6 Overcoming Cancer Tolerance with Immune Checkpoint Blockade

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6.1 Introduction

In 1957, Thomas and Burnet proposed the immunosurveillance theory, contending that the immune system is continuously patrolling, recognizing, and eliminating individual or groups of transformed cells [1]. This theory together with the identification of tumor-associated antigens (TAAs) led to much of the work in cancer vaccines to date. Based on this theory, it stands to reason that if the immune system has failed to recognize or mount a sufficient immune response to cancer, thus allowing a cancer to grow until it is clinically evident, stimulating the immune system sufficiently against the cancer could correct the immune system's failings and destroy the cancer. While there is considerable data in support of this theory, a number of discrepancies have also been noted. Most notably, athymic nude mice, which are T-cell deficient, and immunosuppressed individuals (transplant patients) do not develop neoplasms that are not virally linked at rates much drastically higher than their immunocompetent counterparts [2, 3]. While better models have since confirmed the role of the immune system in protecting against cancer development, it is clear that the immunosurveillance theory alone is not sufficient to explain the role of immune systems in cancer development.

Active immunotherapy for cancer based on the immunosurveillance understanding of cancer has, for the most part, been characterized by promising preclinical and early phase trials with, ultimately, disappointing clinical results in later phase trials [4]. Vaccination techniques have focused on stimulating the immune system by exposure to single or multiple tumor-associated antigens with immunoadjuvants such as cytokines (GM-CSF, IL-2) or toxins. While a variety of different techniques have been tried, with the exception of sipuleucel-T, a cancer vaccine approved for treatment of metastatic prostate cancer, these techniques have largely proven insufficient to overcome the local and systemic immunosuppression of advanced cancer in order to achieve a clinically significant improvement [5]. Historically, various types of active immunotherapy have shown excellent results in eradicating or preventing tumors in relevant murine models. In early phase clinical trials, active immunotherapies have generally had minor, well-tolerated toxicity profiles and shown promising immunologic results; however, these have not translated to clinically meaningful endpoints

when tested in larger-scale controlled trials. As noted above, an exception to this is the sipuleucel-T vaccine, which demonstrated significant benefit in overall survival in castrate-resistant prostate cancer (CRPC) in two phase III trials and has been FDA approved based on these results [5, 6].

The immune system-cancer interaction is now recognized to be more complex than once imagined. The cumulated results of experimental evidence have led to the "immunoediting theory," a modification of the previous immunosurveillance theory that explains how immunocompetent individuals develop cancer and how the immune system can help shape the biologic activity of the cancers themselves. The theory proposes that cancer proceeds though three phases: elimination, equilibrium, and escape. The elimination phase describes the recognition and elimination of nascent cancer cells as in the immunosurveillance theory. The equilibrium phase is a period where the cancer cells that avoid immune destruction are held at bay by the immune system and which, through selective pressure (immunoselection), can change the cancer's phenotype into a less immunogenic and more tolerance-inducing tumor. The escape phase describes the setting in which cancer cells have evolved to evade immune pressure and can replicate to become a clinically apparent neoplasm [7].

Cancer avoids immune destruction in the equilibrium phase and then is able to enter the escape phase through multiple mechanisms that have become increasingly well characterized. Cancer cells can escape immune detection by downregulating production of TAAs or the major histocompatability (MHC) complexes that the antigens are presented on [8, 9]. Tumor tissue can promote lymphocyte anergy, or unresponsiveness, by downregulating necessary co-stimulatory signals, which are necessary for functional lymphocyte activation, or upregulating coinhibitory signals, which are necessary for preventing autoimmunity. Tumors, through contact-mediated and soluble signals, recruit and cause proliferation of inhibitory cell populations such as regulatory T lymphocytes (Tregs), tolerogenic dendritic cells, and myeloid-derived suppressor cells. Additionally, tumors alter the cellular microenvironment through secretion of inhibitory cytokines and metabolic byproducts, all of which hamper effective immune response [10].

Given our increased understanding of how tumor cells actively inhibit and escape host immunity and the disappointing results of most cancer vaccine therapies, it has become increasingly clear that these failures do not stem from lack of ability to stimulate an appropriate immune response but rather from the inability of the immune response to overcome immunosuppressive mechanisms. In other words, regardless of how many stimulated, cancer-specific effector cells are created with a given vaccine, if the cells are rendered ineffective in the "immunoedited" tumor microenvironment, ultimately the therapy will fail [11]. A large amount of research effort is underway to identify, characterize, and target cancer escape mechanisms in hope of delivering more effective immunotherapeutic treatments.

As mentioned earlier, one major mechanism of immune resistance is through multiple costimulatory and inhibitory receptor-ligand combinations (immune checkpoints) that create a context for the effector and target cell (or antigen-presenting cell) interaction. Multiple immune checkpoints have now been identified and have been found to play an integral role in cancer escape (Fig. 6.1). Blockade of two of these checkpoint pathways, CLTA-4 and PD-1/PD-L1, has led to commercially available therapeutic drugs in patients with multiple different types of malignancy. Many other immunomodulatory checkpoints are being actively investigated and will, in all likelihood, lead to further therapeutic options for patients with cancer. In addition, the potential for combination therapy with multiple checkpoints targeted (such as CTLA-4, PD-1, PD-L1) or together with standard therapies or cancer vaccines remains great. This chapter will review the role of therapeutic checkpoint targets to overcome tumor-mediated immune suppression through targeted checkpoint modulation.

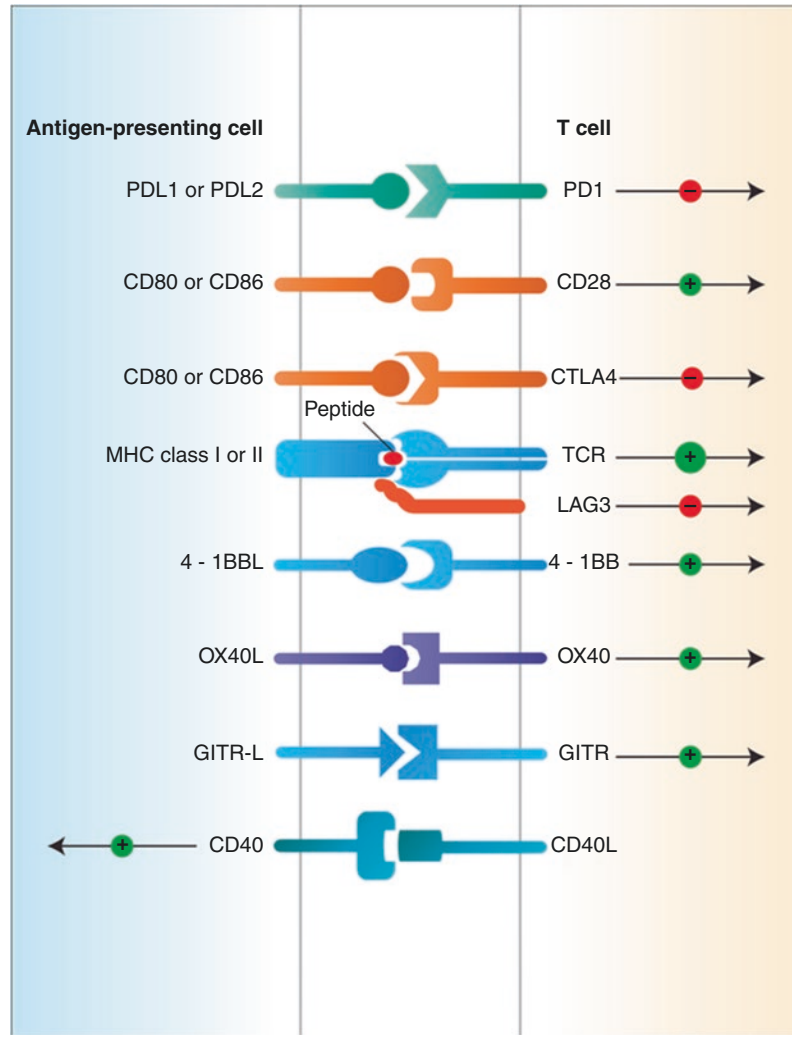

Fig. 6.1 Multiple immunomodulatory coinhibitory and costimulatory receptor-ligand pairs have been identified (although not all are depicted here). These pathways set the immunologic context when an antigen is presented on a T-cell receptor (TCR) to a major histocompatibility (MHC) complex

6.2 Neoantigens: Targets for the Immune System

With the development of multiple commercially available checkpoint blockade drugs, considerable research has been devoted to determining in which tumor types and in which clinical setting the drugs are beneficial. With this new focus, factors that make certain tumors more immunogenic are becoming clearer. All malignancies that become clinically apparent are able to evade immune destruction, but this is often due to immunosuppressive factors (rather than lack of immunogenicity of the tumor itself) that can be countered with checkpoint inhibitors and, potentially, other immunostimulatory drugs in development. Neoantigens are unique antigens generated from gene mutations during neoplastic transformation. Each neoantigen produced represents a potential target for the host immune system to differentiate the tumor from normal tissue. However, not all neoantigens are inherently immunogenic. It is presumably a matter of chance whether the mutations a tumor acquires produce neoantigens immune system is capable of recognizing and targeting. As a consequence, in general, tumors with a higher mutational load, such as melanoma, NSCLC, and microsatellite unstable tumors, are more likely to respond to checkpoint inhibitors [12–17]. However, this is not entirely predictive as tumors with relatively lower somatic mutations (HCC, clear cell carcinoma)

have shown benefit, albeit with lower response rates, to checkpoint inhibitor therapy [18]. Checkpoint inhibitors allow the ineffective immune responses to be more effective (but there has to be an immune response to begin with), illuminating why checkpoint inhibitors are not effective in all patients.

