Advances in Experimental Medicine and Biology 995

Aung Naing
Joud Hajjar *Editors*

Immunotherapy

Second Edition

Springer

Advances in Experimental Medicine and Biology

Volume 995

More information about this series at http://www.springer.com/series/5584

Aung Naing • Joud Hajjar
Editors

Immunotherapy

Second Edition

Springer

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

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-02504-5 ISBN 978-3-030-02505-2 (eBook)
https://doi.org/10.1007/978-3-030-02505-2

Library of Congress Control Number: 2018964072

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Contributors

Hamzah Abu-Sbeih Department of Gastroenterology, Hepatology and Nutrition, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Rana Alqusairi Baltimore, MD, USA

Christian Caglevic Medical Oncology Department, Clínica Alemana Santiago, Santiago, Chile

Luis Corrales Medical Oncology Department, CIMCA / Hospital San Juan de Dios-CCSS, San José, Costa Rica

Naval Daver Department of Leukemia, MD Anderson Cancer Center, Houston, TX, USA

Guillermo Garcia-Manero Department of Leukemia, MD Anderson Cancer Center, Houston, TX, USA

Isabella C. Glitza Oliva The University of Texas MD Anderson Cancer Center, Houston, TX, USA

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

Akash Jain Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Hagop Kantarjian Department of Leukemia, MD Anderson Cancer Center, Houston, TX, USA

Lucia Masarova Department of Leukemia, MD Anderson Cancer Center, Houston, TX, USA

Tito R. Mendoza Department of Symptom Research, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Ken Miller Thoracic Oncology Program, University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA

Julio Oliveira Medical Oncology Department, Portuguese Institute of Oncology of Porto, Porto, Portugal

Omar Pacha Department of Dermatology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Anisha B. Patel Department of Dermatology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Farhad Ravandi Department of Leukemia, MD Anderson Cancer Center, Houston, TX, USA

Christian Rolfo Thoracic Oncology Program, University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA

Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA

Katherine Scilla Thoracic Oncology Program, University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA

Vickie R. Shannon Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Padmanee Sharma Department of Immunotherapy Platform, MD Anderson Cancer Center, Houston, TX, USA

Ajay Sheshadri Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Bettzy Stephen The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Yun Tian Department of Oncology, Shanghai Dermatology Hospital, Tongji University, Shanghai, China

Tongji University Cancer Center, The Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

Yinghong Wang Department of Gastroenterology, Hepatology and Nutrition, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Chapter 1
Overview of Basic Immunology and Translational Relevance 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 crosstalk between the immune cells in the tumor microenvironment influence the nature of immune response, prognosis, and treatment outcomes in cancer patients. Therefore, an understanding of the characteristics of the immune cells and their role in tumor immune surveillance is of paramount importance to identify immune targets and to develop novel immune therapeutics in the war against cancer. In this chapter we provide an overview of the individual components of the human immune system and the translational relevance of predictive biomarkers.

Keywords Adaptive · Biomarkers · CTLA-4 · Immune checkpoints · Immunology · Immunotherapy · Innate · PD-1 · PD-L1 · Resistance · Response · T cells · Translational

The human immune system is an elaborate and dynamic network of cells that work together to defend the human body against attacks by foreign agents including malignant cells. There are two levels of immunity, the innate immunity and the adaptive immunity. The innate immunity constitutes the first line of defense against pathogens, which includes the anatomic and physiologic barriers, phagocytic leukocytes, dendritic cells (DC), natural killer (NK) cells, and the circulating plasma proteins [1]. Elie Metchnikoff, a pathologist and Father of natural immunity, was

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

J. Hajjar (✉)
Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA
e-mail: joud.hajjar@bcm.edu

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_1

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.

Innate Immune System

An association between inflammation and tumorigenesis has long been described, but has been established with turn of the century [5]. The human body is constantly exposed to a highly diverse world of foreign proteins every day, which are rapidly eliminated in a normal healthy individual by the components of the innate immune system. Speed is the essence of innate immune response; however, they are 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].

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 give 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.

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 anti-tumorigenic (N1) or a pro-tumorigenic (N2) phenotype [14, 21]. In untreated tumors, the regulatory cytokine transforming growth factor-beta (TGF-β) in the tumor cells drives the differentiation of TANs 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]. Neutrophils thus assume multiple roles in development and progression

of tumor cells [26]. However, under certain conditions such as TGF-β blockade, TANs assume a N1 phenotype, which are more cytotoxic due to enhanced expression of immune activating cytokines and chemokines, and lower levels of arginase [13]. N1 TANs also communicate with DCs to trigger an adaptive immune response [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, 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 anti-tumorigenic 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 and indoleamine 2,3-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, 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, 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 induce 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 leuckocytes 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 Tie1 receptors on the cell surface. And, activation of basophils releases pro-angiogenic factors VEGF-A and VEGF-B through a crosstalk between the basophils and the mast cells, contributing to neoplastic angiogenesis. Further, the correlation between basophils in the tumor draining lymph node with Th2 inflammation in patients with pancreatic ductal adenocarcinomas and the emergence of basophils as an independent prognostic factor of poor survival after surgery suggests a role for basophils in tumor development and disease recurrence [65].

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), 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 numbers of mast cells found in many tumors may have a double-edged function in tumor development. Infiltration of tumor by mast cells has been associated with poor prognosis in some cancers such as prostate cancer [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 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 crosstalk between mast cells and stromal cells are better predictors of patient survival as they modulate the immune response [1].

Dendritic Cells

DCs are professional APCs that are resident in most tissues of the body and concentrated in the secondary lymphoid tissues [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 and activate the antigen-specific B cells [3]. The lymphoid tissue-resident DCs (CD8+ and CD8− splenic cDCs and thymic cDCs) are immature DCs uniquely located in regions where naïve T cells are activated [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 and facilitate escape of tumor cells

from immune recognition. This may explain why vaccines did not succeed as an effective treatment modality in cancer patients [3]. DCs are aptly called the gatekeepers of the immune system because of their ability to inspect the microenvironment, interpret the cues in the environment, and instruct the immune cells to respond quickly and appropriately between tolerogenic and immunogenic function [83].

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 crosstalk with accessory cells such as DCs, neutrophils, macrophages, and mast cells, and/or a cytokine microenvironment that includes IL-2, IFN-α/β, IL-12, IL-15, IL-18, or IL-21 [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 result 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].

Adaptive Immune System

The hallmark of adaptive immunity, mediated by the T lymphocytes (T cells) and B lymphocytes (B cells), is the specificity of the immune response to antigenic stimuli. Another unique feature of adaptive immunity is its ability to confer lasting immunological memory that results in more rapid and robust immune response with subsequent exposure to the same antigen [2]. Contrary to innate immune response, which is immediate in onset due to the presence of germline-encoded cell surface receptors, the adaptive immune response is a slower process, as the lymphocytes on activation undergo clonal expansion to attain sufficient numbers before the effector cells mount an immune response [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.

Cellular Components of the Adaptive Immune System

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

T Lymphocytes

The lymphoid progenitor cells migrate from the bone marrow to the thymus, where they undergo four stages of differentiation and proliferation, including developmental checkpoints to ensure that cells which 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 that enter the thymus at the cortico-medullary junction do 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 cortico-medullary junction to the capsule, the lymphoid progenitor cells lose their ability to form B cells or NK cells and become committed T cell precursors (DN2) [107]. Following T lineage commitment and expression of recombination-activating gene 1 (RAG1), the TCR-β 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 (positive selection) [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 (CD8+ T cell), while those that express TCRs that bind with MHC Class II molecule mature into a single positive CD4 mature T cell (CD4+ T cell). These naïve T cells then sample the environment in the medulla for antigen presenting DCs. On exposure to antigenic determinants presented by the APCs, the T cells are

activated in the presence of co-stimulation of CD28 by B7 molecules (CD80 and CD86) on the APCs, to form effector T cells that either destroy the pathogenic agent or attract other immune cells to the site. In the absence of antigenic stimuli in the medulla, the naïve T cells enter the blood stream 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 effector T cells (CD4+ helper T cells or CD8+ cytotoxic T cells). Depending on the cytokine milieu and the transcription factors in the TME, the CD4+ helper T cells differentiate into several subtypes that 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]. These helper T cells secrete cytokines and chemokines that regulate the immune response. Th1 cells favor cell-mediated immunity by activation of CD8 T cells to mount an immune response against intracellular pathogens, while Th2 cells favor humoral immunity by activation of B cells against extracellular parasites. On the other hand, CD8+ effector T cells activated by antigen presentation on the MHC class I molecule or through CD4 helper T cells are directly cytotoxic. Hence, they migrate to the tumor and destroy the tumor cells. In addition, some of the activated T cells and B cells differentiate into memory cells that are responsible for the long-lasting immunological memory [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, inducible T cell co-stimulator (ICOS), 4-1BB (CD-137), OX40 (CD-134), and glucocorticoid-induced TNFR-related protein (GITR), while CTLA-4, programmed cell death 1 (PD-1), lymphocyte activation gene-3 (Lag-3), T cell immunoglobulin-3 (Tim-3), and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) are co-inhibitory [118]. CD28 is the primary co-stimulatory molecule constitutively expressed on the surface of naive T cells. On ligand binding with B7-1 and B7-2 on APCs, they provide the essential co-stimulatory signal for T cell activation and downstream signaling [119]. Besides CD28, there are other co-signaling receptors of the TNF receptor superfamily including 4-1BB [120], OX40 [121], and GITR [122] 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. Activated T cells simultaneously express CTLA-4 and PD-1 on their surface as immune checkpoints [123–125]. CTLA-4, a CD28 homologue with a higher affinity to bind with B7 molecules, is an early co-inhibitory signal that regulates T cell activity during the priming phase. On engagement with B7, CTLA-4 blocks CD28 co-stimulation and abrogates T cell activity and cytokine production. On the other hand, PD-1, a CD28 family member, is a late co-inhibitory signal that regulates T cell activity during the effector phase in the peripheral tissue. PD-1 interacts with two ligands, PD-L1 and PD-L2. PD-L1 is expressed on many cells including the tumor cells,

activated B and T cells in response to IFN-γ produced by the activated T cells, while PD-L2 is expressed exclusively on macrophages and DCs [126]. Unlike CTLA-4, the PD-1 to PD-L1 ligand binding does not interfere with co-stimulation, but down-regulates B and T cell proliferation and cytokine production by interfering with signaling pathways downstream of TCRs and B cell receptors (BCRs) [127]. Besides CTLA-4 and PD-1, there are other next generation co-inhibitory receptors such as Lag-3, Tim-3, and TIGIT, which are expressed on distinct lymphocyte subsets that are responsible for differential suppression of immune response [128]. For example, Tim-3 pathway may regulate immune responses in the gut, while TIGIT may regulate in the lungs and Lag-3 in the pancreas. Similarly, they exhibit functional specification in that TIGIT may selectively suppress pro-inflammatory response of Th1 and Th17 cells, while promoting Th2 cell response [129]. Besides immune checkpoints, a chief contributor to this immunosuppressive effect is the Tregs, which are specialized T cells that suppress the cytotoxic function of other T cells [130]. They are classified as thymus-derived natural Tregs (nTregs) and peripherally derived inducible Tregs (iTregs). nTregs characterized by surface expression of the CD4 and CD25 antigens and by the nuclear expression of forkhead box P3 (FOXP3) are positively selected thymocytes with relatively high affinity for self-antigens presented on MHC class II molecules. On the contrary, iTregs differentiate from naïve CD4 T cells in the periphery in the presence of TGF-β. They exert their immunosuppressive action 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 [131]. Thus, under normal conditions, coordinated regulation of immune activation and suppressive pathways play an important role in the maintenance of peripheral tolerance and regulation of the amplitude and duration of T cell responses [132].

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 [133]. 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) [134]. 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-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 [135].

The immature B cells enter the spleen as transitional cells. Very few cells progress from T1 to T2 stage as most of the T1 cells undergo clonal deletion or anergy due to strong reactivity to self-antigens that are expressed only in the peripheral tissue [136]. 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 differentiates into either follicular (FO) B cells with intermediate BCR signals and expression of bruton tyrosine kinase, or marginal zone (MZ) B cell with weak BCR signal and expression of NOTCH2 [136, 137]. 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 [138]. 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 [139]. 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 [140].

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

Each B cell in the body produces only one kind of antibody [140]. 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 [141]. 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 [142]. Later, humanized mAbs that are 85–90% human, where only the CDRs are murine, were developed [143]. Currently, fully human mAbs produced by phage display are available [144]. 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 [145]. 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).

The Immune System in Action!

Summary of the Immune Responses Against Tumor Cells

In the fight against cancer, greater understanding of the immunoregulatory processes of TME is critical for development of immunotherapy. The TME is composed of a variety of cells such as macrophages, DCs, NK cells, mast cells, naïve lymphocytes, B cells, cytotoxic T cells, helper T cells, memory cells, Tregs, myeloid-derived suppressor cells (MDSCs), and stromal cells [146]. Despite the dynamic interaction between these elements in the TME and the tumor, the cancer cells develop cellular processes to subvert the immune attack and become resilient. Thus a comprehensive understanding of the interactions between the tumor and the elements in the TME will help to identify novel targets and therapeutic strategies to combat resistance to therapy.

The human immune system exhibit a dual role in cancer. Though the primary function of the immune system is to eliminate tumor cells, they also shape immunogenicity and promote tumor progression through a dynamic process called cancer immunoediting [147]. 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 [148]. 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. Thus,

innate and adaptive immune cells have the capacity to completely eliminate the tumor cells and halt the immunoediting process.

During the equilibrium phase, there is continuous interaction between the immune cells and tumor cells that have escaped elimination phase. The tumor and the immune cells exist in a state of equilibrium that prevents expansion of the tumor cells. However, this continuous immune pressure selects or promotes the formation of new variants of tumor cells with reduced immunogenicity that escapes recognition by immune system [148]. This is the longest phase in the immunoediting process, when the tumor cell variants reside in a latent form before escaping eventually [149].

During the escape phase, tumor cells adopt several mechanisms to evade immunosurveillance [150]. 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 [151]. Under hypoxic conditions, the TME may release VEGF, which suppresses T cell adhesion to tumor endothelium and impedes T cell infiltration of the tumor. Similarly, TAMs in the presence of IL-4, IL-10, and TGF-β may polarize to assume M2 phenotype and express high levels of IL-10 and low levels of IL-12. These macrophages suppress T cell activity and promote angiogenesis and tumor growth [152]. In addition, MDSCs, which are immature innate immune cells in the TME, utilize various mechanisms such as expression of IL-10, TGF-β, and Tregs to produce immune suppression, resulting in tumor progression [153, 154]. As a result, immunologically sculpted tumor cells with increased resistance emerge, resulting in uncontrolled growth of the tumor with overt clinical disease. It is therefore critical to overcome these barriers to elicit clinical response to therapeutic agents.

Cancer Immunotherapy

Immunotherapy has revolutionized cancer treatment due to its ability to produce durable responses in patients with certain types of advanced cancer. 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 [155] based on their ability to inhibit ligand binding and downstream signaling (cetuximab), target the TME (bevacizumab), and target immunosuppressive cytokines (GC-1008, an anti-TGF-β antibody) [156].

But it is the discovery of immune checkpoint CTLA-4 and a deeper understanding of the immune regulatory pathways that led to a major breakthrough in cancer immunotherapy [157]. Subsequent to the discovery that activated T cells express

CTLA-4, which on binding with B7 molecules on the APC blocks co-stimulation of T cells resulting in immune suppression, a series of experiments were performed to unleash the immune harnessing power of T cells to combat cancer. This led to the development of the concept of immune checkpoint blockade and discovery 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 [158]. The dramatic response with ipilimumab laid the foundation for exploration of other T cell inhibitory pathways. Based on strong preclinical evidence, several clinical trials were conducted to evaluate the efficacy of PD-1/PD-L1 pathway blockade by mAbs [159–163]. As a result of durable responses and survival benefits produced in several tumor types, FDA granted accelerated approval of several immune checkpoint inhibitors (ICPis) as listed in Table 1.1 [164]. This offers proof of concept that checkpoint inhibition provides durable and meaningful response in a subset of patients with responsive tumors.

Besides CTLA-4 and PD-1/PD-L1 signaling pathways, other immune regulatory pathways are being investigated as potential therapeutic targets. IDO is one such immunosuppressive pathway exploited by tumor cells to evade immune surveillance [165]. Several IDO inhibitors are under clinical development including INCB024360 [166, 167], indoximod [168], IDO peptide vaccine [169], BMS-986205 [170], and NLG919 [171]. A robust therapeutic immune response is produced not only by releasing the "brakes" on T cells, but also by stepping on the "gas." T cell co-stimulation through receptors, like OX40 or 4-1BB, provides a potent "go" signal that actively promotes the optimal "killer" CD8 T cell responses [172]. Several ongoing clinical trials are investigating immune checkpoint agonist therapies as single-agent or in combination with other immunotherapies, chemotherapy, targeted therapy, or radiotherapy.

Despite the success with ICPis (CTLA-4, PD-1/PD-L1 blockade) in various tumor types, many patients are primarily resistant or develop resistance to treatment after an initial period of response [173]. Among several mechanistic approaches being investigated in the clinic to overcome primary and secondary resistance to the ICPis, there is growing evidence that combination therapies are far more effective than monotherapies to combat resistance mechanisms as tumors use multiple pathways to evade immune elimination [174]. Further, as these co-inhibitory receptors have non-redundant signaling pathways, a combined blockade of these mechanistically different pathways may be synergistic in restoring T cell-mediated immune response [128]. Recently, FDA approved nivolumab in combination with ipilimumab for the treatment of patients with BRAF V600 wild-type, unresectable or metastatic melanoma and advanced renal cell carcinoma [164]. There is intense research to identify optimal combinations that would increase the response rate and the duration of response. Targeted therapies are known to produce rapid onset of tumor regression [175]. However, the response is short-lived. On the contrary, immunotherapies produce more durable response; but, it takes longer to initiate tumor regression. Due to their complimentary outcomes, combinations of targeted and immunotherapy are being investigated in several clinical trials and emerging

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

Drug	Immune checkpoint(s)	FDA-approved tumor type[b]
Ipilimumab	CTLA-4	Unresectable or metastatic melanoma
Nivolumab	PD-1	Unresectable or metastatic melanoma
		Metastatic non-small cell lung cancer
		Advanced renal cell carcinoma
		Classical Hodgkin lymphoma
		Recurrent or metastatic squamous cell carcinoma of the head and neck
		Locally advanced or metastatic urothelial carcinoma
		Mismatch repair deficient and microsatellite instability high metastatic colorectal cancer
		Hepatocellular carcinoma
Pembrolizumab	PD-1	Unresectable or metastatic melanoma
		PD-L1-positive non-small cell lung cancer
		Recurrent or metastatic squamous cell carcinoma of the head and neck
		Classical Hodgkin lymphoma
		Locally advanced or metastatic urothelial carcinoma
		Unresectable or metastatic microsatellite instability-high or mismatch repair deficient solid tumors
		Recurrent locally advanced or metastatic PD-L1-positive gastric or gastroesophageal junction adenocarcinoma
Atezolizumab	PD-L1	Metastatic urothelial carcinoma
		Metastatic non-small cell lung cancer
Durvalumab	PD-L1	Locally advanced or metastatic urothelial carcinoma
		Unresectable stage III non-small cell lung cancer
Avelumab	PD-L1	Metastatic Merkel cell carcinoma
		Locally advanced or metastatic urothelial carcinoma
Nivolumab in combination with ipilimumab	PD-1 and CTLA-4	Unresectable or metastatic melanoma Advanced renal cell carcinoma

[a]List of FDA-approved immune checkpoint inhibitors as of May 15, 2018, adapted from: https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm
[b]Tumor type must meet the criteria listed in the abovementioned website

data suggests that such combinations may potentially be synergistic [176]. Similarly, radiation-induced immunomodulatory changes provide local control and prolong survival, but are insufficient to shift the balance of the immunosuppressive TME to

achieve tumor rejection [177]. To overcome this limitation, clinical studies evaluating the combination of radiotherapy and ICPis are currently underway [178, 179].

Emerging data suggest that activation of innate immune system could break the immunosuppressive dynamics of TME to evoke an effective antitumor immune response. Importantly, this process leads to initiation of adaptive immune response by enhancement of the T cell priming process. TLRs, the most important receptors in innate immunity exhibit dual role in cancer [180]. While some TLRs on cancer cells favor tumor progression [181, 182] and promote resistance to chemotherapy, most TLRs on immune cells serve as sensors [180]. Activation of these TLRs by foreign antigens triggers a cascade of pro-inflammatory reactions that ultimately initiates an adaptive immune response. Thus TLRs have been identified as potential targets and several TLR agonists (TLR3, TLR4, TLR5, and TLR7 agonists) are being investigated for clinical application [183, 184]. Similarly, an endoplasmic-reticulum-membrane protein STING (Stimulator of Interferon Genes) that is highly expressed in the APCs mediates potent antitumor activity by induction of innate immunity and initiation of adaptive immunity [184]. Typically, self DNA is located in the nucleus or mitochondrion, while microbial/tumor-derived DNA is located in the cytoplasm. By virtue of their location, the tumor-derived DNA is identified by several cytosolic DNA sensors triggering activation of STING signaling in the APCs [185]. The resultant downstream signaling through STING pathway results in phosphorylation of interferon regulatory factor 3 (IRF3) and nuclear factor-κB and subsequent induction of pro-inflammatory molecules, IFN β and cytokines such as TNF, IL-1β, and IL-6. In the process, IFNs also promote cross-priming of T cells by the DCs resulting in initiation of adaptive immune response [186]. As activation of STING pathway promotes T cell priming and induction of adaptive immune mechanism, several STING agonists as vaccine adjuvants and in combination with other immunomodulators are being investigated [187–189]. Thus strategies that bridge the innate and adaptive immune response may have therapeutic utility.

Translational Relevance

Immunotherapeutic agents have revolutionized the treatment paradigm of patients with advanced cancer. However, significant survival benefit has been observed only in a subset of patients. Biomarker-driven drug development is therefore critical, as it may help physicians to preselect patients who are most likely to derive benefit, and more importantly, allow patients who are less likely to benefit to look for alternate therapies and spare them from avoidable immune-related toxicities and cost of treatment [190]. Some of the important biomarkers of response are:

PD-L1 Expression

Early phase I trials suggest that cell surface expression of PD-L1 on tumor cells in pretreatment tissue samples could serve as biomarker of response to treatment with anti-PD-1/PD-L1 therapies. In a phase I study of MDX-1106, an anti-PD-1 inhibitor, in 39 patients with advanced cancers, tumor biopsies from 9 patients were analyzed for PD-L1 expression by immunohistochemistry (IHC) [159]. 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 [191]. 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 [192]. 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, objective response rate (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 [193]. Surprisingly, response to treatment was observed even in patients with PD-L1-negative disease. In addition, the association between response to therapy and PD-L1 status was discordant depending on PD-L1 expression on tumor cells or tumor immune cells. PD-L1 expression on tumor-infiltrating immune cells was significantly associated with response to MPDL3280A ($P = 0.007$), whereas, PD-L1 expression on tumor cells was not significantly associated with response ($P = 0.079$). In addition, in a phase III study, survival benefits were seen in NSCLC patients treated with atezolizumab compared to docetaxel regardless of PD-L1 expression in the tumor or immune cells [194]. There is also marked heterogeneity in PD-L1 expression between samples from the primary and metastatic sites in the same individual [195]. Further, the predictive potential of PD-L1 expression is challenged due to technical issues such as lack of standardized PD-L1 diagnostic assay, use of different PD-L1 antibody clones by multiple immune assays, different staining procedures for IHC staining, different cut-off values and scoring patterns [196]. As a result, there is lack of defined criteria to determine PD-L1-status of the patient. The above findings suggest that though PD-L1 expression in tumor tissue may indicate an increased likelihood of response to treatment with PD-1/PD-L1 inhibitors, it may not be a definitive biomarker to exclude PD-L1-negative patients from therapy [193, 197].

Tumor Infiltrating Lymphocytes (TILs)

There is a broad literature of evidence that infiltration of tumor tissue by T cells, specifically CD8+ T cell density at the invasive tumor edge, is associated with improved survival in patients with melanoma, breast, ovarian, lung, esophageal, gastric, renal cell, colorectal and bladder carcinoma among other solid tumors [198–200]. On the contrary, infiltration of the tumor tissue by Tregs is associated with poor survival in ovarian, breast cancer, hepatocellular carcinoma [201–203]. Interestingly, strong intratumoral infiltration by CD8+ T cells and Th1 cells did not favor immune elimination of tumors in patients with mismatch repair-deficient colorectal cancer [204]. Despite a hostile TME, the tumors survived due to strong co-expression of several immune checkpoints such as PD-1, PD-L1, CTLA-4, Lag-3, and IDO in the invasive margin, stroma, and TILs. This finding suggests that the tumors may be responsive to checkpoint blockade. As a result, MMR status may be predictive of response to checkpoint inhibition.

Further, the type, density, and location of immune cells within the tumor (collectively known as immune contexture) have prognostic value. Multiple immune markers including total T lymphocytes (CD3), T cell effectors (CD8), their associated cytotoxic molecule (GZMB), and memory T cells (CD45RO) in the center of tumor (CT) and the invasive margin (IM) were quantified using IHC in tumors from 415 colorectal cancer patients [205]. The immune cell densities in each tumor region were higher in patients without recurrence than in patients with recurrence and were predictive of disease free survival (DFS) and OS. These results were independent of the staging of the tumor indicating the role of adaptive immune response in preventing tumor recurrence. In addition, presence of markers for Th1 polarization, cytotoxic and memory cells were predictive of low recurrence rate.

Baseline expression of TILs may not always suggest response to immune checkpoint blockade. TILs may not always predict response to ICPis. For example, CD8+ T cells at the IM were positively associated to response with pembrolizumab in patients with metastatic melanoma [206], but not in patients with unresectable stage III/IV melanoma treated with ipilimumab [207]. However, on treatment increase in the levels of tumor infiltrating T cells at the CT and IM were predictive of response to treatment with ICPi in several studies [206–208]. The antitumor activity was largely dependent on pre-existing adaptive immune mechanism as evidenced by the presence of higher numbers of CD8-, PD-1-, and PD-L1-expressing cells in the baseline samples [206].

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 [205]. Marked

variations in clinical outcomes among patients with the same stage of disease were observed with TNM classification, partly due to failure to include the immune cells in the TME in TNM classification of tumors. As the interaction between the tumor cells and the immune cells play an important role in immune escape and progression of the tumor, immune contexture discussed above is a better prognostic indicator than TNM classification [209]. Therefore, a new scoring system was derived from immune contexture called the immunoscore, which is a ratio of the densities of two lymphocyte populations, CD3/CD45RO, CD3/CD8 or CD8/CD45RO, in the CT and IM. Due to difficulty in staining methods, a combination of two markers (CD3+ and CD8+) in CT and IM has been used by the worldwide immunoscore consortium in the development and validation of immunoscore as prognostic markers in different patient populations. The score ranges from immunoscore 0 (I0), when the densities of both the lymphocyte populations are low in both the regions to immunoscore 4 (I4), when the densities of both the lymphocyte populations are high in both the regions. This score is the strongest prognostic indicator of DFS and OS in patients with local and metastatic disease [210]. Recently, the consensus immunoscore was validated in a study conducted by an international consortium of centers in 13 countries [211]. In the analysis that included tissue samples from 2681 colorectal cancer patients, patients with a high immunoscore had the lowest risk of recurrence in 5 years, prolonged DFS and OS, a finding that has been confirmed in both the internal and external validation set. This scoring system will help to stratify patients based on the risk of recurrence. However, the universal application of immunoscore across tumor types has to be determined.

T Cell Receptor Diversity

As T cells play an important role in recognition and eradication of cancer cells, a diverse TCR repertoire will allow for detection of wide range of foreign antigens. On activation, TCRs undergo clonal expansion. Thus characterization and estimation of TCR repertoire diversity by next generation sequencing of CDR3 may provide insight into antitumor activity of ICPis. In a melanoma patient with metastatic lesion to the brain that progressed on ipilimumab, a durable complete clinical response was achieved with sequential whole brain radiation therapy and pembrolizumab [212]. A high-throughput CDR3 sequencing of the intratumoral T cells in the brain metastasis obtained before treatment and the circulating peripheral T cells obtained sequentially during treatment showed that the dominant CD8+ T cell clone in the brain metastasis (pretreatment) had clonally expanded on treatment with pembrolizumab and was detected as the most frequently occurring clone in the blood. This indicates presence of pre-existing but inadequate adaptive immune response that was bolstered by treatment with pembrolizumab. Similar on-treatment clonal expansion of a CD8+ T cell clone present in the metastatic site prior to treatment was seen in a NSCLC patient who experienced pathological complete response with nivolumab [213]. In 10 patients with metastatic melanoma treated with

nivolumab [214], oligoclonal expansion of certain TCR-β clonotypes was observed in post-treatment tumor tissues of responders. Similar results were also observed in 25 patients with metastatic melanoma treated with pembrolizumab [206]. TCR sequencing of pre- and post-treatment samples showed the number of clones that had expanded was ten times more in the responders than in non-responders. Further, clinical response was associated with a more restricted TCR beta chain usage in pre-dosing samples. Thus, a diverse TCR repertoire at baseline and on-treatment tumor antigen-specific clonal expansion may be predictive of response to treatment with ICPis.

Mutation Load and Molecular Alterations

Tumors with high mutational load such as melanoma, NSCLC, head and neck squamous cell carcinoma (HNSCC) are more likely to respond to treatment with ICPis as neoepitomes generated by somatic mutations function as neoantigens and elicit a brisk immune response [215]. In several clinical trials, higher clinical benefit rate and longer progression-free survival have been reported in patients with high mutation burden treated with ICPis [215–217]. It is for the same reason that improved treatment outcomes with ICPis have been reported in patients with solid tumors, colorectal cancer patients in particular, with defects in the mismatch repair (MMR) mechanism [218, 219]. However, Snyder and colleagues described that while high mutational load correlated to sustained response to CTLA-4 blockade, not all melanoma patients with high mutational load responded to therapy [216]. But, the presence of tetrapeptide neoepitope signature in these patients with high mutation load correlated strongly with long-term clinical benefit and OS. On the contrary, tumors with low mutational loads (e.g., pancreatic and prostate cancer) were not responsive to ICPi. Also, molecular alterations in the PI3K pathway may promote tumor immune evasion through constitutive expression of PD-L1 [220]. 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 [199].

Immune Gene Signature

Differential expression of genes may help to identify phenotypes responsive to treatment with ICPis. For example, loss-of-function *BRCA2* mutations with specific mutational signatures were identified in responding melanoma tumors sampled from patients on treatment with anti-PD-1 agents [217]. Likewise, in melanoma patients treated with pembrolizumab, an IFN-γ 10-gene and an expanded immune 28-gene signatures in pretreatment samples were significantly associated with ORR and PFS

[221]. On further evaluation, more refined immune signatures were found to produce similar results in patients with HNSCC and gastric cancer [222]. High pretreatment levels of IFN-γ mRNA and PD-L1 protein expression were associated with increased ORR and longer OS in NSCLC patients treated with durvalumab [223]. A similar association between high expression of T-effector-associated, IFN-γ-associated and PD-L1 genes in tumor tissue and improved OS was seen in NSCLC patients treated with atezolizumab [224]. The T-effector-associated and IFN-γ-associated gene expression was associated with PD-L1 expression on immune cells and not on tumor cells suggesting the role of pre-existing adaptive immune response. On the contrary, a group of 26 innate anti-PD-1 resistance (IPRES) signatures characterized by higher expression of mesenchymal transition, angiogenesis, hypoxia, and wound healing genes were identified in pretreatment melanoma tumors resistant to anti-PD-1 therapy [217]. The IPRES signature was also found in non-responsive pretreatment tumor samples from patients with other solid tumors such as adenocarcinoma of the lung, colon, and pancreas and clear cell carcinoma of kidney. Thus immune-related gene expression signatures may be associated with treatment outcomes.

Cancer Immunogram

The cancer immunogram model was developed to overcome the limitation that no single biomarker can truly reflect the dynamic interaction between the immune cells and tumor. Based on the assumption that T cells are the ultimate effectors of antitumor activity, seven parameters were included in the model to understand the interaction between the tumor and the immune cells in the TME of the patient [225]. The seven parameters and their potential biomarkers in parenthesis are: (1) tumor foreignness (mutation load), (2) general immune status (lymphocyte count), (3) immune cell infiltration (intratumoral T cells), (4) absence of checkpoints (PD-L1), (5) absence of soluble inhibitors (IL-6 and C-reactive protein [CRP]), (6) absence of inhibitory tumor metabolism (lactate dehydrogenase [LDH], glucose utilization), (7) and tumor sensitivity to immune effectors (MHC expression, IFN-γ sensitivity). The data points for each of the seven parameters are plotted in a radar plot and the line joining the individual data points provides a personalized framework reflecting the interaction in the TME. The gaps in the radar plot indicate potential therapeutic strategies that may evoke an effective immune response in the patient.

A modified immunogram has been developed based on the seven steps in the cancer immunity cycle for use in NSCLC patients [226]. The eight axes of the immunogram score (IGS) are: IGS_1, existence of T cell immunity in the tumor; IGS_2, tumor antigenicity (existence of neoantigens and cancer germline antigens); IGS_3, priming and activation (presence of activated DCs); IGS_4, trafficking and T cell infiltration; IGS_5, recognition of tumor antigens; IGS_6, absence of inhibitory cells (Tregs and MDSCs); IGS_7, absence of checkpoint expression (PD-1, PD-L1, etc.); and IGS_8, absence of inhibitory molecules (IDO 1, arginase 1, etc.). High scores for $IGS_{1–5}$ indicate a favorable environment for development of T cell immunity. On the

contrary, high scores for $IGS_{6–8}$ indicate immune suppression. Based on the radar plot, three groups of patients have been identified. Patients with high $IGS_{1–5}$ and low $IGS_{6–8}$ represent T cell-rich phenotype where antitumor activity is dampened by an immunosuppressive TME, patients with low IGS_1, $IGS_{3–5}$ represent T cell-poor phenotype with defects in the T cell priming process, and patients in whom IGS_2, $IGS_{6–8}$ are maintained represent an intermediate phenotype. Thus, the immunogram helps to identify areas of therapeutic focus to elicit an effective antitumor response. Cancer immunograms are promising for personalized approach to immunotherapy.

Serum Biomarkers

Several routinely available peripheral blood parameters have been evaluated as a biomarker of response to treatment with ICPis [208, 227–234]. Most common among them are absolute lymphocyte count (ALC), absolute eosinophil count (AEC), LDH, and CRP. In a compassionate use trial with ipilimumab in patients with advanced refractory melanoma, ALC $\geq$1000/μL after two treatments with ipilimumab was significantly associated with clinical benefit and OS [230, 231]. Though ALC at baseline and after one dose of ipilimumab showed only a trend for improved treatment outcomes, they may be prognostic because a threshold ALC of 1000 cells/μL may be required for adequate activation of the immune system for patients to derive meaningful antitumor response with therapy. Similar results were seen in several clinical trials in patients with melanoma treated with ipilimumab [230–234], where an increase in ALC levels from baseline was associated with improved OS and disease control compared to patients with stable or decreasing levels. Likewise increase in AEC levels after two courses of ipilimumab was associated with OS [230] and was an independent predictor of response in patients with melanoma [235]. On the other hand, elevated level of LDH at baseline was an independent predictor of poor survival [230, 236]. Despite the association between these peripheral blood parameters and treatment outcomes, there is no validated biomarker available for use in the clinic.

Circulating Biomarkers

Serial assessment of circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), which is a measure of tumor burden, may predict response to treatment with checkpoint inhibitors. The association between ctDNA and treatment outcomes was evaluated in three groups of patients treated with PD-1 inhibitors as single agents or in combination with ipilimumab [237]. Group A included patients with undetectable ctDNA at baseline and during treatment, Group B had patients with detectable ctDNA at baseline but undetectable early during therapy, and Group C included patients with detectable ctDNA at baseline and during therapy. Compared

to baseline ctDNA, persistent on treatment levels of ctDNA was associated with decreased ORR and poor survival. On the other hand, increase in circulating levels of immune cells, Ki-67+ T cells, was associated with clinical benefit in NSCLC patients on treatment with PD-1 inhibitors [238]. If these findings are validated in large prospective cohorts, in the context of intratumoral heterogeneity, minimally invasive and easily accessible liquid biopsies may serve as a more comprehensive alternate technique for biomarker assessment.

Microbiome Assessment

Emerging data indicate that gut microbiome may be associated with response to treatment with PD-1 inhibitors. Alpha diversity of gut microbiomes in fecal samples was significantly higher in patients with metastatic melanoma responding (CR/PR/SD ≥6 months) to treatment with PD-1 inhibitors [239]. And, patients with higher alpha diversity had longer PFS compared to patients with low or intermediate diversity. Further, the gut microbiome was enriched for Clostridiales in responders and Bacteroidales in non-responders. And patients with abundance of Faecalibacterium genus in Clostridiales order had significantly longer PFS compared to patients with abundance of Bacteroidales. Thus favorable gut microbiome may enhance antitumor response in patients treated with checkpoint inhibitors.

Due to the dynamic nature of immune response, development of immune oncology biomarkers is challenging. To this end, immune monitoring assays have been developed to perform genomic, proteomic, and functional studies on paired tumor and blood samples obtained before and after treatment with immunotherapeutic agents [197]. 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 [159, 191]. Therefore, identification of a single immunologic biomarker may not be predictive of response [197]. This indicates a need to identify multi-factorial biomarker panels that would help to determine the immunogenic nature of the tumor and predict response or resistance to treatment. For example, presence of intratumoral CD8+ T cells, expression of PD-L1 on tumor cells, and increased mutational load have been associated with greater likelihood of response to PD-1/PD-L1 checkpoint inhibition [240].

Conclusion

Seminal studies have described the different components of the innate and adaptive immune system. Though they are two distinct arms of the human immune system, they are intricately organized in time and space and are critically dependent upon

one another. While the blockade of immune checkpoints by mAbs to unleash the antitumor immune response by T cells has now emerged as a powerful therapeutic tool in the treatment of advanced cancer, components of the innate immune system contribute to the activation and development of adaptive immunity. Improved understanding of the interaction between the tumor cells and the immune cells in the complex TME through rigorous immune profiling will guide the future development of new immunotherapeutic strategies as well as the identification of potential biomarkers of clinical response.

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; 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 CA Jr, 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–E80.
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, DW MG Jr, 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. Arterioscler Throm Vasc Biol. 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(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 anti-tumour 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. 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.
121. 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.
122. Nocentini G, Riccardi C. GITR: a modulator of immune response and inflammation. Adv Exp Med Biol. 2009;647:156–73.
123. 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.
124. 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.

125. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways similarities, differences, and implications of their inhibition. Am J Clin Oncol. 2016;39:98–106.
126. 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.
127. 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.
128. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity. 2016;44:989–1004.
129. Joller N, Lozano E, Burkett PR, Patel B, Xiao S, Zhu C, Xia J, Tan TG, Sefik E, Yajnik V, Sharpe AH, Quintana FJ, Mathis D, Benoist C, Hafler DA, Kuchroo VK. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity. 2014;40:569–81.
130. 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.
131. Croft M. The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol. 2009;9:271–85.
132. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64.
133. Nagasawa T. Microenvironmental niches in the bone marrow required for B-cell development. Nat Rev Immunol. 2006;6:107–16.
134. 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.
135. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. J Exp Med. 1993;177:1009–20.
136. 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.
137. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. J Allergy Clin Immunol. 2013;131:959–71.
138. Shlomchik MJ, Weisel F. Germinal center selection and the development of memory B and plasma cells. Immunol Rev. 2012;247:52–63.
139. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010;125:S41–52.
140. 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.
141. Kung P, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T cell surface antigens. Science. 1979;206:347–9.
142. 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.
143. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature. 1988;332:323–7.
144. 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.
145. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer. 2012;12:278–87.
146. 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.

147. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22:329–60.
148. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
149. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
150. Teng MW, Galon J, Fridman WH, Smyth MJ. From mice to humans: developments in cancer immunoediting. J Clin Invest. 2015;125:3338–46.
151. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480:480–9.
152. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12:253–68.
153. 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.
154. 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.
155. 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.
156. Weiner LM, Surana R, Wang SZ. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol. 2010;10:317–27.
157. 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.
158. 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.
159. 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.
160. 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 GR Jr, 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.
161. 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.
162. 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 G Jr, 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;387:1540–50.
163. 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.
164. U.S. Food and Drug Administration. Hematology/oncology (cancer) approvals & safety notifications. 2018.
165. Moon YW, Hajjar J, Hwu P, Naing A. Targeting the indoleamine 2,3-dioxygenase pathway in cancer. J Immunother Cancer. 2015;3:51.
166. 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.
167. 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.
168. 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.
169. 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;20:221–32.
170. Siu LL, Gelmon K, Chu Q, Pachynski R, Alese O, Basciano P, Walker J, Mitra P, Zhu L, Phillips P, Hunt J, Desai J. Abstract CT116: BMS-986205, an optimized indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, is well tolerated with potent pharmacodynamic (PD) activity, alone and in combination with nivolumab (nivo) in advanced cancers in a phase 1/2a trial. Cancer Res. 2017;77:CT116-CT.
171. Mautino MR, Jaipuri FA, Waldo J, Kumar S, Adams J, Allen CV, Marcinowicz-Flick A, Munn D, Vahanian N, Link CJJ. NLG919, a novel indoleamine-2,3-dioxygenase (IDO)-pathway inhibitor drug candidate for cancer therapy. AACR; 2013. p. 491.
172. Linch SN, McNamara MJ, Redmond WL. OX40 agonists and combination immunotherapy: putting the pedal to the metal. Front Oncol. 2015;5:34.
173. 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.
174. Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. J Clin Oncol. 2011;29:4828–36.
175. Wargo JA, Cooper ZA, Flaherty KT. Universes collide: combining immunotherapy with targeted therapy for cancer. Cancer Discov. 2014;4:1377–86.
176. Sullivan RJ, Gonzalez R, Lewis KD, Hamid O, Infante JR, Patel MR, Hodi FS, Wallin J, Pitcher B, Cha E, Roberts L, Ballinger M, Hwu P. Atezolizumab (a) + cobimetinib (C) + vemurafenib (V) in BRAFV600-mutant metastatic melanoma (mel): updated safety and clinical activity. J Clin Oncol. 2017;35:3063.
177. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. J Natl Cancer Inst. 2013;105:256–65.
178. Golden EB, Chachoua A, Fenton-Kerimian MB, Demaria S, Formenti SC. Abscopal responses in metastatic non-small cell lung cancer (NSCLC) patients treated on a phase 2 study of combined radiation therapy and ipilimumab: evidence for the in situ vaccination hypothesis of radiation. Int J Radiat Oncol. 2015;93:S66–S7.
179. Fiorica F, Belluomini L, Stefanelli A, Santini A, Urbini B, Giorgi C, Frassoldati A. Immune checkpoint inhibitor nivolumab and radiotherapy in pretreated lung cancer patients: efficacy and safety of combination. Am J Clin Oncol. 2018;41:1101–05.

