially when the radiation involves organs with known inflammatory-mediated side effects from immunotherapy, such as the lung or bowel.

Radiation treatment volume also matters in terms of its hematologic effects; in one study [79], the cumulative incidence of grade 2 leukopenia among 27 men who received whole-pelvis irradiation (to 46 Gy) for prostate cancer was higher (15% vs. 2% without pelvic irradiation, $P = 0.02$), as was grade 2 anemia (8% vs. 0% without pelvic irradiation, $P = 0.03$). Because whole-pelvis radiation therapy may be more detrimental hematologically than prostate-only radiotherapy, and because neoadjuvant hormonal therapy reduces hemoglobin levels, one might speculate that negative effects on functioning immune system and tumor oxygenation could explain the disappointing results of RTOG 9413, which enrolled 1323 patients to compare whole-pelvis radiation versus prostate-only radiation therapy [80]. On the other hand, the immunostimulatory effects of radiotherapy (e.g., tumor cell death, changes in antigen availability and inflammatory signals) could have activated lymphocytes and DCs [81]. These and other questions remain to be answered in future trials designed specifically to examine the effects of radiation on the immune system.

3.6.2 Sequence

The optimal sequencing of immunotherapy and radiation therapy is another important consideration that will need to be worked out specifically for each type immunotherapy, as discussed previously. When this chapter was written, only two

checkpoint inhibitors had been approved by the US Food and Drug Administration—anti-CTLA4 and anti-PD1. The need to determine the best sequencing will undoubtedly become increasingly challenging as more immunotherapies enter the clinic in the future because each new mechanism of action will need to be evaluated in the context of how to best combine each agent with radiation.

Perhaps the most extensive experience to date comes from the anti-CTLA4 agent ipilimumab, the first approved checkpoint inhibitor. The rationale for combining anti-CTLA4 with radiation is that radiation can increase the T-cell repertoire and diversity in a tumor, and blocking CTLA4 can promote the expansion of these newly activated T-cells [75]. This rationale also seems to favor the use of concurrent chemotherapy. Yet ipilimumab can also reduce inhibitor T_{reg} populations, and thus pretreatment with ipilimumab could potentially "precondition" the tumor by increasing the CD8/T_{reg} ratio, enabling a more robust T-cell response.

Several case reports have described abscopal responses when ipilimumab is used with radiation therapy; these reports may offer clues as to how this strategy works and the optimal sequencing of these two forms of treatment. In one report, a patient with metastatic non-small cell lung cancer received five fractions of 6 Gy each, starting concurrent ipilimumab on the day after the first fraction; this patient demonstrated an impressive abscopal response that lasted for several months [82]. However, another case report described a patient with metastatic melanoma that had progressed on ipilimumab; in that case, 28.5 Gy was delivered in three fractions to a paraspinal mass [7]. Several months later, that patient developed a systemic abscopal response in the liver, chest, and spleen. Thus giving radiation after disease has progressed on ipilimumab (which can deplete T_{regs}) may have distinct advantages and is the topic of a phase I/II trial testing ipilimumab with SABR for advanced solid tumors (NCT02239900). This trial will evaluate the timing of radiation during ipilimumab therapy (concurrent versus sequential) and will also evaluate different radiation doses and the effect of irradiating metastatic disease at different sites.

Experience with radiation plus anti-PD1/PDL1 therapies is becoming more prevalent as well. Several trials are underway to evaluate this combination, with most starting the anti-PD1 and the radiation at about the same times. Alternatively, other trials are evaluating checkpoint inhibition as adjuvant therapy after radiation for patients who require larger radiation fields (e.g., those with stage III non-small cell lung cancer or mesothelioma). This approach may prove to be safer by reducing the risk of pneumonitis. Radiation has also been shown to increase PDL1 expression in myeloid cells, which could further justify its use as an adjuvant to immunotherapy [76].

3.7 Clinical Trials of Radiation Plus Immunotherapy

3.7.1 Anti-CTLA4 Therapy

Historically, radiation was developed as means of providing local tumor control; immunotherapy, on the other hand, has been tested mainly for patients with metastatic disease, as a final option for tumors that have not responded to conventional

treatments [83–87]. Clinical synergy between these two modalities has been shown in multiple studies of ipilimumab and concurrent radiation given for metastatic melanoma [7, 88]. Another group found mainly partial abscopal responses in 9 (43%) of 21 patients with melanoma, with 2 patients (10%) remaining stable [89]. Combining immune checkpoint blockade with radiation therapy has also shown promise in metastatic prostate cancer; in one phase I/II study of 50 men who received 4- to 10-mg/kg doses of ipilimumab plus 8-Gy fractions to each metastatic lesion [90], one patient had a complete response and six had stabilized disease; these results led to a randomized phase II trial (NCT01689974). Ipilimumab plus SABR is further being tested in several phase II trials for patients with stage IV melanoma and any number of metastases (NCT01970527, NCT02107755, NCT01565837).

In one nonrandomized phase I/II trial of 71 men with metastatic castration-resistant prostate cancer who had experienced disease progression after discontinuing antiandrogen therapy, ipilimumab as monotherapy ($n = 29$) was compared with ipilimumab plus a single radiation dose of 8 Gy ($n = 41$) per bone metastasis, given 24–48 h before the first ipilimumab dose (3 or 10 mg/kg) [90]. Of men with 28 evaluable tumors, ipilimumab at 10 mg/kg, with or without radiation, led to reductions in prostate-specific antigen levels of at least 50% in eight men, and another had a complete response. The phase III randomized, double-blind trial for men with metastatic castrate-resistant prostate cancer that had progressed after docetaxel, involved giving a bone-directed single 8-Gy dose of radiation followed by either ipilimumab 10 mg/kg ($n = 399$) or placebo ($n = 400$) every 3 weeks for up to 4 doses. Results from that trial showed a slightly longer median overall survival time of 11.2 months for men who received ipilimumab versus 10 months for the placebo group ($P = 0.053$); however, this trial did not reach a significant difference between the two cohorts [91]. Ipilimumab is also being tested with cetuximab and intensity-modulated radiation therapy in a phase Ib trial for patients with previously untreated stage III-IVB head and neck cancer (NCT01935921).

3.7.2 Anti-PD1 Therapy

Several trials are ongoing to test anti-PD1 with radiation therapy for patients with melanoma or non-small cell lung cancer that did not respond to at least one regimen of systemic therapy or anti-PD-1 therapy. NCT02608385 is a phase I study of PD1 blockade with pembrolizumab and SABR for advanced solid tumors at the University of Chicago; NCT02303990 or "RADVAX" is a stratified phase I trial of pembrolizumab with hypofractionated radiation therapy for advanced and metastatic cancers at the Abramson Cancer Center of the University of Pennsylvania; and NCT02318771 is a trial of hypofractionated radiotherapy with pembrolizumab for recurrent/metastatic head and neck cancer, renal cell carcinoma, lung cancer, or melanoma.

Immunotherapy with radiation is also being tested to improve long-term local control of high-grade glial tumors. Ongoing trials include a multicenter, open-label nonrandomized phase II trial of MEDI4736 for patients with newly diagnosed, unmethylated MGMT glioblastoma in which patients are given anti-PDL1 (durvalumab)

every 2 weeks with standard radiation therapy (NCT02336165); and a phase I trial of PD1 blockade with pembrolizumab and bevacizumab with 5 days of hypofractionated stereotactic radiotherapy for recurrent high-grade glial tumors has been initiated at the H. Lee Moffitt Cancer Center and Research Institute (NCT02313272).

The PD1 inhibitor pembrolizumab is currently being tested in a phase II trial for patients with surgically resectable squamous cell carcinoma of the head and neck; pembrolizumab is given intravenously approximately 2 or 3 weeks before surgery to be followed by risk-based intensity-modulated radiation therapy to 60 Gy (in daily 2-Gy fractions, NCT02296684); it is also being tested for patients getting reirradiation with inoperable locoregionally recurrent or second primary squamous cell carcinoma (NCT02289209). Another phase I single-arm, open-label trial is testing pembrolizumab in combination with cisplatin-based standard definitive chemoradiation therapy (to a total dose of 70 Gy, in daily 2-Gy fraction) for patients with stage III-IVB squamous cell carcinoma of the head and neck; the accrual goal of that trial is about 39 patients (NCT02586207).

Colorectal cancer, especially tumors of the rectum or anal cancer, has long been treated effectively by radiation therapy; whether immunotherapy could provide a stronger immune response to help destroy tumor cells is being investigated in a phase II trial of pembrolizumab plus radiotherapy or ablation for metastatic colorectal cancer (NCT02437071).

3.7.3 Cytokine Therapy

Intralesional injections of IFN-β (3–5 million units, 3 times/week, before radiation to a total dose of 40–60 Gy) has shown mixed results [92–94]. In one such trial, all 21 patients with metastatic melanoma showed either a complete remission (70%) or a partial remission, with a median survival time of 10 months [95]. In another phase I trial, SABR (one, two, or three doses of 20 Gy/fraction) is followed by high-dose IL-2 at 3 days after the last radiation fraction for metastatic melanoma or renal cell carcinoma. That regimen produced antitumor responses as defined by the Response Evaluation Criteria for Solid Tumors (one complete response and seven partial responses) in nonirradiated target lesions [96]. Ongoing trials combining concurrent high-dose IL-2 with radiation therapy for either renal cell carcinoma or melanoma are focusing on the immunological effects of this treatment [97, 98].

3.7.4 OX40 Therapy

Use of OX40 agents with radiation for breast cancer has been delayed somewhat given the prevalence of open trials of the monoclonal antibodies trastuzumab or pertuzumab for HER2-positive breast cancer. However, a phase I/II study is underway to test the anti-OX40 agent MEDI6469 in combination with SABR for

metastatic liver or lung lesions in patients with progressive metastatic breast cancer (NCT01862900). Another phase I/II trial now ongoing is testing an OX40 agent, which is thought to induce proliferation of memory and effector T-cells, in combination with cyclophosphamide and radiation therapy for patients with progressive metastatic prostate cancer (NCT01303705).

3.8 Conclusions

The combination of immunotherapy plus radiation has great potential to extend the benefit of radiation beyond its current role of local control. As we go forward, we need to consider how our current radiation techniques are best combined with immunotherapy, and in some cases we will likely need to develop unique fields and dosing regimens to expand radiation benefit into new patient populations. Pretreatment assessment of a patient's immune state will become important, as well as minimizing immunosuppressive agents such as steroids. Moving past checkpoint inhibitors we will need to personalize immunotherapy toward patient-specific mechanisms of resistance and XRT-specific immunotherapies. More preclinical studies are needed to evaluate these questions and establish the safety of combination therapy. By working to refine and perfect rational combinations of immunotherapy and radiation we have an opportunity to improve the lives of many patients.

References

1. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. JAMA. 2010;303:1070–6.
2. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys. 2004;58:862–70.
3. Santarpia M, Gonzalez-Cao M, Viteri S, Karachaliou N, Altavilla G, Rosell R. Programmed cell death protein-1/programmed cell death ligand-1 pathway inhibition and predictive biomarkers: understanding transforming growth factor-beta role. Transl Lung Cancer Res. 2015;4:728–42.
4. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008;322:271–5.
5. Qureshi OS, Zheng Y, Nakamura K, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011;332:600–3.
6. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. Clin Cancer Res. 2009;15:5379–88.
7. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366:925–31.
8. Stamell EF, Wolchok JD, Gnjatic S, Lee NY, Brownell I. The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys. 2013;85:293–5.
9. Seyedin SN, Schoenhals JE, Lee DA, et al. Strategies for combining immunotherapy with radiation for anticancer therapy. Immunotherapy. 2015;7(9):967–80.

10. Tang C, Wang X, Soh H, et al. Combining radiation and immunotherapy: a new systemic therapy for solid tumors? Cancer Immunol Res. 2014;2:831–8.
11. Schaue D, Ratikan JA, Iwamoto KS, McBride WH. Maximizing tumor immunity with fractionated radiation. Int J Radiat Oncol Biol Phys. 2012;83:1306–10.
12. Filatenkov A, Baker J, Mueller AM, et al. Ablative tumor radiation can change the tumor immune cell microenvironment to induce durable complete remissions. Clin Cancer Res. 2015;21:3727–39.
13. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. Cancer Treat Rev. 2015;41:503–10.
14. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012;24:207–12.
15. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.
16. Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. J Clin Invest. 2015;125:3384–91.
17. Francisco LM, Salinas VH, Brown KE, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206:3015–29.
18. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793–800.
19. Lee SJ, Jang BC, Lee SW, et al. Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274). FEBS Lett. 2006;580:755–62.
20. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Science Translational Medicine. 2012;4:127ra37.
21. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. Cancer Res. 2014;74:5458–68.
22. Park SS, Dong H, Liu X, et al. PD-1 restrains radiotherapy-induced abscopal effect. Cancer Immunol Res. 2015;3:610–9.
23. Lugade AA, Sorensen EW, Gerber SA, Moran JP, Frelinger JG, Lord EM. Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. J Immunol. 2008;180:3132–9.
24. Wan S, Pestka S, Jubin RG, Lyu YL, Tsai YC, Liu LF. Chemotherapeutics and radiation stimulate MHC class I expression through elevated interferon-beta signaling in breast cancer cells. PLoS One. 2012;7:e32542.
25. Burnette B, Fu YX, Weichselbaum RR. The confluence of radiotherapy and immunotherapy. Front Oncol. 2012;2:143.
26. Kwilas AR, Donahue RN, Bernstein MB, Hodge JW. In the field: exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer. Front Oncol. 2012;2:104.
27. Matsumura S, Wang B, Kawashima N, et al. Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol. 2008;181:3099–107.
28. Melcher A, Gough M, Todryk S, Vile R. Apoptosis or necrosis for tumor immunotherapy: what's in a name? J Mol Med (Berl). 1999;77:824–33.
29. Kotera Y, Shimizu K, Mule JJ. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immunization. Cancer Res. 2001;61:8105–9.
30. McBride WH, Chiang CS, Olson JL, et al. A sense of danger from radiation. Radiat Res. 2004;162:1–19.
31. Friedman EJ. Immune modulation by ionizing radiation and its implications for cancer immunotherapy. Curr Pharm Des. 2002;8:1765–80.
32. Obeid M, Tesniere A, Ghiringhelli F, et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med. 2007;13:54–61.
33. Apetoh L, Ghiringhelli F, Tesniere A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007;13:1050–9.

34. Kachikwu EL, Iwamoto KS, Liao YP, et al. Radiation enhances regulatory T cell representation. Int J Radiat Oncol Biol Phys. 2011;81:1128–35.
35. Canney PA, Dean S. Transforming growth factor beta: a promotor of late connective tissue injury following radiotherapy? Br J Radiol. 1990;63:620–3.
36. Kaur P, Asea A. Radiation-induced effects and the immune system in cancer. Front Oncol. 2012;2:191.
37. Wrzesinski SH, Wan YY, Flavell RA. Transforming growth factor-beta and the immune response: implications for anticancer therapy. Clin Cancer Res. 2007;13:5262–70.
38. Hawinkels LJ, Ten Dijke P. Exploring anti-TGF-beta therapies in cancer and fibrosis. Growth Factors. 2011;29:140–52.
39. Ivanovic V, Todorovic-Rakovic N, Demajo M, et al. Elevated plasma levels of transforming growth factor-beta 1 (TGF-beta 1) in patients with advanced breast cancer: association with disease progression. Eur J Cancer. 2003;39:454–61.
40. Zhang P, Sun Y, Ma L. ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. Cell Cycle. 2015;14:481–7.
41. Chen L, Gibbons DL, Goswami S, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun. 2014;5:5241.
42. Chen Y, Williams J, Ding I, et al. Radiation pneumonitis and early circulatory cytokine markers. Semin Radiat Oncol. 2002;12:26–33.
43. Li J, Mu S, Mu L, Zhang X, Pang R, Gao S. Transforming growth factor-beta-1 is a serum biomarker of radiation-induced pneumonitis in esophageal cancer patients treated with thoracic radiotherapy: preliminary results of a prospective study. Onco Targets Ther. 2015;8:1129–36.
44. Evans ES, Kocak Z, Zhou SM, et al. Does transforming growth factor-beta1 predict for radiation-induced pneumonitis in patients treated for lung cancer? Cytokine. 2006;35:186–92.
45. Stenmark MH, Cai XW, Shedden K, et al. Combining physical and biologic parameters to predict radiation-induced lung toxicity in patients with non-small-cell lung cancer treated with definitive radiation therapy. Int J Radiat Oncol Biol Phys. 2012;84:e217–22.
46. Wang J, Qiao XY, Lu FH, et al. TGF-beta1 in serum and induced sputum for predicting radiation pneumonitis in patients with non-small cell lung cancer after radiotherapy. Chin J Cancer. 2010;29:325–9.
47. Karre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. Nature. 1986;319:675–8.
48. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008;9:503–10.
49. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature. 2005;436:1186–90.
50. Kim JY, Son YO, Park SW, et al. Increase of NKG2D ligands and sensitivity to NK cell-mediated cytotoxicity of tumor cells by heat shock and ionizing radiation. Exp Mol Med. 2006;38:474–84.
51. Mifune M, Sobue T, Arimoto H, Komoto Y, Kondo S, Tanooka H. Cancer mortality survey in a spa area (Misasa, Japan) with a high radon background. Jpn J Cancer Res. 1992;83:1–5.
52. Wei LX, Zha YR, Tao ZF, He WH, Chen DQ, Yuan YL. Epidemiological investigation of radiological effects in high background radiation areas of Yangjiang, China. J Radiat Res. 1990;31:119–36.
53. Kendall GM, Muirhead CR, MacGibbon BH, et al. Mortality and occupational exposure to radiation: first analysis of the National Registry for Radiation Workers. BMJ. 1992;304:220–5.
54. Spycher BD, Lupatsch JE, Zwahlen M, et al. Background ionizing radiation and the risk of childhood cancer: a census-based nationwide cohort study. Environ Health Perspect. 2015;123:622–8.
55. Wall BF, Kendall GM, Edwards AA, Bouffler S, Muirhead CR, Meara JR. What are the risks from medical X-rays and other low dose radiation? Br J Radiol. 2006;79:285–94.
56. Hosoi Y, Ishii K, Yamada S, Ono T, Sakamoto K. Effect of combination treatment of 15 cGy total body irradiation and OK-432 on spontaneous lung metastasis and mitogenic response of splenocytes in mice. Radiat Oncol Investig. 1997;5:283–8.

57. Hosoi Y, Sakamoto K. Suppressive effect of low dose total body irradiation on lung metastasis: dose dependency and effective period. Radiother Oncol. 1993;26:177–9.
58. Ren H, Shen J, Tomiyama-Miyaji C, et al. Augmentation of innate immunity by low-dose irradiation. Cell Immunol. 2006;244:50–6.
59. Kojima S, Nakayama K, Ishida H. Low dose gamma-rays activate immune functions via induction of glutathione and delay tumor growth. J Radiat Res. 2004;45:33–9.
60. Liu SZ. Cancer control related to stimulation of immunity by low-dose radiation. Dose-Response. 2007;5:39–47.
61. Jin SZ, Pan XN, Wu N, Jin GH, Liu SZ. Whole-body low dose irradiation promotes the efficacy of conventional radiotherapy for cancer and possible mechanisms. Dose Response. 2007;5:349–58.
62. Burnette B, Weichselbaum RR. The immunology of ablative radiation. Semin Radiat Oncol. 2015;25:40–5.
63. Okuma K, Yamashita H, Niibe Y, Hayakawa K, Nakagawa K. Abscopal effect of radiation on lung metastases of hepatocellular carcinoma: a case report. J Med Case Reports. 2011;5:111.
64. Zeng J, See AP, Phallen J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys. 2013;86:343–9.
65. Camphausen K, Moses MA, Menard C, et al. Radiation abscopal antitumor effect is mediated through p53. Cancer Res. 2003;63:1990–3.
66. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. J Exp Med. 2006;203:1259–71.
67. Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG, Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol. 2005;174:7516–23.
68. Lee Y, Auh SL, Wang Y, et al. Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment. Blood. 2009;114:589–95.
69. Barker CA, Postow MA, Khan SA, et al. Concurrent radiotherapy and ipilimumab immunotherapy for patients with melanoma. Cancer Immunol Res. 2013;1:92–8.
70. Kiess AP, Wolchok JD, Barker CA, et al. Stereotactic radiosurgery for melanoma brain metastases in patients receiving ipilimumab: safety profile and efficacy of combined treatment. Int J Radiat Oncol Biol Phys. 2015;92:368–75.
71. Mathew M, Tam M, Ott PA, et al. Ipilimumab in melanoma with limited brain metastases treated with stereotactic radiosurgery. Melanoma Res. 2013;23:191–5.
72. Silk AW, Bassetti MF, West BT, Tsien CI, Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. Cancer Med. 2013;2:899–906.
73. Gerber NK, Young RJ, Barker CA, et al. Ipilimumab and whole brain radiation therapy for melanoma brain metastases. J Neurooncol. 2015;121:159–65.
74. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371:2189–99.
75. Twyman-Saint Victor C, Rech AJ, Maity A, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature. 2015;520:373–7.
76. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest. 2014;124:687–95.
77. Tang C, Liao Z, Gomez D, et al. Lymphopenia association with gross tumor volume and lung V5 and its effects on non-small cell lung cancer patient outcomes. Int J Radiat Oncol Biol Phys. 2014;89:1084–91.
78. Hinrichs CS, Palmer DC, Rosenberg SA, Restifo NP. Glucocorticoids do not inhibit antitumor activity of activated CD8+ T cells. J Immunother. 2005;28:517–24.
79. Pinkawa M, Djukic V, Klotz J, et al. Hematologic changes during prostate cancer radiation therapy are dependent on the treatment volume. Future Oncol. 2014;10:835–43.

80. Lawrence YR, Dicker AP. Radiation therapy and the immune system: learning to live together. Future Oncol. 2014;10:777–80.
81. Finkelstein SE, Salenius S, Mantz CA, et al. Combining immunotherapy and radiation for prostate cancer. Clin Genitourin Cancer. 2015;13:1–9.
82. Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res. 2013;1:365–72.
83. Zumwalt TJ, Goel A. Immunotherapy of metastatic colorectal cancer: prevailing challenges and new perspectives. Curr Colorectal Cancer Rep. 2015;11:125–40.
84. Zhang GQ, Zhao H, Wu JY, et al. Prolonged overall survival in gastric cancer patients after adoptive immunotherapy. World J Gastroenterol. 2015;21:2777–85.
85. Zhang GQ, Li F, Sun SJ, et al. Adoptive immunotherapy for small cell lung cancer by expanded activated autologous lymphocytes: a retrospective clinical analysis. Asian Pac J Cancer Prev. 2015;16:1487–94.
86. Wyluda EJ, Cheng J, Schell TD, et al. Durable complete responses off all treatment in patients with metastatic malignant melanoma after sequential immunotherapy followed by a finite course of BRAF inhibitor therapy. Cancer Biol Ther. 2015;16:662–70.
87. Massari F, Santoni M, Ciccarese C, et al. PD-1 blockade therapy in renal cell carcinoma: current studies and future promises. Cancer Treat Rev. 2015;41:114–21.
88. Hiniker SM, Chen DS, Reddy S, et al. A systemic complete response of metastatic melanoma to local radiation and immunotherapy. Transl Oncol. 2012;5:404–7.
89. Grimaldi AM, Simeone E, Giannarelli D, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunol. 2014;3:e28780.
90. Slovin SF, Higano CS, Hamid O, et al. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol. 2013;24:1813–21.
91. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol. 2014;15:700–12.
92. Hazard LJ, Sause WT, Noyes RD. Combined adjuvant radiation and interferon-alpha 2B therapy in high-risk melanoma patients: the potential for increased radiation toxicity. Int J Radiat Oncol Biol Phys. 2002;52:796–800.
93. Nguyen NP, Levinson B, Dutta S, et al. Concurrent interferon-alpha and radiation for head and neck melanoma. Melanoma Res. 2003;13:67–71.
94. Conill C, Jorcano S, Domingo-Domenech J, et al. Toxicity of combined treatment of adjuvant irradiation and interferon alpha2b in high-risk melanoma patients. Melanoma Res. 2007;17:304–9.
95. Paul E, Muller I, Renner H, Bodeker RH, Cochran AJ. Treatment of locoregional metastases of malignant melanomas with radiotherapy and intralesional beta-interferon injection. Melanoma Res. 2003;13:611–7.
96. Seung SK, Curti BD, Crittenden M, et al. Phase 1 study of stereotactic body radiotherapy and interleukin-2--tumor and immunological responses. Sci Transl Med. 2012;4:137ra74.
97. Ridolfi L, De Rosa F, Granato AM, et al. Radiotherapy as an immunological booster in patients with metastatic melanoma or renal cell carcinoma treated with high-dose interleukin-2: interim analysis data. J Clin Oncol. 2015;33(suppl; abstr e14007).
98. Ridolfi L, de Rosa F, Ridolfi R, et al. Radiotherapy as an immunological booster in patients with metastatic melanoma or renal cell carcinoma treated with high-dose Interleukin-2: evaluation of biomarkers of immunologic and therapeutic response. J Transl Med. 2014;12:262.

Chapter 4
Harnessing the Immune System Against Leukemia: Monoclonal Antibodies and Checkpoint Strategies for AML

Lucia Masarova, Hagop Kantarjian, Guillermo Garcia-Mannero, Farhad Ravandi, Padmanee Sharma, and Naval Daver

Abstract Acute myeloid leukemia (AML) is the most common leukemia among adults and is associated with a poor prognosis, especially in patients with adverse prognostic factors, older age, or relapsed disease. The last decade has seen a surge in successful immune-based therapies in various solid tumors; however, the role of immune therapies in AML remains poorly defined. This chapter describes the rationale, clinical data, and toxicity profiles of immune-based therapeutic modalities in AML including naked and conjugated monoclonal antibodies, bispecific T-cell engager antibodies, chimeric antigen receptor (CAR)-T cells, and checkpoint blockade via blockade of PD1/PDL1 or CTLA4. Monoclonal antibodies commonly used in AML therapy target highly expressed "leukemia" surface antigens and include (1) naked antibodies against common myeloid markers such as anti-CD33 (e.g., lintuzumab), (2) antibody-drug conjugates linked to either, (a) a highly potent toxin such as calicheamicin, pyrrolobenzodiazepine, maytansine, or others in various anti-CD33 (gemtuzumab ozogamicin, SGN 33A), anti-123 (SL-401), and anti-CD56 (lorvotuzumab mertansine) formulations, or (b) radioactive particles, such as ^{131}I, ^{213}Bi, or ^{225}Ac-labeled anti-CD33 or CD45 antibodies. Novel monoclonal antibodies that recruit and promote proximity-induced cytotoxicity of tumor cells by T cells (bispecific T-cell engager [BiTE] such as anti CD33/CD3, e.g., AMG 330) or block immune checkpoint pathways such as CTLA4 (e.g., ipilimumab) or PD1/PD-L1 (e.g., nivolumab) unleashing the patients T cells to fight leukemic cells are being

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Naval Daver is responsible for the overall content as guarantor.

L. Masarova • H. Kantarjian • G. Garcia-Mannero • F. Ravandi • N. Daver (✉)
Department of Leukemia, MD Anderson Cancer Center,
1515 Holcombe Blvd, Houston, TX 77030, USA
e-mail: ndaver@mdanderson.org

P. Sharma
Immunotherapy Platform, MD Anderson Cancer Center, Houston, TX, USA

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evaluated in clinical trials in patients with AML. The numerous ongoing clinical trials with immunotherapies in AML will improve our understanding of the biology of AML and allow us to determine the best approaches to immunotherapy in AML.

Keywords Acute myeloid leukemia • Immunotherapy • Monoclonal antibody • Immune checkpoint blockade

4.1 Introduction

Acute myeloid leukemia (AML) is the most prevalent acute leukemia among adults with an annual incidence of 19,000 new cases in the United States. AML comprises a heterogeneous group of diseases with differential behavior and overall survival impacted by numerous clinical, cytogenetic, and molecular factors. Despite extensive research efforts, the therapy of AML has improved modestly over the last four decades. Standard frontline treatment still represents a combination of cytarabine and daunorubicin ("3 + 7") introduced in the 1970s [1]. The 7 + 3 regimen produces complete remissions (CR) in approximately 70% of patients and long-term overall survival (OS) in 40% of young adults with AML. The results are worse in older patients or those with adverse karyotypes, where CR and OS rates are 50% and 15%, respectively [2, 3]. Furthermore, despite intensified consolidation after remission, most patients experience subsequent relapse, likely from persistence of chemorefractory leukemic "stem" cells. Improved induction regimens producing long-term remissions and/or the addition of maintenance therapy to high-risk patients in remission are warranted.

A significant number of patients with AML (especially those with adverse cytogenetic features, adverse molecular mutations, or antecedent hematological disorder) will be refractory or relapse after initial response to induction therapy. Patients with relapsed AML have further dismal outcomes, with response rates ranging from 2 to 30% and OS of 1.5 to 3.8 months with salvage therapy [4, 5]. The only therapy offering long-term survival and a potential for cure in relapsed AML is allogeneic stem cell transplantation (ASCT), but age, performance status, and organ function requirements coupled with considerable morbidity and mortality of this procedure limits routine applicability of this approach. Therefore, improved therapeutic approaches in salvage AML are urgently needed.

Targeted immune therapies such as antibodies, CAR-T cells, and checkpoint inhibitors aim to increase antitumor activity without the burden of systemic toxicities encountered with cytotoxic chemotherapies. Redirecting the patients' own immune system to target cancer cells is a highly attractive treatment option and has become a standard and approved anticancer modality in solid tumors including melanoma, lung cancer, bladder cancer, and renal cancer. Although the role of

antibodies that target CTLA-4 and PD-1, oncolytic viruses and adoptive T-cell therapy is well established in solid tumor malignancies, the experience in incorporating similar immune therapies for the treatment of leukemias remains limited. This is surprising for many reasons. Firstly, leukemias were the first tumor type to demonstrate the success of allogeneic stem cell transplant, an immunotherapeutic approach that depends on graft versus leukemia effect to eradicate leukemia cells [6]. Secondly, having an immune cell lineage, leukemias often express immune checkpoint molecules that are absent in solid tumor cells thereby offering direct targets for immune checkpoint inhibition. In the recent years, a number of immunotherapy approaches are under investigation in numerous clinical trials in patients with hematologic malignancies including AML, myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL). These include monoclonal antibodies, naked or antibody-drug conjugates (ADC) targeting leukemia-specific antigens on AML cells (e.g., anti-CD33, anti-CD38, anti-CD123, anti-56) or immune checkpoint blocking molecules (e.g., anti-PD-1, anti-PD-L1, or anti-CTLA-4), bispecific antibodies (e.g., bispecific T-cell engagers, BiTEs, e.g., CD3/CD33), T-cell adoptive therapy including chimeric antigen receptor (CAR) T cells and adoptively transferred natural killer (NK) cells.

This chapter focuses on the rationale, clinical data, and toxicity profiles of these immunotherapies for patients with AML.

4.2 Monoclonal Antibodies

Antibodies as cancer-targeting therapies have been investigated since the early 1980s and a number of antibodies have successfully been used in the therapy of solid and hematologic malignancies [7]. Monoclonal antibodies work by a number of different mechanisms to target tumor cells, of which one of the most important is antibody-dependent cellular cytotoxicity (ADCC) mediated by activation of NK cells, neutrophils, and macrophages. Following ADCC, fragments of tumor cells are released and taken up by antigen-presenting cells (APCs), where they are presented on the surface by the major histocompatibility complex class II and I (MHC) to cytotoxic T-lymphocytes with subsequent killing of cells containing tumor antigens [8]. An ideal targetable cluster of differentiation (CD) surface antigen has to be highly expressed on leukemic blasts with minimal to no expression on other cells, especially hematopoietic stem cells (HPSC) to allow for recovery of normal hematopoiesis. CD33, CD123, CD32, CD38, CD47, CD44, CD96, and CLL-1 [9] have differential expression on AML and leukemia stem cells (LSC) when compared with normal HPSC and represent potential targets.

Most of the clinical efforts thus far have focused on exploiting CD33, CD123, and CD56 as targets, as they have been shown to be frequently expressed on AML cells including AML stem cells making them ideal markers for eradicating malignant stem cell while sparing normal HPSC [10–12].

4.2.1 Anti-CD33 Antibodies

CD33 is a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs) and is a myeloid differentiation antigen [10] primarily expressed at very early stages on myeloid progenitors. CD33 is highly (>90%) expressed on AML blasts [13]. Unconjugated antibody lintuzumab (SGN-33) and several antibody-drug conjugates (ADCs) such as gemtuzumab ozogamicin (GO), AVE9633, and SGN-33A that target CD33 have been evaluated in the treatment of patients with AML. Conjugated antibodies were engineered with an intention to improve the antitumor efficacy of CD33 antibodies by leveraging the endocytolytic property of CD33.

4.2.1.1 Unconjugated Anti-CD33 Antibodies

Lintuzumab (SGN-33, HuM195), an unconjugated anti-CD33 antibody exerts its anti-leukemic activity through ADCC, complement-dependent cytotoxicity, and inhibition of inflammatory cytokines. Early clinic studies [14] with lintuzumab demonstrated promising activity with good tolerability. Subsequently, a phase 2B trial comparing low-dose cytarabine with or without lintuzumab in the frontline setting and a phase 3 trial comparing mitoxantrone, etoposide, and cytarabine with or without lintuzumab in the salvage setting were conducted and both demonstrated no significant survival benefit. This resulted in cessation of further development of this agent in AML [15, 16].

Despite the initial disappointing results with unconjugated anti-CD33 antibodies in AML, recent research showed promising preclinical anti-leukemic efficacy with a new unconjugated Fc-engineered (enhanced binding affinity to Fcγ receptor IIIa on NK cells), CD33 antibody, *BI836858.* This fully humanized anti-CD33 antibody promoted more robust NK-cell-mediated anti-AML activity in patients treated with 10 day decitabine [17]. The observed higher lysis of AML cells at day 28 post-decitabine was due to up-regulation of NK-activating receptor NKG2D ligands (NKG2DL) by the DNA-methyltransferase inhibitor decitabine resulting in enhanced NK-cell-mediated cytotoxicity against AML blasts. This agent will be entering clinical trials in the United States in late 2016 (Clinicaltrials.gov: NCT02632721).

4.2.1.2 Conjugated Anti-CD33 Antibodies

AVE9633 (ImmunoGen, USA) was the first anti-CD33 antibody conjugated to a cytotoxic toxin to be evaluated in clinical trials for patients with AML. The conjugated toxin was maytansine, a highly potent tubulin inhibitor. *AVE9633* showed limited clinical activity in three phase 1 trials performed on 54 patients with refractory/relapsed AML, with only one CRp (CR with incomplete platelet recovery) and one PR (partial remission) observed [18].

Gemtuzumab ozogamicin (GO; Mylotarg) (Pfizer, USA) is the best-known monoclonal antibody in AML therapy. Thus far, the largest clinical experience with a monoclonal antibody in AML has been with gemtuzumab ozogamicin (GO), a humanized anti-CD33 monoclonal antibody covalently linked to a semisynthetic derivative of a potent DNA-damaging toxin calicheamicin. In 2000, GO was granted accelerated approval by the United States FDA [19] on the basis of a 30% overall response rate (CR + CRi) in phase II clinical trials [20] in 142 and 277 patients with de novo AML in first relapse, respectively [21]. Response duration was difficult to determine due to the high prevalence of post-remission therapies; however, responses were relatively short. However, no difference in OS was observed in the phase III SWOG S0106 trial designed to meet FDA post-approval requirements [22]. The lack of clear clinical benefit, concerns about increased side effects, and slightly increased early death rate with GO in this SWOG trial [22], led to voluntary withdrawal of the drug from US markets in 2008. Particular concerns were related to life-threatening sinusoidal obstruction syndrome or veno-occlusive disease, which was more likely to occur when the drug was used in higher concentration, in combination with hepatotoxic agents, or within 3 months of allogeneic SCT (incidence rate 9–14%) [23]. The mechanisms included either dissociation of calicheamicin from the anti-CD33 antibody causing direct toxic effect to hepatocytes or uptake of GO by CD33(+) cells residing in the hepatic sinusoids [24]. The potential benefits of GO in this trial might have been masked due to a suboptimal dosing schema as well as failure to perform patient subgroup analysis. Subsequently, large randomized trials conducted in the United States and Europe investigated GO in addition to standard induction chemotherapy in adults with newly diagnosed AML. These studies [25–27] showed statistically improved OS when GO was added to standard induction, particularly in younger patients with intermediate and/or favorable risk cytogenetics. In older patients, the addition of GO to cytotoxic induction regimens improved the relapse risk, event-free survival, and overall survival without improving the response rate or early mortality rate [26–27]. In a meta-analysis of these randomized clinical trials, the addition of GO significantly reduced the risk of relapse (HR 0.8; 95%CI 0.72–0.89, $p < 0.001$), improved relapse free (HR 0.8; 95%CI 0.76–0.94, $p = 0.001$) and overall survival (HR 0.89; 95%CI 0.82–0.97, $p = 0.01$), particularly in patients without adverse cytogenetics [28]. These data suggest that the use of GO in AML in the United States and Europe should be reassessed as suggested by experts in the field [29, 30]. Currently, clinical trials are ongoing to evaluate the efficacy and toxicity of GO either as a monotherapy or in combination with chemotherapy in frontline (France) and relapsed (United States) patients with AML, including its addition to standard conditioning prior to ASCT [ClinicalTrials.gov: NCT01869803, NCT02473146, NCT02221310].

SGN33A (vadastuximab talirine, Seattle Genetics, USA) is a promising new anti-CD33 antibody conjugated to a highly potent, synthetic pyrrolobenzodiazepine, producing DNA damage and cell cycle arrest with subsequent leukemic cell apoptosis. In preclinical studies, SGN33A demonstrated greater cytotoxic potency against AML cell lines and primary AML cells than GO, regardless of multi-drug-resistant status or cytogenetic risk group [31]. Furthermore, 5-azacitidine was

shown to significantly enhance the tumor killing ability of SGN33A through enhanced ADCC and phagocytosis [32]. This compound is currently being tested in phase I dose escalation studies as a single agent and in combination with chemotherapy, including DNA-methyltransferase inhibitors (DNMTi) (decitabine or 5-azacitidine) in the pre- and post-ASCT setting, or as a monotherapy in maintenance [Clinicaltrials.gov: NCT02326584, NCT02785900, NCT02706899, NCT02614560]. Initial results from an ongoing phase II study combining SGN-CD33A with a DNMTi in elderly, treatment-naïve patients with AML are promising, with a CR plus CRi rate of 71% (including CR rate of 41%), ≥50% reduction in blasts in 85% of treated patients, and a low early mortality (8-week mortality of 4%) [33].

IMGN 779 (ImmunoGen, USA) is another humanized anti-CD33 antibody conjugated to a novel DNA-alkylating IGN payloads, DGN462 that acts as an alkylating cytotoxic agent without DNA crosslinking [34]. In preclinical studies, the compound showed highly potent activity against AML cell lines in vitro and in primary AML patient samples isolated from peripheral blood or bone marrow. In long-term cultures, it has also demonstrated a dose-dependent decrease in leukemic stem cell colony formation without affecting normal HPSC thereby avoiding prolonged myelosuppression [35, 36]. IMGN 779 is currently being tested in a phase 1 clinical study for patients with relapsed or refractory AML [clinicaltrial.org: NCT02674763].

4.2.2 Anti-CD123

The CD123 antigen is another ideal target for monoclonal antibody-based therapy in patients with AML. Binding of CD123 to interleukin-3 (IL-3Rα) results in increased cell survival and proliferation [37]. Overexpression of the interleukin (IL)-3 receptor α-chain (IL-3 Rα/CD123) on AML cells was found to be associated with enhanced blast proliferation, poor prognosis [38], and a major cause of leukemia relapse and chemotherapy resistance.

The first anti-CD123 antibody *CSL360* was a recombinant, chimeric immunoglobulin G1 against CD123 that prevented IL-3Rα from binding to its receptor. CSL360 had underwhelming clinical efficacy when tested in relapsed, refractory or high-risk AML with only one CR observed among 26 treated patients [39]. These results resulted in cessation of further development of this compound in AML.