At this time, there are five checkpoint inhibitors approved by the US Food and Drug Administration for a variety of cancers, including ipilimumab (melanoma), pembrolizumab (melanoma, non-small cell lung cancer [NSCLC], head and neck squamous cell cancer, classical Hodgkin's lymphoma [cHL], urothelial carcinoma, microsatellite instability [MSI]-high colon cancer, gastric cancer), nivolumab (melanoma, NSCLC, renal cell carcinoma [RCC], cHL, MSI-high colon cancer, hepatocellular carcinoma [HCC]), atezolizumab (urothelial carcinoma, NSCLC), avelumab (Merkel cell carcinoma [MCC], urothelial carcinoma), and durvalumab (urothelial carcinoma) [19].

6.3 Cytotoxic T-Lymphocyte-Associated Antigen-4 (CTLA-4): The First Checkpoint Pathway to Demonstrate Clinical Benefit

Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4, CD152) was the first recognized inhibitory immune checkpoint molecule [20, 21]. CTLA-4 is the target of the first FDA-approved checkpoint-targeting drug, ipilimumab. During the development of CTLA-4 blocking monoclonal antibodies (mAb), much has been learned about dosing, toxicity, combination therapy, and tumor response that are now and will continue to be useful as other immune checkpoint therapies are developed.

6.3.1 CTLA-4 Function

When CTLA-4 (CD152) was first reported in 1987, it was presumed to play a role in controlling T-cell activation given its close sequence homology with CD28, its proximity to CD28 on chromosome 1, and its expression on cytotoxic T lymphocytes (CTLs) coinciding with T-cell activation [20]. The first CTLA-4$^{-/-}$ knockout mice, created in the mid-1990s, confirmed that CTLA-4 played a key role in T-cell homeostasis as the mice quickly succumbed to polyclonal lymphoproliferative disease characterized by massive expansion of activated T cells [22]. Since then, it has become clear that CTLA-4 functions as a negative counterpart to CD28, the required costimulatory signal for the activation and expansion of T cells.

For T lymphocytes to be activated, an antigen-specific T-cell receptor (TCR) must bind to an MHC complex containing the appropriate peptide in its binding grove. While this is necessary, it is not sufficient to complete activation. A number of additional regulatory pathways have since been elucidated that closely control T-cell activation to ensure appropriate, directed immune responses under normal circumstances. Among these pathways, co-stimulation with CD28 (on the T cell) binding to B7-1 (CD80) or B7-2 (CD86) on the antigen-presenting cell (APC) is perhaps the most important and best known. B7-1 and B7-2 are expressed on APCs and are typically upregulated after activation [23, 24].

As a competitively binding counterpart to CD28, CTLA-4 is an inhibitory checkpoint molecule expressed on activated T cells and constitutively expressed on regulatory T cells (Treg) [21]. After TCR-antigen-mediated activation of T lymphocytes, expression of CTLA-4 on the cell membrane increases dramatically. CLTA-4 suppresses immune activation through multiple pathways, and the relative importance of each in overall immune homeostasis and in disease-related autoimmunity and immune suppression is not clear [25].

The CTLA-4 receptor controls effector T-lymphocyte activation by competitive binding with CD28 as well as through internal and external signaling. CTLA-4 binds the same ligands as CD28 (B7-1 and B7-2) but with 20 to 100 times greater avidity and can accommodate two ligands, whereas CD28 can only bind one [26–28]. CTLA-4 appears to blunt T-cell responses by not only competitively binding the CD28 ligands, B7-1 and B7-2, but also by recep-

tor-mediated induction of cell cycle arrest, decreasing production of IL-2, limiting T-cell dwell time, and enhancing Treg function, among other mechanisms [29]. There is evidence that competitive binding of B7-1 and B7-2 by CTLA-4 remains the most important function in counteracting CD28-mediated T-cell stimulation, as treatment of CLTA-4-deficient mouse models with CTLA-4-immunoglobulin fusion protein (CLTA-4Ig) can abrogate the lymphoproliferative autoimmunity which would otherwise be fatal [30]. Additionally, the singular importance of B7-1 and B7-2 in these pathways is demonstrated by the fact that mice deficient in CTLA-4 as well as B7-1 and B7-2 do not demonstrate lymphoproliferative autoimmunity [31]. Unlike CD28, which has some level of constitutive expression on most T cells, CTLA-4 is only expressed in significant quantity on effector T cells after activation. CTLA-4 reaches a maximal expression level as long as 48 h after the T cell is activated serving as a negative feedback loop to turn off or prevent an overly robust immune response as well as to prevent autoimmunity (Fig. 6.2) [27, 32].

In addition to directly and indirectly inhibiting effector T-lymphocyte activation and proliferation, CTLA-4 interacts with Tregs in a manner important to its overall function. As previously stated, CTLA-4 is expressed at some constitutive level on Treg cells, and higher levels of expression may be rapidly mobilized from an intracellular source [25]. The exact role that Treg-mediated immune suppression plays in the overall context of CTLA-mediated immune control is not entirely clear. There is evidence from

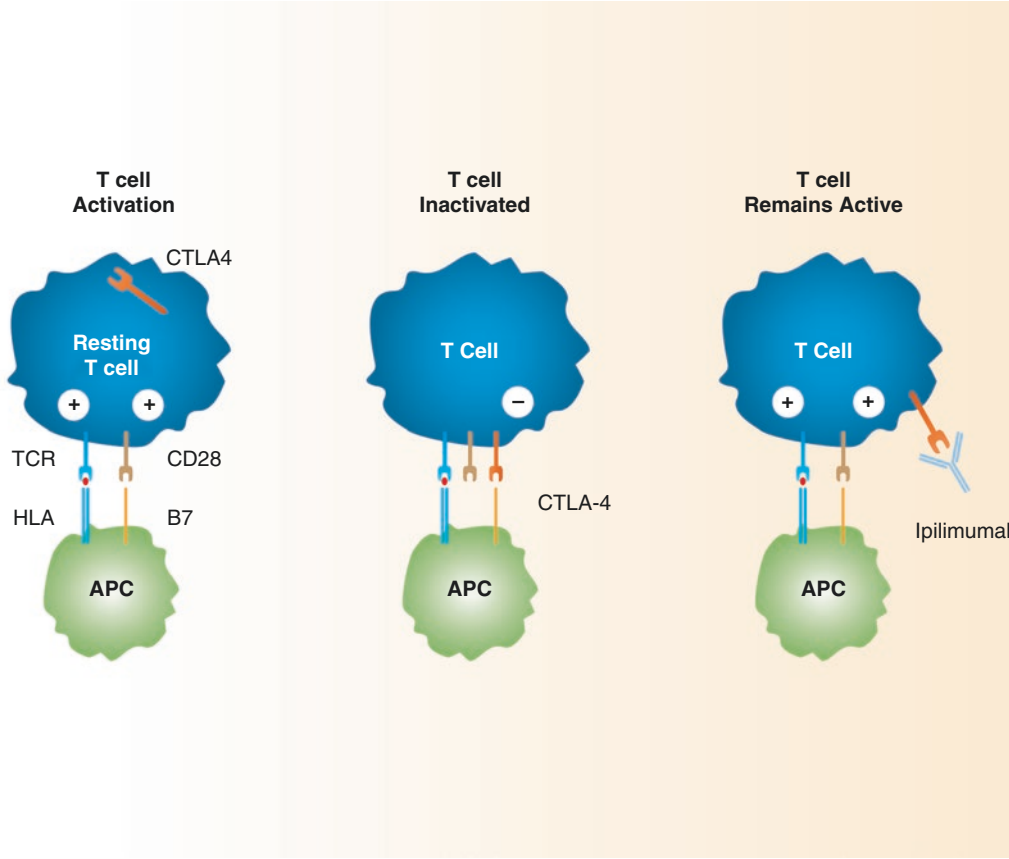

Fig. 6.2 Mechanism of action of CTLA-4 in suppressing activated T cells and proposed mechanism of action for ipilimumab

lymphocytes treated with anti-CTLA-4 monoclonal antibodies (mAbs) in vitro, which suggests that CTLA-4 blockade mediates the immune system by both direct activation of effector T lymphocytes and Treg depletion, dependent on the mAb subtype and its ability to stimulate antibody-dependent cytotoxicity (ADCC) [33, 34].

The important role of CTLA-4 in Treg homeostasis and immune control has become clear in multiple experiments. Treg-mediated CLTA-4 inhibits B7-1 and B7-2 expression on dendritic cells [35]. Murine models with CTLA-4-deficient $CD4^+$ $FOXP3^+$ (Treg) lymphocytes developed lymphoproliferative disease [35]. Additionally, CTLA-4 plays an active role in Treg homeostasis as blocking the receptor with anti-CTLA-4 mAbs leads to a rapid proliferation in peripheral Treg cells [36–38]. This action is thought to be due to CTLA-4 counteraction against CD28-stimulated proliferation of Tregs as blocking both CTLA-4 and CD28 leads to a contraction in the peripheral Treg population [24, 36]. However, expansion of Tregs with CTLA-4 blockade does not appear to lead to increased Treg function [39]. Similarly, in murine organ transplant models, deficiency of CD28 or both B7-1 and B7-2 leads to a significant decrease in the Treg population; however, the mice get paradoxical acceleration of graft rejection inversely proportional to the Treg level [39].