180. Cai Z, Sanchez A, Shi Z, Zhang T, Liu M, Zhang D. Activation of toll-like receptor 5 on breast cancer cells by flagellin suppresses cell proliferation and tumor growth. Cancer Res. 2011;71:2466–75.
181. Wolska A, Lech-Maranda E, Robak T. Toll-like receptors and their role in carcinogenesis and anti-tumor treatment. Cell Mol Biol Lett. 2009;14:248–72.
182. Liu Y, Yan W, Tohme S, Chen M, Fu Y, Tian D, Lotze M, Tang DL, Tsung A. Hypoxia induced HMGB1 and mitochondrial DNA interactions mediate tumor growth in hepatocellular carcinoma through toll-like receptor 9. J Hepatol. 2015;63:114–21.
183. Shi M, Chen X, Ye K, Yao Y, Li Y. Application potential of toll-like receptors in cancer immunotherapy: systematic review. Medicine (Baltimore). 2016;95:e3951.
184. Li K, Qu S, Chen X, Wu Q, Shi M: Promising targets for cancer immunotherapy: TLRs, RLRs, and STING-mediated innate immune pathways. Int J Mol Sci 2017;18. pii: E404.
185. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 2009;461:788–92.
186. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, Gajewski TF. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. J Exp Med. 2011;208:2005–16.
187. Corrales L, Glickman LH, McWhirter SM, Kanne DB, Sivick KE, Katibah GE, Woo SR, Lemmens E, Banda T, Leong JJ, Metchette K, Dubensky TW Jr, Gajewski TF. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell Rep. 2015;11:1018–30.
188. Fu J, Kanne DB, Leong M, Glickman LH, McWhirter SM, Lemmens E, Mechette K, Leong JJ, Lauer P, Liu W, Sivick KE, Zeng Q, Soares KC, Zheng L, Portnoy DA, Woodward JJ, Pardoll DM, Dubensky TW, Kim Y. STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. Sci Transl Med. 2015;7:283ra52.
189. Deng LF, Liang H, Xu M, Yang XM, Burnette B, Arina A, Li XD, Mauceri H, Beckett M, Darga T, Huang XN, Gajewski TF, Chen ZJJ, Fu YX, Weichselbaum RR. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity. 2014;41:843–52.
190. 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.
191. 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.
192. 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.
193. 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.
194. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, Cortinovis DL, Leach J, Polikoff J, Barrios C, Kabbinavar F, Frontera OA, De Marinis F, Turna H, Lee JS, Ballinger M, Kowanetz M, He P, Chen DS, Sandler A, Gandara DR, Grp OS. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet. 2017;389:255–65.
195. 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;28:245–53.
196. Rosell R, Palmero R. PD-L1 expression associated with better response to EGFR tyrosine kinase inhibitors. Cancer Biol Med. 2015;12:71–3.
197. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348:56–61.
198. 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.
199. 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.
200. Ruffini E, Asioli S, Filosso PL, Lyberis P, Bruna MC, Macri L, Daniele L, Oliaro A. Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. Ann Thorac Surg. 2009;87:365–71; discussion 371–2
201. 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.
202. 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.
203. 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.
204. 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.
205. 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.
206. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515:568–71.
207. Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, Guida M, Hyams DM, Gomez H, Bastholt L, Chasalow SD, Berman D. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med. 2011;9:204.
208. Martens A, Wistuba-Hamprecht K, Yuan J, Postow MA, Wong P, Capone M, Madonna G, Khammari A, Schilling B, Sucker A, Schadendorf D, Martus P, Dreno B, Ascierto PA, Wolchok JD, Pawelec G, Garbe C, Weide B. Increases in absolute lymphocytes and circulating CD4+ and CD8+ T cells are associated with positive clinical outcome of melanoma patients treated with ipilimumab. Clin Cancer Res. 2016;22:4848–58.
209. 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.

210. Mlecnik B, Van den Eynde M, Bindea G, Church SE, Vasaturo A, Fredriksen T, Lafontaine L, Haicheur N, Marliot F, Debetancourt D, Pairet G, Jouret-Mourin A, Gigot JF, Hubert C, Danse E, Dragean C, Carrasco J, Humblet Y, Valge-Archer V, Berger A, Pages F, Machiels JP, Galon J. Comprehensive intrametastatic immune quantification and major impact of immunoscore on survival. J Natl Cancer Inst. 2018;110:97–108.
211. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou F-S, Bifulco C, Lugli A, Zlobec I, Rau TT, Berger MD, Nagtegaal ID, Vink-Börger E, Hartmann A, Geppert C, Kolwelter J, Merkel S, Grützmann R, Van den Eynde M, Jouret-Mourin A, Kartheuser A, Léonard D, Remue C, Wang JY, Bavi P, MHA R, Ohashi PS, Nguyen LT, Han S, HL MG, Hafezi-Bakhtiari S, Wouters BG, Masucci GV, Andersson EK, Zavadova E, Vocka M, Spacek J, Petruzelka L, Konopasek B, Dundr P, Skalova H, Nemejcova K, Botti G, Tatangelo F, Delrio P, Ciliberto G, Maio M, Laghi L, Grizzi F, Fredriksen T, Buttard B, Angelova M, Vasaturo A, Maby P, Church SE, Angell HK, Lafontaine L, Bruni D, El Sissy C, Haicheur N, Kirilovsky A, Berger A, Lagorce C, Meyers JP, Paustian C, Feng Z, Ballesteros-Merino C, Dijkstra J, van de Water C, van Lent-van Vliet S, Knijn N, Muşină A-M, Scripcariu D-V, Popivanova B, Xu M, Fujita T, Hazama S, Suzuki N, Nagano H, Okuno K, Torigoe T, Sato N, Furuhata T, Takemasa I, Itoh K, Patel PS, Vora HH, Shah B, Patel JB, Rajvik KN, Pandya SJ, Shukla SN, Wang Y, Zhang G, Kawakami Y, Marincola FM, Ascierto PA, Sargent DJ, Fox BA, Galon J. International validation of the consensus immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018;391:2128–39.
212. Haymaker CL, Kim D, Uemura M, Vence LM, Phillip A, McQuail N, Brown PD, Fernandez I, Hudgens CW, Creasy C, Hwu WJ, Sharma P, Tetzlaff MT, Allison JP, Hwu P, Bernatchez C, Diab A. Metastatic melanoma patient had a complete response with clonal expansion after whole brain radiation and PD-1 blockade. Cancer Immunol Res. 2017;5:100–5.
213. Olugbile S, Park J-H, Hoffman P, Szeto L, Patel J, Vigneswaran WT, Vokes E, Nakamura Y, Klyotani K. Sustained oligoclonal T cell expansion correlates with durable response to immune checkpoint blockade in lung cancer. J Cancer Sci Ther. 2017;9:717–22.
214. Inoue H, Park JH, Kiyotani K, Zewde M, Miyashita A, Jinnin M, Kiniwa Y, Okuyama R, Tanaka R, Fujisawa Y, Kato H, Morita A, Asai J, Katoh N, Yokota K, Akiyama M, Ihn H, Fukushima S, Nakamura Y. Intratumoral expression levels of PD-L1, GZMA, and HLA-A along with oligoclonal T cell expansion associate with response to nivolumab in metastatic melanoma. Oncoimmunology. 2016;5:e1204507.
215. 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.
216. 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.
217. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, Berent-Maoz B, Pang J, Chmielowski B, Cherry G, Seja E, Lomeli S, Kong X, Kelley MC, Sosman JA, Johnson DB, Ribas A, Lo RS. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell. 2016;165:35–44.
218. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube

JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372:2509–20.
219. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjord JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jager N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, Lopez-Otin C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdes-Mas R, van Buuren MM, van't Veer L, Vincent-Salomon A, Waddell N, Yates LR, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR. Signatures of mutational processes in human cancer. Nature. 2013;500:415–21.
220. 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.
221. Ribas A, Robert C, Hodi FS, Wolchok JD, Joshua AM, Hwu WJ, Weber JS, Zarour HM, Kefford R, Loboda A, Albright A, Kang SP, Ebbinghaus S, Yearley J, Murphy E, Nebozhyn M, Lunceford JK, McClanahan T, Ayers M, Daud A. Association of response to programmed death receptor 1 (PD-1) blockade with pembrolizumab (MK-3475) with an interferon-inflammatory immune gene signature. J Clin Oncol. 2015:33.
222. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Albright A, Cheng J, Kang SP, Ebbinghaus S, Yearley J, Shankaran V, Seiwert T, Ribas A, McClanahan T. Relationship between immune gene signatures and clinical response to PD-1 blockade with pembrolizumab (MK-3475) in patients with advanced solid tumors. J Immunother Cancer. 2015;3:P80.
223. Higgs BW, Morehouse C, Streicher K, Rebelatto MC, Steele K, Jin X, Pilataxi F, Brohawn PZ, Blake-Haskins JA, Gupta AK, Ranade K. Relationship of baseline tumoral IFNγ mRNA and PD-L1 protein expression to overall survival in durvalumab-treated NSCLC patients. J Clin Oncol. 2016;34:3036.
224. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, Park K, Smith D, Artal-Cortes A, Lewanski C, Braiteh F, Waterkamp D, He P, Zou W, Chen DS, Yi J, Sandler A, Rittmeyer A, Group PS. 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;387:1837–46.
225. Blank CU, Haanen JB, Ribas A, Schumacher TN. Cancer immunology. The "cancer immunogram". Science. 2016;352:658–60.
226. Karasaki T, Nagayama K, Kuwano H, Nitadori JI, Sato M, Anraku M, Hosoi A, Matsushita H, Morishita Y, Kashiwabara K, Takazawa M, Ohara O, Kakimi K, Nakajima J. An immunogram for the cancer-immunity cycle: towards personalized immunotherapy of lung cancer. J Thorac Oncol. 2017;12:791–803.
227. Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, Wong P, Romano E, Khammari A, Dreno B, Capone M, Ascierto PA, Di Giacomo AM, Maio M, Schilling B, Sucker A, Schadendorf D, Hassel JC, Eigentler TK, Martus P, Wolchok JD, Blank C, Pawelec G, Garbe C, Weide B. Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. Clin Cancer Res. 2016;22:2908–18.
228. Hopkins AM, Rowland A, Kichenadasse G, Wiese MD, Gurney H, McKinnon RA, Karapetis CS, Sorich MJ. Predicting response and toxicity to immune checkpoint inhibitors using routinely available blood and clinical markers. Br J Cancer. 2017;117:913–20.
229. Manson G, Norwood J, Marabelle A, Kohrt H, Houot R. Biomarkers associated with checkpoint inhibitors. Ann Oncol. 2016;27:1199–206.
230. 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.

231. 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.
232. Wilgenhof S, Du Four S, Vandenbroucke F, Everaert H, Salmon I, Lienard D, Marmol VD, Neyns B. Single-center experience with ipilimumab in an expanded access program for patients with pretreated advanced melanoma. J Immunother. 2013;36:215–22.
233. Di Giacomo AM, Danielli R, Calabro L, Bertocci E, Nannicini C, Giannarelli D, Balestrazzi A, Vigni F, Riversi V, Miracco C, Biagioli M, Altomonte M, Maio M. Ipilimumab experience in heavily pretreated patients with melanoma in an expanded access program at the University Hospital of Siena (Italy). Cancer Immunol Immunother. 2011;60:467–77.
234. Simeone E, Gentilcore G, Giannarelli D, Grimaldi AM, Caraco C, Curvietto M, Esposito A, Paone M, Palla M, Cavalcanti E, Sandomenico F, Petrillo A, Botti G, Fulciniti F, Palmieri G, Queirolo P, Marchetti P, Ferraresi V, Rinaldi G, Pistillo MP, Ciliberto G, Mozzillo N, Ascierto PA. Immunological and biological changes during ipilimumab treatment and their potential correlation with clinical response and survival in patients with advanced melanoma. Cancer Immunol Immunother. 2014;63:675–83.
235. Gebhardt C, Sevko A, Jiang HH, Lichtenberger R, Reith M, Tarnanidis K, Holland-Letz T, Umansky L, Beckhove P, Sucker A, Schadendorf D, Utikal J, Umansky V. Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. Clin Cancer Res. 2015;21:5453–9.
236. Kelderman S, Heemskerk B, van Tinteren H, van den Brom RR, Hospers GA, van den Eertwegh AJ, Kapiteijn EW, de Groot JW, Soetekouw P, Jansen RL, Fiets E, Furness AJ, Renn A, Krzystanek M, Szallasi Z, Lorigan P, Gore ME, Schumacher TN, Haanen JB, Larkin JM, Blank CU. Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. Cancer Immunol Immunother. 2014;63:449–58.
237. Lee JH, Long GV, Boyd S, Lo S, Menzies AM, Tembe V, Guminski A, Jakrot V, Scolyer RA, Mann GJ, Kefford RF, Carlino MS, Rizos H. Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. Ann Oncol. 2017;28:1130–6.
238. Kamphorst AO, Pillai RN, Yang S, Nasti TH, Akondy RS, Wieland A, Sica GL, Yu K, Koenig L, Patel NT, Behera M, Wu H, McCausland M, Chen ZJ, Zhang C, Khuri FR, Owonikoko TK, Ahmed R, Ramalingam SS. Proliferation of PD-1+CD8 T cells in peripheral blood after PD-1-targeted therapy in lung cancer patients. Proc Natl Acad Sci U S A. 2017;114:4993–8.
239. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, Cogdill AP, Zhao L, Hudgens CW, Hutchinson DS, Manzo T, de Macedo MP, Cotechini T, Kumar T, Chen WS, Reddy SM, Sloane RS, Galloway-Pena J, Jiang H, Chen PL, Shpall EJ, Rezvani K, Alousi AM, Chemaly RF, Shelburne S, Vence LM, Okhuysen PC, Jensen VB, Swennes AG, McAllister F, Sanchez EMR, Zhang Y, Le Chatelier E, Zitvogel L, Pons N, Austin-Breneman JL, Haydu LE, Burton EM, Gardner JM, Sirmans E, Hu J, Lazar AJ, Tsujikawa T, Diab A, Tawbi H, Glitza IC, Hwu WJ, Patel SP, Woodman SE, Amaria RN, Davies MA, Gershenwald JE, Hwu P, Lee JE, Zhang J, Coussens LM, Cooper ZA, Futreal PA, Daniel CR, Ajami NJ, Petrosino JF, Tetzlaff MT, Sharma P, Allison JP, Jenq RR, Wargo JA. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science. 2018;359:97–103.
240. 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.

Chapter 2
Immunotherapy for Melanoma

Isabella C. Glitza Oliva and Rana Alqusairi

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

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

Introduction

Melanoma is malignant proliferation of melanocytes, which are primarily found in the skin, but can also be identified in the uvea, gastrointestinal mucosa, genitourinary mucosa, as well as meninges/CNS [1]. While it only comprises about 1% of all skin

I. C. Glitza Oliva (✉)
The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: ICGlitza@mdanderson.org

R. Alqusairi
Baltimore, MD, USA

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_2

cancer cases, it is accountable for the majority of all deaths from skin cancer.[1, 2] Furthermore, the annual incidence has been increasing worldwide.[3, 4] While some of the rise may be caused by increased skin cancer awareness and earlier detection, sun-related behaviors such as indoor tanning have been contributing to the incidence [2]. Based on data from the American Cancer Society, 91,270 new cases of melanoma will be diagnosed in 2018 in the United States alone, with 9320 people expected to die of the disease.[5] Melanoma can affect anyone; but risk factors like fair skin, exposure to ultraviolet radiation (sun exposure, tanning beds), history of blistering sunburns in early age, dysplastic or atypical nevi, 50 or more of small nevi, and familial dysplastic nevus syndrome increase the likelihood of melanoma.[6, 7] It is important to note that although melanoma can be associated with preexisting nevi, about 70% of cases can develop de novo (i.e., not from a preexisting pigmented lesion)[8] [2]. Prognosis is related to many components; and late stage, depth (thicker than 4 mm), advanced age, male sex, and location (chest and back) are associated with poorer prognosis [3]. The survival rate depends primarily on the stage, with 98% 5-year survival for stages I and II, 62% for stage III, and it decreases to 18% for stage IV.[9, 10]

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

---

[1] "Melanoma: Statistics | Cancer.Net." https://www.cancer.net/cancer-types/melanoma/statistics. Accessed 6 Feb. 2018.

[2] "Cancer Facts & Figures 2017—American Cancer Society." https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf. Accessed 6 Feb. 2018.

[3] "Melanoma: Statistics | Cancer.Net." https://www.cancer.net/cancer-types/melanoma/statistics. Accessed 6 Feb. 2018.

[4] "Skin Cancer Screening (PDQ®)—NCBI—NIH." 30 Nov. 2017, https://www.ncbi.nlm.nih.gov/books/NBK65861/. Accessed 6 Feb. 2018.

[5] "Key Statistics for Melanoma Skin Cancer—American Cancer Society." 4 Jan. 2018, https://www.cancer.org/cancer/melanoma-skin-cancer/about/key-statistics.html. Accessed 6 Feb. 2018.

[6] "Cancer Facts & Figures 2017—American Cancer Society." https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf. Accessed 6 Feb. 2018.

[7] "Meta-analysis of risk factors for cutaneous melanoma: I. Common and …" http://www.ejcancer.com/article/S0959-8049(04)00832-9/fulltext. Accessed 6 Feb. 2018.

[8] "Skin Cancer Screening (PDQ®)—NCBI—NIH." 30 Nov. 2017, https://www.ncbi.nlm.nih.gov/books/NBK65861/. Accessed 6 Feb. 2018.

[9] "Melanoma: Statistics | Cancer.Net." https://www.cancer.net/cancer-types/melanoma/statistics. Accessed 6 Feb. 2018.

[10] "Cancer Facts & Figures 2017—American Cancer Society." https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf. Accessed 6 Feb. 2018.

It should be mentioned that we also have seen tremendous results with the use of targeted therapy in melanoma, but the focus of this chapter is to summarize the past, present, and future of immunotherapy approaches used in the management of metastatic melanoma.

History of Melanoma Treatment Options Up to 2011

High Dose Interleukin-2

Interleukin-2 (IL-2) is a T cell growth factor, which stimulates T cell proliferation and cytotoxic activity [4]. It was the first immunotherapy to receive regulatory approval in 1998 for the treatment of metastatic melanoma, based on durable objective responses observed in these patients.

In a pooled analysis of 270 melanoma patients treated with high dose IL-2 (HD IL-2) between 1985 and 1993, the overall objective response rate (ORR) was 16% [with complete response (CR) 6%, and partial response 10%]. Importantly, in patients with an ongoing response at 30-months mark no progression was noted [5].

A retrospective chart review of 45 renal cell and 245 melanoma patients treated with HD IL-2 showed median overall survival (OS) of 16.8 months [6]. For patients who experienced a favorable response to treatment, median OS had not been reached, and for patients with stable disease (SD), the median OS was 38.2 months, compared to patients with progressive disease (PD) with a median survival of 7.9 months. In patients who achieved PR or CR, the 3-year OS was 78%.

The significant toxicities observed with HD IL-2 require intensive monitoring and limit its use to specialized centers [7]. The majority of the major side effects, hypotension, renal impairment, shortness of breath, pulmonary and generalized edema, as well as neuropsychiatric alterations are thought to be caused by capillary leak syndrome and lymphoid infiltration. However, toxicities typically resolve after discontinuation of treatment.

Chemotherapy

Since 1975 until 2011, dacarbazine (DTIC) was the only available therapy for most melanoma patients, despite limited efficacy. While ORR of up to 20% has been reported, CRs are rare (~3–4%), and duration of response is fairly short (median 5–6 months), with only 1–2% of patients experiencing long-term survival [8, 9]. Side effects are typically very manageable and include myelosuppression, mild nausea and vomiting, minimal alopecia, and fatigue, but most patients reported that they are able to maintain acceptable quality of life [10, 11].

The oral analog of DTIC, temozolomide (TMZ), has the ability to penetrate blood brain barrier (BBB) and demonstrated modest antitumor activity [11–13]. A randomized clinical phase III trial including 305 treatment naïve metastatic melanoma patients compared DTIC to TMZ and showed similar median OS between the two groups, with 7.7 months for TMZ-treated patients and 6.4 months for those treated with DTIC [Hazard ratio (HR), 1.18; 95% Confidence Interval (CI), 0.92–1.52] [14]. Median progression-free survival (PFS) time was short for both patients treated with TMZ (1.9 month) or DTIC (1.5 month), however statistically significant ($p = 0.012$; HR, 1.37; 95% CI, 1.07–1.75). Importantly, both TMZ and DTIC had similar safety profiles, with mild to moderate nausea and vomiting and noncumulative, transient myelosuppression being some of the most commonly observed toxicities.

Phase II trials of the combination of carboplatin and paclitaxel have shown to also provide modest clinical benefit for patients with metastatic melanoma. In an initial phase II trial, 17 patients were enrolled who had not received prior platinum or taxane agents [15]. The dose for paclitaxel was 175 mg/m^2, with carboplatin [Area under the curve (AUC) 7.5]. Only 15 were evaluable, as two were taken off study for anaphylactic reactions. Partial responses were observed in 20%, and SD in 47% of patients. Grade III or grade IV hematologic toxicities were common ($n = 11$), but all treatment-related toxicities were reversible and no treatment-related deaths were observed.

This regimen also showed some efficacy as second-line therapy for patients. Prior therapies included TMZ or DTIC [16]. This retrospective study evaluated 31 metastatic melanoma patients, who received treatment with weekly paclitaxel (100 mg/m^2) and carboplatin (AUC 2). Partial responses were observed in 26% ($n = 8$) patients and 19% ($n = 6$) had stable disease. Median duration of response was 5.7 months (range, 2.5–7.3 months). No unexpected toxicities were observed.

Nab-paclitaxel (Abraxane) is an albumin-bound preparation of paclitaxel. Its single agent efficacy was evaluated in a phase II trial enrolling either untreated ($n = 37$) or previously treated patients ($n = 37$) [17]. Abraxane was administered either at 100 mg/m^2 weekly for 3 of 4 weeks (in previously treated patients) or at 150 mg/m^2 (in chemotherapy-naive patients). The response rate was higher for the previously untreated patients (21.6% vs. 2.7%); however, median progression-free survival (PFS) was 4.5 months and 3.5 months, and the median OS was 9.6 months and 12.1 months, respectively. Grade III or IV toxicities included neuropathy, alopecia, neutropenia, and fatigue.

The efficacy of combined nab-paclitaxel (100 mg/m^2) and carboplatin (AUC 2) was tested in a parallel phase II trial, which enrolled either previously treated ($n = 34$, with over ~90% of patients previously treated with DTIC or TMZ) or treatment naïve patients ($n = 39$) [18]. Responses were observed in 25.6% of patients (1 CR, 9 PR) in the treatment naïve cohort (90% CI, 16.7–42.3%) and in 8.8% (3 PR) of patients who had received prior chemotherapy (90% CI, 2.5–21.3%). Median PFS was similar for both groups (4.5 months treatment naïve, 4.1 months pretreated), as was median OS (11.1 months vs. 10.9 months, respectively). Toxicities included thrombocytopenia, neurosensory problems, fatigue, nausea, and vomiting.

The triple chemotherapy combination of cisplatin, vinblastine, and dacarbazine (CVD) showed significant antitumor activity in a phase II study [19]. Vinblastine (1.6 mg/m^2/day for 5 days), DTIC (800 mg/m^2 day 1), and cisplatin (20 mg/m^2/day for 4 days) were administered to patients with metastatic melanoma. Of the 50 evaluable patients, 4% patients ($n = 2$) achieved a CR and 36% ($n = 18$) patients a PR. The median duration of response was 9 months; for patients who experienced a response the median OS was 12 months. Significant toxicities were observed, with dose limiting toxicities consisting of peripheral neuropathy.

The addition of interleukin-2 and interferon to the CVD regimen has been labeled biochemotherapy (BCT). BCT was compared directly to CVD in a phase III trial, enrolling patients that were either treatment naïve or had received adjuvant interferon [20]. A total of 395 patients was assessed (CVD, $n = 195$; BCT, $n = 200$). Response rates were only numerically higher for BCT (19.5% vs. 13.8%, $p = 0.140$), but median PFS was significantly longer for BCT than for CVD (4.8 months vs. 2.9 months; $p = 0.015$). It should be mentioned that the improved PFS did not translate into longer OS (9.0 months vs. 8.7 months) or the significantly higher percentage of patients alive at 1 year (41% vs. 36.9%). In addition, grade 3 and 4 toxicities were more commonly observed with BCT (95% vs. 73%; $p = 0.001$).

While chemotherapy is nowadays rarely used in front line, multiple trials are ongoing to explore the efficacy of chemotherapy agents in combination with immunotherapy (e.g., NCT02617849, NCT01827111, NCT01676649).

Finally, melphalan has been used for decades as part of isolated limb perfusion (ISP) for patients with localized in-transit metastases [21]. While its use has significantly diminished in the era of new effective targeted and immunotherapy, it should be pointed out that melphalan-based ILP (M-ILP) led to complete responses (CRs) in 40–50% and overall responses (OR) of 75–80% of patients [22]. The efficacy of this regimen is even more increased when tumor necrosis factor (TNF) is added to melphalan (TM-ILP) [21].

Adoptive Cell Therapy (ACT)

Adoptive cell therapy represents a patient-tailored therapeutic approach for patients, using the autologous derived T cells. While this approach has been used for decades, its use has been limited by the need for specialized laboratories as well as the need for hospital units able to manage the toxicities from HD IL-2, which is most commonly administered in conjunction with the T cell product [23].

Some of the initial trials using autologous derived TIL for metastatic melanoma patients were reported in 1994 [24]. The ORR was 34% for all patients, and side effects stemmed mainly from the HD-IL. Another clinical trial reported a response rate of 51% (9% CR) in 35 patients with metastatic melanoma. Prior lines of therapy with either HD IL-2 and/or chemotherapy were allowed. All patients were pre-treated with fludarabine and cyclophosphamide for lymphodepletion prior to T cell infusion. Mean duration of response was 11.5 ± 2.2 months [25]. Since then, different

approaches have been developed and tested to improve efficacy and toxicity profile of adoptive cell therapy, and multiple clinical trials are currently ongoing (e.g., NCT02652455, NCT01955460) [26, 27].

Immune Checkpoint Inhibitors

The development of checkpoint inhibitors (CPIs) has revolutionized the treatment in metastatic melanoma, and these agents are now successfully used in various other cancer types. However, research to understand the mechanisms of T cell signal transduction was initiated decades ago [28]. The cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) was discovered in 1987, and competes with CD28 to bind to CD80 (B7-1) and CD86 (B7-2) [29]. By binding, CTLA-4 downregulates pathways of T cell activation by competitively binding to B7 proteins (required for stimulation of T cells). Recently, it also has been shown that anti-CTLA-4 induces the expansion of an $ICOS^+$ Th1-like CD4 effector population, which means it engages a different cellular pathway than the programmed cell death protein 1 (PD-1) antibody, which leads to the expansion of specific tumor-infiltrating exhausted-like CD8 T cell subsets [30]. Similarly to CTLA-4, PD-1 negatively regulates the antitumor response.

To date, one CTLA-4 antibody (ipilimumab) and two anti-PD-1 antibodies have received regulatory approval for the treatment of melanoma, as well as in other cancer types.

Ipilimumab

Ipilimumab is a fully human, monoclonal IgG1 antibody that inhibits CTLA-4. Ipilimumab was initially approved in 2011 by the FDA for the treatment of unresectable metastatic melanoma.

In a randomized, double-blind, phase III study, 676 patients were with either ipilimumab plus gp100 peptide vaccine, gp100 alone, or ipilimumab alone in a 3:1:1 ratio [31]. The OS was significantly longer (10.0 months) in the combination arm than for patients treated with gp100 alone (6.4 months, <0.001), and no difference in the OS was noted between the two ipilimumab groups (HR with ipilimumab plus gp100, 1.04; $p = 0.76$). Ipilimumab as single agent resulted in a RR of 10.9%, with a disease control rate of 28.5%. Patients treated with either ipilimumab or ipilimumab plus gp100 experienced immune-related events in about 60% (compared to 32% with gp100). Grade III or IV toxicities were also higher in the ipilimumab groups (10–15% vs. 3%).

In another phase III trial, 502 untreated metastatic melanoma patients were randomly assigned to either ipilimumab (10 mg/kg) plus DTIC (850 mg/m^2) versus DTIC plus placebo ($n = 252$). The response rate (CR + PR) was 15.2% in patients who received ipilimumab/DTIC combination versus 10.3% in DTIC/placebo group

($p = 0.09$). Addition of ipilimumab led to a significantly longer median OS, as survival was 11.2 and 9.1 months for the DTIC group (HR for death with ipilimumab/DTIC 0.72; $p < 0.001$) [32]. The combination therapy resulted in more grade III and IV toxicities (56.3% vs. 27.5%), with the most common grade 4 toxicity being elevation in liver enzymes.

Furthermore, ipilimumab is currently being tested in various combination, including chemotherapy, radiotherapy, vaccines, and cytokines (NCT02644967, NCT02259231, NCT02307149, NCT02203604, NCT02073123, NCT01940809, NCT03297463), as well as in combination with another CPI (nivolumab, see below).

A retrospective study tried to identify if there is a role for HD IL-2 in patients after they received prior CPI therapy. The authors identified 52 metastatic melanoma patients that received prior ipilimumab and HD and 272 patients that did not receive any prior CPI at time of HD IL-2 [33]. The median OS was similar for both the prior ipilimumab versus no prior CPI (19.3 months vs. 19.4 months), but HD IL-2 led to a higher response rate in ipilimumab-exposed patients (21% vs. 12%). Toxicities were somewhat similar between the two groups, but CTLA-4-induced colitis remained a concern.

PD-1 Inhibitors

PD-1, programmed cell death protein 1, is a negative regulator of T cell activity and is expressed by T cells with excessive exposure to antigens. Its primary ligand, PD-L1, is frequently expressed throughout cancerous cells and TILs [34]. The other ligand, PD-L2, is expressed mainly by antigen-presenting cells (APCs). Both ligands are members of B7 protein family. An association between overexpression of PD-1 and PD-L1 on tumor cells and TILs and disease outcomes has been observed in some tumor types [35].

Nivolumab

Nivolumab is a fully human immunoglobulin IgG4 monoclonal antibody directed against PD-1 and was granted regulatory approval in 2014 for the treatment of metastatic melanoma.

In Checkmate-066, a phase III randomized double-blind study, 418 previously untreated patients with metastatic melanoma without a BRAF mutation were randomly assigned to receive either nivolumab (3 mg/kg) and DTIC-matched placebo, or DTIC (1000 mg/m^2) with nivolumab-matched placebo [36]. The overall response rate was 40% (95% CI, 33.3–47.0) in anti-PD-1-treated patients, with over 7% achieving a complete response versus 13.9% overall response (95% CI, 9.5–19.4) and 1% complete response in the DTIC group. Very encouraging was also the 1-year OS for the nivolumab group was 72.9% as compared to 42.1% in the DTIC group. Nivolumab also compared favorable to dacarbazine in regard to grade 3 and 4 adverse events.

Ipilimumab and Nivolumab in Combination

Based on the outcomes of melanoma patients treated with either CTLA-4 or PD-1 CPI monotherapy and a better understanding of activation of T cells, the combination of ipilimumab and nivolumab was tested. Checkmate-069 was a double-blind phase II study, randomly assigned (in a 2:1 ratio) 142 previously untreated patients with metastatic melanoma to receive ipilimumab 3 mg/kg combined with either nivolumab 1 mg/kg or placebo, once every 3 weeks for four doses, followed by nivolumab 3 mg/kg or placebo every 2 weeks [37]. The overall RR for the combination therapy was 56%, with 22% of patients achieving a CR. Similar to prior reports, the RR for patients with ipilimumab was only 11% ($p < 0.0001$, compared to nivolumab) and no patient had a complete response. At median follow-up of 24.5 months (IQR 9.1–25.7), median PFS had not been reached for the ipilimumab/nivolumab group and was 3.0 months (95% CI 2.7–5.1) in the CTLA-4 only group (HR 0.36, 95% CI 0.22–0.56; $p < 0.0001$). In the combination group, 49% of patients discontinued study drug due to toxicities, compared to 22% in the ipilimumab group, and grade 3 or 4 adverse events were reported in 54% of the patients who received the ipilimumab and nivolumab versus 24% of the patients who received ipilimumab monotherapy, respectively.

In a large, randomized, double-blind, phase 3 study (Checkmate-067), a total of 945 previously untreated patients were assigned 1:1:1 ratio to receive nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. Nivolumab was administered at 3mg/kg every 2 weeks (plus ipilimumab-matched placebo), or 1 mg/kg of nivolumab every 3 weeks plus 3 mg/kg of ipilimumab every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg/kg of nivolumab every 2 weeks for cycle 3 and beyond, or 3 mg/kg of ipilimumab every 3 weeks for 4 doses (plus nivolumab-matched placebo) [38]. Overall response rates ranged from 19% (2.2% CR) in the ipilimumab group to 43.7% (8.9% CR) in the nivolumab group to 57.6% (11.5%) in the nivolumab and ipilimumab combination group. PFS was significantly longer in the combination group (11.5 months) compared to the ipilimumab group (2.9 months; HR for death or disease progression, 0.42; 99.5% CI, 0.31–0.57; $p < 0.001$) and the nivolumab group (6.9 months; HR 0.74; 95% CI, 0.60–0.92). As expected, more treatment-related grade 3 and 4 adverse events were observed in the combination group (55.0%) compared to either single agent group [nivolumab group (16.3%); ipilimumab group (27.3%)].

Pembrolizumab

Pembrolizumab is the second fully humanized IgG4 antibody directed against PD-1 receptor that has regulatory approval. It is FDA-approved for multiple different tumor types. In KEYNOTE-002, a multicenter phase II study, 540 previously treated patients were randomly assigned (in a ratio 1:1:1) to receive pembrolizumab 2 mg/kg ($n = 180$), pembrolizumab 10 mg/kg ($n = 181$) given IV every 3 weeks, or investigator-choice chemotherapy (paclitaxel plus carboplatin, paclitaxel,

carboplatin, dacarbazine, or oral temozolomide; $n = 179$) [39]. Higher response rates were observed in both pembrolizumab groups (2 mg/kg group: 21%; 10 mg/kg group: 25%) versus 4% response rate in the group treated with chemotherapy. Grade III and IV adverse events were more frequent (26%) in the chemotherapy group those given pembrolizumab (2 mg/kg group: 11%; 10 mg/kg group: 14%). As expected, the most common grade III or IV treatment-related AEs observed in patients given chemotherapy were anemia, fatigue, neutropenia, and leukopenia, but grade III or IV were extremely rare in the pembrolizumab group.

Pembrolizumab has shown improved objective results compared to ipilimumab. In KEYNOTE-006, a phase III study, 834 metastatic melanoma patients were randomized (1:1:1 ratio) to treatment with either pembrolizumab (10 mg/kg every 2 weeks or every 3 weeks) or four doses of ipilimumab (3 mg/kg every 3 weeks) [40]. The majority of the patients was treatment naïve. Both pembrolizumab arms yielded higher response rate (33.7% for every 2 weeks, 32.9% for every 3 weeks) compared to ipilimumab (11.9%). 6-month PFS was nearly 47% for pembrolizumab versus 26.5% for ipilimumab. In addition to improving overall survival, 12-month OS was 74.1% for pembrolizumab as compared to 58.2% for ipilimumab. No unexpected toxicities were reported, and commonly observed adverse events include fatigue, diarrhea, rash, pruritus, and immune-related endocrine disorders. Endocrine events related to thyroid were more frequent in the pembrolizumab groups, whereas colitis and hypophysitis were more frequent in the ipilimumab group. Grade 3–5 adverse events occurred in 13.3%, 10.1%, and 19.9% of patients treated with pembrolizumab every 2 weeks, every 3 weeks, and ipilimumab, respectively. In general, pembrolizumab has a favorable toxicity profile with fewer high-grade AEs than ipilimumab.

KEYNOTE-029, phase 1b trial, reported the outcomes of 153 melanoma patients who had not received previous CPI therapy [41]. Prior lines of therapy with either targeted therapy or chemotherapy were allowed, but 87% of patients were treatment naïve. Patients were treated with the combination of IV regular dose pembrolizumab (2 mg/kg), but with dose reduced ipilimumab (1 mg/kg) followed by pembrolizumab (2 mg/kg) maintenance therapy. Objective response reached 61% (95% CI 53–69), with 15% complete responses, and estimated 1-year PFS was 69% (95% CI 60–75), and estimated 1-year overall survival was 89% (95% CI 83–93). Grade 3 and 4 toxicities occurred in 45% of patients, with the most common being skin reactions (8%), colitis (7%), and hepatitis (6%). However, while significantly, the frequency of grade 3 and 4 toxicity for the low dose ipilimumab combination with regular dose pembrolizumab was favorable when comparing the toxicity data from low dose nivolumab with regular dose ipilimumab (Checkmate 069).

PD-L1

Antibodies directed at PD-L1 and therefore blocking PD-L1 from binding its receptors PD-1 and B7.1 have also been tested in metastatic melanoma patients. Atezolizumab (or MPDL3280A) is human IgG1 monoclonal antibody and was

tested at various dose levels in a Phase I trial in 45 patients with metastatic melanoma. Nearly 2/3 of all patients had prior systemic therapy. (http://ascopubs.org/doi/abs/10.1200/jco.2013.31.15_suppl.9010). The results showed an overall response rate of 26%, with a 24-week progression-free survival of 35%. Grade 3 and 4 toxicities were observed in 33% of patients, and included hyperglycemia (7%), and elevated ALT/AST (7 and 4%). In addition, MPDL3280A has also been tested in combination with targeted therapy (http://ascopubs.org/doi/abs/10.1200/jco.2013.31.15_suppl.9010). While none of the currently 3 approved PD-L1 agents (atezolizumab, avelumab, and durvalumab) has been approved for the treatment of metastatic melanoma to date, multiple combination trials with PD-L1 inhibitors are ongoing (NCT02535078, NCT02639026, NCT03273153, NCT03178851).

Vaccination and Intratumoral Approaches

Multiple intratumoral and vaccine approaches have been tested in the treatment for advanced melanoma. The vaccines aim to elicit immune response against antigens expressed by melanoma tumor cells, such as tumor-associated antigens (TAAs) or mutation-derived antigens (neoantigens). Various TAAs have been identified such as melanoma antigen A1 (MAGE-A1), gp100, or melanoma antigen recognized by T cells (MART-1/Melan-A) [42]. However, as single agents the results have not been impressive, and combinatorial approaches might be more promising. For example, gp100, a synthetic polypeptide found to carry immunogenic epitopes that can be recognized by T cell lymphocytes to induce antitumor activity was tested in combination with HD dose IL-2 [43]. In this phase III trial, a total of 185 metastatic melanoma patients (prior chemotherapy, interferon and low dose IL-2 were allowed) were randomized to receive either HD IL-2 alone or HD IL-2 with GP100. The response rate was 10% among patients who received HD IL-2 alone and 20% among patients receiving the combination ($p = 0.05$). The median overall survival was 11.1 months among patients receiving HD IL-2 alone and 17.8 months among patients receiving combination therapy ($p = 0.06$). The toxicities were similar in both treatment groups; however, more arrhythmias, lab test abnormalities, and more neurologic events were reported among patients in the vaccine/HD IL-2 group than among patients in HD IL-2 only group.

PV-10 (Rose Bengal)

Rose Bengal (RB) is a water-soluble injectable iodinated fluorescein derivative. After intralesional injection, PV-10 accumulates in tumor lysosomes resulting in rapid lysis of tumor cells, also, it is able to produce cytotoxic reactive oxygen species when exposed to ionizing radiation. PV-10 may also stimulate an antitumor immune response against distant lesions [44]. In a phase II study, 80 patients with refractory stage III and IV melanoma were treated with intralesional PV-10, which resulted in a best overall response rate of 51% (CR in 26%), and 8% of patients still

had no evidence of recurrence after 52 weeks [45]. Importantly, noninjected lesions also showed regression. Toxicity profile was favorable, with no treatment related grade 4. The most recently published prospective phase II trial reported a ORR of 87% (42% CR) in the 45 treated patients [46]. Complete responses were associated with having less than 15 metastases at time of PV-10 injection. PV-10 is currently not FDA approved for the treatment of metastatic melanoma, and clinical trials are also investigating its efficacy in combination with CPIs (NCT02557321).

T-VEC

Talimogene Laherparepvec (T-VEC), a genetically modified herpes simplex virus (HSV) type 1, is currently the only intralesional oncolytic virotherapy with regulatory approval in melanoma. It exerts its effect on regional and systemic antitumor immunity by selective intratumoral replication and expression of GM-CSF (granulocyte macrophage colony-stimulating factor) within the infected melanoma cells [47]. The approval was based on a randomized phase III trial in 436 patients with unresectable stage III or IV melanoma [48]. Patients were randomly assigned at a two-to-one ratio to intralesional T-VEC (up to 4 ml total treatment volume per session) or subcutaneous GM-CSF (125 mg/m^2 daily for 14 days in a 28 day cycle). The overall response rates for T-VEC were higher (26.4%; 95% CI, 21.4–31.5% vs. 5.7%; 95% CI, 1.9–9.5%) and more durable response was observed with T-VEC compared with GM-CSF (16.3% vs. 2.1%) ($p < 0.001$). Median OS was numerically longer with T-VEC than with GM-CSF (23.3 months vs. 18.9 months), but failed to reach statistical significance ($p = 0.051$). T-VEC injections were well tolerated, but reported adverse events included fatigue, chills, pyrexia, nausea, flu-like illness, reaction at injection-site, and vomiting. Incidence of grade 3 and 4 adverse effects was considerably low with 11% (vs. 5% for GM-CSF).

T-VEC also has shown efficacy in combination with CPIs. In a phase Ib trial of T-VEC in combination with ipilimumab in 19 previously untreated melanoma patients (prior adjuvant therapy ≥6 months from last therapy was allowed) [49]. T-VEC was administered intratumorally (up to 4 mL total volume) in week 1 and 4 and then every 2 weeks. Beginning in week 6 ipilimumab (3 mg/kg) was administered every 3 weeks for up to four doses. The objective response rate was 50%; durable responses were seen in 44% of patients lasting ≥6 months. With a median follow-up time of 20 months (1.0–25.4 months), progression-free survival was 50% and overall survival 67% at 18 months. No unexpected toxicities were observed.

T-VEC was also evaluated in combination with pembrolizumab with proven clinical benefit. In MASTERKEY-265, a phase Ib study, 21 advanced melanoma patients with no prior systemic treatment were received T-VEC (up to 4 mL total volume) intralesionally (cutaneous/subcutaneous/nodal) in day 1, day 22 then every 2 weeks, and pembrolizumab (200 mg) on day 36 and then every 2 weeks [50]. Confirmed RR was 62% with a CR rate of 33%, and responses were seen in 43% of noninjected nonvisceral and 33% of noninjected lesions. At time of the report, median PFS and OS had not been reached. No unexpected adverse events were

noted, but the authors described the occurrence of some overlapping pembrolizumab-related toxicities. Grade 3 and 4 toxicities occurred in 36% of patients and included rash ($n = 2$), elevation in liver enzymes ($n = 2$), hyperglycemia ($n = 2$), and squamous cell carcinoma ($n = 2$).