A second-generation anti-CD123 antibody, *CSL362* is a fully humanized, genetically engineered antibody containing a modified Fc-domain to enhance binding to NK cells through Fcγ receptors (FcγR) of CD16 to enhance antibody-dependent cellular toxicity (ADCC). This agent showed potent activity in patients with CD123+ AML with a tolerable safety profile in a phase I study of 25 patients with AML in first or second CR/CRp with adverse risk factors conferring a high risk of early relapse. Among 20 patients evaluable for a response, 10 had maintained their CR, with a median duration of CR of 34+ weeks (range, 26–52 weeks) and ongoing at the last follow up. Furthermore, three out of six patients, who were MRD positive, converted to MRD negative. Related adverse events observed in ≥10% of patients

included infusion reaction/hypotension, hypertension, and increased C-reactive protein, three of these were classified as dose-limiting toxicities. Pharmacodynamic correlative studies showed rapid, complete, and durable in vivo depletion of cells highly expressing CD123 by induced ADCC [40].

Another anti-CD123 antibody currently in phase 2 clinical trials is *SL-401 (DT388IL3) (Stemline Therapeutics, Inc. USA)*—a recombinant fusion protein composed of the truncated diphtheria toxin and a human IL-3 ligand [41], which after binding to CD123 get internalized, and leads to inactivation of protein synthesis, and cell death. Encouraging results were shown in a phase I trial of SL401 in 74 AML/MDS patients (56 with relapsed and refractory AML, 11 with de novo poor risk elderly AML, and 7 high-risk MDS), where ORR was observed in 6 patients (2 CRs and 4 PRs) and a minor response with blasts reduction was observed in 14 patients, including $a > 50\%$ reduction in bone marrow blasts in four patients. Moreover, disease stabilization was observed between 43 and 55% of patients. The median survival and overall survival at 12 months in patients with relapsed AML (≥2nd salvage) was 3.2 months and 22%, respectively, both favorable when compared to historical results. Toxicities did not differ from those observed in patients with BPDCN. Severe grade 3/4 adverse events were only transient and included elevation in transaminases (20%) and capillary leak syndrome (4%) [42, 43]. Recently reported data from the early expansion stages of an ongoing pivotal phase 2 clinical trial confirmed an overall response rate (ORR) of 87% in all patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN) [44]. In the frontline setting, the response rate was 100% with majority of responses CR or CRc. The responses have been durable in all cases. The most common toxicities included fever, chills, hypotension, edema, transaminase elevation, and hypoalbuminemia. The notable toxicity was capillary leak syndrome in 2/18 treated patients; this was reversible in one case and fatal in one case. The study continues to accrue and may be a breakthrough in the management of BPDCN. The phase II study evaluating SL401 in AML shows disease stabilization in heavily pretreated patients with relapsed refractory AML and is ongoing [45]. The results in AML have thus far been less impressive than those see in BPDCN. This agent is also being evaluated in a phase 2 trial designed as a consolidation therapy for patients with high-risk AML in first complete remission to determine whether targeting CD123 improves the duration of response and survival in patients who would traditionally be at a high risk of relapse [clinicaltrials.gov: NCT02270463].

4.2.3 Anti-CD56

CD56, also known as NCAM1, is a member of the neural cell adhesion molecule family [46] that plays an important functional role during nervous system differentiation, and immune surveillance. Although primarily expressed in neuroendocrine, NK, and T cell lineages [47], aberrant CD56 expression is seen in a variety of hematological malignancies [11] as well as solid tumors [48].

IMGN901 (lorvotuzumab mertansine) (ImmunoGen, Inc., USA) is a humanized anti-CD56 antibody conjugated to tubulin inhibitor maytansinoid DM1 via a stable disulfide linker. On binding to the CD56 antigen, IMGN901 is internalized with intracellular release of toxin DM1 with subsequent microtubule disruption, cell cycle arrest, and ultimately cell apoptosis [49]. In preclinical models, IMGN901 demonstrated high-affinity, antigen-specific binding, and antitumor activity in CD56-positive tumors [35]. And, open label phase 1/2 clinical trial was conducted in patients ($n = 97$) with relapsed CD56+ solid tumors in combination with chemotherapy. The drug had an acceptable tolerability profile with CR/PR observed in four patients and disease stabilization in 25% of evaluable patients [50]. This compound is currently being evaluated in a phase 2 clinical trial in CD56+ hematologic malignancies, including AML, myelofibrosis, and BPDCN [clinicaltrials.gov: NCT02420873].

4.2.4 Radioimmunotherapy via Targeted Antibodies Conjugated to Radioactive Particles

The radiosensitive nature of AML best seen in the setting of a stem cell transplantation [51], the diffuse and widespread pattern of involvement, and the high expression of specific antigens on AML blasts, suggests that radioimmunotherapy via targeted antibodies conjugated to radionuclides may be an attractive alternative to antibodies conjugated to toxins. This approach has been explored in patients with AML for over two decades. Since the first phase 1 clinical trial published in 1991 demonstrated the feasibility of using radiolabeled ^{131}I anti-CD33 in patients with relapsed AML [52], several clinical studies have explored antibodies carrying beta (^{131}Iodine, ^{188}Rhenium, ^{90}Yttrium) or alfa (^{213}Bismuth, ^{225}Actinium) emitters, alone or as part of a conditioning regimen for ASCT in patients with relapsed AML against different AML targets (CD33, CD45, or CD66). Early clinical studies with easily accessible radionuclide ^{131}I targeted against CD45 (^{131}I-labeled anti-CD45 antibody) or CD33 (^{131}I-labeled anti-CD33 antibody—murine M195 and humanized Hu195) showed feasibility, efficacy, and acceptable toxicity when used in combination with standard conditioning regimen prior to ASCT in patients with refractory/high-risk AML [53–55]. ^{131}I-labeled anti-CD45 antibody BC8 *(Iomab-B, Actinium Pharmaceuticals, USA)* is currently being tested in a phase 2 [clinicaltrials.gov: NCT00589316] and phase 3 registration trial [clinicaltrials.gov: NCT02665065] to evaluate the efficacy and safety of this agent in patients of all ages with relapsed or refractory AML as a part of myeloablative conditioning regimen prior to ASCT (phase 2) or in older patients with relapsed or refractory AML prior to ASCT in comparison to standard conventional care (phase 3).

In order to reduce the toxicity and improve the efficacy, especially in the settings of minimal residual disease (MRD), several studies have evaluated radionuclides emitting high energy or short range alfa particles, such as ^{213}Bi and ^{225}Ac. Preclinical studies followed by early clinical phase 1 studies showed the safety, feasibility, and

anti-leukemic activity of ^{213}Bi anti-CD33 (^{213}Bi-labeled HuM195). However, a very short half-life of only 48 min limited its widespread clinical testing [56]. To circumvent this problem, second-generation immunoconjugates, such as ^{225}Ac (half-life of 10 days), were developed. A phase 1 clinical trial with ^{225}Ac-labeled anti-CD33 antibody lintuzumab demonstrated clinical activity with reduction of the peripheral blood/bone marrow blasts in 63–67% of 18 evaluable patients with relapsed refractory AML. Dose-limiting toxicities included prolonged myelosuppression and death due to sepsis in three patients [57]. Based on these findings, a multicenter, phase I/II trial is now underway to determine the toxicity and efficacy of fractionated-dose ^{225}Ac-lintuzumab (A*ctimab-A*) (*Actinium Pharmaceuticals, USA*) in combination with low-dose cytarabine in untreated older (>60) patients with AML [clinitaltrials.gov: NCT02575963].

4.3 T-Cell-Engaging Antibodies

A novel class of antibody-based immunotherapy in AML includes monoclonal antibodies designed to promote antitumor activity by engaging and enhancing T- cell activation. These agents are called bispecific T-cell engagers (BiTEs). BiTE antibodies are able to effectively recruit antigen-experienced T cells, without the requirement of pre- or co-stimulation, and lead to direct killing of tumor-associated antigen cell (TAA) [58]. BiTEs are composed of a single polypeptide chain consisting of two light and heavy chains of targeted antibodies. The first-in-class BiTE antibody, anti-CD19/CD3 Blinatumomab, demonstrated significant clinical activity against CD19-positive malignancies [59]. Single agent blinatumomab tested in phase 2 clinical study in 189 relapsed refractory ALL patients showed 43% CR/CRi rate (95% CI 36–50), with median OS and RFS of 6.1 and 5.9 months, respectively, and served as an excellent bridge to potentially curable allo-SCT in 40% of patients who achieved CR/CRi. These data resulted in the FDA approval of blinatumomab for the treatment of relapsed/refractory B-ALL. Based on these promising results, a similar construct targeting CD3/CD33 has been developed to target AML, *AMG 330 (Amgen, USA)* [60]. In preclinical studies, AMG 330 demonstrated potent CD33-dependent cytolytic activity in vitro [61]. The drug is currently being evaluated in phase 1 clinical trial in patients with relapsed/refractory AML [clinicaltrials.gov: NCT02520427]. Another CD123/CD3 BiTE, *JNJ-63709178 (Janssen, USA)* is soon to enter phase 1 clinical trials in patients with relapsed refractory AML [clinicaltrial.gov: NCT02715011].

In an effort to improve the efficacy, stability, and valency of BiTEs, a novel class of Bivalent Dual Affinity Re-Targeting Bispecific Antibodies (DARTs) has been developed. DARTs are composed of heavy and light chain variable domains of two antigen-binding specificities connected to two independent polypeptide chains via a disulfide linker [62]. Recently, a CD123/CD3 DART has been developed for AML (MGD006) and demonstrated promising anti-leukemic activity in preclinical studies [63]. This compound is currently being evaluated in a first-in-human phase I dose escalation study in patients with relapsed AML or International Prognostic Scoring system (IPSS) intermediate-2/high-risk MDS [clinicaltrials.gov: NCT02152956].

4.4 Adoptive T-Cell Therapy

Adoptive cell therapy (ACT) is a highly personalized therapy that involves transfer of ex vivo expanded cytotoxic T-lymphocytes (CTLs) capable of targeting TAA into tumor-bearing patients. It was first recognized >20 years ago that some T cells from patients with cancer could immunologically recognize and kill the patients cancer cells [64]. Researchers found that patient lymphocytes stimulated in vitro with interleukin 2 and tumor cells were able to lyse autologous tumor cell lines through major histocompatibility complex II (MHC II). These tumor-reactive T cells have been extensively investigated over the past years and may revolutionize out current approach to cancer therapy in hematologic and possibly in solid malignancies. The biggest advantage of ACT is that a large number (up to 10^{11}) of lymphocytes can be grown in vitro and genetically engineered to express the binding site of specific antibodies. These T cells with engineered chimeric antigen receptor, also called CAR-T cell, are then able to directly bind to a specific TAA producing highly targeted and robust tumor killing [65].

CARs consist of an extracellular domain created by the fusion between the variable region of heavy and light chains of an antigen-specific monoclonal antibody (ScFv) separated by a short peptide linker and an intracellular T cell-activating domain, usually CD3-ζ of the TCR receptor, and a co-stimulator molecule. This allows CAR-T cells to manifest the tumor specificity of monoclonal antibodies while simultaneously activating effector T-cells independent of MHC [66]. Various CAR-T constructs have different co-stimulatory molecules to increase their efficacy and longevity (CD28, OX40, or 4-1BB in the second and third-generation constructs; additional cytokines such as IL-2, IL-15, IL-12, and IL-21 in the fourth-generation constructs) [67]. Anti-CD19 CAR-Ts have already shown remarkable success in the treatment of B-cell malignancies [68], and it remains to be established whether similar activity can be reproduced in AML.

Only one clinical study testing anti-LeY CAR-T cells (Australia) in patients with AML has been completed and reported to date. This study reported the feasibility, safety, and persistence of CAR-T cells for up to 10 months post infusion as tested in five patients with relapsed AML (first salvage). Two patients achieved stable disease (duration of 23 months in one patient), and an additional two had transient response (blasts reduction/cytogenetic remission). Overall, infusion were well tolerated with no severe (grade 3 or 4) adverse events or tumor lysis syndrome observed [69]. A number of phase I clinical trials with CAR-T cells in relapsed, refractory AML patients are ongoing including anti-CD33, CD7, and CD133 CAR-T cell studies in China [clinicaltrials.gov: NCT01864902, NCT02799680, NCT02742727, NCT02541370] and anti-CD123 and anti-NKG2D ligand CAR-T cell studies in the United States [clinicaltrials.gov: NCT02159495, NCT02623582, NCT02203825].

Alternative T-cell-engaging antibody constructs, cytokine-induced killers (CIK), involving CD56 + NK like cells with a potent killing activity, showed activity in reducing refractory AML blasts and cell lines in preclinical studies when combined with anti-CD33 and/or anti-CD123 CAR-T [70].

4.5 Checkpoint Inhibitors

Maintenance of immune homeostasis, self-tolerance, and prevention of autoimmunity requires strict regulation of immune response, especially its quality and amplitude, provided by T cells and multiple interactions between co-stimulatory and co-inhibitory signals [71]. T-cell-mediated immunity includes many steps involving initial presentation of antigen peptide on MHC through the T-cell receptor (TCR) with sequential activation of T cells. All the steps in this pathway are regulated by careful counterbalancing of the co-stimulatory and co-inhibitory signals and receptors, resulting in appropriate T-cell effector function. The most important receptors promoting final activation of T cells are co-stimulatory signals CD28, 4-1BB (CD137), and CD27 (expressed on T cells), and CD80, CD86 (expressed on APC). These stimulatory signals are antagonized by inhibitory receptors (the so-called checkpoint inhibitors)—cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed cell-death protein (PD-1).

A major impediment to cancer immunotherapy with the previously discussed antibody-based approaches in this chapter is tumor-induced immune suppression and evasion of anti-tumor immune responses, rendering the host tolerant to tumor-associated antigens [72, 73]. The true potential of cancer immunotherapy came to the fore with James Allison's breakthrough discovery of cytotoxic T-lymphocyte antigen 4 (CTLA-4), a receptor on the surface of T cells that blocks the immune response by inhibiting T-cell activation and the subsequent development of an anti-CTLA-4 antibody, ipilimumab, that blocks this "immune checkpoint" protein, thereby freeing the immune system to attack tumors [74].

Under normal physiological conditions, immune checkpoints regulate self-tolerance and protect tissues from damage by restraining the immune systems response to pathogenic infection. Deregulation of immune checkpoint proteins including up-regulation of negative co-stimulatory receptors and downregulation of positive co-stimulatory receptors plays a central role in tumor-mediated evasion of T-cell immune response [71]. Targeting CTLA4 and other immune checkpoint molecules represented a major breakthrough for immunotherapy in solid tumors and more recently in hematologic malignancies. These agents target inhibitory pathways on T cells thereby unleashing antitumor immune responses.

The two major approaches to immune checkpoint blockade that have been clinically investigated in large numbers of patients, primarily in solid tumors and more recently in hematologic malignancies, focus on targeting the co-inhibitory receptors, CTLA4 and PD-1, or its ligands PD-L1/PD-L2. These two inhibitory molecules work on different levels and by different mechanisms. CTLA4 is expressed predominantly on the T cells in lymph nodes where it primarily regulates early T-cell activation. CTLA4 is sequestered in intracellular vesicles in T cells and is transported to the surface only after antigen recognition. The level of CTLA4 induction depends on the amplitude of the initial T-cell receptor (TCR)-mediated signaling, further amplified by co-stimulatory receptor CD28. The stronger the stimulation through the TCR, the greater the amount of CTLA4 deposited on the T-cell surface.

CTLA4 then binds to the same ligands as the co-stimulatory receptor CD28, namely, CD80 and CD86 and counteracts the stimulatory activity of CD28 by competitive inhibition. CTLA4 has higher affinity to CD80 and CD86 ligands and serves as a signal dampener to maintain a consistent level of T-cell activation, primarily by downregulation of T-helper cells and up-regulation of T-regulatory cells [74, 75]. In contrast, the major role of the PD-1 pathway (PD-1 receptor and its ligands) is to regulate inflammatory responses in the peripheral tissues by inhibiting effector T cells [76]. Inflammatory signals activate T cells and up-regulate expression of PD-1 and PD-1L in the tissue. PD-1 expression inhibits the T-cell effector activity by decreasing the duration of interaction between the T cell <– > APC or T cell <– > target cell and enhancing Treg proliferation [77]. Moreover, chronic inflammation leads to excessive production of inhibitory co-signals in tumor cells or their microenvironmental components, resulting in an exhausted or anergic state among co-signaling antigen-specific Tcells leading to immune escape of the tumor [78]. This may possibly be reversed by PD-1 or PD-L1 pathway blockade [79].

Clinical trials with anti-CTLA-4 antibodies, the first immune checkpoint targeted antibody [74], have shown encouraging responses in melanoma, advanced mesothelioma, gastric cancer, non-small cell lung cancer, bladder cancer, and prostate cancer [80–82]. CTLA4 inhibitor, ipilimumab, demonstrated overall survival benefit in patients with metastatic melanoma, and more importantly, revealed an important concept of immune-based therapies which seem to re-educate the immune system to keep tumors under control even in patients with multiple prior therapeutic intervention as was noticed by increased proportion of long-term survivors [80]. The responses with anti-CTLA4 occurred slowly after treatment initiation, in many patients were delayed up to 6 months, and were often maintained for many years after completion of a relatively short course of treatment. Toxicity mostly involved immune-mediated pneumonitis, colitis, hepatitis, or thyroiditis, and seemed to be manageable with steroids. Identification and targeting of additional positive co-stimulatory receptors (4-1BB, CD27, ICOS, OX40, GITR) and negative co-stimulatory receptors (PD-1, CTLA4, TIGIT, BTLA, LAG3, TIM3) regulating T-cell activation and dual blockade of concurrently expressed receptors produced synergistic antitumor responses in mouse models [78].

Since the basic immunologic principles behind immune checkpoint therapy can be applied to other tumor types, it is plausible that immune checkpoint therapy can also be beneficial for patients with leukemias and other hematologic malignancies, specifically AML. Firstly, leukemias are one of the first tumor types to be successfully treated with immunotherapy approaches as proven by the success of allogeneic stem cell transplantation. Secondly, leukemias have an immune cell lineage and may express immune checkpoint molecules thereby offering direct targets for immune checkpoint therapy. For example, there is frequent expression of PD-L1 and PD-L2 ligands on various hematopoietic cells—activated and non-activated T cells, B cells, and NK cells [83]. Similarly, markers typically associated with antigen-presenting cells, such as CD80 and CD86, are commonly overexpressed in hematologic malignancies owing to a common lineage shared by leukemia cells and APC [84–88]. Thirdly, a number of studies have demonstrated encouraging results

with immune checkpoint inhibition in other hematologic malignancies including Hodgkin's lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, and multiple myeloma. Specifically in leukemia, PD-1 and CTLA4 have been shown to play a role in leukemia, and graft versus host disease (GvHD), and their overexpression was clearly associated with a more aggressive leukemia [89, 90]. Researchers have demonstrated that PD1 plays a role in immune evasion and exhaustion of tumor-infiltrating lymphocytes (TILs) and that blocking CTLA4 and PD-1/PD-L1 pathways enhances the anti-leukemia responses with decreased tumor burden and increased survival in murine models [91, 92, 93, 94]. Additionally, PD-1 positive T cells were shown to be significantly increased in the bone marrow aspirates of patients with relapsed AML as compared to healthy adult donors [95].

The initial clinical results of a phase I study of PD-1/PDL-1 inhibitor *pidilizumab (MDV9300)* in patients with various solid and hematologic malignancies included a small number of patients with AML. Among eight patients with AML and one patient with MDS, minimal response was seen in one patient with AML in the form of a decrease in the blast percentage from 50 to 5% [96]. In order to improve the response rate and the durability of response in patients with AML treated with checkpoint inhibitors, combinations of these agents with standard anti-leukemic therapy may be needed. 5-azacitidine, an epigenetic drug approved by FDA for the treatment of MDS, up-regulated PD-1, PD-L1, and PD-L2 ($\geq$ 2-fold) in >50% of 61 evaluable patients with AML/MDS during their first course of therapy. There was a trend toward increased expression of all three genes in azacytidine-resistant patients compared with sensitive patients, suggesting up-regulation of immune makers as a potential mechanism of resistance to 5-azacytidine and that concomitant inhibition of the PD-1/PD-L1 axis may be a potential mechanism to prevent or overcome resistance to 5-azacytidine [97]. These data have resulted in currently ongoing clinical trials combining epigenetic therapy with PD-1/PD-L1 inhibitor *nivolumab* (*Opdivo, BMS-936558*) (*Bristol-Myers Squibb, USA*) in relapsed and frontline elderly AML, relapsed and frontline MDS and epigenetic therapy in combination with CTLA4 inhibitor *ipilimumab (Yervoy, BMS-734016)* in relapsed and frontline MDS patients [ClinicalTrials.gov: NCT02397720, NCT02530463]. Phase 1/2 trials are evaluating the combination of PD-1 inhibitor nivolumab with standard induction chemotherapy in newly diagnosed AML patients [CTI: NCT02464657] or single agent nivolumab as a maintenance in high-risk AML patients to reduce the incidence of relapse (clinicaltrials.gov: NCT02532231). CTLA4 inhibitor is being evaluated as a monotherapy in high-risk MDS failing HMA therapy and AML with minimal residual disease [CTI: NCT01757639]. Both PD1 and CTLA4 are also being tested in phase 1 trials for patients with AML after ASCT [CTI: NCT01822509]. Results from ongoing phase 1/2 trial of PD1 inhibitor nivolumab with 5-azacitidine [NCT02397720] are encouraging. Preliminary data on the 22 evaluable patients were recently presented by Daver et al. [98] and showed significantly improved overall response rate, 8-week mortality, and median progression-free survival as compared to historical outcomes with 5-azacitidine-based therapies from the same institution. A phase 1 trial with CTLA4 inhibitor ipilimumab [CTI: NCT00060372] in patients with solid and hematologic malignancies, including patients with relapsed AML after allo-SCT

has been completed. Results on 28 patients with hematologic malignancies after stem cell transplant, including 12 patients with AML and 2 with MDS, were recently presented, and showed very encouraging activity with a CR/CRi rate of 33% and an overall disease reduction in 48%. Five of 12 (42%) patients with AML achieved CR, including 4 patients with chemorefractory leukemia cutis and/or myeloid sarcoma with the longest duration of response of 8 months and still ongoing. Typical immune-related grade 2–4 toxicities were observed in four patients, three of them were able to resume the therapy after management with steroids. One patient died due to sepsis presumably related to severe adverse events (pneumonitis and colitis), four others had to be withdrawn from the study due to treatment-related adverse events (acute and chronic GVHD of gastrointestinal tract) [99].

Evaluation of the clinical efficacy of targeting immune checkpoint pathways beyond PD-1/PD-L1 and CTLA4, such as 4-1BB, OX40, and ICOS, is currently ongoing in patients with advanced or metastatic carcinomas [CTI: NCT02315066]. Agonistic antibodies to these co-stimulatory signals, such as 4-1BB, or OX40 may result in increased immune effector cytotoxicity. OX40 (CD134), 4-1BB (CD137) receptors, and the inducible co-stimulator receptor (ICOS) belong to tumor necrosis factor (TNF) receptors family members, and are potent co-stimulators in T-cell activation and promote expansion and proliferation of CD8+ and CD4+ T cells. They are transiently up-regulated on APC, B cells, macrophages, and T cells following their activation, and play a significant role in the functional maturation of T cells [100–102]. They were also found to be overexpressed on leukemic cells [103], and in the bone marrows of patients with AML [95] as compared to healthy donors. These data suggest that evaluation of these immune system accelerators in hematologic malignancies, especially leukemias is warranted and may improve the responses when rationally administered in combination with checkpoint inhibitors. A number of ongoing phase 1/2 of clinical trials are evaluating these molecules (anti-4-1BB antibody PF-05082566; anti-OX40 antibody MEDI-6469, and anti-ICOS antibody MEDI-570) in patients with advanced solid malignancies or lymphomas as single agents or in combinations [ClinicalTrials.gov: NCT02554812, NCT02559024, NCT02315066, NCT02520791]. Hopefully, these will soon be evaluable in hematologic malignancies.

Currently, ongoing clinical trials testing checkpoint inhibitors and monoclonal antibodies in patients with AML are summarized in Table 4.1 and Figs. 4.1 and 4.2.

4.6 Discussion

Immunotherapy is undoubtedly a breakthrough in cancer therapy, and emerging data suggests that immunotherapeutic approaches hold the potential to become one of the cornerstones of treatment strategies in AML. In spite of the rapid development of monoclonal antibodies and other immunotherapeutic agents for AML in clinical trials, none of these agents are approved for standard use and there remains limited experience in incorporating these therapies in routine clinical practice. Historic

Table 4.1 Ongoing trials of monoclonal antibodies and immune checkpoint blockade in AML

Type	Therapy	Primary endpoint	Inclusion[a]	Clinicaltrials.gov Identifier
Phase 2	GO	Efficacy—ORR, Toxicity	R/R AML	NCT01869803
Phase 2,3	GO + Cytarabine vs "7 + 3"	Efficacy—ORR, OS, Toxicity	Frontline AML, >65 years	NCT02473146
Phase 2	GO + Busulfan + CFA → ASCT	Efficacy—ORR, OS	Salvage High-Risk AML, MDS—1st CR or R/R	NCT02221310
Phase 1b	SGN-CD33A + "7 + 3", 1SGN-CD33A + HDAC; SGN-CD33A	Toxicity/DLT	Frontline AML Maintenance	NCT02326584
Phase 3	SGN-CD33A + DAC/AZA[b]	Efficacy—OS	Frontline AML	NCT02785900
Phase 1/2	SGN-CD33A + AZA[b]	Toxicity, Efficacy—ORR	Frontline Int-2/High-Risk MDS	NCT02706899
Phase 1/2	SGN-CD33A + Fludarabine + Melphalan → ASCT; SGN-CD33A	Toxicity, Efficacy—OS	R/R AML pre-ASCT Post-ASCT maintenance	NCT02614560
Phase 1/2	SL 401	Toxicity, Efficacy—OS	Consolidation—High-Risk AML, or MRD+ AML in 1st CR	NCT02270463
Phase 2	IMGN 901	Efficacy—ORR	R/R CD56+ AML	NCT02420873
Phase 1	AMG 330	Toxicity	R/R AML	NCT02520427
Phase 1	JNJ-63709178	Toxicity	R/R AML	NCT02715011
Phase 1	IMGN 779	Toxicity	R/R AML	NCT0267463
Phase 1	MGD 006	Toxicity	R/R AML, Int-2 and HR MDS	NCT02152956
Phase 1/2	CAR-T CD33	Toxicity	R/R AML	NCT01864902
Phase 1/2	CAR-T CD7 (NK cells)	Toxicity	R/R AML	NCT02742727
Phase 1	CAR-T CD123	Toxicity	R/R AML	NCT02159495
Phase 0, pilot	CAR-T CD123	Toxicity	R/R AML	NCT02623582
Phase 1	CAR-T NKG2DL	Toxicity	R/R AML, MDS RAEB	NCT02203825
Phase 1	CAR-T CD33	Toxicity	R/R AML	NCT02799680
Phase 1	CAR-T CD133	Toxicity	R/R AML	NCT02541370

(continued)

Table 4.1 (continued)

Type	Therapy	Primary endpoint	Inclusion[a]	Clinicaltrials.gov Identifier
Phase 2	Iomab-B, ^{131}I anti-CD45 + conditioning prior SCT	Toxicity	R/R AML pre allo-SCT	NCT00589316
Phase 3	Iomab-B, ^{131}I anti-CD45 vs. standard care	Efficacy—ORR, OS	Active, R/R AML >55 years Pre allo-SCT	NCT02665065
Phase 1/ 2	Actimab-B, ^{225}Ac anti-CD33 + LDAC	Toxicity	Frontline AML, >60 years	NCT02575963
Phase 1	Ipilimumab	Toxicity	R/R AML, High-Risk MDS	NCT01757639
Phase 1/1b	Ipilimumab or Nivolumab	Toxicity, MTD	R/R AML after ASCT	NCT01822509
Phase 2	Pidilizumab + DC vaccine	Toxicity	AML in CR prior to cell collection for DC generation	NCT01096602
Phase 2	Nivolumab + AZA	Efficacy—ORR	R/R AML Frontline AML, >65 years	NCT02397720
Phase 2	Nivolumab + "7 + 3"	EFS	Frontline AML, <60 years	NCT02464657
Phase 2	Nivolumab	EFS	AML in CR	NCT02532231

Abbreviations: *R/R* relapsed/refractory, *AML* acute myeloid leukemia, *ORR* overall response rate, *OS* overall survival, *EFS* event-free survival, *GVHD* graft versus host disease, *ASCT* allogeneic stem cell transplantation, *MDS* myelodysplastic syndrome, *DC* dendritic cells, *DAC* decitabine, *AZA* azacitidine, *HDAC* high-dose Cytarabine, *GO* gemtuzumab ozogamicin, Data was compiled from ClinicalTrials.gov (https://clinicaltrials.gov) assessed on 6/14/2016

[a]Only AML indications listed

[b]Placebo controlled trials

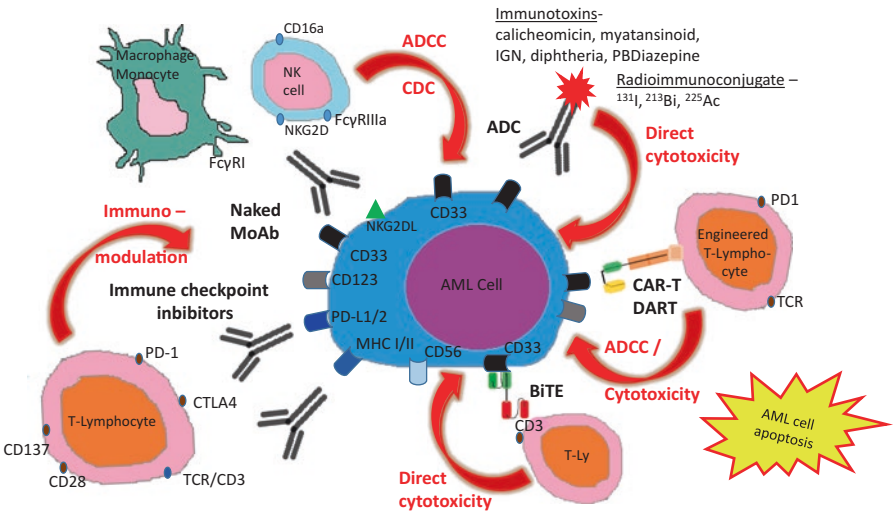

Fig. 4.1 Mechanism of action of immunotherapy in AML. Abbreviations: *ADCC* antibody-dependent cellular cytotoxicity, *CDC* complement-dependent cytotoxicity, *NK cell* natural killer, *MoAb* monoclonal antibody, *BiTE* bispecific T-cell engager

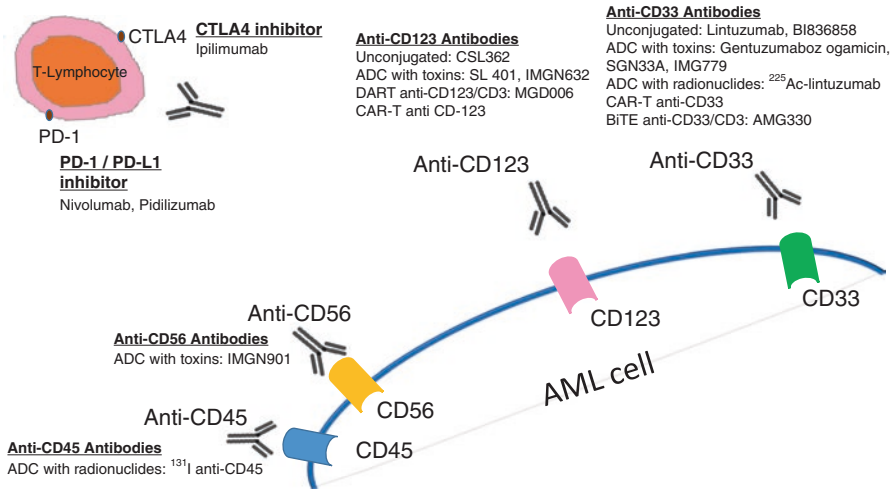

Fig. 4.2 Current immunotherapeutic in development for AML

monoclonal antibodies showed encouraging efficacy albeit with a potential for significant toxicity. The new generation of monoclonal antibodies with more effective payloads and better-selected targets are showing further enhanced activity with abrogated toxicity profiles. BiTEs, CAR-T cells, and other T-cell engaging agents along with immune checkpoint inhibitors are designed to harness the patient's own immune system to target and kill leukemic cells, and the preclinical and early

clinical data are very promising. With rationally designed biomarker driven clinical trials these agents may well find a place in frontline treatment of high-risk AML as well as in salvage or maintenance setting.

Immunotherapy research in solid tumors has significantly enhanced our understanding of solid tumor cancer biology, and we hope that the ongoing research in leukemia will similarly help us better understand the underlying mechanisms of AML. However, several critical issues need to be addressed before immunotherapy is widely used in clinical practice for AML, including (1) defining the best targets in order to eradicate the disease while sparing the normal tissue, (2) accurately timing systemic therapy which may be lymphodepleting and may limit the efficacy of T cells required for immunotherapy effect, (3) timing of the immunotherapy in the context of high tumor burden with rapid proliferation often seen in hematologic malignancies and leukemias, (4) identification of ideal T-cell antigens to enable the development of targeted adoptive T-cell strategies with maximum potency and limited collateral organ damage, (5) improving the technology of targeted therapies to ensure better stability, delivery, and efficacy, (6) defining the ideal approach for combining immunotherapy with standard chemotherapy or other anti-leukemic therapies, (7) recognizing and developing standardized management of immune-mediated toxicities in leukemias, (8) and identification of resistance mechanisms with development of strategies to overcome such mechanisms. The gamut of ongoing and future clinical trials with extensive biomarker assays will likely help answer a number of these questions and allow immunotherapy to find its true niche in AML therapy.

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References

1. Yates JW, Wallace Jr HJ, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. Cancer Chemother Rep. 1973;57(4):485–8.
2. Kantarjian H, et al. Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. Cancer. 2006;106(5):1090–8. doi:10.1002/cncr.21723.
3. Kantarjian HM. Therapy for elderly patients with acute myeloid leukemia: a problem in search of solutions. Cancer. 2007;109(6):1007–10. doi:10.1002/cncr.22502.
4. Giles F, et al. Outcome of patients with acute myelogenous leukemia after second salvage therapy. Cancer. 2005;104(3):547–54. doi:10.1002/cncr.21187.
5. Ravandi F, et al. (2010) Characteristics and outcome of patients with acute myeloid leukemia refractory to 1 cycle of high-dose cytarabine-based induction chemotherapy. Blood 116(26):5818–23; quiz 6153. doi:10.1182/blood-2010-07-296392
6. Horowitz MM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75(3):555–62.
7. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol. 2005;23(9):1147–57. doi:10.1038/nbt1137.
8. Shuptrine CW, Surana R, Weiner LM. Monoclonal antibodies for the treatment of cancer. Semin Cancer Biol. 2012;22(1):3–13. doi:10.1016/j.semcancer.2011.12.009.

9. Saito Y, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med. 2010;2(17):17ra9. doi:10.1126/scitranslmed.3000349.
10. Freeman SD, Kelm S, Barber EK, Crocker PR. Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules. Blood. 1995;85(8):2005–12.
11. Guo W, et al. Numbers and cytotoxicities of CD3 + CD56+ T lymphocytes in peripheral blood of patients with acute myeloid leukemia and acute lymphocytic leukemia. Cancer Biol Ther. 2013;14(10):916–21. doi:10.4161/cbt.25938.
12. Munoz L, et al. Interleukin-3 receptor alpha chain (CD123) is widely expressed in hematologic malignancies. Haematologica. 2001;86(12):1261–9.
13. Hauswirth AW, et al. Expression of the target receptor CD33 in CD34+/CD38−/CD123+ AML stem cells. Eur J Clin Invest. 2007;37(1):73–82. doi:10.1111/j.1365-2362.2007.01746.x.
14. Raza A, et al. Complete remissions observed in acute myeloid leukemia following prolonged exposure to lintuzumab: a phase 1 trial. Leuk Lymphoma. 2009;50(8):1336–44. doi:10.1080/10428190903050013.
15. Feldman EJ, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. J Clin Oncol. 2005;23(18):4110–6. doi:10.1200/jco.2005.09.133.
16. Sekeres MA, et al. Randomized phase IIb study of low-dose cytarabine and lintuzumab versus low-dose cytarabine and placebo in older adults with untreated acute myeloid leukemia. Haematologica. 2013;98(1):119–28. doi:10.3324/haematol.2012.066613.
17. Vasu SHS, Cheney C, Gopalakrishnan B, Mani R, Lozanski G, Mo X. Decitabine upregulates surface expression of ligands for NK activating receptors in AML blasts resulting in increased efficacy of the novel CD33-specifci Fc-engineered antibody BI 836858 against primary AML blasts – a rational for combination therapy. Blood. 2016;127(23):2879–89.
18. Lapusan S, et al. Phase I studies of AVE9633, an anti-CD33 antibody-maytansinoid conjugate, in adult patients with relapsed/refractory acute myeloid leukemia. Invest New Drugs. 2012;30(3):1121–31. doi:10.1007/s10637-011-9670-0.
19. Bross PF, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clin Cancer Res. 2001;7(6):1490–6.
20. Sievers EL, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. J Clin Oncol. 2001;19(13):3244–54.
21. Larson RA, et al. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. Cancer. 2005;104(7):1442–52. doi:10.1002/cncr.21326.
22. Petersdorf SH, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. Blood. 2013;121(24):4854–60. doi:10.1182/blood-2013-01-466706.
23. McKoy JM, et al. Gemtuzumab ozogamicin-associated sinusoidal obstructive syndrome (SOS): an overview from the research on adverse drug events and reports (RADAR) project. Leuk Res. 2007;31(5):599–604. doi:10.1016/j.leukres.2006.07.005.
24. Rajvanshi P, Shulman HM, Sievers EL, McDonald GB. Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy. Blood. 2002;99(7):2310–4.
25. Burnett AK, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. J Clin Oncol. 2011;29(4):369–77. doi:10.1200/jco.2010.31.4310.
26. Burnett AK, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy improves survival in older patients with acute myeloid leukemia. J Clin Oncol. 2012;30(32):3924–31. doi:10.1200/jco.2012.42.2964.
27. Castaigne S, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet (London, England). 2012;379(9825):1508–16. doi:10.1016/s0140-6736(12)60485-1.