As work progresses in deciphering the mechanisms of the CTLA-4 receptor's complex interplay within broader immune homeostasis, the CTLA-4 receptor remains an active target of investigation for modulating the immune system for therapeutic purposes. The identified roles that CTLA-4 plays in human disease are substantial and ever-growing. There is evidence that CTLA-4 polymorphisms plays a role in autoimmune conditions such as type 1 diabetes, thyroiditis autoimmune hypothyroidism, and Graves' disease [40–43].

6.3.2 Tremelimumab

Tremelimumab (formerly CP-675, 206, ticilimumab, previously licensed to Pfizer, New York, NY, now licensed to AstraZeneca, London, UK) is another humanized anti-CTLA-4 mAb that has been evaluated in human clinical trials [29, 44]. Tremelimumab is an IgG2 antibody that, similar to ipilimumab, blocks the binding site of CLTA-4. It has a longer half-life of approximately 22 days compared to 12–14 days for ipilimumab [44]. In vitro testing of tremelimumab revealed enhanced T-cell activation, demonstrated by increased cytokine production. Based on this, as well as initial experience with ipilimumab, the drug proceeded with human trials.

The first dose escalation phase I trial of tremelimumab enrolled metastatic melanoma ($n = 34$), renal cell carcinoma ($n = 4$), and colon cancer patients ($n = 1$). The trial did note dose-limiting autoimmune toxicity, but determined that the drug was tolerated up to 15 mg/kg in a single dose. The trial also noted complete or partial response in 4 of the 29 patients with measurable melanoma [45]. Ongoing evaluation of tremelimumab is occurring in a phase II hepatocellular carcinoma study in combination with durvalumab (NCT02519348).

A phase I/II trial further evaluated dosing in metastatic melanoma patients and recommended dosing at 15 mg/kg every 3 months for further study given equivalent efficacy and better safety to more frequent dosing [46]. A subsequent single-arm, phase II trial of tremelimumab was conducted in 251 patients with relapsed or refractory metastatic melanoma. Patients were treated with tremelimumab at 15 mg/kg every 90 days (as recommended in the previous trial) for 4 doses and allowed up to 4 additional doses in patients with a tumor response or stable disease. The trial revealed an objective response rate of 6.6%. The trial reported an overall OS of 10.0 months, which is comparable with what was found in the previously described phase III trial of ipilimumab in similar patients. Serious adverse events (≥grade 3) were seen in 21% of patients [47].

The phase III trial of tremelimumab monotherapy in treatment-naïve unresectable stage III or stage IV melanoma began enrolling in March 2006. Patients were randomized to receive tremelimumab at 15 mg/kg every 90 days until symptomatic disease progression or standard-of-care

chemotherapy (temozolomide or dacarbazine) for 12 weeks or until disease progression. The primary end-point was OS. The trial was terminated by the data safety monitoring board at the second interim analysis (after two-thirds of planned events had occurred) because the test statistic crossed the prespecified futility boundary [48]. Survival follow-up continued after the trial was stopped. At final analysis, the median overall survival was 12.6 months in the tremelimumab arm compared to 10.7 months in the chemotherapy arm ($p = 0.127$). Objective response rates were similar in both arms (10.7% vs. 9.8%, respectively). Grade 3 or 4 adverse events occurred in 52% of tremelimumab patients compared to 37% of chemotherapy patients [49]. More recent work has suggested that the lack of tremelimumab efficacy may stem from the fact that it is an IgG2 isotype mAb, thus less able to produce reduction in intratumoral Tregs than ipilimumab, an IgG1 mAb [34]. Despite its lack of proven effect in this trial, tremelimumab remains under active investigation in other patient populations (discussed further below).

6.3.3 Toxicity

As previously described, CTLA-4 blocking antibodies can lead to unique, immunologic toxicities termed "immune-related adverse events" (irAEs) through nonspecific activation of the immune system. While the majority of these are minor and manageable, they occur relatively frequently, particularly at higher doses and can be severe. In the first phase III trial of ipilimumab, with treatment at 3 mg/kg, 14 patients (2.1%) receiving ipilimumab died from causes deemed treatment-related, with 7 of the deaths were from irAEs [50]. In a pooled analysis of 325 patients treated with ipilimumab at 10 mg/kg every 3 weeks for 4 doses, 72.3% experienced irAEs and 25.2% were ≥grade 3 [51]. In the phase III trial combining ipilimumab with dacarbazine for treatment naïve melanoma, 56.3% of patients in the combination arm experienced grade 3 or 4 adverse events. The most frequent irAEs are of the skin, gastrointestinal tract, liver, and endocrine system. These adverse events tend to occur at predictable times after receiving CTLA-4 blocking antibodies [51].

Skin toxicity is the most frequent irAE in some series, with roughly half of the patients receiving ipilimumab experiencing some form of rash. The rashes can typically be managed with symptom control and topical medication until they become more severe when systemic steroids and/or withholding or discontinuing treatment may be necessary. There are rare reported cases of toxic epidermal necrolysis that have been fatal [52].

Diarrhea is another frequent adverse event seen in CTLA-4 blockade treatment, occurring in between 32.8% and 51% of patients in phase III trials of ipilimumab and tremelimumab [49, 50, 53]. Severe diarrhea, colitis, and perforation are less common but can occur. Like skin toxicity, initial management is symptomatic. A high degree of suspicion for colitis with a low threshold for endoscopic evaluation is necessary for more severe (≥grade 2) diarrhea. The diagnosis of colitis or grade 3 or higher diarrhea necessitates more aggressive treatment with fluid replacement, systemic steroids, and treatment cessation. Infliximab treatment has been effective for severe colitis. A high index of suspicion for perforation with involvement of gastroenterology and surgery is also warranted in these cases [52].

Hepatotoxicity is seen less frequently (3–9%) with CTLA-4 blocking antibodies but can be severe. In general, liver function tests should be followed during treatment, and ≥grade 3 hepatotoxicity requires systemic treatment with systemic steroids and occasionally mycophenolate mofetil along with drug cessation [51].

Endocrine toxicities consist of hypophysitis and, less frequently, autoimmune thyroid dysfunction and adrenal insufficiency. Hypophysitis appears to occur in less than 5% of cases but typically has permanent sequelae and can lead to life-threatening adrenal insufficiency if not properly recognized and managed. Suspicion for hypophysitis should lead to pituitary MRI and laboratory testing. Treatment consists of systemic steroids and withholding CTLA-4 blocking treatment. Monitoring of

serum chemistries and thyroid function panels is recommended with ipilimumab treatment [54].

Other less frequent irAEs seen with CTLA-4 blocking therapies include episcleritis, uveitis, pancreatitis, neuropathies, and lymphadenopathy. Screening for a history of autoimmune disease and consideration of risk factors and expected benefits are recommended given the potential for serious toxicity with CTLA-4 blocking antibodies. National Comprehensive Cancer Network (NCCN) guidelines recommend participation in a risk evaluation and mitigation strategy (REMS) program when using ipilimumab [55].

Interestingly, multiple phase I and II trials of ipilimumab have noted a higher rate of clinical response in patients with irAEs and, in particular, grade 3 and 4 irAEs [52, 56–62]. A similar correlation was not addressed in the phase III trials of CLTA-4 blockade antibodies, and further evaluation may help clarify this as well as the underlying mechanisms.

6.4 Programmed Death 1 (PD-1) Pathway

6.4.1 Function

Programmed death 1 (PD-1) is a more recently discovered immune checkpoint receptor that has generated considerable excitement based on favorable preclinical profiling and initial clinical results. PD-1 was first discovered in 1992 by subtractive mRNA hybridization in an attempt to identify genes involved in programmed cell death [63]. Its protein structure was deduced based on the mRNA sequence obtained; however, its function remained unclear until PD1$^{-/-}$ knockout mice were noted to develop lupus-like autoimmune disease [64]. At that time, it was correctly suspected that PD-1 played a role in inducing peripheral tolerance.

Since its discovery, the function and significance of PD-1 has become more clear [65]. Like CTLA-4, PD-1 is a transmembrane protein expressed on effector immune cells [66]. Also like CTLA-4, expression of PD-1 is inducibly expressed with lymphocyte activation, although it is expressed more broadly than CTLA-4 as it is also found on activated B lymphocytes and NK cells [67–69]. PD-1 is bound principally by programmed death ligand 1 (PD-L1, B7-H1) but also, to a lesser degree, by programmed death ligand 2 (PD-L2, B7-DC) [70]. PD-L1 is constitutively expressed in certain tissues such as lung and placental macrophages [71]. Its high level of expression in the placenta has been implicated in mediating maternofetal tolerance [72, 73]. PD-L1 expression can also be induced on a broad range of hematopoietic, endothelial, and epithelial tissues in response to proinflammatory cytokines, such as interferon, GM-CSF, IL-4, and IL-19 [67, 74–77]. PD-L2 expression is more limited as it is inducibly expressed on dendritic cells, macrophages, and mast cells [71].