Multiple clinical trials are currently ongoing and investigating the efficacy of T-VEC in combination with CPI, targeted therapy as well as radiation (NCT02263508, NCT03088176, NCT02819843, NCT02965716).

T-VEC is contraindicated in pregnant women and severely immunocompromised patients, as it is consisted of live virus and may cause disseminated herpetic infection (https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM469575.pdf). Patients treated with T-VEC have been found to shed live virus; hence, strict precautionary guidelines have been established, particularly with infants, pregnant women, and immunocompromised patients.

Melanoma Brain Metastases and Immunotherapy

Despite the advances with immunotherapy and targeted therapy, a significant number of patients will develop brain metastasis (MBM) during their course of treatment [51].

However, a recent phase II study in patients with melanoma ($n = 18$) or non-small cell lung cancer ($n = 34$) has reported a response rate of 22% for single agent pembrolizumab in MBM, with responses being durable [52]. No unexpected extracranial toxicities were reported, but three patients in the melanoma cohort experienced transient neurological adverse events.

Importantly, two recent studies have shown that in patients with untreated MBM, the combination of ipilimumab and nivolumab can yield intracranial response rates similar to extracranial response rates as observed in Checkmate-067. In Checkmate-204, 75 MBM patients were treated with the ipilimumab (3 mg/kg) in combination with nivolumab (1 mg/kg combination with ipilimumab), followed by nivolumab (3 mg/kg). At over 9 months of follow-up, 21% of patients had reached a CR in the brain, and the median PFS was not reached. In addition to 33% that had a PR, 5% of patients benefited by having SD. Importantly, the median time to response was only 2.8 months (range 1–11 months), and duration of response had not been reached at time of report. Similarly to Checkmate-067, 52% of patients experienced grade 3 or 4 toxicities, and 25% had to discontinue study drug. Importantly, treatment-related nervous system adverse events were rare, and grade 3 and 4 toxicities only occurred in 8% [53].

The second phase III trial led by the Australian group (ABC trial) randomized patients with MBM to receive either combination therapy with ipilimumab and nivolumab (same dosing regimen as Checkmate-204) or to receive single agent nivolumab. Compared to Checkmate-204, patients had a higher number of brain metastases, but in treatment naïve patients the observed RR for patients receiving the combination was similar with 50% (15% CR, 35% PR), and SD was noted in

10%. Of the 26 patients treated in this cohort, 46% experienced grade 3 and 4 toxicities, leading to 27% discontinuation rate.

As these results are very promising, multiple clinical trials are currently ongoing, with a specific focus of the efficacy of immunotherapy or combined therapy approaches for MBM patients, and in patients who require corticosteroids for their MBM (NCT03175432, NCT02460068, NCT02621515, NCT02681549, NCT02716948).

Adjuvant Therapies

The goal of systemic adjuvant therapy is to decrease the risk for high-risk melanomas to recur after surgery. Traditionally, this approach has focused mainly on patients with stage III disease, which is defined as the presence of lymph node and/or in-transit metastasis. An increasing number of involved lymph nodes, but also an increase in primary tumor depth, mitotic rate as well as the presence of ulceration in the primary tumor are all associated with worse outcomes [54].

Adjuvant Therapy with Interferon

Interferon alpha-2 (INF)-α has been studied in patients with stage II or III melanoma in different dose levels, and multiple review articles summarize these results [55].

The ECOG E1684 trial which enrolled 287 patients with stage II/III melanoma has the longest follow-up, and patients were either treated with high-dose interferon (HD INF), or observed [56]. Median RFS of 1.72 (HD INF) versus 0.98 years (observation, $p = 0.0023$), and median OS of 3.82 versus 2.78 years (observation, $p = 0.0237$) were significantly improved for the HD INF arm. The 5-year survival rate was also higher in the HD INF arm (46% versus 37% with observation). However, at a median follow-up of 12.1 years, the OS benefit was no longer observed, and the authors proposed competing causes of death in an elderly cohort impacting the OS analysis.

The ECOG E1690 trial tested two different dose levels of INF and did not show an improvement of OS with either regimen versus observation, but patient crossover analysis might have altered survival analysis [57].However, 5-year RFS rates for HD INF were 44%, 40% for low dose IFN, and 35% for observation ($p = 0.3$). Pooled analysis of both E1684 and E1690 showed indeed a RFS, but no OS survival benefit from HD INF. Other pooled analysis showed that increased benefit was observed in patients with ulcerated primary melanomas [58].

Pegylated interferon has slightly more favorable side effect profile compared to HD INF, and has the advantage of longer half-life, with less injections per week. However, while showing improvement in RFS similar to HD INF, there was no improvement in OS. Furthermore, the positive impact on RFS appeared to decrease over time [59]. Patients with ulcerated primary and micro-metastatic nodal disease derived the biggest clinical benefit.

Adjuvant Biochemotherapy

In an effort to increase the efficacy of adjuvant therapy, a shorter course of biochemotherapy (up to three cycles) was compared to standard HD INF monotherapy. In this SWOG S0008 phase III study, 402 patients who had undergone completion lymph node resection for stage III melanoma were randomly assigned to either biochemotherapy (CVD as previously described, IL-2 at 9 MU/m^2 administered as a 96-h continuous IV infusion on days 1 through 4, and IFN at 5 MU/m^2 administered on days 1 through 5; treatment was repeated every 21 days for a total of three cycles), or to HD INF (20 MU/m^2 IV per day for 5 days for 4 weeks, followed by 10 MU/m^2 subcutaneously three times per week for 48 weeks) monotherapy [60]. In the HD-Interferon group, 43% of patients were able to complete therapy, where in the biochemotherapy group 80% of patients were able to receive all three planned treatment cycles ($p < 0.001$). With a median follow-up of 7.2 years, the median PFS was 4.0 years versus 1.9 years for biochemotherapy and HD INF, respectively ($p = 0.029$). The 5-year RFS was 48% versus 39%, respectively; however, despite numerical longer OS in the biochemotherapy group (9.9 years) versus the HD INF group (6.7 years) this was not statistically significant (HR, 0.98; 95% CI, 0.74–1.31; two-sided $p = 0.55$). As expected, both treatment groups experienced different toxicities, patients treated with INF have higher rates of LFTs abnormalities, and patients in biochemotherapy group experienced hypotension, hematologic, metabolic, and gastrointestinal toxicities more frequently. No unexpected new toxicities for either treatment arm was reported.

Checkpoint Inhibitors in the Adjuvant Setting

The improvement of overall survival and durable responses that were observed with ipilimumab in unresectable advanced melanoma patients led to study its efficacy in the adjuvant therapy. EORTC 18071 was a phase III double-blind randomized study comparing high-dose ipilimumab (10 mg/kg, every 3 weeks for four doses, then every 3 months for up to 3 years) to placebo in patients with fully resected stage III melanoma who had not received any other prior systemic therapy. At a median follow-up of 2.74 years (IQR 2.28–3.22), improvement of the median RFS was noted in the ipilimumab group (26.1 months) versus the placebo group (17.1 months, p = 0.0013) [61]. An improvement of the RFS was also observed at 3 years for ipilimumab (46.5%; 95% CI 41.5–51.3) versus the placebo (34.8%; 95% CI 30.1–39.5) in the placebo group. As expected, toxicities in the treatment group were much more common and included hepatic and endocrine toxicities. It should be noted that five (1%) participants developed significant drug-related adverse events in the ipilimumab group and died.

In an update of this trial and with an overall median follow-up of 5.3 years, the 5 years was 65.4% (95% CI, 60.8–69.6) in the ipilimumab group, as compared with

54.4% (95% CI, 49.7–58.9) in the placebo group [62]. Overall survival was also significantly improved for patients treated with ipilimumab compared to placebo (HR for death from any cause, 0.72; 95.1% CI, 0.58–0.88; $p = 0.001$), and this benefit was observed in all subgroups.

In a randomized double-blind phase 3 trial (Checkmate-238), 906 patients with complete resection of stage IIIB, IIIC, or IV melanoma were randomized to either ipilimumab (10 mg/kg) or nivolumab (3 mg/kg), with the primary end point of recurrence-free survival (RFS) [63]. The 12-month RFS was remarkably higher in nivolumab group (70.5%) versus (60.8%) in the ipilimumab group ($P < 0.001$). Median RFS was not reached in either arm of the trial at time of analysis. Similar to previous reports, nivolumab had a favorable toxicity profile, as only 14.4% of patients experiencing grade 3 and 4 toxicities, compared to 45.9% patients in the ipilimumab group. Based on Checkmate-238, nivolumab received regulatory approval in December of 2017 as an adjuvant therapy treatment option for metastatic melanoma patients.

The results of a recently presented phase III trial have shown that pembrolizumab might represent a promising choice in the adjuvant setting (Need reference for SITC 2018, Eggermont). All of the 1019 patients were stage III post resection and received treatment with pembrolizumab (200 mg Q3W) or placebo for up to 1 year (13 doses total) or until disease recurrence. At a median follow-up of 15 months, a significant reduction in risk of death or relapse (43%, HR = 0.57, 95% CI: 0.43–0.74; p less than 0.0001) was observed in the pembrolizumab group compared to placebo. Adverse events were similar to nivolumab in the adjuvant setting. As the first time ever in the adjuvant setting, this trial allows patients within the placebo cohort to "cross-over" to receive pembrolizumab post-relapse, and will deepen our understanding of efficacy in the post-relapse pembrolizumab efficacy.

The Future of Melanoma Treatment

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

Indoleamine Dioxygenase Inhibitors

Indoleamine dioxygenase (IDO) inhibitors have recently emerged as new and exciting class of anticancer drugs. IDO is one of the enzymes involved in the catalyzing tryptophan into kynurenine and that regulates the first and rate limiting step. T cells need tryptophan for function, and research has shown that tumors can increase IDO levels, thereby suppressing the function of cytotoxic T cells, and activation of T regulatory cells [64].

Epacadostat is a selective inhibitor of the IDO1 enzyme, and while it has not shown great efficacy as single agent, it has been studied in combination with CPIs [65]. It is interesting that epacadostat has essentially no independent antitumor activity; however, it demonstrated great efficacy when added to other checkpoint inhibitors such as anti-PD-1.

A phase I/II study (ECHO-202/KEYNOTE-037) recently reported the phase 1 and 2 efficacy and safety data for melanoma patients treated with epacadostat ((25, 50, 100, or 300 mg by mouth twice daily, PO BID) and pembrolizumab 2 mg/kg or 200 mg) combination during phase 1, with 100 mg BID epacadostat and 200 mg pembrolizumab every 3 weeks selected for phase 2 (https://academic.oup.com/annonc/article/28/suppl_5/mdx377.001/4109288). Prior CPI was not allowed. The ORR was 56% (11% CR) among 54 efficacy-evaluable patients, and disease control rate (CR + PR + SD) was 78%. Median PFS was 12.4 months, and progression-free survival rates were encouraging (6 months: 70%, 12 months: 54%, and 18 months: 50%). No significant added toxicities were noted, and grade 3 and 4 toxicities were observed in 17.2% of patients. These results support the ongoing phase III trial of epacadostat plus pembrolizumab in patients with advanced melanoma (NCT02752074). Furthermore, epacadostat is also being evaluated in combination with nivolumab (NCT02327078).

BMS-986205 is another selective IDO1 inhibitor that is being evaluated in combination with nivolumab and ipilimumab (NCT02658890).

Lymphocyte-Activation Gene 3 (LAG-3)

LAG-3 is an immune checkpoint receptor (CD223) found on the surface of activated CD4 and CD8 T cells, NK cells, B cells, and plasmacytoid dendritic cells [66]. LAG-3's main ligand is MHC class II, and it has various biologic effects on T cell function, including the negative regulation of T cell proliferation, activation, and homeostasis, and LAG-3 becomes upregulated during T cell exhaustion. Recently, its role in the maturation and activation of dendritic cells has also been described [67]. The development of LAG-3 blockade has now moved into clinical testing. In a phase I/IIa clinical trial, 43 melanoma patients who progressed on prior PD-1/PD-L1 exposure were treated with relatlimab (previously known as BMS-986016) in combination with nivolumab (http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.9520). Disease control rate was 45%, and overall response rate was 16% in the 31 efficacy-evaluable patients. Benefit was even observed in patients refractory to prior PD-1. Importantly, relatlimab did not appear to add toxicity, as grade 3 or 4 toxicities were only observed in 9% of the treated patients. Multiple clinical trials are currently evaluating the efficacy of anti-LAG-3 in combination with other immunotherapies and in other tumor types (NCT02676869, NCT01968109, NCT03250832, NCT03219268).

T Cell Immunoglobulin-3 (Tim-3)

TIM-3 is a co-inhibitory receptor which is expressed on specific subtypes of IFN-γ-producing CD4+ and CD8+ as well as dendritic cells, NK, and monocytes [68]. It was shown that a subset of T cells in patients with advanced melanoma upregulates Tim-3 expression, and that cells positive for this marker appear to be dysfunctional [69]. It was also shown that concurrent blockade with anti-PD-1 acted synergistic in reversing tumor-induced T cell exhaustion and dysfunction.

Currently, a few Tim-3 antagonists are in early-phase clinical development, either as single agent or in combination with anti-PD-1 or PD-L1 (NCT03099109, NCT03489343, NCT02817633, NCT02608268). While most of these trials focus on safety, the results are eagerly awaited.

OX40

OX40 (or CD134) is a member of tumor necrosis factor (TNF) receptor superfamily (TNFRSF), and in vitro studies have shown that stimulation of its ligand can lead to proliferation, improved effector function, and prolonged survival of T cells, and treatment with OX40 agonists can increase antitumor immunity [70].

In an initial phase one trial using an OX40 agonistic murine monoclonal antibody, regression of metastatic lesions was noted in 12 out of 30 patients, with 7 patients with metastatic melanoma. Grade 3 and 4 lymphopenia was noted in 7 patients, and other grade 1 and 2 toxicities included fatigue, nausea, vomiting, rash, and flu-like symptoms. Multiple clinical trials are currently ongoing, including in combination with atezolizumab (NCT02410512), durvalumab (NCT02705482), or tremelimumab (anti-CTLA-4; NCT02705482). In preclinical models, MEDI6383, a human OX40 ligand fusion protein, can initiate an intracellular signaling pathway to enhance T cell survival and activity, and proliferation, and is being evaluated in combination with durvalumab (NCT02221960) [71].

4-1BB

4-1BB (CD137) is another member of TNFRSF, is an inducible costimulatory receptor expressed on T cells and other immune cells, and can restore effector function [72]. 4-1BB and 4-1BBL interaction results in cytokine secretion and increased survival of CD8+ T cells. Urelumab (BMS-663513) is a fully humanized 4-1BB agonist mAb that has been tested in a phase I dose-escalation study. Only 3 out of 54 melanoma patients had a response to the monotherapy (http://ascopubs.org/doi/abs/10.1200/jco.2008.26.15_suppl.3007). However, because of the synergistic activity of urelumab with nivolumab in preclinical data, this combination is currently being evaluated in a phase I dose-escalation clinical trials. In addition,

PF-05082566, another 4-1BB agonist mAb, has also been evaluated in combination of pembrolizumab in patients with solid tumors (NCT02253992, NCT02179918). Another interesting combination is being studied, PF-04518600 (OX40 agonist) and PF-05082566 (4-1BB agonist), in select advanced or metastatic carcinomas (NCT02315066).

PF-05082566 (4-1BB agonist) is also being studied in combination with avelumab in advanced melanoma patients (NCT02554812).

Toll-Like Receptors (TLRs)

Toll-like receptors are members of immune recognition receptor family and were initially discovered through their role within the innate as well as adaptive immune response [73]. Furthermore, it was discovered that many tumor types express functional TLRs, leading to tumor proliferation, formation of metastases, and resistance to apoptosis. Numerous studies are now underway to see if a TLR-based therapeutic approach can increase the efficacy of anticancer immunotherapies (NCT02644967, NCT03052205, NCT00960752, NCT03445533).

Conclusion

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

References

1. Tas F, Keskin S, Karadeniz A, et al. Noncutaneous melanoma have distinct features from each other and cutaneous melanoma. Oncology. 2011;81(5–6):353–8.
2. McCourt C, Dolan O, Gormley G. Malignant melanoma: a pictorial review. Ulster Med J. 2014;83(2):103–10.
3. Lideikaite A, Mozuraitiene J, Letautiene S. Analysis of prognostic factors for melanoma patients. Acta Med Litu. 2017;24(1):25–34.
4. Jiang T, Zhou C, Ren S. Role of IL-2 in cancer immunotherapy. Oncoimmunology. 2016;5(6):e1163462.
5. Atkins MB, Lotze MT, Dutcher JP, 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.
6. Hughes T, Klairmont M, Broucek J, Iodice G, Basu S, Kaufman HL. The prognostic significance of stable disease following high-dose interleukin-2 (IL-2) treatment in patients with metastatic melanoma and renal cell carcinoma. Cancer Immunol Immunother. 2015;64(4):459–65.

7. Schwartzentruber DJ. Guidelines for the safe administration of high-dose interleukin-2. J Immunother. 2001;24(4):287–93.
8. Serrone L, Zeuli M, Sega FM, Cognetti F. Dacarbazine-based chemotherapy for metastatic melanoma: thirty-year experience overview. J Exp Clin Cancer Res. 2000;19(1):21–34.
9. Hill GJ II, Krementz ET, Hill HZ. Dimethyl triazeno imidazole carboxamide and combination therapy for melanoma. IV. Late results after complete response to chemotherapy (Central Oncology Group protocols 7130, 7131, and 7131A). Cancer. 1984;53(6):1299–305.
10. Bajetta E, Del Vecchio M, Bernard-Marty C, et al. Metastatic melanoma: chemotherapy. Semin Oncol. 2002;29(5):427–45.
11. Bhatia S, Tykodi SS, Thompson JA. Treatment of metastatic melanoma: an overview. Oncology (Williston Park). 2009;23(6):488–96.
12. Li RH, Hou XY, Yang CS, et al. Temozolomide for treating malignant melanoma. J Coll Physicians Surg Pak. 2015;25(9):680–8.
13. Quirt I, Verma S, Petrella T, Bak K, Charette M. Temozolomide for the treatment of metastatic melanoma: a systematic review. Oncologist. 2007;12(9):1114–23.
14. Middleton MR, Grob JJ, Aaronson N, et al. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol. 2000;18(1):158–66.
15. Hodi FS, Soiffer RJ, Clark J, Finkelstein DM, Haluska FG. Phase II study of paclitaxel and carboplatin for malignant melanoma. Am J Clin Oncol. 2002;25(3):283–6.
16. Rao RD, Holtan SG, Ingle JN, et al. Combination of paclitaxel and carboplatin as second-line therapy for patients with metastatic melanoma. Cancer. 2006;106(2):375–82.
17. Hersh EM, O'Day SJ, Ribas A, et al. A phase 2 clinical trial of nab-paclitaxel in previously treated and chemotherapy-naive patients with metastatic melanoma. Cancer. 2010;116(1):155–63.
18. Kottschade LA, Suman VJ, Amatruda T III, et al. A phase II trial of nab-paclitaxel (ABI-007) and carboplatin in patients with unresectable stage IV melanoma: a North Central Cancer Treatment Group Study, N057E(1). Cancer. 2011;117(8):1704–10.
19. Legha SS, Ring S, Papadopoulos N, Plager C, Chawla S, Benjamin R. A prospective evaluation of a triple-drug regimen containing cisplatin, vinblastine, and dacarbazine (CVD) for metastatic melanoma. Cancer. 1989;64(10):2024–9.
20. Atkins MB, Hsu J, Lee S, et al. Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alfa-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group. J Clin Oncol. 2008;26(35):5748–54.
21. Grunhagen DJ, Verhoef C. Isolated limb perfusion for stage III melanoma: does it still have a role in the present era of effective systemic therapy? Oncology (Williston Park). 2016;30(12):1045–52.
22. Eggermont AM, van Geel AN, de Wilt JH, ten Hagen TL. The role of isolated limb perfusion for melanoma confined to the extremities. Surg Clin North Am. 2003;83(2):371–84, ix.
23. Lotze MT, Rosenberg SA. Results of clinical trials with the administration of interleukin 2 and adoptive immunotherapy with activated cells in patients with cancer. Immunobiology. 1986;172(3–5):420–37.
24. Rosenberg SA, Yannelli JR, Yang JC, et al. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst. 1994;86(15):1159–66.
25. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol. 2005;23(10):2346–57.
26. Baruch EN, Berg AL, Besser MJ, Schachter J, Markel G. Adoptive T cell therapy: an overview of obstacles and opportunities. Cancer. 2017;123(S11):2154–62.
27. Merhavi-Shoham E, Itzhaki O, Markel G, Schachter J, Besser MJ. Adoptive cell therapy for metastatic melanoma. Cancer J. 2017;23(1):48–53.
28. Page DM, Kane LP, Allison JP, Hedrick SM. Two signals are required for negative selection of CD4+CD8+ thymocytes. J Immunol. 1993;151(4):1868–80.

29. Brunet JF, Dosseto M, Denizot F, et al. The inducible cytotoxic T-lymphocyte-associated gene transcript CTLA-1 sequence and gene localization to mouse chromosome 14. Nature. 1986;322(6076):268–71.
30. Wei SC, Levine JH, Cogdill AP, et al. Distinct cellular mechanisms underlie anti-CTLA-4 and Anti-PD-1 checkpoint blockade. Cell. 2017;170(6):1120–33.e1117.
31. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
32. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517–26.
33. Buchbinder EI, Gunturi A, Perritt J, et al. A retrospective analysis of high-dose interleukin-2 (HD IL-2) following ipilimumab in metastatic melanoma. J Immunother Cancer. 2016;4:52.
34. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
35. Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015;21(1):24–33.
36. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–30.
37. Hodi FS, Chesney J, Pavlick AC, et al. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol. 2016;17(11):1558–68.
38. Larkin J, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(13):1270–1.
39. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908–18.
40. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521–32.
41. Long GV, Atkinson V, Cebon JS, et al. Standard-dose pembrolizumab in combination with reduced-dose ipilimumab for patients with advanced melanoma (KEYNOTE-029): an open-label, phase 1b trial. Lancet Oncol. 2017;18(9):1202–10.
42. Hirayama M, Nishimura Y. The present status and future prospects of peptide-based cancer vaccines. Int Immunol. 2016;28(7):319–28.
43. Schwartzentruber DJ, Lawson DH, Richards JM, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011;364(22):2119–27.
44. Thompson JF, Hersey P, Wachter E. Chemoablation of metastatic melanoma using intralesional Rose Bengal. Melanoma Res. 2008;18(6):405–11.
45. Thompson JF, Agarwala SS, Smithers BM, et al. Phase 2 study of intralesional PV-10 in refractory metastatic melanoma. Ann Surg Oncol. 2015;22(7):2135–42.
46. Read TA, Smith A, Thomas J, et al. Intralesional PV-10 for the treatment of in-transit melanoma metastases-results of a prospective, non-randomized, single center study. J Surg Oncol. 2018;117(4):579–87.
47. Conry RM, Westbrook B, McKee S, Norwood TG. Talimogene laherparepvec: first in class oncolytic virotherapy. Hum Vaccin Immunother. 2018;14(4):839–46.
48. Andtbacka RH, Kaufman HL, Collichio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33(25):2780–8.
49. Puzanov I, Milhem MM, Minor D, et al. Talimogene laherparepvec in combination with ipilimumab in previously untreated, unresectable stage IIIB-IV melanoma. J Clin Oncol. 2016; 34(22):2619–26.
50. Ribas A, Dummer R, Puzanov I, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. Cell. 2017;170(6):1109–19.e1110.
51. Cohen JV, Tawbi H, Margolin KA, et al. Melanoma central nervous system metastases: current approaches, challenges, and opportunities. Pigment Cell Melanoma Res. 2016;29(6):627–42.
52. Goldberg SB, Gettinger SN, Mahajan A, et al. Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. Lancet Oncol. 2016;17(7):976–83.

53. Tawbi HA, Forsyth PAJ, Algazi PA, Hamid O, Hodi FS, Moschos S, Khushalani N, Margolin KA. Efficacy and safety of nivolumab (NIVO) plus ipilimumab (IPI) in patients with melanoma (MEL) metastatic to the brain: results of the phase II study CheckMate 204. J Clin Oncol. 2012;30 (Suppl; abstr 8584). 2017;35:(Suppl; abstr 9507).
54. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009;27(36):6199–206.
55. Agha A, Tarhini AA. Adjuvant therapy for melanoma. Curr Oncol Rep. 2017;19(5):36.
56. Kirkwood JM, Resnick GD, Cole BF. Efficacy, safety, and risk-benefit analysis of adjuvant interferon alfa-2b in melanoma. Semin Oncol. 1997;24(1 Suppl 4):S16–23.
57. Kirkwood JM, Ibrahim JG, Sondak VK, et al. High- and low-dose interferon alfa-2b in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. J Clin Oncol. 2000;18(12):2444–58.
58. Ives NJ, Suciu S, Eggermont AMM, et al. Adjuvant interferon-alpha for the treatment of high-risk melanoma: an individual patient data meta-analysis. Eur J Cancer. 2017;82:171–83.
59. Eggermont AM, Suciu S, Testori A, et al. Long-term results of the randomized phase III trial EORTC 18991 of adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma. J Clin Oncol. 2012;30(31):3810–8.
60. Flaherty LE, Othus M, Atkins MB, et al. Southwest Oncology Group S0008: a phase III trial of high-dose interferon Alfa-2b versus cisplatin, vinblastine, and dacarbazine, plus interleukin-2 and interferon in patients with high-risk melanoma--an intergroup study of cancer and leukemia Group B, Children's Oncology Group, Eastern Cooperative Oncology Group, and Southwest Oncology Group. J Clin Oncol. 2014;32(33):3771–8.
61. Eggermont AM, Chiarion-Sileni V, Grob JJ, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol. 2015;16(5):522–30.
62. Eggermont AM, Chiarion-Sileni V, Grob JJ, et al. Prolonged survival in stage III melanoma with ipilimumab adjuvant therapy. N Engl J Med. 2016;375(19):1845–55.
63. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. N Engl J Med. 2017;377(19):1824–35.
64. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. Nat Med. 2005;11(3):312–9.
65. Yue EW, Sparks R, Polam P, et al. INCB24360 (Epacadostat), a highly potent and selective Indoleamine-2,3-dioxygenase 1 (IDO1) inhibitor for immuno-oncology. ACS Med Chem Lett. 2017;8(5):486–91.
66. Goldberg MV, Drake CG. LAG-3 in cancer immunotherapy. Curr Top Microbiol Immunol. 2011;344:269–78.
67. Catakovic K, Klieser E, Neureiter D, Geisberger R. T cell exhaustion: from pathophysiological basics to tumor immunotherapy. Cell Commun Signal. 2017;15(1):1.
68. Hahn AW, Gill DM, Pal SK, Agarwal N. The future of immune checkpoint cancer therapy after PD-1 and CTLA-4. Immunotherapy. 2017;9(8):681–92.
69. Fourcade J, Sun Z, Benallaoua M, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Exp Med. 2010;207(10):2175–86.
70. Buchan SL, Rogel A, Al-Shamkhani A. The immunobiology of CD27 and OX40 and their potential as targets for cancer immunotherapy. Blood. 2018;131(1):39–48.
71. Oberst MD, Auge C, Morris C, et al. Potent immune modulation by MEDI6383, an engineered human OX40 ligand IgG4P Fc fusion protein. Mol Cancer Ther. 2018;17(5):1024–38.
72. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. Blood. 2018;131(1):49–57.
73. Huang B, Zhao J, Unkeless JC, Feng ZH, Xiong H. TLR signaling by tumor and immune cells: a double-edged sword. Oncogene. 2008;27(2):218–24.

Chapter 3
Immunotherapy in Lung Cancer: A New Age in Cancer Treatment

Luis Corrales, Katherine Scilla, Christian Caglevic, Ken Miller, Julio Oliveira, and Christian Rolfo

Abstract The management of Non-Small Cell Lung Cancer (NSCLC) has changed dramatically in the last 10 years with an increase in the understanding of the biology and with the development of new and multiple treatments. Chemotherapy being the first systemic treatment used in the setting of advanced disease, proving benefit for patients over palliative care. With the identification of oncogenic drivers, innovative targeted therapies were developed and tested, leading to important changes in the management of certain patients and giving to some of them the possibility to be treated in first line with oral inhibitors. Immunotherapy was then explored as a potential option, with promising results, and data of impact in important endpoints in lung cancer treatments. This chapter explores the different CTLA-4 inhibitors that have been investigated in NSCLC: ipilimumab and tremelimumab, as well as the different immune checkpoint inhibitors: anti PD-1 (nivolumab and pembrolizumab) and PD-L1 (atezolizumab, durvalumab,

Luis Corrales and Katherine Scilla contributed equally to this work.

L. Corrales
Medical Oncology Department, CIMCA / Hospital San Juan de Dios-CCSS, San José, Costa Rica

K. Scilla · K. Miller
Thoracic Oncology Program, University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA

C. Caglevic
Medical Oncology Department, Clínica Alemana Santiago, Santiago, Chile

J. Oliveira
Medical Oncology Department, Portuguese Institute of Oncology of Porto, Porto, Portugal

C. Rolfo (✉)
Thoracic Oncology Program, University of Maryland Greenebaum Comprehensive Cancer Center, Baltimore, MD, USA

Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, MD, USA
e-mail: christian.rolfo@umm.edu

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_3

avelumab, BMS-936559) medications. It also analyzes the different studies that have been developed for NSCLC with these medications, the evidence obtained, and the possible role in the management of patients. Immunotherapy has definitely changed the paradigm on NSCLC treatment, and the future is promising for the benefit of patients.

Keywords NSCLC · Immunotherapy · PD-L1 and PD1 · Precision oncology · Pembrolizumab · Nivolumab · Atezolizumab · Immunotoxicity

Introduction

Non-small cell lung cancer (NSCLC) represents approximately 85% of all lung cancer cases. Most NSCLC patients are diagnosed with advanced stage disease and lung cancer is the leading cause of cancer-related death worldwide. Tobacco consumption is the most important risk factor associated with this disease and can account for regional differences in its epidemiology [1]. Environmental pollution and some mineral exposures are also associated with NSCLC; for example, some northern cities in Chile have a very high incidence of lung cancer and mortality due to lung cancer, which is thought to be related to arsenic concentrations in drinking water [2].

Until some years ago, metastatic NSCLC has been an incurable malignancy and only palliative treatments have been offered to patients with the purpose of improving quality of life and prolonging survival. In the late 1980s, a Canadian prospective randomized trial demonstrated that cisplatin-based chemotherapy combinations had a modest benefit in overall survival when compared with best supportive care in metastatic NSCLC patients; however, these treatments were associated with high toxicity [3]. Twenty years later, a meta-analysis showed a 9% benefit in 1-year overall survival in advanced NSCLC patients that received chemotherapy plus best supportive care compared with best supportive care alone [4]. The importance of histology in the treatment of advanced NSCLC patients has been highlighted by a randomized trial in which differences in overall survival were noted depending on the histologic subtype and type of cisplatin-based chemotherapy combination used [5].

After failure of first line of cytotoxic chemotherapy for metastatic NSCLC, docetaxel may be used as second-line treatment for patients with good performance status, with an overall survival benefit of 3 months when compared with best supportive care [6]. In patients with adenocarcinoma histology who were not treated with pemetrexed in the first-line setting, pemetrexed can be used as second-line therapy with similar overall survival outcomes when compared with docetaxel but with a significantly lower toxicity profile [7]. Patients whose disease progressed through second-line chemotherapy without significant worsening of their performance status can be considered for subsequent lines of treatment, but with unclear results and less literature to support it [8].

Adding an antiangiogenic drug to cytotoxic chemotherapy has become a strategy to improve survival in metastatic non-squamous NSCLC. Bevacizumab received approval by the FDA for this subset of patients based on the results of several clinical trials [9]. Nintedanib, an oral antiangiogenic drug that simultaneously inhibits VEGFR, FGFR,

PDGFR, and also RET [10], has received approval in Europe in combination with docetaxel for second-line metastatic non-squamous NSCLC patients. Among advanced lung adenocarcinoma patients, treatment with the combination of docetaxel plus nintedanib led to significantly improved median overall survival of 12.6 months, compared to 10.3 months with docetaxel plus placebo [1, 11].

Until the early 2000s, pathologic differentiation between NSCLC and small cell lung cancer was the main determining factor to guide oncologic treatment decisions. Among patients with NSCLC, it later became important to also distinguish between squamous and non-squamous histologies, with non-squamous comprised primarily of adenocarcinoma and large cell carcinoma subtypes. This classification allowed for appropriate chemotherapy regimens to be recommended for metastatic NSCLC patients based on histologic subtype. The subsequent discovery of new specific genetic and molecular alterations with potential targeted therapies found mainly in the non-squamous population led to further changes in treatment algorithms for advanced NSCLC. Mutations of the epidermal growth factor receptor (EGFR) and the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene [12] are the most clinically relevant given their epidemiologic frequency and the availability of targeted therapies. Other less frequent mutations in NSCLC include ROS-1, BRAF, HER2, MEK, MET, and RET.

Cancers are characterized by different genetic and epigenetic alterations. High rates of somatic mutations in lung cancer generate a variety of tumor-specific antigens and may contribute to increased 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, one factor which contributes to cancer development is 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 of recognizing 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 recognizing and eliminating tumors already formed [16].

It is important to remember that the innate immune system is composed of dendritic cells, macrophages, natural killer (NK) cells, granulocytes (basophils, eosinophils, and neutrophils), complement proteins, chemokines and cytokines, among others. The innate immune system produces a rapid, nonspecific response to an antigen. In contrast, the adaptive IR, constituted by B lymphocytes, CD4 and CD8 T lymphocytes and antibodies, is a specific response toward a particular antigen which occurs more slowly, with the ability to leave immunological memory. The antitumor IR has been divided into seven stages [14–17], which make up 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 a specific recognition receptor within their membrane (T cell receptor (TCR) or B cell receptor (BCR), respectively). However, this single signal is not sufficient to achieve lymphocyte activation and simultaneous presence of costimulatory molecules is required (interaction between CD80/CD28, CD40/CD40-ligand, CD86/CTLA-4, ICOS/ICOS ligand, among others). 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, certain mechanisms are important: the participation of regulatory T cells (Tregs), the expression of inhibitory receptors (called checkpoints), the activation of apoptosis, and cell depletion [18].

Parallel to these events, tumors develop mechanisms to evade or to inhibit the IR, which include downregulation of antigen presentation (downregulation of the major histocompatibility complex—MHC), upregulation of inhibitors of apoptosis (Bcl-XL, FLIP), and expression of inhibitory cell surface molecules (programmed cell death 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].

Based on these principles, immunotherapy was explored as a potential treatment option for malignancy. In NSCLC, initial vaccine trials failed to demonstrate benefit [2]. More recently, several immunotherapy agents have been developed which have proven beneficial in patients with NSCLC. These medications now have an established role in the management of NSCLC. Initial immunotherapy studies which evaluated agents that block the CTLA-4 pathway failed to show benefit in overall survival in NSCLC patients. However, anti-PD-1 and anti-PD-L1 treatment have shown impressive positive results for NSCLC patients when used as monotherapy, or in combination with other immunotherapy drugs or chemotherapy.

Pathways and Immunotherapy Drugs in NSCLC Treatment

CTLA-4 Pathway

The IS has counterregulatory mechanisms that limit potentially harmful amplification of the IR. Specifically, following antigen exposure, there is an upregulation of different molecules on the surface of the T cells, aimed at ending 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. It functions to inhibit the activation of these cells.

Once T cells are activated by the interaction between the MHC of the APC and the TCR, associated with costimulatory molecules (for example, 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 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 effector T cells and a dramatic reduction of the intratumoral Tregs [22, 23].

CTLA-4 Inhibitors

Ipilimumab

Currently, the most established 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 developed at the University of California, ipilimumab currently is under license of Bristol-Myers Squibb [25]. Ipilimumab was the first checkpoint inhibitor ever approved for cancer treatment. Hodi et al. published positive overall survival results in unresectable and metastatic melanoma patients when comparing ipilimumab with or without the combination of 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 the same positive results.

The assumption that tumor necrosis due to cytotoxic chemotherapy releases tumor antigens and may enhance the response to immunotherapy has been the basis of the rationale to combine carboplatin plus paclitaxel doublet chemotherapy with ipilimumab [27]. The interactions between ipilimumab and cytotoxic chemotherapy were tested by Weber in treatment-naïve melanoma patients in a phase I trial. Ipilimumab was given at a dose of 10 mg/kg intravenous every 3 weeks for a maximum of four doses; carboplatin was given at AUC of 6 and paclitaxel at 175 mg/m^2 every 3 weeks. Patients without limiting toxicity were allowed to receive maintenance ipilimumab starting at week 24 every 12 weeks until limiting toxicity or disease progression. No relevant pharmacodynamics or pharmacokinetics findings were found between both arms [28].

A phase 2 clinical trial that combined ipilimumab plus carboplatin/paclitaxel doublet chemotherapy was developed for chemotherapy-naïve stage IIIB/IV NSCLC patients whose disease was not amenable for curative treatment. The trial was a three-arm study (1:1:1) including 204 patients. The control arm was the doublet of carboplatin and paclitaxel for up to six cycles. Experimental arms included ipilimumab at a dose of 10 mg/kg given concurrently with the carboplatin/paclitaxel for four cycles followed by two doses of placebo; or two doses of placebo plus carboplatin/paclitaxel followed by ipilimumab plus the combination of carboplatin/paclitaxel for four cycles. 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 endpoint, irPFS using immune-related RECIST criteria was met for the phased ipilimumab plus chemotherapy doublet (HR 0.72, $p = 0.05$) but not for the concurrent ipilimumab plus chemotherapy combination (HR 0.83, $p = 0.13$). Median irPFS 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. PFS using modified WHO criteria was also statistically significant in favor of the phased ipilimumab arm when compared with the 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 overall survival advantage 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 irPFS 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 three 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/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].

A phase III study was recently published evaluating the efficacy and safety of first-line ipilimumab or placebo plus paclitaxel and carboplatin in advanced squamous NSCLC. Patients with stage IV or recurrent chemotherapy-naïve squamous NSCLC were assigned (1:1) to receive paclitaxel and carboplatin plus ipilimumab 10 mg/kg or placebo every 3 weeks on an induction schedule comprised of six chemotherapy cycles, with ipilimumab or placebo from cycles 3 to 6 followed by

ipilimumab or placebo maintenance every 12 weeks for patients with stable disease or response. The primary endpoint was overall survival (OS). Nine hundred and fifty-six patients were included, with 749 received at least one dose therapy (chemotherapy plus ipilimumab, $n = 388$; chemotherapy plus placebo, $n = 361$). Median OS was 13.4 months for chemotherapy plus ipilimumab and 12.4 months for chemotherapy plus placebo (hazard ratio, 0.91; 95% CI, 0.77–1.07; $p = 0.25$) [3]. 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) [30]. Results from both trials will be very important to confirm the potential benefit of combining ipilimumab with cytotoxic chemotherapy in squamous NSCLC, or combining ipilimumab with target therapies in NSCLC patients that have an EGFR common mutation or an ALK translocation.

Ipilimumab in combination with other immunotherapy drugs will be discussed later in this chapter.

Tremelimumab

Tremelimumab is an anti-CTLA-4 IgG2 fully humanized monoclonal antibody [31]. Despite the similar mechanism of action than ipilimumab, tremelimumab as monotherapy has not shown benefit in NSCLC patients. In a phase 2 clinical trial for locally advanced or metastatic NSCLC patients with good performance status that had received four or more cycles of a platinum-based chemotherapy and had responded were randomized to tremelimumab or to best supportive care. The primary endpoint of the trial, progression-free survival, was not met, with an objective response rate of only 4% 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 [32].

Currently, a phase 1 clinical trial that studies tremelimumab plus gefitinib combination is ongoing for pretreated patients with stage IIIB and IV EGFR-mutated NSCLC (NCT02040064) [33].

Tremelimumab in combination with other immunotherapy drugs will be discussed ahead in this chapter.

PD-1/PD-L1 Pathway

The PD-1 receptor (Programmed Cell Death-1) is expressed in T/B cells, NK, and 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 Ligand 1 and 2, respectively), which downregulates the activity of T cells, limiting collateral tissue damage and maintaining the self-tolerance.

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 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].

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 IgG4 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 withdrawal of the 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. Eighty-five percent of the NSCLC patients had received at least two prior lines of treatment including a 34% of patients receiving a tyrosine kinase inhibitor. The maximum tolerated dose for nivolumab was not reached. In the NSCLC expansion cohort, regardless of the histologic subtype, patients were randomized to nivolumab at doses of 1, 3, or 10 mg/kg. There were 11 deaths (4%) related to serious adverse events, none of which were secondary to nivolumab according to the investigators' reports. Fourteen NSCLC patients that underwent treatment had an objective response, 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 an objective response had responses that lasted 24 or more weeks. Seven percent of the patients that had stable disease as the best response had not have 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-L1 status; no objective responses were found in 17 patients with PD-L1 negative tumors, while objective responses were seen in 36% of patients with PD-L1 positive tumors [42].

A second publication of the same phase I trial focused only on the NSCLC cohort with updated results in overall survival, durability of response, and long-term safety published in 2015. The total number of NSCLC patients enrolled was 129. Patients received one of the three doses described above every 2 weeks, in 8-week cycles, for up to 96 weeks. The median of age was 65 years, 42% had a squamous and 57% had a non-squamous histology, 98% had an ECOG performance status of 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 patients included, 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% with no statistical difference between histologic 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%, decreased appetite 12%, and diarrhea 10%) but only 14% had a grade 3 or 4 toxicity (most frequent: fatigue 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 given every 2 weeks in 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 endpoint 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 reported median time to response was 3.3 months. Median duration of response was not reached. Twenty-six percent of the patients achieved a stable disease as the best radiological response with a median duration of 6 months. The median PFS was 1.9 months, 6-month PFS was 25.9%, and 1-year PFS was 20%. The median OS was 8.2 months with 1-year OS of 40.8%. From patients that provided tumor samples to evaluate PD-1 expression, cutoff points of less or higher than 5%, patients with a higher expression achieved 24% of partial response, 24% of stable disease, and 44% of progressive disease as best response; patients with a

lower PD-1 expression had a 14% of partial response, 20% of stable disease, and 49% of progressive 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%), and rash, pruritus, myalgia and anemia (1% each). Twelve percent of treatment-related adverse events 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 multiple comorbidities and progression of their disease [44]. In a longer follow-up of at least 11 months, median duration of response was still not reached, and no new deaths due to nivolumab were reported [45].