28. Hills RK, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. Lancet Oncol. 2014;15(9):986–96. doi:10.1016/s1470-2045(14)70281-5.
29. Ravandi F, et al. Gemtuzumab ozogamicin: time to resurrect? J Clin Oncol. 2012;30(32):3921–3. doi:10.1200/jco.2012.43.0132.
30. Rowe JM, Lowenberg B. Gemtuzumab ozogamicin in acute myeloid leukemia: a remarkable saga about an active drug. Blood. 2013;121(24):4838–41. doi:10.1182/blood-2013-03-490482.
31. Kung Sutherland MS, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. Blood. 2013;122(8):1455–63. doi:10.1182/blood-2013-03-491506.
32. Sutherland MK, et al. 5-azacytidine enhances the anti-leukemic activity of lintuzumab (SGN-33) in preclinical models of acute myeloid leukemia. mAbs. 2010;2(4):440–8.
33. Fathi A Erba HP, Lancet JE, Stein B, Walter R, DeAngelo D. (2015) SGN-CD33A plus hypomethylating agents: a novel, well-tolerated regimen with high remission rate in frontline unfit AML. Blood 126(Abst. 454).
34. Watkins K WR, Fishkin N, Audette Ch, Kovtun Y, Romanelli A (2015) IMGN779, a CD33-targeted antibody-drug conjugate (ADC) with a novel DNA-alkylating effector molecule, induces DNA damage, cell cycle arrest, and apoptosis in AML cells. ASH Abstract No 1366.
35. Whiteman KR, et al. Lorvotuzumab mertansine, a CD56-targeting antibody-drug conjugate with potent antitumor activity against small cell lung cancer in human xenograft models. mAbs. 2014b;6(2):556–66. doi:10.4161/mabs.27756.
36. Whiteman KR NP, Walker R, Watkins K, Kovtun Y, Harvey L, Wilhelm A, et al. (2014a) The antibody-drug conjugate (ADC) IMGN779 is highly active in vitro and in vivo against acute myeloid leukemia (AML) with FLT3-ITD mutations. ASH Abstract No 2321.
37. Bagley CJ, Woodcock JM, Stomski FC, Lopez AF. The structural and functional basis of cytokine receptor activation: lessons from the common beta subunit of the granulocyte-macrophage colony-stimulating factor, interleukin-3 (IL-3), and IL-5 receptors. Blood. 1997;89(5):1471–82.
38. Testa U, et al. Elevated expression of IL-3Ralpha in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. Blood. 2002;100(8):2980–8. doi:10.1182/blood-2002-03-0852.
39. He SZ, et al. A Phase 1 study of the safety, pharmacokinetics and anti-leukemic activity of the anti-CD123 monoclonal antibody CSL360 in relapsed, refractory or high-risk acute myeloid leukemia. Leuk Lymphoma. 2015;56(5):1406–15. doi:10.3109/10428194.2014.956316.
40. Smith BD RJ, Walter RB, et al. (2014) 120 First-in Man, Phase 1 Study of CSL362 (Anti-IL3Rα/Anti-CD123 monoclonal antibody) in patients with CD123+ acute myeloid leukemia (AML) in CR at high risk for early relapse. Blood. Abstract 616
41. Frankel AE, Ramage J, Kiser M, Alexander R, Kucera G, Miller MS. Characterization of diphtheria fusion proteins targeted to the human interleukin-3 receptor. Protein Eng. 2000;13(8):575–81.
42. Frankel AE, et al. Activity of SL-401, a targeted therapy directed to interleukin-3 receptor, in blastic plasmacytoid dendritic cell neoplasm patients. Blood. 2014;124(3):385–92. doi:10.1182/blood-2014-04-566737.
43. Konopleva M HD, Rizzieri D, Cirrito T, Liu JS, Kornblau S, Grable M, Hwang IL, Borthakur G, et al. (2010) Phase I trial results for SL-401, a novel cancer stem cell (CSC) targeting agent, demonstrate clinical efficacy at tolerable doses in patients with heavily pre-treated AML, poor risk elderly AML, and high risk MDS. 53th ASH Annual Meeting and Exposition Abstract No 3298.
44. Pemmaraju N, Lane A, Sweet K, Stein A, Vasu S, Konopleva M, et al. Results from ongoing phase 2 registration study of SL-401 in patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN). EHA 2016, Abstract S812.
45. Sweet K PN, Lane A, Stein A, Vasu S, Blum W, Rizzieri DA, et al. (2015) Lead-in stage results of a pivotal trial of SL-401, an interleukin-3 receptor (IL-3R) targeting biologic, in

patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN) or acute myeloid leukemia (AML) ASH 2015 Abstract No 3795
46. Zeromski J, Nyczak E, Dyszkiewicz W. Significance of cell adhesion molecules, CD56/NCAM in particular, in human tumor growth and spreading. Folia Histochem Cytobiol. 2001;39(Suppl 2):36–7.
47. Cunningham BA, Hemperly JJ, Murray BA, Prediger EA, Brackenbury R, Edelman GM. Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. Science (New York, NY). 1987;236(4803):799–806.
48. Patel K, et al. Neural cell adhesion molecule (NCAM) is the antigen recognized by monoclonal antibodies of similar specificity in small-cell lung carcinoma and neuroblastoma. Int J Cancer. 1989;44(4):573–8.
49. Erickson HK, Lambert JM. ADME of antibody-maytansinoid conjugates. AAPS J. 2012;14(4):799–805. doi:10.1208/s12248-012-9386-x.
50. Shah MH, et al. Phase I study of IMGN901, a CD56-targeting antibody-drug conjugate, in patients with CD56-positive solid tumors. Invest New Drugs. 2016;34(3):290–9. doi:10.1007/s10637-016-0336-9.
51. Clift RA, et al. Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. Blood. 1990;76(9):1867–71.
52. Scheinberg DA, et al. A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: specific bone marrow targeting and internalization of radionuclide. J Clin Oncol. 1991;9(3):478–90.
53. Burke JM, et al. Cytoreduction with iodine-131-anti-CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. Bone Marrow Transplant. 2003;32(6):549–56. doi:10.1038/sj.bmt.1704201.
54. Matthews DC, et al. Phase I study of (131)I-anti-CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. Blood. 1999;94(4):1237–47.
55. Pagel JM, et al. 131I-anti-CD45 antibody plus busulfan and cyclophosphamide before allogeneic hematopoietic cell transplantation for treatment of acute myeloid leukemia in first remission. Blood. 2006;107(5):2184–91. doi:10.1182/blood-2005-06-2317.
56. Rosenblat TL, et al. Sequential cytarabine and alpha-particle immunotherapy with bismuth-213-lintuzumab (HuM195) for acute myeloid leukemia. Clin Cancer Res. 2010;16(21):5303–11. doi:10.1158/1078-0432.ccr-10-0382.
57. Jurcic JG RT, McDevitt MR, et al. (2011) Phase I trial of the targeted alpha-particle nano-generator Actinium-225(225Ac)-Lintuzumab (anti-CD33; HuM195) in acute myeloid leukemia (AML). ASH Abstract No 768
58. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 2009;69(12):4941–4. doi:10.1158/0008-5472.can-09-0547.
59. Topp MS, et al. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol. 2011;29(18):2493–8. doi:10.1200/jco.2010.32.7270.
60. Krupka C, et al. CD33 target validation and sustained depletion of AML blasts in long-term cultures by the bispecific T-cell-engaging antibody AMG 330. Blood. 2014;123(3):356–65. doi:10.1182/blood-2013-08-523548.
61. Laszlo GS, et al. Cellular determinants for preclinical activity of a novel CD33/CD3 bispecific T-cell engager (BiTE) antibody, AMG 330, against human AML. Blood. 2014;123(4):554–61. doi:10.1182/blood-2013-09-527044.
62. Rader C. DARTs take aim at BiTEs. Blood. 2011;117(17):4403–4. doi:10.1182/blood-2011-02-337691.
63. Chichili GR, et al. A CD3xCD123 bispecific DART for redirecting host T cells to myelogenous leukemia: preclinical activity and safety in nonhuman primates. Sci Transl Med. 2015;7(289):289ra82. doi:10.1126/scitranslmed.aaa5693.

64. Knuth A, Danowski B, Oettgen HF, Old LJ. T-cell-mediated cytotoxicity against autologous malignant melanoma: analysis with interleukin 2-dependent T-cell cultures. Proc Natl Acad Sci U S A. 1984;81(11):3511–5.
65. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science (New York, NY). 2015;348(6230):62–8. doi:10.1126/science.aaa4967.
66. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci U S A. 1989;86(24):10024–8.
67. Pegram HJ, Park JH, Brentjens RJ. CD28z CARs and armored CARs. Cancer journal (Sudbury, MA). 2014;20(2):127–33. doi:10.1097/ppo.0000000000000034.
68. Grupp SA, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368(16):1509–18. doi:10.1056/NEJMoa1215134.
69. Ritchie DS, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013;21(11):2122–9. doi:10.1038/mt.2013.154.
70. Pizzitola I, et al. Chimeric antigen receptors against CD33/CD123 antigens efficiently target primary acute myeloid leukemia cells in vivo. Leukemia. 2014;28(8):1596–605. doi:10.1038/leu.2014.62.
71. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64. doi:10.1038/nrc3239.
72. Motz GT, Coukos G. Deciphering and reversing tumor immune suppression. Immunity. 2013;39(1):61–73.
73. Kirkwood JM, Tarhini AA, Panelli MC, Moschos SJ, Zarour HM, Butterfield LH, Gogas HJ. Next generation of immunotherapy for melanoma. J Clin Oncol. 2008;26(20):3445–55.
74. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271(5256):1734–6.
75. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity. 1994;1(9):793–801.
76. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999;11(2):141–51.
77. Velu V, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature. 2009;458(7235):206–10. doi:10.1038/nature07662.
78. Dong H, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8(8):793–800. doi:10.1038/nm730.
79. Barber DL, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439(7077):682–7. doi:10.1038/nature04444.
80. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23. doi:10.1056/NEJMoa1003466.
81. Page DB, Postow MA, Callahan MK, Allison JP, Wolchok JD. Immune modulation in cancer with antibodies. Annu Rev Med. 2014;65:185–202. doi:10.1146/Annurev-Med-092012-112807.
82. Robert C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517–26. doi:10.1056/NEJMoa1104621.
83. Andorsky DJ, Yamada RE, Said J, Pinkus GS, Betting DJ, Timmerman JM. Programmed death ligand 1 is expressed by non-hodgkin lymphomas and inhibits the activity of tumor-associated T cells. Clin Cancer Res. 2011;17(13):4232–44. doi:10.1158/1078-0432.ccr-10-2660.
84. Costello RT, Mallet F, Sainty D, Maraninchi D, Gastaut JA, Olive D. Regulation of CD80/B7-1 and CD86/B7-2 molecule expression in human primary acute myeloid leukemia and their role in allogenic immune recognition. Eur J Immunol. 1998;28(1):90–103. doi:10.1002/(SICI)1521-4141(199801)28:01<90::AID-IMMU90>3.0.CO;2-5.
85. Graf M, et al. High expression of costimulatory molecules correlates with low relapse-free survival probability in acute myeloid leukemia (AML). Ann Hematol. 2005;84(5):287–97. doi:10.1007/s00277-004-0978-0.

86. Re F, et al. Expression of CD86 in acute myelogenous leukemia is a marker of dendritic/monocytic lineage. Exp Hematol. 2002;30(2):126–34.
87. Vollmer M, et al. Expression of human leucocyte antigens and co-stimulatory molecules on blasts of patients with acute myeloid leukaemia. Br J Haematol. 2003;120(6):1000–8.
88. Whiteway A, Corbett T, Anderson R, Macdonald I, Prentice HG. Expression of co-stimulatory molecules on acute myeloid leukaemia blasts may effect duration of first remission. Br J Haematol. 2003;120(3):442–51.
89. Fevery S, et al. CTLA-4 blockade in murine bone marrow chimeras induces a host-derived antileukemic effect without graft-versus-host disease. Leukemia. 2007;21(7):1451–9. doi:10.1038/sj.leu.2404720.
90. Mumprecht S, Schurch C, Schwaller J, Solenthaler M, Ochsenbein AF. Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression. Blood. 2009;114(8):1528–36. doi:10.1182/blood-2008-09-179697.
91. Zhang L, Gajewski TF, Kline J. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. Blood. 2009;114(8):1545–52. doi:10.1182/blood-2009-03-206672.
92. Zhou Q, et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. Blood. 2011;117(17):4501–10. doi:10.1182/blood-2010-10-310425.
93. Koestner W, et al. PD-L1 blockade effectively restores strong graft-versus-leukemia effects without graft-versus-host disease after delayed adoptive transfer of T-cell receptor gene-engineered allogeneic CD8+ T cells. Blood. 2011;117(3):1030–41. doi:10.1182/blood-2010-04-283119.
94. Zhou Q, et al. Program death-1 signaling and regulatory T cells collaborate to resist the function of adoptively transferred cytotoxic T lymphocytes in advanced acute myeloid leukemia. Blood. 2010;116(14):2484–93. doi:10.1182/blood-2010-03-275446.
95. Daver N, Basu S, Garcia-Manero G, Cortes J, Ravandi F, Ning J, Sharma P, et al. Defining the Immune Checkpoint Landscape in Patients with Acute Myeloid Leukemia. Blood 2016; 128:2900.
96. Berger R, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res. 2008;14(10):3044–51. doi:10.1158/1078-0432.CCR-07-4079.
97. Yang H, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. Leukemia. 2013; doi:10.1038/leu.2013.355.
98. Daver N, Basu S, Garcia-Manero G, Cortes J, Ravandi F, Sharma P, et al. Phase IB/II Study of Nivolumab in Combination with 5-Azacitidine in Patients with Relapsed Acute Myeloid Leukemia. Blood 2016;128:763
99. Davids MS KH, Costello CL, McSweeney PA, Liguori R, Lukez A, et al. (2015) A multicenter phase I/Ib study of ipilimumab for relapsed hematologic malignancies after allogeneic hematopoietic stem cell transplantation. ASH Abstract No 723
100. Richter G, Burdach S. ICOS: a new costimulatory ligand/receptor pair and its role in T-cell activion. Onkologie. 2004;27(1):91–5. doi:10.1159/000075612.
101. Richter G, et al. Tumor necrosis factor-alpha regulates the expression of inducible costimulator receptor ligand on CD34(+) progenitor cells during differentiation into antigen presenting cells. J Biol Chem. 2001;276(49):45686–93. doi:10.1074/jbc.M108509200.
102. Wang C, Lin GH, McPherson AJ, Watts TH. Immune regulation by 4-1BB and 4-1BBL: complexities and challenges. Immunol Rev. 2009;229(1):192–215. doi:10.1111/j.1600-065X.2009.00765.x.
103. Imura A, et al. OX40 expressed on fresh leukemic cells from adult T-cell leukemia patients mediates cell adhesion to vascular endothelial cells: implication for the possible involvement of OX40 in leukemic cell infiltration. Blood. 1997;89(8):2951–8.

Chapter 5
Immunotherapy in NSCLC: A Promising and Revolutionary Weapon

Christian Rolfo, Christian Caglevic, Mariacarmela Santarpia, Antonio Araujo, Elisa Giovannetti, Carolina Diaz Gallardo, Patrick Pauwels, and Mauricio Mahave

C. Rolfo, M.D., Ph.D., M.B.Ah (✉)
Phase I—Early Clinical Trials Unit, Oncology Department, University Hospital Antwerp, Edegem, Belgium

Center or Oncological Research (CORE), Antwerp University, Antwerp, Belgium
e-mail: christian.rolfo@uza.be

C. Caglevic, M.D.
Department of Investigational Cancer Drugs, Medical Oncology Department, Instituto Oncológico Fundación Arturo López Pérez, Santiago, Chile

Medical Oncology Department, Instituto Oncológico Fundación Arturo López Pérez, Santiago, Chile

M. Santarpia, M.D.
Medical Oncology Unit, Department of Human Pathology 'G. Barresi', University of Messina, Messina, Italy

A. Araujo, M.D., Ph.D.
Medical Oncology Department, Centro Hospitalar do Porto, Porto, Portugal

E. Giovannetti, M.D., Ph.D.
Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

C.D. Gallardo, M.D.
Immunology Unit, Internal Medicine Service, Clinicas Las Condes, Santiago, Chile

P. Pauwels, M.D., Ph.D.
Molecular Pathology Unit, Pathology Department, Antwerp University Hospital, Edegem, Belgium

M. Mahave, M.D.
Medical Oncology Department, Instituto Oncológico Fundación Arturo López Pérez, Santiago, Chile

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Abstract Lung cancer is the leader malignancy worldwide accounting 1.5 millions of deaths every year. In the United States the 5 year-overall survival is less than 20% for all the newly diagnosed patients. Cisplatin-based cytotoxic chemotherapy for unresectable or metastatic NSCLC patients in the first line of treatment, and docetaxel in the second line, have achieved positive results but with limited benefit in overall survival. Targeted therapies for EGFR and ALK mutant patients have showed better results when compared with chemotherapy, nevertheless most of patients will fail and need to be treated with chemotherapy if they still have a good performance status.

Immunotherapy recently has become the most revolutionary treatment in solid tumors patients. First results in unresectable and metastatic melanoma patients treated with an anti CTLA-4 monoclonal antibody showed an unexpected 3-year overall survival of at least 25%.

Lung cancer cells have multiple immunosuppressive mechanisms that allow to escape of the immune system and survive, however blocking CTLA-4 pathway with antibodies as monotherapy treatment have not achieved same results than in melanoma patients. PD-1 expression has been demonstrated in different tumor types, suggesting than PD-1 / PD-L1 pathway is a common mechanism used by tumors to avoid immune surveillance and favoring tumor growth. Anti PD-1 and anti PD-L1 antibodies have showed activity in non-small cell lung cancer patients with significant benefit in overall survival, long lasting responses and good safety profile, including naïve and pretreated patients regardless of the histological subtype. Even more, PD-1 negative expression patients achieve similar results in overall survival when compared with patients treated with chemotherapy. In the other side high PD-1 expression patients that undergo immunotherapy treatment achieve better results in terms of survival with lesser toxicity. Combining different immunotherapy treatments, combination of immunotherapy with chemotherapy or with targeted treatment are under research with some promising PRELIMINARY results in non-small cell lung cancer patients.

This chapter attempts to summarize the development of immunotherapy treatment in non-small cell lung cancer patients and explain the results that have leaded immunotherapy as a new standard of treatment in selected NSCLC patients.

Keywords Immunotherapy • PDL1 • PD1 • NSCLC • Immune checkpoints

5.1 Introduction

Non-small cell lung cancer (NSCLC) represents around 85% of all the lung cancer types; most of the patients are diagnosed at uncurable stages; and it is the leader cause of mortality by cancer worldwide. Tobacco consumption is the most important risk factor related with this disease and represents the major reason of regional differences of its epidemiology [1]. Environmental pollution and some mineral exposures are also related with NSCLC, as an example some northern cities of Chile have a very high incidence and mortality due to lung cancer, thus it is presumed to be related with water contamination by arsenic [2].

Until the current times, metastatic NSCLC has been an incurable malignancy and only palliative treatments can be offered to patients with the purpose to improve quality of life and prolong survival.

By late 1980s it was reported, in a Canadian prospective randomized trial, that cisplatin-based combinations had a modest benefit in overall survival when compared with best supportive care in NSCLC metastatic patients, but treatments were associated to a high toxicity [3].

Twenty years later, a meta-analysis showed a 9% benefit in 1-year overall survival in advanced NSCLC patients that underwent chemotherapy plus best supportive care when compared with best supportive care alone [4].

Differences in overall survival have been found depending of the histologic subtypes and depending on the type of cisplatin-based chemotherapy combination used in advanced or metastatic NSCLC patients [5].

After failure to a first line of cytotoxic chemotherapy for metastatic disease, docetaxel may be used as a second line of treatment for patients that are still in good performance status, with a benefit of 3 months in overall survival when compared with best supportive care [6]. Patients that have not previously used pemetrexed can use it as a second-line treatment with similar outcomes in overall survival when compared with docetaxel but with a significant lower toxicity profile [7]. Patients that have failed to a second line of chemotherapy, without significative clinical worsening, can be considered for a third or further lines of treatment, but with uncertain results and with lesser support by literature [8].

Adding an antiangiogenic drug to cytotoxic chemotherapy has become a strategy looking forward to improve survival in metastatic non-squamous NSCLC. Bevacizumab has got approval by FDA for this group of patients based on several clinical trials despite controversial results and interpretation of them [9]. In Europe, Nintedanib, an oral antiangiogenic drug that simultaneously inhibits VEGFR, FGFR, PDGFR, and also RET [10], has got approval in combination with docetaxel for second-line metastatic non-squamous NSCLC patients achieving improve overall survival in 12.6 months for nintedanib plus docetaxel combination versus 10.3 months for the docetaxel plus placebo arm [11].

Until the earliest 2000s, clinicians just needed to know if the patient with lung cancer had a non-small cell lung cancer or a small cell lung cancer. For patients with NSCLC, it had clinical implicance to know if the histology was squamous or non-squamous NSCLC, non-squamous subtypes correspond mainly to adenocarcinoma and large cell tumors. Based on this classification, most of the decisions were taken in order to define the chemotherapy treatment in metastatic NSCLC patients. The decision-making treatment's algorithm had to be rebuilt due to the discover of new specific genetic changes with potential target treatments, in special in non-squamous NSCLC patients with local advanced and metastatic disease. There are two types of mutations in NSCLC that have been targeted with a novel type of anticancer drugs: kinase inhibitors. The mutations involved are Epidermal Growth Factor Receptor (EGFR) mutations and the fusion gene Echinoderm Microtubule-Associated Protein-Like 4 (EML4) with the Anaplastic Lymphoma Kinase (ALK) gene, creating the EML4/ALK fusion gene [12]. There are three drugs that have got

approval for NSCLC with EGFR mutations: gefitinib, erlotinib, and afatinib, and two drugs that have also got approval that target the EML4/ALK fusion gene: crizotinib and ceritinib. In selected population with specific mutations, those drugs have been tested against chemotherapy with better outcomes (overall survival, progression-free survival, response rate), that is the reason why when EGFR or EML4/ALK mutations are detected in NSCLC patients, targeted treatment should be preferred over chemotherapy. Unfortunately only one-fourth of the total of patients, considering epidemiological and regional disparities, will have one type of these mutations, mainly EGFR mutations. Thus, targeted therapy to EGFR and EML4/ALK mutations became the new standard of care since late 2000s in mutated patients. Recently, for specific anti-EGFR-treatment resistance, new generation drugs are available.

Cancers are characterized by different genetic and epigenetic alterations; specifically high rates of somatic mutations in lung cancer generate a variety of tumor-specific antigens and may contribute to increase immunogenicity [13]. Unfortunately, often oncogenic processes are studied independently of the antitumoral immune response (IR), which is a paradox, since one of the fundamental roles of the immune system (IS) is to distinguish self from foreign elements. Specifically, cancer is consolidated as a result of the failure of various immunological mechanisms intended to eliminate altered antigens [14, 15]. With the aim of preventing the development of neoplasia, the immune system has different ways to recognize cells that have escaped from the intrinsic suppressor mechanisms, identifying and destroying clones of transformed cells before they grow and form tumors, as well as recognize and eliminate tumors already formed [16].

It is important to remember that innate immune system is composed by dendritic cells, macrophages, Natural Killer (NK) cells, granulocytes (basophils, eosinophils, and neutrophils), complement proteins, chemokines and cytokines, among others. It corresponds to a rapid response system to an antigen but unspecific. Meanwhile, adaptive IR, constituted by B and T lymphocytes, both CD4 and CD8, in addition to the antibodies, is a specific response but with slower installation, with the ability to leave immunological memory.

The antitumor–IR has been pedagogically divided into seven stages [14–17] (Fig. 5.1), which are the Cancer-immunity Cycle: (a) Release of cancer cells antigens (tumor cell death); (b) Cancer antigens presentation (fundamental role of dendritic antigen-presenting cells and professionals—APC); (c) APC and T cells priming and activation; (d)Trafficking of cytotoxic T-cells to tumor; (e) T lymphocyte infiltration into the tumor (cytotoxic T lymphocytes, endothelial cells); (f) Recognition of tumor cells by T lymphocytes; and finally (g) Death of the tumor cells.

During the presentation phase, the APC presents the antigen to either T or B cells, which have in their membrane a specific recognition receptor (TCR and BCR, respectively). However, this single signal is not sufficient to achieve lymphocyte activation and simultaneous presence of co-stimulatory molecules is required (interaction between CD80/CD28, CD40/CD40-ligand, CD86/CTLA-4, ICOS/ICOS ligand, among other).

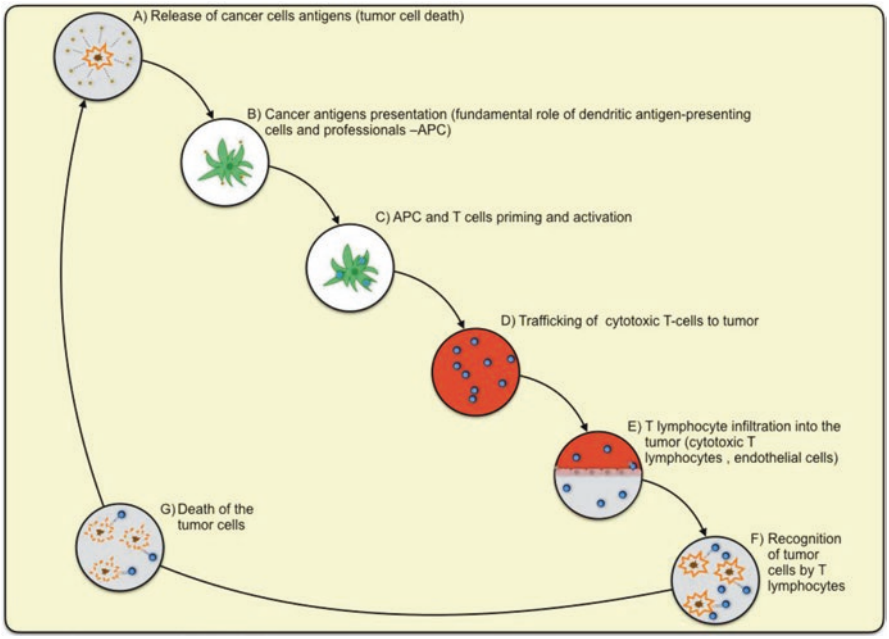

Fig. 5.1 The cancer-immunity cycle

In addition, we must consider that every normal IR has mechanisms intended to prevent its perpetuation and the consequent damage associated with an exaggerated response. In this process, participate the regulatory T cells (Tregs), the expression of inhibitory receptors (called checkpoints), the activation of apoptosis, and cell depletion [18].

Parallel to these events, tumors evolve mechanisms to elude or to inhibit the IR, for example, the downregulation of the antigen presentation (downregulation of the major histocompatibility complex—MHC), upregulation of inhibitors of apoptosis (Bcl-XL, FLIP), or expression of inhibitory cell surface molecules that directly kill cytotoxic T cells (programmed cell death 1 ligand 1-PD-L1, FasL). In addition, tumor cells secrete factors that inhibit effector immune cell functions (TGF-β, IL-10, VEGF, LXR-L, IDO, gangliosides, or soluble MICA) or recruit regulatory cells to generate an immunosuppressive microenvironment (IL-4, IL-13, GM-CSF, IL-1β, VEGF, or PGE2). Once recruited, regulatory cells attenuate antitumor immunity through the liberation of immunosuppressive cytokines and by altering the nutrient content of the microenvironment. Specifically, secretion of IL-4 and IL-13 leads to recruitment and polarization of M2 macrophages, which express TGF-β, IL-10, and PDGF that inhibit T cells. The release of colony-stimulating factors, IL-1β, VEGF, or PGE2 by tumor cells results in the accumulation of myeloid-derived suppressor cells (MDSCs) that can block T cell function by expressing TGF-β, ARG1, and iNOS. Tregs can also inhibit effector T cells through multiple mechanisms, including expression of CTLA-4 [16].

Recently, an unexpected weapon against NSCLC appeared in action. First positive results were published in 2015, immunotherapy "came to stay" and has taken an unequivocal place in the treatment of this malignancy. First attempts, using drugs, as monodrug therapy that blocked CTLA-4 pathway, failed in showed benefit in overall survival in NSCLC patients. Nevertheless, anti-PD-1 treatment have shown impressive results that have turned in some countries the upfront treatment for NSCLC patients. Combination of immunotherapy drugs with different mechanisms of action are under study but early results are already promising.

5.2 Pathways and Drugs in Immunotherapy NSCLC Treatment

5.2.1 CTLA-4 Pathway

The IS has counterregulatory mechanisms that limit potentially harmful amplification of the IR. Specifically, posterior to antigen exposure, an upregulation of different molecules on the surface of the T cells is evidenced, aimed to end the IR. These molecules are known as checkpoints, i.e., CTLA-4, LAG-3, PD-1/2, TIM-3. In some tumors, including lung cancer, the expression of these molecules is altered [19, 20]. CTLA-4 is constitutively expressed in Tregs but only upregulated in conventional T cells after activation. Its function is to inhibit the activation of these cells.

Once the T-cells are activated by the interaction of MHC of the APC with the TCR of these cells, associated with co-stimulatory molecules (e.g., CD28 binding to CD80/86), the CTLA-4 expression occurs at the level of the cell membrane. CD28 and CTLA-4 share identical ligands, CD80 and CD86. However, CTLA-4 has a higher overall affinity for both ligands. This interaction ends the IR. The critical role of CTLA-4 in maintaining the self-tolerance is demonstrated by a rapidly lethal systemic immune-hyperactivation phenotype in knockout mice [21].

CTLA-4 was the first immune checkpoint targeted for cancer therapy in clinical practice. The anti-CTLA-4 antibodies interpose and prevent the interaction between CTLA-4 and its receptor, thereby inhibiting the completion of the IR and allowing the maintenance of the antitumoral IR. This is associated with the increase of the effectors T-cells and a dramatic reduction of the intratumoral Tregs [22, 23].

5.2.1.1 CTLA-4 Inhibitors

Ipilimumab

Currently, the most famous CTLA-4 inhibitor is ipilimumab. This drug is a fully humanized IgG1 anti-cytotoxic T-lymphocyte antigen CTLA-4 monoclonal antibody that has the potential to block the binding of CTLA-4 to its ligand. By blocking the

regulatory mechanisms of the T-cell regulator CTLA-4, ipilimumab allows the immune system to attack the tumor cells [24].

First originated in the University of California, ipililumab currently is under license of Bristol-Myers Squibb [25].

Ipilimumab was the first check point inhibitor ever approved for cancer treatment. Hodi et col. published the positive results in unresectable and metastatic melanoma patients in terms of overall survival when comparing ipilimumab with or without combination with glycoprotein 100 peptide vaccine (gp100) against gp100 alone [26].

Despite the great favorable outcomes in unresectable or metastatic melanoma, NSCLC patients that have undergone treatment with ipilimumab monotherapy have not achieved those same results.

The assumption that tumor necrosis, due to cytotoxic chemotherapy releases tumor antigens may enhance the response to immunotherapy, has been the base of the rationality to intent the combination treatment using carboplatin plus paclitaxel duplet in combination with ipilimumab [27]. The interactions between ipilimumab with cytotoxic chemotherapy, such as carboplatin plus paclitaxel duplet and between ipilimumab with dacarbazine, were tested by Weber in melanoma naïve treated patients in a phase I trial. The used dose of ipilimumab was 10 mg/kg intravenously every 3 weeks for a maximum of four doses; the combination dose of carboplatin was six AUC intravenously and paclitaxel dose was 175 mg/m^2 every 3 weeks. Patients without limiting toxicity were allowed to receive maintenance ipilimumab since week 24 every 12 weeks until limiting toxicity or until disease progression. No relevant pharmacodynamics or pharmacokinetics findings were found between ipilimumab either in combination with carboplatin/paclitaxel duplet or between ipilimumab with dacarbazine combination [28].

A phase 2 clinical trial that combined ipilimumab plus carboplatin/paclitaxel duplet was developed for NSCLC patients, chemotherapy naïve, stage III B and IV not amenable for curative treatment. In this study, the same doses of carboplatin and paclitaxel than in the phase 1 trial for melanoma unresectable and metastatic patients were used. This NSCLC phase 2 trial was a 3 arms study (1:1:1) that included 204 patients. The control arm was the duplet of carboplatin and paclitaxel up to 6 cycles. Ipilimumab in a dose of 10 mg/kg was given as follow: four doses of concurrent ipilimumab plus carboplatin—paclitaxel doublet followed by two doses of placebo; or given as phased—ipilimumab (two doses of placebo plus carboplatin—paclitaxel followed by ipilimumab plus the carboplatin—paclitaxel combination for four doses. Patients without limiting toxicity and or without disease progression were allowed to receive ipilimumab/placebo treatment beyond the regular end of the treatment every 12 weeks as a maintenance therapy. Immune-related response criteria and modified WHO criteria were used to assess response. Immune-related progression-free survival (irPFS) was the primary endpoint of this trial; secondary endpoints were progression-free survival, overall survival, best overall response rate, immune-related best overall response rate and safety.

The primary end point, immune-related progression-free survival (irPFS using immune-related RECIST criteria) was met for the phased ipilimumab plus chemotherapy duplet (HR 0.72, $p = 0.05$) but not for the concurrent ipilimumab plus

chemotherapy combination (HR 0.83, $p = 0.13$). Median ir-progression-free survival was 4.6 months for the carboplatin plus paclitaxel combination, 5.5 months when adding concurrent ipilimumab and up to 5.7 months when adding phased ipilimumab regimen. Progression-Free Survival using modified WHO criteria was also statistical significant in favor of the phased ipilimumab arm when compared with control arm but not for the concurrent ipilimumab arm. Median overall survival was 8.3 months for the control arm and 12.2 months for the phased group (HR 0.87 $p = 0.23$), no advantages in order of overall survival was reached in the concurrent—ipilimumab group (9.7 months HR 0.99 $p = 0.48$). The subgroup analysis showed a trend of benefit in ir-progression-free survival and in overall survival in patients treated in the phased arm that had squamous histology when compared with non-squamous histology. Regarding toxicity, grade 3 and grade 4 adverse events were similar in the 3 arms: 37% in the control arm, 41% in the concurrent arm, and 39 in the phased arm, hematological adverse events were similar in the ipilimumab-containing groups when compared with the carboplatin plus paclitaxel group. Non-hematological, any grade (>15%), adverse events were most frequent in the control arm and included fatigue, alopecia, peripheral sensory neuropathy, nausea, and vomiting. Rash, diarrhea, and pruritus were higher in the ipilimumab groups than in the control arm. Immune-related grade 3–4 toxicities such as colitis, elevated transaminases and hypophysitis were higher in the ipilimumab containing arms (20% for concurrent and 15% for phased ipilimumab groups) when compared with the control arm (6%). Two deaths related to treatment were reported, one of them was in the control group and the other in the concurrent group [29].

There is a phase 3 clinical trial currently ongoing, limited to squamous NSCLC histology, that is looking to answer if adding ipilimumab to the combination of carboplatin plus paclitaxel is beneficial for patients when compared with the cytotoxic chemotherapy combination (NCT01285609) [30]. Another phase 1 clinical trial that combines either erlotinib or crizotinib, depending if patients have EGFR- or ALK-mutated status, plus ipilimumab is also currently ongoing (NCT01998126) [31]. Results from both trials will be very important to know the potential benefit in order to combine ipilimumab with cytotoxic chemotherapy in squamous NSCLC, or to combine ipilimumab with target therapies in NSCLC patients that have EGFR- or ALK-common mutations.

Ipilimumab in combination with other immunotherapy drugs will be discussed ahead in this chapter (see in Nivolumab and Pembrolizumab).

Tremelimumab

Tremelimumab is an anti-CTLA4 IgG2 fully humanized monoclonal antibody [32]. Despite the similar mechanism of action than ipilimumab, tremelimumab, as mono-immunotherapy drug, has not shown a benefit in NSCLC patients.

In a phase 2 clinical trial for local advanced or metastatic NSCLC patients with good performance status, that had received four or more cycles of a platinum-based chemotherapy and that had achieved stable disease or any-grade response as best

overall response, were randomized to tremelimumab or to best supportive care. The primary end point of the trial, progression-free survival, was not met, with only 4% of objective response rate in the treated group. Grade 3–4 adverse events were reported in 20% of patients (including 9% of immune-related toxicities) versus none in the best supportive care arm [33].

Currently a phase 1 clinical trial that studies tremelimumab plus gefitinib combination is ongoing for pretreated patients with stage III B and IV EGFR-mutated—NSCLC (NCT02040064) [34].

Tremelimumab in combination with other immunotherapy drugs will be discussed ahead in this chapter (see in Durvalumab).

5.2.2 PD-1/PD-L1 Pathway

The PD-1 molecule (Cell Death Protein—Programmed 1) is expressed in T/B cells, NK, MDSCs after their activation. Its main function is to limit the activity of T cells in peripheral tissues, where the effector phase takes place (in contrast to the anti-CTLA-4 antibodies that fulfill their role in the initial activation of T cells). Excessive induction of PD-1 in the setting of a chronic antigenic exposure can induce anergy or exhaustion [19–35]. Inflammatory signals in tissues, mainly IFN-y, induce the expression of two ligands of this molecule, PD-L1 and PD-L2 (Programmed cell death protein 1 ligand 1 and 2 respectively), which downregulate the activity of T cells, limiting collateral tissue damage and maintaining the self-tolerance (Fig. 5.2).

Numerous tumor types express high PD-L1 levels, including NSCLC, suggesting that PD-1/PD-L1 pathway activation is a common mechanism used by tumors to avoid immune surveillance and growth [36, 37].

Specifically, the effects of PD-1/PD-L1 interaction include inhibition of T-cell proliferation, survival, and effector functions (cytokine release and cytotoxicity) and promotion of differentiation of CD4+ T cells into Tregs. PD-1 is expressed on

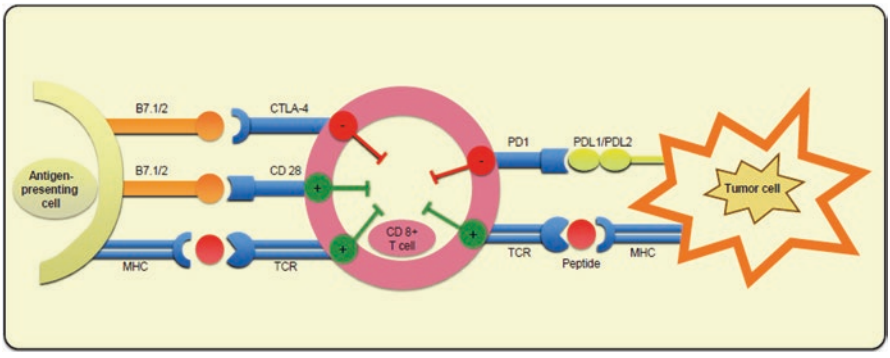

Fig. 5.2 The programmed cell death 1 (PD-1)/PD-L1 pathway. *MHC* major histocompatibility complex, *TCR* T-cell receptor, *CTLA-4* cytotoxic T-lymphocyte antigen-4

a large proportion of tumor-infiltrating lymphocytes (TILs) which appear to be "exhausted," functionally inhibited, due to chronic antigen stimulation. This exhausted state was partially reversible by PD-1 pathway blockade in murine models of chronic viral infections [19].

Blockade of PD-1 signaling can restore CD8+ T-cell functions and cytotoxic capabilities from the exhausted phenotype and enhance antitumor immunity, as demonstrated in preclinical studies [38, 39].

5.2.2.1 Anti-PD-1 Drugs

Nivolumab

Nivolumab (Opdivo®, Bristol Mayer Squibb) is a genetically engineered, fully human immunoglobulin G4 (IgG4) monoclonal antibody specific for human PD-1 [40].

The Ig G4 isotype was engineered to obviate antibody-dependent cellular cytotoxicity (ADCC). An intact ADCC has the potential to deplete activated T cells and tumor-infiltrating lymphocytes and diminish activity as PD-1 is expressed on T effector cells and other immune cells. Nivolumab binds PD-1 with high affinity and blocks its interactions with both PD-L1 and PD-L2 [41].

In the CA 209–003 study, a phase 1 clinical trial that included patients with NSCLC, melanoma, castration-resistant prostatic cancer, renal cancer, and colorectal cancer, patients were enrolled to receive nivolumab at a dose of 0.1–10 mg/kg every 2 weeks to a maximum of 12 doses or until a complete response was achieved, limiting toxicity, progressive disease or withdrawn of consent for this trial. The primary objectives were to evaluate safety and tolerability. The trial was designed as a dose escalation and cohort expansion that included 122 NSCLC patients (47 squamous, 73 non-squamous, 2 unknown) from a total of 296 patients that were enrolled in the trial. 85% of the NSCLC patients had received at least two lines of prior treatment including 34% of patients that had received a tyrosine kinase inhibitor. The maximum tolerated dose for nivolumab was not reached. In the cohort expansion, patients with NSCLC, regardless of the histology subtype, were randomized to nivolumab at doses of 1, 3, or 10 mg/kg dose. There were 11 deaths (4%) related to serious adverse events but no one of them was secondary to nivolumab according with investigators' reports. 14 NSCLC patients that underwent treatment had objective response rate, 6% at dose of 1 mg/kg, 32% at dose of 3 mg/kg, and 18% at dose of 10 mg/kg. The global response rate for squamous and non-squamous non-small cell lung cancer was 33% and 12%, respectively. Eight patients that achieved objective response had responses that lasted 24 or more weeks. 7% of the patients that had stable disease as the best response had not disease progression for at least 24 weeks. When considering all the patients that participate in the trial regardless of the primary tumor, 42 samples were analyzed for PD-1 status; of the 17 patients with PD-1 negative no objective responses were found; in the other hand, 36% of the patients that were PD-1 positive had an objective response [42].