The PD-1 receptor pathway is an important negative regulator of the immune system. PD-1 appears to play a role primarily in dampening immune response in the setting of peripheral inflammation as opposed to CTLA-4, which plays a greater role in regulating T-cell activation [71]. As mentioned before, PD-1 knockout mice helped initially reveal the function of PD-1. The initial B6-PD-1$^{-/-}$congenic mice developed varying degrees of autoimmune arthritis and glomerulonephritis by 6 months of age and exaggerated inflammatory response to infection, in contrast to CTLA-4 knockout mice who die of diffuse lymphoproliferative disease shortly after birth [22, 64, 78]. Remarkably, later PD-1$^{-/-}$ knockout mouse models (BALB/c-PD-1$^{-/-}$ and MLR-PD-1$^{-/-}$) developed fatal autoimmune dilated cardiomyopathy early in life due to production of autoantibodies [79, 80]. In contrast, mice deficient in PD-L1 do not manifest autoimmunity, but can have increased accumulation of CD8^{+} lymphocytes in the liver and increased tissue destruction with experimental autoimmune hepatitis [81].

Ligation of PD-1, which again is found primarily on immunologic cells, counters CD28-mediated signaling through multiple mechanisms. PD-1 is phosphorylated upon ligand engagement, initiating a cascade of intracellular events [82, 83]. PD-1 signaling decreases the production of several proinflammatory cytokines such as IFN-

γ, TNF-α, and IL-2 [71]. It may also serve to retard cell activation mediated via CD28 and IL-2. PD-1 ligation has also been implicated in inhibiting transcription factors and initiation of several cell death pathways [84–86]. Importantly, PD-1 and its ligands also appear to play a role in shifting lymphocyte response from activation to tolerance when exposed to antigens, an attribute that is particularly significant for cancer immunotherapy [87]. Interestingly, PD-L1 was discovered to function not only as a ligand for PD-1 but also as a receptor bound by B7-1 (CD80) capable of delivering an inhibitory signal [88]. This finding not only demonstrates the complexity of lymphocyte regulation but suggests that blockade of these molecules could result in functionally different outcomes [78].

The PD-1 and PD-L pathways have been implicated in a variety of human diseases. Higher than normal expression levels of PD-1 and single nucleotide polymorphisms of PD-1 have been implicated in multiple autoimmune diseases such as systemic lupus erythematosus, Sjogren's disease, type 1 diabetes, and rheumatoid arthritis. As such, this pathway remains an active therapeutic target in these conditions [65]. In infectious diseases, the PD-1 and PD-L pathways play an important role in preventing unnecessary immune-mediated tissue destruction and have also been implicated in preventing the clearance of chronic viral, bacterial, and parasitic infections [71, 89].

6.4.2 PD-1 Pathway in Cancer

Just as the PD-1 pathway plays a central role in tolerance of chronic infections, it also appears to have a primary role in cancer tolerance and immune escape. PD-1 ligand expression, particularly of PD-L1 expression, has been demonstrated at various levels on a large variety of human cancer tissues. Higher expression of PD-L1 on tumor cells is associated with worse prognosis, more aggressive features, and/or resistance to immunotherapy in the large majority of cancers in which it has been characterized [90–101]. However, in some cases higher expression appears to have little influence on prognosis, as was found in NSCLC, and has even been associated with a more favorable prognosis, as found in colorectal cancer without mismatch repair (MMR) deficiency [102, 103]. $CD8^+$ tumor-infiltrating lymphocytes ($CD8^+$ TILs) have been noted to have high levels of PD-1 expression in many cases; nonetheless, correlation between PD-L expression and prognosis is mixed [97, 102, 104, 105]. Circulating NK cells in cancer patients have been noted to express PD-1, while healthy control NK cells do not [106]. Furthermore, preclinical data demonstrates that increasing tumor expression of PD-L1 makes it less susceptible to immunotherapy, while blocking it increases its vulnerability to immune-mediated destruction [107–110].

Some of the differences observed in tumor PD-L1 expression and correlation with cancer prognosis may be due to tumor-host interaction. Two recent studies examining human melanocytic lesions and colorectal cancer found a strong positive correlation between tumor PD-L1 expression and patient survival, in contrast to the majority of tissue types previously examined. However, in addition to this, higher PD-L1 expression was associated with both increased tumor infiltrating lymphocytes and interferon gamma (INF-γ) levels or gene expression in the tumor microenvironment [103, 111]. In these cases, the higher levels of PD-L1 expression may be in response to INF-γ signaling, as observed in normal human tissue [112, 113]. Thus, upregulation of PD-L1 expression may represent an adaptive tumor response to tumor-specific immunity, termed "adaptive resistance." [111, 114] The effective host immune response may explain the more favorable outcomes observed in these patients. Other evidence implicates different transcriptionally related oncogenic pathways in the upregulation of PD-1, which may or may not be related to external inflammatory signaling [92]. The adaptive resistance hypothesis may help further explain how tumors are able to escape immune stimulation from active immunotherapy and lead to blockade of the PD-1 pathway of particular therapeutic interest.

6.4.3 PD-1 Blockade

In preclinical studies with murine cancer models, anti-PD-1 and anti-PD-L1 blockade demonstrated antitumor effect as monotherapy and augmented the effects when given comitant with cancer vaccination [115–120]. Similarly, ex vivo blockade of PD-1 or PD-L1 improved the ability of human lymphocytic function against tumor tissue in multiple studies [107, 121–123]. Based on the functional importance of PD-1 in cancer as well as promising preclinical therapeutic results, several blocking mAbs have proceeded to human clinical trials.

6.4.4 Nivolumab

Nivolumab (MDX-1106, BMS-936558, Bristol-Myers Squibb, New York, NY) is a fully humanized IgG4 mAb that binds to PD-1, blocking its binding site. It was initially tested in a phase I, dose escalation trial on 296 patients with heavily pretreated advanced melanoma (n = 104), colorectal cancer (n = 19), CRPC (n = 17), NSCLC (n = 122), and renal cell carcinoma (n = 34). Nivolumab was given at 0.3, 1, 3, or 10 mg/kg in six patient cohorts followed by expansion cohorts at 10 mg/kg. Patients were initially given a single dose and allowed additional doses if they demonstrated clinical benefit; however, the trial transitioned into a phase Ib where patients were dosed every 2 weeks and reassessed every 8 weeks. Treatment was continued for up to 96 weeks or until disease progression or complete response. Overall, treatment with nivolumab was better tolerated than treatment with CTLA-4 blocking antibodies with no maximum tolerated dose achieved. Only 14% experienced serious (≥grade 3) drug toxicity, leading to the discontinuation of therapy in only 5%. There were drug-related adverse events in 41% and serious drug-related adverse events in 6% of patients that were likely irAEs, including pneumonitis, diarrhea, colitis, hepatitis, hypophysitis, and vitiligo. Pneumonitis, which occurred in 3% of patients, is of special interest, since it was not typically seen with CTLA-4 blocking mAbs and led to only three treatment-related deaths [124]. This toxicity may be secondary to constitutive expression of PD-L1 in alveolar macrophages.

Nivolumab treatment demonstrated substantial antitumor effect, with partial or complete responses (by RECIST criteria) observed in patients with melanoma, NSCLC, and renal cell carcinoma but not colorectal cancer or CRPC. Responses were observed across various doses at rates of 19–41% in melanoma, 6–32% in NSCLC, and 24–31% in renal cell carcinoma. One patient with melanoma and one with renal cell carcinoma had complete response to treatment. Responses tended to be durable with over half of melanoma and renal cell responses lasting for greater than 1 year. In addition, disease stability and mixed response (as described in irRC) were observed in a substantial portion of patients. Further analysis of PD-L1 expression from 61 patients who had pretreatment specimens available demonstrated an objective response in 36% of tumors expressing PD-L1 and none in PD-L1-negative tumors [124].

This data raises the possibility that PD-L1 could serve as a biomarker for response to therapy, an idea that is being actively investigated. PD-L1 has been shown to be a prognostic biomarker in the tumor cells of head and neck squamous cell cancer [125]; however, a recent review indicates that PD-L1 expression alone is insufficient for patient selection for most malignancies, both as monotherapy and combination therapy [126]. Another group showed the association between the mutational load of >100 nonsynonymous somatic mutations or neoantigens and ipilimumab or tremelimumab therapy with long-term clinical benefit in patients with advanced melanoma [127]. Another study in melanoma patients showed an association between that same mutational load and clinical benefit (complete or partial response or stable disease with overall survival longer than 1 year). Interestingly, only 0.04% of the identified antigens were present in more than one patient who showed clinical benefit, suggesting that most neoantigens associated with immunotherapy success are patient specific. Most recently, however, a systematic review and meta-analysis of 6664

patients found that PD-L1 expression was predictive of favorable response across tumor types including non-small cell lung cancer, melanoma, bladder cancer, renal cell carcinoma, gastroesophageal cancer, head and neck cancer, merkel cell carcinoma, and small cell lung cancer (OR 2.26, 95% CI, 1.85–2.75, $p < 0.001$), with the greatest effect observed in non-small cell lung cancer, where quantitative PD-L1 testing is now recommended prior to treatment (OR 2.51, 95% CI 1.99–3.17, $p < 0.001$) [12, 127].

Nivolumab has now been approved by the US Food and Drug Administration for use in humans in multiple cancer types. It was first approved in 2014 for patients with unresectable or metastatic melanoma and disease progression following ipilimumab and a BRAF inhibitor if applicable. Approximately 1 year later, nivolumab was approved for metastatic squamous and nonsquamous NSCLC with progression on or after platinum-based chemotherapy, unresectable or metastatic melanoma in combination with ipilimumab in BRAF V600 wild-type patients, and renal cell carcinoma in patients who received prior antiangiogenic therapy. In 2016, approval was granted for classical Hodgkin lymphoma (cHL) that progressed after hematopoietic stem cell transplantation and recurrent or metastatic head and neck squamous cell carcinoma that progressed on or after platinum-based chemotherapy. To date, additional approvals have been granted in locally advanced or metastatic urothelial carcinoma on or following platinum-based chemotherapy, adult and pediatric microsatellite high (MSI-H) or mismatch repair-deficient metastatic colon cancer that has progressed following chemotherapy, and HCC in patients previously treated with sorafenib [17, 19, 128–134].