The phase 3 clinical trial CheckMate 017 was a study evaluating stage IIIB or IV squamous NSCLC patients whose disease had progressed through first-line platinum-based doublet chemotherapy. This trial compared nivolumab 3 mg/kg IV every 2 weeks with docetaxel 75 mg/m^2 IV every 3 weeks, with both treatments given until disease progression or unacceptable toxicity. The primary endpoint was OS. Two hundred and sixty patients with an ECOG performance status of 0–1 were randomized. Median age was 62 years in the nivolumab arm and 64 years in the docetaxel arm, and most of the patients included in the study were male. 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 PFS 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. PD-L1 expression was evaluated using an immunohistochemical assay, Dako North America, from rabbit monoclonal antihuman (Clone 28-8, Epitomics). Any staining at any level was considered as positive. Three levels of positivity for PD-L1 expression were prespecified: 1, 5, and 10%. The 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 were 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, the FDA approved nivolumab as a second-line treatment for squamous NSCLC patients that have failed first-line platinum-based doublet chemotherapy.

CheckMate 057, with a similar design as CheckMate 017, was a phase 3 clinical trial that compared nivolumab and docetaxel but in non-squamous NSCLC that had progressed during or after platinum-based doublet chemotherapy. Secondary endpoints

included objective response rate, PFS, and efficacy according to PD-L1 expression. Five hundred and eighty-two patients were randomized to receive nivolumab or docetaxel in a 1:1 randomization model. Median overall survival, 12-month overall survival, and 18-month 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 vs. 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. In contrast to the squamous NSCLC patients treated in CheckMate 017, PD-L1 expression using the same immunohistochemical assay mentioned before was predictive of outcome for all the endpoints. Subgroup analysis showed also benefit in current or former smokers and in KRAS-mutated patients if being treated with nivolumab, nevertheless, patients that had EGFR mutations, older than 75 years and or never smokers had no clear benefit of the treatment with the monoclonal antibody when compared with docetaxel [47]. Based on the results of this trial, the 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. Two-year overall survival in CheckMate 017 was 23% for nivolumab versus 8% for docetaxel in squamous NSCLC patients. Two-year overall survival for non-squamous NSCLC patients from CheckMate 057 was 29% for nivolumab and 16% for docetaxel, respectively [48].

In the first-line setting, nivolumab was assessed in the CheckMate 026 trial. This phase 3 trial randomized untreated stage IV or recurrent NSCLC patients in a 1:1 ratio to receive nivolumab at a dose of 3 mg/kg every 2 weeks or a platinum-based chemotherapy every 3 weeks for up to six cycles. Crossover from the chemotherapy arm to the nivolumab arm was permitted. Primary endpoint was the independent central review PFS among patients with a PD-L1 expression of more than 5%. Four hundred and twenty-three patients with a PD-L1 expression level of 5% or more were included. The median progression-free survival was 4.2 months in the nivolumab arm versus 5.9 months with chemotherapy (HR = 1.15; 95% CI, 0.91–1.45; $P = 0.25$), and the median OS was 14.4 months versus 13.2 months (HR = 1.02; 95% CI, 0.80–1.30). A total of 128 of 212 patients (60%) in the chemotherapy group received nivolumab as subsequent therapy. Grade 3–4 treatment-related adverse events occurred in 18% of the patients who received nivolumab and in 51% of those who received chemotherapy. Therefore, nivolumab did not result in a better PFS or OS when compared to chemotherapy in this population [4].

Combination strategies were also investigated in the first-line setting. CheckMate 012 is a phase 1 trial multi-arm that assessed nivolumab as first-line treatment in combination with ipilimumab for NSCLC patients. Patients were randomly assigned (1:1:1) to receive nivolumab 1 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks, nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 12 weeks, or nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks until

disease progression, unacceptable toxicities, or withdrawal of consent [49]. Results of the last two arms were presented where objective responses were achieved in 18 (47% [95% CI 31–64]) patients in the ipilimumab every-12-weeks cohort and 15 (38% [95% CI 23–55]) patients in the ipilimumab every-6-weeks cohort. The median duration of response was not reached in either cohort, with median follow-up times of 12.8 months (IQR 9.3–15.5) in the ipilimumab every-12-weeks cohort and 11.8 months (6.7–15.9) in the ipilimumab every-6-weeks cohort. In patients with PD-L1 of 1% or greater, confirmed objective responses were achieved in 12 (57%) of 21 patients in the ipilimumab every-12-weeks cohort and 13 (57%) of 23 patients in the ipilimumab every-6-weeks cohort. Grade 3–4 treatment-related adverse events occurred in 14 (37%) patients in the ipilimumab every-12-weeks cohort and 13 (33%) patients in the every-6-weeks cohort; the most commonly reported grade 3 or 4 treatment-related adverse events were increased lipase (three [8%] and no patients), pneumonitis (two [5%] and one [3%] patients), adrenal insufficiency (one [3%] and two [5%] patients), and colitis (one [3%] and two [5%] patients) [5].

The CheckMate 227 was an open-label, phase 3 trial, evaluating the combination of nivolumab plus ipilimumab versus chemotherapy among patients with a high tumor mutational burden that was defined as ≥10 mutations per megabase. Patients with previously untreated stage IV or recurrent NSCLC were analyzed for tumor mutational burden using FoundationOne CDx assay. Additionally, patients with a PD-L1 expression of at least 1% were randomly assigned in a 1:1:1 ratio to receive nivolumab plus ipilimumab, nivolumab monotherapy, or chemotherapy. Those with PD-L1 expression of less than 1% were randomly assigned in a 1:1:1 ratio to receive nivolumab plus ipilimumab, nivolumab plus chemotherapy, or chemotherapy. PFS among patients with a high tumor mutational burden was significantly longer with nivolumab plus ipilimumab than with chemotherapy. The 1-year PFS rate was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy, and the median PFS was 7.2 months (95% CI, 5.5–13.2) versus 5.5 months (95% CI, 4.4–5.8). The HR obtained was 0.58; 97.5% CI, 0.41–0.81; $p < 0.001$ and the objective response rate was 45.3% with nivolumab plus ipilimumab and 26.9% with chemotherapy [6]. The high tumor mutation has become a possible marker to evaluate efficacy of immunotherapy, dissecting the population that will respond better to treatment.

Pembrolizumab

Pembrolizumab (MK-3475, Keytruda®, Merck Sharp & Dohme) is a highly selective IgG4 kappa isotype monoclonal antibody against PD-1. This highly selective antibody binds PD-1 and blocks the PD-1, PD-L1/PD-L2 axis, thus overcoming this major immune checkpoint inhibitor [50]. It was first approved in 2014 for unresectable and metastatic melanoma.

Advanced non-small cell lung cancer patients were assigned to multiple expansion cohorts as part of the phase 1 Keynote 001 clinical trial. Patients with an ECOG performance status of 0–1, adequate organ function, no history of pneumonitis or autoimmune diseases, and no 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 coprimary endpoint 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 70% of the patients, grade 3 or higher adverse events were reported in 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 37, 6% of patients and higher of 50% was present in 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 [51].

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 [52].

Based on these results, in October 2015, FDA approved pembrolizumab for metastatic NSCLC patients that failed to a first line of cytotoxic chemotherapy and presented with a positive 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-based doublet 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 endpoints 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). Nine hundred and ninety-one NSCLC patients (22% squamous) received at least one dose of pembrolizumab or docetaxel. Twenty-eight percent 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 subgroups 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 pembrolizumab 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 immune-related adverse events 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 treatment-related deaths 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) [53].

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 [54].

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) [55].

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 were 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 [56].

Keynote 024 is a phase 3 trial that included 305 patients not previously treated for an advanced NSCLC with PD-L1 expression on at least 50% of tumor cells and no sensitizing mutation of the EGFR gene or ALK translocation to receive either pembrolizumab (at a fixed dose of 200 mg every 3 weeks) or the investigator's choice of platinum-based chemotherapy. Crossover from the chemotherapy group to the pembrolizumab group was permitted. The primary endpoint, PFS, was assessed by means of blinded, independent, central radiologic review. Secondary endpoints were overall survival, objective response rate, and safety. Median PFS was 10.3 months (95% CI, 6.7 to not reached) in the pembrolizumab group versus 6.0 months (95% CI, 4.2–6.2) in the chemotherapy group with a HR = 0.50; 95% CI, 0.37–0.68; $p < 0.001$. The estimated rate of overall survival at 6 months was 80.2% in the pembrolizumab group versus 72.4% in the chemotherapy group (HR = 0.60; 95% CI, 0.41–0.89; $p = 0.005$). The response rate was higher in the pembrolizumab group than in the chemotherapy group (44.8% vs. 27.8%), and the median duration of response was longer (not reached [range, 1.9+ to 14.5+ months] vs. 6.3 months [range, 2.1+ to 12.6+]). Regarding toxicity, treatment-related adverse events of any grade were less frequent in the pembrolizumab arm, occurring in 73.4% versus 90.0% of patients, were grade 3, 4, or 5 treatment-related adverse events, and were present in 26.6% versus 53.3% [7]. An updated analysis after 25 months of follow-up was later presented showing a OS with pembrolizumab of 30.2 months versus 14.2 months with chemotherapy, representing a 37% reduction in the risk of death (hazard ratio, 0.63; 95% CI, 0.47–0.86; $p = 0.002$). The 24-month OS rate was 51.5% versus 34.5% favoring the pembrolizumab arm. At 12 months, the OS rate was 70.3% in the pembrolizumab arm compared with 54.8% in the chemotherapy group. The ORR was 45.5% (95% CI, 37.4–53.7) with pembrolizumab compared with 29.8% (95% CI, 22.6–37.8) in the chemotherapy group. Median duration of response was not reached in the pembrolizumab group (range, 1.8+ to 20.6+ months) compared with 7.1 months (range, 2.1+ to 18.1+ months) in the chemotherapy group [8].

Keynote 042 is a phase 3 clinical trial for the first-line metastatic or unresectable NSCLC (squamous and non-squamous histology), in patients that are not amenable for curative treatment and had a PD-L1 expression of at least 1%. Patients were assigned to receive pembrolizumab as a monotherapy versus chemotherapy

(carboplatin plus paclitaxel or carboplatin plus pemetrexed). PD-L1 levels were assessed by tumor proportion score (TPS). The primary endpoint was OS with TPS of ≥50%, ≥20%, and ≥1%. The study has met its endpoint and the result will be presented in a near future [57, 58].

Combination trials have also been evaluated with pembrolizumab. Keynote 189 is a double-blind, phase 3 trial, that assigned 616 metastatic non-squamous NSCLC patients without sensitizing EGFR or ALK mutations who had received no previous treatment for metastatic disease in a 2:1 ratio to receive pemetrexed and a platinum-based drug in combination with either 200 mg of pembrolizumab or placebo every 3 weeks for four cycles, followed by pembrolizumab or placebo for up to a total of 35 cycles plus pemetrexed maintenance therapy. Crossover to pembrolizumab monotherapy was permitted among the patients in the placebo-combination group who had verified disease progression. The primary endpoints were overall survival and progression-free survival, as assessed by blinded, independent central radiologic review. Overall survival at 12 months was 69.2% (95% CI, 64.1–73.8) in the pembrolizumab-combination group versus 49.4% (95% CI, 42.1–56.2) in the placebo-combination group (HR = 0.49; 95% CI, 0.38–0.64; $p < 0.001$) after a median follow-up of 10.5 months. The benefit of the pembrolizumab combination was observed in all subgroups that were analyzed, including those with a PD-L1 tumor proportion score of less than 1% (12-month OS rate, 61.7% vs. 52.2%; HR = 0.59; 95% CI, 0.38–0.92), a score of 1–49% (12-month OS rate, 71.5% vs. 50.9%; HR = 0.55; 95% CI, 0.34–0.90), and a score of 50% or greater (12-month OS rate, 73.0% vs. 48.1%; HR = 0.42; 95% CI, 0.26–0.68). Median PFS was 8.8 months (95% CI, 7.6–9.2) in the pembrolizumab-combination group and 4.9 months (95% CI, 4.7–5.5) in the placebo-combination group (HR = 0.52; 95% CI, 0.43–0.64; $p < 0.001$). Adverse events of grade 3 or higher occurred in 67.2% of the patients in the pembrolizumab-combination group and in 65.8% of those in the placebo-combination group. The frequency of deaths attributed to pneumonitis in this trial was consistent with the frequency previously observed with pembrolizumab monotherapy in advanced NSCLC [9].

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 [59].

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 PD-L1 and CD80, can help to suppress another negative control on T cells that can theoretically maximize the monoclonal antibody's activity [60]. This has not been proven clinically.

There have been several drugs under research.

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. Forty-three percent of patients presented grade 1–2 adverse events; however, no grade 3–5 pneumonitis was reported and no differences in toxicity between pretreated or treatment-naïve patients were seen. Preliminary results of 13 first patients that underwent treatment in the different cohorts showed 3 partial responses and 2 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 [61].

Recently an update from the phase 1–2 clinical trial was reported in which 198 NSCLC patients (116 non-squamous and 82 squamous histology) were treated 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%), decreased appetite (9%), and nauseas (8%). Six percent of patients had a grade 3–4 toxicity and only 2% of patients were discontinued treatment due to toxicity. From the total of patients treated, there was only two pneumonitis reported [62].

A recent report based on a treatment-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 [63].

As monotherapy, durvalumab has shown the most promising results in locally advanced stage III patients after receiving chemoradiotherapy. This phase 3 study randomly assigned patients in a 2:1 ratio to receive durvalumab at a dose of 10 mg per kilogram or placebo every 2 weeks for up to a year. These treatments were given between 1 and 42 days after a definitive treatment of chemoradiotherapy. Two primary endpoints were explored: PFS and OS. The study included 709 patients that received treatment, 473 receiving durvalumab, and 236 receiving placebo. The results were published in which median PFS was 16.8 months (95% CI, 13.0–18.1) with durvalumab versus 5.6 months (95% CI, 4.6–7.8) with placebo (HR = 0.52; 95% CI, 0.42–0.65; $p < 0.001$); the 12-month PFS rate was 55.9% versus 35.3%, and the 18-month PFS rate was 44.2% versus 27.0%. The median duration of response was longer for the durvalumab arm (72.8% vs. 46.8% of the patients had an ongoing response at 18 months). The median time to distant metastasis or death was longer with durvalumab 23.2 months than with placebo (14.6 months;

$p < 0.001$). Adverse events were also important to evaluate given the nature of the study, in which a treatment was given after a definitive management where there was no prior recommendation of treatment continuation. Grade 3 or 4 adverse events occurred in 29.9% of the patients who received durvalumab and 26.1% of those who received placebo; the most common adverse event of grade 3 or 4 was pneumonia (4.4% and 3.8%, respectively). Also, a total of 15.4% of patients in the durvalumab group and 9.8% of those in the placebo group discontinued the study drug because of adverse events [10]. The positivity of this trial has been possibly the most important advance in locally advanced disease in the last decade.

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 mg/kg, 10 mg/kg, 15 mg/kg 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%). Three 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 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 [64].

Licensed by Astra Zeneca, durvalumab is currently under study in different clinical trials for NSCLC patients, including the TATTON trial where durvalumab is evaluated 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 [65].

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%. Twenty-four-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% [66].

The expanded trial which included 85 NSCLC patients with both squamous and non-squamous histology, within a study that included other cancer types such as melanoma and renal cell carcinoma, was performed. NSCLC patients were treated with atezolizumab every 3 weeks, achieving an objective response rate of 21%. Current and former smoker had a higher response rate than never smokers (42% vs. 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 70% of the patients. The most common adverse events reported were fatigue (24%), decreased 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%) [67].

Clinical outcomes in distinct cancer types with high levels of PD-L2 expression have also showed a superior benefit with atezolizumab treatment [68].

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 [69].

The phase 2 clinical trial BIRCH was an open-label multicenter 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 score that included both components and were reported as TC 0 (TC0 < 1%), 1 (TC1 ≥ 1% and <5%), 2 (TC2 ≥ 5% and <50%) or 3 (TC3 ≥ 50%) and IC 0 (IC0 < 1%), 1 (IC1 ≥ 1% and <5%), 2 (IC2 ≥ 5% and <10%) or 3 (IC3 ≥ 10%). Eligible patients for this trial were patients with a TC 2/3 or IC 2/3. Patients included received atezolizumab at 1200 mg intravenously every 3 weeks. The primary endpoint 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% vs. 19%), second line (24% vs. 17%), and third line or further of treatment (27% vs. 17%) [70].

The POPLAR trial was a phase 2 study that compared atezolizumab versus docetaxel in locally advanced or metastatic NSCLC that had progressed after a first line of treatment, regardless of the PD-L1 status assessed by the same immunohistochemical assay that was mentioned above. Two hundred and eighty-seven patients were enrolled in the trial receiving atezolizumab at a fixed dose of 1200 mg every

3 weeks. POPLAR's primary endpoint 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 months versus 9.2 months (HR 0.59 $p = 0.005$), medium (TC2/3 or IC2/3) and high (TC3 or IC3) expression 15.1 months versus 7.4 months (HR 0.54 $p = 0.014$), high expression 15.5 months 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) [71].

A recent update of POPLAR trial showed an increase in the separation of curves with improved overall survival in favor of atezolizumab when compared with docetaxel (ITT population median overall survival 12.6 months versus 9.7 months ($p = 0.011$); TC3 or IC3 median overall survival not reached versus 11.1 months ($p = 0.033$). Regarding histology, there was no significant difference between histologies, with both histologic subtypes (squamous vs. non-squamous) favoring atezolizumab over docetaxel in overall survival [72].

The OAK trial was a phase 3, open-label, second or higher line international trial. Patients included had a stage IIIB or IV squamous or non-squamous NSCLC who had received one or two previous chemotherapy regimens and no previous anti-CTLA-4, anti-PD-L1, or anti-PD-L1 therapy. Patients were randomly assigned in a 1:1 to either atezolizumab 1200 mg or docetaxel 75 mg/m^2 every 3 weeks. Coprimary endpoints were OS in the intention-to-treat (ITT) and PD-L1-expression population TC1/2/3 or IC1/2/3 (≥1% PD-L1 on tumor cells or tumor-infiltrating immune cells). One thousand two hundred and twenty-five patients were recruited where 425 patients were randomly assigned to receive atezolizumab and 425 patients were assigned to receive docetaxel. OS was significantly longer in patients who had received atezolizumab in both the ITT and PD-L1-expression populations. In the ITT population, OS was improved with atezolizumab compared with docetaxel where the median OS was 13.8 months (95% CI 11.8–15.7) versus 9.6 months (8.6–11.2); HR = 0.73 (95% CI 0.62–0.87); $p = 0.0003$. OS in the TC1/2/3 or IC1/2/3 population was improved with atezolizumab ($n = 241$) compared to docetaxel ($n = 222$); median OS was 15.7 months (95% CI 12.6–18.0) with atezolizumab versus 10.3 months (8.8–12.0) with docetaxel; HR = 0.74 (95% CI 0.58–0.93); $p = 0.0102$. Patients in the PD-L1 with TC0 and IC0 also had a positive result with improved survival favoring atezolizumab with a median OS of 12.6 months versus 8.9 months; HR = 0.75 (95% CI 0.59–0.96). OS improvement difference was similar in the squamous and non-squamous populations. Regarding side effects, fewer patients had treatment-related grade 3 or 4 adverse events with atezolizumab (15% of patients) versus docetaxel (43% of patients). One treatment-related death from a respiratory tract infection was reported in the docetaxel group [11].

Atezolizumab has also been recently evaluated in combination with bevacizumab and chemotherapy among patients with previously untreated metastatic non-squamous NSCLC regardless of PD-L1 expression. The IMpower 150 trial is an international, open-label, phase III study which randomized 1202 patients in a 1:1:1 ratio into three treatment arms: atezolizumab plus carboplatin plus paclitaxel (ACP), atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP), or bevaci-

zumab plus carboplatin plus paclitaxel (BCP), each administered for 4–6 cycles. After induction chemotherapy, patients continued to receive atezolizumab, bevacizumab, or both until disease progression or intolerable toxicity. Primary endpoints were progression-free survival in the intention-to-treat population with wild type (WT) genotype (no EGFR or ALK genomic alterations) and among patients in the WT population with high expression of an effector T cell gene signature (Teff-high WT population), as well as overall survival in the WT population. The Teff gene signature was defined as the expression of PD-L1, CXCL9 and IFN-γ messenger RNA. In the WT population, median progression-free survival was significantly longer in the ABCP arm than in the BCP arm (8.3 vs. 6.8 months, HR 0.62, 95% CI 0.52–0.74, $p < 0.001$). In the Teff-high WT population, median progression-free survival was significantly longer in the ABCP group compared to the BCP group (11.3 vs. 6.8 months, HR 0.51, 95% CI 0.38–0.68, $p < 0.001$). In subgroup analysis, prolonged progression-free survival was also noted irrespective of PD-L1 status, including those with no PD-L1 expression, low PD-L1 expression, and low Teff gene signature expression. Notably, in an analysis of patients with EGFR mutations or ALK translocations ($n = 108$), median progression-free survival was also longer in the ABCP arm compared to the BCP arm (9.7 vs. 6.1 months, HR 0.59, 95% CI 0.37–0.94). Among the wild type population, OS was found to be significantly longer in the ABCP arm compared to the BCP arm (19.2 months vs. 14.7 months, HR 0.78, 95% CI 0.64–0.96, $p = 0.02$). Grade 3 or 4 treatment-related adverse events occurred in 55.7% of patients in the ABCP arm and 47.7% of the BCP group. The safety profile of the ABCP arm was felt to be consistent with the known safety risks of each of the individual drugs [73]. The data from this study suggest that the addition of cytotoxic chemotherapy to immune checkpoint inhibitors may enhance the effects of PD-1/PD-L1 inhibition.

Avelumab

Avelumab (MSB0010718C) is a fully human anti-PD-L1 IgG1 monoclonal antibody and has a native Fc receptor for ADCC [74].

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. Four hundred and eighty 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%). Thirty-four patients were discontinued of treatment due to adverse events including eight patients that presented infusion reactions. Drug-related toxicity grade 3 or higher was reported 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%) [75].

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. One hundred and eighty-four NSCLC patients were included (62% adenocarcinoma, 29% squamous carcinoma). Seventy-five percent 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 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) [76].

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 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 [77].

Currently, a phase 3 clinical trial comparing avelumab with docetaxel as second line of treatment for PD-L1 positive NSCLC patients is ongoing [78].

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 [59].

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 1 year [79]. Despite this drug is not currently being studied in cancer patients, there are clinical trials ongoing for sepsis treatment.

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

Non-small cell lung cancer is unfortunately the most common malignancy worldwide. Official records by Globocan showed that 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 with lung cancer there will be 87 persons that will die due to lung cancer in a 12 month time period. For both sexes together and in men, non-small cell lung cancer is the leading cause of mortality by cancer and the second cause of mortality by cancer in women [80]. 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 [81].

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-year survival rates. By the second half of the 2000s targeted therapies, in the beginning directed against EGFR mutations and years later against ALK translocations, 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 to 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 showed 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 that 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 needed 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 not 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 with squamous and non-squamous histology (Tables 3.1 and 3.2). Moreover patients not expressing PD-L1 in their tumors, when 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.

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 [23–88]. 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

Table 3.1 Pivotal second-line phase III immunotherapy trials in advanced NSCLC

Trial	Histology, PD-L1 expression requirement	Drugs	Number of patients	Median PFS (months)	Median OS (months)
CheckMate 017	Squamous	Nivolumab	135	3.5	9.2
		Docetaxel	137	2.8	6.0
CheckMate 057	Non-squamous	Nivolumab	292	2.3	12.2
		Docetaxel	290	4.2	9.4
KEYNOTE-010	NSCLC, ≥1%	Pembrolizumab 2 mg/kg	344	3.9	10.4
		Pembrolizumab 10 mg/kg	346	4.0	12.7
		Docetaxel	343	4.0	8.5
OAK	NSCLC	Atezolizumab	425	2.8	13.8
		Docetaxel	425	4.0	9.6

NSCLC non-small cell lung cancer, *PFS* progression-free survival, *OS* overall survival

Table 3.2 Pivotal first-line phase III immunotherapy trials in advanced NSCLC

Trial	Histology, PD-L1 expression requirement	Drug	Number of patients	Median PFS (months)	Median OS (months)
KEYNOTE-024	NSCLC, ≥50%	Pembrolizumab	154	10.3	30.2
		Platinum-based chemotherapy	151	6.0	14.2
KEYNOTE-189	Non-squamous	Pembrolizumab + platinum + pemetrexed	410	8.8	NR
		Placebo + platinum + pemetrexed	206	4.9	11.3
IMpower 150	Non-squamous	Atezolizumab + carboplatin + paclitaxel	348	N/A	N/A
		Atezolizumab + bevacizumab + carboplatin + paclitaxel	356	8.3	19.2
		Bevacizumab + carboplatin + paclitaxel	336	6.8	14.7

NSCLC non-small cell lung cancer, *PFS* progression-free survival, *OS* overall survival, *NR* not reached, *N/A* not available

should be considered as PD-L1 positive, which ought to be the cutoff point and then how to define the best treatment for every patient [89]. 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 [90]. 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, it 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 cutoff 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 chemo-radiation.

It is well known that the toxicity profile of immunotherapy is different from that of 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 [91].

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 have ethical concerns. Indubitably, 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 approved in 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 mainstay of cancer treatment and it will likely help in the future to provide a powerful hand in cancer cure.

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. 2008;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 Bazo I, Marquez-Medina D. Chapter 14. Chemotherapy beyond the second line of treatment in non-small cell lung cancer: new drug development. In: Fighting lung cancer with conventional therapies. Hauppauge, NY: 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;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 Immunol Rev. 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: Cellular and molecular immunology. Philadelphia: 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/ct2/show/NCT01998126.
31. 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.
32. 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.
33. https://clinicaltrials.gov/ct2/show/NCT02040064.
34. https://clinicaltrials.gov/show/NCT01285609.
35. Nguyen L, Ohashi P. Clinical blockade of PD1 and LAG3-potencial mechanisms of action. Nat Immunol Rev. 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. Najjar Y, Kirkwood J. Pembrolizumab: pharmacology and therapeutics. Am J Hematol Oncol. 2014;10(5):17–9.
51. 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.
52. 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.
53. 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.
54. 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.
55. 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.
56. 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.
57. https://clinicaltrials.gov/ct2/show/NCT02220894.
58. https://clinicaltrials.gov/ct2/show/NCT02343952.
59. Philips GK, Atkins M. Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies. Int Immunol. 2015;27(1):39–46.
60. 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.
61. 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(Suppl):5s, abstr 8021.
62. 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, May 20 Supplement):8032.
63. 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.

64. 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.
65. Reichert J. Antibodies to watch in 2016. MAbs. 2016;8(2):197–204.
66. 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.
67. 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.
68. 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.
69. 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.
70. 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, 25–29 Sept, Vienna, Austria. Abstract 16LBA.
71. 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.
72. Smith D, Vansteenkiste J, Fehrenbacher L, et al. Updated survival and biomarker analyses of a randomized phase II study of atezolizumab vs docetaxel in 2L/3L NSCLC (POPLAR). J Clin Oncol. 2016;34(Suppl), abstr 9028.
73. Socinski MA, Jotte RM, Cappusso F, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med. 378(24):2288–301.
74. Hamanishi J, Mandai M, Konishi I. Immune checkpoint inhibition in ovarian cancer. Int Immunol. 2016;7. pii: dxw020.
75. 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.
76. 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.
77. 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.
78. https://clinicaltrials.gov/ct2/show/NCT02395172.
79. 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.
80. Globocan. Estimated cancer incidence, mortality and prevalence worldwide 2012. 2012. http://globocan.iarc.fr/Pages/fact_sheets_population.aspx.
81. NIH, National Cancer Institute: surveillance, epidemiology and end results. http://seer.cancer.gov/statfacts/html/lungb.html.
82. 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(Suppl):5s, abstr 8113.
83. 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(Suppl):5s, abstr 8024.
84. Gettinger SN. Presented at European Society for Medical Oncology (ESMO), 25–29 Sept 2015, Vienna, Austria.
85. 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(Suppl):5s, abstr 8023.

86. Rizvi NA, Gettinger SN, Goldman JW, et al. Safety and efficacy of first-line nivolumab and ipilimumab in non-small cell lung cancer. In: 16th World Conference on Lung Cancer. Abstract ORAL02.05. Presented 7 Sept 2015.
87. 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.
88. Santarpia M, Karachaliou N. Tumor immune microenvironment characterization and response to anti-PD-1 therapy. Cancer Biol Med. 2015;12(2):74–8.
89. 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. https://doi.org/10.1371/journal.pone.0130142.
90. Kerr K, Hirsch F. Programmed death ligand-1 immunohistochemistry. Friend or Foe? Arch Pathol Lab Med. 2016;140:326–31.
91. 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 4
Update on Immunotherapy in AML and MDS: Monoclonal Antibodies and Checkpoint Inhibitors Paving the Road for Clinical Practice

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

Abstract In the past few years, our improved understanding of the pathogenesis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) has led to remarkable advances in the development of novel therapeutic approaches for these diseases. This chapter summarizes the available clinical data with immune-based therapeutic modalities in AML and MDS, focusing on monoclonal antibodies, T cell engager antibodies, chimeric antigen receptor (CAR)-T cells, and checkpoint blockade via blockade of PD-1/PD-L1 or CTLA4. Numerous clinical trials are currently ongoing in patients with AML and MDS, both in the frontline and relapsed refractory setting. Given the natural diversity of AML blasts, it became apparent that the best responses would be achieved with rationally designed combination strategies of immune therapy, molecular therapy, and chemotherapy. A number of such combinations are enrolling patients with AML in various clinical settings. Biomarkers to select the optimal combination regimen for individual patients are critical.

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

---

Lucia Masarova and Naval Daver collected and reviewed the data and wrote the paper. All authors participated in the discussion, have reviewed and approved the current version of the manuscript. Naval Daver is responsible for the overall content as guarantor.

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

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

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_4

Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous disease, characterized by clonal proliferation of myeloid precursors. Despite our improved understanding of the biology underlying AML, the therapeutic approach to AML had not substantially changed over the last 40 years. Standard frontline therapy comprises 3 + 7 like induction chemotherapy, introduced in the 1970s [1], followed by consolidation cycles or allogeneic stem cell transplantation (alloSCT), based on the patient's risk of relapse. Unfortunately, prognosis remains relatively poor with long-term overall survival (OS) achieved in approximately 40% of young adults, and only 10–15% of elderly patients (>65 years) with AML [2]. Most patients are either primary refractory to induction therapy or subsequently relapse following a brief remission likely due to persistence of chemo-resistant leukemia stem cells or low volume minimal residual disease.

Targeting specific tumor-related antigens with antibody-based therapies and engaging the patient's own immune system to attack cancer cells have recently become areas of significant clinical research in many hematologic malignancies, including AML and myelodysplastic syndrome (MDS).

In this chapter, we focus on mechanism-based overview of novel "immunotherapeutic" agents in AML currently being evaluated in clinical trials, particularly monoclonal antibodies, and T- cell engaging therapies. Currently ongoing clinical trials are summarized in Tables 4.1 and 4.2, and the chapter focuses on the most recent clinical advances in the development of immune-based therapies in patients with AML and MDS.

Monoclonal Antibodies

Monoclonal antibodies (MoAb) based therapies have become an integral part of cancer treatment, and leukemia is well suited to this approach because of the accessibility to malignant cells in blood and bone marrow. Ideal targets represent surface antigens expressed primarily on leukemic blasts while sparing hematopoietic stem cells. In AML, putative targets for antibody targeted therapy include antigens such as CD33, CD123, CD32, CD25, CD44, CD96, CLL-1, and TIM-3 [3].

Most clinical studies in AML have focused on CD33 and CD123 with various MoAb currently in clinical development. As unconjugated MoAb showed limited activities, most recent approaches have focused on MoAb conjugated with a toxic payload, also called "antibody drug conjugates" (ADC). Furthermore, novel approach in MoAb development includes constructs that bring cytotoxic T cells (by binding to CD3) in proximity with leukemia cells (by binding to a specific leukemia antigen) resulting in T cell activation and leukemia cell destruction, such as bispecific T- cell engagers (BiTEs), bispecific/trispecific killer cell engagers designed to target CD16 on NK cells (BiKE/TriKE), or dual affinity retargeting (DART) molecules.

Table 4.1 Clinical trials of monoclonal antibodies in AML and MDS

Phase	Target	Therapy	Primary endpoint	Inclusion	Clinicaltrials.gov identifier
II	CD33	GO	ORR, toxicity	R/R AML (approved for marketing)	NCT01869803
II/III		GO + Cytarabine vs. "7 + 3"	ORR, OS, toxicity	Frontline elderly AML	NCT02473146
II		GO + Busulfan + CFA → alloSCT	ORR, OS	AML in CR1 high risk, HR MDS, R/R MDS	NCT02221310
III[a]		CHT with ATRA ± GO	OS	New AML, NPM1 mut.	NCT00893399
I/II[a]		GO and AZA	Toxicity—MTD, ORR	R/R AML	NCT00766116
II		GO as consolidation post alloSCT	Graft failure incidence, OS, toxicity	AML in CR1/2, MDS < 5% blasts	NCT02117297
OBS		GO with CHT (MYLOR program)	ORR, toxicity	R/R AML	NCT03287128
Ib/II		GO/PF-04518600/avelumab/AZA/utomilumab/glasdegib	Toxicity, ORR	R/R AML	NCT03390296
III		GO/daunorubicin/cladribine/AC220/ganetespib	OS, ORR, toxicity	Frontline elderly; R/R AML	NCT02272478
Ib[a]		*SGN-CD33A + "7 + 3," SGN-CD33A + HDAC; SGN-CD33A single*	*Toxicity—MTD*	*All AML*	*NCT02326584*
III[a]		*SGN-CD33A + DAC/AZA*	*OS*	*Frontline AML*	*NCT02785900*
I/II[a]		*SGN-CD33A + AZA*	*Toxicity, ORR*	*Frontline Int-2/high risk MDS*	*NCT02706899*
I/II[a]		*SGN-CD33A and FluMel → autoSCT; SGN-CD33A*	*Toxicity, ORR, OS*	*R/R AML pre-ASCT; post-ASCT maintenance*	*NCT02614560*
I		AMG330	Toxicity	R/R AML	NCT02520427
I		AMG673	Toxicity—MTD	R/R AML	NCT03224819
II		BI 836858 and DAC	Toxicity—MTD, ORR	R/R AML, elderly unfit frontline AML	NCT02632721
		BI 836858 and F16IL2	Toxicity—MTD	R/R AML post SCT	NCT03207191

(continued)

Table 4.1 (continued)

Phase	Target	Therapy	Primary endpoint	Inclusion	Clinicaltrials.gov identifier
I/II		BI 836858	Toxicity—MTD; time to first PRBC	Low/int-1 risk MDS ± PRBC dependent	NCT02240706
I[a]		BI 836858	Toxicity	R/R AML or in CR1 HR for relapse	NCT01690624
I	CD123	SL-401 and AZA	Toxicity—MTD	Frontline unfit AML; R/R AML, HR MDS	NCT03113643
I/II		SL-401	Toxicity, OS	HR AML, MRD+ in CR1 (consolidation)	NCT02270463
I		XmAb14045	Toxicity—MTD	R/R AML	NCT02730312i
I		IMGN779	Toxicity—MTD	R/R AML,	NCT02674763
I	CD123 × CD3	JNJ-63709178	Toxicity	R/R AML	NCT02715011
I		MGD006	Toxicity	R/R AML, Int-2, and HR MDS	NCT02152956
II	CD38	Daratumumab	ORR	R/R AML, HR MDS	NCT03067571
I	CD25	ADCT-301	ORR	R/R AML	NCT02588092
I	CD47	Hu5F9-G4	Toxicity—MTD	R/R AML, HR MDS	NCT02678338
I		CC-90002	Toxicity—MTD	R/R AML, HR MDS	NCT02641002
I		TTI-621/SIRPαFc ± nivolumab ± rituximab	Toxicity	R/R AML, MDS	NCT02663518
I/II	CXCR4	Ulocuplumab + LD Ara-C	Toxicity—MTD, ORR	Frontline AML	NCT02305563

Abbreviations: R/R, relapsed/refractory; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ORR, overall response rate; OS, overall survival; PFS, progression free survival; alloSCT, allogeneic stem cell transplantation; autoSCT, autologous stem cell transplantation; GO, gemtuzumab ozogamicin; FluMel, Fludarabine & Melphalan; MTD, maximum tolerable dose; LD Ara-c, low dose cytarabine; HR, high risk; PRBC, packed red blood cells; DAC, decitabine; AZA, azacitidine; HiDAC, high dose cytarabine; CHT, chemotherapy, data was compiled from ClinicalTrials.gov (https://clinicaltrials.gov) assessed on 6/1/2018

[a]Active, but not recruiting; *italic—on hold by FDA*

Table 4.2 Clinical trials of immune checkpoint inhibitors and CAR-T/NK cells in AML and MDS

Phase	Target	Therapy	Primary endpoint	Inclusion	Clinicaltrials.gov identifier
I	CTLA4	Ipilimumab	Toxicity	R/R AML, HR MDS	NCT01757639
I		Ipilimumab and DAC	Toxicity	Frontline elderly, R/R AML	NCT02890329
II	PD-1	Pidilizumab + DC vaccine	Toxicity	AML in CR prior to collection for DC cells	NCT01096602
II		Nivolumab + "7 + 3"	EFS	Frontline AML, <60 years	NCT02464657
II		Nivolumab	EFS	AML in CR	NCT02532231
II		Nivolumab vs. OBS	PFS	AML in CR with MRD	NCT02275533
II		Pembrolizumab and AZA	Toxicity—MTD	R/R AML, frontline elderly AML	NCT02845297
I/II		Pembrolizumab and DAC	Feasibility	R/R AML	NCT02996474
II		Pembrolizumab	Consolidation of CR, ORR	Elderly AML in CR as consolidation	NCT02708641
0/pilot I		Pembrolizumab	ORR, OS	R/R AML after induction	NCT03291353
II		Pembrolizumab and HiDAC	ORR	R/R AML	NCT02768792
II		Pembrolizumab	Feasibility	R/R AML post SCT	NCT03286114
0/pilot I		Pembrolizumab	Toxicity—MTD	R/R AML post SCT	NCT02981914
II		Pembrolizumab and FluMel →autoSCT	ORR/2 years	HR AML in CR	NCT02771197
I		Entinostat and pembrolizumab	Toxicity—MTD	HMA failed MDS	NCT02936752
I/II	PD-1 and CTLA4	Ipilimumab/nivolumab/combination of both	ORR, CR duration	AML in CR, HR for relapse	NCT02846376
I/Ib		Ipilimumab/nivolumab	Toxicity—MTD	R/R AML after SCT	NCT01822509
II		Ipilimumab and nivolumab and AZA	ORR	HMA failed, HR MDS	NCT02530463
II		Ipilimumab ± nivolumab + AZA	ORR	R/R AML; frontline elderly AML	NCT02397720
I	CLL1 and CD3	MCLA-117	Toxicity—MTD	Frontline elderly AML, R/R AML	NCT03038230

(continued)

Table 4.2 (continued)

Phase	Target	Therapy	Primary endpoint	Inclusion	Clinicaltrials.gov identifier
I	PD-L1 and TIM-3	PDR001/MBG453 + DAC	Toxicity—MTD	R/R AML, elderly frontline AML, HR MDS	NCT03066648
I/II	PD-L1	Atezolizumab and SGI-110	Toxicity—MTD, ORR	HMA failure MDS	NCT02935361
I		Durvalumab ± AZA and Tremelimumab	Toxicity—MTD	HMA failure MDS	NCT02117219
II		Durvalumab with AZA	ORR	Elderly AML, frontline HR MDS	NCT02775903
I/II		Avelumab with AZA	Toxicity—MTD	R/R AML	NCT02953561
I		Avelumab with DAC	Toxicity—MTD	Frontline elderly AML	NCT03395873
II	KIR	Lirilumab and AZA	ORR	R/R AML	NCT02399917
II		Lirilumab and AZA and nivolumab	ORR	Frontline MDS, all	NCT02599649
I/II	CD33	CAR-T	Toxicity	R/R AML	NCT01864902
I	CD33	CAR-T	Toxicity	R/R AML	NCT02799680
I	CD123	CAR-T	Toxicity	R/R AML	NCT02159495
0/pilot I	CD123	CAR-T	Toxicity	R/R AML	NCT02623582
I	CD133	CAR-T	Toxicity	R/R AML	NCT02541370
I	CD33/CD56	CAR-NK	Toxicity	R/R AML	NCT02944162
I/II	CD7	CAR-NK	Toxicity	R/R AML	NCT02742727
I	CIK	CIK cells stim. IL-2	Toxicity	R/R AML, MDS	NCT01898793
I	CD123	UCAR-T (allogeneic cells)	Toxicity	R/R AML	NCT03190278

Abbreviations: R/R, relapsed/refractory; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ORR, overall response rate; OS, overall survival; EFS, event free survival; PFS, progression free survival; SCT, allogeneic stem cell transplantation; autoSCT, autologous stem cell transplantation; FluMel, Fludarabine & Melphalan; MTD, maximum tolerable dose; LD Ara-c, low dose cytarabine; HR, high risk; DC, dendritic cells; DAC, decitabine; AZA, azacitidine; HiDAC, high dose cytarabine; data was compiled from ClinicalTrials.gov (https://clinicaltrials.gov) assessed on 6/1/2018

Anti-CD33 Antibodies

CD33 is a myeloid differentiation antigen primarily expressed at very early stages on myeloid progenitors, as well as >90% on AML blasts [4].

Gemtuzumab Ozogamicin (GO; Mylotarg)

The development of the best-known MoAb in AML therapy, gemtuzumab ozogamicin (GO), a humanized anti-CD33 MoAb conjugated with a DNA-damaging toxin calicheamicin, has been a mixture of successes and disappointments. The FDA first granted GO an accelerated approval in 2000 for older patients with AML in first relapse on the bases of a 30% overall response rate (complete remission [CR]+ complete remission with incomplete counts recovery [CRi]) in a large phase II clinical trials [5, 6]. Ten years later, GO was voluntarily withdrawn from the US market, when the phase III SWOG S0106 trial showed no survival benefit, increased early mortality, and increased rate of sinusoidal obstruction syndrome or veno-occlusive disease (VOD) in the patients who received GO [7]. The GO dose used in this study was a non-fractionated, higher dose of 6 mg/m^2. Subsequently, four large randomized trials showed improved overall survival (OS) without increased early mortality or VOD with the addition of fractionated doses of GO to standard induction chemotherapy, particularly in patients with favorable or intermediate cytogenetics [8–10].