A second publication related with the just mentioned phase 1 dose-escalation cohort expansion trial, focused only in NSCLC patients was reported on 2015, giving an update of the overall survival, durability of response, and long-term safety. The total number of enrolled patients with NSCLC was 129, whom were randomized to receive nivolumab at doses of 1, 3, or 10 mg/kg every 2 weeks, in 8 weeks cycles, for up 96 weeks. The median of age was 65 years, 42% squamous, and 57 non-squamous NSCLC histology, 98% ECOG 0–1 and 54% of all the patients had received at least three lines of prior treatment before the first dose of nivolumab. The median overall survival was 9.9 months and the progression-free survival was 2.3 months for all the patients. For all doses 1-year survival was 42%, 2-year survival 24% and 3-year survival 18%, respectively. The chosen doses for further development was nivolumab 3 mg/kg every 2 weeks and the 1-, 2-, and 3-year survival reported for this dose was 56%, 42%, and 27%, respectively, with a median overall survival of 14.9 months. The overall response rate was 17% without differences between histology subtypes, with a median duration of response of 17 months and a median progression-free survival of 20.6 months. Among all patients, 71% presented an adverse event of any grade (most frequent: fatigue 24%, decrease appetite 12% and diarrhea 10%) but only 14% had a grade 3 or 4 toxicity being the most frequent fatigue with 3%. Defined as adverse event that needed a more frequent monitoring or use of immune suppression treatment or hormonal replace treatment due nivolumab toxicity, 41% of patients presented a "select adverse event" but only 4.7% were grade 3 or 4. Two grade 3–4 and one grade 5 pneumonitis were reported as related with nivolumab. There were three deaths (2%) related with treatment, all of them were associated with pneumonitis [43].

A phase 2 trial, CheckMate 063, was a single arm trial of nivolumab at 3 mg/kg dose intravenously every 2 weeks, for squamous NSCLC patients that had received at least two previous lines of treatment for metastatic or unresectable disease. A total of 117 patients participated in this study. The primary end point of this study was to evaluate the objective response rate assessed by an independent radiologic review committee. The objective response rate was 14.5% including one patient that achieved a complete response. The median time to response reported was 3.3 months. Median duration of response was not reached. 26% of the patients achieved stable disease as best radiological response with a median duration of 6 months. The median progression-free survival was 1.9 months, 6-months progression-free survival was 25.9%, and 1-year progression-free survival was 20%. The median overall survival was 8.2 months with 1-year overall survival of 40.8%. From patients that provided tumor samples to evaluate PD-1 expression, considering cut off points of 5% or greater and lower of 5%, those evaluated that had 5% of greater PD-1 expression achieved 24% of partial response, 24% of stable disease, and 44% of progression disease as best response; patients with PD-1 expression lower than 5% had 14% of partial responses, 20% of stable disease, and 49% of progression disease as best response to nivolumab treatment. Grade 3–4 adverse events were reported in 17% of patients, the most common were fatigue 4%, diarrhea 3%, pneumonitis 3%, rash, pruritus, myalgia, and anemia 1% each. 12% of treatment-related adverse event led to discontinuation. Two deaths were attributed to nivolumab by investigators, one

due to pneumonia and the other to an ischemic stroke; however, both patients had multiples comorbidities and progression disease [44]. In a longer term follow-up of at least 11 months median duration of response was still not reached, and no new deaths due to nivolumab were reported. Peripheral increases in serum interferon-γ-stimulated cytokines were found [45].

The phase 3 clinical trial CheckMate 017 was a study focused on squamous NSCLC stage III B or IV patients that have failed to a first-line platinum-based doublet. This trial compared in a 1:1 model nivolumab at 3 mg/kg IV every 2 weeks with docetaxel 75 mg/m^2 IV every 3 weeks, both treatments until progression disease or unacceptable toxicity. The primary end point was overall survival. 260 patients were randomized to be treated. The median of age was 62 years in the nivolumab arm and 64 years in the docetaxel arm, most of the patients were men, and all had an ECOG 0–1. The median overall survival was 9.2 months for nivolumab and 6 months for docetaxel group, 1-year survival for nivolumab and docetaxel were 42% and 24%, respectively. The progression-free survival was 2.8 months for docetaxel and 3.5 months for nivolumab. The objective response rate was 20% for nivolumab and 9% for docetaxel. The median duration of response was 8.4 months for docetaxel and not reached for nivolumab (2.9–20.5 + months). PD-L1 expression was evaluated using an immunohistochemical assay, Dako North America, from rabbit monoclonal antihuman (Clone 28–8, Epitonics). Any staining at any level was considered as positive. Three levels of positivity for PDL1 expression were prespecified: 1, 5, and 10%. Authors concluded that PD-L1 expression was neither prognostic nor predictive of benefit for nivolumab. Despite that conclusion, when analyzing the graphics of the original publication it seems to be a trend to benefit in patients treated with nivolumab that had PD-L1 expression greater of 10% when compared with patients with lower levels, the same analysis may be done for patients with PD-L1 expression greater than 5% when compared with patients with lower expression of PD-L1. All grades and grade 3–4 toxicities were much higher for docetaxel arm when compared with nivolumab: 87% versus 59% for all grades, and 56% versus 8% for grade 3–4 adverse events, respectively. Fatigue, decreased appetite and diarrhea were the most common grade 3–4 adverse event reported for nivolumab. Immune-mediated adverse events by organ category was presented in gastrointestinal, pulmonary, and renal in one case each [46].

Due to the benefit in overall survival, the Independent Data Monitoring Committee recommended to stop the trial in January 2015. In March 2015, FDA-approved nivolumab as a second-line treatment for squamous NSCLC that have failed to a first line of chemotherapy cisplatin-base doublet.

Similar in design, arms and primary end point to Checkmate 017, Checkmate 057 was a phase 3 clinical trial that compared nivolumab and docetaxel in non-squamous NSCLC that had progressed during or after platinum-based doublet chemotherapy. Secondary end points included objective response rate, progression-free survival, and efficacy according to PD-L1 expression. 582 patients were randomized to receive nivolumab or docetaxel in a 1:1 randomization model. Median overall survival, 1-year overall survival and 18-months overall survival was 12.2 months, 51% and 39% for nivolumab-treated patients and 9.4 months, 39% and 23% for

docetaxel, respectively. The response rate was 19% and 12% for nivolumab and docetaxel. Despite median progression-free survival was higher for docetaxel (4.2 versus 2.3 months), 1-year progression-free survival was 8% for docetaxel and 19% for nivolumab. Grade 3–4 adverse events were much higher for docetaxel (54%) when compared with nivolumab (10%). Fatigue, diarrhea, and nausea were the most common adverse events reported related with nivolumab. As a difference from squamous NSCLC patients treated in Checkmate 017, PD-L1 expression using the same immunohistochemical assay mentioned before was predictive of outcome for all the end points. Subgroup analysis showed also benefit in current or former smokers and in KRAS-mutated patients if they had been treated with nivolumab, nevertheless, patients that had EGFR mutations, older than 75 years and or never smokers had no clear trend to benefit of the treatment with the monoclonal antibody when compared with docetaxel [47]. Thanks to the results of this trial FDA-approved nivolumab for non-squamous NSCLC-pretreated patients in October 2015.

An update in 2-year survival for Checkmate 017 and Checkmate 057 was recently presented. 2-year overall survival in Checkmate 017 was 23% for nivolumab versus 8% for docetaxel squamous NSCLC-treated patients. 2-year overall survival for non-squamous NSCLC patients from Checkmate 067 was 29% for nivolumab and 16% for docetaxel, respectively [48].

Currently ongoing, Checkmate 012 is a phase 1 trial multi-arm that assesses nivolumab in the first line of treatment for NSCLC patients either in combination with standard chemotherapy, in combination with bevacizumab, in combination with erlotinib, in combination with ipilimumab or as monotherapy [49]. Preliminary results of nivolumab in combination with platinum-based doublets in the first line of treatment have been presented. Treatment was given as follow: for squamous patients, nivolumab 10 mg/kg every 3 weeks in combination with cisplatin–gemcitabine doublet, for non-squamous NSCLC patients, nivolumab 5 or 10 mg/kg every 3 weeks in combination with carboplatin–paclitaxel doublet, or nivolumab 10 mg/kg every 3 weeks in combination with cisplatin–pemetrexed doublet. Cytotoxic chemotherapy was given for 4 cycles, nivolumab was given until unacceptable toxicity or until disease progression. Reported response rates ranged from 33 to 47%, progression-free survival at 24 weeks 38 to 71%, 1-year overall survival from 50 to 87%. Grade 3–4 toxicities were reported in 45% of the patients and include pneumonitis (7%), fatigue (5%), and acute renal failure (5%) [50].

As part of Checkmate 012, first line of treatment for NSCLC stage III B or IV with nivolumab monodrug is under study. Patients with squamous and non-squamous histology were considered for this study and received nivolumab 3 mg/kg dose every 2 weeks. Preliminary report of the first 20 patients and after a follow-up of 6 or more months, showed an objective response rate by RECIST criteria of 30%. PD-L1-positive patients (9/20) had an objective response rate of 67%; however, no objective responses were found in patients with PD-L1-negative status (6/20). Grade 3–4 adverse events were presented in three patients (15%), and they were elevated AST or ALT, rash, and hyperglycemia [51].

An update of this study was presented at the European Society for Medical Oncology Annual Meeting 2015. 52 patients were assessed for survival. Median

overall survival for all the patients was 22.6 months, when evaluated by histology median overall survival was 16.8 months for squamous NSCLC, for non-squamous NSCLC median overall survival was not reached by the time of the presentation [52].

Combining immunotherapy drugs with different mechanisms of action is an interesting strategy that has also been study in Checkmate 012. As part of this phase 1 trial, nivolumab (anti-PD-1) has been studied in combination with an anti-CTLA-4, ipilimumab. Initially, four cohorts were included in order to look forward the best dose for NSCLC patients, 2 for squamous and 2 for non-squamous carcinoma. The cohorts for squamous and non-squamous NSCLC patients were nivolumab 1 mg/kg plus ipilimumab 3 mg/kg every 3 weeks for 4 doses followed by nivolumab 3 mg/kg every 2 weeks; nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for 4 doses followed by Nivolumab 3 mg/kg every 2 weeks. Treatment was given until unacceptable toxicity or disease progression. After the study was already ongoing an amendment allowed including a new cohort of nivolumab 1 mg/kg in combination with ipilimumab 1 mg/kg every 3 weeks for 4 cycles. Results from the first 46 patients showed a confirmed response rate ranging between 7 and 25% and stable disease ranging between 19 and 50% among the patients from the first four cohorts. The overall response rate was 22% and was higher, regardless of the histology, for patients that were included in the nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks for 4 cycles followed by nivolumab 3 mg/kg every 2 weeks. Antitumor activity was seen in patients with either PD-L1-positive or negative expression. 48% of patients had a grade 3–4 adverse event, 16 patients were discontinued from the trial due to toxicity. Three deaths related with investigational treatment were report: one due to respiratory failure, one due to bronchopulmonary hemorrhage, and one due to toxic epidermal necrolysis [53].

Other studied cohorts and their preliminary results have been presented: nivolumab 1 mg/kg every 3 weeks plus ipilimumab 1 mg/kg every 3 weeks for 4 cycles followed by nivolumab 3 mg/kg every 2 weeks (a); nivolumab 1 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (b), nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 12 weeks, (c) and nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks (d). A fifth cohort of nivolumab 3 mg/kg every 3 weeks (e) has been compared with the other cohorts. For cohorts mentioned in this chapter as a, b, c, d, and e objective preliminary response rate reported were: 13%, 25%, 39%, 31%, and 23% respectively; disease control rate 55%, 58%, 74%, 51%, and 50%; median progression-free survival 10.6 months, 4.9 months, 8 months, 8.3 months, and 3.6 months. Median overall survival for nivolumab monodrug was 22.6 months and not reached for the other four cohorts. Reported grade 3 and 4 adverse events that included endocrine, gastrointestinal, hepatic, pulmonary, renal and skin disorders were higher for all the combination cohorts when compared with nivolumab alone [54].

Updates of results from cohorts c, d, and e have been recently presented. Grade 3 and 4 toxicities and discontinuation rate due to toxicity were 37%, 33%, and 19% for cohort c, d, and e, respectively. Response rate and progression-free survival was 47% and 8.1 months for cohort c, 39% and 3.9 months for cohort d, 23% and

3.6 months for cohort e. 1-year survival was 73% for cohort e, 69% for cohort d, and not calculated for cohort c (censored results). Response rate for PD-L1-negative patients was 14% for cohort e and 17% for cohorts (c + d), for patients with PD-L1 expression higher or equal to 1% the response rate was 28% for cohort e and 57% for cohorts (c + d), and for PD-L1 expression greater than 50% the response rate was 50% for cohort e and 92% for cohorts (c + d) [55].

Note: for better understanding, in order to clarify results, the different arms of this part of the phase 1 trial Checkmate 012 have been mentioned by letters a-b-c-d-e.

Pembrolizumab

Pembrolizumab (MK-3475, Keytruda®, Merck Sharp & Dohme) is a highly selective IgG4 kappa isotype monoclonal antibody against PD-1. This antibody, highly selective, binds PD-1 and blocks the PD-1, PD-L1/PD-L2 axis, thus overcoming this major immune checkpoint inhibitor [56]. It was firstly approved in 2014 for unresectable and metastatic melanoma.

Metastatic or advanced, not amenable to curative treatment, non-small cell lung cancer patients were assigned to multiple expansion cohorts as part of the phase 1 Keynote 001 clinical trial. Patients with ECOG 0–1, adequate organ function and without medical history of pneumonitis, autoimmune diseases, and without active use of systemic immunosuppressive therapy were considered to participate in this trial. The primary objectives of this trial were to evaluate the safety, toxicity profile, and activity of pembrolizumab in NSCLC patients. After an amendment, a co-primary end point was added to assess the efficacy in patients with NSCLC that expressed high levels of PD-L1. PD-L1 expression was assessed by immunohistochemical 22C3 antibody pharm DX test. Patients were randomized to either pembrolizumab 2 mg/kg every 3 weeks, pembrolizumab 10 mg/kg every 3 weeks, or pembrolizumab 10 mg/kg every 2 weeks, intravenously in a 30 min perfusion.

Of the 495 randomized patients that received at least one dose of pembrolizumab, any-grade adverse events were presented in the 70% of the patients, grade 3 or higher adverse events were reported in the 9.5% of patients. The most common any-grade adverse events were fatigue, pruritus, and decreased appetite. Most frequent treatment-related adverse events reported were infusion reactions 2%, hypothyroidism 6.9%, and pneumonitis 3.6% including 1.8% grade 3 and 1 death for this reason. Regardless of the dose, schedule, and histology, similar response rate were found among the three arms. The overall response rate was 19.4% (18% for previous treated and 24.8% for untreated patients) and overall stable disease was 21.8%. Response rate was also higher in current or former smokers (22.5%) as compared with never smoker patients (10.3%). Median duration of response was 12.5 months (10.4 months for previous treated and 23.3 months for untreated patients). Overall median progression-free survival and median overall survival was 3.7 months (3 months for previous treated and 6 months for untreated patients) and 12 months (9,3 months for previous treated and 16.2 months for previous untreated patients), respectively. Tumor samples assessment showed that PD-L1 expression 1–49% was

present in the 37, 6% of patients and higher of 50% was present in the 23.2% of patients. The objective response rate (45.2%) was higher in patients that overexpressed PD-L1 (50% or higher) when compared with patients that had PD-L1 expression of 1–49% or less than 1%. Median progression-free survival for the group with high PD-L1 expression was 6.3 months and median overall survival was not reached [57].

Recent update from Keynote 001 regarding overall survival in patients with PD-L1 expression of 1–49% showed a median overall survival of 11.3 months in previous treated and 22.1 months in untreated patients. Median overall survival for PD-L1 expression of 50% or higher was 15.4 months for previous treated and still not reached for untreated patients [58].

Based on these results, in October 2015, FDA-approved pembrolizumab for metastatic NSCLC patients that failed to a first line of cytotoxic chemotherapy and present PD-L1 expression.

Conducted in 24 countries, Keynote 010 was an open label phase 2–3 trial that compared, in NSCLC patients that had failed to at least one prior line of platinum-doublet-based chemotherapy, pembrolizumab with docetaxel. All patients had to have at least 1% of PD-L1 expression in their tumors evaluated by immunohistochemical assay (22C3 antibody pharm DX test) and measurable disease according to RECIST 1.1. Patients were randomized to receive pembrolizumab 2 mg/kg every 3 weeks, pembrolizumab 10 mg/kg every 3 weeks, or docetaxel 75 mg/m^2 every 3 weeks. Primary end points were overall survival and progression-free survival in the total population and in the group of patients that have a high expression of PD-L1 (50% or higher). 991 NSCLC patients (22% squamous) received at least one dose of pembrolizumab or docetaxel. 28% of patients had a PD-L1 expression of at least 50%. In the total population group, overall survival was higher in both groups of pembrolizumab-treated patients when compared with docetaxel with a HR 0.71 for pembrolizumab 2 mg/kg dose ($p = 0.0008$) and a HR 0.61 for pembrolizumab 10 mg/kg dose ($p = 0.0001$). Median overall survival and 1-year survival was 10.4 months and 43.2%, 12.7 months and 52.3%, 8.5 months and 34.6% for pembrolizumab 2 mg, pembrolizumab 10 mg, and docetaxel arms, respectively. No differences in overall survival were between both arms containing pembrolizumab. In subgroup analysis, there was a clear benefit for the adenocarcinoma patients; however there was not a clear benefit in overall survival for squamous NSCLC patients.

Benefit in overall survival was higher in patients treated with pembrolizumab with high expression of PD-L1 (at least 50%). When compared with docetaxel, the HR of pembrolizumab 2 mg was 0.54 ($p = 0.0002$) and HR 0.5 ($p = 0.0001$) for 10 mg/kg dose. Median overall survival in patients with high expression of PD-L1 was for pembrolizumab 2 mg/kg, for pembrolizumab 10 mg, and for docetaxel, 14.9 months, 17.3 months, and 8.2 months, respectively. Progression-free survival was not statistically superior for the pembrolizumab arms when compared with docetaxel in the total population; however, it was significantly higher in patients with high expression of PD-L1 (HR 0.59) for both groups of pembrolizumab. Median progression-free survival was 5 months for pembroli-

zumab 2 mg/kg, 5.2 months for pembrolizumab 5.3 mg/kg, and 4.1 months for docetaxel. Objective response rate was significantly higher either for both pembrolizumab arms than for docetaxel. That was seen in the total study population and in patients with PD-L1 expression of 50% or higher as well. For pembrolizumab 2 mg, pembrolizumab 10 mg and docetaxel, response rates for the total population and for higher PD-L1 population were 18 and 30%, 18 and 29%, 9 and 8%, respectively. There were no complete responses in none of the three treated groups. Toxicity was significantly lower in both pembrolizumab arms when compared with docetaxel. Grade 3–5 adverse events and toxicity that led to treatment discontinuation was reported as follows: 13 and 4% for pembrolizumab 2 mg, 16 and 5% for pembrolizumab 10 mg, 35 and 10% for docetaxel arm. Immune-related toxicity was similar for pembrolizumab 2 mg (20%) and for pembrolizumab 10 mg (19%). Most common adverse events immune-related reported were hypothyroidism, hyperthyroidism, and pneumonitis. Grade 3–5 adverse events reported in more than 1% in both pembrolizumab arms were pneumonitis and skin reactions. Two deaths treatment related were reported for pembrolizumab 2 mg (one pneumonitis and one pneumonia) and three deaths for pembrolizumab 10 mg (one myocardial infarction, one pneumonia, and one pneumonitis) [59].

Recent updated reports of Keynote 010 showed a statistically greater outcome in overall survival, progression-free survival, and response rate for patients that present PD-L1 expression of 75% or higher when compared with subgroups with lower expression (PD-L1 expression 50–74%, 25–49%, and 1–24%). No differences in these outcomes were reported for docetaxel-treated group regardless of the level of PD-L1 expression [60].

Benefit in overall survival in pembrolizumab-treated patients was not driven solely by the PD-L1 expression of 50% or higher. A recent report confirmed that patients from Keynote 010, that were treated with pembrolizumab, had benefit in overall survival when compared with docetaxel (HR 0.79 with 9.4 months in median overall survival for pembrolizumab 2 mg/kg dose, HR 0.71 with median overall survival of 10.8 months for pembrolizumab 10 mg/kg dose, versus median overall survival of 8.6 months for docetaxel arm) [61].

About the importance to provide a new tissue sample or not, to evaluate the PD-L1 expression versus using archived samples to assess this expression by immunohistochemistry, no differences in overall survival was seen between patients with archived or new samples and not significantly difference in PD-L1 expression of 50% or higher was found regardless if the biopsy provided was archived or from a fresh tissue sample [62].

Keynote 042 is a phase 3 clinical trial for the first-line metastatic or unresectable NSCLC (squamous and not squamous histology), in patients that are not amenable for curative treatment and have PD-L1 expression positive. The accrual for this study is already advanced and probably preliminary data will be reported in a near future [63]. Consolidation treatment with pembrolizumab after chemoradiation for patients with unresectable stage III NSCLC is also under research [64].

5.2.2.2 Anti-PD-L1 Inhibitors

An interesting strategy, similar to PD-1 blockade, is the chance to block PD-L1 using monoclonal antibodies that bind this ligand. The PD-L1 antibodies do not prevent PD-1 from interacting with PD-L2 and CD80, which seems to play a role in controlling inflammation and protect normal lung tissue from excessive damage when immune system is activated [65].

This different mechanism of action of the anti-PD-L1 inhibitors, when compared with PD-1 inhibitors, can lead to a more reduced immune-related toxicity and also, by blocking the interaction between PDL-1 with CD80, can help to suppress another negative control on T cells that can theoretically maximize the monoclonal antibody activity [66].

Despite there is less data that supports the use of anti-PD-L1 inhibitors when comparing with PD-1 inhibitors in NSCLC patients, there are several drugs that are under research. Recently, FDA has approved the first anti-PD-L1, atezolizumab, in local advanced or metastatic urothelial carcinoma that failed to a platinum-based first-line chemotherapy. FDA is also reviewing data that might lead to the first approval for an anti-PD-L1 metastatic or local advanced NSCLC indication.

Durvalumab (MEDI4736)

Durvalumab is a high affinity human IgG1 that selectively blocks PD-L1 binding to PD-1 and CD80 without binding to PD-L2, decreasing the risk of immune-related toxicity due to PD-L2 inhibition.

In a phase 1 dose escalation, cohort expansion, clinical trial, safety, and efficacy of durvalumab was assessed in NSCLC pretreated and treatment naïve patients. 43% of patients presented grade 1–2 adverse events, however no grade 3–4–5 toxicity was reported without relevant different in previous treated and treatment naïve patients. Preliminary results of the 13 first patients that underwent treatment in the different cohorts showed three partial responses and two other patients that achieved tumor shrinkage without resulting in partial response using immune-RECIST criteria. Expansion cohort was opened to recruit at least 300 patients [67].

Recently was presented an update report in NSCLC patients from this phase1–2 clinical trial, in 198 NSCLC patients (116 non-squamous and 82 squamous histology), using durvalumab in a dose of 10 mg/kg intravenously every 2 weeks, until disease progression, unacceptable toxicity or after 1-year of treatment, whatever first, with the chance to retreat patients if they failed after 12 months of treatment. The objective response rate was 14% but it was higher in the PD-L1-positive patients (23%). By histology, response rate was higher in squamous than in non-squamous histology (21 and 10%, respectively). Duration of response range was from 0.1 to 35 weeks. Any-grade toxicity was reported in 48% of patients, most common reported adverse events were fatigue (14%), decrease appetite (9%), and nauseas (8%). 6% of patients had a grade 3–4 toxicity and only 2% of patients were discon-

tinued of treatment due to toxicity. From the total of patients treated there was only two pneumonitis reported [68].

Recent report based on the treated naïve population showed an objective response rate of 25% (26% in squamous and 25% in non-squamous NSCLC) and a disease control rate of 12 or more weeks of 56%. Grade 3 or higher toxicity was reported in 9% of patients with 7% of treatment discontinuation due to toxicity with two cases of diarrhea that led to stop treatment [69].

Combining an anti-PD-L1 with an anti-CTLA-4 antibody is a promising alternative in NSCLC patients that is under evaluation. A multicenter non-randomized, open label phase 1b study assessed the safety and antitumor activity of durvalumab plus tremelimumab in 102 locally advanced or metastatic NSCLC patients. Durvalumab was given in doses of 3, 10, 15, or 20 mg/kg every 4 weeks or in a dose of 10 mg/kg every 2 weeks; tremelimumab was given in doses of 1, 3 or 10 mg/kg every 4 weeks for six doses, then after every 12 weeks for three doses. The maximum tolerated dose was exceeded in the cohort that received durvalumab 20 mg/kg every 4 weeks plus tremelimumab 3 mg/kg every 4 weeks with two of six patients with dose-limiting toxicity (one patient with grade 3 elevated transaminases and one patient with grade 4 increased lipase). Toxicity led to discontinuation of treatment in 26% of the patients. The most common any-grade adverse events reported were diarrhea (32%), fatigue (24%), and pruritus (21%). Most common grade 3 or grated reported toxicities were diarrhea (11%), colitis (9%), and increased lipase (8%). 3 of 22 deaths during the study period were reported as attributed to treatment. Based on safety data the dose chosen for the expansion phase dose was durvalumab 20 mg/kg plus tremelimumab 1 mg/kg. Of the 63 patients that were assessed for tumor response, 17% achieved an objective response (including 5% in PD-L1-negative patients), and disease control rate was achieved in the 29% of patients. Based on this, the authors of this trial concluded that PD-L1 status might not predict the response to durvalumab plus tremelimumab combination [70].

Licensed by Astra Zeneca, durvalumab is currently under study in different clinical trials for NSCLC patients, including the TATTON trial in combination with Osimertinib, either as monotherapy or in combination with tremelimumab.

Atezolizumab (MPDL3280A)

Another anti-PD-L1 agent is atezolizumab, a human IgG1 monoclonal antibody that contains a mutated Fc domain designed to avoid Fc-receptor binding and therefore any PD-L1-targeted ADCC [71].

In a phase I expansion study, squamous and non-squamous-pretreated NSCLC patients were treated with atezolizumab at doses between 1 and 20 mg/kg. Reported grade 3–4 adverse events included pericardial effusion (6%), dehydration (4%), dyspnea (4%), and fatigue (4%). No treatment-related deaths occurred. The reported objective response rate by RECIST 1.1 was 24%. 24-week progression-free survival was 48%. Four over four patients that had PD-L1-positive status achieved objective

response (100%), nevertheless PD-L1-negative patients (4/26) achieved an overall response rate of 15% with progression disease of 58% [72].

The expanded trial, that included 85 NSCLC patients with both squamous and non-squamous histology, inside a study that included other cancer types such as melanoma and renal cell carcinoma, has been reported. Atezolizumab-treated NSCLC patients every 3 weeks, achieved an objective response rate of 21%. Current and former smoker had higher response rate than never smokers (42% versus 10%, respectively). Patients with higher expressions of PD-L1 levels achieved better responses compared to whom did not. For all the patients treated in this trial, including NSCLC and other tumor types, any-grade toxicities were reported in the 70% of the patients. The most common adverse events reported were fatigue 24%, decrease appetite 11%, nauseas 11%, pyrexia 11%, diarrhea 10%, and rash 10%; grade 3–4 toxicities were reported in 39% of patients and included dyspnea 4%, anemia 3.6%, fatigue 3.2%, and hyperglycemia 2.5% [73].

Clinical outcomes in distinct cancer types with high levels of PD-L2 expression have also showed an improved benefit with atezolizumab treatment [74].

The combination of atezolizumab plus chemotherapy in the first line of treatment in NSCLC patients has been tested in a phase 1b trial. Patients received atezolizumab 15 mg/kg intravenously every 3 weeks plus 4–6 doses of platinum-based chemotherapy followed of atezolizumab as maintenance therapy. Up to 13% of patients presented grade 3–4 toxicity, most of them hematological and related with chemotherapy. One death due to candidemia after a prolonged neutropenia was reported. Overall response rate was different into groups of chemotherapy treatment but it ranged between 60 and 75%, responses were considered as not related to PD-L1 status [75].

The phase 2 clinical trial BIRCH was an open label multicentre study that assessed the safety and efficacy of atezolizumab in NSCLC patients that express PD-L1.This trial included 667 treatment naïve and pretreated patients. PD-L1 status was assessed by an immunohistochemical assay developed by Roche Diagnostics that measures tumor cells (TCs) and tumor infiltrating immune cells (ICs); therefore, its results are interpreted by a mixture score that includes both components and are informed as TC 0,1,2, or 3 and IC 0,1,2, or 3. Eligible patients for this trial were who had TC 2/3 or IC 2/3. Patients received, in the first line of treatment or further, atezolizumab at 1200 mg intravenously every 3 weeks. The primary end point was objective response rate. Patients that scored TC 3/IC 3 had higher responses rates than patients that presented TC 2/3 or IC 2/3 in the first line (26% versus 19%), second line (24% versus 17%), and third line or further of treatment (27% versus 17%) [76].

POPLAR trial was a phase 2 study that compared atezolizumab versus docetaxel in local advanced or metastatic NSCLC that progressed after a first line of treatment, regardless of the PD-L1 status assessed by the same immunohistochemical assay that was mentioned above. 287 patients were enrolled in the trial. POPLAR's primary end point was overall survival. Atezolizumab achieved higher survival than docetaxel in all the subgroups of patients that were PD-L1 positive: median overall survival for any expression 15.5 versus 9.2 months (HR 0.59 $p = 0.005$), medium and high expression 15.1 versus 7.4 months (HR 0.54 $p = 0.014$), high expression

15.5 versus 11.1 months (HR 0.49 $p = 0.068$). For PD-L1-negative patients (TC 0 and IC 0), there was no difference in median overall survival for atezolizumab and docetaxel (9.7 months for both groups) [77].

A recent update of POPLAR trial shows a further separation of curves with improve in overall survival when atezolizumab is compared with docetaxel (ITT population median overall survival 12.6 months versus 9.7 months ($p = 0.011$); TC 3 or IC 3 median overall survival not reached versus 11.1 months ($p = 0.033$). By histology median overall survival favors atezolizumab for both squamous and non-squamous patients over docetaxel [78].

Due to the positive results from both BIRCH and POPLAR trials, FDA granted priority review for atezolizumab in NSCLC; however, until the date of this publication this drug is still under revision by regulatory agencies.

Avelumab

Avelumab (MSB0010718C) is a fully human anti-PD-L1 IgG1 monoclonal antibody and has a native Fc receptor for ADCC [79].

A phase I, open-label, parallel-group expansion study of avelumab was conducted to assess the tolerability and safety of avelumab in metastatic or local advanced solid tumors that included NSCLC patients but also gastric, ovarian, melanoma, and breast cancer patients. Avelumab was given a 10 mg/kg dose every 2 weeks. 480 patients were treated in this trial and 68% of them present an adverse event any grade, most frequent toxicities reported were fatigue (20%), nausea (13%), infusion-related reaction (9%), diarrhea (7%), chills (7%), decreased appetite (6%), pyrexia (5%), influenza-like illness (5%), and arthralgia (5%). 34 patients were discontinued of treatment due to adverse events including 8 patients that presented infusion reactions. Drug-related toxicity grade 3 or higher was reports in 12% of patients and the most common toxicities reported were anemia (5), fatigue (5), increased GGT (4), infusion reactions (4), increased lipase (4), and decreased lymphocytes (3). Immune-related toxicities were reported in 11.7% of patients, and the most common were hypothyroidism (4.0%) and pneumonitis (1.5%) [80].

Inside this study, stage III B or IV NSCLC patients previously treated with a platinum-based doublet were considered to receive avelumab 10 mg/kg every 2 weeks until complete response, disease progression, or unacceptable toxicity. 184 NSCLC patients were included (62% adenocarcinoma, 29% squamous carcinoma). 75% of patients presented at least one any-grade adverse event. Most common toxicities reported were fatigue, nausea, infusion-related reactions, chills, decreased appetite, and diarrhea. Drug-related toxicity grade 3–4 was present in the 12% of patients including four cases of infusion reactions. Three drug-related deaths were reported (radiation pneumonitis, acute respiratory failure, and disease progression). Response rate and stable disease were observed in 12 and 38% of patients (14.4% of response rate in PD-L1-positive and 10% in PD-L1-negative patients). Overall progression-free survival was 11.6 weeks (11.7 weeks in PD-L1-positive and 5.9 weeks in PD-L1-negative patients) [81].

In a phase 1b trial avelumab was tested as first line of treatment in 145 local advanced or metastatic NSCLC patients (63% adenocarcinoma, 27 squamous) without EGFR or ALK mutations, regardless of the PD-L1 status.

Patients received avelumab 10 mg/kg intravenously every 2 weeks until progression or unacceptable toxicity. All grade toxicities were reported in the 56% of patients. Most common adverse events were infusion reactions (16%) and fatigue (14%). Grade 3–4 toxicities were reported in 9% of the patients. No deaths related to treatment were observed. Overall response rate, assessed by RECIST 1.1 was reported in 18.7% of patients (1 complete response and 13 partial responses), stable disease was reported in 45% of patients. All reported responses were achieved in PD-L1-positive patients without any response in PD-L1-negative patients. Median progression-free survival was 11.6 weeks for all the treated population [82].

Currently, a phase 3 clinical trial comparing avelumab with docetaxel as second line of treatment for NSCLC patients—PD-L1 positive is ongoing [83].

BMS-936559

BMS-936559 is a fully human IgG4 antibody that inhibits binding of PD-L1 to PD-1 and CD80, binding PD-L1 but also CTLA-4 and CD28 with high affinity [65].

This drug was tested in a phase 1 dose escalation and cohort expansion trial including melanoma, NSCLC, renal cell carcinoma patients, and others (ovarian, pancreatic, colorectal cancer). There was 8.6% of grade 3–4 toxicity without deaths due to treatment. Some adverse events of special interest reported were hypothyroidism, hepatitis, sarcoidosis, endophthalmitis, and myasthenia gravis. Objective responses were observed in heavily pretreated patients including responses lasting longer than one year [84]. Despite this drug is not currently being studied in cancer patients, there are clinical trials ongoing for sepsis treatment.

5.3 Immunotherapy and NSCLC: Milestones, Concerns, Fears, and Challenges

Non-small cell lung cancer is unfortunately the leader malignancy worldwide. Official records by Globocan show than in 2012 there was an incidence, including both sexes, of 1.824.701 new cases around the world and 1.589.925 deaths in the same year for this disease. In other words for every 100 persons that have been diagnosed of a lung cancer there will be 87 persons that will die due to lung cancer in a period of time of 12 months. For both sexes together and in men, non-small cell lung cancer is the leader cause of mortality by cancer and the second cause of mortality by cancer in women [85]. In the United States, there is a trend to decrease in incidence and mortality due to NSCLC since 2012. Anti-tobacco laws and regulations are playing probably a major role in this trend to “improve” of the curves; however, there was reported in the United States an 5-year survival for lung cancer of only 17.7% for the period

2006–2012, with 224.390 new cases estimated for 2016 and 158.080 deaths in the same year representing 26.5% of mortality for cancer in this country [86].

Since 1980s and until the first half of the 2000s decade, very few steps that had a real impact in the prognosis of unresectable or metastatic NSCLC patients were given: some new chemotherapy regimens (always in first-line platinum-based doublets); attempts to add antiangiogenics to chemotherapy regimens; development of second-line cytotoxic chemotherapies. However, those steps did not achieve a great impact in overall survival and obviously lesser impact in 5-years survival rates. By the second half of the 2000s targeted therapies, in the beginning directed to anti-EGFR mutations and years later to anti-ALK mutations, have taken a place in the treatment of this malignancy, achieving a high impact in overall survival in this population of patients, that represents approximately one-fourth–one-fifth of the entire population of non-small cell lung cancer worldwide, with disparities by regions probably due to genetics and tobacco consumption.

We have been witnesses of the most revolutionary milestone of the systemic cancer treatment: the emergence of immunotherapy. Unexpected first results in melanoma patients were published in 2010, changing the paradigm of how to treat this malignancy. Pooled analysis show that one-fourth of the patients that had been treated with ipilimumab are alive for more than 3 years, with a clear plateau in the survival curve. It is too early yet to talk about "the cure of cancer," nonetheless it seems immunotherapy in general is given an approach to this scenario. We are currently under a storm of information that many times exceeds the capability of analysis and comprehension. New drugs are emerging and clinical trials that are looking for testing them are under development.

First reports and approval in NSCLC of immunotherapy drugs are relatively new, time will be need to assess a longer term benefit, however, with the current information we already can say that there must be a change in the paradigm of how to treat NSCLC patients that are nor amenable for curative options.

Lung cancer cells have multiple immunosuppressive mechanisms that are critical to escape of the immune system and survive. Anti-CTLA-4 such as ipilimumab, drug that changed the paradigm in melanoma treatment, when tested in clinical trials did not show the expected benefit in non-small cell lung cancer patients. Nevertheless, other checkpoint inhibitors such as anti-PD-1 and anti-PD-L1 are emerging. These drugs do not attack directly the tumor cell as cytotoxic chemotherapy does; they work by suppression of the main mechanisms involved in immune-tolerance and tumor evasion from immune response.

In NSCLC, anti-PD-1 and anti-PD-L1 monoclonal antibodies have shown significant activity, significant outcomes in survival, long lasting responses and good safety profile when compared with cytotoxic chemotherapy, including naïve and pretreated patients, squamous and non-squamous histology. Moreover, patients that not express PD-L1 in their tumors, when are treated with anti-PD-1 drugs, achieve similar responses to patients treated with chemotherapy, but patients with high levels of PD-L1 expression have much better results when compared with standard treatment.

In NSCLC, the only immunotherapy drugs that have approval by FDA are two anti-PD-1s: Nivolumab and Pembrolizumab. As mentioned above nivolumab,

thanks to two phase three clinical trials, was the first anti-PD-1 to get approval for treatment of NSCLC patients that failed to a first line of standard chemotherapy, first in squamous and after in non-squamous histology. A few time later, pembrolizumab was also approved for patients with NSCLC, PD-L1 positive, that have progression after a first line of platinum-based doublet.

Until now, no other immunotherapy drug has received FDA approval for treatment of NSCLC patients. There are other anti-PD-1 drugs and anti-PD-L1 that are under research and waiting for FDA review. Nivolumab has been approved regardless of the PD-1 status; however, pembrolizumab was approved only for PD-L1-positive patients.

Identification of predictive biomarkers to select patients most likely responding to immunotherapies is currently being investigated. Because of the critical role of PD-1/PD-L1 pathway activation in downregulating T-cell activity, several investigations have focused on tumor microenvironment components [23–87]. PD-L1 is upregulated in selected solid tumors, including squamous and non-squamous non-small cell lung cancers, and it can be detected by immunohistochemistry on tumor cells (TCs) and immune cells (ICs).

Both anti-PD-1 pembrolizumab and anti-PD-L1 atezolizumab show a greater impact in outcomes in PD-L1-positive patients. Nivolumab, however, got approval without needing PD-L1-positive demonstration, even though there is a trend of benefit in PD-L1-positive patients, mainly in adenocarcinoma histology. One big problem is how to translate the results of the different trials in order to define what should be considered as PD-L1 positive, which ought to be the cut-off point and then how to define the best treatment for every patient [88]. This is a confusing situation. We cannot affirm if an anti-PD-1 is more effective than the other just for the published results of the different trials. All the anti-PD-1s approved and the anti-PD-1s and anti-PD-L1s under research and development use different assays to measure the levels of PD-L1 expression [89]. Probably in a short time, some of the immunotherapy drugs under development will be approved and the decision of treatment will become harder. PD-L1 seems to be a predictive biomarker; however, when there are several immunohistochemical assays for just one biomarker is difficult to decide which one to use, and it is also important to understand that currently every assay is linked to a specific drug. In most of the clinical trials, PD-L1 expression has been assessed in tumor cells; however, atezolizumab's trials have also incorporated the determination of PD-L1 in immune cells. It is not possible to provide different samples of tissue in order to define the treatment that fits the best for just one single patient. It is extremely necessary that the regulatory agencies can take part of this issue in order that the pharmaceutical industry can define one universal assay to evaluate PD-L1 expression and can define similar cut-off points to be able to compare the different drugs for the same indication.