6.4.5 Pembrolizumab

Pembrolizumab (Keytruda, Merck, Whitehouse Station, NJ) is a humanized monoclonal antibody that binds to PD-1 and blocks interaction with PD-L1 and PD-L2. At this time, it is FDA approved in patients with unresectable or metastatic melanoma, select NSCLC, recurrent head and neck squamous cancer, refractory cHL, locally advanced or metastatic urothelial carcinoma, and select gastric cancers. Most notably, pembrolizumab has received a broad indication for all adults and pediatric MSI-H or mismatch repair deficient solid tumors who have progressed following prior treatment, and colorectal cancer that has progressed following chemotherapy.

Deserving special mention is the first-of-its-kind MSI-H, and mismatch repair deficient (dMMR) indication was obtained in five uncontrolled, open-label, multi-cohort, multicenter, single-arm trials[45], known respectively as KEYNOTE-016, −164, −012, −028, −158. A total of 149 MSI-H or dMMR patients met inclusion criteria, and 98% had metastatic disease. Most had received two or more prior therapies. Patients received either 200 mg every 3 weeks or 10 mg/kg every 2 weeks. The majority (60%) of patients had colorectal cancer, and the remainder consisted of multiple solid tumors most commonly endometrial, biliary, and gastric/GE junction tumors. The overall response rate was 39.6% (95% CI 31.7–47.9), with 78% of patients demonstrating a durable response at 6 months [19, 135–140].

6.4.6 PD-L1 Blockade

Initial results of the PD-1 pathway blockade are very encouraging. The findings of objective clinical responses of up to 41% of subgroups of patients with nivolumab and relatively high response rates in NSCLC, a disease historically resistant to immunotherapy, are unprecedented in cancer immunotherapy. Additionally, lower rates of toxicity, in particular, serious irAEs, compared to CTLA-4 blockade have given hope that this pathway will yield more widely applicable and better-tolerated therapies. Much work remains and is currently in progress to bring these therapies into general clinical use. Determination of optimal dosing, duration of treatment, and the subsets of patients who benefit from treatment are all underway. As with CLTA-4 blockade, preclinical data supports a possible synergistic effect when PD-1 pathway blockade is combined with other cancer

treatments such as chemotherapy, radiation, and immunotherapy; this deserves and is receiving further investigation [107, 119, 121, 141]. As these investigations move forward, one area of particular interest will be whether PD-L1 expression on tumors continues to serve as a reliable biomarker for predicted therapeutic benefit, thus increasing the ever-growing trend of more personalized, tailored treatment for individual tumors.

6.4.7 Atezolizumab

Atezolizumab is an Fc-engineered, humanized, monoclonal antibody that binds to PD-L1, blocking its interaction with PD-1 and B7-1 receptors. It is now FDA approved in patients with unresectable or metastatic urothelial carcinoma who are not eligible for platinum-based chemotherapy or who progressed on such therapy and metastatic NSCLC with progression on or after platinum-based chemotherapy. The urothelial carcinoma indication was granted accelerated approval in 2015 based on early-phase results in 310 patients who had disease progression after platinum-based therapy. Compared to historical controls with a 10% overall response rate, an objective response rate of 15% with a median follow-up of 11.7 months was achieved. In addition, increased levels of PD-L1 expression on immune cells were associated with increased response [142–145].

NSCLC approval was based on two randomized, open-label clinical trials (POPLAR and OAK) where atezolizumab 1200 mg IV every 3 weeks was compared with docetaxel and an overall survival benefit of 2.9 months in POPLAR at a median survival of 12.6 months and 4.2 months in OAK at a median survival of 13.8 months [144, 146].

6.4.8 Durvalumab

Durvalumab (MEDI-4736) was recently approved for locally advanced or metastatic urothelial carcinoma who progressed after platinum-based chemotherapy. It was approved under accelerated approval based on a phase I/II open-label study in 182 patients who had disease progression on or after platinum-based chemotherapy and received durvalumab 10 mg/kg IV every 2 weeks for 12 weeks. 31 patients (17%) demonstrated clinical responses, with 5 complete responses at a median follow-up of 5.6 months [147].

Additional approval has been granted for patients with unresectable stage III NSCLC without disease progression following platinum-based chemotherapy and radiation. This approval was granted based on the PACIFIC study, a multicenter, randomized, double-blind, placebo-controlled study enrolling 713 patients who had completed at least two cycles of platinum-based chemotherapy and definitive radiation. Patients who received durvalumab demonstrated a statistically significant overall response rate of 28.4% compared to 16% in the placebo group ($p < 0.001$), with a longer median duration of response in the durvalumab group (72.8% vs. 46.8% had an ongoing response at 18 months post-randomization). Median progression-free survival was 16.8 months for durvalumab versus 5.6 months for placebo (95% CI 4.7–7.8) [148].

6.4.9 Avelumab

Avelumab is another PD-L1 blocking antibody that received accelerated FDA approval in 2017 for metastatic Merkel cell carcinoma in adults and children age 12 and older. This approval was granted based on a prospective, open-label, phase II trial in patients with stage IV, chemotherapy-refractive Merkel cell carcinoma who were given avelumab 10 mg/kg every 2 weeks. 88 patients received at least one dose, and 28 (32%) patients achieved an objective response (20 partial, 8 complete) at a median follow-up of 10.4 months [149, 150].

6.5 Immune-Related Response Criteria

Initial WHO response criteria and later RECIST criteria, which have undergone many revisions over the years, were developed to identify and

Table 6.1 Comparison of World Health Organization (WHO) and immune-related response criteria (irRC) for tumor response

	Word Health Organization (WHO)	Immune-related response criteria (irRC)
CR	Disappearance of all lesions in two observations at least 4 weeks apart	Disappearance of all lesions in two observations at least 4 weeks apart
PR	≥50% decrease in SPD of all index lesions in the absence of progression of nonindex lesions or new lesions in two observations at least 2 weeks apart	≥50% decrease in total tumor burden in two observations at least 4 weeks apart
SD	<50% decrease compared to baseline and <25% increase compared to nadir measurements of the SPD of index lesions, in the absence of progression of nonindex lesions or new lesions	<50 decrease compared to baseline and <25% increase compared to nadir
PD	≥25% increase in SPD compared with nadir or progressions of nonindex lesions or appearance of new lesions	≥25% increase in tumor burden compared to nadir in two observations at least 4 weeks apart

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *SPD* sum of the products of the largest dimensions of lesions

standardize definitions of tumors responsive to cytotoxic therapy and not as a surrogate for survival [151]. They have been used in early phase clinical trials as a surrogate for response to therapy. The use of these criteria assumes that tumors will shrink or stabilize at the outset of therapy. Tumor growth or the appearance of new metastases constitutes progressive disease and, therefore, lack of response. In immunotherapy trials, including those evaluating ipilimumab, it has been shown that tumors often progress or remain stable before responding, therefore making RECIST criteria less helpful in predicting treatment response. Based on these observations, new immune-related response criteria (irRC) were proposed (Table 6.1). The new criteria do not necessarily consider the appearance of new lesions or growth of isolated lesions as progressive disease but, instead, consider overall tumor burden. Based on retrospective observations of 487 metastatic melanoma patients in three phase II trials of ipilimumab at 10 mg/kg dosing, 9.7% of treated patients initially classified as progressive disease under WHO criteria later had evidence of response to therapy. In retrospective reclassification by irRC, response to therapy appears to correlate better with overall survival than WHO criteria [152]. Immune-related response criteria have been used alongside WHO criteria in multiple ipilimumab trials since it was first introduced [153, 154]. Further prospective validation will be needed to determine to what degree it correlates with overall survival.

6.6 CTLA-4 Blockade Monotherapy

Two mAbs, ipilimumab and tremelimumab, were developed in parallel. The therapies underwent phase III trials that ultimately led to approval for ipilimumab for treating metastatic melanoma and showed disappointing results for tremelimumab.

6.6.1 Ipilimumab

Based on the work in murine models, fully humanized IgG1 CTLA-4 mAbs were created by Medarex Inc. (Princeton, NJ; purchased by Bristol-Myers Squibb, New York, NY, in 2009) using a transgenic hybridoma HuMAb mouse model. The proprietary mouse model has multiple genetic modifications designed to facilitate production of high-avidity human IgG mAbs [155]. The mAb used for initial in vivo testing was selected based on affinity and specificity for CTLA-4 as well as ability to block the binding site. The antibody, called 10D1 (later designated MDX-010 and ipilimumab), also had cross-reactivity with macaques monkey CTLA-4. It was initially tested in this setting where it was shown to increase antibody response to hepatitis

surface antigen as well as a human melanoma cell vaccine. Additionally, the macaques did not demonstrate polycolonal T-cell activation or autoimmunity [156]. Based on this work, ipilimumab proceeded with human trials.