More recently, the efficacy of GO was confirmed in a multicenter, phase 3, randomized (1:1) study of 237 older patients with newly diagnosed AML, comparing single agent GO to best supportive care (BSC). GO demonstrated an improved median OS over BSC (4.9 vs. 3.6 months, $p = 0.005$, HR 0.69, CI 0.53–0.90), with 1-year OS rates of 24.3% with GO and 9.7% with BSC. More importantly, the OS benefit with GO was consistent across most subgroups with the best activity in patients with high CD33 expression, in those with favorable/intermediate cytogenetics, and in women. Overall response (CR/CRi) occurred in 30 of 111 (27%) GO recipients [11]. After another phase 2 study demonstrated the ability of GO to induce CR in 26% of patients with relapsed AML after only one course [12], the efficacy of GO has become undeniable.

Finally, in September of 2017, FDA re-approved GO for the treatment of adults with newly diagnosed CD33+ AML and relapsed refractory CD33+ AML in patients older than 2 years in combination with chemotherapy or as a monotherapy. Currently, multiple clinical trials are ongoing to gain additional knowledge regarding the efficacy, toxicity, and best clinical use of GO in patients with AML in the frontline or relapsed setting (Table 4.1).

SGN33A (Vadastuximab Talirine)

After preclinical studies of SGN33A showed encouraging cytotoxic potency against AML cell lines (>30 more potent than GO) [13, 14], the agent entered numerous phase I and II clinical trials in treatment naïve, relapsed/refractory, or elderly patients with CD33+ AML, as a single agent or in combination with cytotoxic chemotherapy or hypomethylating agents (HMA; azacitidine [AZA] or decitabine [DAC]).

Initial phase I–II studies were promising and showed rapid and deep remissions, and a tolerable safety profile. As a monotherapy, SGN33A produced overall response rate (CR/CRi) of 28% with a 47% blast clearance, at the recommended dose of 40 μg/kg, in patients with relapsed/refractory AML or older treatment naïve AML (n = 131). Myelosuppression (>G3 neutropenia 15%; anemia 25%; and thrombocytopenia in 31%) was the most common adverse event (AE). The 8-week mortality rate was 8% [15].

SGN33A was shown to be more effective when combined with induction chemotherapy (7 + 3) or HMA, producing overall response rate (CR/CRi) of 78% [16] and 73% [17] in newly diagnosed patients with AML, respectively. The early mortality rates in the phase II studies were similar to what would be expected with standard chemotherapy or HMA alone. These encouraging results led to a global, double-blinded, placebo-controlled phase 3 successor trial (CASCADE) investigating SGN33A with and without HMA in frontline AML patients not fit for induction chemotherapy.

However, further development of SGN33A has been disappointing. Initially, FDA placed some of the SGN33A trials (especially those that administered peri-transplant SGN33A) on intermittent clinical hold between 12/2016 and 3/2017 due to occurrence of hepatotoxicity/VOD when the agent was administered close to the time of SCT. Finally, the development of SGN33A was suspended in all clinical trials in June 2017 after an independent panel observed a higher rate of deaths, including fatal infections, on the SGN33A arm of phase 3 CASCADE trial [18]. The data from the CASCADE trial are currently being analyzed, but it is unlikely that SGN33A will move forward in the AML space.

IMGN779 (ImmunoGen)

Another anti-CD33 antibody with promising preliminary clinical activity is IMGN779, a humanized antibody conjugated to DGN462, a novel DNA-alkylating agent consisting of an indolino-benzodiazepine dimer (IGN payloads) [19].

In preclinical studies, IMGN779 showed potent activity against AML cells, including those harboring mutations in FMS-like tyrosine kinase 3 (FLT3) [20]. Furthermore, recent reports suggest that cytarabine might potentiate the activity of IMGN779 by increasing the surface CD33 levels on AML cells, leading to improved DNA damage response, cell cycle arrest, and apoptosis. This observation favors evaluation of combination of these two agents in clinical trials [21].

Preliminary results of phase I dose finding study of IMNG779 in 26 patients with relapsed refractory AML (including 19% patients after alloSCT) are promising. The agent was safe with no drug limiting toxicities at doses up to 0.7 mg/kg. Grade ≥3 AEs observed in more than 10% of patients included febrile neutropenia (39%), pneumonia (19%), anemia (19%), respiratory failure (15%), and hypophosphatemia (12%). At doses between 0.39 and 0.7 mg/kg, all nine patients showed decreased peripheral blasts with a median maximal reduction of 67% (range: 15–100%). Additionally, three patients showed a substantial decrease in bone marrow blasts within the first three cycles by 96, 90, and 48% [22].

Anti-CD123

The second most common clinically exploited target for moAb for patients with AML is CD123, which after binding to interleukin-3 (IL-3Rα) promotes increased cell survival and proliferation [23], as well as leukemia relapse and resistance to chemotherapy [24].

JNJ-56022473 (Talacotuzumab, Variant of Former CSL-362)

A second-generation anti-CD123 antibody, JNJ-56022473 (talacotuzumab), a fully humanized antibody with enhanced cellular toxicity due to binding to NK cells (CD16), demonstrated activity and safety as a maintenance therapy in a phase 1 study of patients with CD123+ AML in first or second CR/CRi and at a high risk of relapse. Fifty percent of patients (10/20) maintained their CR with a median duration of CR of 34+ weeks. Hypertension and infusion reaction were the most common treatment emergent and dose limiting toxicities [25]. A randomized phase II/III trial of DAC with or without talacotuzumab in patients with untreated AML who are not candidates for intensive chemotherapy was initiated (n = 326). In October of 2017, the company (J&J) decided to stop further development of this compound for an unreleased reason.

SL-401 (DT388IL3)

Currently, one of the most promising anti-CD123 antibodies is SL-401 (DT388IL3), which has been granted breakthrough drug designation by the FDA and EU for the treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN) in October of 2017. SL-401 is a recombinant fusion protein composed of the truncated diphtheria toxin and a human IL-3 ligand [26], which after binding to CD123 gets internalized, leading to the inactivation of protein synthesis, and cell death.

A phase 2 clinical trial for patients with BPDCN showed excellent activity of SL-401 with an overall response rate (ORR) of 84% in all patients (and ORR of 95% in the frontline settings) [27].

Results in patients with AML were not that impressive. The agent produced 2 complete and 4 partial remissions, with 4 additional patients having a >50% bone marrow blasts reduction in a phase I trial involving 74 AML/MDS patients (56 with relapsed and refractory AML). The median survival and overall survival at 12 months in patients with relapsed AML (≥2nd salvage) were 3.2 months and 22%, respectively, both favorable when compared to historical results. Grade ≥3 AEs were only transient and included elevation in transaminases (20%), and capillary leak syndrome (4%) [28, 29]. The phase II study evaluating SL-401 in patients with relapsed refractory AML is ongoing, and so far has shown stable disease in 3/6 patients for 12+ cycles [30]. The agent is also being evaluated as a maintenance therapy in a phase 2, multicenter, two-stage study in patients with AML in a first or second CR with a high risk of relapse. The first dose finding stage was successfully completed ($n = 9$) without any drug limited toxicity, and with a recommended dose for phase 2 at 12 mcg/kg in this maintenance setting. The most common grade ≥3 AEs included ALT/AST increase (up to 31%), thrombocytopenia (19%), and cytokine release syndrome (CRS, 13%). Five patients were relapse-free for at least 5+ months [31]. Translational data presented from the previous studies showed a potential mechanism of resistance to SL-401 by the loss or decreased expression of DPH1 enzyme that converts histidine to diphthamide—the direct target for ADP ribosylation—which could be overcome by combining SL-401 with AZA [32]. Based on these data, a multicenter phase 1 trial of the combination of SL-401 and AZA in patients with AML or MDS has been initiated and is currently enrolling (Table 4.1).

Anti-KIR

Another intriguing approach to immunotherapy for AML represents an antibody against killer-cell immunoglobulin receptor (KIR) on NK cells, *lirilumab.* Lack of KIR interaction with HLA class I has been associated with augmented NK cell-mediated antitumor activity in patients with AML [33].

Lirilumab was found to be safe in a phase 2, randomized, double-blinded, placebo-controlled maintenance trial in elderly patients with AML in first CR ($n = 153$). Patients were randomly allocated to receive placebo ($n = 51$), or lirilumab as an intermittent (0.1 mg/kg, $n = 50$) or continuous infusion (1 mg/kg; $n = 51$) for up to 2 years of therapy. The median time to randomization from CR was 3.3 months. Due to excess early relapses, the continuous arm (1 mg/kg dose) was discontinued at interim analysis. The other two cohorts continued to accrue with a mean number of cycles at the time of final report of 14.7 and 13.8 for the lirilumab (0.1 mg/kg) and placebo, respectively. After a median follow-up of 36.6 months, lirilumab was well

tolerated. Ten percent of patients discontinued therapy due to AE, most of which were grade 1 or 2. Leukemia free survival was similar between lirilumab and placebo (LFS lirilumab intermitted versus placebo: 17.6 (range 11.2–25) versus 13.9 (range, 7.9–27.9) months, respectively; HR 0.96 [95% CI 0.61–1.56]) [34].

Similarly, safety of lirilumab in combination with AZA has been shown in an ongoing phase 2 study in patients with heavily pretreated refractory AML. Thirty-five patients with relapsed AML (12 with secondary AML, 7 post-alloSCT) who had received a median of three previous therapies (range, 1–8) were treated with AZA (75 mg/m^2 × 7 days) and lirilumab (1 and 3 mg/kg Q4 weeks in two consecutive cohorts of six patients each). Lirilumab 3 mg/kg was established as the recommended phase 2-dose. Four patients (11%) achieved CR/CRi and 1 (3%) achieved hematologic improvement (HI) for an overall response rate of 14%. Additionally three patients (9%) had a ≥50% reduction in blast count. The 4-week and 8-week mortality were 7% and 15%, respectively. With a median follow-up of 3.6 months (range, 1.1–15.1 months), the median overall survival among all patients was 4.2 months (range, 0.4–15.1). Grade ≥3 AEs were similar to those expected with AZA-based salvage therapies. Immune-related grade ≥3 AEs were observed in three patients (pneumonitis in one and colitis in two); however, all responded rapidly to steroids. Furthermore, there were no grade ≥3 immune-related AE observed in seven post-alloSCT patients treated on this regimen [35].

T Cell Engaging Antibodies

A novel class of antibody-based immunotherapy in AML includes MoAb constructs that combine the specificities and biologic functions of two antibodies by targeting tumor-associated antigens and T-effector cells, effectively bringing T cells in proximity to tumor cells resulting in enhanced T cell activation and antitumor activity [36]. Recent data suggests that these antibodies may enhance the quantity and quality of immune responses not only through direct T cell-mediated cytotoxicity, but also by circumventing immune evasion by targeting myeloid derived suppressor cells, which are involved in hampering antitumor immune activity [37, 38].

Bispecific T cell engagers (BiTEs) consist of four variable domains of heavy and light chain linked to each other by a polypeptide linker, and represent the first in class T cell engaging MoAb. After promising preclinical data, three compounds—anti CD3/CD33, AMG-330; anti CD123/CD3, JNJ-63709178; and anti CD3/CD123, XmAb—entered phase 1 clinical trials in patients with relapsed refractory AML and are currently enrolling (Table 4.1). Data on clinical efficacy and tolerability of these agents are expected to be presented in late 2018.

To improve the stability, safety, and efficacy of BiTEs, novel compounds of T cell engaging MoAb are in various stages of preclinical and clinical development, such as bispecific or trispecific killer cell engagers (BiKE/TriKE) designed to target CD16 on NK cells [39], or bivalent dual affinity re-targeting bispecific antibodies

(DARTs) composed of two antigen-binding specificities connected to two independent polypeptide chains [40].

DARTs may have a slightly improved safety profile as compared to BiTEs as recently demonstrated in clinical trial with *CD123/CD3 DART flotetuzumab (MGD006)*.

After promising preclinical data [41], flotetuzumab was evaluated in a first-in-human trial in patients with relapsed/refractory AML or intermediate-2/high risk MDS. Preliminary results were recently reported from the phase 1 dose-escalation portion of the study, involving 45 patients (89% AML). The median age of the patients was 64 years. Overall, flotetuzumab demonstrated a manageable toxicity profile; grade ≥3 drug-related AEs were observed in 44% patients, and the most common were infusion-related reactions or cytokine release syndrome (CRS, 76% total, 13% grade ≥3). Among 14 patients treated at the established threshold dose (500 ng/kg/day) for at least one cycle, anti-leukemic activity was documented in 8 of 17 (57%) patients (including 3 CR). This study is currently enrolling patients in the expansion cohort at the 500 ng/kg/day dose in the USA and Europe [42]. Additional data from this study confirmed that stepwise lead-in dose strategies during the first week of flotetuzumab administration, in conjunction with early intervention with tocilizumab, could decrease the severity of CRS by mean 0.54 grade. Preliminary data also showed a positive correlation between baseline circulating T cell number and maximum early CRS grade [43]. The investigators showed that primary AML samples with higher levels of PD-L1 on malignant blasts were less susceptible to flotetuzumab-mediated killing in vitro. Furthermore, patients that progressed early on flotetuzumab treatment had higher baseline levels of PD-L1 on AML cells. Synergistic cytotoxicity was observed after treatment of AML cell lines with flotetuzumab and anti-PD-1 inhibitor in vitro [44].

Adoptive T Cell Therapy

Adoptive cell therapy (ACT) is a highly personalized therapy that involves transfer of ex-vivo expanded cytotoxic T-lymphocytes into tumor-bearing patients. These tumor-reactive T cells genetically engineered to express the binding site of specific antibodies (chimeric antigen receptor, CAR-T) are capable of targeted tumor killing [45]. CARs are made of antigen binding element consisting of the extracellular single-chain immunoglobulin variable fragments (scFvs), a trans-membrane short peptide linker, and an intracellular T cell signaling domain, usually CD3-ζ of the TCR receptor, and various co-stimulator molecules, such as CD28, OX40, or 4-1BB (second- and third-generation CARs); or additional cytokines (IL-2, IL-15, IL-12, IL-21; in the fourth-generation constructs) [46].

Clinical trials with CAR-T in patients with AML are in the early phases of development. The first clinical trial to show the safety and feasibility of CAR-T in relapsed AML patients evaluated an *anti-LeY CAR-T*. Among five patients treated

on this trial, two achieved stable disease, with a maximal duration of 23 months in one of the patients. More importantly, no grade ≥3 AE or CRS was reported [47].

Recently, another CAR-T compound directed against CD123 demonstrated safety and promising clinical activity in patients with relapsed/refractory AML and BPDCN. Six patients who had relapsed/refractory AML following alloSCT with a median of four prior lines of therapy, received 1–2 doses of *CD123 CAR-T* cells, and two of them achieved CR with successful bridge to second alloSCT. Additional two patients achieved blasts reduction not classified as CR. All toxicities were reversible and manageable with only one grade 3 AE (rash), and no treatment limiting AE [48].

A recent innovative and exciting approach in adaptive T cell therapy is the development of CARs redirecting CD56+ NK cells towards specific antigens on AML blasts (CD33, CD23, CD7, etc.). NK cells are an attractive cell population due to their natural killer ability of attacking malignant cells without prior antigen presentation, which would allow them to be used from allogeneic donors *(CAR-NK cells)* [49]. Another method of deriving NK cells is by cultivating them from peripheral blood mononuclear cells in the presence of cytokines *(cytokine-induced killers, CIK)* [50].

Recently, Zhang et al. reported a single center experience using CIK and NK cells in patients with low- and intermediate-risk AML over a period of 11 years. One hundred and fifty-two patients were treated with combined chemotherapy (fludarabine, cyclophosphamide, and cytarabine) and immunotherapy (53 with CIK and 67 with alternating CIK and NK cells). Overall survival and disease-free survival at 80 months were up to 92% and 72%, respectively. Survival rates were superior in patients treated with CIK alternating with NK to those treated with the CIK alone (OS 95.5% vs. 71.4%, $p < 0.001$), (DFS 85% vs. 63.5%, $p = 0.001$). Side effects were mild with some fever, chills, and fatigue [51].

Checkpoint Inhibitors

Harnessing the immune system to target cancer by using checkpoint inhibitors has been a major breakthrough in cancer research in solid tumors and Hodgkin's lymphoma. Checkpoint inhibitors, including cytotoxic T-lymphocyte-associated-protein 4 (CTLA4) and programmed cell-death protein (PD-1), are antibodies that block inhibitory signals on T cells resulting in the release of "brakes" on anticancer cytotoxic T cells. Immune checkpoints play a central role in the regulation of immune homeostasis and self-tolerance, and represent an important mechanism for tumor cells to escape immune surveillance [52].

Overexpression of PD-1 and CTLA4 on AML blasts was shown to be clearly associated with a more aggressive leukemia, likely due to a suboptimal antitumor T cell response [53, 54]. Blockade of CTLA4 and PD-1/PD-L1 pathways enhanced the anti-leukemia responses and increased survival in murine models [55, 56]. We

and others have recently shown that patients with AML have a significantly higher frequency of PD1+ T cells, including PD1+ CD8+, PD1+ T-effector, and PD1+ Tregs in their bone marrows ($n = 107$) compared to healthy donors ($n = 8$). The frequency of Tregs increased progressively from healthy donors to newly diagnosed AML to relapsed AML (1.6% vs. 2.8%, $p < 0.01$, vs. 4.5% $p < 0.01$). Furthermore, an increased Treg infiltration correlated with higher proportion of $CD8^+$ T cells expressing PD-1, as well as a significantly higher PD-L1/L2 expressing AML blasts [57]. These findings point towards the exhausted T cell immunity in patients with AML suggesting a role for checkpoint inhibitor based therapies.

Recent clinical trials have demonstrated encouraging response rates and durable responses in patients with relapsed AML treated with PD-1/PD-L1 inhibitors *nivolumab (Opdivo, BMS-936558)(Bristol-Myers Squibb, USA)* or *pembrolizumab (Keytruda, MK-3475/former lambrolizumab, Merck, USA)* and *CTLA4 inhibitor ipilimumab (Yervoy, BMS-734016)* based therapies, either as a monotherapy or in combinations with other agents in patients with AML and MDS. Monotherapy with checkpoint inhibitors has shown only limited responses in patients with AML. Rational combinations with other standard anti-leukemic agents are needed to improve the response rates, and the durability of responses. HMAs (AZA and DAC), epigenetic drugs approved by FDA for the treatment of MDS, have been shown to upregulate inhibitory immune checkpoint proteins such as PD-1, PD-L1, and PD-L2, thereby potentially sensitizing T cells to PD-1/PD-L1 blocking antibodies. The effect of HMAs (AZA and DAC) on the immune system is diverse as these agents possess both immune-stimulatory as well as immune-suppressive properties. HMAs are capable of enhancing the immune response by augmenting antigenicity (upregulating tumor cell antigen expression, antigen presentation with MHC-I), overexpression of co-stimulatory molecules (including PD-1, PD-L1, and PD-L2), and inducing T cell priming and effector function [58]. Conversely, PD-1 upregulation may be involved in resistance to AZA, which might be potentially overcome by concomitant inhibition with the PD-1/PD-L1 axis [59].

The most impressive results from single agent immune checkpoint therapy in leukemia were with CTLA4 inhibitor ipilimumab in patients with relapsed AML in the post-alloSCT setting. The original report included CR/CRi in 5 of 14 patients with post-SCT relapsed/refractory AML/MDS (median of 3 prior salvage) with ipilimumab at 10 mg/kg [60]. This was recently updated with a median follow-up of 15 months. In this report, among the five responding AML/MDS patients in the original ipilimumab 10 mg/kg cohort, ongoing responses have been seen in two of the three responding patients with leukemia cutis and one responding patient with marrow AML for a duration of 30, 32, and 34 months, respectively. Response data were not presented on the ongoing expansion cohort with single agent ipi 5 mg/kg (6 AML and 1 MDS patients), or single agent nivolumab at dose 0.5–1 mg/kg cohort (4 AML, 1 MDS patients). Reported toxicity data included some serious grade ≥4 immune-related AE, including fatal myocarditis (1), pneumonitis (1) and sepsis (2), and grade 4 fever (1) and grade 4 AIHA (1). Additionally, due to the toxicity

observed at 1 mg/kg nivolumab, only the 0.5 mg/kg cohort is currently ongoing without any significant toxicities observed thus far ($n = 2$) [61].

The single agent activity of ipilimumab (3 mg/kg) was also shown in patients with refractory MDS post-HMA failure wherein single agent ipilimumab produced an overall response in 22% of the patients (2/9) with acceptable toxicity (Grade ≥3 AE in 33% of patients). In the same study, single agent nivolumab (3 mg/kg) showed no activity in 15 patients in the refractory setting.

The best results of this study with the highest response rate were observed on the third, combinational arm of nivolumab (3 mg/kg) with AZA (75 mg/m^2 × 5 days), where these two agents led to response rates of 80% in the frontline high risk MDS (9/11; 2 CR, 5 mCR and HI and 2 HI) with acceptable 27% of grade ≥3 AE [62].

Daver et al. recently reported encouraging results of AZA (75 mg/m^2 days 1–7) with nivolumab (3 mg/kg every 2 weeks)/ipilimumab (3 mg/kg monthly) or their combination in patients with AML. Cohort 1 evaluating AZA and nivolumab in relapsed/refractory AML was completed after accrual of 70 patients. Two subsequent cohorts are now enrolling: AZA with nivolumab in frontline AML ≥65 years and not suitable for induction therapy (cohort 2), and AZA with nivolumab and ipilimumab in relapsed/refractory AML (first and second salvage) (cohort 3). Results from cohort 1 on 70 patients with relapsed/refractory AML (34% with poor risk cytogenetics) and a median age of 70 years (range, 22–90) showed overall CR/CRi rate of 22% (4 CR, 11 CRi), 10% hematologic improvement, and 24% with ≥50% BM blast reduction. Patients with diploid cytogenetics, and those without prior HMA therapy or with ASXL1 mutations had higher response rate. The 8-week mortality was 7%. The median OS among the CR/CRi patients was 15.3 months (range, 2.29–17.25+), which compares favorably to historical median OS with AZA-based salvage protocols from the same institution ($p = 0.004$). Grade 3/4 immune-related AEs were observed in eight (12%) patients, and mostly included pneumonitis, colitis, nephritis, and skin rash. The median time to onset of immune-related AEs was 6 weeks (range, 4 days to 14 weeks). Preliminary results from currently enrolling cohort 2 evaluating AZA and nivolumab in patients with frontline AML ≥65 years of age ($n = 9$) showed 5 CR/CRp (including 2 CR), and 1 partial remission. Patients achieving CR/CRi had higher pretherapy total CD3+ and CD8$^+$ T cells in the BM, as well as progressive increase in BM CD8$^+$ and CD4$^+$ infiltrate during therapy [63].

Feasibility of combination of nivolumab and high dose chemotherapy in AML patients was shown by Ravandi et al. Thirty-two patients with newly diagnosed AML ($n = 30$) or high risk MDS (≥10% blasts, $n = 2$) were treated with idarubicin (12 mg/m^2 × 3 days) and cytarabine (1.5 g/m^2 over 24 h × 4 days) followed by nivolumab 3 mg/kg started on day 24 ± 2 days for up to 2 years. Overall response rate (CR/CRi) was 23 (72%). Early 8-weeks mortality was 6%, and 16% experienced grade ≥3 AE. Median overall survival has not been reached (median follow-up of 8.3 months). Similar to previous observations, there was a positive correlation between achievement of CR/CRi and a higher frequency of pretherapy CD3$^+$ total T

cell infiltrate, and between non-response and a higher frequency of pretherapy CD4+ PD1+/TIM3+ T-effector cells [64].

Encouraging results were presented in a phase 2, multicenter study evaluating a combination of pembrolizumab (200 mg) and high dose cytarabine (1.5–2 g/m^2 × 5 days) in patients with relapsed/refractory AML (n = 13). The median age was 54 years and ~50% patients were either adverse risk by ELN or secondary AML. Among the 10 evaluable patients, the overall response rate (CR/CRi) was 50% with 4 CRs. The toxicity profile has been manageable, and 2 immune-related grade 3 AEs (elevation in hepatic enzymes, and rash) were noted. Two patients underwent an alloSCT in CR, without any serious post-SCT AE. The 4-week and 8-week morality was 0% and 10%, respectively [65].

Discussion

Over the past decades, an improved understanding of the biology of AML has led to breakthroughs in AML therapy especially in the field of targeted therapies. Many such targeted therapies (FLT3 inhibitors, IDH inhibitors, BCL-2 inhibitors) have improved outcomes in patients with AML either in combination with frontline chemotherapy or hypomethylating agents, as salvage therapies, or in the post-transplant setting. Monoclonal antibodies, T cell engaging agents, and immune checkpoint inhibitors represent promising immune approaches with encouraging clinical data for a number of these modalities. Critical step in future development of these drugs will be identifying and implementing biomarkers enabling the selection of patients with AML/MDS most likely to benefit from immunotherapy. Furthermore, timing, dosing, optimal combinations, and sequencing of these therapies is an active area of research, and will hopefully improve our ability to safely and effectively deliver these therapies.

Funding Source This review was supported in part by the MD Anderson Cancer Center Support Grant (CCSG) CA016672.

Conflict of Interest L.M. and G.G.M.—none. N.D.—research funding from BMS, Pfizer, Immunogen, AbbVie, Astellas, Servier, Daiichi-Sankyo, Nohla, Genentech; advisor/consultant to Novartis, BMS, Daiichi-Sankyo, Astellas, AbbVie, Pfizer, Jazz, Agios, and Celgene. H.K.—research grants from AbbVie, Agios, Amgen, Ariad, Astex, BMS, Cyclacel, Daiichi-Sankyo, Immunogen, Jazz Pharma, Novartis, Pfizer; honoraria: AbbVie, Actinium (advisory board), Agios, Amgen, Immunogen, Orsinex, Pfizer, Takeda. F.R.—research support and honoraria from BMS. P.S.—stock/ownership for Jounce, Neon, Constellation, Oncolytics, BioAtla, Forty-Seven, Apricity, Polaris, Marker Therapeutics, Codiak; advisory board on Constellation, Jounce, Kite Pharma, Neon, BioAtla, Pieris Pharmaceuticals, Oncolytics Biotech, Merck, BioMx, Forty-Seven, Polaris, Apricity, Marker Therapeutics, Codiak.

References

1. Yates JW, Wallace HJ Jr, Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. Cancer Chemother Rep. 1973;57:485–8.
2. Kantarjian HM. Therapy for elderly patients with acute myeloid leukemia: a problem in search of solutions. Cancer. 2007;109:1007–10. https://doi.org/10.1002/cncr.22502.
3. Saito Y, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med. 2010;2:17ra19. https://doi.org/10.1126/scitranslmed.3000349.
4. Hauswirth AW, et al. Expression of the target receptor CD33 in CD34+/CD38-/CD123+ AML stem cells. Eur J Clin Invest. 2007;37:73–82. https://doi.org/10.1111/j.1365-2362.2007.01746.x.
5. 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;104:1442–52. https://doi.org/10.1002/cncr.21326.
6. 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;19:3244–54.
7. 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;121:4854–60. https://doi.org/10.1182/blood-2013-01-466706.
8. 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;29:369–77. https://doi.org/10.1200/jco.2010.31.4310.
9. Burnett AK, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy improves survival in older patients with acute myeloid leukemia. J Clin Oncol. 2012;30:3924–31. https://doi.org/10.1200/jco.2012.42.2964.
10. 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. 2012;379:1508–16. https://doi.org/10.1016/s0140-6736(12)60485-1.
11. Amadori S, et al. Gemtuzumab ozogamicin versus best supportive care in older patients with newly diagnosed acute myeloid leukemia unsuitable for intensive chemotherapy: results of the randomized phase III EORTC-GIMEMA AML-19. J Clin Oncol. 2016;34:972–9. https://doi.org/10.1200/jco.2015.64.0060.
12. Taksin AL, et al. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukemia: a prospective study of the alfa group. Leukemia. 2007;21:66–71. https://doi.org/10.1038/sj.leu.2404434.
13. 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;122:1455–63. https://doi.org/10.1182/blood-2013-03-491506.
14. Sutherland MK, et al. 5-azacytidine enhances the anti-leukemic activity of lintuzumab (SGN-33) in preclinical models of acute myeloid leukemia. MAbs. 2010;2:440–8.
15. Stein EM, et al. A phase 1 trial of vadastuximab talirine as monotherapy in patients with CD33-positive acute myeloid leukemia. Blood. 2018;131:387–96. https://doi.org/10.1182/blood-2017-06-789800.
16. Erba HP, Levy MY, Vasu S, et al. A phase 1b study of vadastuximab talirine in combination with 7+3 induction therapy for patients with newly diagnosed acute myeloid leukemia (AML). Blood. 2016;128:211. Abstract 906
17. Fathi AT, Erba HP, Lancet JE, et al. Vadastuximab talirine plus hypomethylating agents: a well-tolerated regimen with high remission rate in frontline older patients with acute myeloid leukemia. Leukemia. 2016;128(22):591.
18. Bothell W. Seattle genetics. 2017.

19. Watkins K, Walker R Fishkin N, Audette C, Kovtun Y, Romanelli A. 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. Blood. 2015;126. Abstract No 1366.
20. Whiteman KR, Noordhuis P Walker R, Watkins K, Kovtun Y, Harvey L, Wilhelm A, et al. The antibody-drug conjugate (ADC) IMGN779 is highly active in vitro and in vivo against acute myeloid leukemia (AML) with FLT3-ITD mutations. Blood. 2014. Abstract No 2321.
21. Adams S, Watkins K, McCarthy R, Wilhelm A, et al. IMGN779, a next generation CD33-targeting ADC, combines effectively with cytarabine in acute myeloid leukemia (AML) pre-clinical models, resulting in increased DNA damage response, cell cycle arrest and apoptosis in vitro and prolonged survival in vivo. Blood. 2017;130:1357.
22. Cortes J, Traer E, Wang E, Erba HP, et al. IMGN779, a next-generation CD33-targeting antibody-drug conjugate (ADC) demonstrates initial antileukemia activity in patients with relapsed or refractory acute myeloid leukemia. Blood. 2017;130:1312.
23. 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;89:1471–82.
24. 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;100:2980–8. https://doi.org/10.1182/blood-2002-03-0852.
25. Smith BD, Roboz GJ, Walter RB, et al. 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. 2014. Abstract 616.
26. 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;13:575–81.
27. Pemmaraju N, Sweet KL, Lane AA, Stein AS, et al. Results of pivotal phase 2 trial of SL-401 in patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN). Blood. 2017;130:1298.
28. 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;124:385–92. https://doi.org/10.1182/blood-2014-04-566737.
29. Konopleva M, Hogge DE, Rizzieri D, Cirrito T, Liu JS, Kornblau S, Grable M, Hwang IL, Borthakur G, et al. 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. In: 53th ASH Annual Meeting and Exposition Abstract No 3298; 2010.
30. Sweet K, Pemmaraju N, Lane A, Stein A, Vasu S, Blum W, Rizzieri DA, et al. 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). Blood. 2015;126. Abstract No 3795.
31. Lane AA, Sweet KL, Wang ES, et al. Results from ongoing phase 2 trial of SL-401 as consolidation therapy in patients with acute myeloid leukemia (AML) in remission with high relapse risk including minimal residual disease (MRD). Blood. 2016;128:215.
32. Stephansky J, Togami K, Ghandi M, Montero J, et al. Resistance to SL-401 in AML and BPDCN is associated with loss of the diphthamide synthesis pathway enzyme DPH1 and is reversible by azacitidine. Blood. 2017;130:797.
33. Romagne F, Andre P, Spee P et al. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. Blood 2009;114:2667–2677.
34. Vey N, Dumas P-Y, Recher C, Gastaud L, et al. Randomized phase 2 trial of lirilumab (anti-KIR monoclonal antibody, mAb) as maintenance treatment in elderly patients (pts) with acute myeloid leukemia (AML): Results of the Effikir Trial. Blood. 2017;130:889.

35. Daver N, Boddu P, Garcia-Manero G, Ravandi F, et al. Phase IB/II study of lirilumab with azacytidine (AZA) in relapsed AML. Blood. 2017;130:2634.
36. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 2009;69:4941–4. https://doi.org/10.1158/0008-5472.can-09-0547.
37. Cheng P, Eksioglu E, Chen X, et al. Immunodepletion of MDSC By AMV564, a novel tetravalent bispecific CD33/CD3 T cell engager restores immune homeostasis in MDS in vitro. Blood. 2017;130:51.
38. Mougiakakos D, Saul D, Braun M, et al. CD33/CD3-bispecific T-cell engaging (BiTE®) antibody constructs efficiently target monocytic CD14+ hla-DRlow IDO+ aml-MDSCs. Blood. 2017;130:1363.
39. Vallera DA, et al. IL15 trispecific killer engagers (TriKE) make natural killer cells specific to CD33+ targets while also inducing persistence, in vivo expansion, and enhanced function. Clin Cancer Res. 2016;22:3440–50. https://doi.org/10.1158/1078-0432.ccr-15-2710.
40. Rader C. DARTs take aim at BiTEs. Blood. 2011;117:4403–4. https://doi.org/10.1182/blood-2011-02-337691.
41. 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;7:289ra282. https://doi.org/10.1126/scitranslmed.aaa5693.
42. Uy GL, Godwin J, Rettig PM, et al. Preliminary results of a phase 1 study of flotetuzumab, a CD123 × CD3 Bispecific Dart® protein, in patients with relapsed/refractory acute myeloid leukemia and myelodysplastic syndrome. Blood. 2017;130:637.
43. Jacobs K, Godwin J, Foster M, Vey N, et al. Lead-in dose optimization to mitigate cytokine release syndrome in AML and MDS patients treated with flotetuzumab, a CD123 × CD3 Dart® molecule for T-cell redirected therapy. Blood. 2017;130:3856.
44. Rettig M, Godwin J, Vey N, Fox B, et al. Preliminary translational results from an ongoing phase 1 study of flotetuzumab, a CD123 × CD3 Dart®, in AML/MDS: rationale for combining flotetuzumab and anti-PD-1/PD-L1 immunotherapies. Blood. 2017;130:1365.
45. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015;348:62–8. https://doi.org/10.1126/science.aaa4967.
46. Pegram HJ, Park JH, Brentjens RJ. CD28z CARs and armored CARs. Cancer J. 2014;20:127–33. https://doi.org/10.1097/ppo.0000000000000034.
47. Ritchie DS, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013;21:2122–9. https://doi.org/10.1038/mt.2013.154.
48. Budde L, Song JY, Blanhcard S, Wagner J, et al. Remissions of acute myeloid leukemia and blastic plasmacytoid dendritic cell neoplasm following treatment with CD123-specific CAR T cells: a first-in-human clinical trial. Blood. 2017;130:811.
49. Hermanson DL, Kaufman DS. Utilizing chimeric antigen receptors to direct natural killer cell activity. Front Immunol. 2015;6:195. https://doi.org/10.3389/fimmu.2015.00195.
50. Yang XY, Zeng H, Chen FP. Cytokine-induced killer cells: a novel immunotherapy strategy for leukemia. Oncol Lett. 2015;9:535–41. https://doi.org/10.3892/ol.2014.2780.
51. Zhang X, Yang J, Zhang G, Lu P. A 11-year clinical summary of DC—CIK/NK cell immunotherapy for 152 patients with acute myeloid leukemia. Blood. 2017;130:1369.
52. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–64. https://doi.org/10.1038/nrc3239.
53. 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;21:1451–9. https://doi.org/10.1038/sj.leu.2404720.
54. 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;114:1528–36. https://doi.org/10.1182/blood-2008-09-179697.

55. Zhang L, Gajewski TF, Kline J. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. Blood. 2009;114:1545–52. https://doi.org/10.1182/blood-2009-03-206672.
56. 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;117:4501–10. https://doi.org/10.1182/blood-2010-10-310425.
57. Williams P, Basu S, Garcia-Manero G, Cortes J, et al. Checkpoint expression by acute myeloid leukemia (AML) and the immune microenvironment suppresses adaptive immunity. Blood. 2017;130:185.
58. Heninger E, Krueger TE, Lang JM. Augmenting antitumor immune responses with epigenetic modifying agents. Front Immunol. 2015;6:29. https://doi.org/10.3389/fimmu.2015.00029.
59. 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. 2014;28(6):1280–8. https://doi.org/10.1038/leu.2013.355.
60. Davids MS, et al. Ipilimumab for patients with relapse after allogeneic transplantation. N Engl J Med. 2016;375:143–53. https://doi.org/10.1056/NEJMoa1601202.
61. Davids MS, Kim HT, Costello C, Herrera AF, et al. Optimizing checkpoint blockade as a treatment for relapsed hematologic malignancies after allogeneic hematopoietic cell transplantation. Blood. 2017;130:275.
62. Garcia-Manero G, Daver NG, Montalban-Bravo G, Jabbour EJ, et al. A phase II study evaluating the combination of nivolumab (Nivo) or ipilimumab (Ipi) with azacitidine in Pts with previously treated or untreated myelodysplastic syndromes (MDS). Blood. 2016;128:344.
63. Daver N, Basu S, Garcia-Manero G, Cortes EJ, et al. Phase IB/II study of nivolumab with azacytidine (AZA) in patients (pts) with relapsed AML. J Clin Oncol. 2017;35:7026–702.
64. Ravandi F, Daver N, Garcia-Manero G, Benton CB, et al. Phase 2 study of combination of cytarabine, idarubicin, and nivolumab for initial therapy of patients with newly diagnosed acute myeloid leukemia. Blood. 2017;130:815.
65. Zeidner FJ, Vincent BG, Ivanova A, Foster M, et al. Phase II study of high dose cytarabine followed by pembrolizumab in relapsed/refractory acute myeloid leukemia (AML). Blood. 2017;130:1349.

Chapter 5
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. 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 three 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, at our institution, 20% of them had a dose interruption as a result. This means potentially 9% of patients having dose interruption of ipilimumab because of their cutaneous AEs. 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-PD-1 · Anti-CTLA-4 · Dermatitis · Rash · Immunotherapy · Pruritus

The novelty of immune checkpoint inhibitors has only recently led to the characterization of cutaneous adverse events (AEs). This, along with the substantial rate of cutaneous reactions, has left many clinicians 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 three 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, at our institution, 20% of them had a dose interruption as a result. This means potentially 9% of patients having dose interruption of ipilimumab because of

A. B. Patel (✉) · O. Pacha
Department of Dermatology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: APatel11@mdanderson.org; opacha@mdanderson.org

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_5

their cutaneous AEs [1]. In the following chapter, we will review the categories of these drugs, common cutaneous effects, their grading, and management options.

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

Common Cutaneous Adverse Events Seen with Immune Checkpoint Inhibitors

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

Morbilliform drug eruption (commonly identified as "maculopapular") clinically presents with erythematous macules and thin 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 the distribution is more diffuse and mucosal surfaces are involved, but body surface area (BSA) remains below 10%, this is SJS. When the BSA is greater than 30%, this is called TEN, which can rapidly progress. For morbilliform eruptions, topical steroids with drug continuation are often sufficient. For EM, depending on the severity, oral or IV steroids can be used with drug cessation. For SJS and TEN, drug cessation and supportive care are critical, possibly with the addition of intravenous steroids, or intravenous immunoglobulin therapy.

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

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

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

It is important to distinguish between the inflammatory skin reactions as they have different treatment options for the more severe presentations. Although mild presentations may be treated with topical steroids, diffuse presentations require systemic treatments, some of which are specific to the type of inflammatory reaction (Figs. 5.1 and 5.2).

Eczema appears as pruritic, ill-defined, edematous and erythematous papules coalescing into plaques occasionally with vesicles in exuberant cases. As it evolves, the plaques are rough, erythematous, and have visible excoriation. Distribution is diffuse, affecting the trunk and extremities more than the face with a flexural predominance, as is typical with atopic dermatitis. Scalp and genital areas are often involved in diffuse presentations. Plaques are very pruritic with pain in areas of microfissures or superinfection. The histology shows prominent spongiosis and the variable presence of eosinophils [13]. Treatment consists of topical steroids, usually mid-strength creams such as triamcinolone 0.1% to begin with and graduating to super-potent formulations such as clobetasol 0.05% cream. The face, axilla, and groin are usually treated with mild and low potency steroids such as hydrocortisone 2.5% or desonide 0.05% creams. Patients can be effectively controlled with a regimen of topical steroids involving twice daily application for flares and twice weekly application for maintenance. Supplementation with first-generation oral antihistamines such as diphenhydramine or hydroxyzine is a mainstay. In the author's experience, the addition of

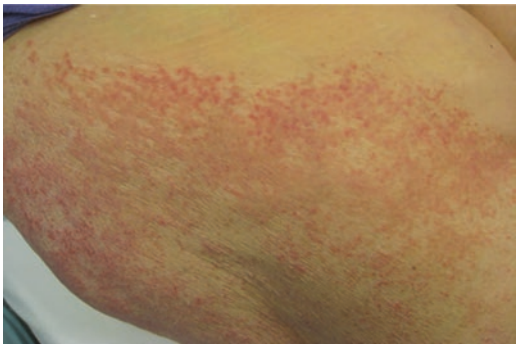

Fig. 5.1 Eczema—erythematous papules coalescing into plaques that are rough and have minimal scale

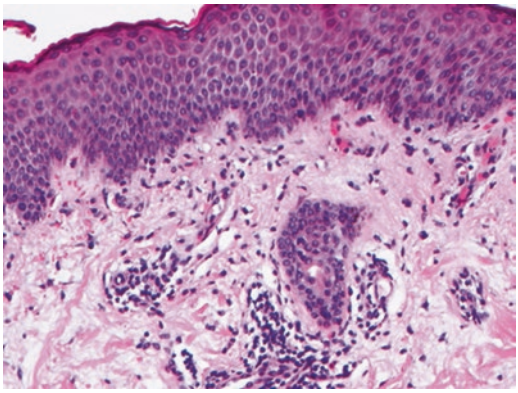

Fig. 5.2 Eczemaspongiotic dermatitis with dermal eosinophils

second-generation nonsedating antihistamines such as cetirizine or loratadine in the morning is also beneficial. In patients with grade 3 AEs, involving >30% of BSA, and refractory to topical therapies, the addition of oral steroids such as prednisone at 1 mg/kg is usually effective and can be slowly tapered. The slow taper is often effectively weaned with topical steroid maintenance.

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

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

For pruritus without rash, clinical presentation is variable. Most often patients have normal-appearing skin, although they can have skin changes secondary to manipulation masquerading as a primary rash. Geometric erosions and ulcerations, prurigo nodules, and linear erosions are secondary to the pruritus. Prurigo nodules are ill-defined, discrete, erythematous, hyperpigmented acanthotic papules often with central erosion. Histology shows fibrosis and vertically oriented blood vessels in the superficial dermis with an overlying acanthotic epidermis. The first step in management is to eliminate a primary inflammatory condition. For primary pruritus, a stepwise approach depending on severity is best. For mild cases, a first-generation antihistamine is oftentimes sufficient with the added benefit of sedation that can help patients sleep when pruritus is usually most severe—right before bed. As intensity increases, the addition of tricyclic antidepressant doxepin nightly and GABA agonists like gabapentin at increasing doses have been effectively used (Figs. 5.3 and 5.4).