Beside PD-L1 expression, other biomarkers are under investigation. Tumor heterogeneity and mutational density in lung cancer, and also the tumor microenvironment play a role in the variability of responses and outcomes in immunotherapy-treated patients regardless of the PD-L1 status. Probably, PD-L1 expression is the first approach to define a biomarker that can predict response; however, it is insufficient to understand several mechanisms of resistance to drugs and also to understand why PD-L1-negative patients can achieve response to treatment.

Combining anti-PD-1s or anti-PD-L1s with anti-CTLA-4 drugs seems to be an interesting strategy to improve the outcomes in NSCLC. Clinical trials are already ongoing and preliminary reports are auspicious. Other strategies under development, related with immunotherapy in NSCLC, include combination of immunotherapy plus chemotherapy, antiangiogenics, and specific mutation-targeted therapy (such as anti-EGFR or anti-ALK mutations). Immunotherapy is also under research in patients with local advanced disease as adjuvant treatment after chemoradiation.

Well is known that the toxicity profile of immunotherapy is different than chemotherapy. Immunotherapy has a lower incidence of adverse events but it can be severe in some opportunities, hard to predict and with unusual forms of presentation. This scenario needs that oncologists have to be trained in immune-related adverse events recognition and their specific treatments [90].

Many of the NSCLC patients treated with immunotherapy worldwide have been able to access to these drugs because they have been enrolled in a clinical trial, or they have been supported in a compassionate use of a specific drug. However, the commercial value of these treatments is an issue that has ethical concerns. Indubitable, pharmaceutical companies make a big investment in drug's development; nevertheless, the current costs of the drugs will limit the possibility of the patients to be treated, and or will affect the economy of several countries in case of they were command to provide them by law. Even more, current combination of immunotherapy treatments, if they are approve in a future for NSCLC, could cost up to one million dollars per patient per year. This economical and ethical issue will force to select very well whom will be the patients that will have a real positive impact with immunotherapy treatment, and to look for biomarkers that can ensure in a correct manner a good and prolonged response to treatment.

In a short period of time, not only in NSCLC but also in several malignancies, immunotherapy became a main stone in cancer treatment, and it will probably help in the future to provide a powerful hand in cancer cure.

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References

1. Molina J, Yang P, Cassivi S, et al. Non–small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc. 2008;83(5):584–94.
2. Ferreccio C, González C, Milosavjlevic V, et al. Lung cancer and arsenic concentrations in drinking water in Chile. Epidemiology. 2000;11(6):673–9.
3. Rapp E, Pater JL, Willan A, et al. Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer--report of a Canadian multicenter randomized trial. J Clin Oncol. 1988;6(4):633–41.
4. NSCLC Meta-Analyses Collaborative Group. Chemotherapy in addition to supportive care improves survival in advanced non–small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials. J Clin Oncol. 26:4617–25.
5. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol. 2008;26(21):3543–51.
6. Fossella FV, Lynch T, Shepherd FA. Second line chemotherapy for NSCLC: establishing a gold standard. Lung Cancer. 2002;38(Suppl 4):5–12.
7. Hanna N, Shepherd FA, Fossella FV, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J Clin Oncol. 2004;22(9):1589–97.
8. Rolfo C, Caglevic C, Mahave M, Bustamante E, Castañon E, Gil BI, Marquez-Medina D. Chapter 14: Chemotherapy beyond the second line of treatment in non-small cell lung cancer: new drug development. In: Marquez-Medina D, editor. Fighting lung cancer with conventional therapies. New York: Nova Science Publishers; 2015. p. 229–40.
9. Vokes E, Salgia R, Karrison R. Evidence-based role of bevacizumab in non-small cell lung cancer. Ann Oncol. 2013;24(1):6–9.
10. Caglevic C, Grassi M, Raez L, Listi A, Giallombardo M, Bustamante E, Gil-Bazo I, Rolfo C. Nintedanib in non-small cell lung cancer: from preclinical to approval. Ther Adv Respir Dis. 2015;9(4):164–72.
11. Reck M, Kaiser R, Mellemgaard A, et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): a phase 3, double-blind, randomised controlled trial. Lancet Oncol. 2014 Feb;15(2):143–55.
12. Horn L, Pao W. EML4-ALK: honing in on a new target in non–small-cell lung cancer. J Clin Oncol. 2009;27(26):4232–5.
13. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499(7457):214–8.
14. Györki D, Callahan M, Wolchock J, Ariyan C. The delicate balance of melanoma immunotherapy. Clin Transl Immunol. 2013;2:e5.
15. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol. 2007;7:41–51.
16. Vesely M, Kershaw M, Schreiber R, Smyth M. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011;29:235–71.
17. Chen D, Mellman I. Oncology meets immunology: “The Cancer-Immunity Cycle.”. Immunity. 2013;39(25):1–10.
18. Abbas A, Lichtman A, Pillai S. Immunity to tumors. In: Abbas AK, Lichtman AH, Pillai S, editors. Celular and molecular immunology. Philadelphia, PA: Elsevier; 2015. p. 383–97.
19. Pardoll D. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.
20. Cebon J, Behren A. Evolving role of tumor antigens for future melanoma therapies. Future Oncol. 2014;10:1457–68.
21. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell. 2015;27(4):450–61.

22. Boussiotis V. Somatic mutations and immunotherapy outcome with CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):30–2.
23. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015;33:1974–82.
24. Weber J. Anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. Oncologist. 2007;12:864–72.
25. Cameron F, Whiteside G, Perry C. Ipilimumab: first global approval. Drugs. 2011;71(8): 1093–104.
26. Hodi F, O'Day S, McDermott D, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
27. Tomasini P, Khobta N, Greillier L, Barlesi F. Ipilimumab: its potential in non-small cell lung cancer. Ther Adv Med Oncol. 2012;4(2):43–50.
28. Weber J, Hamid O, Amin A, et al. Randomized phase I pharmacokinetic study of ipilimumab with or without one of two different chemotherapy regimens in patients with untreated advanced melanoma. Cancer Immun. 2013;13:7.
29. Lynch T, Bondarenko I, Luft A. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non–small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J Clin Oncol. 2012;30(17):2046–54.
30. https://clinicaltrials.gov/show/NCT01285609
31. https://clinicaltrials.gov/ct2/show/NCT01998126
32. Ribas A, Hanson D, Noe D, et al. Tremelimumab (CP-675,206), a cytotoxic T lymphocyte–associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. Oncologist. 2007;12(7):873–83.
33. Zatloukal P, Heo DS, Park K, et al. Randomized phase II clinical trial comparing tremelimumab (CP-675,206) with best supportive care (BSC) following first-line platinum-based therapy in patients (pts) with advanced non-small cell lung cancer (NSCLC). J Clin Oncol (Meeting Abstracts). 2009;27(15S):8071.
34. https://clinicaltrials.gov/ct2/show/NCT02040064
35. Nguyen L, Ohashi P. Clinical blockade of PD1 and LAG3- Potential mechanisms of action. Nat Rev Immunol. 2015;15:45–56.
36. Zou W, Chen L. Inhibitory B7-family molecules in the tumor microenvironment. Nat Rev Immunol. 2008;8(6):467–77.
37. Konishi J, Yamazaki K, Azuma M, et al. B7-H1 expression on non small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res. 2004;10(15):5094–100.
38. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends Immunol. 2015;36(4):265–76.
39. Chinai JM, Janakiram M, Chen F, et al. New immunotherapies targeting the PD-1 pathway. Trends Pharmacol Sci. 2015;36(9):587–95.
40. Wang C, Thudium K, Han M, et al. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res. 2014;2:846.
41. Sundar R, Cho B-C, Brahmer JR, Soo RA. Nivolumab in NSCLC: latest evidence and clinical potential. Ther Adv Med Oncol. 2015;7(2):85–96.
42. Topalian SL, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–54.
43. Gettinger SN, et al. Overall survival and long-term safety of nivolumab (anti-programmed death 1 antibody, BMS-936558, ONO-4538) in patients with previously treated advanced non-small-cell lung cancer. J Clin Oncol. 2015;33:2004–12.
44. Rizvi NA, Mazieres J, Planchard D, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol. 2015;16:257–65.
45. Horn L, Rizvi N, Mazieres J et al. Longer-term follow-up of a phase 2 study (CheckMate 063) of nivolumab in patients with advanced refractory squamous (SQ) non-small cell lung cancer (NSCLC). J Thor Oncol. 2015;10(9 suppl 2), abstract 02.03.

46. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. N Engl J Med. 2015;373:123–35.
47. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced non-squamous non-small-cell lung cancer. N Engl J Med. 2015;373(17):1627–39.
48. Borghaei H, Brahmer J., Horn L. et al. Nivolumab vs docetaxel in patients with advanced NSCLC: CheckMate 017/057 2-y update and exploratory cytokine profile analyses. J Clin Oncol. 2016;34(suppl; abstr 9025).
49. https://clinicaltrials.gov/ct2/show/NCT01454102
50. Antonia S, Brahmer J, Gettinger S, et al. Nivolumab (anti-PD-1; BMS-936558, ONO-4538) in combination with platinum-based doublet chemotherapy in advanced non-small cell lung cancer (NSCLC). J Clin Oncol. 2014;32:5s(suppl; abstr 8113).
51. Gettinger S, Shepherd F, Antonia S, et al. First-line nivolumab (anti-PD-1; BMS-936558, ONO-4538) monotherapy in advanced NSCLC: safety, efficacy, and correlation of outcomes with PD-L1 status. J Clin Oncol. 2014;32:5s(suppl; abstr 8024).
52. Gettinger SN. Presented at European Society for Medical Oncology (ESMO), September 25–29, 2015, Vienna, Austria.
53. Antonia S, Gettinger S, Quan Man Chow L, et al. Nivolumab (anti-PD-1; BMS-936558, ONO-4538) and ipilimumab in first-line NSCLC: interim phase I results. J Clin Oncol. 2014;32:5(suppl; abstr 8023).
54. Rizvi NA, Gettinger SN, Goldman JW, et al.: Safety and efficacy of first-line nivolumab and ipilimumab in non-small cell lung cancer. 16th World conference on lung cancer. Abstract ORAL02.05. Presented September 7, 2015.
55. Hellmann M., Gettinger S., Goldman J., et al. CheckMate 012: "Safety and efficacy of first-line nivolumab and ipilimumab in advanced NSCLC". J Clin Oncol. 2016; 34(suppl; abstr 3001).
56. Najjar Y, Kirkwood J. Pembrolizumab: pharmacology and therapeutics. Am J Hematol Oncol. 2014;10(5):17–9.
57. Garon E, Rizvi N, Hui R, et al. Pembrolizumab for the treatment of non–small-cell lung cancer. N Engl J Med. 2015;372:2018–28.
58. Hui R., Gandhi L., Carcereny Costa E, et al. Long-term OS for patients with advanced NSCLC enrolled in the KEYNOTE-001 study of pembrolizumab. J Clin Oncol. 2016;34 (suppl; abstr 9026)
59. Herbst R, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540–50.
60. Baas P, Garon E, Herbst R., et al. Relationship between level of PD-L1 expression and outcomes in the KEYNOTE-010 study of pembrolizumab vs docetaxel for previously treated, PD-L1–Positive NSCLC. J Clin Oncol. 2016;34(suppl; abstr 9015).
61. Garon E., Herbst R, Kim DW, et al. Pembrolizumab vs docetaxel for previously treated advanced NSCLC with a PD-L1 tumor proportion score (TPS) 1%–49%: results from KEYNOTE-010. J Clin Oncol. 2016;34(suppl; abstr 9024).
62. Herbst R, Baas P, Perez-Gracia JL et al. Archival vs new tumor samples for assessing PD-L1 expression in the KEYNOTE-010 study of pembrolizumab vs docetaxel for previously treated advanced NSCLC. J Clin Oncol. 2016;34(suppl; abstr 3030).
63. https://clinicaltrials.gov/ct2/show/NCT02220894
64. https://clinicaltrials.gov/ct2/show/NCT02343952
65. Philips GK, Atkins M. Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies. Int Immunol. 2015;27(1):39–46.
66. Haile S, Dalal S, Clements V. Soluble CD80 restores T cell activation and overcomes tumor cell programmed death ligand-1-mediated immune suppression. J Immunol. 2013;191(5):2829–36.
67. Brahmer J, Rizvi N, Lutzky J, et al. Clinical activity and biomarkers of MEDI4736, an anti-PD-L1 antibody, in patients with NSCLC. J Clin Oncol. 2014;32:5(suppl; abstr 8021).
68. Rizvi N, Brahmer J, Ou SH, et al. Safety and clinical activity of MEDI4736, an anti-programmed cell death-ligand 1 (PD-L1) antibody, in patients with non-small cell lung cancer (NSCLC). J Clin Oncol. 2015;33(15 Suppl):8032.

69. Antonia S., Kim SW, Spira A., et al. Safety and clinical activity of durvalumab (MEDI4736), an anti-PD-L1 antibody, in treatment-naïve patients with advanced non–small-cell lung cancer. J Clin Oncol. 2016;34(suppl; abstr 9029).
70. Antonia S, Goldberg S, Balmanoukian A, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. Lancet Oncol. 2016;17(3):299–308.
71. Reichert J. Antibodies to watch in 2016. MAbs. 2016;8(2):197–204.
72. Spigel D, Gettinger S., Horn L., et al. Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). J Clin Oncol. 2013;31(suppl; abstr 8008).
73. Herbst R, Soria J-C, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563–7.
74. Schmid P., Hegde P., Zou W., et al. Association of PD-L2 expression in human tumors with atezolizumab activity. J Clin Oncol. 2016;34(suppl; abstr 11506).
75. Liu S., Powderly J., Camidge R. et al. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with platinum-based doublet chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). J Clin Oncol. 2015;33(suppl; abstr 8030).
76. Besse B, Johnson M, Jänne PA, et al. Phase II, single-arm trial (BIRCH) of atezolizumab as first-line or subsequent therapy for locally advanced or metastatic PD-L1-selected non-small cell lung cancer (NSCLC). Presented at 2015 European Cancer Congress, September 25–29, Vienna, Austria. Abstract 16LBA.
77. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016;387(10030):1837–46.
78. Smith D.,Vansteenkiste J., Fehrenbacher L., et al. Updated survival and biomarker analyses of a randomized phase II study of atezolizumab vs docetaxel in 2 L/3 L NSCLC (POPLAR). J Clin Oncol. 2016;34(suppl; abstr 9028).
79. Hamanishi J, Mandai M, Konishi I. Immune checkpoint inhibition in ovarian cancer. Int Immunol. 2016;7. pii:dxw020.
80. Kelly K, Patel M., Infante J. et al. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with metastatic or locally advanced solid tumors: assessment of safety and tolerability in a phase I, open-label expansion study. J Clin Oncol. 2015;33(suppl; abstr 3044).
81. Gulley J, Spigel D, Kelly K, et al. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in advanced NSCLC patients: a phase 1b, open-label expansion trial in patients progressing after platinum-based chemotherapy. J Clin Oncol (Meeting Abstracts). 2015;33(15_suppl):8034.
82. Verschraegen C., Chen F., Spigel D., et al. Avelumab (MSB0010718C; anti-PD-L1) as a first-line treatment for patients with advanced NSCLC from the JAVELIN Solid Tumor phase 1b trial: safety, clinical activity, and PD-L1 expression. J Clin Oncol. 2016;34(suppl; abstr 9036).
83. https://clinicaltrials.gov/ct2/show/NCT02395172
84. Brahmer J, Tykodi S, Chow L, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65.
85. Globocan 2012: "Estimated Cancer Incidence, Mortality and Prevalence Worlwide 2012." http://globocan.iarc.fr/Pages/fact_sheets_population.aspx
86. NIH, National Cancer Institute: surveillance, epidemiology and end results. http://seer.cancer.gov/statfacts/html/lungb.html
87. Santarpia M, Karachaliou N. Tumor immune microenvironment characterization and response to anti-PD-1 therapy. Cancer Biol Med. 2015;12(2):74–8.
88. Carbognin L, Pilotto S, Milella M, et al. Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. PLoS One. 10(6):e0130142. doi:10.1371/journal.pone.0130142.
89. Kerr K, Hirsch F. Programmed death ligand-1 immunohistochemistry. Friend or foe? Arch Pathol Lab Med. 2016;140:326–31.
90. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016;54:139–48.

Chapter 6
Immune Therapy for Sarcomas

Peter M. Anderson

Abstract Absolute lymphocyte count (ALC) recovery rapidly occurring at 14 days after start of chemotherapy for osteosarcoma and Ewing sarcoma is a good prognostic factor. Conversely, lymphopenia is associated with significantly decreased sarcoma survival. Clearly, the immune system can contribute towards better survival from sarcoma. This chapter will describe treatment and host factors that influence immune function and how effective local control and systemic interventions of sarcoma therapy can cause inflammation and/or immune suppression but are currently the standard of care. Preclinical and clinical efforts to enhance immune function against sarcoma will be reviewed. Interventions to enhance immune function against sarcoma have included regional therapy (surgery, cryoablation, radiofrequency ablation, electroporation, and radiotherapy), cytokines, macrophage activators (mifamurtide), vaccines, natural killer (NK) cells, T cell receptor (TCR) and chimeric antigen receptor (CAR) T cells, and efforts to decrease inflammation. The latter is particularly important because of new knowledge about factors influencing expression of checkpoint inhibitory molecules, PD1 and CTLA-4, in the tumor microenvironment. Since these molecules can now be blocked using anti-PD1 and anti-CTLA-4 antibodies, how to translate this knowledge into more effective immune therapies in the future as well as how to augment effectiveness of current interventions (e.g., radiotherapy) is a challenge. Barriers to implementing this knowledge include cost of agents that release immune checkpoint blockade and coordination of cost-effective outpatient sarcoma treatment. Information on how to research clinical trial eligibility criteria and how to access current immune therapy trials against sarcoma are shared, too.

Keywords Sarcoma • Absolute lymphocyte count • Lymphopenia • Mifamurtide • CAR T cells • PD1 • CTLA-4 • Immune checkpoint blockade • (Mal)adaptive immune response • Glutamine disaccharide (Healios)

P.M. Anderson, M.D., Ph.D. (✉)
Department of Pediatric Hematology/Oncology/BMT, Cleveland Clinic S20, 9500 Euclid Ave, Cleveland, OH 44195, USA
e-mail: andersp@ccf.org

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Table 6.1 Immune function (lymphocytes) and sarcoma survival

Parameter	Observation	Reference
ALC[a]	ALC >500, then better EWS[b] survival	[2, 3]
On d14 after		
Initial cycle of chemotherapy	ALC >800, then better osteosarcoma survival	[4, 5]
Lymphopenia at diagnosis	Significantly decreased survival	[1]
PMN/Lymph ratio	High PMN/Lymph ratio has worse survival in STS[c]	[6]

ALC absolute lymphocyte count, *EWS* Ewing sarcoma, *STS* soft tissue sarcoma

6.1 Background

Lymphopenia is frequent in advanced cancers including advanced soft tissue sarcomas and has been associated with poor survival (5 vs 10 months; $p < 0.01$; Ref. [1]). Better lymphocyte recovery or resilience after starting chemotherapy for Ewing sarcoma or osteosarcoma is also predictive of better survival [2–5]. The higher pretreatment neutrophil to lymphocyte ratio predicts a worse prognosis; conversely, more lymphocytes (i.e., a lower neutrophil:lymphocyte ratio) were associated with significantly better survival ($p < 0.05$) for patients with soft tissue sarcomas [6].

So if immune function contributes to better survival, how can this be realized? The promise and prospect of having increased immune response for not only destruction of existing macroscopic >3 mm deposits seen on imaging, but also for therapy of micrometastases and surveillance to prevent recurrences has been recently reviewed for childhood sarcomas [7]. This chapter applies to patients with sarcomas of all ages (Table 6.1).

6.2 Factors Influencing Immune Function

Medical and physical (local control) treatments for sarcoma can contribute to immune dysfunction. A recent randomized trial of epidural versus general versus combined epidural + general anesthesia for osteosarcoma limb salvage surgery showed the combination as associated with more prompt recovery of t-lymphocyte subsets and restoration of immune function [8]. Chemotherapy and radiation commonly are associated with lymphopenia. The severity of lymphopenia associated with therapy has significantly inferior outcomes for a variety of cancers including pancreatic adenocarcinoma ($p = 0.001$; Ref. [9]). In patients with newly diagnosed solid tumors, >40% developed severe and persistent treatment related lymphopenia (TRL) within 2 months; TRL was associated with poor survival (HR 2.1; $p < 0.0001$; Ref. [10]). Commonly used cytotoxic agents used against sarcomas which are associated with immune suppression and lymphopenia include alkylators (cyclophosphamide, ifosfamide, cisplatin), anthracyclines (doxorubicin), taxanes (docetaxel), and vincristine. Dexamethasone is also often used as a short 1–3 day "pulse" to

counteract acute side effects of chemotherapy including nausea and anaphylactoid reactions to docetaxel.

Because of location and difficulty in achieving complete resection with adequate margins, radiation is a commonly used modality in the treatment of high-grade soft tissue sarcomas (6308/10,290)—and is associated with significantly improved survival compared to the no radiotherapy group ($p < 0.001$; Ref. [11]). However, radiotherapy (RT) is associated with lymphopenia and galectin-1 secretion by tumors [12, 13]. Sometimes lymphopenia related to radiation is long-lasting. Galectin-1, a potential mediator of radiation-induced lymphopenia, can be detected in blood. Research detailing effect of location, dose, and schedule of radiation associated with galectin-1 may be instructive.

6.3 Cytokines and Inflammation

Cytokine action is most effective at short distances and regionally. However, if "supra-physiologic doses" of a cytokine such as IL-2, G-CSF, GM-CSF, or erythropoietin are given repeatedly and/or using long-acting formulations, inflammatory effects associated with white blood cell proliferation and activation may possibly become counterproductive. This is because of recent evidence showing that inflammation contributes to an "adaptive immune response," the production of PD1 and CTLA-4 [14–19]. Programmed cell death ligand (PD-L1) and PD-1 interaction is the immune system's checkpoint to decrease potential autoimmune "off-target" effects. In sarcomas, there is evidence of variable expression of both tumor infiltrating lymphocytes with PD-L1 expression and PD1 in the tumor microenvironment [20, 21]. One could hypothesize that if inflammation occurs in a tissue harboring sarcoma micrometastases such as lung, this could potentially be counterproductive. Interestingly, it appears that metastatic, but primary osteosarcomas express PD-L1 [22]. Figure 6.1 illustrates how inflammation including iatrogenic inflammation (surgery, radiation, chemotherapy) may contribute towards less immune function for the control of sarcomas during current therapy as well as new agents to block immune checkpoint inhibitory molecules.

6.4 Nutrition and Immune Function: Glutamine Appears to be a Key Player

Nutrition can contribute toward better or worse immune function. The major fuel for both lymphocytes and enterocytes is glutamine. Catabolic situations (poor appetite, nausea, NPO for medical procedures) lead towards a "glutamine shuttle" in which muscle must produce glutamine to maintain enteral health and immune function. Glutamine-enriched diets support muscle glutamine metabolism without

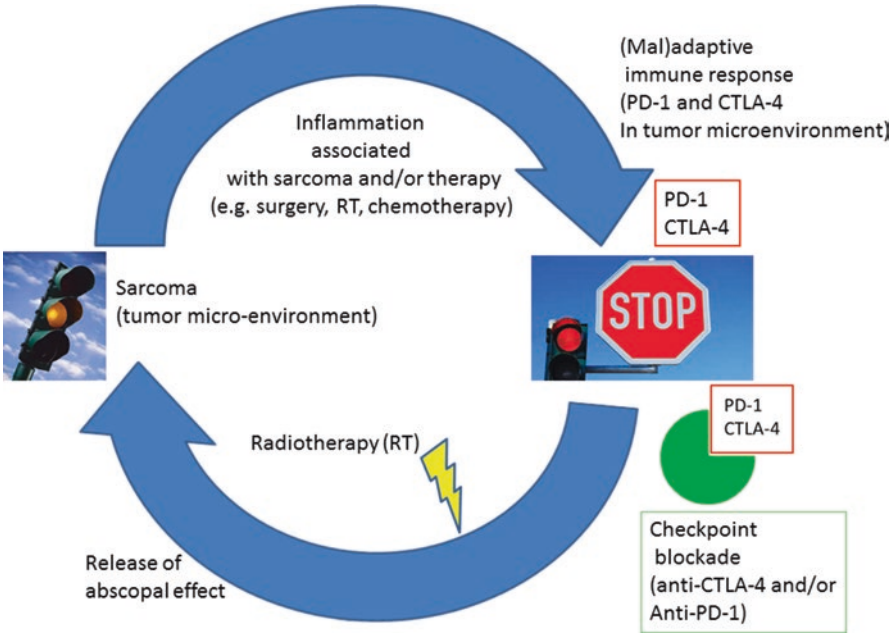

Fig. 6.1 Paradigm of inhibition of immune via inflammation from interventions and tumor growth check immune function versus release of checkpoint inhibition by anti-PD-1 and/or anti-CTLA-4 (checkpoint blockade) to facilitate abscopal (out-of–field) responses with radiation. Thus RT may possibly act like a "tumor vaccine"

stimulating tumor growth [23, 24]. Glutamine can accelerate healing of small intestine and improve outcome after radiation including whole abdominal radiation [25–28]. Elegant studies by Klimberg's group [29, 30] have shown that not only does glutamine improve tolerance of chemotherapy but may also improve methotrexate efficacy. Glutamine is particularly effective in reduction of stomatitis and oral, pharyngeal, and esophageal mucositis if it is in a suspension with a disaccharide that facilitates mucosal absorption [31–33]. A powder containing glutamine and trehalose is now commercially available (Healios). Concerns about glutamine "feeding the tumor" were not born out using a genetically engineered mouse model in which mice routinely developed cancer, glutamine supplementation did not "feed the tumor"; supplementation was associated with upregulation of p53 signaling, inhibition of Akt, lower levels of IGF-1R, and higher levels of PTEN and mdm-2 proteins [34]. Lim et al. showed glutamine supplementation prevented DMBA-induced squamous cell cancers [35]. Thus better nutrition which could include glutamine supplementation may not only reduce chemotherapy-associated toxicity, but also may result in a favorable therapeutic index against cancer [36–39]. Finally, oral glutamine could reduce radiation morbidity in breast conservation [40]. Whether a similar result could be obtained after pre-op radiation for sarcomas remains to be determined.

Iatrogenic factors that contribute to inflammation are many. Chemotherapy alone is an ineffective approach to control osteosarcoma [41] and other sarcomas except GIST. Although chemotherapy may become the main therapeutic intervention for months before or after surgery, chemotherapy cycles can be associated with repeated bouts of poor appetite, catabolic states, and inflammation (e.g., mucositis, enteritis, skin toxicity). C-reactive protein (CRP), a biomarker of inflammation, is associated with the diagnosis, prognosis, and causes of cancer [42]. Surgery also invariably elicits an inflammatory response. Elevated CRP before sarcoma surgery has been associated with decreased survival in patients with soft tissue sarcoma and bone sarcomas including chondrosarcoma, osteosarcoma, and Ewing sarcoma [43–47].

CRP level has been recently correlated with failure-free survival after prostate cancer radiotherapy [48]. Inflammation from radiation is also "part of the package deal" of an adequate local control plan for sarcoma. It appears that radiotherapy is a mixed blessing for sarcoma control. Sharma et al. found that radiotherapy of human sarcoma promotes an intratumoral immune effector signature [49]. Although radiotherapy (RT) is recommended for large, deep, high-grade soft tissue sarcomas, only 6308 of 10,290 soft tissue sarcoma patients received RT. Lack of RT was associated with lower long-term survival ($p < 0.001$; Ref. [11]). Similarly, in metastatic Ewing sarcoma, patients that received adequate local control, especially those with both RT and surgery had better outcomes [50].

With chemotherapy and radiation, there may be tumor evolution to become resistant to apoptosis that is chemotherapy-related, but also to evade immune surveillance (e.g., loss of HLA expression, loss of antigen expression, and/or selection for more stem cell-like phenotype such as aldehyde dehydrogenase expression [51–55]).

6.5 Current Sarcoma Treatment Paradigm

In order to successfully eliminate sarcoma stem cells, local control measures remain the cornerstone for elimination of primary and metastatic disease. Local control measures can be thought of as "physical" and include surgery, RT, heat (radiofrequency ablation, RFA), freezing (cryoablation), and electric current (electroporation).

Control of sarcoma micrometastases has relied on antiproliferative agents in chemotherapy sensitive bone and soft tissue sarcomas [56–62] and now targeted tyrosine kinase inhibitors and agents including pazopanib [63–69].

If adjuvant therapy is actually eliminating all micrometastases or assisting the immune system by control of rapidly proliferating cells and buying time for immune system to finally effectively "mop-up" remaining non-proliferating cancer stem cells is a matter of conjecture. The following will summarize and detail only some of the immune approaches against sarcoma.

Regional therapies may not only kill tumor stem cells but also leave antigen in place to facilitate local and systemic immune responses [11, 70–87]. Cytokines and

macrophage activators act on different immune cells to facilitate more sustained and possibly effective immune responses [88–102]. Augmentation of immune response against sarcoma using antibodies has been tried against osteosarcoma and chondrosarcoma [103–118]. There have been "FANG" now known as VIGIL vaccine trials in Ewing sarcoma [119, 120] and NY-ESO vaccine has been used in sarcoma [121]. The above efforts are summarized in Table 6.2.

Perhaps the most complex, yet promising approach with potential for systemic immune surveillance against cancer involves transfer of immune cells with anti-sarcoma specificity. Table 6.3 summarizes some current investigational efforts (from clinicalTrials.gov and Ref. [103, 122–126]).

The final section of this chapter will describe the potential for RT to augment anti-sarcoma immune function. An abscopal response refers to an out-of-field effect of radiotherapy that is systemic, not just local [127]. Preclinical models and clinical observations using radiotherapy including stereotactic ablative radiotherapy have shown that PD-1 and/or CTLA-4 restrains radiotherapy-induced abscopal effects [13, 49, 128–132]. Perhaps the most elegant demonstration of the synergy of dual checkpoint blockade with anti-PD-1 + anti-CTLA-4 with RT was by Minn's group [133]. In this study both apparently durable complete responses including abscopal responses after RT in three different models systems were significantly better using dual checkpoint blockade with both anti-PD-1 and anti-CTLA-4 [133]. Thus it would appear that combining radiation and checkpoint inhibition may possibly become a new systemic therapy for solid tumors [127, 131, 132, 134–137]. Use of these agents in sarcomas is just beginning (Table 6.4). In 2015, there are no clinical trials of dual checkpoint inhibition and RT in sarcoma open yet. Thus, enhancing immune function within the current paradigm of RT may become an important part of a multidisciplinary approach towards sarcoma (Fig. 6.1).

6.6 Summary and Conclusion

Better immune function can improve sarcoma survival. Sarcoma experts and caregivers will need to become forward observers call in the most effective means to treat this group patients with rare cancers in a variety of locations. The future is to reconcile, translate, and integrate our knowledge that immune function is very important to survival from sarcoma with known benefit from surgery, chemotherapy, and radiotherapy (RT). This will result in new treatments and improved paradigms when developing sarcoma multidisciplinary plans.

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Table 6.2 Agents and therapies which affect immune function against sarcomas: physical means, activators, antibodies, and vaccines

Agent or therapy	Effects on immune function	References
Physical means		
Modality	Comment and reference(s)	
Surgery	Part of multidisciplinary approach [70]	
RFA	Feasible, may improve disease free survival but results in denatured tumor antigens [71–74]	
Electroporation	Seems effective in Kaposi sarcoma [75], nonthermal [76–78]	
Cryoablation	Tumor cell death and antigen preservation [79, 80]	
Ultrasound	Specialized equipment needed [81, 82]	
Radiotherapy (RT)	Preservation of tumor antigens and a common pre-op modality [11, 70, 85]; increase in size during RT does not affect prognosis [86]. Stereotactic body radiotherapy is a reasonable option for metastases [83, 84]. Immune response to RB1-regulated senescence limits radiation-induced osteosarcoma [87]	
Activators of immune function against sarcomas		
Activator	Mechanism and reference(s)	
GM-CSF + Furin	Macrophages increase and antigen presentation; furin decreases TGF-beta in vaccine microenvironment	
Aerosol GM-CSF	Aerosol decreases toxicity but was ineffective against osteosarcoma [88]	
G-CSF	Granulocyte increases; Ewing sarcoma expresses G-CSF and the receptor for G-CSF [89]	
IL-2	NK and T cell activation and proliferation against sarcoma [90, 91]	
	Works with NK cells as aerosol [92, 93]	
Mifamurtide	Macrophage activator requires prolonged schedule of administration for best effects [94–96]. L-MTP-PE phosphatidyl serine lipid is an address signal for "apoptosis" [102]; Improved osteosarcoma survival [97–102]	
Antibodies and fusion proteins against sarcomas		
Antibody	Disease, reference(s)	
Anti-GD2 antibody	Osteosarcoma [103–105]	
Anti-TP-3-PAP	Preclinical antibody x immunotoxin conjugate [106, 107]	
Apo2L/TRAIL	Possible activity in chondrosarcoma [108, 109]	
Denosumab	Giant cell tumor [110–112]	
Anti-IGF-1R	Ewing sarcoma, osteosarcoma, sarcoma [94, 113–118] NCT02306161	
Olaratumab	FDA approved for relapsed sarcomas. See also NCT02677116 and NCT02659020	
Vaccines		
Sarcoma	Antigen/adjuvant and reference(s)	
Ewing Sarcoma	bi-shRNAfurin and GM-CSF [119, 120]	
Sarcoma	NY-ESO+ dendritic cell [121]	

Table 6.3 Cellular therapy against sarcomas

Sarcoma type	Cells recognizing antigen(s)	Reference
Osteosarcoma	CAR T cells against GD2	NCT-02107963 (NCI)
Osteosarcoma	iC9-GD2-CAR-VZV-CTL T Cells	NCT-01953900 (Baylor/TCH)
Osteosarcoma	CAR T cells against Her-2	[103, 122–124]
Osteosarcoma	Activated T cells armed with GD2 x CD3 bispecific antibody	NCT 02173093
Synovial Sarcoma	TCR T cells against NY-ESO	[125, 126] NCT01343043 and TATCTASOM (NCT02239861)
Sarcoma	SCT and NK cells	NCT02100891
		NCT01847468
		NCT01287104
Sarcoma	Expanded, Activated NK cells	NCT02409576

Table 6.4 Clinical trials of checkpoint blockade in sarcomas: anti-PD1 and/or anti-CTLA-4 (November 2015)

Intervention(s)	Disease	ClinicalTrials.gov info
Nivo + ipi	Kaposi sarcoma	NCT02408861
		NCT02408861
Nivo +/− ipi	Metastatic or unresectable sarcoma (adults)	NCT02500797
Nivo +/− ipi	Recurrent or refractory sarcomas (younger patients)	NCT02304458
Nivolumab	Uterine leiomyosarcoma	NCT02428192
Ipi + dasatinib	GIST, stage IV soft tissue sarcoma	NCT01643278
Pem	Advanced sarcomas	NCT02301039
Pem + cyclophos	Advanced sarcomas	NCT02406781
Pem + Chemo	Advanced sarcomas	NCT02331251
Pem + p53 vaccine	Sarcoma	NCT02439263

Nivo Nivolumab (anti-PD-1), *Pem* Pembrolizumab (anti-PD-1), *Ipi*: Ipilimumab, (anti-CTLA-4)

References

1. Ray-Coquard I, et al. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. Cancer Res. 2009;69(13):5383–91.
2. De Angulo G, et al. Early lymphocyte recovery as a prognostic indicator for high-risk Ewing sarcoma. J Pediatr Hematol Oncol. 2007;29(1):48–52.
3. DuBois SG, Elterman K, Grier HE. Early lymphocyte recovery in Ewing sarcoma. J Pediatr Hematol Oncol. 2007;29(5):351–2.
4. Anderson P. Predicting and facilitating survival of pediatric cancer patients: the ALC story. Pediatr Blood Cancer. 2010;55(6):1041–2.
5. Moore C, et al. Prognostic significance of early lymphocyte recovery in pediatric osteosarcoma. Pediatr Blood Cancer. 2010;55(6):1096–102.
6. Idowu OK, et al. Clinical implication of pretreatment neutrophil to lymphocyte ratio in soft tissue sarcoma. Biomarkers. 2012;17(6):539–44.

7. Roberts SS, Chou AJ, Cheung NK. Immunotherapy of childhood sarcomas. Front Oncol. 2015;5:181.
8. Wei L, Meng QG, Bi ZG. Result of a randomized clinical trial comparing different types of anesthesia on the immune function of patients with osteosarcoma undergoing radical resection. Panminerva Med. 2013;55(2):211–6.
9. Wild AT, et al. The association between chemoradiation-related lymphopenia and clinical outcomes in patients with locally advanced pancreatic adenocarcinoma. Am J Clin Oncol. 2015;38(3):259–65.
10. Grossman SA, et al. Survival in patients with severe lymphopenia following treatment with radiation and chemotherapy for newly diagnosed solid tumors. J Natl Compr Cancer Netw. 2015;13(10):1225–31.
11. Hou CH, et al. The use of radiation therapy in localized high-grade soft tissue sarcoma and potential impact on survival. Ann Surg Oncol. 2015;22(9):2831–8.
12. Kuo P, et al. Galectin-1 mediates radiation-related lymphopenia and attenuates NSCLC radiation response. Clin Cancer Res. 2014;20(21):5558–69.
13. Welsh JW, et al. Galectin-1 and immune suppression during radiotherapy. Clin Cancer Res. 2014;20(24):6230–2.
14. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348(6230):56–61.
15. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161(2):205–14.
16. Momtaz P, Postow MA. Immunologic checkpoints in cancer therapy: focus on the programmed death-1 (PD-1) receptor pathway. Pharmgenomics Pers Med. 2014;7:357–65.
17. Tumeh PC, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–71.
18. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
19. Homet Moreno B, et al. Anti-PD-1 therapy in melanoma. Semin Oncol. 2015;42(3):466–73.
20. D'Angelo SP, et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol. 2015;46(3):357–65.
21. Kim JR, et al. Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One. 2013;8(12):e82870.
22. Lussier DM, et al. Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions. J Immunother. 2015;38(3):96–106.
23. Klimberg VS, et al. Glutamine-enriched diets support muscle glutamine metabolism without stimulating tumor growth. J Surg Res. 1990;48(4):319–23.
24. Klimberg VS, McClellan JL. Claude H. Organ, Jr. Honorary lectureship. Glutamine, cancer, and its therapy. Am J Surg. 1996;172(5):418–24.
25. Klimberg VS, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. Arch Surg. 1990;125(8):1040–5.
26. Klimberg VS, et al. Prophylactic glutamine protects the intestinal mucosa from radiation injury. Cancer. 1990;66(1):62–8.
27. Souba WW, Klimberg VS, Copeland 3rd EM. Glutamine nutrition in the management of radiation enteritis. JPEN J Parenter Enteral Nutr. 1990;14(4 Suppl):106S–8S.
28. Klimberg S. Prevention of radiogenic side effects using glutamine-enriched elemental diets. Recent Results Cancer Res. 1991;121:283–5.
29. Klimberg VS, et al. Glutamine facilitates chemotherapy while reducing toxicity. JPEN J Parenter Enteral Nutr. 1992;16(6 Suppl):83S–7S.
30. Rubio IT, et al.. Effect of glutamine on methotrexate efficacy and toxicity. Ann Surg. 1998; 227(5):772–8; discussion 778–80.
31. Skubitz KM, Anderson PM. Oral glutamine to prevent chemotherapy induced stomatitis: a pilot study. J Lab Clin Med. 1996;127(2):223–8.
32. Anderson PM, Schroeder G, Skubitz KM. Oral glutamine reduces the duration and severity of stomatitis after cytotoxic cancer chemotherapy. Cancer. 1998;83(7):1433–9.