6.6.1.1 Ipilimumab in Uveal Melanoma

Uveal melanoma is a rare cancer that, like cutaneous melanoma, shares melanocyes as the cell of origin but has different pathogenesis and clinical behavior. Similar to melanoma, it has a very poor prognosis when it has metastasized (typically to the liver) and is resistant to systemic chemotherapy [156, 157]. Three open-label, multicenter, single arm phase II trials have been conducted using ipilimumab in uveal melanoma. The GEM-1 trial enrolled 32 patients treated with 10 mg/kg ipilimumab. At a median follow-up of 5.5 months, 13 patients had evaluable responses, with 1 having a partial response (7.7%) and 6 having stable disease (46.2%) [158].

The DeCOG treated 53 pretreated and treatment-naïve patients with metastatic uveal melanoma with ipilimumab at a dose of 3 mg/kg. Overall, they reported a relatively disappointing median progression-free survival (2.8 months) and overall survival (6.8 months) [159].(NCT01585194). The GEM-1402 trial is a phase I/II trial looking at ipilimumab in combination with nivolumab in the adjuvant setting for high-risk uveal melanoma after completion of standard treatment. In an interim analysis, it showed progression-free survival of 4.99 months at a median follow-up of 4.6 months (NCT02626962).

6.6.2 Phase III Trials of Checkpoint Inhibitors in Melanoma

The first phase III study of ipilimumab, sponsored by Bristol-Meyers Squibb, began enrolling patients in September 2004. The trial enrolled 676 HLA-A*0201$^+$ patients with pretreated, unresectable stage III or IV melanoma. The patients were randomized 3:1:1 to receive either ipilimumab with gp100 peptide vaccine, ipilimumab alone, or gp100 alone. The gp100 peptide had demonstrated effectiveness in previous phase II trials in melanoma, particularly when combined with ipilimumab [56–58, 160]. Ipilimumab was dosed at 3 mg/kg every 3 weeks for four doses. Patients were not routinely offered maintenance therapy; however, those who progressed after responding to therapy or who had stable disease after 12 weeks were allowed "reinduction" therapy. The primary endpoint of the trial was OS. The trial demonstrated an OS benefit in all patients who received ipilimumab (median OS: 10.0 months for ipilimumab with gp100, 10.0 months for ipilimumab alone, and 6.4 months for gp100 alone; $p < 0.003$). There was no difference in survival in patients who received ipilimumab with gp100 and those who received ipilimumab alone. There were four cases of complete responses and multiple cases of long-term disease control in patients who received ipilimumab. Approximately, 60% of patients treated with ipilimumab experienced some irAE, with the rates of serious irAEs (≥grade 3) of 10–15% in the ipilimumab groups [50]. Of the 31 patients who met criteria for and received "reinduction" therapy (progression after complete or partial response or stable disease), 19% achieved a complete or partial response and 68% achieved disease control with similar toxicity to the original induction therapy [161]. Based on this study, ipilimumab achieved FDA approval at a dose of 3.0 mg/kg to treat unresectable stage III and stage IV melanoma.

When ipilimumab was approved for therapy, it generated considerable interest because it represented a therapeutic success for nonspecific immunostimulation, a new modality in cancer treatment. In addition to this, it raised hope for future successes for cancer immunotherapy, particularly coming on the heels of the FDA approval of another cancer immunotherapy, sipuleucel T (Provenge; Dendreon, Seattle, WA), the first therapeutic cellular immunotherapy to prove effective in phase III trials [5, 6]. It gave hope to clinicians treating and patients with metastatic melanoma, as this was the first therapy to show an overall survival benefit in a randomized,

phase III trial for metastatic melanoma [162]. Significant questions remain and are currently under evaluation regarding the treatment of melanoma with ipilimumab. As discussed previously, a randomized, double-blind phase II trial comparing the dosing of ipilimumab demonstrated the superiority of 10 mg/kg dosing over 3 mg/kg dosing (used in the phase III trial and currently approved) in pretreated patients [163]. This data was not available at the initiation of the phase III trial.

The randomized, double-blind, multicenter phase III trial comparing 10 mg/kg versus 3 mg/kg ipilimumab in 727 patients with previously untreated or previously treated unresectable stage III/IV melanoma without previous treatment with BRAF inhibitors or immune checkpoint inhibitors showed a significant overall survival advantage with 10 mg/kg therapy over 3 mg/kg therapy (15.7 vs. 11.5 months, $p = 0.04$). The 10 mg/kg group did demonstrate a higher frequency of treatment-related adverse events and adverse events leading to discontinuation [164].

An additional question raised by the previous trials is the duration of treatment. Many of the previous phase II trials included maintenance dosing every 3 months after completion of the "induction" phase [52, 153, 163, 165]. The phase III trial of ipilimumab monotherapy applied a somewhat different approach, using "reinduction" therapy, in which the patients were redosed every 3 weeks for four doses if they had evidence of progression after initial response to treatment. Both long-term dosing schedules appear to be well tolerated. It remains to be seen if one is clearly superior. Ipilimumab monotherapy in metastatic melanoma has largely been replaced by combination therapy of ipilimumab with PD-1 inhibitors pembrolizumab and nivolumab. Phase III data for pembrolizumab was obtained in the KEYNOTE-006 study, in which 834 ipilimumab-naïve patients with advanced melanoma were randomized 1:1:1 to receive pembrolizumab 10 mg/kg every 2 weeks or 3 weeks or four doses of ipilimumab 3 mg/kg every 3 weeks. In the final analysis, pembrolizumab in both dosages provided a superior overall survival to ipilimumab at a median follow-up of 22.9 months. Median overall survival was not reached in either pembrolizumab group and was 16 months in the ipilimumab group. Twenty-four month overall survival was 55% in both the 2 and 3 weeks pembrolizumab dosing group and 43% in the ipilimumab group [138, 166]. In addition, patient-reported health-related quality-of-life scores were superior for patients who received pembrolizumab [167].

Nivolumab was evaluated in a phase III trial in ipilimumab-refractory melanoma patients who had unresectable or metastatic disease, comparing nivolumab to the investigator's choice of chemotherapy. In an analysis after 120 patients were enrolled in the nivolumab arm, there was an objective response rate of 31.7% (95% CI 23.5–40.8%) in the nivolumab arm versus 10.6% (95% CI 3.5–23.1%) in the chemotherapy arm. Additionally, nivolumab was associated with fewer toxic effects than chemotherapy [132].
Another study, known as CheckMate-066, examined untreated patients in a phase III study in previously untreated melanoma patients without a BRAF mutation and compared nivolumab with dacarbazine. Nivolumab was associated with improved overall survival at 1 year (72.9% vs. 42.1% respectively, $p < 0.001$) and progression-free survival (median 5.1 vs. 2.2 months, respectively, $p < 0.001$) [134].

6.6.3 Adjuvant Checkpoint Inhibitors

Ipilimumab was first approved as adjuvant therapy for melanoma due to results from a double-blind, phase III trial in patients with stage III cutaneous melanoma after resection, who received 10 mg/kg ipilimumab or placebo every 3 weeks for four doses and then every 3 months for up to 3 years.

951 patients were randomized, and median recurrence-free survival was 26.1 months (95% CI 19.3–39.3) in the ipilimumab group vs. 17.1 months (95% CI 13.4–21.6) in the placebo group. In patients who received ipilimumab, 52% discontinued therapy due to adverse events, most commonly gastrointestinal, hepatic, and endocrine [168].

Ipilimumab (10 mg/kg) was compared to nivolumab (3 mg/kg) in resected stage IIIB/IIIC/IV melanoma patients. 12-month recurrence-free survival was 70.5% (95% CI 66.1–74.5%) in the nivolumab group versus 60.8% (95% CI 56.0–65.2%) in the ipilimumab group. Grades 3 and 4 treatment-related adverse events were significantly worse in the ipilimumab group (45.9% vs. 14.4% in the nivolumab group), with two deaths in the ipilimumab group. The hazard ratio for death or recurrence favored nivolumab over ipilimumab (HR 0.65, 0.51–0.83, $P < 0.001$) [169].

Pembrolizumab was evaluated in a phase III double-blind trial in patients with completely resected stage III melanoma. Patients were randomized to receive either 200 mg pembrolizumab IV every 3 weeks for 18 doses or placebo. Pembrolizumab was associated with significantly longer recurrence-free survival at 1 year, 75.4% (95% CI 71.3–78.9) versus 61.0% (56.5–65.1) for placebo. Grades 3–5 trial-related adverse events were reported in 14.7% that received pembrolizumab compared to 3.4% in the placebo group [170].

Combination therapy involving checkpoint inhibitors is an active area of study. Recently, improved survival was observed using ipilimumab in combination with nivolumab in late-stage melanoma [129]. This will be covered in more detail in a later section.

6.7 Checkpoint Inhibitors as Combination Therapy

While CTLA-4 blockade, specifically ipilimumab, has found success as monotherapy in metastatic melanoma, and more trials are underway to test its effectiveness in a variety of malignancies and different clinical scenarios, its greatest potential may lie in combining it with other antineoplastic agents. The hope is that by combining CTLA-4 blocking therapy with other antineoplastic therapies that carry different toxicity profiles, a synergistic effect of the agents will be achieved. Recognizing these issues, researchers have been actively pursuing combination therapy with CTLA-4 blockade since its inception. The primary areas of research focus on combining CTLA-4 blockade with chemotherapy, radiation, surgery, and other immunotherapy.