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-CTLA-4 and anti-PD-1 therapies [3]. Histology shows loss of melanocytes at the dermal–epidermal junction. Patients are usually

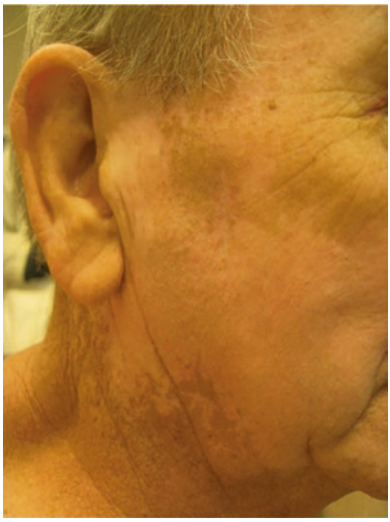

Fig. 5.3 Vitiligo-depigmented patches of head and neck

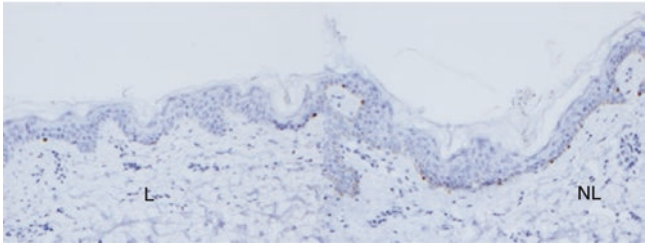

Fig. 5.4 Vitiligo-MART1 immunostain in lesional skin (L) showing decreased melanocytes at the dermal–epidermal junction compared to MART1 immunostain of non-lesional (NL) skin

asymptomatic, but can have occasional preceding pruritus. Treatment for vitiligo includes a combination of topical steroids and ultraviolet (UV) light therapy; however, in melanoma patients with this drug-induced side effect, treatment is not usually undertaken because of the risk of further skin cancers with increased UV exposure.

Common Cutaneous Adverse Events for Anti-CTLA-4

The most commonly reported adverse events in patients receiving ipilimumab are "rash" from one quarter to more than one half of patients and pruritus from a quarter to one third [16]. The type of rash varied from mild eczema to toxic epidermal necrolysis [17] with the majority experiencing a more traditional morbilliform drug eruption or an eczematous atopic dermatitis-like eruption [16]. The onset of rash has been reported to appear at about 3 weeks and then usually resolves at about 2.5 months [16]. Although in our institutional review, complete resolution was usually not

obtained for most patients until drug cessation (unpublished data Patel). The most common CAEs seen with this class of medication are discussed above. Less frequent eruptions include acneiform eruption [12] and granulomatous dermatitis [18].

Its mechanism of action through the activation of T cells by the prevention of T cell blockade leads to an upregulation of the body's immune system and therefore its antitumor activity as described elsewhere in this text. It appears that the cutaneous AE is independent of dosing with those on 10 mg/kg developing similar CAEs as those on 3 mg/kg. Fortunately, high grade rash as defined by the common terminology criteria as grade 3 or higher was substantially lower at 2.4% [19].

CAE in Anti-PD-1

In addition to the shared inflammatory skin reactions discussed earlier, psoriasis [20, 21] and bullous pemphigoid have been induced by anti-PD-1 antibodies [22, 23]. More recently, eruptive keratoacanthomas have been reported in patients receiving anti-PD-1 therapy [24] (Figs. 5.5 and 5.6).

Psoriasiform dermatitis can appear clinically as classic psoriasis vulgaris with well-demarcated erythematous slightly indurated plaques with adherent fine scale and areas of sparing in a focal to diffuse distribution. It is often worse on extremities than trunk and has a predilection for the scalp. It can also present in inverse distribution with prominence in intertriginous areas [21], or in the pustular variant (Patel unpub). It can be pruritic or painful, induce microfissures, and contribute to edema of extremities. Histology shows a spongiotic psoriasiform dermatitis with subcorneal pustules with variable eosinophils. The authors have found psoriasis to be more

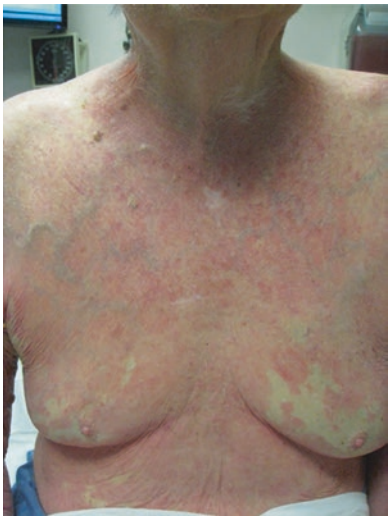

Fig. 5.5 Psoriasiform dermatitis—erythematous well-demarcated plaques with fine adherent scale

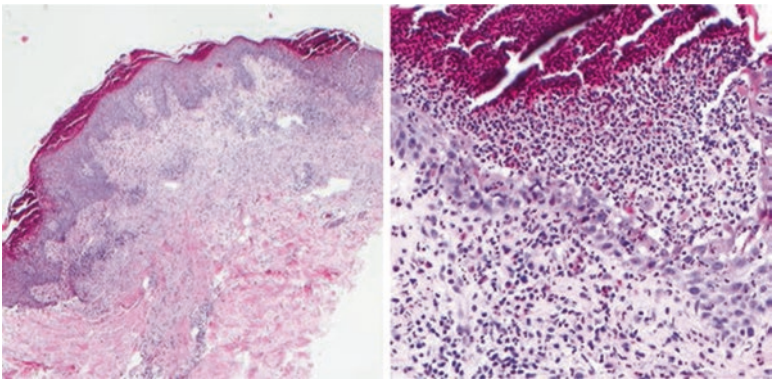

Fig. 5.6 Spongiotic psoriasiform dermatitis with subcorneal pustules, irregular acanthosis, and numerous eosinophils

resistant to treatment than eczema, making distinguishing between the two a prognostic indicator of rash outcome. Treatment should start with topical steroids with antihistamines, if indicated. Escalation of treatment includes oral acitretin, oral apremilast, ultraviolet-B (UV-B) therapy, or oral steroids. Biological medications such as interleukin-17 (IL-17) inhibitors are a potential therapy for refractory cases and have been used anecdotally with success.

Bullous pemphigoid is an antibody-mediated bullous disorder presenting with tense bullae. The bullae vary in size, are filled with serous fluid, and are extremely pruritic. Histology shows a subepidermal vesicular dermatitis with prominent eosinophils in the superficial dermis and within the bullae. The dermal–epidermal split is cleaved and the epidermal roof is intact. Dyskeratosis is not a feature. Direct immunofluorescence highlights IgG deposition at the dermal–epidermal junction. Topical and oral steroids as well as rituximab have been used successfully in this slow-to-appear cutaneous AE [25].

Eruptive keratoacanthoma appears to be relatively well demarcated and a low grade of squamous cell carcinoma. They were treated conservatively in this report without treatment interruption for the patients [24].

Grading

Grading has nearly been universally based upon the Common Terminology Criteria for Adverse Events and more recently a modified version produced by the American Society of Clinical Oncology as their “Practice Guideline” that focuses on symptoms and quality of life rather than extent of involvement. This appears to be a more useful measure as relatively small body surface area involvement can still be dose limiting (Table 5.1 and Fig. 5.7).

Table 5.1 Common terminology criteria for adverse events [26]

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		

CAE as Prognostic Indicators

Vitiligo is a relatively innocuous adverse event as it is largely asymptomatic and untreated. It is, however, associated with increased progression free survival and tumor response when occurring in patients on immune checkpoint inhibitors. Vitiligo is widely believed to be an underreported side effect as it can be easily missed if a full body skin exam is not performed. Vitiligo has only been reported in patients being treated with melanoma [2, 3, 28, 29]. Incidence of rash was also associated with increased survival and tumor response [2].

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

Fig. 5.7 Management of skin irAEs in patients treated with ICPIs [27]

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

Fig. 5.7 (continued)

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

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

Diagnostic work-up

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

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

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

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

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

Fig. 5.7 (continued)

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, Vujic I, Daud A, et al. Pembrolizumab cutaneous adverse events and their association with disease progression. JAMA Dermatol. 2015;151(11):1206–12.
3. Teulings HE, Limpens J, Jansen SN, 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, Phan GQ, Maker AV, 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. Sundaresan S, Nguyen KT, Nelson KC, Ivan D, Patel AB. Erythema multiforme major in a patient with metastatic melanoma treated with nivolumab. Dermatol Online J. 2017;23(9).
6. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711–23.5.
7. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364:2517–26.
8. 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;384:1109–7.7.
9. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372:320–30.
10. 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.
11. 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.
12. 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.
13. 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.
14. Fujii T, Colen RR, Bilen MA, et al. Incidence of immune-related adverse events and its association with treatment outcomes: the MD Anderson Cancer Center experience. Invest New Drugs. 2018;36(4):638–46.
15. Horvat TZ, Adel NG, Dang TO, et al. Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. J Clin Oncol. 2015;33(28):3193–8.
16. Lacouture ME, Wolchok JD, Yosipovitch G, Kähler KC, Busam KJ, Hauschild A. Ipilimumab in patients with cancer and the management of dermatologic adverse events. J Am Acad Dermatol. 2014;71(1):161–9.
17. Nayar N, Briscoe K, Fernandez Penas P. 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.
18. Kubicki SL, Welborn ME, Garg N, Aung PP, Patel AB. Granulomatous dermatitis associated with ipilimumab therapy (Ipilimumab associated granulomatous dermatitis). J Cutan Pathol. 2018;45(8):636–8.
19. 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.

20. 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.
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. Jour G, Glitza IC, Ellis RM, et al. Autoimmune dermatologic toxicities from immune checkpoint blockade with anti-PD-1 antibody therapy: a reporton bullous skin eruptions. J Cutan Pathol. 2016;43(8):688–96.
23. 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.
24. Freites-martinez A, Kwong BY, Rieger KE, Coit DG, Colevas AD, Lacouture ME. Eruptive keratoacanthomas associated with pembrolizumab therapy. JAMA Dermatol. 2017;153(7):694–7.
25. Sowerby L, Dewan AK, Granter S, Gandhi L, Leboeuf NR. Rituximab treatment of nivolumab-induced bullous pemphigoid. JAMA Dermatol. 2017;153(6):603–5.
26. Common Terminology Criteria for Adverse Events (CTCAE) v4.0. 2008. http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. Accessed 26 Jul 2016.
27. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2018;36(17):1714–68. https://doi.org/10.1200/JCO.2017.77.6385.
28. 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.
29. 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.

Chapter 6
Immune-Related Adverse Events: Pneumonitis

Akash Jain, Vickie R. Shannon, and Ajay Sheshadri

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

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

Introduction

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

A. Jain · V. R. Shannon · A. Sheshadri (✉)
Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: asheshadri@mdanderson.org

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_6

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

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

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

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

Immune Checkpoint Inhibition as a Therapeutic Strategy in Cancer

Cancer cells harness checkpoint activation through the PD-1 and CTLA-4 pathways to induce anergy in anti-tumor lymphocytes. Inhibition of these pathways can lead to tumor regression. In this section, we will briefly discuss the CTLA-4 inhibitor ipilimumab, the PD-1 inhibitors nivolumab and pembrolizumab, and the PD-L1 inhibitors atezolizumab, avelumab, and durvalumab. These drugs have been approved by the Federal Drug Administration (FDA) to treat several cancers, and several more trials of ICPI therapy are underway.

Ipilimumab is the only CTLA-4 inhibitor approved by the FDA. Ipilimumab binds the front β-sheet of CTLA-4 and interferes with the formation of CTLA-4:B7 complexes [36]. The Federal Drug Administration approved ipilimumab in 2011 after a pivotal studied showed improved survival in metastatic melanoma [37]. Another CTLA-4 inhibitor, tremelimumab, is in development, but not yet approved by the FDA and is beyond the scope of this chapter.

Inhibitors of the PD-1 pathway broadly fall into two categories: inhibitors of PD-1 function and inhibitors of PD-L1 function. Nivolumab and pembrolizumab bind competitively to PD-1 to form PD-1:monoclonal antibody complexes [38]. However, the two drugs bind PD-1 in slightly different orientations. Nivolumab was approved by the FDA for use in melanoma in 2014, squamous cell lung cancer and advanced renal cell cancer in 2015, non-Hodgkin's lymphoma and classical Hodgkin's lymphoma in 2016, and in combination with ipilimumab for treatment of advanced renal cell cancer in 2018. Pembrolizumab was approved by the FDA for use in melanoma in 2014, metastatic non-small cell lung cancer in 2015, advanced head and neck cancers in 2016, and solid tumors with mismatch repair deficiencies or microsatellite instability in 2017.

Avelumab, atezolizumab, and durvalumab competitively bind to PD-L1 in slightly different orientations [39]. In 2017, avelumab was approved by the FDA for use in urothelial cell cancer and Merkel cell carcinoma. In 2016, the FDA approved atezolizumab for use in urothelial cell cancer and non-small cell lung cancer. Durvalumab was approved by the FDA for use in metastatic urothelial cell cancer in 2017, non-small cell lung cancer in 2018. Several other PD-1 and PD-L1 inhibitors are in development but beyond the scope of this chapter.

Clinical and Radiologic Patterns of Pneumonitis

In the following section, we discuss presentations of pneumonitis after immune checkpoint inhibitor (ICI) therapy. Pneumonitis is a rare irAE after ICI therapy that presents as an interstitial lung disease [40]. Pneumonitis after ICI therapy presents in four patterns: organizing pneumonia (OP), nonspecific interstitial pneumonia (NSIP), hypersensitivity pneumonitis (HP), and diffuse alveolar damage (DAD).

For the purposes of this chapter, we will combine NSIP and HP into one category, due to similarities in presentation and in therapeutic approaches. Table 6.1 summarizes the clinical, radiological, and pathological features associated with each pattern of pneumonitis, and Fig. 6.1 shows characteristic images from chest computed tomography (CT) scans. A more complete discussion of the clinical features and pathophysiology of various ILDs is available elsewhere [41, 42].

OP: OP is a common manifestation of pneumonitis after ICI therapies [43]. OP primarily affects distal bronchioles, respiratory bronchioles, alveolar ducts, and alveolar walls [44]. Symptoms of OP may include low-grade fever, malaise, and cough, and the onset of symptoms in idiopathic cases is often subacute [45–48]. Respiratory infections are often associated with the development of OP though the mechanism remains unclear [49]. Thoracic CT imaging of patients with OP primarily appears as ground-glass or consolidative opacities which are more predominant in the lung periphery in sub-pleural regions [50]. The reverse halo sign, which is characterized by ground-glass opacities surrounded by denser consolidative opacities, can be seen in OP but is not pathognomonic [51]. The extent of radiological involvement can vary substantially from case to case. The histology of OP is characterized by excessive proliferation of plugs of granulation tissue (Fig. 6.2) in distal airspaces with infiltration by lymphocytes and plasma cells [50]. These plugs consist of loose collage, fibroblasts, and myofibroblasts. Bronchoalveolar lavage (BAL) is often performed in OP to rule out infection though a BAL inflammatory signature is not sufficient to diagnose OP [50]. The treatment of OP depends upon the severity of the disease. We recommend use of the Common Terminology Criteria for Adverse Events (CTCAE, Table 6.2) to grade the severity of pneumonitis [52]. Mild cases (Grade 1) of OP may resolve spontaneously, but close monitoring for early signs of pulmonary impairment is imperative [53]. Patients with pneumonitis of grade 2 or higher should be treated with corticosteroid therapy. Corticosteroids are highly efficacious in OP, and treatment doses typically start at 0.5–1 mg/kg/day of prednisone or equivalent for 3–6 months. Interruptions in corticosteroid treatment may result in relapse of OP [54]. Non-corticosteroid therapies, such as cyclophosphamide, cyclosporine, rituximab, and macrolides, have been associated with anecdotal success in small case series of steroid-refractory patients, but are not typically used [55–58]. Infliximab has been reported to be effective in severe pneumonitis, but this requires validation in a prospective study [43]. In general, at least temporary cessation of ICI therapy is recommended to allow for resolution of pneumonitis.

NSIP: NSIP is a rare ILD that is often associated with autoimmune diseases or human immunodeficiency virus infection, and along with OP is a common manifestation of pneumonitis after ICI therapy [59]. NSIP typically presents with nonspecific symptoms of cough and dyspnea though the duration of symptoms may vary from case to case. Thoracic CT imaging of NSIP typically reveals ground-glass opacities, reticular infiltrates, and traction bronchiectasis [60–62]. Sub-pleural sparing of lung infiltrates may help distinguish NSIP from idiopathic pulmonary fibrosis [63]. The HP variant of ICI-related pneumonitis may be characterized by air trap-

Table 6.1 Clinical, radiological, and histopathological features of common patterns of pneumonitis

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

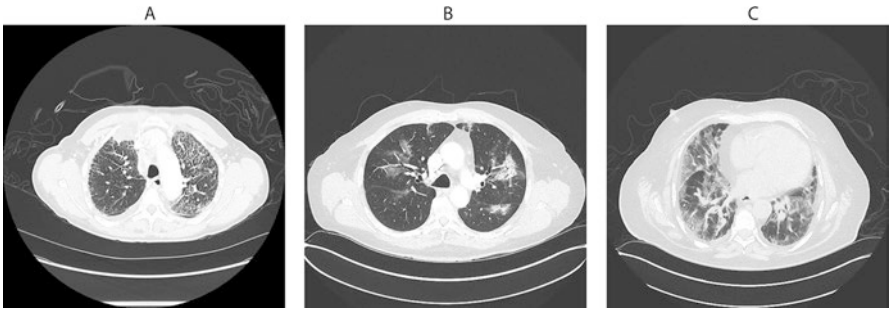

Fig. 6.1 Representative images of (**a**) nonspecific interstitial pneumonitis, (**b**) organizing pneumonia, and (**c**) diffuse alveolar damage in patients receiving precision oncology therapies

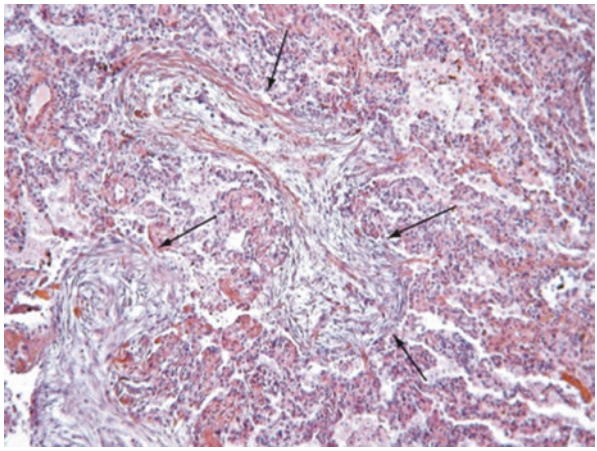

Fig. 6.2 Buds of granulation tissue (*arrows*) in the lumen of alveoli. Reproduced with permission from *Clinical Respiratory Medicine*, Cottin V. and Cordier J., 2012, Elsevier Publishing

Table 6.2 Grading of pneumonitis as outlined by the Common Terminology Criteria for Adverse Events v5.0

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

ping on expiratory chest CT imaging [64]. However, unlike HP that occurs in the general population, there is no clear link to pulmonary exposures such as aerosolized molds [65] or toxic chemicals [66]. Histologically, NSIP is characterized by dense fibrosis with diffuse inflammatory cell infiltration and uniform and diffuse thickening of alveolar walls, but unlike idiopathic pulmonary fibrosis, there is no loss of alveolar integrity [67]. Fibroblastic foci may be present, but are less common in cases of NSIP [68]. The HP variant of pneumonitis may be characterized by poorly formed non-caseating granulomas [64]. In general, patients who develop NSIP after ICI therapy require corticosteroid therapy (0.5–1 mg/kg/day of prednisone or equivalent) for 8–12 weeks. Steroid-refractory disease is more commonly seen in NSIP than in OP and may require further therapy with intravenous corticosteroids and/or cytotoxic therapies [53]. For ICI-related NSIP, interruption of ICI therapy is generally recommended [69].

DAD: DAD is a severe form of pneumonitis caused by widespread alveolar injury that results in severe capillary leak and non-cardiogenic pulmonary edema [69, 70]. Clinically, the presentation is similar the acute respiratory distress syndrome, characterized by tachypnea, severe hypoxemia, and widespread alveolar infiltrates. Typically, this occurs more rapidly than OP or NSIP, with the onset of symptoms rapidly progressing in days. Though histology is difficult to obtain due to the severity of illness, the histopathologic appearance of diffuse alveolar damage (DAD) is characterized by the formation of thickened alveolar membranes, hyaline membrane deposition, and infiltration with inflammatory cells (Fig. 6.3) [71, 72]. The acute phase of DAD is characterized by inflammation and edema of alveolar structures, while the organizing phase is characterized by the deposition of collagen by fibroblasts [73]. Thoracic CT images of DAD show widespread airspace opacities, which may be more prominent in the dependent areas of the lung [74–76]. Other diseases may mimic drug-induced DAD and should be ruled out. Pulmonary infections and eosinophilic pneumonias may be ruled out by analysis of BAL fluid, while congestive heart failure should be ruled out with a thorough clinical examination, echocardiography, and potentially right heart catheterization. Supportive therapies, including noninvasive or invasive mechanical ventilation are often necessary to treat respiratory failure associated with DAD. Early initiation of high-dose systemic corticosteroids is generally recommended although data supporting this practice is very limited. Mortality rates despite aggressive therapy remain high [77].

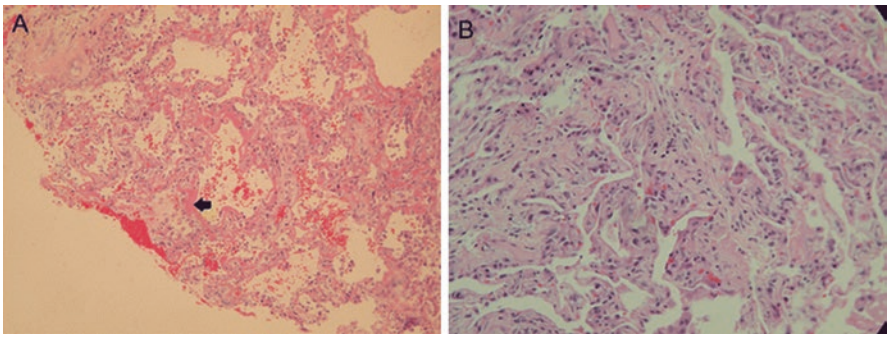

Fig. 6.3 Pathological findings of diffuse alveolar damage. (**a**) Diffuse alveolar damage in the acute phase. The interstitium is edematous. Hyaline membrane (arrow) is seen lining the alveolar ducts (hematoxylin and eosin stain, ×100). (**b**) Diffuse alveolar damage in the organizing phase. The interstitium is thickened with organizing connective tissue. Prominent type 2 pneumocyte hyperplasia is seen (hematoxylin and eosin stain, ×200) [73]

Clinical Approach to the Evaluation of ICI-Related Pneumonitis

Because symptoms of pneumonitis may be subtle and masked by other comorbid symptoms associated with the underlying cancers (e.g., large lung cancers or widespread pulmonary metastases), we advise that clinicians that evaluate and treat patients who are on ICI therapies have a low threshold for initiating a thorough evaluation for pneumonitis. Symptoms such as dyspnea, cough, fever, and chest pain should raise the suspicion for pneumonitis [78, 79]. We recommend thoracic imaging and pulmonary function testing. Chest radiography is not sufficiently sensitive to detect subtle findings of pneumonitis; therefore, symptomatic patients should be referred for thoracic CT imaging [80]. Radiation doses associated with thoracic CT are low with modern scanners, making serial thoracic imaging a safe and effective method to evaluate progression or resolution of pneumonitis [81]. Pulmonary function testing should be performed at the time of evaluation, as early impairment in pulmonary function may herald the onset of pneumonitis [82]. Furthermore, in patients with confirmed pneumonitis, pulmonary function should be monitored serially to evaluate for progression or resolution of pneumonitis. Early consultation with pulmonary experts is recommended, and bronchoscopy with BAL should be performed early in the course of the evaluation of patients who are suspected of having ICI-related pneumonitis in order to rule out alternative diagnoses, such as infectious pneumonia. Surgical biopsies of the involved lung parenchyma should be considered in select patients to evaluate the histopathological features of pneumonitis. Transbronchial biopsies are generally not recommended due to poor sensitivity for the detection of ILD [83].

Incidence and Clinical Characteristics of Pneumonitis After ICI Therapy

The incidence of pneumonitis varies with the specific agent. For example, pneumonitis occurs in about 1% of patients treated with ipilimumab, while the incidence with PD-1 and PD-L1 inhibitor monotherapy is 3–5%, and the incidence with combination therapy with PD-1 or PD-L1 inhibitors and CTLA-4 inhibitors is as high as 10% [84–88]. In general, the median onset of pneumonitis is about 3 months [43, 89–91]. Pneumonitis after ICI therapy generally presents as OP or NSIP, but may rarely present as DAD and can have a fulminant course. In this section, we discuss incidence rates and specific forms of pneumonitis that occur with each FDA-approved ICI therapy.

CTLA-4 Inhibitors

Ipilimumab is the only CTLA-4 inhibitor approved by the FDA at the time of this writing. The incidence of pneumonitis with ipilimumab is low, with pneumonitis of any grade occurring in 1.3% of treated patients, and high-grade (grades 3 or 4) pneumonitis occurring in 0.3% of treated patients [92]. The median time from treatment initiation to the onset of pneumonitis has been reported to be around 2.3 months, and the most common pattern of pneumonitis is OP [93]. While some irAEs are more common with CTLA-4 inhibitors than PD-1 or PD-L1 inhibitors [94, 95], pneumonitis is less common, though the mechanism for this difference is unclear [96]. Pneumonitis occurs at about one-third the rate in patients treated with ipilimumab for melanoma treatment as compared to those being treated for renal cell cancer or non-small cell lung cancer [96]. One possibility for this may be the presence of lung disease from cigarette smoking, as has been described in other ILDs [97].

PD-1 and PD-L1 Inhibitors

In this section, we will discuss the PD-1 inhibitors nivolumab and pembrolizumab and the PD-L1 inhibitors atezolizumab, avelumab, and durvalumab. Pneumonitis after PD-1 inhibition occurs as much three times more frequently as compared to conventional chemotherapy regimens across several types of cancers [98]. A recent meta-analysis of clinical trials of nivolumab and pembrolizumab found that the overall incidence of pneumonitis due to anti-PD-1 therapy is around 3% overall and 1.5% for high-grade pneumonitis [98]. However, the incidence in individual trials ranged from around 0.5% in melanoma [94] to around 5% in non-small cell lung cancer [99]. Similar to ipilimumab, the incidence of pneumonitis after PD-1

inhibition seems to be higher in smoking-related cancers. The rate of any-grade pneumonitis and high-grade (grade 3 or higher by CTCAE criteria) pneumonitis in renal cell cancer (any: 4.4%, high: 1.7%) and non-small cell lung cancer (any: 4.3%, high: 2.0%) are higher than in studies of melanoma (any: 1.4%, high: 0.9%) [98]. Similarly, in a case-control study of patients who developed pneumonitis after PD-1 inhibitor therapy, smoking status was not associated with the risk of pneumonitis, but a history of COPD or lung radiotherapy was predictive of pneumonitis [100]. However, there does not appear to be any difference in the incidence of pneumonitis by PD-1 inhibitor dosage, suggesting that irAEs are not directly tied to these therapies in a dose-dependent fashion [98]. This is consistent with our observation that pneumonitis after checkpoint inhibitor therapy appears to be an idiosyncratic phenomenon. Pneumonitis after PD-L1 inhibitor therapy may occur less frequently than after PD-1 inhibitor therapy. In non-small cell lung cancers, the overall incidence of any-grade and high-grade pneumonitis was higher in patients treated with PD-1 inhibitors as compared to PD-L1 inhibitors (PD-1 vs. PD-L1: any: 3.6% vs. 1.3%; high: 1.1% vs. 0.4%) [85].

One key caveat is that because many of these trials were single-arm, open-label studies, these results could be prone to bias. In fact, in patients treated with PD-1 and PD-L1 inhibitors in clinical practice at two high-volume institutions, the rates of pneumonitis after PD-1 or PD-L1 inhibition appear to be similar in those with melanoma (5%) and those with non-small cell lung cancer (4%) [86]. The median time to pneumonitis in that study was 2.8 months from the time of treatment initiation. Further studies are needed to better understand the incidence of pneumonitis, particularly as these therapies are approved for new cancers. For example, in a small sub-cohort, Naidoo et al. found an 11% incidence rate of pneumonitis in patients with hematologic cancers, markedly higher than in melanoma or non-small cell lung cancer [86].

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

By inhibiting both the CTLA-4 and PD-1 pathways, it is possible to achieve greater immune activation that may increase anti-tumor responses in certain cancers [101]. However, this also increases the risk for irAEs, including pneumonitis. Compared to monotherapy, the incidence of pneumonitis with combination therapy may be as high as 10%, and the time to onset is usually sooner [86]. Naidoo et al. found that the median time to pneumonitis onset was 2.7 months in patients receiving combination ICI therapy as opposed to 4.6 months in those receiving ICI monotherapy [86]. Wu et al. found a similarly higher incidence of pneumonitis with combination ICI therapy as compared to ICI monotherapy. In combination ICI therapy, the incidence of pneumonitis was almost 7%, and the incidence of high-grade pneumonitis was almost 2% [98]. This suggests that when compared to ICI monotherapy, combination ICI therapy results in a higher risk for any-grade and high-grade pneumonitis, and a faster onset to pneumonitis in patients in whom this

develops. ICI therapies often have durable effects due the induction of immunologic memory [102]. As a result, sequential treatment with PD-1/PD-L1 inhibitors and CTLA-4 inhibitors may have a similar increase in the risk of pneumonitis as with combination ICI therapy where both PD-1/PD-L1 inhibitors are given at the same time. In a small study of 40 patients who received nivolumab or pembrolizumab followed by ipilimumab, Bowyer et al. found that 8% of patients experienced high-grade pneumonitis [103]. This finding needs to be confirmed in a larger study cohort, but suggests that when ICI therapies are given sequentially, the risk of pneumonitis is similar to combination therapy.

Radiologic Patterns of Pneumonitis After ICI Therapy

Pneumonitis after ICI therapy typically presents as NSIP or COP. In clinical practice, in a cohort of 915 patients who received ICI monotherapies or combination therapies, the most common pattern of pneumonitis was NSIP (18/27), followed by COP (5/27). Others have shown that COP is more common after PD-1 [43] or CTLA-4 inhibitor therapy [93]. DAD reactions are rarer and typically have a more severe clinical course, but may still be managed with prompt initiation of immunosuppression.

Other manifestations of pulmonary irAEs have been described in the literature. Airway inflammation with bronchiolitis has been described in a patient who was receiving nivolumab for non-small cell lung cancer [104]. Rapidly recurrent pleural and pericardial effusions were reported in two patients within 8 weeks of initiating nivolumab therapy [105]. An increased incidence of pleural effusions was also noted in the early clinical trials of nivolumab therapy in patients with non-small cell lung cancer, although these effusions could not be definitely attributed to nivolumab, as opposed to progression of disease [106]. ICI-related pleural and pericardial fluid accumulation may be a form of irAE or a form of pseudoprogression. Drug interruption and management of pleural/pericardial drainage procedures are the primary focus of treatment. Initiation of immunosuppressive therapy for recalcitrant effusions is reasonable although the role of steroids in this setting has not been established.

Sarcoid-like reactions have been observed with ipilimumab [93, 107, 108] and with PD-1 inhibition [109, 110]. Sarcoid-like reactions are rare irAEs, and the manifestations vary from case to case. Presentations may include mediastinal lymphadenopathy, pulmonary infiltrates, skin rashes, and renal disease. While these reactions may resemble sarcoidosis clinically, the immunology is not necessarily identical to sarcoidosis that occurs in the general population [107, 111]. However, inhibition of immune checkpoint pathways may increase the population of Th17 cells, which are thought to be involved in non-ICI-related sarcoidosis [112, 113]. Therefore, there is a plausible biological basis for the incidence of sarcoid-like reactions in patients treated with ICI inhibitors. Treatment includes interruption of ICI treatment and systemic steroids. Further work is necessary to understand the incidence of sarcoid-like reactions after ICI therapies.

Areas of Uncertainty

Re-challenge with ICI Therapies After the Occurrence of Pneumonitis

A key question in patients receiving ICI therapy is whether the onset of irAEs such as pneumonitis may indicate a more favorable response to treatment. Some groups have found that patients who experience irAEs have a better treatment response [91, 114], while others have not [115]. Therefore, re-challenge with ICI therapies after the occurrence ICI-related pneumonitis may be desirable. Several groups have reported the safety of resuming ICPI therapy after irAEs [116, 117]. However, the overall incidence of irAEs is higher upon drug re-challenge, with about half of patients experience any-grade irAEs. Furthermore, about 20% of patients experience irAEs which are different from the initial irAE [117]. In other words, patients who develop pneumonitis after ICI therapies may experience a non-pneumonitis irAE upon drug re-challenge. Generally, these events are treatable with corticosteroids and are not fatal [91] though rare fatalities have been reported [117]. However, it is not clear whether ICI re-challenge is of sufficient clinical benefit to warrant the risk of recurrent irAEs [35]. The Society for Immunotherapy of Cancer recommends that drug re-challenge can remain an option in patients with grade 2 pneumonitis that has resolved completely, as well as in select patients with grade 3 pneumonitis that have resolved completely and in whom the benefits of ICI therapies outweigh the risks of recurrent irAEs [118]. Patients with grade 4 pneumonitis should not undergo re-challenge with ICI therapies. Further work in this area is necessary to guide practice algorithms.

Biomarkers to Identify Patients at Risk for Pneumonitis

As noted earlier in this chapter, certain patients may be at higher risk for the initiation of pneumonitis. In particular, patients with pre-existing lung injury from smoking or from radiation may bear a higher risk for ICI-related pneumonitis. Recent advances in imaging techniques have allowed thoracic CT images to be analyzed at the voxel level to detect textural features which are associated with disease or health [119]. A similar approach led to the development of a radiomic-based algorithm which predicted the onset of pneumonitis from pre-treatment thoracic CT scans of patients who underwent ICI therapies [120]. These findings need to be externally validated, but highlight the power of imaging as a biomarker of disease risk.

Interleukin-17 is an inflammatory cytokine that is upregulated in many autoimmune diseases, including inflammatory bowel disease [121]. Elevated serum IL-17 levels were predictive of colitis in patients with melanoma treated with ipilimumab [122]. Similarly, in patients with leukemia, Th1/Th17 cells are expanded in bronchoalveolar lavage fluid from patients with leukemia who developed pneumonitis

after ICI therapy as compared to control patients with leukemia who had not received ICI therapy [123]. Further work is necessary to identify inflammatory biomarkers in the blood or in bronchoalveolar lavage fluid that can help predict the onset of pneumonitis after ICI therapy.

Conclusions

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

References

1. Ahmad AS, Ormiston-Smith N, Sasieni PD. Trends in the lifetime risk of developing cancer in Great Britain: comparison of risk for those born from 1930 to 1960. Br J Cancer. 2015;112(5):943–7.
2. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66(4):271–89.
3. Baxevanis CN, Perez SA, Papamichail M. Cancer immunotherapy. Crit Rev Clin Lab Sci. 2009;46(4):167–89.
4. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? BMC Med. 2016;14:73.
5. Dillman RO. Cancer immunotherapy. Cancer Biother Radiopharm. 2011;26(1):1–64.
6. Oiseth SJ, Aziz MS. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. J Cancer Metastasis Treat. 2017;3(10):250–61.
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. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161(2):205–14.
9. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439(7077):682–7.
10. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219–42.
11. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677–704.
12. Fife BT, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. Ann N Y Acad Sci. 2011;1217:45–59.

13. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11(11):3887–95.
14. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25(21):9543–53.
15. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. 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(7):1027–34.
16. Riley JL. PD-1 signaling in primary T cells. Immunol Rev. 2009;229(1):114–25.
17. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol. 2001;2(3):261–8.
18. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206(13):3015–29.
19. Amarnath S, Mangus CW, Wang JC, Wei F, He A, Kapoor V, et al. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. Sci Transl Med. 2011;3(111):111ra20.
20. Wang X, Teng F, Kong L, Yu J. PD-L1 expression in human cancers and its association with clinical outcomes. Onco Targets Ther. 2016;9:5023–39.
21. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545(7655):495–9.
22. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. Am J Clin Oncol. 2016;39(1):98–106.
23. Sharpe AH, Abbas AK. T-cell costimulation—biology, therapeutic potential, and challenges. N Engl J Med. 2006;355(10):973–5.
24. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. Nat Immunol. 2002;3(7):611–8.
25. Teft WA, Kirchhof MG, Madrenas J. A molecular perspective of CTLA-4 function. Annu Rev Immunol. 2006;24:65–97.
26. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182(2):459–65.
27. Walunas TL, Bakker CY, Bluestone JA. CTLA-4 ligation blocks CD28-dependent T cell activation. J Exp Med. 1996;183(6):2541–50.
28. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995;3(5):541–7.
29. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science. 1995;270(5238):985–8.
30. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, et al. CTLA-4 can function as a negative regulator of T cell activation. Immunity. 1994;1(5):405–13.
31. Darrasse-Jèze G, Deroubaix S, Mouquet H, Victora GD, Eisenreich T, Yao K-H, et al. Feedback control of regulatory T cell homeostasis by dendritic cells in vivo. J Exp Med. 2009;206(9):1853.
32. Mandelbrot DA, McAdam AJ, Sharpe AH. B7-1 or B7-2 is required to produce the lymphoproliferative phenotype in mice lacking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). J Exp Med. 1999;189(2):435–40.
33. Piccirillo CA, Shevach EM. Naturally-occurring CD4+CD25+ immunoregulatory T cells: central players in the arena of peripheral tolerance. Semin Immunol. 2004;16(2):81–8.
34. Syn NL, Teng MWL, Mok TSK, Soo RA. De-novo and acquired resistance to immune checkpoint targeting. Lancet Oncol. 2017;18(12):e731–e41.

35. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol. 2015;33(17):1889–94.
36. Ramagopal UA, Liu W, Garrett-Thomson SC, Bonanno JB, Yan Q, Srinivasan M, et al. Structural basis for cancer immunotherapy by the first-in-class checkpoint inhibitor ipilimumab. Proc Natl Acad Sci U S A. 2017;114(21):E4223–E32.
37. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
38. Tan S, Zhang H, Chai Y, Song H, Tong Z, Wang Q, et al. An unexpected N-terminal loop in PD-1 dominates binding by nivolumab. Nat Commun. 2017;8:14369.
39. Tan S, Chen D, Liu K, He M, Song H, Shi Y, et al. Crystal clear: visualizing the intervention mechanism of the PD-1/PD-L1 interaction by two cancer therapeutic monoclonal antibodies. Protein Cell. 2016;7(12):866–77.
40. Antoniou KM, Margaritopoulos GA, Tomassetti S, Bonella F, Costabel U, Poletti V. Interstitial lung disease. Eur Respir Rev. 2014;23(131):40–54.
41. Lim G, Lee KH, Jeong SW, Uh S, Jin SY, Lee DH, et al. Clinical features of interstitial lung diseases. Korean J Intern Med. 1996;11(2):113–21.
42. Glasser SW, Hardie WD, Hagood JS. Pathogenesis of interstitial lung disease in children and adults. Pediatr Allergy Immunol Pulmonol. 2010;23(1):9–14.
43. Nishino M, Ramaiya NH, Awad MM, Sholl LM, Maattala JA, Taibi M, et al. PD-1 inhibitor-related pneumonitis in advanced Cancer patients: radiographic patterns and clinical course. Clin Cancer Res. 2016;22(24):6051–60.
44. Epler GR. Bronchiolitis obliterans organizing pneumonia: definition and clinical features. Chest. 1992;102(1 Suppl):2S–6S.
45. Epler GR, Colby TV, McLoud TC, Carrington CB, Gaensler EA. Bronchiolitis obliterans organizing pneumonia. N Engl J Med. 1985;312(3):152–8.
46. Cordier JF, Loire R, Brune J. Idiopathic bronchiolitis obliterans organizing pneumonia. Definition of characteristic clinical profiles in a series of 16 patients. Chest. 1989;96(5):999–1004.
47. Guerry-Force ML, Muller NL, Wright JL, Wiggs B, Coppin C, Pare PD, et al. A comparison of bronchiolitis obliterans with organizing pneumonia, usual interstitial pneumonia, and small airways disease. Am Rev Respir Dis. 1987;135(3):705–12.
48. King T Jr. Organizing pneumonia. In: Schwarz M, King T, editors. Interstitial lung disease. Shelton, CT: People's Medical Publishing House; 2011.
49. Cordier JF. Organising pneumonia. Thorax. 2000;55(4):318–28.
50. Cordier JF. Cryptogenic organising pneumonia. Eur Respir J. 2006;28(2):422–46.
51. Godoy MCB, Viswanathan C, Marchiori E, Truong MT, Benveniste MF, Rossi S, et al. The reversed halo sign: update and differential diagnosis. Br J Radiol. 2012;85(1017):1226–35.
52. Friedman CF, Proverbs-Singh TA, Postow MA. Treatment of the immune-related adverse effects of immune checkpoint inhibitors: a review. JAMA Oncol. 2016;2(10):1346–53.
53. Wells AU, Hirani N. Interstitial lung disease guideline. Thorax. 2008;63(Suppl 5):v1–v58.
54. Bradley B, Branley HM, Egan JJ, Greaves MS, Hansell DM, Harrison NK, et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. Thorax. 2008;63(Suppl 5):v1–58.
55. Pathak V, Kuhn JM, Durham C, Funkhouser WK, Henke DC. Macrolide use leads to clinical and radiological improvement in patients with cryptogenic organizing pneumonia. Ann Am Thorac Soc. 2014;11(1):87–91.
56. Ding QL, Lv D, Wang BJ, Zhang QL, Yu YM, Sun SF, et al. Macrolide therapy in cryptogenic organizing pneumonia: a case report and literature review. Exp Ther Med. 2015;9(3):829–34.
57. Purcell IF, Bourke SJ, Marshall SM. Cyclophosphamide in severe steroid-resistant bronchiolitis obliterans organizing pneumonia. Respir Med. 1997;91(3):175–7.