33. Peterson DE, Jones JB, Petit 2nd RG. Randomized, placebo-controlled trial of Saforis for prevention and treatment of oral mucositis in breast cancer patients receiving anthracycline-based chemotherapy. Cancer. 2007;109(2):322–31.
34. Todorova VK, et al. Modulation of p53 and c-myc in DMBA-induced mammary tumors by oral glutamine. Nutr Cancer. 2006;54(2):263–73.
35. Lim V, et al. Glutamine prevents DMBA-induced squamous cell cancer. Oral Oncol. 2009;45(2):148–55.
36. Cao Y, et al. Glutamine enhances gut glutathione production. JPEN J Parenter Enteral Nutr. 1998;22(4):224–7.
37. Todorova VK, et al. Effect of dietary glutamine on tumor glutathione levels and apoptosis-related proteins in DMBA-induced breast cancer of rats. Breast Cancer Res Treat. 2004;88(3):247–56.
38. Todorova VK, et al. Oral glutamine protects against acute doxorubicin-induced cardiotoxicity of tumor-bearing rats. J Nutr. 2010;140(1):44–8.
39. Todorova VK, et al. Tamoxifen and raloxifene suppress the proliferation of estrogen receptor-negative cells through inhibition of glutamine uptake. Cancer Chemother Pharmacol. 2011;67(2):285–91.
40. Rubio I, et al. Oral glutamine reduces radiation morbidity in breast conservation surgery. JPEN J Parenter Enteral Nutr. 2013;37(5):623–30.
41. Jaffe N, et al. Can cure in patients with osteosarcoma be achieved exclusively with chemotherapy and abrogation of surgery? Cancer. 2002;95(10):2202–10.
42. Allin KH, Nordestgaard BG. Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer. Crit Rev Clin Lab Sci. 2011;48(4):155–70.
43. Nakamura T, et al. The value of C-reactive protein and comorbidity in predicting survival of patients with high grade soft tissue sarcoma. Eur J Cancer. 2013;49(2):377–85.
44. Nakamura T, et al. The prognostic value of the serum level of C-reactive protein for the survival of patients with a primary sarcoma of bone. Bone Joint J. 2013;95-B(3):411–8.
45. Nakamura T, et al. The role of C-reactive protein in predicting post-metastatic survival of patients with metastatic bone and soft tissue sarcoma. Tumour Biol. 2015;36(10):7515–20.
46. Nakamura T, et al. The combined use of the neutrophil-lymphocyte ratio and C-reactive protein level as prognostic predictors in adult patients with soft tissue sarcoma. J Surg Oncol. 2013;108(7):481–5.
47. Szkandera J, et al. Validation of the prognostic relevance of plasma C-reactive protein levels in soft-tissue sarcoma patients. Br J Cancer. 2013;109(9):2316–22.
48. Hall WA, et al. The association between C-reactive protein (CRP) level and biochemical failure-free survival in patients after radiation therapy for nonmetastatic adenocarcinoma of the prostate. Cancer. 2013;119(18):3272–9.
49. Sharma A, et al. Radiotherapy of human sarcoma promotes an intratumoral immune effector signature. Clin Cancer Res. 2013;19(17):4843–53.
50. Haeusler J, et al. The value of local treatment in patients with primary, disseminated, multifocal Ewing sarcoma (PDMES). Cancer. 2010;116(2):443–50.
51. D'Andrea FP. Intrinsic radiation resistance of mesenchymal cancer stem cells and implications for treatment response in a murine sarcoma model. Dan Med J. 2012;59(2):B4388.
52. Canter RJ, et al. Anti-proliferative but not anti-angiogenic tyrosine kinase inhibitors enrich for cancer stem cells in soft tissue sarcoma. BMC Cancer. 2014;14:756.
53. Awad O, et al. High ALDH activity identifies chemotherapy-resistant Ewing's sarcoma stem cells that retain sensitivity to EWS-FLI1 inhibition. PLoS One. 2010;5(11):e13943.
54. Emori M, et al. High expression of CD109 antigen regulates the phenotype of cancer stem-like cells/cancer-initiating cells in the novel epithelioid sarcoma cell line ESX and is related to poor prognosis of soft tissue sarcoma. PLoS One. 2013;8(12):e84187.
55. Lohberger B, et al. Aldehyde dehydrogenase 1, a potential marker for cancer stem cells in human sarcoma. PLoS One. 2012;7(8):e43664.

56. Jaffe N. Historical perspective on the introduction and use of chemotherapy for the treatment of osteosarcoma. Adv Exp Med Biol. 2014;804:1–30.
57. Jaffe N, et al. Control of primary osteosarcoma with chemotherapy. Cancer. 1985;56(3):461–6.
58. D'Adamo DR. Appraising the current role of chemotherapy for the treatment of sarcoma. Semin Oncol. 2011;38(Suppl 3):S19–29.
59. Wesolowski R, Budd GT. Use of chemotherapy for patients with bone and soft-tissue sarcomas. Cleve Clin J Med. 2010;77(Suppl 1):S23–6.
60. Schuetze SM. Chemotherapy in the management of osteosarcoma and Ewing's sarcoma. J Natl Compr Cancer Netw. 2007;5(4):449–55.
61. Linch M, et al. Systemic treatment of soft-tissue sarcoma-gold standard and novel therapies. Nat Rev Clin Oncol. 2014;11(4):187–202.
62. Movva S, Verschraegen C. Systemic management strategies for metastatic soft tissue sarcoma. Drugs. 2011;71(16):2115–29.
63. Harwood JL, et al. Targeted chemotherapy in bone and soft-tissue sarcoma. Orthop Clin North Am. 2015;46(4):587–608.
64. Ranieri G, et al. Pazopanib a tyrosine kinase inhibitor with strong anti-angiogenetic activity: a new treatment for metastatic soft tissue sarcoma. Crit Rev Oncol Hematol. 2014;89(2):322–9.
65. Rajendra R, Jones RL, Pollack SM. Targeted treatment for advanced soft tissue sarcoma: profile of pazopanib. Onco Targets Ther. 2013;6:217–22.
66. Riedel RF, Maki RG, Wagner AJ. Targeted therapy in sarcoma: should we be lumpers or splitters? Am Soc Clin Oncol Educ Book. 2012:652–7.
67. Shor AC, et al. Dasatinib inhibits migration and invasion in diverse human sarcoma cell lines and induces apoptosis in bone sarcoma cells dependent on SRC kinase for survival. Cancer Res. 2007;67(6):2800–8.
68. Radaelli S, et al. Emerging therapies for adult soft tissue sarcoma. Expert Rev Anticancer Ther. 2014;14(6):689–704.
69. Walczak BE, Irwin RB. Sarcoma chemotherapy. J Am Acad Orthop Surg. 2013;21(8):480–91.
70. Siegel GW, et al. The multidisciplinary management of bone and soft tissue sarcoma: an essential organizational framework. J Multidiscip Healthc. 2015;8:109–15.
71. Jiang L, et al. Significance of local treatment in patients with metastatic soft tissue sarcoma. Am J Cancer Res. 2015;5(6):2075–82.
72. Jones RL, et al. Radiofrequency ablation is a feasible therapeutic option in the multi modality management of sarcoma. Eur J Surg Oncol. 2010;36(5):477–82.
73. Koelblinger C, Strauss S, Gillams A. Outcome after radiofrequency ablation of sarcoma lung metastases. Cardiovasc Intervent Radiol. 2014;37(1):147–53.
74. Anderson P. Non-surgical treatment of pulmonary and extra-pulmonary metastases. Cancer Treat Res. 2009;152:203–15.
75. Di Monta G, et al. Electrochemotherapy as "new standard of care" treatment for cutaneous Kaposi's sarcoma. Eur J Surg Oncol. 2014;40(1):61–6.
76. Yu Z, et al. Therapeutic potential of irreversible electroporation in sarcoma. Expert Rev Anticancer Ther. 2012;12(2):177–84.
77. de Bree R, et al. Electroporation therapy in soft tissue sarcoma: a potentially effective novel treatment. Sarcoma. 2006;2006:85234.
78. Hyacinthe M, et al. Electrically enhanced drug delivery for the treatment of soft tissue sarcoma. Cancer. 1999;85(2):409–17.
79. Lippa N, et al. Standardization of selection criteria for percutaneous image-guided cryoablation of recurrent soft-tissue sarcomas. Diagn Interv Imaging. 2014;95(11):1071–7.
80. Ahlmann ER, et al. Cryoablation and resection influences patient survival for soft tissue sarcomas: impact on survivorship and local recurrence. Clin Orthop Relat Res. 2007;459:174–81.

81. Avedian RS, et al. Magnetic resonance guided high-intensity focused ultrasound ablation of musculoskeletal tumors. Curr Orthop Pract. 2011;22(4):303–8.
82. Chen W, et al. Primary bone malignancy: effective treatment with high-intensity focused ultrasound ablation. Radiology. 2010;255(3):967–78.
83. Brown LC, et al. Stereotactic body radiotherapy for metastatic and recurrent ewing sarcoma and osteosarcoma. Sarcoma. 2014;2014:418270.
84. Dhakal S, et al. Stereotactic body radiotherapy for pulmonary metastases from soft-tissue sarcomas: excellent local lesion control and improved patient survival. Int J Radiat Oncol Biol Phys. 2012;82(2):940–5.
85. Ozaki T. Diagnosis and treatment of Ewing sarcoma of the bone: a review article. J Orthop Sci. 2015;20(2):250–63.
86. Delisca GO, et al. Tumor size increase following preoperative radiation of soft tissue sarcomas does not affect prognosis. J Surg Oncol. 2013;107(7):723–7.
87. Kansara M, et al. Immune response to RB1-regulated senescence limits radiation-induced osteosarcoma formation. J Clin Invest. 2013;123(12):5351–60.
88. Arndt CA, et al. Inhaled granulocyte-macrophage colony stimulating factor for first pulmonary recurrence of osteosarcoma: effects on disease-free survival and immunomodulation. a report from the Children's Oncology Group. Clin Cancer Res. 2010;16(15):4024–30.
89. Morales-Arias J, et al. Expression of granulocyte-colony-stimulating factor and its receptor in human Ewing sarcoma cells and patient tumor specimens: potential consequences of granulocyte-colony-stimulating factor administration. Cancer. 2007;110(7):1568–77.
90. D'Angelo SP, et al. Sarcoma immunotherapy: past approaches and future directions. Sarcoma. 2014;2014:391967.
91. Anderson PM, et al. Increased local antitumor effects of interleukin 2 liposomes in mice with MCA-106 sarcoma pulmonary metastases. Cancer Res. 1990;50(6):1853–6.
92. Guma SR, et al. Aerosol interleukin-2 induces natural killer cell proliferation in the lung and combination therapy improves the survival of mice with osteosarcoma lung metastasis. Pediatr Blood Cancer. 2014;61(8):1362–8.
93. Guma SR, et al. Natural killer cell therapy and aerosol interleukin-2 for the treatment of osteosarcoma lung metastasis. Pediatr Blood Cancer. 2014;61(4):618–26.
94. Anderson P, et al. Novel bone cancer drugs: investigational agents and control paradigms for primary bone sarcomas (Ewing's sarcoma and osteosarcoma). Expert Opin Investig Drugs. 2008;17(11):1703–15.
95. Kleinerman ES, Jaffe N. Liposomal MTP-PE for the adjuvant therapy of osteosarcoma. Prog Clin Biol Res. 1990;343:263–79.
96. Kleinerman ES, et al. Phase II study of liposomal muramyl tripeptide in osteosarcoma: the cytokine cascade and monocyte activation following administration. J Clin Oncol. 1992;10(8):1310–6.
97. Meyers PA. Muramyl tripeptide (mifamurtide) for the treatment of osteosarcoma. Expert Rev Anticancer Ther. 2009;9(8):1035–49.
98. Meyers PA. Systemic therapy for osteosarcoma and ewing sarcoma. Am Soc Clin Oncol Educ Book. 2015;35:e644–7.
99. Meyers PA, Chou AJ. Muramyl tripeptide-phosphatidyl ethanolamine encapsulated in liposomes (L-MTP-PE) in the treatment of osteosarcoma. Adv Exp Med Biol. 2014;804:307–21.
100. Meyers PA, et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group. J Clin Oncol. 2008;26(4):633–8.
101. Chou AJ, et al. Addition of muramyl tripeptide to chemotherapy for patients with newly diagnosed metastatic osteosarcoma: a report from the Children's Oncology Group. Cancer. 2009;115(22):5339–48.
102. Anderson PM, et al. Mifamurtide in metastatic and recurrent osteosarcoma: a patient access study with pharmacokinetic, pharmacodynamic, and safety assessments. Pediatr Blood Cancer. 2014;61(2):238–44.

103. Ahmed M, Cheung NK. Engineering anti-GD2 monoclonal antibodies for cancer immunotherapy. FEBS Lett. 2014;588(2):288–97.
104. Navid F, Santana VM, Barfield RC. Anti-GD2 antibody therapy for GD2-expressing tumors. Curr Cancer Drug Targets. 2010;10(2):200–9.
105. Frost JD, et al. A phase I/IB trial of murine monoclonal anti-GD2 antibody 14.G2a plus interleukin-2 in children with refractory neuroblastoma: a report of the Children's Cancer Group. Cancer. 1997;80(2):317–33.
106. Anderson PM, et al. In vitro and in vivo cytotoxicity of an anti-osteosarcoma immunotoxin containing pokeweed antiviral protein. Cancer Res. 1995;55(6):1321–7.
107. Ek O, et al. Antitumor activity of TP3(anti-p80)-pokeweed antiviral protein immunotoxin in hamster cheek pouch and severe combined immunodeficient mouse xenograft models of human osteosarcoma. Clin Cancer Res. 1998;4(7):1641–7.
108. Herbst RS, et al. Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. J Clin Oncol. 2010;28(17):2839–46.
109. Subbiah V, et al. Targeting the apoptotic pathway in chondrosarcoma using recombinant human Apo2L/TRAIL (dulanermin), a dual proapoptotic receptor (DR4/DR5) agonist. Mol Cancer Ther. 2012;11(11):2541–6.
110. Skubitz KM. Giant cell tumor of bone: current treatment options. Curr Treat Options in Oncol. 2014;15(3):507–18.
111. Thomas D, et al. Denosumab in patients with giant-cell tumour of bone: an open-label, phase 2 study. Lancet Oncol. 2010;11(3):275–80.
112. Thomas DM, Skubitz KM. Giant cell tumour of bone. Curr Opin Oncol. 2009;21(4):338–44.
113. Pappo AS, et al. A phase 2 trial of R1507, a monoclonal antibody to the insulin-like growth factor-1 receptor (IGF-1R), in patients with recurrent or refractory rhabdomyosarcoma, osteosarcoma, synovial sarcoma, and other soft tissue sarcomas: results of a sarcoma alliance for research through collaboration study. Cancer. 2014;120(16):2448–56.
114. Tabernero J, et al. Anticancer activity of the type I insulin-like growth factor receptor antagonist, ganitumab, in combination with the death receptor 5 agonist, conatumumab. Target Oncol. 2015;10(1):65–76.
115. Anderson, PM, et al. A Phase II study of clinical activity of SCH717454 (robatumumab) in relapsed osteosarcoma and Ewing Sarcoma. Clin Cancer Immunol. 2015; in review.
116. Naing A, et al. Phase I trial of cixutumumab combined with temsirolimus in patients with advanced cancer. Clin Cancer Res. 2011;17(18):6052–60.
117. Naing A, et al. Insulin growth factor receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with metastatic adrenocortical carcinoma. Br J Cancer. 2013;108(4):826–30.
118. Naing A, et al. Insulin growth factor-receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with refractory Ewing's sarcoma family tumors. Clin Cancer Res. 2012;18(9):2625–31.
119. Ghisoli M, et al. Pilot trial of FANG Immunotherapy in Ewing's sarcoma. Mol Ther. 2015;23(6):1103–9.
120. Nemunaitis J, et al. Summary of bi-shRNA/GM-CSF augmented autologous tumor cell immunotherapy (FANG) in advanced cancer of the liver. Oncology. 2014;87(1):21–9.
121. Krishnadas DK, et al. A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. Cancer Immunol Immunother. 2015;64(10):1251–60.
122. Ahmed N, et al. Immunotherapy for osteosarcoma: genetic modification of T cells overcomes low levels of tumor antigen expression. Mol Ther. 2009;17(10):1779–87.
123. Rainusso N, et al. Immunotherapy targeting HER2 with genetically modified T cells eliminates tumor-initiating cells in osteosarcoma. Cancer Gene Ther. 2012;19(3):212–7.
124. Ahmed N, et al. Human epidermal growth factor receptor 2 (HER2)—specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol. 2015;33(15):1688–96.

125. Lai JP, et al. NY-ESO-1 expression in synovial sarcoma and other mesenchymal tumors: significance for NY-ESO-1-based targeted therapy and differential diagnosis. Mod Pathol. 2012;25(6):854–8.
126. Robbins PF, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29(7):917–24.
127. Hanna GG, Coyle VM, Prise KM. Immune modulation in advanced radiotherapies: targeting out-of-field effects. Cancer Lett. 2015;368(2):246–51.
128. Grimaldi AM, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology. 2014;3:e28780.
129. Park SS, et al. PD-1 restrains radiotherapy-induced abscopal effect. Cancer Immunol Res. 2015;3(6):610–9.
130. Shahabi V, et al. Immune-priming of the tumor microenvironment by radiotherapy: rationale for combination with immunotherapy to improve anticancer efficacy. Am J Clin Oncol. 2015;38(1):90–7.
131. Verbrugge I, et al. Enhancing the antitumor effects of radiotherapy with combinations of immunostimulatory antibodies. Oncoimmunology. 2012;1(9):1629–31.
132. Verbrugge I, et al. Radiotherapy increases the permissiveness of established mammary tumors to rejection by immunomodulatory antibodies. Cancer Res. 2012;72(13):3163–74.
133. Twyman-Saint Victor C, et al. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature. 2015;520(7547):373–7.
134. Tang C, et al. Combining radiation and immunotherapy: a new systemic therapy for solid tumors? Cancer Immunol Res. 2014;2(9):831–8.
135. Okwan-Duodu D, et al. Role of radiation therapy as immune activator in the era of modern immunotherapy for metastatic malignant melanoma. Am J Clin Oncol. 2015;38(1):119–25.
136. Seyedin SN, Tang C, Welsh JW. Author's view: radiation and immunotherapy as systemic therapy for solid tumors. Oncoimmunology. 2015;4(3):e986402.
137. Barbee MS, et al. Current status and future directions of the immune checkpoint inhibitors ipilimumab, pembrolizumab, and nivolumab in oncology. Ann Pharmacother. 2015;49(8):907–37.

Chapter 7
Cancer Imaging in Immunotherapy

Yousra Eleneen and Rivka R. Colen

Abstract Immune therapeutics are revolutionizing cancer treatments. In tandem, new and confounding imaging characteristics have appeared that are distinct from those typically seen with conventional cytotoxic therapies. In fact, only 10% of patients on immunotherapy may show tumor shrinkage, typical of positive responses on conventional therapy. Conversely, those on immune therapies may initially demonstrate a delayed response, transient enlargement followed by tumor shrinkage, stable size, or the appearance of new lesions. New imaging response criteria such as the immune-related Response Evaluation Criteria in Solid Tumors (irRECIST) and immune-related Response Criteria (irRC) are being implemented in many trials. However, FDA approval of emerging therapies including immunotherapies still relies on the current RECIST criteria. In this review, 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 Cancer imaging • irRC • Immune imaging criteria • irRECIST • Immunotherapy

7.1 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

Y. Eleneen, M.B.B.Ch. • R.R. Colen, M.D. (✉)
Department of Cancer Systems Imaging and Diagnostic Radiology—Neuro Imaging, The University of Texas MD Anderson Cancer Center, T. Boone Pickens Academic Towe (FCT16.5037), 1515 Holcombe Blvd., Unit 1482, Houston, TX 77030, USA
e-mail: rcolen@mdanderson.org

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originally created to assess the effects of cytotoxic chemotherapy and are dependent on tumor shrinkage and absence of new lesions; however, these criteria do not perform well in assessing the effects of drugs with other mechanisms of action such as antiangiogenic therapies or immune therapies [1, 4]. Evaluation of tumor response to cytotoxic chemotherapy depends on tumor shrinkage within a few weeks of initiating treatment. In fact, in addition to the appearance of new lesions and increased tumor size, stable disease was at one point considered a treatment failure [4]. On the other hand, new tumor therapies with recombinant cytokines, cancer vaccines, and immunomodulatory monoclonal antibodies may demonstrate a delayed response, transient enlargement (transit flair up phase) followed by tumor shrinkage, stable size, or the appearance of new lesions [4]. Unique challenges associated with immunotherapy reflect delays in response and therapy-induced inflammation. Cancers after immunotherapy demonstrate confounding radiographic appearances with only 10% showing regression [4]. Typically, these tumors initially demonstrate a delay in response, including none or slow decrease in tumor size, increase in tumor size, and/or the appearance of new lesions which overtime become stable, decrease, or resolve without further treatment (Fig. 7.1). 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

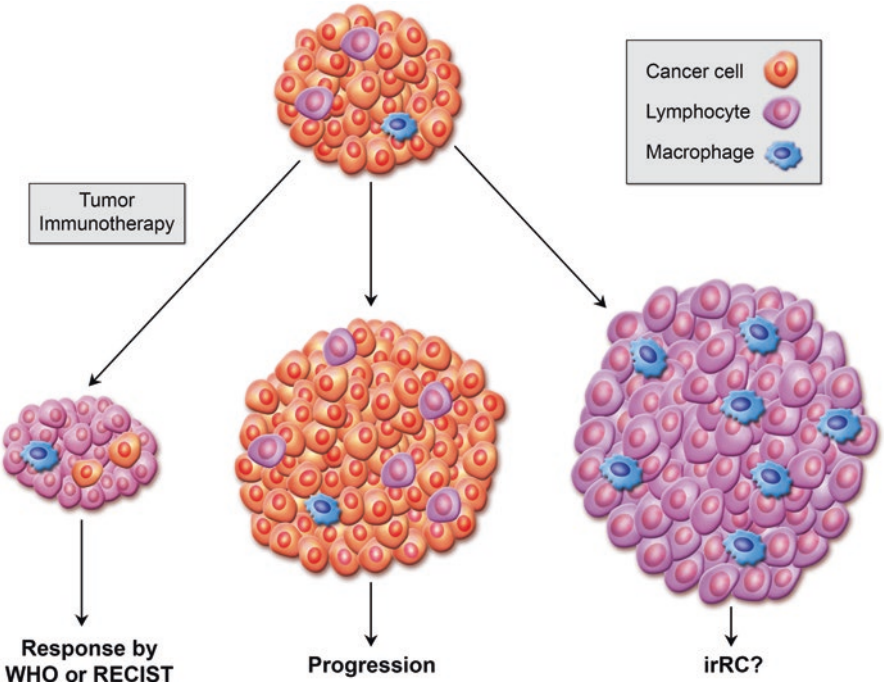

Fig. 7.1 Cancer imaging in immunotherapy

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 review, we discuss the use of a variety of traditional and new immunotherapy criteria for the evaluation of tumor response in patients who are undergoing immunotherapy. We will also briefly discuss some of the immunotherapy-induced adverse events.

7.2 Conventional Imaging Response Criteria (Table 7.1)

The WHO and the RECIST criteria were the first criteria developed to assess tumor responses to traditional cancer treatment which included cytotoxic chemotherapy, radiation therapy, or surgical resection. These criteria depend on reduction in tumor size and do not take in consideration appearance of new lesions when evaluating responses that may be related to treatment [4].

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 [6]. The WHO criteria introduced the concept of assessing tumor burden using the sum of the products of diameters (SPD) (i.e., longest overall tumor diameter and longest diameter perpendicular to the longest overall diameter) and determining response to therapy by evaluating the changes from baseline during treatment [6]. These criteria were categorized into four tumor response groups: complete response (tumor not detected for at least 4 weeks); partial response (≥50% reduction in the SPD from baseline also confirmed at 4 weeks); progressive disease (≥25% increase in tumor size in one or more lesions); and no change (stable) in disease (neither partial response, complete response, nor progressive disease) (Table 7.1) [7]. However, the WHO has a few major pitfalls (*discussed below*); in particular, because tumor measurements are based on SPD, small increases in tumor size may result in a sufficiently overall increase in tumor size (≥25% increase) to consider it as progressive disease [5].

7.2.2 RECIST 1.0, 1.1 and mRECIST Criteria

7.2.2.1 RECIST 1.0

In 2000, the RECIST criteria were established and addressed some of the pitfalls of the WHO criteria. 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 7.1) [6].

Table 7.1 Comparison between the basis of WHO, RECIST 1.0, RECIST 1.1, irRC, and irRECIST criteria [1, 2, 4]

Criterion	WHO	RECIST 1.0	RECIST 1.1	irRC	irRECIST
Method of measurement	SPD	Longest diameter	Longest diameter (except in lymph nodes)	SPD	Single longest diameter (except in lymph nodes)
Measurable lesions	Should be measurable in two dimensions, no minimum lesion size	Minimum size = 10 mm at spiral CT, 20 mm at conventional CT	Minimum size = 10 mm at CT	Minimum size of the lesion is 5 mm × 5 mm	Minimum size = 10
Number of lesions measured	No assessment	Ten lesions (≤5 in any one organ)	Five lesions (≤2 in any one organ)	Ten lesions (≤5 in any organ)	Five lesions (≤2 in any one organ)
Progressive disease	≥25% increase in SPD	20% increase in SLD or new lesions, unequivocal progression considered to indicate progressive disease	>20% increase in SLD; ≥5-mm increase in size; new lesions; detailed description of unequivocal progression	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart
Lymph nodes	Unspecified	Unspecified	Short axis: target lesions ≥15 mm, nontarget lesions = 10–15 mm, nonpathologic lesions <10 mm	Unspecified	Short axis: target lesions ≥15 mm, nontarget lesions = 10–15 mm, nonpathologic lesions <10 mm
New lesions	No assessment	No assessment	Provides guidance as to when a lesion is considered new (i.e., representative of progressive disease)	Does not constitute progressive disease in itself, but is rather added to the SPD and contributes to progression	Does not constitute progressive disease in itself, but is rather added to the sum of longest diameter and contributes to progression
Guidance for imaging studies	No assessment	CT, MRI, chest radiography	CT, MRI, FDG PET	CT, MRI, chest radiography, FDG PET	CT, MRI, chest radiography, FDG PET

7.2.2.2 RECIST 1.1

In 2009, the RECIST 1.1 were developed. RECIST 1.1 addressed multiple questions regarding the assessment of lymph nodes, number of lesions to be assessed, and use of new imaging modalities such as multidetector 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 uses 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 [5] (Table 7.1).

7.2.2.3 Modified RECIST (mRECIST)

Modified RECIST (mRECIST) was created to measure the response rate in hepatocellular carcinoma (HCC). Similar to RECIST 1.0 and 1.1, mRECIST uses tumor size as an index of tumor response; however, in contrast, mRECIST takes into account treatment-induced tumor necrosis, and changes in size are determined by assessing for viable tumor, referred to an uptake of contrast agent in the arterial phase on CT or MRI [9, 10]. For example, a complete tumor response is defined as the disappearance of arterial phase enhancement in all target lesions which should be classified as a measurable lesion according to 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.

7.2.3 Choi Response Criteria

The Choi criteria were initially proposed for assessment of GIST tumors on imatinib, a tyrosine kinase receptor inhibitor. This study found that GISTs on treatment may initially increase in size due to internal hemorrhage, necrosis, or myxoid degeneration. Some may show a minimal decrease in tumor size but not sufficient enough to be classified as having a positive response to therapy according to RECIST criteria [11]. The Choi criteria focuses on changes in density (Hounsfield units on CT) rather than tumor shrinkage to assess response. A decrease in tumor density on CT is often seen in these tumors responding to imatinib and is related to tumor necrosis or myxoid degeneration. There are two main limitations of the Choi criteria; these cannot be applied to MRI and there is lack of sufficient validation in other tumors.

7.2.4 PERCIST Criteria

Based on the premise that newer cancer therapies are more cytostatic than cytocidal, tumor response can manifest with a decrease in metabolism without a notable tumor size reduction [12]. In 2009, the PERCIST criteria were proposed and is based mainly on FDG uptake to evaluate tumor response [13]. PERCIST focuses on the percentage of change in metabolic activity from baseline and the number of weeks from initiation therapy. The standardized uptake value (SUV) corrected for lean body mass (SUL) is used for the assessment of tumor response. The SUL peak is measured within a spherical region of interest of 1.2 cm in diameter (or 1 cm^3 for volume) within the area of highest uptake in the tumor [5]. PERCIST defines four metabolic response categories. In brief, according to these criteria, complete response means disappearance of all metabolically active tumors while partial metabolic response is defined as a 0.8-unit (>30%) decline in SUL peak between the most intense lesion before treatment and the most intense lesion after treatment. Of note, the lesion at follow-up may be a different lesion than previously measured since the most active lesion needs to be followed. Progressive disease is defined as an increase (>30%) in SUL peak or the appearance of a new metabolically active lesion [5].

7.3 Immunotherapy Imaging Response Criteria

Evaluating tumor responses during immune therapy in solid cancers remains a challenge [5, 14]. The mechanism of action in immunotherapy differs substantially from cytotoxic agents, thus a well-tailored set of criteria to capture accurate and exact response to this new line of therapeutic agents is needed [4, 5, 14]. To this end, Wolchok et al. presented a set of criteria to evaluate immune-related responses, adopting a bidimensional approach similar to the WHO criteria and measuring a maximum number of five lesions per organ (Table 7.2) [4]. Although these criteria were widely accepted, it still harbors some challenges. For instance, assessing a relatively large number of lesions per organ could be relatively time consuming in cases of extreme tumor burdens [2, 15]. Furthermore, evaluation of excessive number of lesions impacts

Table 7.2 Summary of immune-related response criteria (irRC) [4]

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

(continued)

Table 7.2 (continued)

Summary of immune-related response criteria (irRC)	
New nontarget lesions	If the new lesions fail to fulfill the criteria of target lesions, they do not contribute to total tumor burden
	However, complete remission of such lesions is essential for establishing a complete response
Imaging modalities	Almost all current imaging modalities could be used to assess tumors in a longitudinal manner. 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
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

the reproducibility of the results [2, 15]. As such, Nishino et al. proposed a modification to the immune-related response criteria (irRC) in the light of RECIST 1.1 guidelines [2, 8, 15]. With regard to brain tumors, the Immunotherapy Response Assessment for Neuro-Oncology (iRANO) criteria are a set to tumor metrics to assess brain tumors in patients undergoing immune therapies.

7.3.1 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 2005. In 2009, the immune-related Response Criteria (irRC) published by Wolchok et al. were based on observed patterns in treatment response from phase II clinical trials in advanced melanoma patients who were receiving ipilimumab, a human monoclonal antibody that blocks cytotoxic T lymphocyte antigen–4 (CTLA-4). In this study [4], four patterns of treatment responses were recognized: (1) a decrease in the size of the lesion and without new tumors, similar to what is seen after conventional cytotoxic therapy; (2) stable disease after completion of treatment; (3) a delay in tumor response to therapy after an initial increase in total tumor burden; (4) the appearance of new lesions that precede tumor shrinkage.

In contrast to the WHO and RECIST criteria, irRC takes into account both the index and new measurable lesions to assess the "total tumor burden," a new concept from prior criteria, and compared to the baseline scan [4]. The irRC was derived from WHO criteria and, therefore, the thresholds of response remain the 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 10 visceral lesions and five cutaneous index lesions). At every time point, the index lesions and any new measurable lesions are added together to accurately measure the total tumor burden (TTB) [$(\text{TTB} = \text{SPD}_{\text{index lesions}} + \text{SPD}_{\text{new, measurable lesions}})$]. This is a major difference from the WHO criteria which considers all new measurable lesions as progressive disease [5]. Further, a confirmatory examination at least 4 weeks from the initial scan documenting progression is required by the irRC prior to declaring progressive disease, as there can be a delay in response in patients on immunotherapy. In addition, decreases in tumor burden must be assessed relative to baseline measurements (i.e., the SPD of all index lesions at screening). The overall response according to the irRC is derived from time-point response assessments based on tumor burden as described in Table 7.2.

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].

7.3.2 Immune-Related RECIST Criteria

The newly proposed irRECIST 1.1 (Table 7.3) and adopted irRC [4] set thresholds for determining different possible responses including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [2, 15]. Nishino et al. demonstrated that such changes did not result in any statistically significant variation of the response evaluation in patient with melanoma receiving

Table 7.3 Summary of immune-related RECIST 1.1 [2]

Summary of immune-related RECIST1.1 (irRECIST)	
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 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 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)
Types of overall response	Complete response (CR), partial response (pr), stable disease (SD), and progressive disease (PD)
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

immunotherapy [2, 15]. They also demonstrated that irRECIST 1.1 measurements were relatively more reproducible than the more involved bidimensional irRC measurements [2, 15]. However, those studies were performed on relatively small cohorts of patients and better evaluation of irRECIST 1.1 is still required.

7.3.3 *Immunotherapy Response Assessment for Neuro-Oncology Criteria*

The iRANO criteria are used to assess brain lesions in patients undergoing immunotherapy [3]. In order that misclassification of patient with stable or increasing tumor size and new lesions as progressive disease does not occur when the therapy is actually effective and the patient is receiving clinical benefit, the iRANO criteria were published. In brief, the iRANO follow the same guidelines as the RANO criteria. However, in those cases of appearance of disease in the absence of clinical deterioration within 6 months of immunotherapy, continuation of immunotherapy and repeat assessment in 3 months is recommended (Table 7.4). As with all current imaging assessment criteria, the iRANO guidelines will require future amendments, including the possible incorporation of volumetrics, advanced imaging sequences, and other types of imaging analytics. A recent study by our group demonstrated that radiomics can discriminate between patients who have pseudoprogression versus true tumor progression with high sensitivity (97%), specificity (79%), and accuracy (95%) in patients with glioblastoma [17].

Table 7.4 Summary of immune therapy Response Assessment in Neuro-Oncology (iRANO) [3]

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 3 more months taking in reference the time point at which progression was initially reported
Imaging modalities	MRI is the gold standard in evaluation of intracranial neoplasms; however, the criteria could be also used to evaluate CT scan with relative restrictions
Target lesions criteria	Target lesions should measure at least 10 × 10 mm. A maximum of five target lesions could be selected
Time-point response assessment	The growth kinetics of target and new lesions are determined. Percentage change of tumor growth is then calculated referencing baseline assessment as well as the smallest reported tumor burden (nadir)
Types of overall response	Complete response (CR), partial response (PR), minor response (MR), stable disease (SD), and progressive disease (PD)

(continued)

Table 7.4 (continued)

Summary of immune therapy response assessment in neuro-oncology (iRANO)	
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
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

7.4 Future Directions for Immune Therapy Imaging Assessment

Although irRECIST and irRC represent an improvement over the conventional WHO criteria and RECIST to evaluate tumor response in immunotherapy, there remains limitations and challenges and further refinements are warranted [4]. Plans for improving imaging response criteria include volumetric (3D) imaging, dynamic contrast imaging, and functional (molecular) imaging. More recently, radiomics is a more recent developing field within imaging that can help in more precise tumor assessments that are un-related to tumor size or burden. Further, radiogenomics, the linkage between imaging phenotypes and tumor genomics, might help develop more robust stratification and end-point imaging biomarkers for molecular targeted clinical trials.

7.5 Immune-Related Adverse Events

Immune-related adverse events (irAE) can represent a serious complication and can be challenging for any imager. Thus, it is important to be aware and take into consideration the possibility of its occurrence so that early management is undertaken [18]. Treatment of adverse events is typically based on published guidelines and includes delaying treatment dosing, administering corticosteroids, or terminating

therapy depending on the severity of the event. However, success in outcome lies heavily on correctly identifying and interpreting these complications.

Severe colitis has the highest mortality and worst outcome associated with irAE [18]. Because the possibility of misdiagnosis of autoimmune colitis, the patient can take antibiotic therapy instead of corticosteroid therapy, which can result in a delayed diagnosis and complicated by colonic bowel perforation [18]. Other common immune adverse events are sarcoid-like adenopathy and pancreatitis. It is important to recognize and accurately diagnose these events in order to avoid misdiagnosis as metastatic disease [1]. There are also many other events which can occur with immunotherapy for example autoimmune hepatitis, pneumonitis, thyroiditis, myocarditis, pericarditis, temporal arteritis, conjunctivitis, sarcoid-like reaction such as lymphocytic vasculitis, organizing pneumonia, and fasciitis [19, 20]. Endocrinopathies such as autoimmune hypophysitis and thyroiditis can also be seen. A recent study by our group demonstrated that specific radiomic imaging features were able to predict those patients that will subsequently develop pneumonitis (Fig. 7.2).[21] This study highlights the ability of imaging to identify those patients that might be most susceptible to irAE before the irAE even occurs.

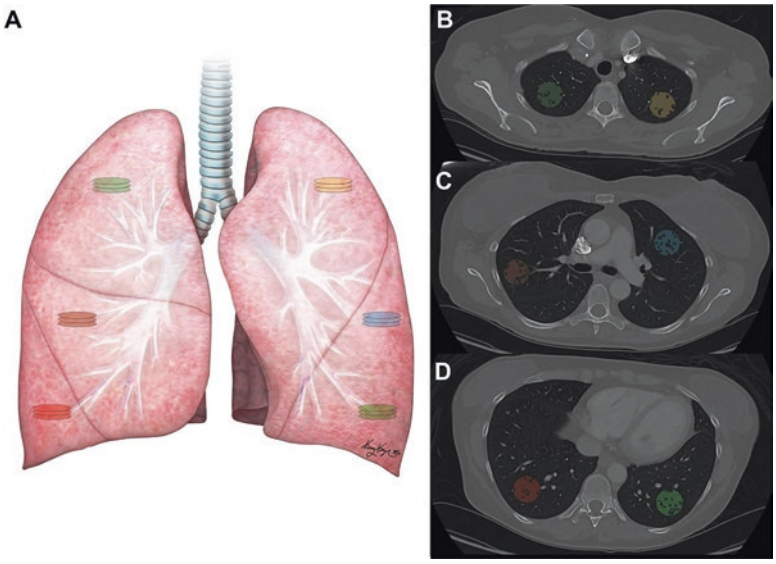

Fig. 7.2 (**a**) An illustration of the outlined ROIs in the lungs. An ROI containing three consecutive slices, taken in each lobe in the right lung and ROIs outlined in the left lung correspond to the same level as the right lung ROIs. Post-contrast lung CT images depicting the segmented ROIs in upper (**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

References

1. Kwak JJ, et al. Cancer immunotherapy: imaging assessment of novel treatment response patterns and immune-related adverse events. Radiographics. 2015;35(2):424–37.
2. Nishino M, et al. Cancer immunotherapy and immune-related response assessment: the role of radiologists in the new arena of cancer treatment. Eur J Radiol. 2015;84(7):1259–68.
3. Okada H, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. Lancet Oncol. 2015;16(15):e534–42.
4. Wolchok JD, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412–20.
5. Tirkes T, et al. Response criteria in oncologic imaging: review of traditional and new criteria. Radiographics. 2013;33(5):1323–41.
6. Miller AB, et al. Reporting results of cancer treatment. Cancer. 1981;47(1):207–14.
7. Organization, W.H. WHO handbook for reporting results of cancer treatment; 1979.
8. Eisenhauer EA, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228–47.
9. Bruix J, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the study of the liver. J Hepatol. 2001;35(3):421–30.
10. Llovet JM, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst. 2008;100(10):698–711.
11. Van den Abbeele AD, Badawi RD. Use of positron emission tomography in oncology and its potential role to assess response to imatinib mesylate therapy in gastrointestinal stromal tumors (GISTs). Eur J Cancer. 2002;38(Suppl 5):S60–5.
12. Vossen JA, Buijs M, Kamel IR. Assessment of tumor response on MR imaging after locoregional therapy. Tech Vasc Interv Radiol. 2006;9(3):125–32.
13. Wahl RL, et al. From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. J Nucl Med 2009;50(Suppl 1):122s–50s.
14. Ribas A, Chmielowski B, Glaspy JA. Do we need a different set of response assessment criteria for tumor immunotherapy? Clin Cancer Res. 2009;15(23):7116–8.
15. Nishino M, et al. Optimizing immune-related tumor response assessment: does reducing the number of lesions impact response assessment in melanoma patients treated with ipilimumab? J Immunother Cancer. 2014;2:17.
16. Aarntzen EH, et al. In vivo imaging of therapy-induced anti-cancer immune responses in humans. Cell Mol Life Sci. 2013;70(13):2237–57.
17. Srishti Abrol GT, Hassan A, Kotrotsou A, Alfaro-Munoz KD, de Groot JF, Zinn P, Colen RR. Radiomics differentiate between true progression and pseudo-progression in GBM patients. In: Annual meeting of the American society of neuroradiology—ASNR, Washington, DC; 2016.
18. Weber JS, et al. Patterns of onset and resolution of immune-related adverse events of special interest with ipilimumab: detailed safety analysis from a phase 3 trial in patients with advanced melanoma. Cancer. 2013;119(9):1675–82.
19. Barjaktarevic IZ, et al. Organizing pneumonia as a side effect of ipilimumab treatment of melanoma. Chest. 2013;143(3):858–61.
20. Bronstein Y, et al. Radiologic manifestations of immune-related adverse events in patients with metastatic melanoma undergoing anti-CTLA-4 antibody therapy. AJR Am J Roentgenol. 2011;197(6):W992–w1000.
21. Takeo Fujii RRC, Bilen AM, Hess KR, Hajjar J, Suarez-Almazor ME, Alshawa A, Hong DS, Stephen B, Janku F, Subbiah V, Kato S, Karp DD, Piha-Paul SA, Tsimberidou AM, Fu S, Sharma P, Meric-Bernstam F, Naing A. Incidence of immune-related adverse events in patients who received immunotherapy and radiographic analysis to predict pneumonitis in EORTC-NCI-AACR molecular targets and cancer therapeutics symposium, Munich, Germany; 2016.