6.7.1 Checkpoint Inhibitors and Chemotherapy

Given the known immunosuppressive effects of most chemotherapeutic agents, it has been thought that combining chemotherapy with immunotherapy would be unsuccessful. However, there is increasing evidence for a possible synergistic role between the two modalities. The immune system appears to play an important role in antitumor activity of chemotherapy, an effect which may be further augmented by immune checkpoint blockade [171, 172]. In murine models of mesothelioma, CTLA-4 blockade given between cycles of chemotherapy has been demonstrated to increase tumor-infiltrating lymphocytes and inflammatory cytokines and inhibit cancer cell repopulation [173]. Additionally, chemotherapy, when given appropriately, may enhance the effect of specific immunotherapy [174]. Evidence from clinical trials reveals that combining chemotherapy with cancer vaccination can be more effective than either therapy alone [175–177]. The mechanisms by which chemotherapy may increase anticancer immunity include reduction of immunosuppressive influences by decreasing tumor mass, inducing the expression of TAAs on the cell surface, exposing the immune system to TAAs through cell death, and "resetting" the immune posture through depletion of inhibitory cell populations (i.e., Tregs and myeloid-derived suppressor cells) [171]. Indeed, there is growing evidence that the success of certain chemotherapy regimens is dependent on the drug's ability to cause immunogenic cell death of tumors, where TAAs are presented in the appropriate context to elicit a broader immune response [178]. While this is a promising area for future development, clearly the timing of drug administration, chemotherapeutic regimen used, and dosing are integrally important to successful application. Highly dosed cytotoxic treatment has the

potential to quash a developing therapeutic immune response. Optimizing these factors will be necessary in future trials of combining checkpoint blockade with chemotherapy.

Clinical trials have been performed combining chemotherapy with CTLA-4 blockade. A randomized phase II trial testing the combination of chemotherapy with ipilimumab was conducted in patients with treatment-naïve metastatic melanoma. Seventy-two patients with unresectable, metastatic melanoma were randomized to receive ipilimumab at 3 mg/kg every 4 weeks for four doses with dacarbazine compared to ipilimumab monotherapy. The trial demonstrated an increased objective response rate (14.3% vs. 5.4%, by RECIST criteria) and increased median OS (14.3 vs. 11.4 months) for the combination therapy group, although neither reached statistical significance due to the smaller number of patients. Toxicity was higher in the combination group, including 17.1% ≥ grade 3 irAEs compared to 7.7% in the monotherapy arm [179].

Based on these results, the concept was tested in a randomized phase III trial evaluating ipilimumab with dacarbazine versus dacarbazine alone [163]. Additionally, based on the results of the phase II ipilimumab monotherapy trial that showed a benefit of higher dosing, 10 mg/kg of ipilimumab was used in combination with dacarbazine. Five hundred two patients were enrolled and randomized 1:1 to receive ipilimumab plus dacarbazine every 3 weeks for four doses followed by dacarbazine every 3 weeks until week 22 or placebo plus dacarbazine at the same schedule. Patients with stable disease or RECIST criteria objective responses were able to receive maintenance ipilimumab or placebo every 12 weeks. Of note, based on emerging consensus from previous work with CTLA-4 blockade and other immunotherapy, the primary endpoint was changed, with FDA approval, from progression-free survival to OS prior to unblinding of the treatment groups or data analysis [152, 180]. Ultimately, the trial showed that patients who received the combination of ipilimumab with dacarbazine survived longer (11.2 months) compared to dacarbazine alone (9.2 months, $p < 0.001$). The difference became more pronounced with time, as the combination arm had 20.8% of patients alive at 3 years compared to 12.2% in the chemotherapy only arm. Toxicities were greater in the combination arm and also greater than in many of the previous ipilimumab studies (56% ≥ grade 3), likely secondary to the higher dose (10 mg/kg) of ipilimumab used as well as the addition of chemotherapy. Interestingly, the toxicity profile was different. There were lower rates of gastrointestinal toxicities, such as diarrhea and colitis, and endocrine toxicity but a higher rate of hepatic toxicity compared with previous ipilimumab trials. No treatment-related death was reported [53]. Differences may reflect the effect of the combination therapy; however, clinician's experience managing the drug may have affected the outcome as well. Based on the results of this study, the combination of ipilimumab and dacarbazine is approved as the first-line therapy for unresectable melanoma.

However, the potential for unanticipated toxicity exists with combining CTLA-4 blockade, particularly with other targeted therapies. Initial results from a phase I study of combination therapy with both ipilimumab (dosed at 3 mg/kg) and vemurafenib, a BRAF inhibitor approved for treatment of BRAF-V600E-mutated melanoma, demonstrated an unacceptably high level of hepatotoxicity, leading to early termination of the trial [181].

Additional trials of combination chemotherapy and ipilimumab were conducted in patients with advanced non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Advanced-stage NSCLC carries a poor prognosis with a median survival of 8–12 months despite first-line chemotherapy [172, 182]. In a phase II trial, 204 patients with stage IIIB or IV NSCLC were enrolled in a randomized, double-blind trial of ipilimumab plus chemotherapy (paclitaxel and carboplatin) given concurrently, ipilimumab plus chemotherapy given phased with two doses of chemotherapy given prior to starting ipilimumab and chemotherapy given together, or placebo plus chemotherapy. Ipilimumab was dosed at 10 mg/kg every 3 weeks for up to 18 weeks with the option for

maintenance therapy (or maintenance placebo) every 12 weeks. The primary endpoint was immune-related progression-free survival (irPFS). The concept of immune-response criteria for immunotherapy in cancer (different from classic World Health Organization RECIST criteria) came from observations with ipilimumab and other immunotherapies (discussed further below) [152]. The trial showed improved irPFS with phased ipilimumab and chemotherapy (median: 5.7 months, HR: 0.72, $p = 0.05$), while concurrent ipilimumab and chemotherapy did not reach statistical significance (median: 5.5 months, HR: 081, $p = 0.13$) compared to the control regimen (median 4.6 months). Improvement was also noted in PFS by WHO criteria ($p = 0.02$), and an improvement in OS by 3.9 months ($p = 0.23$) was observed for phased ipilimumab over chemotherapy alone. Overall toxicity was similar across the treatment arms; however, there was more severe toxicity (grade ≥ 3) in the combination arms. A phase III trial was conducted using phased ipilimumab and chemotherapy in patients with squamous NSCLC, the group that derived the greatest benefit in subset analyses [154]; however, the addition of ipilimumab to first-line chemotherapy consisting of paclitaxel and carboplatin did not prolong OS [183].

A similar phase II trial was conducted in patients with extensive disease-small cell lung cancer (ED-SCLC). Chemotherapy remains the first-line and only effective therapy in this disease process with a median overall survival of 8–11 months [184]. Eligible patients ($n = 130$) were randomized to receive concurrent therapy with ipilimumab and chemotherapy (paclitaxel and carboplatin), the phased combination, or placebo with chemotherapy. In this trial, again the phased combination of ipilimumab and chemotherapy was superior with an improvement in irPFS (median: 6.4 months, $p = 0.03$), while concurrent therapy did not improve irPFS (median: 5.7 months, $p = 0.11$), compared to the control arm (median: 5.3 months). There was no significant difference in mWHO PFS or OS. The combination of ipilimumab plus etoposide and platinum chemotherapy versus etoposide and platinum alone has been evaluated in a phase III trial. 954 patients were randomized with no significant OS benefit (11.0 vs. 10.9 months), with increased rates of diarrhea, colitis, and rash in the ipilimumab group [185].

The combination of ipilimumab has been further studied in a phase II trial in prostate cancer. Forty-three patients with CRPC were randomized to receive either ipilimumab monotherapy at 3 mg/kg every 4 weeks for four doses or ipilimumab (dosed the same) with a single dose of docetaxel at the start of therapy. The number of responses to therapy were small with three patients having a decrease of >50% in each arm [186]. However, this study may be limited by underdosing of both the ipilimumab and docetaxel, concurrent (instead of phased) administration of the two drugs, as well as the small number of patients tested.

The combination of tremelimumab and sunitinib, an oral small-molecule tyrosine kinase inhibitor, was tested in a phase I dose escalation trial in patients with metastatic renal cell carcinoma. Unexpectedly, the trial demonstrated a high (4/28 patients) rate of sudden onset grade 3 renal failure in addition to other toxicity associated with CTLA-4 blockade. Further testing of this combination at doses of tremelimumab >6 mg/kg with sunitinib was not recommended by the study authors [187].

6.7.1.1 PD-1/PD-L1 Inhibitors and Chemotherapy

Pembrolizumab in combination with chemotherapy recently received FDA approved based on results of a double-blind phase III trial in which 616 patients with metastatic NSCLC without sensitizing EGFT or ALK mutations with no previous treatment were randomized to receive pemetrexed and a platinum-based drug plus either 200 mg pembrolizumab or placebo every 3 weeks for 4 cycles, followed by maintenance pemetrexed and pembrolizumab or placebo for 35 cycles. At a median follow-up of 10.5 months, estimated overall survival at 12 months was 69.2% (95% CI, 64.1–73.8) in the pembrolizumab group versus 49.4% (95% CI, 42.1–56.2)

in the placebo group, corresponding to a hazard ratio for death of 0.49 (95% CI, 0.38–0.64, $p < 0.001$). In addition, progression-free survival was significantly greater in the pembrolizumab arm: 8.8 versus 4.9 months. Adverse events of grade 3 or higher were comparable between arms (67.2% for pembrolizumab vs. 65.8% for placebo) [188].

There are no current FDA indications for nivolumab in combination with chemotherapy; however, multiple clinical trials are evaluating this (NCT02477826, NCT03101566).