58. Koinuma D, Miki M, Ebina M, Tahara M, Hagiwara K, Kondo T, et al. Successful treatment of a case with rapidly progressive Bronchiolitis obliterans organizing pneumonia (BOOP) using cyclosporin A and corticosteroid. Intern Med. 2002;41(1):26–9.
59. Romagnoli M, Nannini C, Piciucchi S, Girelli F, Gurioli C, Casoni G, et al. Idiopathic nonspecific interstitial pneumonia: an interstitial lung disease associated with autoimmune disorders? Eur Respir J. 2011;38(2):384–91.
60. Park IN, Jegal Y, Kim DS, Do KH, Yoo B, Shim TS, et al. Clinical course and lung function change of idiopathic nonspecific interstitial pneumonia. Eur Respir J. 2009;33(1):68–76.
61. Silva CI, Muller NL, Lynch DA, Curran-Everett D, Brown KK, Lee KS, et al. Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. Radiology. 2008;246(1):288–97.
62. Travis WD, Hunninghake G, King TE Jr, Lynch DA, Colby TV, Galvin JR, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med. 2008;177(12):1338–47.
63. Akira M, Inoue Y, Kitaichi M, Yamamoto S, Arai T, Toyokawa K. Usual interstitial pneumonia and nonspecific interstitial pneumonia with and without concurrent emphysema: thin-section CT findings. Radiology. 2009;251(1):271–9.
64. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med. 2002;165(2):277–304.
65. Malmberg P, Rask-Andersen A, Rosenhall L. Exposure to microorganisms associated with allergic alveolitis and febrile reactions to mold dust in farmers. Chest. 1993;103(4):1202–9.
66. Zeiss CR, Kanellakes TM, Bellone JD, Levitz D, Pruzansky JJ, Patterson R. Immunoglobulin E-mediated asthma and hypersensitivity pneumonitis with precipitating anti-hapten antibodies due to diphenylmethane diisocyanate (MDI) exposure. J Allergy Clin Immunol. 1980;65(5):347–52.
67. Hashisako M, Fukuoka J. Pathology of idiopathic interstitial pneumonias. Clin Med Insights Circ Respir Pulm Med. 2015;9(Suppl 1):123–33.
68. Flaherty KR, Martinez FJ, Travis W, Lynch JP 3rd. Nonspecific interstitial pneumonia (NSIP). Semin Respir Crit Care Med. 2001;22(4):423–34.
69. Schwaiblmair M. Drug induced interstitial lung disease. Open Respir Med J. 2012;6:63–74.
70. Kaarteenaho R, Kinnula VL. Diffuse alveolar damage: a common phenomenon in progressive interstitial lung disorders. Pulm Med. 2011;2011:1.
71. Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. Annu Rev Pathol. 2011;6:147–63.
72. Spira D, Wirths S, Skowronski F, Pintoffl J, Kaufmann S, Brodoefel H, et al. Diffuse alveolar hemorrhage in patients with hematological malignancies: HRCT patterns of pulmonary involvement and disease course. Clin Imaging. 2013;37(4):680–6.
73. Kao KC, Hu HC, Chang CH, Hung CY, Chiu LC, Li SH, et al. Diffuse alveolar damage associated mortality in selected acute respiratory distress syndrome patients with open lung biopsy. Crit Care. 2015;19:228.
74. Goodman LR. Congestive heart failure and adult respiratory distress syndrome. New insights using computed tomography. Radiol Clin N Am. 1996;34(1):33–46.
75. Gattinoni L, Presenti A, Torresin A, Baglioni S, Rivolta M, Rossi F, et al. Adult respiratory distress syndrome profiles by computed tomography. J Thorac Imaging. 1986;1(3):25–30.
76. Pelosi P, Crotti S, Brazzi L, Gattinoni L. Computed tomography in adult respiratory distress syndrome: what has it taught us? Eur Respir J. 1996;9(5):1055–62.
77. Rogers S. Spencer's pathology of the lung. Histopathology. 1999;34(5):470.
78. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol. 2015;26(12):2375–91.

79. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016;54:139–48.
80. Claessens YE, Debray MP, Tubach F, Brun AL, Rammaert B, Hausfater P, et al. Early chest computed tomography scan to assist diagnosis and guide treatment decision for suspected community-acquired pneumonia. Am J Respir Crit Care Med. 2015;192(8):974–82.
81. Hammond E, Sloan C, Newell JD Jr, Sieren JP, Saylor M, Vidal C, et al. Comparison of low- and ultralow-dose computed tomography protocols for quantitative lung and airway assessment. Med Phys. 2017;44(9):4747–57.
82. Franzen D, Schad K, Kowalski B, Clarenbach CF, Stupp R, Dummer R, et al. Ipilimumab and early signs of pulmonary toxicity in patients with metastastic melanoma: a prospective observational study. Cancer Immunol Immunother. 2018;67(1):127–34.
83. Raghu G, Mageto YN, Lockhart D, Schmidt RA, Wood DE, Godwin JD. The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: a prospective study. Chest. 1999;116(5):1168–74.
84. Nishino M, Giobbie-Hurder A, Hatabu H, Ramaiya NH, Hodi FS. Incidence of programmed cell death 1 inhibitor-related pneumonitis in patients with advanced cancer: a systematic review and meta-analysis. JAMA Oncol. 2016;2(12):1607–16.
85. Khunger M, Rakshit S, Pasupuleti V, Hernandez AV, Mazzone P, Stevenson J, et al. Incidence of pneumonitis with use of programmed death 1 and programmed death-ligand 1 inhibitors in non-small cell lung Cancer: a systematic review and meta-analysis of trials. Chest. 2017;152(2):271–81.
86. Naidoo J, Wang X, Woo KM, Iyriboz T, Halpenny D, Cunningham J, et al. Pneumonitis in patients treated with anti–programmed death-1/programmed death ligand 1 therapy. J Clin Oncol. 2017;35(7):709–17.
87. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–30.
88. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung Cancer. N Engl J Med. 2016;375(19):1823–33.
89. Nishino M, Hatabu H, Hodi FS, Ramaiya NH. Drug-related pneumonitis in the era of precision Cancer therapy. JCO Precision Oncol. 2017;1:1–12.
90. Shohdy KS, Abdel-Rahman O. Risk of pneumonitis with different immune checkpoint inhibitors in NSCLC. Ann Transl Med. 2017;5(17):365.
91. Fujii T, Colen RR, Bilen MA, Hess KR, Hajjar J, Suarez-Almazor ME, et al. Incidence of immune-related adverse events and its association with treatment outcomes: the MD Anderson Cancer Center experience. Investig New Drugs. 2018;36(4):638–46.
92. Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, 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(7):700–12.
93. Tirumani SH, Ramaiya NH, Keraliya A, Bailey ND, Ott PA, Hodi FS, et al. Radiographic profiling of immune-related adverse events in advanced melanoma patients treated with ipilimumab. Cancer Immunol Res. 2015;3(10):1185–92.
94. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521–32.
95. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373(1):23–34.
96. Khoja L, Day D, Wei-Wu Chen T, Siu LL, Hansen AR. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: a systematic review. Ann Oncol. 2017;28(10):2377–85.

97. Ryu JH, Colby TV, Hartman TE, Vassallo R. Smoking-related interstitial lung diseases: a concise review. Eur Respir J. 2001;17(1):122–32.
98. Wu J, Hong D, Zhang X, Lu X, Miao J. PD-1 inhibitors increase the incidence and risk of pneumonitis in cancer patients in a dose-independent manner: a meta-analysis. Sci Rep. 2017;7:44173.
99. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, 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.
100. Cui P, Liu Z, Wang G, Ma J, Qian Y, Zhang F, et al. Risk factors for pneumonitis in patients treated with anti-programmed death-1 therapy: a case-control study. Cancer Med. 2018;7:4115.
101. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2017;377(14):1345–56.
102. Ribas A, Shin DS, Zaretsky J, Frederiksen J, Cornish A, Avramis E, et al. PD-1 blockade expands intratumoral memory T cells. Cancer Immunol Res. 2016;4(3):194–203.
103. Bowyer S, Prithviraj P, Lorigan P, Larkin J, McArthur G, Atkinson V, et al. Efficacy and toxicity of treatment with the anti-CTLA-4 antibody ipilimumab in patients with metastatic melanoma after prior anti-PD-1 therapy. Br J Cancer. 2016;114(10):1084–9.
104. Balagani A, Arain M, Sheshadri A. Bronchiolitis obliterans after combination immunotherapy with pembrolizumab and ipilimumab. J Immunother Precision Oncol. 2018;1(1):49–52.
105. Kolla BC, Patel MR. Recurrent pleural effusions and cardiac tamponade as possible manifestations of pseudoprogression associated with nivolumab therapy—a report of two cases. J Immunother Cancer. 2016;4:80.
106. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373(17):1627–39.
107. Berthod G, Lazor R, Letovanec I, Romano E, Noirez L, Mazza Stalder J, et al. Pulmonary sarcoid-like granulomatosis induced by ipilimumab. J Clin Oncol. 2012;30(17):e156–9.
108. Bronstein Y, Ng CS, Hwu P, Hwu WJ. 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.
109. Reuss JE, Kunk PR, Stowman AM, Gru AA, Slingluff CL Jr, Gaughan EM. Sarcoidosis in the setting of combination ipilimumab and nivolumab immunotherapy: a case report & review of the literature. J Immunother Cancer. 2016;4:94.
110. Tetzlaff MT, Nelson KC, Diab A, Staerkel GA, Nagarajan P, Torres-Cabala CA, et al. Granulomatous/sarcoid-like lesions associated with checkpoint inhibitors: a marker of therapy response in a subset of melanoma patients. J Immunother Cancer. 2018;6(1):14.
111. Ramstein J, Broos CE, Simpson LJ, Ansel KM, Sun SA, Ho ME, et al. IFN-gamma-producing T-helper 17.1 cells are increased in sarcoidosis and are more prevalent than T-helper type 1 cells. Am J Respir Crit Care Med. 2016;193(11):1281–91.
112. Facco M, Cabrelle A, Teramo A, Olivieri V, Gnoato M, Teolato S, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. Thorax. 2011;66(2):144–50.
113. von Euw E, Chodon T, Attar N, Jalil J, Koya RC, Comin-Anduix B, et al. CTLA4 blockade increases Th17 cells in patients with metastatic melanoma. J Transl Med. 2009;7:35.
114. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, 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.
115. Horvat TZ, Adel NG, Dang TO, Momtaz P, Postow MA, Callahan MK, et al. Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. J Clin Oncol. 2015;33(28):3193–8.

116. Santini FC, Rizvi H, Wilkins O, van Voorthuysen M, Panora E, Halpenny D, et al. Safety of retreatment with immunotherapy after immune-related toxicity in patients with lung cancers treated with anti-PD(L)-1 therapy. J Clin Oncol. 2017;35:9012.
117. Pollack MH, Betof A, Dearden H, Rapazzo K, Valentine I, Brohl AS, et al. Safety of resuming anti-PD-1 in patients with immune-related adverse events (irAEs) during combined anti-CTLA-4 and anti-PD1 in metastatic melanoma. Ann Oncol. 2018;29(1):250–5.
118. Puzanov I, Diab A, Abdallah K, Bingham CO 3rd, Brogdon C, Dadu R, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. J Immunother Cancer. 2017;5(1):95.
119. Cunliffe A, Armato SG 3rd, Castillo R, Pham N, Guerrero T, Al-Hallaq HA. Lung texture in serial thoracic computed tomography scans: correlation of radiomics-based features with radiation therapy dose and radiation pneumonitis development. Int J Radiat Oncol Biol Phys. 2015;91(5):1048–56.
120. Colen RR, Fujii T, Bilen MA, Kotrotsou A, Abrol S, Hess KR, et al. Radiomics to predict immunotherapy-induced pneumonitis: proof of concept. Invest New Drugs. 2018;36(4):601–7.
121. Abraham C, Cho J. Interleukin-23/Th17 pathways and inflammatory bowel disease. Inflamm Bowel Dis. 2009;15(7):1090–100.
122. Tarhini AA, Zahoor H, Lin Y, Malhotra U, Sander C, Butterfield LH, et al. Baseline circulating IL-17 predicts toxicity while TGF-beta1 and IL-10 are prognostic of relapse in ipilimumab neoadjuvant therapy of melanoma. J Immunother Cancer. 2015;3:39.
123. Kim S, Shannon V, Sheshadri A, Kantarjian HM, Garcia-Manero G, Im J, et al. Th1/Th17 hybrid CD4+ cells in bronchial alveolar lavage fluid from leukemia patients with checkpoint inhibitor-induced pneumonitis. J Clin Oncol. 2017;36(5 suppl):204.

Chapter 7
Immune Checkpoint Inhibitors-Induced Colitis

Yun Tian, Hamzah Abu-Sbeih, and Yinghong Wang

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

Keywords Immune checkpoint inhibitors · Colitis · Diarrhea · Corticosteroids Infliximab · Steroids · Immunotherapy

The Incidence of ICI-Induced Colitis

ICI-induced colitis, which shares some similarities with inflammatory bowel diseases (IBDs), is observed in 25–30% of patients receiving anti CTLA-4 agents [1–3]. Anti-PD-1 antibodies are associated with lower rate of gastrointestinal (GI) adverse events, approximately 10% [4]. However, combination therapy with both

Y. Tian
Department of Oncology, Shanghai Dermatology Hospital, Tongji University, Shanghai, China

Tongji University Cancer Center, The Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

H. Abu-Sbeih · Y. Wang (✉)
Department of Gastroenterology, Hepatology and Nutrition, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: YWang59@mdanderson.org

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_7

CTLA-4 and PD-1 blockers raised the risk of GI toxicities to about 45% which is much higher than monotherapy [5]. Grade 3 or 4 diarrhea was reported to be among the most commonly reported serious adverse events and occurs in 10% of cases receiving ICIs [3, 6].

Clinical Presentation of ICI-Induced Colitis

Among all the clinical symptoms of ICI-induced GI toxicities, the most common presentation is watery diarrhea followed by abdominal pain, hematochezia, nausea/vomiting, and fever [1, 2, 7]. Weight loss has also been found in patients with ICI-induced colitis [1]. Many patients often have only non-bloody self-limiting diarrhea without other associated symptoms [8, 9], whereas severe colitis may result in colonic perforation and death [10–12]. The severity of diarrhea and colitis is graded based on the Common Terminology Criteria for Adverse Events (version 4.03). Details of CTCAE criteria for diarrhea and colitis are shown in Table 7.1 [13].

Diarrhea adverse event generally occurs around 6–7 weeks following commencement of ICI treatment [11, 14]. However, the onset can range from immediately after the first dose to more than 4 months after the last dose [7, 15, 16].

Diagnostic Tools for the Evaluation of ICI-Induced Colitis

Patients on ICI treatment who develop acute onset of diarrhea should be evaluated for infectious etiology first [12]. Stool tests for bacterial infection, *C. difficile*, viral, parasitic, or fungus should be performed to rule out infectious causes before conferring a diagnosis of ICI-induced diarrhea or colitis [17, 18]. It was noted that in some cases, ICI-induced colitis and GI infection can coexist [19].

Currently, there are no available specific serologic or fecal markers for ICI-induced colitis [20]. Fecal calprotectin is a stool inflammatory marker that has been widely used in the clinical practice for patients with inflammatory bowel disease. It has also been reported as a diagnostic or predictive tool for ICI-induced colitis [2]. However, the association between the increased fecal calprotectin level and ICI-induced colitis is not well established [20].

For patients who have ≥grade 2 diarrhea and colitis symptoms, endoscopy with biopsies is highly recommended to further evaluate the severity of ICI-induced GI toxicity [21, 22]. Endoscopic manifestations often reveal erythema, edema, erosions, ulcers, exudates, granularity, loss of vascular pattern, and bleeding [23]. About 43% colonic inflammation is distributed throughout the ileum and colon, while 34% is limited to left colon alone. The rest is normal colon exam [24]. The inflammation pattern can vary from diffuse circumferential, patchy, segmental, to

Table 7.1 Common terminology criteria for adverse events of diarrhea and colitis

Gastrointestinal disorders					
Adverse events	Grade				
	1	2	3	4	5
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4–6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of ≥7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated	Death
Colitis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Abdominal pain; mucus or blood in stool	Severe abdominal pain; change in bowel habits; medical intervention indicated; peritoneal signs	Life-threatening consequences; urgent intervention indicated	Death

isolated and focal type. For patients who had normal appearing colon on the exam, routine biopsy is required to rule out a subtype of colitis, which mimics microscopic colitis [7, 25]. Although colitis presented with significant endoscopic inflammation accounts for 79% and normal endoscopic exam in 21% [24].

Microscopic findings from inflamed colon are presented with three categories: acute, chronic, and microscopic inflammation [22, 25]. Acute inflammation features include neutrophil and/or eosinophil infiltration, epithelium apoptosis, cryptitis, and crypt microabscesses, which account for 23% of colitis; chronic inflammation features include crypt architectural distortion, basal lymphoplasmocytosis, granuloma, and Paneth cell metaplasia, that account for 60% of colitis; and microscopic colitis can present with features of lymphocytic infiltration in the epithelium and/or subepithelial collagen band deposition which is 8% [24]. Chronic histologic features share fair similarity with both Crohn's disease and ulcerative colitis. In addition, the absence of cytomegalovirus infection on histopathological examination of the colon tissue should be confirmed [2].

Radiology especially CT scan is important to evaluate bowel perforation, obstruction, and toxic megacolon that are complications of severe ICI-induced colitis. Features of colonic inflammation on imaging include diffuse wall thickening, mesenteric vessel engorgement, peri-colic fat stranding, and mucosal enhancement in patients with ICI-induced colitis [2, 26]. Free intraperitoneal air indicates the presence of bowel perforation [27]. However, the sensitivity of detecting evidence of colitis on imaging is only 50% if endoscopy is used as the gold standard for

inflammation [24]. For selected patients with high suspicion for toxic megacolon or perforation, abdominal imaging should be obtained to provide early guidance for further management.

Management and Clinical Outcomes of ICI-Induced Colitis

Current management of ICI-induced diarrhea and colitis depends on the severity of the symptoms [28]. For patients with grade 1 diarrhea, usually conservative managements with over the counter anti-diarrheal agents, adequate oral hydration, diet modification, and close follow-up monitoring are recommended. It has also been reported that 5-ASA may be effective in those with milder grade diarrhea [29]. Usually, ICI can be continued for grade 1 symptoms. If patients fail conservative management, or symptoms progress to higher grade level, more aggressive management strategy is required.

For grade 2 and above diarrhea and colitis, holding immunotherapy is highly recommended [30, 31]. The main treatment options for higher grade of ICI-induced diarrhea/colitis are immunosuppressants to reverse the effect of ICI, and hamper the inflammation. These include corticosteroids and other nonsteroidal immunosuppressants, e.g., infliximab and/or vedolizumab [3, 32, 33]. The forms of corticosteroid reported to be used for ICI-colitis include hydrocortisone enema, oral budesonide, and systematic use of corticosteroids (intravenous form of steroid and oral prednisone). Intravenous corticosteroid is indicated in patients who have severe symptoms that require hospitalization especially for grade 3 and above toxicities. Long steroid taper duration over 4–6 weeks is recommended to minimize the rebound symptoms. The standard dose of initial steroid treatment is 1 mg/kg/day, but can be increased to 2 mg/kg if symptoms are refractory within 2–3 days. The use of steroid enema and budesonide was reported in case studies only [14, 17, 29, 34]. For cases refractory to corticosteroid treatment, anti-TNF agents such as infliximab and adhesion molecule blocker, e.g., vedolizumab had been reported to be successful in case studies [3, 32, 35]. Indeed, early use of infliximab is associated with shorter duration of immunosuppressant treatment and improved clinical outcome [32, 33, 36]. The contraindications for biological agents include bowel perforation and infection, especially sepsis [11]. The response to infliximab therapy is usually within 1–3 days [7], while some patients may need more than one dose [29]. The reported response rate to infliximab was as high as 83–100% [2].

When symptoms resolve or improve to grade 1 or less after steroid treatment, resuming checkpoint inhibitor may be considered especially non-CTLA-4 agents [11]. Recurrent GI symptoms after the initial episode can occur months after successful treatment and may require complete evaluation for the same etiology [17].

Other immunosuppressive agents such as tacrolimus or mycophenolate mofetil have also been reported in case studies for the treatment of ICI-induced colitis [33]. It should be noted that, for patients with high suspicion of bowel perforation or toxic megacolon, steroids should be withheld and a surgical consultation should be

obtained [11]. Surgery with colectomy is usually reserved for patients with serious GI complications, e.g., colonic perforation [33, 37, 38]. Avoidance of nonsteroidal anti-inflammatory drugs (NSAID) is usually recommended to prevent exacerbation of gastrointestinal symptoms based on case reports [1, 39].

Conclusion

The recognition of ICI-induced colitis is increasing with the wide use of ICIs in the past few years. It shares some characteristics with IBD; however, presents with much broader range of manifestations than IBD. The diagnosis and the severity measures of ICI-induced colitis are based on multiple evaluation modalities. Early use of immunosuppressants, e.g., corticosteroids, infliximab and/or vedolizumab can lead to quick symptom improvement in severe cases. The ultimate goal is to provide maintenance treatment to keep the colitis in remission while keeping patients on ICI treatment to maximize its benefit if they are deemed to be good responders. Further studies are still required to further improve the management strategy.

Disclosure The authors declared no financial conflict of interest.

References

1. Marthey L, Mateus C, Mussini C, et al. Cancer immunotherapy with Anti-CTLA-4 monoclonal antibodies induces an inflammatory bowel disease. J Crohns Colitis. 2016;10:395–401.
2. Gupta A, De Felice KM, Loftus EV Jr, Khanna S. Systematic review: colitis associated with anti-CTLA-4 therapy. Aliment Pharmacol Ther. 2015;42:406–17.
3. 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.
4. Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current diagnosis and management of immune related adverse events (irAEs) induced by immune checkpoint inhibitor therapy. Front Pharmacol. 2017;8:49.
5. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23–34.
6. Wang Y, Abu-Sbeih H, Mao E, Ali N, Ali FS, Qiao W, et al. Immune-checkpoint inhibitor-induced diarrhea and colitis in patients with advanced malignancies: retrospective review at MD Anderson. J Immunother Cancer. 2018;6(1):37.
7. Beck KE, Blansfield JA, Tran KQ, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol. 2006;24:2283–9.
8. Johnston RL, Lutzky J, Chodhry A, Barkin JS. Cytotoxic T-lymphocyte-associated antigen 4 antibody-induced colitis and its management with infliximab. Dig Dis Sci. 2009;54:2538–40.
9. de Guillebon E, Roussille P, Frouin E, Tougeron D. Anti program death-1/anti program death-ligand 1 in digestive cancers. World J Gastrointest Oncol. 2015;7:95–101.
10. 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.

11. Howell M, Lee R, Bowyer S, Fusi A, Lorigan P. Optimal management of immune-related toxicities associated with checkpoint inhibitors in lung cancer. Lung Cancer. 2015;88:117–23.
12. Ledezma B, Binder S, Hamid O. Atypical clinical response patterns to ipilimumab. Clin J Oncol Nurs. 2011;15:393–403.
13. Common terminology criteria for adverse events (CTCAE) version 4.0. US Department of Health and Human Services.
14. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol. 2012;30:2691–7.
15. Weber J. Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. Oncologist. 2007;12:864–72.
16. Lord JD, Hackman RC, Moklebust A, et al. Refractory colitis following anti-CTLA4 antibody therapy: analysis of mucosal FOXP3+ T cells. Dig Dis Sci. 2010;55:1396–405.
17. Pernot S, Ramtohul T, Taieb J. Checkpoint inhibitors and gastrointestinal immune-related adverse events. Curr Opin Oncol. 2016;28:264–8.
18. Eggermont AM, Chiarion-Sileni V, Grob JJ. Correction to Lancet Oncol 2015; 16: 522-30. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol. 2015;16:e262.
19. McCutcheon JL, McClain CM, Puzanov I, Smith TA. Infectious colitis associated with ipilimumab therapy. Gastroenterol Res. 2014;7:28–31.
20. Berman D, Parker SM, Siegel J, et al. 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.
21. Abu-Sbeih H, Ali FS, Luo W, Qiao W, Raju GS, Wang Y. Importance of endoscopic and histological evaluation in the management of immune checkpoint inhibitor-induced colitis. J Immunother Cancer. 2018;6(1):95.
22. Wang Y, Abu-Sbeih H, Mao E, Ali N, Qiao W, Trinh VA, et al. Endoscopic and histologic features of immune checkpoint inhibitor-related colitis. Inflamm Bowel Dis. 2018;24(8):1695–705.
23. Di Giacomo AM, Biagioli M, Maio M. The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications. Semin Oncol. 2010;37:499–507.
24. Wang Y, Abu-Sbeih H, Mao E, et al. Endoscopic and histologic features of immune checkpoint inhibitor-related colitis. Inflamm Bowel Dis. 2018;24(8):1695–705.
25. Choi K, Abu-Sbeih H, Samdani R, Nogueras Gonzalez G, Raju GS, Richards DM, et al. Can immune checkpoint inhibitors induce microscopic colitis or a brand new entity? Inflamm Bowel Dis. 2018.
26. Kim KW, Ramaiya NH, Krajewski KM, et al. Ipilimumab-associated colitis: CT findings. Am J Roentgenol. 2013;200:W468–74.
27. Mitchell KA, Kluger H, Sznol M, Hartman DJ. Ipilimumab-induced perforating colitis. J Clin Gastroenterol. 2013;47:781–5.
28. Singal AK, Jampana SC, Singal V, Kuo YF. Hepatocellular carcinoma predicts in-hospital mortality from acute variceal hemorrhage among patients with cirrhosis. J Clin Gastroenterol. 2012;46:613–9.
29. Tarhini A, Lo E, Minor DR. Releasing the brake on the immune system: ipilimumab in melanoma and other tumors. Cancer Biother Radiopharm. 2010;25:601–13.
30. Puzanov I, Diab A, Abdallah K, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. J Immunother Cancer. 2017;5:95.
31. Hwang JH, Shergill AK, Acosta RD, et al. The role of endoscopy in the management of variceal hemorrhage. Gastrointest Endosc. 2014;80:221–7.
32. Dadu R, Zobniw C, Diab A. Managing adverse events with immune checkpoint agents. Cancer J. 2016;22:121–9.
33. Spain L, Diem S, Larkin J. Management of toxicities of immune checkpoint inhibitors. Cancer Treat Rev. 2016;44:51–60.
34. Collins M, Michot JM, Danlos FX, et al. Inflammatory gastrointestinal diseases associated with PD-1 blockade antibodies. Ann Oncol. 2017;28:2860–5.

35. Minor DR, Chin K, Kashani-Sabet M. Infliximab in the treatment of anti-CTLA4 antibody (ipilimumab) induced immune-related colitis. Cancer Biother Radiopharm. 2009;24:321–5.
36. Johnson DH, Zobniw CM, Trinh VA, Ma J, Bassett RL Jr, Abdel-Wahab N, et al. Infliximab associated with faster symptom resolution compared with corticosteroids alone for the management of immune-related enterocolitis. J Immunother Cancer. 2018;6(1):103.
37. Yang JC, Hughes M, Kammula U, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother. 2007;30:825–30.
38. Burdine L, Lai K, Laryea JA. Ipilimumab-induced colonic perforation. J Surg Case Rep. 2014;2014:rju010.
39. Ananthakrishnan AN, Higuchi LM, Huang ES, et al. Aspirin, nonsteroidal anti-inflammatory drug use, and risk for Crohn disease and ulcerative colitis: a cohort study. Ann Intern Med. 2012;156:350–9.

Chapter 8
Immune Checkpoint Inhibitors-Induced Hepatitis

Yun Tian, Hamzah Abu-Sbeih, and Yinghong Wang

Abstract Immune checkpoint inhibitors (ICIs) have been increasingly used for multiple cancer types in the past decade. ICIs include CTLA-4 inhibitors (e.g., ipilimumab) and the PD-1 and PD-L1 inhibitors (e.g., nivolumab and pembrolizumab). Hepatotoxicity is not uncommon secondary to ICI treatment. It can occur 8–12 weeks after the initiation of ICI and presents with elevation of aspartate transaminase and alanine transaminase. ICI-induced hepatitis is usually asymptomatic but may present with fever, malaise, and even death in rare cases. It is a diagnosis of exclusion after other etiologies are excluded based on medical history, laboratory evaluation, and imaging and histological findings. ICI-induced hepatitis might require discontinuation of ICI and/or treatment with immunosuppressants.

Keywords Immune checkpoint inhibitors · Hepatitis · Anti-CTLA-4 · Anti-PD-1/anti-PD-L1 · Corticosteroids · Transaminitis · Liver injury

The Incidence of ICI-Induced Hepatitis

Immune checkpoint inhibitor (ICI)-induced liver injury occurs in 5–30% of patients [1, 2]. Compared with patients treated with PD-1/PD-L1-blocking antibodies, patients receiving CTLA-4-blocking antibodies are associated with higher risk of liver toxicity, which can be up to 15% [3, 4]. On the other hand, the incidence of hepatic injury associated with anti-PD-1/PD-L1 agents is 5–10%. However,

Y. Tian
Department of Oncology, Shanghai Dermatology Hospital, Tongji University, Shanghai, China

Tongji University Cancer Center, The Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

H. Abu-Sbeih · Y. Wang (✉)
Department of Gastroenterology, Hepatology and Nutrition, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: YWang59@mdanderson.org

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_8

hepatotoxicity raises up to 30% in patients treated with combination therapy with anti-CTLA-4 and anti-PD-1/PD-L1 inhibition [3–5].

The most common pattern of hepatocellular injury induced by ICI is panlobular hepatitis [5–12]. Grade 3–4 hepatitis has been reported in 1–3% of patients receiving ICI monotherapy and in 8–14% of patients treated with anti-PD-1 and anti-CTLA-4 combination [5, 7–10, 13–16].

Clinical Presentation of ICI-Induced Hepatitis

ICI-induced hepatitis develops through an immune-mediated mechanism which manifests as either hepatocellular or cholestatic injury [14, 17–19]. The presentation of ICI-induced hepatitis remains highly heterogeneous, ranging from complete asymptomatic with mild rise in aminotransferases to death due to hepatic failure [6, 20, 21]. Certain patients with ICI-induced hepatitis could present with fever, malaise, jaundice, and changes of stool color [17, 22]. The increased level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin can be attributed to any ICI agent including CTLA-4 and PD-1/PD-L1 classes [13, 17, 20, 23].

ICI-induced hepatitis can occur at any time, but often becomes clinically evident 8–12 weeks after initiation of ICI therapy [16, 20, 24]. Patients present with delayed onset hepatitis tend to have milder disease [14, 25]. It should be noted that the sudden onset of fulminant hepatitis can occur despite patient has tolerated long-term ICI treatment [26].

Diagnostic Tools for the Evaluation of ICI-Induced Hepatitis

CTCAE grading system for biochemical markers of hepatitis and hepatic failure is shown in Table 8.1 [27].

The exclusion of other causes of liver injury such as medications, autoimmunity, viral infection, and alcohol is the initial approach for the management of suspected ICI-induced hepatitis [13, 28]. In addition to monitoring hepatic function closely, the evaluation for other etiologies includes diagnostic laboratory and imaging studies. Liver biopsy should be considered for cases that fail the standard immunosuppressive treatments [29].

Diagnostic laboratory biochemistry can help to evaluate for viral and other autoimmunity-related causes. Computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) imaging findings are usually nonspecific for the diagnosis of ICI-induced hepatitis [30]. However, imaging modalities can be of value to detect other etiologies that lead to abnormal liver enzymes, e.g., liver metastatic disease and thromboembolic event [17, 31]. Radiological features of ICI-induced hepatitis include periportal edema, hepatomegaly, periportal MRI T2-hyperintensity, attenuated liver parenchyma, and enlarged periportal lymph nodes on CT and MRI in

Table 8.1 Hepatobiliary disorders

Grade					
Adverse events	1	2	3	4	5
Hepatitis					
1. ALT and AST	1–3xULN	3–5xULN	5–20xULN	>20xULN	–
2. Total bilirubin	1–1.5xULN	1.5–3xULN	3–10xULN	>10xULN	
Hepatic failure	–	–	Asterixis; mild encephalopathy; limiting self-care ADL	Moderate to severe encephalopathy; coma; life-threatening consequences	Death

severe hepatitis [17, 25, 32]. Mild hepatitis usually has normal appearance of the liver on imaging [17, 33]. ICI-induced hepatitis treatment has been reported to improve hepatomegaly and periportal lymphadenopathy on imaging [17].

Histological examination of ICI-induced hepatitis demonstrated nonspecific features of panlobular hepatitis and bile duct injury [22] including fibrin ring granulomas [34], central vein endotheliitis [20, 35], prominent sinusoidal lymphohistiocytic infiltrates, and endothelialitis involving central veins [20]. The histology of anti-PD-1/PD-L1-induced hepatitis is different from that of anti-CTLA4. PD-1/PD-L1 antibody-induced hepatitis causes lobular non-granulomatous hepatitis [16], whereas CTLA4 antibody-induced hepatitis causes granulomatous hepatitis with fibrin deposits [16]. In addition, ICI-induced hepatitis has increased numbers of $CD3^+$ and $CD8^+$ lymphocytes and decreased $CD20^+$ B cells and $CD4^+$ T cells compared with autoimmune hepatitis and drug-induced liver injury [35].

Management and Outcomes of ICI-Induced Hepatitis

For mild cases, e.g., grade 1 hepatitis, expectant management with close laboratory monitoring is recommended [36]. ICI can be continued in these cases. For grade 2 and above hepatitis, after other apparent causes are excluded, immunosuppressants, e.g., corticosteroid should be initiated and ICI should be held. The dosage of corticosteroids that has been recommended with over 4 weeks taper range from 0.5 to 2 mg/kg/day [11, 13]. ICI can be resumed when corticosteroid has been tapered down to 10 mg/day (toxicity grade $\leq$ 1) for grade 2. Permanent discontinuation of ICI and corticosteroids treatment are recommended for grades 3 and 4 hepatitis [36]. Usually, corticosteroids lead to the normalization or improvement of liver enzymes in most patients [20, 26, 35]. Some patients might need multiple cycles of corticosteroid treatment [17]. The median time from corticosteroids initiation to resolution is approximately 8 weeks [37]. In clinical practice, spontaneous improvement of liver biochemistry following ICI cessation without any corticosteroid

therapy has been reported [16]. Patients with ICI-induced hepatitis that is refractory to high dose corticosteroids may need a trial of mycophenolate mofetil based on some case studies [6, 21]. Because of its potential hepatotoxic effect (very rare), infliximab is not recommended for the treatment of ICI-induced hepatitis [22, 24]. Antithymocyte globulin therapy was also reported as an alternative treatment in the event of corticosteroid intolerance [21].

For ICI-induced hepatitis, ICI therapy can be resumed after the resolution of transaminitis to grade 1 or below. In the event of persistent grade 3 or 4 hepatitis, it may require more than 1 month to the resolution of hepatic injury, and this can lead to permanent termination of ICI treatment. The liver function panel should be monitored as some patients may have rebound elevation of AST and ALT even after completion of corticosteroids therapy and clinical resolution [20].

Conclusion

The high incidence of ICI-induced hepatitis has been reported in the literature considering the wide use of ICI in the past few years. ICI-induced hepatitis often occurs 8–12 weeks after the initiation of ICI. The presentation of ICI-induced hepatitis is usually asymptomatic and shares a few characteristics with viral hepatitis, displaying elevated levels of AST, ALT, and total bilirubin, but may co-present with fever, malaise, and even death in rare cases. The diagnosis of ICI-induced hepatitis is usually made after the exclusion of other etiologies of hepatitis. For the management of ICI-induced hepatitis, the discontinuation of ICI treatment and the early use of immunosuppressants, e.g., corticosteroids, can lead to quick improvement in severe cases. The ultimate goal is to maintain normal hepatic function panel while continuing ICI treatment to maximize the benefit of ICI in good responders. Future studies are still required to further improve the management of ICI-induced hepatitis.

Disclosure The authors declared no financial conflict of interest.

References

1. Foller S, Oppel-Heuchel H, Fetter I, Winkler Y, Grimm MO. [Adverse events of immune checkpoint inhibitors]. Der Urologe Ausg A. 2017;56:486–91.
2. Ali AK, Watson DE. Pharmacovigilance assessment of immune-mediated reactions reported for checkpoint inhibitor cancer immunotherapies. Pharmacotherapy. 2017;37:1383–90.
3. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32:1020–30.
4. Bernardo SG, Moskalenko M, Pan M, et al. Elevated rates of transaminitis during ipilimumab therapy for metastatic melanoma. Melanoma Res. 2013;23:47–54.

5. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23–34.
6. O'Day SJ, Maio M, Chiarion-Sileni V, et al. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. Ann Oncol. 2010;21:1712–7.
7. Schachter J, Ribas A, Long GV, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet. 2017;390:1853–62.
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 (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16:375–84.
10. 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.
11. Hofmann L, Forschner A, Loquai C, et al. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. Eur J Cancer. 2016;60:190–209.
12. van den Eertwegh AJ, Versluis J, van den Berg HP, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol. 2012;13:509–17.
13. Spain L, Diem S, Larkin J. Management of toxicities of immune checkpoint inhibitors. Cancer Treat Rev. 2016;44:51–60.
14. Boutros C, Tarhini A, Routier E, et al. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. Nat Rev Clin Oncol. 2016;13:473–86.
15. Sznol M, Ferrucci PF, Hogg D, et al. Pooled analysis safety profile of nivolumab and ipilimumab combination therapy in patients with advanced melanoma. J Clin Oncol. 2017;35:3815–22.
16. De Martin E, Michot JM, Papouin B, et al. Liver injury from cancer immunotherapy using monoclonal immune checkpoint inhibitors. J Hepatol. 2018 Oct 5. pii: S0168-8278(18)32379-1. doi: 10.1016/j.jhep.2018.09.006. PMID: 30297274.
17. Kim KW, Ramaiya NH, Krajewski KM, et al. Ipilimumab associated hepatitis: imaging and clinicopathologic findings. Investig New Drugs. 2013;31:1071–7.
18. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol. 2012;30:2691–7.
19. Kwak JJ, Tirumani SH, Van den Abbeele AD, Koo PJ, Jacene HA. Cancer immunotherapy: imaging assessment of novel treatment response patterns and immune-related adverse events. Radiographics. 2015;35:424–37.
20. Johncilla M, Misdraji J, Pratt DS, et al. Ipilimumab-associated hepatitis: clinicopathologic characterization in a series of 11 cases. Am J Surg Pathol. 2015;39:1075–84.
21. Chmiel KD, Suan D, Liddle C, et al. Resolution of severe ipilimumab-induced hepatitis after antithymocyte globulin therapy. J Clin Oncol. 2011;29:e237–40.
22. Cramer P, Bresalier RS. Gastrointestinal and hepatic complications of immune checkpoint inhibitors. Curr Gastroenterol Rep. 2017;19:3.
23. 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.
24. Friedman CF, Proverbs-Singh TA, Postow MA. Treatment of the immune-related adverse effects of immune checkpoint inhibitors: a review. JAMA Oncol. 2016;2:1346–53.
25. Tirumani SH, Ramaiya NH, Keraliya A, et al. Radiographic profiling of immune-related adverse events in advanced melanoma patients treated with ipilimumab. Cancer Immunol Res. 2015;3:1185–92.
26. Imafuku K, Yoshino K, Yamaguchi K, Tsuboi S, Ohara K, Hata H. Successful treatment of sudden hepatitis induced by long-term nivolumab administration. Case Rep Oncol. 2017;10:368–71.

27. Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. US Department of Health and Human Services. 2009
28. Suzuki A, Brunt EM, Kleiner DE, et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. Hepatology. 2011;54:931–9.
29. Kleiner DE, Berman D. Pathologic changes in ipilimumab-related hepatitis in patients with metastatic melanoma. Dig Dis Sci. 2012;57:2233–40.
30. Mortele KJ, Segatto E, Ros PR. The infected liver: radiologic-pathologic correlation. Radiographics. 2004;24:937–55.
31. Widmann G, Nguyen VA, Plaickner J, Jaschke W. Imaging features of toxicities by immune checkpoint inhibitors in cancer therapy. Curr Radiol Rep. 2016;5:59.
32. Kumar V, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current diagnosis and management of immune related adverse events (irAEs) induced by immune checkpoint inhibitor therapy. Front Pharmacol. 2017;8:49.
33. Alessandrino F, Tirumani SH, Krajewski KM, et al. Imaging of hepatic toxicity of systemic therapy in a tertiary cancer centre: chemotherapy, haematopoietic stem cell transplantation, molecular targeted therapies, and immune checkpoint inhibitors. Clin Radiol. 2017;72:521–33.
34. Everett J, Srivastava A, Misdraji J. Fibrin ring granulomas in checkpoint inhibitor-induced hepatitis. Am J Surg Pathol. 2017;41:134–7.
35. Zen Y, Yeh MM. Hepatotoxicity of immune checkpoint inhibitors: a histology study of seven cases in comparison with autoimmune hepatitis and idiosyncratic drug-induced liver injury. Mod Pathol. 2018;31:965–73.
36. Puzanov I, Diab A, Abdallah K, et al. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. J Immunother Cancer. 2017;5:95.
37. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372:2006–17.

Chapter 9
Symptoms as Patient-Reported Outcomes in Cancer Patients Undergoing Immunotherapies

Tito R. Mendoza

Abstract Cancer therapies are toxic. Newer oncological treatments such as immunotherapy produce unconventional adverse events that are collectively referred to as immune-related adverse events (irAEs). These irAEs are clinician-rated and typically reported via tabulation of adverse events from the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE). However, the symptomatic effects of treatment and the severity of disease are best reported by the patient themselves. Although many pivotal trials for immunotherapeutic agents include health-related quality-of-life measures, symptom-focused assessments are more proximal to the effects of treatment and disease burden. This chapter discusses how best to measure symptoms, describes the desirable properties of a psychometrically valid symptom assessment tool, reviews available symptom assessment tools, provides methods to assist in the interpretation of PRO data, elucidates the feasibility and benefit of incorporating PRO in several cancer cohorts, describes the current use of PROs in immunotherapy, and identifies areas where further research are needed to enhance the use of PROs in cancer patients undergoing immunotherapy.

Keywords Patient-reported outcomes · Symptoms · Immunotherapy · Cancer

Introduction

Cancer is a disease with symptoms that profoundly impair a patient's quality of life and ability to function. Symptoms are further exacerbated by newer cancer treatments such as immunotherapies that have revolutionized the treatment of various cancers by reinvigorating a suppressed immune system. Because of this disruption in immune balance, a unique set of side effects referred to as immune-related adverse events (irAEs) have emerged. These irAEs are typically clinician-rated and may not

T. R. Mendoza (✉)
Department of Symptom Research, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: tmendoza@mdanderson.org

A. Naing, J. Hajjar (eds.), *Immunotherapy*, Advances in Experimental Medicine and Biology 995, https://doi.org/10.1007/978-3-030-02505-2_9

be consistent with patient's reports of their symptoms. In order to accurately measure symptoms, we must rely on the use of patient-reported outcomes (PRO).

Symptoms, like health-related quality of life, is a PRO because the patients themselves are the best source of information. However, unlike health-related quality of life, symptom is more proximal to the effect of treatment and the disease. Health-related quality of life is a much broader concept than symptom.

This chapter describes how best to measure symptoms using patient-reported outcomes, discusses the desirable properties of a psychometrically valid symptom assessment tool, reviews available symptom assessment tools, provides methods to assist in the interpretation of PRO data, elucidates the feasibility and benefit of incorporating PRO in several cancer cohorts, describes the current use of PROs in immunotherapy, and identifies areas where further research are needed to enhance the use of PROs in cancer patients undergoing immunotherapy.