Chapter 8
Adverse Events in Cancer Immunotherapy

Noha Abdel-Wahab, Anas Alshawa, and Maria E. Suarez-Almazor

Abstract Cancer immunotherapy has resulted in durable responses in patients with metastatic disease, unseen with traditional chemotherapy. Several therapies have been approved by the Food and Drug Administration for the treatment of various cancers, including: immune checkpoint inhibitors, cytokines - interleukin 2 (IL-2) and interferon alpha (IFN), and the cancer vaccine sipuleucel-T. These therapies upregulate the immune system to enhance antitumor responses. As a consequence, they can cause inflammatory and immune-related adverse events that can affect one or more organs, can be serious, and on occasion life-threatening. The management of these adverse events is complex, and requires a multidisciplinary approach involving not only oncologists, but also other internal medicine specialists, to ensure prompt diagnosis and optimal management of these complications.

Keywords Immune-related adverse events • Immune checkpoint inhibitors • Cytokines • Cancer vaccine • Cancer immunotherapy

N. Abdel-Wahab, M.D., Ph.D.
Section of Rheumatology and Clinical Immunology, Department of General Internal Medicine, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

Rheumatology and Rehabilitation Department, Assiut University Hospitals, Assiut 71526, Egypt

A. Alshawa, M.D.
Department of Investigational Cancer Therapeutics, University of Texas MD Anderson Cancer Center, Houston, TX, USA

M.E. Suarez-Almazor, M.D., Ph.D. (✉)
Section of Rheumatology and Clinical Immunology, Department of General Internal Medicine, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA
e-mail: msalmazor@mdanderson.org

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Immunotherapy has resulted in impressive benefits in the treatment of various cancers. Yet, while the immune upregulation provoked by these therapies results in an enhanced antitumoral response, it can also cause a myriad of inflammatory and autoimmune manifestations that can affect different organs, are often severe, and on occasion, fatal. In order to minimize harms, it is crucial to be aware of, and promptly recognize, the broad spectrum of immune-related adverse events (irAEs) that may occur in patients receiving these therapies, identifying patients at increased risk, and adequately monitoring and treating toxicity. The scope of inflammatory and autoimmune adverse events seen with immunotherapy is quite distinct from what is reported with traditional chemotherapy and targeted agents, and therefore requires careful management [1, 2]. In this chapter, we will describe the spectrum of irAEs related to biologic immunotherapies approved by the Food and Drug Administration (FDA) for the treatment of cancer, including immune checkpoint inhibitors, cytokines—interleukin 2 (IL-2) and interferon alpha (IFN), and the cancer vaccine sipuleucel-T.

8.1 Immune Checkpoint Inhibitors

There are currently four checkpoint inhibitors approved for cancer therapy: ipilimumab, a CTLA-4 inhibitor, nivolumab and pembrolizumab, PD-1 inhibitors, and atezolizumab, a PD-L1 inhibitor. Immune checkpoint inhibition has led to major breakthroughs in the treatment of cancer, but can be hampered by frequent irAEs, which can affect almost any organ or system. Up to 80% of patients receiving these therapies can experience irAEs, which generally occur within the first 3–4 months of therapy, but may be also seen later [3–5]. Constitutional symptoms such as fatigue, and skin manifestations are the most common irAEs and can arise early on. Other adverse events such as endocrinopathies or pneumonitis can occur insidiously. The majority of the reported events are transient and may be self-limited, but long-lasting effects and sequelae have been reported [6]. Most irAEs can occur with any checkpoint inhibitor, but some are more frequent with a particular agent. Overall, ipilimumab has a higher incidence of adverse events than anti-PD-1 agents [5]. Combination of checkpoint inhibitors (e.g., ipilimumab and nivolumab), while more efficacious, is significantly associated with increased toxicity compared to either agent alone [7]. Atezolizumab, an anti-PD-L1 antibody, is the most recently approved inhibitor; its toxicity profile is not well defined yet but is expected to be similar to the observed with anti-PD-1 agents [8].

We describe below the most frequent irAEs reported in clinical trials, as well as uncommon irAEs described in case series and reports.

Dermatitis. Skin toxicity is the earliest and most frequent irAE seen with either ipilimumab [9] or anti-PD-1 agents [10]. Most frequently, patients develop a maculopapular rash, predominantly on the trunk and extremities, often sparing the face, and which may present with Koebner phenomenon [11–14]. The lesions are usually mild to moderate (grade 1 or 2), involving less than 30% of the body. They can occur early on, and while they can worsen with each subsequent dose, they generally remain self-limited, and do not require discontinuation of the immunotherapy.

Pruritus can be seen alone or in association with the rash, shortly after the start of treatment [11, 13]. Vitiligo is common, more frequently reported with nivolumab and pembrolizumab than with ipilimumab [11, 13, 15]. Exacerbation of preexisting psoriasis or de novo disease has been described with all checkpoint inhibitors [16–21]. Lichenoid dermatitis has been reported, and can be associated with severe pruritus and present later, compared to the more common forms of nonspecific dermatitis [22, 23]. Other irAEs infrequently reported, primarily as case reports, include Sweet syndrome, poliosis, alopecia universalis, Grover's disease, bullous pemphigoid, and pyoderma gangrenosum [24]. Stevens–Johnson's syndrome and toxic epidermal necrolysis have rarely been reported, but are among the most severe irAEs. Meticulous evaluation and prompt management of patients developing mucosal ulcerations, blisters, and a positive Nikolsky sign are critical [10].

Skin biopsy is generally unnecessary, but has been recommended in patients with persistent or recurrent severe rash, or if atypical lesions are observed. The histopathology of the nonspecific dermatitis seen with ipilimumab is well described and typically shows superficial perivascular CD4+ T lymphocyte infiltrates that may be associated with epidermal spongiosis and eosinophilic infiltration in the upper dermis. The histopathology is not as clearly described in anti-PD-1 induced dermatitis [25].

Mucosal involvement with dry eyes, dry mouth, and mucositis has been reported primarily with the use of anti-PD-1 agents [26].

Enterocolitis. Diarrhea occurs more frequently in patients receiving ipilimumab compared to those receiving anti-PD-1 therapy, and its incidence is significantly increased when ipilimumab is combined with nivolumab. Many patients develop grade 1 or 2 diarrhea, usually after the third dose of ipilimumab, later than dermatitis. Patients can also present with nausea, vomiting, and abdominal pain, and occasionally rectal bleeding. Severe acute colitis, with abdominal pain and grade 3 or 4 diarrhea, is a medical emergency since it may lead to life-threatening intestinal perforation if the diagnosis is delayed. Rectosigmoidoscopy or colonoscopy, and imaging with computed tomography (CT) scans may be required for persisting grade 3 and 4 diarrhea and/or abdominal pain [11]. Colonoscopic findings include edema, erythema, erosion, ulceration, bleeding, and loss of vascularization [27]. The histopathology typically shows acute or chronic inflammation resembling, but not identical, to that observed in patients with idiopathic inflammatory bowel disease, with inflammatory cell infiltrates, predominantly neutrophils, eosinophils, and CD4 lymphocytes, and most frequently in the descending colon [9]. Screening for *Clostridium Difficile* and cytomegalovirus infections is recommended, especially in patients with colonic ulceration [28].

Endocrinopathies. The spectrum of endocrinopathies reported in patients receiving checkpoint inhibitors is quite broad, including hypophysitis, thyroid disease, and less frequently, primary adrenal insufficiency, hypogonadism, pancreatitis, hypercalcemia, and type 1 diabetes. Hypophysitis is more frequently reported in male patients receiving ipilimumab, and typically occurs 2–4 months after treatment initiation. It can result in hypopituitarism, with anterior pituitary hormonal deficiencies. Its frequency significantly increases with combination therapy. Patients usually present with nonspecific symptoms such as headache, fatigue, nausea, and vomiting. They can also

complain of anorexia, insomnia, changes in mental status, temperature intolerance, erectile dysfunction, and decreased libido. Diplopia due to pituitary enlargement compressing the optic chiasm is rare [29–34]. The histopathology shows lymphocytic infiltration, resembling idiopathic autoimmune hypophysitis. Hypophysitis should be suspected in the presence of related persistent symptoms, and can be confirmed by laboratory evidence of hypopituitarism, and magnetic resonance imaging (MRI) demonstrating pituitary enlargement and/or thickening of the stalk. The most common hormonal deficiency seen with hypophysitis is central hypothyroidism, with reduced levels of thyroid stimulating hormone (TSH). Patients can also develop hypogonadotropic hypogonadism, and central adrenal insufficiency. Hypophysitis can resolve, and thyroid and gonadal functions may recover. However, patients can develop long-term sequelae with permanent hormonal deficiencies, especially adrenal insufficiency, which may require lifetime hormonal replacement.

Primary thyroiditis can occur at any time during therapy, with or without associated autoantibodies. It can initially present with transient hyperthyroidism, especially in patients receiving anti-PD-1 agents, followed by severe hypothyroidism (low free T4 and high TSH). Case reports of full Graves' disease have been reported.

Acute adrenal insufficiency secondary to adrenalitis (or to hypophysitis) can be a medical emergency. Patients present with hypotension, dehydration, and hyponatremia in central insufficiency, and with added hyperkalemia in primary insufficiency [35].

The FDA recommends testing for thyroid function before treatment initiation, before each treatment cycle, and as recommended by the treating physician based on the patients clinical symptoms. Baseline screening for other endocrine problems is not recommended at this time [36].

Autoimmune hepatitis. Hepatotoxicity is occasionally seen with any of the checkpoint inhibitors. Its prevalence increases in patients receiving combination therapy, with grade 3/4 toxicity. Elevated aminotransferases are the initial signs of liver inflammation, preceding symptoms. Hyperbilirubinemia, jaundice, and fatigue may develop as the hepatitis progresses. Autoimmune hepatitis can be severe and life-threatening, so regular monitoring of liver function tests is generally recommended before each treatment cycle [36]. Differential evaluation for other causes of hepatitis is recommended if liver enzymes remain persistently elevated [37]. Liver biopsy might be appropriate if there is no response to treatment. Histopathology shows lymphocytic infiltrates [36].

Pneumonitis. Pneumonitis can occur with both anti-CTLA-4 and anti-PD-1 agents. Although relatively less frequent than other irAEs, it can result in dire complications and death. Its frequency increases in patients receiving combination therapy [11, 12]. Concomitant pulmonary comorbidities are a major risk factor for pneumonitis. It has therefore been reported more frequently in patients with lung cancer receiving nivolumab, conceivably not because of differences among the agents, but primarily because patients with lung cancer (for which nivolumab is approved) often have coexistent lung disease. Symptoms are usually nonspecific including dry persistent cough, dyspnea, or tachypnea. Physical examination may reveal fine crackles. Patients may deteriorate rapidly, and therefore, close observation and careful follow-up is recommended even for mild grade 1 symptoms. Differential diagnoses need to be carefully

evaluated, including cancer progression, lymphangitis carcinomatosa, infections (e.g., bacterial, cytomegalovirus, or pneumocystis pneumonia), or exacerbation of preexisting chronic obstructive pulmonary disease [38]. Patients with suspected pneumonitis should undergo a CT scan, pulmonary function tests, and measurement of carbon monoxide diffusion capacity. Bronchoscopy and biopsy may be indicated to exclude other etiologies. Imaging usually shows reticular infiltration with ground-glass lesions and/or also consolidation. Occasionally, other patterns such as cryptogenic organizing pneumonia or multiple nodules have been reported [39, 40].

Arthritis. Data from selected systematic reviews and original trials reported arthralgia and arthritis in patients receiving checkpoint inhibitors, primarily with anti-PD-1 agents [41], and in patients treated with the combination of ipilimumab and nivolumab [11]. Rheumatoid-like polyarthritis and reactive arthritis were recently reported in a series of patients who received ipilimumab and/or nivolumab [42]. A few of these patients had positive antinuclear antibodies (ANA), but none had positive rheumatoid factor or anti-cyclic citrullinated antibodies. Additionally, persistent polyarthritis was reported in few cases several months after discontinuation of the checkpoint inhibitor.

Sicca syndrome. Severe dry mouth with evidence of salivary glands hypofunction, with or without parotid gland enlargement, and positive ANA and/or low titer of antibodies against La/SSB were recently reported in a case series of patients with cancer receiving ipilimumab, nivolumab, or a combination of both agents [42].

Other rarely reported irAEs. Many other inflammatory and autoimmune events have been reported as cases or in small series. Our recent systematic review of case reports of irAEs associated with the use of checkpoint inhibitors in patients with cancer identified various syndromes affecting different organs and systems including sarcoidosis (pulmonary, cutaneous, muscular, and neurological), celiac disease, polymyalgia rheumatica/giant cell arteritis, lupus-like nephritis, interstitial nephritis, acute tubular necrosis, inflammatory myopathies, neurologic disorders (myasthenia, transverse myelitis), uveitis, episcleritis, pericarditis, Takotsubo-like cardiomyopathy, Vogt–Koyanagi–Harada-like syndrome, and various other rare diseases [24].

8.1.1 Pathophysiology of irAEs

The exact pathways mediating irAEs in checkpoint inhibition are not completely understood. Checkpoint blockade targets the regulatory molecules that inhibit T cell activation to enhance antitumor immune response, with aberrant T cell activation and loss of self-tolerance with off-target inflammation and autoimmunity [43]. There is evidence of activated T cells with augmented production of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), and interleukins (IL-6 and IL-17) at the site of irAEs [44, 45]. Murine and human studies of checkpoint inhibition show impaired function and survival of regulatory T cells (Tregs) [46], and altered T cell–B cell interactions with pathogenic antibody production associated to the development of irAEs [47–52].

Both CTLA-4 and PD-1 inhibition share clear similarities in the immune system upregulation leading to abnormal Treg function and humoral immunity. However, the respective differences in the frequency and phenotypes of irAEs between both classes of agents are not clearly understood. Furthermore, many patients do not develop irAEs despite continuous use of a particular agent, and some who present with an adverse event with anti-CTLA4 therapy do not develop repeated toxicity with PD-1 inhibition [51]. Therefore, host factors especially genetic predisposition to autoimmunity, or combined host/environmental factors such as the microbiota, may play a substantial role in the development of irAEs.

Several genetic markers have been associated with autoimmunity in general. Mutations in CTLA-4 and PD-1 genes associated with specific single nucleotide polymorphisms (SNPs) have been linked to various autoimmune and inflammatory diseases including thyroiditis, neurologic disorders, type 1 diabetes, inflammatory bowel disease, rheumatoid arthritis (RA), ankylosing spondylitis, and systemic lupus erythematosus [53–60]. The functional consequences of the identified SNPs in the patients with cancer receiving checkpoint inhibitor therapies are not fully explicit, yet they could play a part in the development of irAEs and may exacerbate underlying autoimmunity.

Conceivably, an individual's microbiota could also contribute to the development of specific irAEs. Dubin et al. examined the intestinal microbiota of patients receiving ipilimumab and observed a lower odds of immune-related colitis in patients with increased *Bacteroidetes phylum* [61].

8.1.2 Management of irAEs

A multidisciplinary patient-centered approach with risk evaluation of each patient before starting immune checkpoint blockade inhibitors is recommended for prompt recognition and monitoring of irAEs. In general, preventing the deleterious events and sequelae without impairing the beneficial effect of the checkpoint inhibitor therapies remains the crucial goal in management of irAEs [4, 36, 62]. The Risk Evaluation and Mitigation Strategy (REMS) developed by the FDA for managing ipilimumab-associated toxicity, based on the common terminology criteria for adverse events (CTCAE) grading (U.S. Food and Drug Administration [63]), is commonly used in clinical practice for treatment of irAEs in checkpoint blockade. Most patients are initially treated with supportive care and corticosteroids, with the initial steroid dose determined according to the severity of the irAE. No clear evidence is available regarding whether high-dose corticosteroids regimens or more targeted therapies as TNF inhibitors are appropriate early on, but they are recommended for severe manifestations. For severe irAEs, discontinuation of the checkpoint inhibitor therapy is recommended, along with oral prednisone at 1 mg/kg/day, and possible hospitalization. For life-threatening events, hospitalization and intravenous methylprednisolone at 2 mg/kg/day are recommended. Treatment with infliximab at 5 mg/kg has been considered, primarily for colitis, and could be repeated in 2 weeks if needed. Treatment rechallenge is not usually recommended in severe

cases, although a different class of checkpoint inhibitor can occasionally be considered, especially if there are no other therapies for the cancer.

Specific algorithms for the most frequently reported irAEs are presented below.

Dermatitis. Grade 1 and 2 dermatitis can be managed with topical glucocorticoids and oral antihistaminics. High potency topical steroids should generally be avoided, primarily on the face, axilla, and groin. Oral corticosteroids should be used for grade 3 and 4 dermatitis.

Enterocolitis. Electrolyte replacement, intravenous hydration, and balanced diet are recommended as needed. For grade 1 and 2 colitis, anti-motility agents are started, followed by oral prednisone if symptoms persist. Intravenous methylprednisolone should be considered immediately for grade 3 and 4 toxicity. Early use of infliximab is also recommended and has occasionally been considered as first-line therapy, because it is significantly associated with better clinical outcomes including earlier resolution of symptoms and shorter duration of steroid treatment. When intestinal perforation is suspected, immediate surgical intervention is required.

Endocrinopathies. Physiologic hormonal replacement is appropriate for most cases with endocrinopathies. Cortisol and thyroid hormone replacement should be started immediately; androgen replacement can be considered at a later stage. For patients with persistent inflammatory symptoms or visual changes, high-dose steroid therapy might be needed. Patients with suspected adrenal crisis should be hospitalized immediately, and need prompt intravenous fluids and corticosteroid therapy.

Autoimmune hepatitis. In persistent grade 2 and grade 3 toxicity, oral steroids are recommended. For refractory cases, there are reports of successful use of mycophenolate mofetil, tacrolimus, and antithymocyte globulin.

Pneumonitis. Corticosteroids remain the cornerstone for treatment. Infliximab, mycophenolate mofetil, and cyclophosphamide have also been recommended in severe cases.

Arthritis. No clear guidelines are currently available. An initial dose of 20–30 mg of oral prednisone with gradual tapering might be adequate. In a case series of arthritis after ipilimumab or nivolumab therapy, a few patients required higher doses of prednisone up to 120 mg [42]. Disease modifying antirheumatic drugs, including biologic therapy, may be required if there is no response to oral steroids, or, if these cannot be tapered successfully.

8.1.3 Challenges in Patients with Cancer and Preexisting Autoimmune Disease

Patients with preexisting autoimmune diseases were not included in the original trials evaluating checkpoint inhibitor therapies because of the risk of exacerbation of the underlying autoimmune disease. Thus, much of the available information is derived mainly from case series and sporadic reports of cases with cancer and preexisting

autoimmune disease who received the checkpoint inhibitor in clinical practice. The majority of the reported cases included patients with melanoma and various concomitant autoimmune diseases treated with ipilimumab. Only few cases were treated with either nivolumab or pembrolizumab [16, 18, 64–81]. Exacerbation of the autoimmune disease and/or de novo irAEs were reported in some patients with two treatment-related deaths. However, many patients were easily managed with corticosteroids, with a few requiring more aggressive immunosuppressant therapy. Half of the patients did not require discontinuation of checkpoint inhibitor therapy. Notably, about a third of the patients did not develop any irAEs.

8.2 Recombinant Interleukin-2 Therapy

Human recombinant IL-2 (aldesleukin) has been approved by the FDA for the treatment of metastatic renal cell carcinoma and melanoma. IL-2 has been proved to be efficacious for a small proportion of patients with these tumors, but its use may decrease with the availability of checkpoint inhibitors, which seem more efficacious. Its biological activity is mediated through activation of cellular immunity, including lymphocyte cytotoxicity, and killer cell activity, and the induction of cytokine production including TNF, IL-1, and gamma interferon. Toxicity with IL-2 agents is dose-dependent and can limit its use [82]. Lower dose regimens have not been as effective as higher dose ones, and therefore, toxicity remains a limiting factor. The indirect role of IL-2 in the production of other cytokines (e.g., IFN-gamma, TNF-alpha) seems to be involved in many toxicities [83].

The most common reported high-grade toxicities have been hypotension, pyrexia, nausea or vomiting, diarrhea, and cardiac toxicity [82].

Constitutional symptoms. The most frequent adverse events for IL-2 therapy are fever and malaise, which can be severe, and require discontinuation of therapy [84].

Cardiovascular. Cardiovascular complications are the most concerning toxicities with the use of high-dose IL-2 regimens. These toxicities include vascular leak syndrome, hypotension, angina, myocardial infarction, myocarditis, hypocontractility, and arrhythmia [83]. Vascular leak syndrome is a common adverse event of high-dose IL-2 therapy which presents with peripheral edema, weight gain, ascites, and/or pleural effusion [83]. This syndrome is a result of multiple factors that involve cytokine release leading to increased capillary permeability with decreased vascular resistance, causing extravascular fluid shift [85]. Moreover, the hypocontractility occurring with IL-2 therapy can aggravate the vascular leak syndrome [83].

Myocardial toxicity can occur secondary to IL-2 related release of IL-1 and TNF-alpha [83]. Preexisting coronary artery disease might increase the risk of IL-2 induced myocardial infarction [86].

Patients receiving high-dose IL-2 therapy can develop hypotension as a result of cardiac hypocontractility, and vascular leak syndrome among other factors.

Pulmonary edema can result from multifactorial causes including myocardial dysfunction and vascular leak syndrome and is more severe in patients with reduced pulmonary reserve [83].

Gastrointestinal. The most frequently reported adverse events are nausea, vomiting, anorexia, diarrhea, and stomatitis [83]. While anorexia might be caused by the increased release of TNF-alpha [86], the diarrhea is multifactorial in etiology and may be caused bowel edema from capillary leak syndrome, or other cytokine effects [87]. Patients on high-dose IL-2 are predisposed to peptic ulcer disease because of increased gastric acid secretion due to the stress and because of the use of nonsteroidal anti-inflammatory drugs (NSAIDS) to control fever [83].

Intrahepatic cholestasis is common. However, its pathogenesis is still unknown [88].

Renal. Renal toxicity can result from high-dose IL-2 therapy, manifesting as oliguria and elevated serum creatinine and blood urea nitrogen. It can be either due to prerenal hypotension [85] or intrinsic renal injury [89]. This toxicity is usually reversible [87]. Elevated creatinine level (above 3 mg/dL) warrants stopping any NSAIDS or nephrotoxic antibiotics, and discontinuing IL-2 therapy [84, 87].

Neurologic. Neurologic toxicity includes confusion, somnolence, disorientation, anxiety, and dizziness [84]. These symptoms usually have late onset, and worsen immediately after stopping the drug, disappearing later within hours or days [85]. Neurotoxicity might also occur as a side effect of concomitant medications [84]. Peripheral neural toxicity including carpal tunnel syndrome [90], hyperesthesia, and paresthesia [91] have been reported.

Hematologic. Hematologic toxicity is generally mild, reversible, and not dose-limiting [87]. Thrombocytopenia can occur, possibly due to IL-2 induced splenomegaly and sequestration [87, 92]. Leukopenia and lymphopenia possibly due to lymphocyte redistribution and sequestration have also been reported [84, 87] and may involve IL-2 induced impaired chemotaxis [93]. Other hematological abnormalities may include eosinophilia (along with pruritus and skin rash) [92], coagulopathy (may be a result of decreased coagulation factors production in the liver) [94], and mild anemia that rarely requires transfusion [87].

Skin. Cutaneous adverse events include rash, dry skin, pruritus, hair thinning or loss, and vitiligo [85, 87]. For the rash, lotions or emollient, that does not contain alcohol or steroid, can be used [87]. Meanwhile, pruritus can be managed by antihistamines, gabapentin, or opioids [87, 95].

Endocrinopathies. The use of IL-2 therapy may be complicated by hypo- or hyperthyroidism, sometimes requiring pharmacological management [96].

Infections. IL-2 therapy can be associated with infectious complications that occur mostly at the site of venous catheter placement or in the urinary tract, conceivably because of impairment of neutrophil chemotaxis [84, 93]. Prophylactic antibiotics can be used in patients with central venous catheters who are receiving IL-2.

8.2.1 Management of Adverse Events

Despite its toxicity profile, therapy with IL-2 can be tolerated [82], even in elderly patients [97]. Screening before therapy initiation and guidelines for treatment of toxicity established by specialized centers have improved the management of patients receiving IL-2 therapy [82], which is now even administered in community hospital settings [98].

The fever can be minimized by giving the patients antipyretics (including acetaminophen and NSAIDS) before or during the course of treatment [83, 84]. While steroids can help in managing fever, they are generally not recommended because they can block the therapeutic effect of IL-2 [99].

In order to prevent cardiac complications, patients should be screened for any underlying cardiac disease prior to initiating IL-2 treatment, usually through EKG exercise treadmill stress testing or echocardiography [87]. If the screening is abnormal, a cardiac consultation should be requested for determining eligibility for treatment [87].

Hypotension necessitates dose reductions and therapy in most patients [83, 84]. It is recommended to stop antihypertensive medication 24 h before starting therapy [84]. Regular checking of blood pressure should be performed for patients receiving IL-2 therapy [87]. The goal of monitoring is to establish a blood pressure baseline and try to maintain it throughout the treatment. A systolic pressure of at least 80–90 mmHg is an appropriate goal if there are no cardiac risk factors [87]. If hypotension is detected, with signs of hemodynamic instability, therapy should be discontinued and adequate fluid resuscitation and vasopressor therapy should be implemented [84]. To minimize the risk of pulmonary toxicity, it is advisable to select patients with forced expiratory volume 1 (FEV1) >75% of predicted [87]. It is recommended to regularly check cardiac indices prior to the administration of each dose of IL-2 [84].

8.3 Recombinant Interferon Alpha-2b

Recombinant interferon alpha-2b (IFN-alpha-2b) therapy has been approved for the treatment of hairy cell leukemia, Kaposi sarcoma, non-Hodgkin lymphoma, and melanoma. Its adverse events profile can affect almost any organ systems, often requiring dose adjustments or even cessation of therapy [100]. The incidence and severity of INF-Alpha-2b toxicities are related to the dose and duration of treatment [101]. These adverse events can be reversible [101, 102].

Because of its frequent toxicity, a careful assessment of benefits and harms needs to be considered when recommending IFN-alpha-2b therapy, and patients receiving this treatment need to be carefully monitored [101, 103].

Constitutional symptoms. Flu-like symptoms including fatigue, fever, chills, myalgia, arthralgia, headache, and nausea are common [101, 102], and can last for several hours [100]. Acute toxicity is more frequent with intravenous compared to subcutaneous administration [101], and can be reduced by gradual dose increase

over several days, to reach higher, more effective dosages [102]. These adverse events can subside gradually and eventually disappear [102]. Some symptoms, especially fatigue, can persist and become chronic [101].

The mechanism causing fever with IFN-alpha-2b therapy is not well recognized. It might be due to an intrinsic pyrogenic effect (increasing prostaglandins in the hypothalamus), rather than being mediated by leukocytic pyrogens [104, 105], but clearly involves the release of cytokines and prostaglandins that affect the central nervous system [100]. It is important to also consider infection if there is any change in the pattern of the fever [102].

Dermatologic. Skin manifestations are not as common, but may include dermatitis with rash and alopecia [106].

Cardiovascular. Acute side effects may include cardiovascular symptoms caused by an acute febrile reaction, manifesting as tachycardia, vasoconstriction, distal cyanosis, diaphoresis, and hypotension. Coronary vasospasm may also provoke acute angina-like symptoms [107]. Congestive heart failure might develop in those with preexisting heart disease [102]. Long-term treatment may cause dilated cardiomyopathy, arrhythmia, and ischemic heart disease [108]. These effects might be due to mitochondrial dysfunction or inflammatory infiltrates inducing electrical conduction abnormalities [109].

Neurologic. Neurologic side effects are rare and include seizures and EEG abnormalities that resemble those seen in diffuse encephalitis [100–102]. These toxicities are thought to be autoimmune in origin, and usually require cessation of therapy and, possibly, administration of steroids [100].

More rarely, retinopathy or optic neuropathy may occur, especially in patients with preexisting diabetes or hypertension. Ocular toxicity is usually reversible after discontinuation of treatment. However, some cases are permanent [110].

Depression is an important psychiatric effect thought to be secondary to changes in neurotransmitters in the brain, but is sometimes considered part of the "sickness behavior" that may accompany the somatic side effects [100]. Other reported psychiatric side effects include cognitive impairment, such as disturbed memory and attention, and delirium [101].

Hematologic. Hematological side effects include neutropenia, granulocytopenia and rarely, anemia and thrombocytopenia [100–102]. They may be caused by cellular growth inhibition, redistribution of white blood cells, and changes in leukocyte adhesion molecules [100]. Rarely, when severe, these toxicities may indicate cessation of therapy [100].

Renal. IFN-alpha-2b is occasionally associated with renal toxicity ranging from asymptomatic proteinuria [102] to severe renal failure in some patients [111].
Endocrine. Endocrine effects include thyroid dysfunction, diabetes mellitus, sex hormone irregularity, or hypopituitarism.

Gastrointestinal. Gastrointestinal symptoms can include anorexia, nausea, vomiting or diarrhea [100, 102]. Abnormalities in liver function tests (mild to moderate elevation of AST and ALT) have also been described [100–102].

Respiratory. Pulmonary side effects have occasionally been reported including pleural effusion or pneumonitis.

8.3.1 Pathophysiology

Direct cytokine effects probably cause many of the constitutional symptoms seen with IFN-alpha-2b. Autoimmunity is suspected to be responsible for other irAEs such as thyroid disease [100]. Conceivably, autoimmunity could be increased in patients with latent autoimmune diseases. Additionally, IFN-alpha-2b may induce an increase in the expression of HLA antigens, and other antigens linked to autoimmune diseases [100].

Autoimmune-related toxicity is considered a prognostic marker for antitumor activity [112]. Furthermore, the presence of IFN-induced thyroid autoantibodies can have a beneficial prognostic value for patients with renal cell carcinoma [113].

8.3.2 Management of Adverse Events

Management of IFN toxicity is primarily based on clinical experience [100]. Recommendations have been published [110]. Reducing the dose of INF-Alpha-2b is generally recommended, especially in patients with hematologic or liver toxicity [101].

Acute constitutional symptoms can be managed with dose decreases and/or administering acetaminophen or NSAIDS before initiating therapy [100]. Bedtime administration and adequate hydration can also reduce constitutional symptoms [110]. As fatigue may impair performance status, it is recommended to assess patient performance both before starting the treatment and regularly during the course of therapy [110]. Other possible causes of fatigue (e.g., anemia, depression, or hypothyroidism) need to be considered as well [114]. Pharmacologic treatment includes methylphenidate, megestrol acetate, or antidepressants [101].

Cardiovascular. Cardiac toxicity often requires therapy discontinuation and pharmaceutical management [115].

Hematologic. Monitoring of hematologic indices is recommended. Neutropenia tends to be mild and rarely requires therapy discontinuation [110].

Neurologic. Adequate monitoring for any emerging neuropsychiatric symptoms is crucial to ensure early detection and treatment [110]. Depression may be managed with antidepressants [110]. Baseline psychiatric evaluations and use of prophylactic antidepressants have been proposed to decrease psychiatric complications [110]. While baseline eye exam is indicated for all patients, a regular eye exam is indicated only for those with preexisting retinopathy [116]. New or worsening retinal abnormalities necessitate drug discontinuation [110].

Gastrointestinal. Liver function should be regularly monitored due to the risk of fatal hepatic failure [110]. It is also important to avoid using other hepatotoxic agents and limit alcohol consumption [110]. Hepatotoxicity is usually managed by dose adjustment.

Endocrine. Before initiating IFN-alpha therapy, patients should undergo screening tests for thyroid dysfunction [110]. IFN-induced thyroid dysfunction may be reversible upon drug discontinuation, and if it is not, it is usually manageable with hormonal replacement (hypothyroidism) or beta blockers or corticosteroids for hyperthyroidism [110].

8.4 Vaccines: Sipuleucel-T

Sipuleucel-T is the only currently approved vaccine for the treatment of cancer. Unlike other immunotherapies, this vaccine does not appear to carry a risk for autoimmunity due to the absence of cross-reactivity of prostatic acid phosphatase antigen, targeted by the vaccine, and normal tissues, and the lack of expression of this antigen in organs other than the prostate [117–119].

In a 2012 meta-analysis [120], the pooled relative risk for all adverse events for Sipuleucel-T compared to placebo was not statistically significant (RR = 1.03; 95% CI: 1.00–1.05; *p*-value = 0.06), with similar results for grade 3 to 5 adverse events alone (RR = 0.98; 95% CI: 0.79–1.22; *p*-value = 0.86), and cerebrovascular events alone (RR = 1.93; 95% CI: 0.73–5.09; *p*-value = 0.18). The analysis concluded that this vaccine is safe. The safety of Sipuleucel-T is not affected by the age of patients [121]. Non-Caucasian patients were not adequately represented in the trials, and the safety of Sipuleucel-T cannot be generalized across all races [121, 122]. Drugs interactions with Sipuleucel-T have not been studied, neither its carcinogenicity or mutagenicity [121]. Concomitant administration of the vaccine with checkpoint inhibitors does not result in additional toxicity [123].

Sipuleucel-T is generally very well tolerated with most of the adverse events happening during or shortly after the infusion, and resolving within 48 h [124].

Acute infusion reactions. Infusion reactions occur in many patients receiving Sipuleucel-T vaccines, and include fever, chills, dyspnea, nausea, vomiting, headache, flu-like symptoms, myalgia, fatigue, hypertension and/or tachycardia. Most occur within the first day of infusion, are mild to moderate in intensity, and resolve within 24–48 h after drug administration [124]. Grade 3 or greater infusion adverse events have been reported in about 7% of patients within the first day of vaccine administration [124].

Neurologic. There has been a debate about the possible role of Sipuleucel-T in causing adverse cerebrovascular events. Several studies have reported an increased incidence of cerebrovascular events in the vaccine group compared to the placebo group [123–125], suggesting a possible increased risk [123]. However, the differences were not statistically significant [120, 124], and an adequate temporal relationship was not clearly established [125].

Other adverse events. Infrequent adverse events include hypoxia, cyanosis, hypoxemia, wheezing, bronchospasm, and cardiac arrhythmias.

8.4.1 Management of Adverse Events

Because the mild acute infusion reactions are the most common adverse events, prophylactic treatment with acetaminophen and diphenhydramine and infusion rate adjustments are recommended [121, 122, 125]. More severe infusion reactions can be managed with meperidine hydrochloride [125]. It is important to monitor patients with preexisting cardiac or pulmonary disease during and after the infusion.

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References

1. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348(6230): 56–61.
2. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol. 2012;30(21):2691–7.
3. Chen WW, Razak ARA, Bedard PL, Siu LL, Hansen AR. A systematic review of immune-related adverse event (irAE) reporting in clinical trials of immune checkpoint inhibitors (ICIs). J Clin Oncol. 2016;34(15).
4. Gao J, He Q, Subudhi S, Aparicio A, Zurita-Saavedra A, Lee DH, Jimenez C, Suarez-Almazor M, Sharma P. Review of immune-related adverse events in prostate cancer patients treated with ipilimumab: MD Anderson experience. Oncogene 2015;34(43):5411–7.
5. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, Berdelou A, Varga A, Bahleda R, Hollebecque A, Massard C, Fuerea A, Ribrag V, Gazzah A, Armand JP, Amellal N, Angevin E, Noel N, Boutros C, Mateus C, Robert C, Soria JC, Marabelle A, Lambotte O. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016;54:139–48.
6. Johnson DB, Friedman DL, Berry EG, Decker I, Ye F, Zhao S, Morgans AK, Puzanov I, Sosman JA, Lovly CM. Survivorship in immune therapy: assessing chronic immune toxicities, health outcomes, and functional status among long-term ipilimumab survivors at a single referral center. Cancer Immunol Res. 2015;3(5):464–9.
7. Hammers HJ, Plimack ER, Sternberg C, McDermott DF, Larkin JMG, Ravaud A, Rini BI, Sharma P, Bhagavatheeswaran P, Gagnier P, Motzer R. CheckMate 214: a phase III, randomized, open-label study of nivolumab combined with ipilimumab versus sunitinib monotherapy in patients with previously untreated metastatic renal cell carcinoma. J Clin Oncol. 2015;33(15 Suppl 1).
8. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y, Srinivas S, Retz MM, Grivas P, Joseph RW, Galsky MD, Fleming MT, Petrylak DP, Perez-Gracia JL, Burris HA, Castellano D, Canil C, Bellmunt J, Bajorin D, Nickles D, Bourgon R, Frampton GM, Cui N, Mariathasan S,

Abidoye O, Fine GD, Dreicer R. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909–20.
9. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. BMC Med 2015;13(1):211.
10. Sibaud V, Meyer N, Lamant L, Vigarios E, Mazieres J, Delord JP. Dermatologic complications of anti-PD-1/PD-L1 immune checkpoint antibodies. Curr Opin Oncol. 2016;28(4):254–63.
11. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(1):23–34.
12. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD, Hodi FS. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372(21):2006–17.
13. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A, K.-. investigators. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521–32.
14. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, Korman AJ, Wigginton JM, Gupta A, Sznol M. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122–33.
15. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbe C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, Ascierto PA. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–30.
16. Johnson DB, Sullivan RJ, Ott PA, Carlino MS, Khushalani NI, Ye F, Guminski A, Puzanov I, Lawrence DP, Buchbinder EI, Mudigonda T, Spencer K, Bender C, Lee J, Kaufman HL, Menzies AM, Hassel JC, Mehnert JM, Sosman JA, Long GV, Clark JI. Ipilimumab therapy in patients with advanced melanoma and preexisting autoimmune disorders. JAMA Oncol. 2015;2(2):1–7.
17. Kato Y, Otsuka A, Miyachi Y, Kabashima K. Exacerbation of psoriasis vulgaris during nivolumab for oral mucosal melanoma. J Eur Acad Dermatol Venereol. 2015;30(10):e89–91.
18. Matsumura N, Ohtsuka M, Kikuchi N, Yamamoto T. Exacerbation of psoriasis during nivolumab therapy for metastatic melanoma. Acta Derm Venereol. 2016;96(2):259–60.
19. Ohtsuka M, Miura T, Mori T, Ishikawa M, Yamamoto T. Occurrence of psoriasiform eruption during nivolumab therapy for primary oral mucosal melanoma. JAMA Dermatol. 2015;151(7):797–9.
20. Sanlorenzo M, Vujic I, Daud A, Algazi A, Gubens M, Luna SA, Lin K, Quaglino P, Rappersberger K, Ortiz-Urda S. Pembrolizumab cutaneous adverse events and their association with disease progression. JAMA Dermatol. 2015;151(11):1206–12.
21. Totonchy MB, Ezaldein HH, Ko CJ, Choi JN. Inverse psoriasiform eruption during pembrolizumab therapy for metastatic melanoma. JAMA Dermatol. 2016;152(5):590–2.
22. Joseph RW, Cappel M, Goedjen B, Gordon M, Kirsch B, Gilstrap C, Bagaria S, Jambusaria-Pahlajani A. Lichenoid dermatitis in three patients with metastatic melanoma treated with anti-PD-1 therapy. Cancer Immunol Res. 2015;3(1):18–22.
23. Schaberg KB, Novoa RA, Wakelee HA, Kim J, Cheung C, Srinivas S, Kwong BY. Immunohistochemical analysis of lichenoid reactions in patients treated with anti-PD-L1 and anti-PD-1 therapy. J Cutan Pathol. 2016;43(4):339–46.
24. Abdel-Wahab N, Shah M, Suarez-Almazor ME. Adverse events associated with immune checkpoint blockade in patients with cancer: a systematic review of case reports. PLoS One. 2016;11(7):e0160221.

25. Minkis K, Garden BC, Wu S, Pulitzer MP, Lacouture ME. The risk of rash associated with ipilimumab in patients with cancer: a systematic review of the literature and meta-analysis. J Am Acad Dermatol. 2013;69(3):e121–8.
26. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, Hodi FS, Schachter J, Pavlick AC, Lewis KD, Cranmer LD, Blank CU, O'Day SJ, Ascierto PA, Salama AK, Margolin KA, Loquai C, Eigentler TK, Gangadhar TC, Carlino MS, Agarwala SS, Moschos SJ, Sosman JA, Goldinger SM, Shapira-Frommer R, Gonzalez R, Kirkwood JM, Wolchok JD, Eggermont A, Li XN, Zhou W, Zernhelt AM, Lis J, Ebbinghaus S, Kang SP, Daud A. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908–18.
27. Gupta A, De Felice KM, Loftus EV, Khanna S. Systematic review: colitis associated with anti-CTLA-4 therapy. Aliment Pharmacol Ther. 2015;42(4):406–17.
28. Pernot S, Ramtohul T, Taieb J. Checkpoint inhibitors and gastrointestinal immune-related adverse events. Curr Opin Oncol. 2016;28(4):284–9.
29. Blansfield JA, Beck KE, Tran K, Yang JC, Hughes MS, Kammula US, Royal RE, Topalian SL, Haworth LR, Levy C, Rosenberg SA, Sherry RM. Cytotoxic T-lymphocyte-associated antigen-4 blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother. 2005;28(6):593–8.
30. Corsello SM, Barnabei A, Marchetti P, De Vecchis L, Salvatori R, Torino F. Endocrine side effects induced by immune checkpoint inhibitors. J Clin Endocrinol Metab. 2013;98(4):1361–75.
31. Di Giacomo AM, Biagioli M, Maio M. The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications. Semin Oncol. 2010;37(5):499–507.
32. Dillard T, Yedinak CG, Alumkal J, Fleseriu M. Anti-CTLA-4 antibody therapy associated autoimmune hypophysitis: serious immune related adverse events across a spectrum of cancer subtypes. Pituitary. 2010;13(1):29–38.
33. Faje A. Immunotherapy and hypophysitis: clinical presentation, treatment, and biologic insights. Pituitary. 2015;19(1):82–92.
34. Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, Suri KB, Levy C, Allen T, Mavroukakis S, Lowy I, White DE, Rosenberg SA. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother. 2007;30(8):825–30.
35. Torino F, Corsello SM, Salvatori R. Endocrinological side-effects of immune checkpoint inhibitors. Curr Opin Oncol. 2016;28(4):278–87.
36. Dadu R, Zobniw C, Diab A. Managing adverse events with immune checkpoint agents. Cancer J. 2016;22(2):121–9.
37. Kim KW, Ramaiya NH, Krajewski KM, Jagannathan JP, Tirumani SH, Srivastava A, Ibrahim N. Ipilimumab associated hepatitis: imaging and clinicopathologic findings. Invest New Drugs. 2013;31(4):1071–7.
38. Tabchi S, Messier C, Blais N. Immune-mediated respiratory adverse events of checkpoint inhibitors. Curr Opin Oncol. 2016;28(4):269–77.
39. Berthod G, Lazor R, Letovanec I, Romano E, Noirez L, Mazza Stalder J, Speiser DE, Peters S, Michielin O. Pulmonary sarcoid-like granulomatosis induced by ipilimumab. J Clin Oncol. 2012;30(17):e156–9.
40. Tirumani SH, Ramaiya NH, Keraliya A, Bailey N, Ott PA, Hodi FS, Nishino M. Radiographic profiling of immune-related adverse events in advanced melanoma patients treated with ipilimumab. Cancer Immunol Res. 2015;3(10):1185–92.
41. Boutros C, Tarhini A, Routier E, Lambotte O, Ladurie FL, Carbonnel F, Izzeddine H, Marabelle A, Champiat S, Berdelou A, Lanoy E, Texier M, Libenciuc C, Eggermont AM, Soria JC, Mateus C, Robert C. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. Nat Rev Clin Oncol. 2016;13(8):473–86.
42. Cappelli LC, Gutierrez AK, Baer AN, Albayda J, Manno RL, Haque U, Lipson EJ, Bleich KB, Shah AA, Naidoo J, Brahmer JR, Le D, Bingham 3rd CO. Inflammatory arthritis and sicca syndrome induced by nivolumab and ipilimumab. Ann Rheum Dis. 2016;76(1):43–50.
43. Calabrese L, Velcheti V. Checkpoint immunotherapy: good for cancer therapy, bad for rheumatic diseases. Ann Rheum Dis. 2017;76(1):1–3.

44. Dulos J, Carven GJ, van Boxtel SJ, Evers S, Driessen-Engels LJ, Hobo W, Gorecka MA, de Haan AF, Mulders P, Punt CJ, Jacobs JF, Schalken JA, Oosterwijk E, van Eenennaam H, Boots AM. PD-1 blockade augments Th1 and Th17 and suppresses Th2 responses in peripheral blood from patients with prostate and advanced melanoma cancer. J Immunother. 2012;35(2):169–78.
45. von Euw E, Chodon T, Attar N, Jalil J, Koya RC, Comin-Anduix B, Ribas A. CTLA4 blockade increases Th17 cells in patients with metastatic melanoma. J Transl Med. 2009;7:35.
46. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev. 2014;13(6):668–77.
47. Berman D, Parker SM, Siegel J, Chasalow SD, Weber J, Galbraith S, Targan SR, Wang HL. Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. Cancer Immun. 2010;10:11.
48. Delyon J, Mateus C, Lambert T. Hemophilia A induced by ipilimumab. N Engl J Med. 2011;365(18):1747–8.
49. Fadel F, El Karoui K, Knebelmann B. Anti-CTLA4 antibody-induced lupus nephritis. N Engl J Med. 2009;361(2):211–2.
50. Iwama S, De Remigis A, Callahan MK, Slovin SF, Wolchok JD, Caturegli P. Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. Sci Transl Med 2014;6(230): 230ra245.
51. Kong YC, Flynn JC. Opportunistic autoimmune disorders potentiated by immune-checkpoint inhibitors anti-CTLA-4 and anti-PD-1. Front Immunol. 2014;5:206.
52. Min L, Vaidya A, Becker C. Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy. Eur J Endocrinol. 2011;164(2):303–7.
53. Barreto M, Santos E, Ferreira R, Fesel C, Fontes MF, Pereira C, Martins B, Andreia R, Viana JF, Crespo F, Vasconcelos C, Ferreira C, Vicente AM. Evidence for CTLA4 as a susceptibility gene for systemic lupus erythematosus. Eur J Hum Genet. 2004;12(8):620–6.
54. Lee YH, Bae SC, Kim JH, Song GG. Meta-analysis of genetic polymorphisms in programmed cell death 1. Associations with rheumatoid arthritis, ankylosing spondylitis, and type 1 diabetes susceptibility. Z Rheumatol. 2015;74(3):230–9.
55. Lee YH, Kim JH, Seo YH, Choi SJ, Ji JD, Song GG. CTLA-4 polymorphisms and susceptibility to inflammatory bowel disease: a meta-analysis. Hum Immunol. 2014;75(5):414–21.
56. Lee YH, Woo JH, Choi SJ, Ji JD, Song GG. Association of programmed cell death 1 polymorphisms and systemic lupus erythematosus: a meta-analysis. Lupus. 2009;18(1):9–15.
57. Li G, Shi F, Liu J, Li Y. The effect of CTLA-4 A49G polymorphism on rheumatoid arthritis risk: a meta-analysis. Diagn Pathol. 2014;9:157.
58. Liu JL, Zhang FY, Liang YH, Xiao FL, Zhang SQ, Cheng YL, Yuan CD, Chen QP, Yang S, Zhang XJ. Association between the PD1.3A/G polymorphism of the PDCD1 gene and systemic lupus erythematosus in European populations: a meta-analysis. J Eur Acad Dermatol Venereol. 2009;23(4):425–32.
59. Scalapino KJ, Daikh DI. CTLA-4: a key regulatory point in the control of autoimmune disease. Immunol Rev. 2008;223:143–55.
60. Wu J, Zhang L, Zhou Y. The association between CTLA-4 (+49 A/G) polymorphism and susceptibility to ankylosing spondylitis: a meta-analysis. Int J Rheum Dis. 2015.
61. Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, No D, Gobourne A, Littmann E, Huttenhower C, Pamer EG, Wolchok JD. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun. 2016;7:10391.
62. Champiat S, Lambotte O, Barreau E, Belkhir R, Berdelou A, Carbonnel F, Cauquil C, Chanson P, Collins M, Durrbach A, Ederhy S, Feuillet S, Francois H, Lazarovici J, Le Pavec J, De Martin E, Mateus C, Michot JM, Samuel D, Soria JC, Robert C, Eggermont A, Marabelle A. Management of immune checkpoint blockade dysimmune toxicities: a collaborative position paper. Ann Oncol. 2016;27(4):559–74.
63. U.S. Food and Drug Administration. Yervoy: risk evaluation and mitigation strategy (REMS). 2016. http://www.fda.gov/downloads/drugs/drugsafety/postmarketdrugsafetyinformationforpatientsandproviders/ucm249435.pdf. Accessed 5 Oct 2016.

64. Bagley SJ, Kosteva JA, Evans TL, Langer CJ. Immune thrombocytopenia exacerbated by nivolumab in a patient with non-small-cell lung cancer. Cancer Treatment Commun. 2016;6:20–3.
65. Batacchi Z, Alarcon-Casas Wright L. Hyperthyroidism followed by worsening primary hypothyroidism in a patient treated with ipilimumab and pembrolizumab. Thyroid. 2015;25:A215.
66. Bostwick AD, Salama AK, Hanks BA. Rapid complete response of metastatic melanoma in a patient undergoing ipilimumab immunotherapy in the setting of active ulcerative colitis. J Immunother Cancer. 2015;3:19.
67. Chia PL, John T. Severe psoriasis flare after anti-programmed death ligand 1 (PD-L1) therapy for metastatic non-small cell lung cancer (NSCLC). J Immunother. 2016;39(5):202–4.
68. Gettings EJ, Hackett CT, Scott TF. Severe relapse in a multiple sclerosis patient associated with ipilimumab treatment of melanoma. Mult Scler. 2015;21(5):670.
69. Hughes J, Vudattu N, Sznol M, Gettinger S, Kluger H, Lupsa B, Herold KC. Precipitation of autoimmune diabetes with anti-PD-1 immunotherapy. Diabetes Care. 2015;38(4):e55–7.
70. Izzedine H, Gueutin V, Gharbi C, Mateus C, Robert C, Routier E, Thomas M, Baumelou A, Rouvier P. Kidney injuries related to ipilimumab. Invest New Drugs. 2014;32(4):769–73.
71. Kamil F, Cohen M, Kumta N, Wan D. Ipilimumab-induced colitis in a patient with ulcerative colitis and lung cancer. Am J Gastroenterol. 2013;108:S404.
72. Kyi C, Carvajal RD, Wolchok JD, Postow MA. Ipilimumab in patients with melanoma and autoimmune disease. J Immunother Cancer. 2014;2(1):35.
73. Lau KHV, Kumar A, Yang I, Nowak R. A case of myasthenia gravis exacerbation in a patient with melanoma treated with pembrolizumab. Neurology 2016;86(16 Suppl 1).
74. Maul LV, Weichenthal M, Kahler KC, Hauschild A. Successful anti-PD-1 antibody treatment in a metastatic melanoma patient with known severe autoimmune disease. J Immunother. 2016;39(4):188–90.
75. Menzies AM, Johnson DB, Ramanujam S, Atkinson V, Wong ANM, Park JJ, McQuade JL, Shoushtari AN, Tsai KK, Eroglu Z, Klein O, Hassel JC, Sosman JA, Guminski A, Sullivan RJ, Ribas A, Carlino MS, Davies MA, Sandhu SK, Long GV. Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders (AD) or major toxicity with ipilimumab (IPI). J Clin Oncol. 2016;34(15).
76. Narita T, Oiso N, Taketomo Y, Okahashi K, Yamauchi K, Sato M, Uchida S, Matsuda H, Kawada A. Serological aggravation of autoimmune thyroid disease in two cases receiving nivolumab. J Dermatol. 2015;43(2):210–4.
77. Pedersen M, Andersen R, Norgaard P, Jacobsen S, Thielsen P, Thor Straten P, Svane IM. Successful treatment with Ipilimumab and interleukin-2 in two patients with metastatic melanoma and systemic autoimmune disease. Cancer Immunol Immunother. 2014;63(12):1341–6.
78. Plachouri KM, Mohr M, Sunderkotter C, Weishaupt C. Induction of muscular sarcoidosis in a metastastic melanoma patient treated with ipilimumab. J German Soc Dermatol. 2012;10(11):861.
79. Sahuquillo-Torralba A, Ballester-Sanchez R, Pujol-Marco C, Botella-Estrada R. Pembrolizumab: a new drug that can induce exacerbations of psoriasis. [Spanish]. Actas Dermosifiliogr. 2016;107(3):264–6.
80. Weishaupt C, Mohr M, Sunderkotter C. Muscular sarcoidosis in a metastastic melanoma patient treated with ipilimumab. J German Soc Dermatol. 2012;10(9):683.
81. Zmeili O, Samantray J. Anti-CTLA-4 antibody therapy causing autoimmune hypophysitis in a patient with a longstanding history of hashimotos's thyroiditis. Endocrine Rev 2013;1.
82. Hotte S, Waldron T, Canil C, Winquist E. Interleukin-2 in the treatment of unresectable or metastatic renal cell cancer: a systematic review and practice guideline. Can Urol Assoc J. 2007;1(1):27–38.
83. Siegel JP, Puri RK. Interleukin-2 toxicity. J Clin Oncol. 1991;9(4):694–704.
84. Schwartz RN, Stover L, Dutcher J. Managing toxicities of high-dose interleukin-2. Oncology (Williston Park). 2002;16(11 Suppl 13):11–20.
85. Schwartzentruber D. Interleukin-2: clinical applications. Principles of administration and management of side effects. In: Rosenberg SA, editor. Principles and practice of the biologic therapy of cancer Philadelphia: Lippincott Williams & Wilkins; 2000. p. 32–50.
86. Mier JW, Vachino G, van der Meer JW, Numerof RP, Adams S, Cannon JG, Bernheim HA, Atkins MB, Parkinson DR, Dinarello CA. Induction of circulating tumor necrosis factor

(TNF alpha) as the mechanism for the febrile response to interleukin-2 (IL-2) in cancer patients. J Clin Immunol. 1988;8(6):426–36.
87. Dutcher JP, Schwartzentruber DJ, Kaufman HL, Agarwala SS, Tarhini AA, Lowder JN, Atkins MB. High dose interleukin-2 (Aldesleukin)—expert consensus on best management practices-2014. J Immunother Cancer. 2014;2(1):26.
88. Fisher B, Keenan AM, Garra BS, Steinberg SM, White DE, DiBisceglie AM, Hoofnagle JH, Yolles P, Rosenberg SA, Lotze MT. Interleukin-2 induces profound reversible cholestasis: a detailed analysis in treated cancer patients. J Clin Oncol. 1989;7(12):1852–62.
89. Shalmi CL, Dutcher JP, Feinfeld DA, Chun KJ, Saleemi KR, Freeman LM, Lynn RI, Wiernik PH. Acute renal dysfunction during interleukin-2 treatment: suggestion of an intrinsic renal lesion. J Clin Oncol. 1990;8(11):1839–46.
90. Sikora S, Sarnsonov M, Dookeran K, Edington H, Lotze M. Peripheral nerve entrapment: an unusual adverse event with high-dose interleukin-2 therapy. Ann Oncol. 1996;7(5):535–6.
91. Sarna G, Figlin R, Pertcheck M, Altrock B, Kradjian S. Systemic administration of recombinant methionyl human interleukin-2 (Ala 125) to cancer patients: clinical results. J Biol Response Mod. 1989;8(1):16–24.
92. MacFarlane MP, Yang JC, Guleria AS, White Jr RL, Seipp CA, Einhorn JH, White DE, Rosenberg SA. The hematologic toxicity of interleukin-2 in patients with metastatic melanoma and renal cell carcinoma. Cancer. 1995;75(4):1030–7.
93. Klempner MS, Noring R, Mier JW, Atkins MB. An acquired chemotactic defect in neutrophils from patients receiving interleukin-2 immunotherapy. N Engl J Med. 1990;322(14):959–65.
94. Oleksowicz L, Strack M, Dutcher JP, Sussman I, Caliendo G, Sparano J, Wiernik PH. A distinct coagulopathy associated with interleukin-2 therapy. Br J Haematol. 1994;88(4):892–4.
95. Lee SH, Baig M, Rusciano V, Dutcher JP. Novel management of pruritus in patients treated with IL-2 for metastatic renal cell carcinoma and malignant melanoma. J Immunother. 2010;33(9):1010–3.
96. Krouse RS, Royal RE, Heywood G, Weintraub BD, White DE, Steinberg SM, Rosenberg SA, Schwartzentruber DJ. Thyroid dysfunction in 281 patients with metastatic melanoma or renal carcinoma treated with interleukin-2 alone. J Immunother Emphasis Tumor Immunol. 1995;18(4):272–8.
97. Quan Jr W, Ramirez M, Taylor C, Quan F, Vinogradov M, Walker P. Administration of high-dose continuous infusion interleukin-2 to patients age 70 or over. Cancer Biother Radiopharm. 2005;20(1):11–5.
98. Payne R, Glenn L, Hoen H, Richards B, Smith 2nd JW, Lufkin R, Crocenzi TS, Urba WJ, Curti BD. Durable responses and reversible toxicity of high-dose interleukin-2 treatment of melanoma and renal cancer in a Community Hospital Biotherapy Program. J Immunother Cancer. 2014;2:13.
99. Dutcher J, Atkins MB, Margolin K, Weiss G, Clark J, Sosman J, Logan T, Aronson F, Mier J. Kidney cancer: the Cytokine Working Group experience (1986-2001): part II. Management of IL-2 toxicity and studies with other cytokines. Med Oncol. 2001;18(3):209–19.
100. Sleijfer S, Bannink M, Van Gool AR, Kruit WH, Stoter G. Side effects of interferon-alpha therapy. Pharm World Sci. 2005;27(6):423–31.
101. Kirkwood JM, Bender C, Agarwala S, Tarhini A, Shipe-Spotloe J, Smelko B, Donnelly S, Stover L. Mechanisms and management of toxicities associated with high-dose interferon alfa-2b therapy. J Clin Oncol. 2002;20(17):3703–18.
102. Quesada JR, Talpaz M, Rios A, Kurzrock R, Gutterman JU. Clinical toxicity of interferons in cancer patients: a review. J Clin Oncol. 1986;4(2):234–43.
103. Menkes DB, MacDonald JA. Interferons, serotonin and neurotoxicity. Psychol Med. 2000;30(2):259–68.
104. Ackerman SK, Hochstein HD, Zoon K, Browne W, Rivera E, Elisberg B. Interferon fever: absence of human leukocytic pyrogen response to recombinant alpha-interferon. J Leukoc Biol. 1984;36(1):17–25.
105. Dinarello CA, Bernheim HA, Duff GW, Le HV, Nagabhushan TL, Hamilton NC, Coceani F. Mechanisms of fever induced by recombinant human interferon. J Clin Invest. 1984;74(3):906–13.

106. Guillot B, Blazquez L, Bessis D, Dereure O, Guilhou JJ. A prospective study of cutaneous adverse events induced by low-dose alpha-interferon treatment for malignant melanoma. Dermatology. 2004;208(1):49–54.
107. Salman H, Bergman M, Bessler H, Alexandrova S, Djaldetti M. The effect of interferon on mouse myocardial capillaries: an ultrastructural study. Cancer. 1999;85(6):1375–9.
108. Sonnenblick M, Rosin A. Cardiotoxicity of interferon. A review of 44 cases. Chest. 1991;99(3):557–61.
109. Kondo Y, Yukinaka M, Nomura M, Nakaya Y, Ito S. Early diagnosis of interferon-induced myocardial disorder in patients with chronic hepatitis C: evaluation by myocardial imaging with 123I-BMIPP. J Gastroenterol. 2000;35(2):127–35.
110. Hauschild A, Gogas H, Tarhini A, Middleton MR, Testori A, Dreno B, Kirkwood JM. Practical guidelines for the management of interferon-alpha-2b side effects in patients receiving adjuvant treatment for melanoma: expert opinion. Cancer. 2008;112(5):982–94.
111. Willson RA. Nephrotoxicity of interferon alfa-ribavirin therapy for chronic hepatitis C. J Clin Gastroenterol. 2002;35(1):89–92.
112. Ferrantini M, Capone I, Belardelli F. Interferon-alpha and cancer: mechanisms of action and new perspectives of clinical use. Biochimie. 2007;89(6–7):884–93.
113. Franzke A, Peest D, Probst-Kepper M, Buer J, Kirchner GI, Brabant G, Kirchner H, Ganser A, Atzpodien J. Autoimmunity resulting from cytokine treatment predicts long-term survival in patients with metastatic renal cell cancer. J Clin Oncol. 1999;17(2):529–33.
114. Malik UR, Makower DF, Wadler S. Interferon-mediated fatigue. Cancer. 2001;92(6 Suppl):1664–8.
115. Khakoo AY, Halushka MK, Rame JE, Rodriguez ER, Kasper EK, Judge DP. Reversible cardiomyopathy caused by administration of interferon α. Nat Clin Pract Cardiovasc Med. 2005;2(1):53–7.
116. FDA. Product information INTRON® A Interferon alfa-2b, recombinant. 2014. http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/103132s5190lbl.pdf. Accessed 25 Sept 2016
117. Cunha AC, Weigle B, Kiessling A, Bachmann M, Rieber EP. Tissue-specificity of prostate specific antigens: comparative analysis of transcript levels in prostate and non-prostatic tissues. Cancer Lett. 2006;236(2):229–38.
118. Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, Valone FH. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J Clin Oncol. 2000;18(23):3894–903.
119. Solin T, Kontturi M, Pohlmann R, Vihko P. Gene expression and prostate specificity of human prostatic acid phosphatase (PAP): evaluation by RNA blot analyses. Biochim Biophys Acta. 1990;1048(1):72–7.
120. Kawalec P, Paszulewicz A, Holko P, Pilc A. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. A systematic review and meta-analysis. Arch Med Sci. 2012;8(5):767–75.
121. FDA. Approved products—PROVENGE (sipuleucel-T). 2010. http://www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts/approvedproducts/ucm210012.htm
122. Anassi E, Ndefo UA. Sipuleucel-T (provenge) injection: the first immunotherapy agent (vaccine) for hormone-refractory prostate cancer. P T. 2011;36:197–202.
123. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, Provost N, Frohlich MW. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer. 2009;115(16):3670–9.
124. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
125. Hall SJ, Klotz L, Pantuck AJ, George DJ, Whitmore JB, Frohlich MW, Sims RB. Integrated safety data from 4 randomized, double-blind, controlled trials of autologous cellular immunotherapy with sipuleucel-T in patients with prostate cancer. J Urol. 2011;186(3):877–81.

Chapter 9
Skin Reactions to Immune Checkpoint Inhibitors

Anisha B. Patel and Omar Pacha

Abstract 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 without sufficient familiarity to diagnose and treat. In the following chapter, we will review the categories of these drugs, common cutaneous effects, their grading, and management options.

Keywords Immune checkpoint inhibitors • Dermatitis • Ipilimumab • Nivolumab • Anti-PD1 • Anti-CTLA4 • Dermatitis • Rash • Immunotherapy • Pruritus

The novelty of immune checkpoint inhibitors has only recently led to the characterization of cutaneous adverse events (AEs). This, along with the substantial rate of cutaneous reactions, has left many clinicians without sufficient familiarity to diagnose and treat. Pruritus and rash are among the top five immune-related AEs reported in clinical trials for this class of therapy. Incidence varies between 35 and 50% for cutaneous AEs among the 3 FDA-approved drugs. Although only 2% are reported as grade 3 or 4 events, the quality of life impact can be significant for these patients, and is best described in ipilimumab trials. 43.5% of ipilimumab patients have a cutaneous AE and, of these patients at our institution, 20% of them had a dose interruption because of this AE, which extrapolates to potentially 9% of patients having dose interruption of ipilimumab because of their cutaneous AEs [1]. In the following chapter, we will review the categories of these drugs, common cutaneous effects, their grading, and management options.

A.B. Patel, M.D. (✉) • O. Pacha, M.D.
Department of Dermatology, The University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Unit 1452, Houston, TX 77030, USA
e-mail: APatel11@mdanderson.org; opacha@mdanderson.org

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In general, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade and those drugs that bind the programmed cell death receptor-1 (PD-1) have similar reactions although PD-1 receptor inhibitors are usually better tolerated with fewer reported skin AEs (43.5% and 18%, respectively) [1]. Additionally, it appears that the reactions both tend to be delayed, with CTLA-4s causing a rash after about a month of therapy and PD-1s slightly later [2]. Interestingly, skin toxicities have been associated with improved responses and paradoxically, if well managed, can be an indicator of a good prognosis [1, 3, 4].

9.1 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's Syndrome (SJS)/Toxic Epidermal Necrolysis (TEN) on the severe end.

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

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

9.2 Cutaneous Adverse Events Shared by Anti-CTLA4 and Anti-PD1 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 psoriasis, eczema, and morbilliform drug eruption. Compared to anti-CTLA4 antibodies, the anti-PD1 antibodies have a lower incidence of rash; however, the incidence of severe (Grade 3 and 4) cutaneous AEs is the same (2.4% and 2.6%, respectively). Eczema, pruritus, and vitiligo are seen with both classes of immune checkpoint inhibitors [5–11].

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. 9.1 and 9.2).

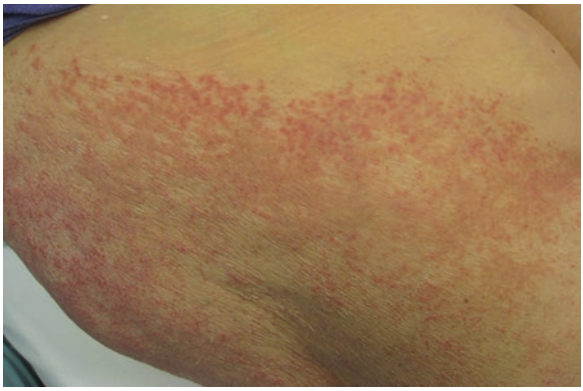

Fig. 9.1 Eczema—erythematous papules coalescing into plaques that are rough and have minimal scale

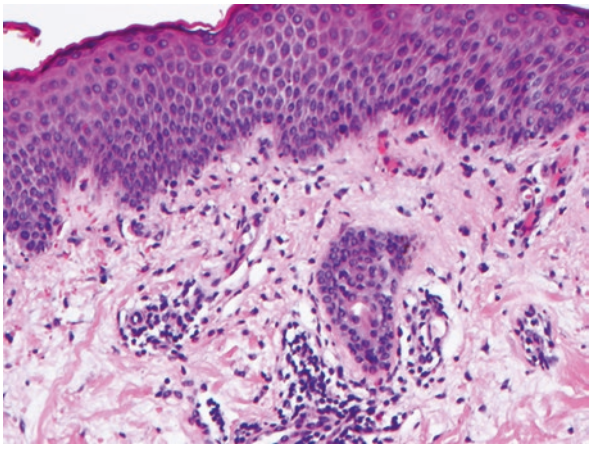

Fig. 9.2 Eczema-spongiotic dermatitis with dermal eosinophils

Eczema appears as pruritic, ill-defined, edematous, and erythematous papules coalescing into plaques occasionally with vesicles in exuberant cases. As it evolves, the plaques are rough, erythematous, and have visible excoriation. Distribution is diffuse, affecting the trunk and extremities more than the face, and can have 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 [12]. Treatment consists of topical steroids, usually mid-strength creams like triamcinolone to begin with and graduating to super-potent formulations such as clobetasol cream. The face, axilla, and groin are usually treated with mild and low potency steroids such as hydrocortisone or desonide 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 non-sedating antihistamines such as cetirizine in the morning is also beneficial. In patients with grade 3 AEs, involving >30% of BSA, and refractory to topical therapies, the addition of oral steroids such as prednisone at 1 mg/kg is usually effective and can be slowly tapered. The slow taper is often effectively weaned with topical steroid maintenance.

Biological therapy for atopic dermatitis is undergoing clinical trials currently, targeting the interleukin-4 receptor alpha subunit (IL-4Ra). Biological therapies are potential treatment options for severe refractory eczema in patients requiring continuing therapy with immune checkpoint inhibitors.

For pruritus without rash, the 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 the pruritus. Prurigo nodules are ill-defined, discrete, erythematous, hyperpigmented keratotic papules often with central erosion. Histology shows fibrosis and vertically- oriented blood vessels in the superficial dermis with an overlying acanthotic epidermis. The first step in management is to eliminate a primary inflammatory condition. For primary pruritus, a stepwise approach depending on severity is best. For mild cases, a first-generation antihistamine is oftentimes sufficient with the added benefit of sedation that can help patients sleep when pruritus is usually most severe—right before bed. As intensity increases, the addition of the tricyclic antidepressant doxepin QHS and GABA agonists like gabapentin at increasing doses have been effectively used.

Vitiligo presents as depigmented well-demarcated macules coalescing into patches, occasionally preceded by erythema and pruritus, exclusively reported in melanoma patients. Incidence is about 2% for anti-CTLA4 and anti-PD1 therapies [3]. Histology shows loss of melanocytes at the dermal–epidermal junction. Patients are usually asymptomatic, but can have occasional preceding pruritus. Treatment for vitiligo includes a combination of topical steroids and ultraviolet (UV) light

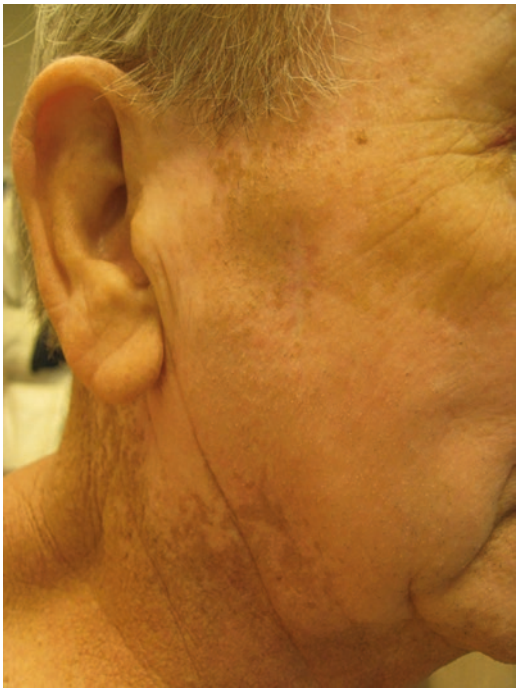

Fig. 9.3 Vitiligo- Depigmented patches of head and neck

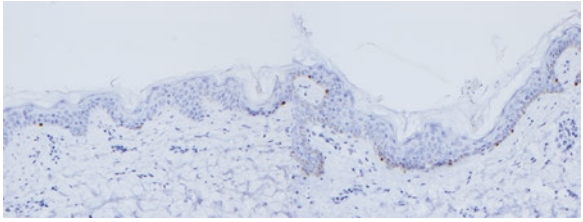

Fig. 9.4 Vitiligo- MART1 immunostain in lesional skin (L) showing decreased melanocytes at the dermal–epidermal junction compared to MART1 immunostain of non-lesional (NL) skin

therapy; however, in melanoma patients with this drug-induced side effect, treatment is not usually undertaken because of the risk of further skin cancers with increased UV exposure (Figs. 9.3 and 9.4).

9.3 Common Cutaneous Adverse Events for Anti-CTLA4

The most common reported adverse event in patients receiving ipilimumab is "rash," with one quarter to more than one half of patients experiencing rash and a quarter to one third experiencing pruritus [13]. The type of rash varied from mild

eczema to toxic epidermal necrolysis [14] with the majority experiencing a more traditional morbilliform drug eruption or an eczematous atopic dermatitis-like eruption [13]. The onset of rash has been reported to appear at about 3 weeks and then usually resolves at about 2.5 months [13] although in our institutional review, complete resolution was usually not obtained for most patients until drug cessation [unpublished data Patel AB]. Less frequent eruptions include acneiform eruption [11] and unpublished Patel AB] and granulomatous dermatitis [unpublished data Patel AB].

Its mechanism of action through the activation of T-cells by the prevention of T-cell blockade leads to an upregulation of the body's immune system and therefore its antitumor activity as described elsewhere in this text. It appears that the skin reaction 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 than overall rash incidence at 2.4% [15].

9.4 CAE in Anti-PD1

In addition to the shared inflammatory skin reactions discussed earlier, psoriasis [16, 17] and bullous pemphigoid have been induced by anti-PD1 antibodies [18, 19] (Figs. 9.5 and 9.6).

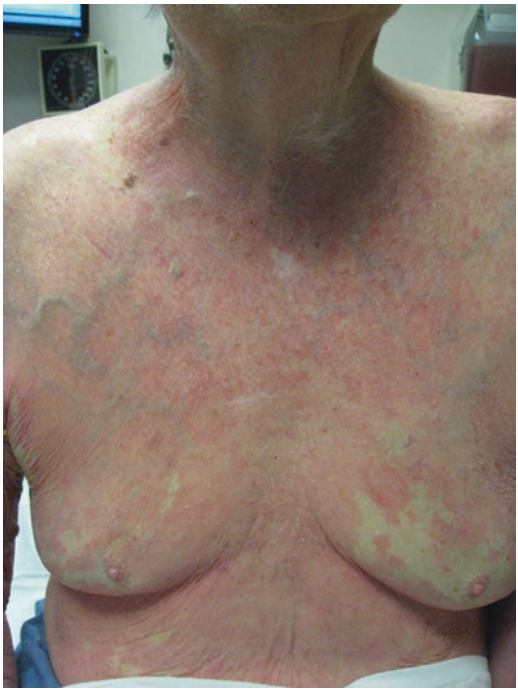

Fig. 9.5 Psoriasiform dermatitis—erythematous well-demarcated plaques with fine adherent scale

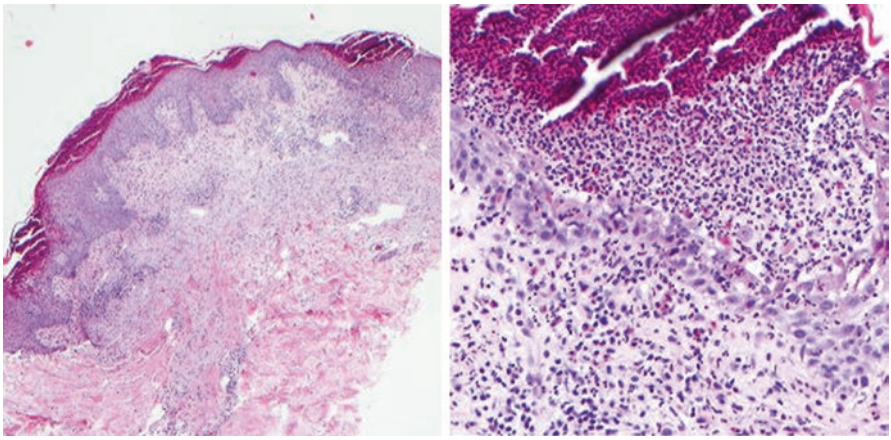

Fig. 9.6 Spongiotic psoriasiform dermatitis with subcorneal pustules, irregular acanthosis, and numerous eosinophils

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 [17], or in the pustular variant [Patel AB unpublished]. 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 presence of 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 oral antihistamines, if indicated. Escalation of treatment includes oral acitretin, oral apremilast, UV-B therapy, or oral steroids. Biologic medications such as IL-17 inhibitors are a potential therapy for refractory cases.

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.

9.5 Grading

Grading has nearly been universally based upon the Common Terminology Criteria for Adverse Events as seen in the table below.

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		

Common Terminology Criteria for Adverse Events (CTCAE) v4.0. 2008. 26 Jul. 2016 http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

9.6 CAE as Prognostic Indicators

Vitiligo is a relatively innocuous AE 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 under reported side effect as it can be easily missed if a full body skin exam is not performed. Vitiligo has only been reported in patients being treated with melanoma ([2, 3, 20, 21]). Incidence of rash was also associated with increased survival and tumor response [2].

9.7 CAE as Indicators of Other irAEs

Cutaneous AEs are usually the earliest irAEs to present; however, their presence has not been linked to further or more severe systemic AEs [1].

References

1. Villadolid J, Amin A. Immune checkpoint inhibitors in clinical practice: update on management of immune-related toxicities. Transl Lung Cancer Res. 2015;4(5):560–75.
2. Sanlorenzo M. Pembrolizumab cutaneous adverse events and their association with disease progression. JAMA Dermatol. 2015;151(11):1206–12.
3. Teulings H-E, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. J Clin Oncol. 2015;33(7):773–81.
4. Attia P, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti–cytotoxic T-lymphocyte antigen-4. J Clin Oncol. 2005;23(25):6043–53.
5. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.
6. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364:2517–26.
7. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet. 2014;384:1109–17.
8. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372:320–30.
9. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16:375–84.
10. Rizvi NA, Mazières J, Planchard D, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. Lancet Oncol. 2015;16:257–65.
11. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372:2018–28.

12. Giacomo D, Maria A, Biagioli M, Maio M. The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications. Semin Oncol. 2010;37:499–507.
13. Lacouture ME, et al. Ipilimumab in patients with cancer and the management of dermatologic adverse events. J Am Acad Dermatol. 2014;71(1):161–9.
14. Nayar N, Briscoe K, Penas PF. Toxic epidermal necrolysis-like reaction with severe satellite cell necrosis associated with nivolumab in a patient with ipilimumab refractory metastatic melanoma. J Immunother. 2016;39(3):149–52.
15. Minkis K, et al. The risk of rash associated with ipilimumab in patients with cancer: a systematic review of the literature and meta-analysis. J Am Acad Dermatol. 2013;69(3):e121–8.
16. Ohtsuka M, Miura T, Mori T, Ishikawa M, Yamamoto T. Occurrence of psoriasiform eruption during nivolumab therapy for primary oral mucosal melanoma. JAMA Dermatol. 2015;151(7):797–9.
17. Totonchy MB, Ezaldein HH, Ko CJ, Choi JN. Inverse psoriasiform eruption during pembrolizumab therapy for metastatic melanoma. JAMA Dermatol. 2016;152(5):590–2.
18. Jour G, Glitza IC, Ellis RM, et al. Autoimmune dermatologic toxicities from immune checkpoint blockade with anti-PD-1 antibody therapy: a report on bullous skin eruptions. J Cutan Pathol. 2016;43(8):688–96.
19. Naidoo J, Schindler K, Querfeld C, et al. Autoimmune bullous skin disorders with immune checkpoint inhibitors targeting PD-1 and PD-L1. Cancer Immunol Res. 2016;4(5):383–9.
20. Hua C, Boussemart L, Mateus C, et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. JAMA Dermatol. 2016;152(1):45–51.
21. Freeman-keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. Nivolumab in resected and unresectable metastatic melanoma: characteristics of immune-related adverse events and association with outcomes. Clin Cancer Res. 2016;22(4):886–94.