Importance of Symptom Assessment

Patient's inability to tolerate treatment-related symptoms often precludes full and effective treatment, and residual symptoms of treatment may limit the functioning of those who may be in remission. Most symptom-focused interventions are typically designed with the goal of usually reducing the severity and impact of symptoms. Because patients commonly face choices among treatments that are similarly effective for tumor control and prolonging survival, differences in the patient's symptoms during the survival period is a major factor in making individualized treatment choices and in developing new therapies. Hence, the ability to compare treatment-related symptoms provides a benchmark for evaluating various cancer treatments. Quality assurance also depends on information about the extent and severity of symptoms. All of these approaches require accurate symptom measurement.

Symptoms and Patient-Reported Outcomes

A symptom report is the patient's statement of their perception of disturbance in normal function that is caused by disease or treatment of disease. Although symptoms are based on complex biological and behavioral phenomena, as subjective experiences their measurement is typically restricted to self-report. Because a symptom can only be known through the patient's *subjective* report, it is by definition a patient-reported outcome (PRO). In contrast, a sign or laboratory value, such as elevated white blood cell count or reduced hemoglobin, is *objective* evidence of the presence of a disease or toxicity of therapy.

The use of PROs continues to increase over the years for several reasons. First, the National Institutes of Health (NIH), as part of its Roadmap Program, has made a significant investment in the development of a measurement system called the

Patient-Reported Outcomes Measurement Information System to increase the measurement precision of patient self-report questionnaires [1]. Second, the US Food and Drug Administration (FDA) has issued guidance for the pharmaceutical industry entitled *patient-reported outcome measures: use in medical product development to support labeling claims*, which provides guidance on how self-report measures are to be used for making claims about the effectiveness of agents for which approval is being sought [2]. Third, the National Cancer Institute realized the shortcomings of their Common Terminology Criteria for Adverse Events (CTCAE) and therefore commissioned contract work to develop a patient-reported outcome version of the CTCAE coined as the PRO-CTCAE [3].

Symptom Reports as Proximal Measure of Disease and Treatment

Patient-reported outcomes can assume many forms such as health status, patient satisfaction, symptom severity, and functional impact. As alluded to earlier, symptoms are generally seen as a subset of health-related quality of life (HRQOL). HRQOL is a multidimensional construct comprising at least four dimensions: physical function (e.g., daily activities, self-care), psychological function (e.g., emotional or mental state, mood), social role function (e.g., social interactions, family dynamics), and disease-related or treatment-related symptoms (e.g., pain, nausea) [4]. Commonly used HRQOL measures, including the Medical Outcomes Study Short Form-36 (SF-36) [5], the Functional Assessment of Cancer Therapy (FACT) [6], and the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) [7], address major symptoms such as pain, depression, fatigue, and nausea. In the EORTC QLQ-C30, 18 of 30 items are self-reported symptoms. HRQOL measures also ask questions about various dimensions of patient perception, such as societal role function and concerns about social support. In most conceptualizations of HRQOL, symptoms can be viewed as the patient report closest to the physical and psychological perceptions of the disease process and the immediate effects of treatment on these perceptions [8].

Symptom Measurement

Symptoms are only known by what people tell us. Statements about symptoms (such as, “I have terrible back pain”) are reports of experiences that have common meaning to the person (patient) reporting the symptom and to the person (clinician or caregiver) receiving the report. A person who has never experienced pain might find a pain report hard to comprehend. Unlike height or weight, pain, fatigue, or feeling sad cannot be measured with a measuring stick or a weighing scale.

Symptom measurement depends on our understanding of how symptoms are communicated between the person experiencing the symptom and the people who need to know about it. Because self-reported symptoms are subjective, they are typically described using "constructs," or internal mental states that we cannot measure directly. Rather, we deduce that construct through a set of questions or items that underlie that construct. For example, to understand the construct of pain, we ask questions about the severity of pain and how pain impact daily functions. The measurement of such constructs as symptoms depends on the science of psychometrics, a field of study that originated in educational testing because of the need to know how best to measure intelligence and educational achievement. We can ask many questions with some seemingly more relevant than others. The primary goal of psychometrics is in managing the precision of self-report. Psychometrics concerns itself with reducing the measurement error so that each item provides maximum information about the construct that we are trying to approximate [9]. Two commonly used psychometric metrics are reliability and validity of a scale.

Desirable Properties of a Symptom Measure

Measures of Reliability

Test-retest reliability. If patients are asked about their symptoms more than once within a short time frame and symptoms are not expected to change, symptom ratings should be very similar each time. In general, the correlations between the ratings of the same item at these various times are considered adequate if they equal or exceed 0.70 [10]. This type of reliability is known as "test-retest reliability." Because the symptoms of patients with cancer can change quite rapidly, test-retest reliability should be assessed in patients whose symptoms and disease status are relatively stable during the specified assessment times.

Internal consistency reliability. Another measure of reliability is internal consistency, or the degree to which individual items in a measure correlate with the total score to which the item contributes. One of the most widely used measures of internal consistency reliability is the Cronbach alpha [11]. The Cronbach alpha can be thought of as the average correlation calculated from all possible combinations of items when split into two half-tests.

Measures of Validity

Content validity. Self-report measures need to be more than just stable or reliable. The term "validation" is sometimes used broadly to include all the steps used to evaluate a self-report instrument. However, in a more technical psychometric sense, "validity"

refers to evidence that the assessment instrument is actually capturing the concept or concepts it is designed to measure. An assessment instrument has content validity if it appears to measure the construct of interest. Content validity is related to face validity, which reflects the judgment of stakeholders who will use the measurement tool (health care professionals and patients) that the instrument appropriately represents what it is intended to measure. Experts and clinicians have long been traditionally consulted on item selection, but the incorporation of patient input into the measurement process is becoming a new standard of validation not found in educational measurement standards [12]. The FDA's guidance imposes the common-sense criteria that a PRO measurement needs to "make sense" to the patients who will be asked to complete the measure and should incorporate symptoms relevant to the disease/treatment to be evaluated [2, 13]. This typically involves patient interviewing and commenting at several steps in the item-development process, a method known as "qualitative research" or "cognitive interviewing." If a new measurement tool is being created, this partially assures that the items and scales are meaningful and understood by patients [14]. If an existing assessment tool is to be used in a study, cognitive debriefing supports the appropriateness of using the tool in that particular study or trial. The FDA guidance recommends that cognitive debriefing studies be included in the medical product's dossier including those of new immunotherapeutic agents to support labeling claims [2].

Convergent validity. Convergent validity indicates whether scores agree with results from a similar-but-independent measure. Convergent-related validity is determined by correlating the new assessment measure with a known "gold standard" for assessing the variable of interest (the symptom). Unfortunately, few gold standards are available for measuring symptoms. Some studies of convergent validity have used previously validated measures of the symptom or symptom-specific subscales from validated HRQOL measures, such as the pain items from the SF-36 or the fatigue subscale of the Profile of Mood States, to estimate measurement convergence.

Known-group validity. Known-group validity refers to the ability of the instrument to differentiate between groups in a predictable way. For example, cancer patients with poor performance status or late-stage disease should demonstrate higher symptom burden on the measurement instrument compared with patients who have good performance status or early-stage disease, respectively. Similarly, patients undergoing aggressive therapy should have higher severity levels of treatment-related symptoms (such as fatigue) later on in their treatment, compared with pretreatment symptom severity.

Sensitivity to Change

Whereas known-group validity is cross-sectional in nature, a measure's sensitivity is assessed repeatedly over the time that symptoms are expected to change. Sensitivity always includes a time component in that changes can be demonstrated in the expected direction. For example, pain severity ratings should improve when

the patient receives appropriate analgesics for pain in a pre-post study design. Similarly, patients undergoing aggressive cancer therapy are expected to experience worsening symptoms as they progress through their treatment regimen, and a symptom assessment tool should be able to detect those expected changes.

Practical Characteristics of a Symptom Measure

In addition to being sensitive to change and having acceptable reliability and validity, an ideal symptom assessment measure should also be brief and easy to complete, so as to reduce patient burden. Conciseness is particularly important if the symptom measure is to be used repeatedly to monitor changes in symptoms over time. A symptom measure must also be easy to understand, preferably written at around fifth grade level so that a patient with poor education can still complete it with minimal assistance. Availability in multiple languages is also important, especially in settings where patients come from different countries and linguistic background. Finally, the scores derived from the measure should be easy to interpret and intuitively meaningful to both patients reporting symptoms and to the clinicians and researchers making decisions about them.

Commonly Used Symptom Assessment Tools

Pain assessment instruments. A measure of pain should reflect (1) important aspects of what a person with pain experiences, and (2) how pain is expected to change as a result of the study to be conducted or the treatment to be administered. These issues have been the focus of a long-standing working group called the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT, see www.immpact.org). The collective publications of this working group, available on its Web site, are an important resource for persons planning symptom trials. IMMPACT has specified domains of measurement that should be considered in a clinical pain study, such as pain severity, pain interference, and effects of the treatment on other symptoms, including mood [15]. One single-symptom, multi-item measure that assesses these recommended dimensions is the Brief Pain Inventory (BPI) [16, 17].

Other tools that are commonly used for pain assessment in cancer are the Short-Form McGill Pain Questionnaire (recently revised) [18], the bodily pain subscale of the SF-36 [5], and the EORTC QLQ-C30 pain scale [7].

Fatigue assessment instruments. Fatigue, the most common symptom described by patients with cancer, is endemic during cancer treatment and in advanced disease. Substantial debate is being waged over how best to measure fatigue, which many agree is multidimensional, having physical, mental, and, perhaps, emotional components.

It has been argued that single-item fatigue measures and short single-symptom, multi-item measures are too simplistic to represent the complex construct of fatigue; conversely, measures that attempt to capture the complexity of fatigue have many more items and take longer to complete, making them more burdensome for longitudinal administration than the shorter measures.

The Brief Fatigue Inventory (BFI) [19] is a single-symptom, multi-item measure that evolved from the Brief Pain Inventory. The BFI is useful for rapid assessment of fatigue severity in clinical screening and clinical trials. We developed the BFI along the lines of the BPI and examined its psychometric properties in inpatients and outpatients with cancer and in a comparison sample of community-dwelling adults. As with the BPI, the BFI asks patients to rate their fatigue or tiredness on three items assessing fatigue severity and six items assessing how much fatigue interferes with daily functioning. Although our aim in constructing the BFI was to capture both fatigue severity and interference, several studies have demonstrated that the underlying structure of the BFI items suggests a single dimension underlying all items. This single-factor result for the BFI is consistent with the report of Lai et al. [20] that, on the basis of results from 555 patients with cancer who responded to 72 fatigue items, cancer-related fatigue can be considered unidimensional.

Other single-symptom, multi-item measures for fatigue include the Cancer Fatigue Scale [21], Fatigue Symptom Inventory [22], the FACT fatigue [23], Lee Fatigue Scale [24], Multidimensional Fatigue Inventory [25], the revised Piper Fatigue Scale [26], and the Schwartz Cancer Fatigue Scale [27].

Item banks for individual symptoms. The Patient-Reported Outcomes Measurement Information System (PROMIS) is an NIH-funded initiative tasked with developing a more fluid, yet consistent, measurement system for PROs. PROMIS has developed and continues to test a large bank of items that measure various PROs that allows for efficient, psychometrically robust assessment of PROs in clinical research [1]. PROMIS is using item response theory (IRT) to generate a list of patient self-report questions based on initial cues.

Although the PROMIS measures represent a major advance in the development of PROs because of item banking and its methodical IRT approach, much work remains to be done to provide evidence for the utility of the PRO measures that would lead to clinicians' acceptance of their use.

Item library for adverse events reporting. In order to complement the Common Terminology Criteria for Adverse Events (CTCAE), the US National Cancer Institute contracted work to develop its patient-reported outcomes version (PRO-CTCAE). The validated PRO-CTCAE consists of 124 items reflecting 78 symptomatic adverse events, and each adverse event is assessed relative to one or more attributes, specifically presence or absence, frequency, severity, and/or interference with usual or daily activities [28]. PRO-CTCAE captures a full range of symptomatic treatment effects across a full range of cancer treatment modalities. Frequency, severity, and interference with daily activities are scored using a 0–4 rating scale (i.e., frequency: 0 indicates never, 1 rarely, 2 occasionally, 3 frequently, and 4 almost constantly; severity: 0 indicates none, 1 mild, 2 moderate, 3 severe, and 4 very severe; and interference with

daily activities: 0 indicates not at all, 1 a little bit, 2 somewhat, 3 quite a bit, and 4 very much). The response options for presence or absence are 0 for no or 1 for yes. The recall period for all items is the past 7 days. Intended to complement the CTCAE, the PRO-CTCAE is primarily used to describe and elucidate the toxicity profile of an investigational agent. The PRO-CTCAE has been shown to be feasible to use in large multicenter trials [29] but because the PRO-CTCAE was only recently developed, work remains to be done to determine clinically meaningful differences in PRO-CTCAE scores.

Multisymptom assessment tools. Immunotherapies produce a host of symptoms. An ideal multisymptom assessment tool should include the symptoms that occur most frequently and are most distressing to patients. At the same time, the assessment should be short, easy to understand. Multisymptom inventories can be used to identify symptoms that are prevalent and distressing across various cancers and treatments. For example, the M. D. Anderson Symptom Inventory (MDASI*)* is a brief measure of the severity and impact of cancer-related symptoms regardless of cancer or treatment type [30]. The MDASI was developed on the basis of our previous efforts to assess the severity and interference of single symptoms, including the development of the Brief Pain Inventory and the Brief Fatigue Inventory [16, 19]. The MDASI asks patients to rate the severity of 13 symptoms that are common in patients with cancer once treatment begins: fatigue, disturbed sleep, pain, drowsiness, poor appetite, nausea, vomiting, shortness of breath, numbness, difficulty remembering, dry mouth, distress, and sadness. Patients rate each symptom's presence and greatest severity in the previous 24 h on an 11-point (0–10) scale, with 0 representing "not present" and 10 representing "as bad as you can imagine." The MDASI also contains six items that assess the degree to which symptoms have interfered with aspects of the patient's life in the previous 24 h: general activity, mood, walking ability, normal work (including work outside the home and housework), relations with other people, and enjoyment of life. Each interference item is also rated on an 11-point scale, with 0 signifying "did not interfere" and 10 signifying "interfered completely."

Other most commonly used multisymptom assessment tools include the EORTC QLQ C30 [7], the Rotterdam Symptom Checklist [31], the Symptom Distress Scale [32], the MSAS [33], the ESAS [34], and the symptom monitor [35].

Interpretation of Patient-Reported Symptom Data and Methods of Determining Minimally Important Difference

Widespread use of an instrument depends on how well clinicians and researchers can use and interpret scores derived from the tool. Once a tool's validity has been established, the next step is to determine the instrument's minimal clinically important difference (MCID; or minimally important difference, MID) in symptom scores. With large enough sample sizes, very small differences in symptom ratings

can be statistically significant, yet offer little value to patients and health care providers making treatment decisions. Determining the MCID in the field of health-related quality of life can facilitate the interpretability of symptom scores. Two approaches are used to determine the MCID: distribution-based methods and the anchor-based methods [36]. One approach is not preferred over the other, and one clinical significance consensus panel [37] suggested that the procedures within each method are not sufficient by themselves but are complementary, especially when their respective results are consistent.

Distribution-based methods. Distribution-based methods compare the change in symptom scores seen in a clinical trial to measures of variability in score distributions, such as the standard deviation, the effect size, or the standard error of measurement (SEM). For effect sizes, variability of symptom reports at baseline for all trial patients is typically used. However, estimates of variability can potentially vary from one study to another depending on the heterogeneity of the patient sample.

One approach for the distribution-based method is to set the MCID as one-half standard deviation of the symptom scores at baseline [38, 39]. Cohen's effect-size guidelines, which attach values to the magnitude of an effect, can also be used to aid interpretation of symptom scores [40]. The SEM can be calculated to further minimize the impact of population heterogeneity. This is computed as the baseline standard deviation multiplied by the square root of (1 − the reliability of the symptom scores); for any longitudinal study, either of two estimates of reliabilities, internal consistency and test-retest reliability, can be used. Wyrwich et al. [41] demonstrated that a criterion of 1 SEM was closely related to the anchor-based approach when determining the MCID for the Chronic Respiratory Questionnaire and the Chronic Heart Failure Questionnaire.

Anchor-based methods. As the name implies, this method requires the use of an "anchor," which typically is a question or set of questions designed to compare the patient's judgment of degree of change in a variable (e.g., a rating of health status) that is logically associated with the change. The anchor can either be individual-focused (single anchor) or population-focused (multiple anchors). Both approaches require that the anchor by itself is interpretable and that the anchor is related to symptoms. An example of the single-anchor method might be an item stating, "Compared with your last treatment, how do you rate your symptom now?" with possible response options of "better," "no change," or "worse." The average symptom score that falls into each value of this item constitutes an MCID. This strategy is consistent with approaches used in developing MCIDs for the Chronic Heart Failure Questionnaire [42]. For the multiple-anchor method, this procedure can be extended by using candidate variables such as disease severity, disease progression, response to treatment, or treatment discontinuation.

Using cut points to determine treatment responders. Categorizing symptoms as mild, moderate, or severe may be useful for interpreting clinically significant changes in symptom levels in the clinic and in determining the amount of change that constitutes a response to treatment in a clinical trial. Serlin et al. [43] showed how

cancer "pain at its worst" measured on a 0–10 NRS can be categorized into mild (1–4), moderate (5, 6), or severe (7–10) levels using cut points determined by multivariate analysis of variance. Previous studies have shown that patients whose pain is moderate to severe (i.e., 5 or greater on the 0–10 NRS) report significantly greater pain-related interference with function than do patients with mild or no pain. The derivation of cut points has also been applied to fatigue using the 0–10 NRS scale of the Brief Fatigue Inventory. Several researchers have employed this methodology using "average pain" rather than "pain at its worst" and with non-malignant disease conditions (e.g., diabetic neuropathy [44], low back pain [45]). Cut-point-defined categories such as mild, moderate, and severe are a simple way for clinicians to assess patient symptoms within the practice setting.

This cut-point method can also be used to compare treatment groups in clinical trials [46, 47]. For example, a responder can be defined as a patient whose "pain at its worst" changed from moderate or severe at intake to none or mild at follow-up after an intervention.

Feasibility and Utility of Incorporating PRO in Different Cancer Cohorts

This section discusses the feasibility and added benefit of including PRO objectives specifically the MDASI, presented earlier in this chapter, in evaluating the toxicity of treatment and understanding symptom trajectory over the treatment period. The patient cohorts include lung, hematological and head and neck cancers receiving various cancer treatments.

Symptom severity is predictive of the development of radiation-induced pneumonitis. In a study of 152 patients with non-small cell lung cancer treated with concurrent chemoradiation, the MDASI was administered before the start of chemoradiation and then weekly up to 6 months after therapy was completed. After controlling for the effects of sex, age, and radiation dose/volume, the authors found that increases in the severity levels of shortness of breath and coughing were associated with high-grade radiation-related pneumonitis at 6 months after therapy completion [48]. In short, concurrent chemoradiation therapy for locally advanced non-small cell lung cancer was found to be associated with the development of clinically significant radiation-related pneumonitis.

Symptom severity and symptom interference predict survival in advanced lung cancer. In a study in which we followed 94 patients with advanced-stage non-small cell lung cancer, we collected symptom data with the MDASI before and after the first cycle of chemotherapy [49]. We found that moderate to severe levels of cough (ratings $\geq$5 on a 0–10 scale) at baseline predicted poor overall survival. In addition, increases in fatigue and shortness of breath from baseline to the end of the first chemotherapy cycle predicted poor overall survival. In a separate cohort of patients with advanced-stage non-small cell lung cancer, we found that patient-

reported symptom interference with daily activities, as measured by the MDASI, added prognostic information to Eastern Cooperative Oncology Group performance status and cancer stage in the prediction of overall survival [50].

Symptom burden in hematopoietic stem cell transplantation recipients. We used the blood and marrow transplantation module of the MDASI (i.e., MDASI-Bone Marrow Transplantation) in 192 patients who had undergone hematopoietic stem cell transplantation to assess symptom severity and symptom interference with daily activities. Data were collected at 20 time points from the day of stem cell infusion to 100 days after hematopoietic stem cell transplantation. Symptom severity and symptom interference with daily activities were calculated using the arithmetic average of MDASI-Bone Marrow Transplantation items for symptom severity or symptom interference with daily activities. Those who had acute graft-versus-host disease (GVHD) had higher symptom severity and greater symptom interference with daily activities than patients without GVHD [51]. Symptoms are initially expected to increase but will eventually decrease over time. These changes in symptoms can be reliably and validly measured using MDASI-Bone Marrow Transplantation. It is worth noting the commonality between GVHD and immunotherapy. GVHD is one of the major complications of allogeneic hematopoietic stem cell transplantation [52]. For both GVHD and immunotherapy, symptoms are reported because of the immune response.

We have also shown that long-term collection of symptom data is feasible. In a study of patients with chronic myeloid leukemia, symptoms were assessed via MDASI-Chronic Myeloid Leukemia every 2 weeks for 1 year using an interactive voice response system. Compliance was excellent: 80% of patients completed at least 50% of assessments and 51% of patients completed 80% of the assessments [53].

Symptom burden in patients with head and neck cancer. In a prospective study [54], we examined the pattern of patient-reported symptoms during radiation therapy and concurrent chemotherapy for patients with head and neck cancer so that future symptom interventions and clinical investigations could be more effectively designed. A cohort consisting of 149 patients completed the head and neck module of the MDASI weekly during the course of radiation therapy-based treatment. Overall symptom severity ($p < 0.001$) and symptom interference with daily activities ($p < 0.001$) became progressively worse over the treatment course and were worse for those receiving concurrent chemotherapy ($p < 0.001$). Fatigue, drowsiness, lack of appetite, mouth and throat mucus, and problems tasting food were more severe for those receiving concurrent chemotherapy. By the end of 6–7 weeks of treatment, about 67% of patients experienced high symptom burden. Multivariable analysis showed that low patient baseline performance status and receipt of concurrent chemotherapy were associated with increased symptom burden. In conclusion, the study identified the pattern of both local and systemic symptoms, and the degree of symptom interference with daily activities was temporally distinct, marked by increased magnitudes and shifts in individual symptom rankings, as well as identifiable symptom clusters.

Symptom PRO and Immunotherapies

While there are multiple ongoing clinical trials that are testing the safety and efficacy of immunotherapy either singly or in combination with other forms of therapy, patient-reported symptom data related to new immune-based oncology treatments are lacking. Although a few studies [55, 56] reported HRQOL associated with immunotherapy, symptom-focused PRO is more relevant owing to its proximity to the effects of immunotherapy. A recent study by Bordoni et al. [57] did use the EORTC-QLQ-C30 that includes many symptoms as a PRO measure. However, the frequency of assessments may not lend itself to precise symptom tracking. In Bordoni et al. study, PROs were collected on day 1 of every cycle up to the end of treatment visit. Weekly PRO assessments up until the first restaging may provide useful data. As presented later in this chapter, PRO assessments do not have to coincide with clinic visits but can be accomplished through various modes of administration. This frequent assessment is vital for clinicians because it allows tracking of the patient's ability to tolerate the intended oncologic therapies and allows for improved patient-centered care [58]. Because the FDA is also concerned on how cancer patients feel and function, in addition to prolonging survival of cancer patients, the role of symptom PRO is even more critical in drug development especially for newer immunotherapeutic agents. However, the lack of symptom data collected rather frequently over time for patients undergoing treatment with immunotherapy hinders our understanding of these changes in symptoms and their associated interference with daily functions.

PRO in patients in early-phase trials. In 52 patients with advanced cancer enrolled in a phase I clinical trial of the first-in-human true human monoclonal antibody, MABp1, patients completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire, a PRO measure, at three time points over the course of the trial [59]. The PRO measure was able to capture longitudinal changes in symptoms over time. PRO assessments at baseline and week 8 showed significant improvements on day 1 of cycle 3 in social ($p = 0.042$), emotional ($p = 0.032$), and role function scores ($p = 0.006$). Fatigue ($p = 0.0084$), pain ($p = 0.025$), and appetite loss ($p = 0.020$) also improved. Patients reported a significant improvement in global quality-of-life scores, from 4.8 to 5.4 ($p = 0.021$). These results indicate that PRO changes can be observed in patients in phase I clinical trials undergoing treatment with monoclonal antibodies.

In a recent cross-sectional study, George et al. [60] explored symptom patterns and patient clusters based on symptom severity and examined associated factors. The researchers approached 248 patients in phase I clinical trials and only two patients declined to participate. Patients in a phase I clinical trial reported less dyspnea ($p < 0.001$) and vomiting ($p < 0.029$) than did patients who were not enrolled, but the patient groups did not differ in terms of other symptoms. The researchers also assessed the relationships among sleep quality, symptom burden,

and mood in patients with advanced cancer who were enrolled in early-phase clinical trials. Results showed that sleep quality was poor among most patients, and poor sleep was associated with an increased likelihood of high symptom burden and symptom-related interference with daily activities.

Feasibility of obtaining multiple baseline symptom assessments and frequent assessments in patients in phase I clinical trial settings. In a recent study of cancer patients enrolled in phase I clinical trials at MD Anderson [61], 37 patients receiving immunotherapy were assessed daily for about 2 weeks before beginning treatment and twice per week for 4–6 weeks before the end of cycle 2 or disease progression. Patients were given the option to respond on paper, through an interactive voice response system, or electronically through web-based platforms. Most patients preferred responding electronically. With 15 potential maximum baseline assessments, the mean was 10.2 and the standard deviation was 2.8. The median number of baseline assessments was 11 with a mode of 12 from 8 patients. With 22 potential maximum on-treatment assessments, the mean was 11.8, standard deviation 6.1, median 13, and mode 15.

Mode of PRO Administration

With technological advancement, there are many options to collect PRO data. Patient reports can be obtained either via the use of interactive voice response system or various web-based version of data collection. A major advantage of these various options is the ability to collect more frequent and real-time assessment and without having the need for the patient to be in the clinic or hospital. In addition, missing data is minimized which is critically important in longitudinal studies.

Potential Issues in the Incorporation of PRO in Immunotherapy Studies

Issues of practicability, ease of administration, level of patient (assessment) burden, and interpretability are critical factors to consider in considering the use of PRO in immunotherapy studies. Immunotherapy is known to prolong survival in many cases, but the patient's experience and function with this survival benefit is less clear. PRO focusing on symptom burden will improve understanding of the impact of immunotherapy. Many symptom measures are available to suit a variety of needs but require critical thinking about how they will be used. We can ask similar questions to those used for other treatment modalities. Will the treatment reduce symptoms that are present (e.g., shortness of breath in lung cancer) or prevent symptoms normally expected to occur (e.g., neuropathy from certain cancer treatments)? Will the treatment have rapid effects on symptoms, requiring repeated assessments over

a short period, perhaps daily or three times per week? Or will the treatment have more gradual effects on the symptom, such as the pain reduction associated with palliative radiotherapy? If the effects on symptoms are rapid, repeated use of a brief and easily administered symptom measure is probably the best choice, whereas if symptoms change more gradually, assessment should be less frequent and might include additional symptom items.

Selection of symptom items for assessment in immunotherapy poses another challenge. Many symptom measures, including the MDASI, were further improved by including items specific to the disease or treatment. For example, the head and neck module of the MDASI included items such as difficulty swallowing and problems with mouth sores to underscore the nature of the cancer affecting the head and neck region. However, a comprehensive list of symptoms associated with immunotherapy has yet to be uncovered. Although the list of immune-related adverse events provides a good indication of the symptomatic effects of immunotherapy, we need to ask the patients themselves via qualitative interviewing, a well-accepted approach favored by regulatory agencies.

Conclusions

We have discussed how symptom or collectively symptom burden is more proximal to the effect of the disease and treatment compared to the more general health-related quality of life. In developing or even using symptom measures, we need to be cognizant of the desirable properties of a psychometrically valid symptom assessment tool. We reviewed available symptom assessment tools focusing first singly on pain and fatigue and then emphasizing the need for a multisymptom assessment because cancer and its treatment produce multiple symptoms. We described two main methods in deriving minimally important difference, anchor-based and distribution-based methods, to help in the interpretation of PRO data.

We have shown the importance of symptom assessment. It can no longer be argued that we cannot use patient report to represent patients' symptoms with a relatively high degree of precision or to meet the standards of "assay sensitivity" that are expected of standard clinical assessments and laboratory tests. Changes in symptom status as measured by patient report are critical for clinical care and for implementation of clinical guidelines for symptom control. Quality assurance and clinical effectiveness research increasingly demand assessment of symptom status as a representation of what the patient experiences in a clinical trial or clinical encounter.

Finally, we described the utility of incorporating PROs in several cancer cohorts, discussed the current use of PROs in immunotherapy and identify areas where further research is needed to enhance the use of PROs in cancer patients undergoing immunotherapy. With the emergence of immunotherapies, regulatory agencies such as the FDA are increasingly interested not only in prolonging survival of cancer

patients but also in how these patients feel and function while undergoing cancer treatment. Understanding patient's experiences is best accomplished by directly asking them about their symptoms with the use of PRO. Many studies involving the use of immunotherapeutic agents have started to incorporate PRO in the study design. However, many of these studies are still in their infancy. Many issues involved in symptom assessment have yet to be resolved, such as frequency of administration and adequacy of the chosen symptom list to cover both known and unknown effects of immunotherapy. These areas offer a potentially rich agenda for future research.

Conflicts of Interest The author reports no conflict of interest in this work.

References

1. Patient-Reported Outcomes Measurement Information System. Welcome to PROMIS. Available from: http://www.nihpromis.org. Accessed 26 June 2018.
2. US Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, Center for Devices and Radiological Health. Guidance for industry. Patient-reported outcome measures: use in medical product development to support labeling claims. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071975.pdf. Accessed 26 June 2018.
3. Basch E, Reeve BB, Mitchell SA, Clauser SB, Minasian LM, Dueck AC, et al. Development of the National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). J Natl Cancer Inst. 2014;106(9). pii: dju244.
4. de Haes JC. Quality of life: conceptual and theoretical considerations. In: Watson M, Greer S, Thomas C, editors. Psychosocial oncology. Oxford: Pergamon Press; 1988. p. 61–70.
5. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I Conceptual framework and item selection. Med Care. 1992;30(6):473–83.
6. Cella DF, Tulsky DS, Gray G, et al. The Functional Assessment of Cancer Therapy Scale: development and validation of the general measure. J Clin Oncol. 1993;11(3):570–9.
7. Aaronson NK, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst. 1993;85(5):365–76.
8. Cleeland CS. Symptom burden: multiple symptoms and their impact as patient-reported outcomes. J Natl Cancer Inst Monogr. 2007;(37):16–21.
9. Crocker LM, Algina J. Introduction to classical and modern test theory. Pacific Grove: Wadsworth Publishing; 2006.
10. Litwin MS. How to measure survey reliability and validity, vol. 7: The survey kit. Thousand Oaks: Sage; 1995.
11. Nunnally JC, Bernstein IH. Psychometric theory, vol. 3. New York: McGraw-Hill; 1994. McGraw-Hill Series in Psychology
12. American Educational Research Association, American Psychological Association, National Council on Measurement in Education, Joint Committee on Standards for Educational and Psychological Testing. Standards for educational and psychological testing. 2nd ed. Washington, DC: American Educational Research Association; 1999.
13. Turner RR, Quittner AL, Parasuraman BM, Kallich JD, Cleeland CS. Patient-reported outcomes: instrument development and selection issues. Value Health. 2007;10(s2):S86–93.

14. Willis GB, Reeve BB, Barofsky I, Invited Paper C. The use of cognitive interviewing techniques in quality of life and patient-reported outcomes assessment. In: Lipscomb J, Gotay CC, Snyder C, editors. Outcomes assessment in cancer: measures, methods and applications. Cambridge: Cambridge University Press; 2004. p. 610–22.
15. Dworkin RH, Turk DC, Farrar JT, et al. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain. 2005;113(1–2):9–19.
16. Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. Ann Acad Med Singap. 1994;23(2):129–38.
17. Cleeland CS. Measurement of pain by subjective report. In: Chapman CR, Loeser JD, editors. Issues in pain measurement, vol. 12: Advances in pain research and therapy. New York: Raven Press; 1989. p. 391–403.
18. Dworkin RH, Turk DC, Revicki DA, et al. Development and initial validation of an expanded and revised version of the Short-form McGill Pain Questionnaire (SF-MPQ-2). Pain. 2009;144(1–2):35–42.
19. Mendoza TR, Wang XS, Cleeland CS, et al. The rapid assessment of fatigue severity in cancer patients: use of the Brief Fatigue Inventory. Cancer. 1999;85(5):1186–96.
20. Lai JS, Crane PK, Cella D. Factor analysis techniques for assessing sufficient unidimensionality of cancer related fatigue. Qual Life Res. 2006;15(7):1179–90.
21. Okuyama T, Akechi T, Kugaya A, et al. Development and validation of the cancer fatigue scale: a brief, three-dimensional, self-rating scale for assessment of fatigue in cancer patients. J Pain Symptom Manage. 2000;19(1):5–14.
22. Hann DM, Jacobsen PB, Azzarello LM, et al. Measurement of fatigue in cancer patients: development and validation of the Fatigue Symptom Inventory. Qual Life Res. 1998;7(4):301–10.
23. Cella D. The Functional Assessment of Cancer Therapy-Anemia (FACT-An) Scale: a new tool for the assessment of outcomes in cancer anemia and fatigue. Semin Hematol. 1997;34(3 Suppl 2):13–9.
24. Lee KA, Hicks G, Nino-Murcia G. Validity and reliability of a scale to assess fatigue. Psychiatry Res. 1991;36(3):291–8.
25. Smets EM, Garssen B, Bonke B, de Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res. 1995; 39(3):315–25.
26. Piper BF, Dibble SL, Dodd MJ, Weiss MC, Slaughter RE, Paul SM. The revised Piper Fatigue Scale: psychometric evaluation in women with breast cancer. Oncol Nurs Forum. 1998;25(4):677–84.
27. Schwartz AL. The Schwartz Cancer Fatigue Scale: testing reliability and validity. Oncol Nurs Forum. 1998;25(4):711–7.
28. Dueck AC, Mendoza TR, Mitchell SA, Reeve BB, Castro KM, Rogak LJ, et al. Validity and reliability of the US National Cancer Institute's Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE). JAMA Oncol. 2015; 1(8):1051–9.
29. Basch E, Pugh SL, Dueck AC, Mitchell SA, Berk L, Fogh S, et al. Feasibility of patient reporting of symptomatic adverse events via the Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE) in a Chemoradiotherapy Cooperative Group Multicenter Clinical Trial. Int J Radiat Oncol Biol Phys. 2017;98(2):409–18.
30. Cleeland CS, Mendoza TR, Wang XS, et al. Assessing symptom distress in cancer patients: the M.D. Anderson Symptom Inventory. Cancer. 2000;89(7):1634–46.
31. de Haes JC, van Knippenberg FC, Neijt JP. Measuring psychological and physical distress in cancer patients: structure and application of the Rotterdam Symptom Checklist. Br J Cancer. 1990;62(6):1034–8.
32. McCorkle R, Young K. Development of a symptom distress scale. Cancer Nurs. 1978;1(5):373-378.
33. Portenoy RK, Thaler HT, Kornblith AB, et al. The Memorial Symptom Assessment Scale: an instrument for the evaluation of symptom prevalence, characteristics and distress. Eur J Cancer. 1994;30A(9):1326–36.

34. Bruera E, Kuehn N, Miller MJ, Selmser P, Macmillan K. The Edmonton Symptom Assessment System (ESAS): a simple method for the assessment of palliative care patients. J Palliat Care. 1991;7(2):6–9.
35. Hoekstra J, Bindels PJ, van Duijn NP, Schadé E. The symptom monitor. A diary for monitoring physical symptoms for cancer patients in palliative care: feasibility, reliability and compliance. J Pain Symptom Manage. 2004;27(1):24–35.
36. Revicki D, Hays RD, Cella D, Sloan J. Recommended methods for determining responsiveness and minimally important differences for patient-reported outcomes. J Clin Epidemiol. 2008;61(2):102–9.
37. Guyatt GH, Osoba D, Wu AW, Wyrwich KW, Norman GR. Methods to explain the clinical significance of health status measures. Mayo Clin Proc. 2002;77(4):371–83.
38. Norman GR, Sloan JA, Wyrwich KW. Interpretation of changes in health-related quality of life: the remarkable universality of half a standard deviation. Med Care. 2003;41(5):582–92.
39. Sloan JA, Dueck A. Issues for statisticians in conducting analyses and translating results for quality of life end points in clinical trials. J Biopharm Stat. 2004;14(1):73–96.
40. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale: Lawrence Earlbaum; 1988.
41. Wyrwich KW, Tierney WM, Wolinsky FD. Further evidence supporting an SEM-based criterion for identifying meaningful intra-individual changes in health-related quality of life. J Clin Epidemiol. 1999;52(9):861–73.
42. Guyatt G, Walter S, Norman G. Measuring change over time: assessing the usefulness of evaluative instruments. J Chronic Dis. 1987;40(2):171–8.
43. Serlin RC, Mendoza TR, Nakamura Y, Edwards KR, Cleeland CS. When is cancer pain mild, moderate or severe? Grading pain severity by its interference with function. Pain. 1995;61(2):277–84.
44. Zelman DC, Gore M, Dukes E, Tai KS, Brandenburg N. Validation of a modified version of the brief pain inventory for painful diabetic peripheral neuropathy. J Pain Symptom Manag. 2005;29(4):401–10.
45. Keller S, Bann CM, Dodd SL, Schein J, Mendoza TR, Cleeland CS. Validity of the brief pain inventory for use in documenting the outcomes of patients with noncancer pain. Clin J Pain. 2004;20(5):309–18.
46. Cleeland CS, Portenoy RK, Rue M, et al. Does an oral analgesic protocol improve pain control for patients with cancer? An intergroup study coordinated by the Eastern Cooperative Oncology Group. Ann Oncol. 2005;16(6):972–80.
47. Wong GY, Schroeder DR, Carns PE, et al. Effect of neurolytic celiac plexus block on pain relief, quality of life, and survival in patients with unresectable pancreatic cancer: a randomized controlled trial. JAMA. 2004;291(9):1092–9.
48. Wang XS, Shi Q, Yue J, Chen TY, Xu T, Komaki R, et al. Predictive value of patient-reported outcomes on radiation pneumonitis related normal lung uptake of FDG on PET in patients with non-small cell lung cancer treated with concurrent chemoradiation [abstract 3015]. International Society for Quality of Life Research 23rd annual conference; Copenhagen, Denmark; Oct 19-22, 2016. Qual Life Res. 2016;25(Suppl 1):157–8.
49. Wang XS, Shi Q, Lu C, Basch EM, Johnson VE, Mendoza TR, et al. Prognostic value of symptom burden for overall survival in patients receiving chemotherapy for advanced nonsmall cell lung cancer. Cancer. 2010;116(1):137–45.
50. Barney BJ, Wang XS, Lu C, Liao Z, Johnson VE, Cleeland CS, et al. Prognostic value of patient-reported symptom interference in patients with late-stage lung cancer. Qual Life Res. 2013;22(8):2143–50. PMCID: PMC3724766.
51. Williams LA, Giralt SA, Wang XS, Mobley GM, Mendoza TR, Cohen MZ, et al., editors. Measuring the symptom burden of allogeneic hematopoietic stem cell transplantation in patients with and without acute graft-versus-host disease [abstract 49]. Biol Blood Marrow Transplant. 2009;15(Suppl 2):20–1.
52. Stewart BL, Storer B, Storek J, Deeg HJ, Storb R, Hansen JA, et al. Duration of immunosuppressive treatment for chronic graft-versus-host disease. Blood. 2004;104(12):3501–6.

53. Williams LA, Garcia Gonzalez AG, Ault P, Mendoza TR, Sailors ML, Williams JL, Huang F, Nazha A, Kantarjian HM, Cleeland CS, Cortes JE. Measuring the symptom burden associated with the treatment of chronic myeloid leukemia. Blood. 2013;122(5):641–7.
54. Rosenthal DI, Mendoza TR, Fuller CD, Hutcheson KA, Wang XS, Hanna EY, et al. Patterns of symptom burden during radiotherapy or concurrent chemoradiotherapy for head and neck cancer: a prospective analysis using The University of Texas MD Anderson Cancer Center Symptom Inventory-Head and Neck Module. Cancer. 2014;120(13):1975–84.
55. Long GV, Atkinson V, Ascierto PA, Robert C, Hassel JC, Rutkowski P, et al. Effect of nivolumab on health-related quality of life in patients with treatment-naive advanced melanoma: results from the phase III CheckMate 066 study. Ann Oncol. 2016;27:1940–6.
56. Schadendorf D, Dummer R, Hauschild A, Robert C, Hamid O, Daud A, et al. Health-related quality of life in the randomised KEYNOTE-002 study of pembrolizumab versus chemotherapy in patients with ipilimumab-refractory melanoma. Eur J Cancer. 2016;67:46–54.
57. Bordoni R, Ciardiello F, von Pawel J, Cortinovis D, Karagiannis T, Ballinger M, Sandler A, Yu W, He P, Matheny C, Felizzi F, Rittmeyer A. Patient-reported outcomes in OAK: a phase III study of Atezolizumab versus Docetaxel in advanced non-small-cell lung cancer. Clin Lung Cancer. 2018. pii: S1525-7304(18)30131-1. https://doi.org/10.1016/j.cllc.2018.05.011.
58. Basch E, Iasonos A, McDonough T, Barz A, Culkin A, Kris MG, et al. Patient versus clinician symptom reporting using the National Cancer Institute common terminology criteria for adverse events: results of a questionnaire-based study. Lancet Oncol. 2006;7:903–9.
59. Hong DS, Hui D, Bruera E, Janku F, Naing A, Falchook GS, Piha-Paul S, Wheler JJ, Fu SQ, Tsimberidou AM, Stecher M, Mohanty P, Simard J, Kurzrock R. MABp1, a first-in-class true human antibody targeting interleukin-1 alpha in refractory cancers: an open-label, phase 1 dose-escalation and expansion study. Lancet Oncol. 2014;15:656–66.
60. George GC, Iwuanyanwu EC, Anderson KO, Yusuf A, Zinner RG, Piha-Paul SA, et al. Sleep quality and its association with fatigue, symptom burden, and mood in patients with advanced cancer in a clinic for early-phase oncology clinical trials. Cancer. 2016;122:3401–9.
61. Mendoza TR, George G, Williams L, Shi Q, Naing A, Hong DS, et al, editors. Feasibility and added value of multiple baseline symptom assessments in early-phase clinical trials [abstract]. Presented at the International Society for Quality of Life Research (ISOQOL) 24th annual conference, Philadelphia; 2017.