6.7.2 Checkpoint Inhibitors and Radiation

Much like chemotherapy, there is evidence that the local and systemic effects of radiation therapy can increase the effectiveness of immunotherapy, in general, and CTLA-4 blockade, specifically. Radiation therapy damages tumor cells that are in the path of the focused energy, which, like chemotherapy, can result in cell death and antigen cross-presentation, leading to an effective, targeted immune response toward remaining tumor cells [189]. Radiation-induced cell damage may lead to several cellular changes that promote effective presentation of TAAs such as the release of high mobility box group 1 (HMBG1), which signals migration of immune cells to the tumor microenvironment, and upregulation of MHC I complexes, Fas, and ICAM-1, all of which increase susceptibility to T-cell-mediated death [189–192]. Additionally, localized radiation does not typically produce the same level of lymphodepletion and immunosuppression associated with high-dose chemotherapy. As with chemotherapy, reduction in the mass of a viable tumor may help decrease cancer-related immunosuppression. All of these factors make the combination of radiation with immunotherapy appealing [193]. The concept of combining radiation with immune checkpoint blockade is particularly attractive. Unlike more specific, directed immunotherapy (cancer vaccines), CTLA-4 blockade helps overcome cancer immunosuppression, but ultimately relies on the body's preexisting immunity toward a neoplasm. Radiation, by damaging cancer cells and releasing a wide array of TAAs in an inflammatory context, especially with immunosuppression checked, may allow the immune system to mount a response that is appropriate both for the individual and the tumor.

There is considerable preclinical data that supports the combination of CTLA-4 blockade and radiation. In one study, a mouse model of poorly immunogenic mammary carcinoma, 4T1, was treated with control IgG, CLTA-4 blocking IgG (9H10), radiation therapy, or a combination of 9H10 IgG and radiation. CTLA-4 blockade alone did not affect tumor growth or mouse survival. Radiation therapy slowed tumor growth but did not affect survival. The combination of CTLA-4 blockade and radiation therapy inhibited metastases and increased survival compared to the control [193]. Subsequent studies in this model revealed that treatment with the combination in mice deficient in invariant natural killer (NK) T-cell lymphocytes led to an even more effective response with some mice becoming disease-free and resistant to tumor rechallenge, highlighting the important role for this cell type in regulation of cancer immune responses [194]. Finally, an additional study in TSA mouse mammary carcinoma and MCA38 mouse colon carcinoma models again demonstrated the effectiveness of combining radiation and CTLA-4 blocking antibody; moreover, they showed that the use of a fractionated radiation schedule (but not single dose radiation) along with CTLA-4 blockade could significantly inhibit tumor foci out of the radiation field, a phenomenon known as the abscopal effect [195].

The abscopal effect refers to the regression of tumors in remote areas following localized radiation of tumors. This phenomenon has been documented in melanoma, renal cell carcinoma, and lymphoma [196–198]. Several cases of this occurrence have been documented in patients receiving ipilimumab. In one notable case, a patient with recurrent melanoma with paraspinal, right hilar lymphadenopathy, and splenic metastases was enrolled in an ipilimumab monotherapy trial in September 2009. She received treatment at 10 mg/kg dosing per protocol with

slow progression of her disease over the subsequent 15 months. In December 2010, she received directed, external beam radiation to her symptomatic paraspinal lesion followed by an additional dose of ipilimumab in February 2011. Surprisingly, follow-up imaging revealed significant regression of metastatic lesions outside the radiation field, which remained stable at minimal disease for at least 10 months after her radiation treatment. Along with this clinical effect, the patient was noted to have a marked increase in peripheral antibodies to the tumor antigen NY-ESO-1, an increase in $ICOS^{high}$ T cells, and a decrease in myeloid derived suppressor cells [199]. Similar cases of abscopal regression of metastatic melanoma in patients on ipilimumab have since been reported [200].

A phase I/II study examined the effects of ipilimumab with radiation therapy (RT) in patients with metastatic CRPC. Patients were treated with dose escalation ipilimumab monotherapy (3, 5, or 10 mg/kg) or ipilimumab (3 mg/kg or 10 mg/kg) with external beam RT, although the trials were not designed to directly compare the two arms. Ipilimumab was given every 3 weeks for a total of 4 weeks [201]. An overall of 71 patients were treated; 33 patients were treated in the dose escalation phase, and the 10 mg/kg arm was expanded to a total of 50 patients. At the 10 mg/kg dosing level, 16 were given ipilimumab monotherapy and 34 received ipilimumab with radiation. In the 10 mg/kg dosing group, there were four (25%) PSA declines >50% in the ipilimumab monotherapy arm and four (12%) PSA declines >50% in the ipilimumab with radiation group; however, a higher proportion of patients in the monotherapy group were chemotherapy naïve. A phase III trial examining radiation with ipilimumab compared to radiation alone in advanced CRPC has not shown a difference in overall survival [202].

A retrospective study was performed analyzing patients treated with pembrolizumab for NSCLC on the phase I KEYNOTE-001 study to determine the effect of previous radiotherapy on clinical outcomes. Of 98 patients that received pembrolizumab, 43% received previous radiotherapy. At a median follow-up of 32.5 months for surviving patients, progression-free survival was significantly increased in patients that received previous radiotherapy (4.4 months; 95% CI, 2.1–8.6) versus no radiotherapy (2.1 months; 95% CI, 1.6–2.3), corresponding to a hazard ratio of 0.56 (95% CI 0.34–0.91), $p = 0.019$. Median overall survival was increased in patients who received any radiotherapy (10.7 months; 95% CI, 6.5–18.9) versus no radiotherapy (5.3 months; 95% CI, 2.7–7.7), corresponding to a hazard ratio of HR 0.58 (95% CI 0.36–0.94), $p = 0.026$ [203].

There are no current FDA indications for PD-1/PD-L1 inhibitors in combination with radiation; however, multiple clinical trials are attempting to answer this question (NCT02830594 in pembrolizumab, NCT03148327 in durvalumab).

6.8 Combination Immunotherapy

Results from trials of CTLA-4 and PD-1 pathway blocking mAbs as monotherapy or in combination with conventional therapies are encouraging. Immune checkpoint blockade has delivered clinical responses in patients with limited or no therapeutic options remaining. However, in all of the immune checkpoint blockade trials covered, only a minority of patients have responded which is usually transient. It is true that the vast majority of the patients treated in these trials have advanced disease, are immunosuppressed, and have limited time and options remaining. Targeting earlier stage disease and combining immune checkpoint blockade with other therapies will undoubtedly yield more impressive results. However, it is naïve to think that targeting any one checkpoint will be a "silver bullet" therapy. Just as cancer, under immunologic pressure, learns to evade the immune system to become a clinically evident disease initially, as we modulate coinhibitory and costimulatory receptors, some cancers will adapt to escape through alternative pathways. Combining active immunization (cancer vaccines) with checkpoint blockade may ultimately prove effective; nonetheless, initial

results have not been convincing. Other techniques under investigation, targeting multiple checkpoints simultaneously or in sequence, may limit the escape routes.

6.8.1 CTLA-4 Blockade and Vaccination

Early on in the development of CTLA-4 blocking therapy, anti-CTLA-4 antibodies were combined with cancer vaccines in preclinical models [204]. In multiple cancer animal models, tumors, which were poorly responsive to CTLA-4 blocking therapy alone or active immunotherapy alone, responded significantly better to the combination of the two [37, 204–216]. These studies have helped elucidate the function and significance of the CTLA-4 receptor and have led to clinical trials in patients.

Some of the first human trials of ipilimumab used a combination of peptide vaccines from gp100, a tumor-associated antigen expressed by the majority of malignant melanomas [217]. Gp100 peptides have been shown to be immunogenic and elicit an antigen-specific T-cell response in the majority of melanoma patients [160]. One peptide, gp100:209–217(210M), when combined with IL-2 therapy, has also been shown in a randomized phase III trial to significantly increase clinical response and PFS compared to IL-2 alone in HLA*A0201$^+$ metastatic melanoma patients [218]. Three phase I and II trials were conducted using ipilimumab combined with gp100 in unresectable melanoma patients. While these trials did not directly compare the efficacy of the addition of the peptide vaccines to ipilimumab monotherapy, they did show impressive response rates and manageable toxicity [56–58]. Based on these (and other) results, ipilimumab proceeded to the phase III trial comparing ipilimumab monotherapy, ipilimumab plus two gp100 peptides (gp100:209–217 and gp100:280–288), or the gp100 peptides alone. As previously detailed, the trial demonstrated a survival advantage for ipilimumab therapy but also showed that the addition of the peptide vaccine to ipilimumab offered no improvement over ipilimumab monotherapy [50]. It is not clear why the peptide vaccine did not prove efficacious in this setting, particularly given its proven efficacy when given with IL-2 therapy in a similar patient population. There is speculation that CTLA-4 blockade may augment CD4$^+$ lymphocyte activity more, while gp100 peptides preferentially generate a CD8$^+$ lymphocyte response, a hypothesis that has mixed preclinical data to support it. Another proposed possibility is that the antitumor effect of ipilimumab may stem largely from its ability to deplete intratumoral Tregs, a mechanism which may not function synergistically with MHC class I peptide vaccination [34]. Certainly, there are other possibilities to explain the results; further studies will be necessary to clarify.

Additional trials on combining CTLA-4 blocking antibodies with cancer vaccines have been conducted in melanoma and prostate cancer. In melanoma, the combination of multiple tumor-associated antigen peptides (gp100, MART-1, tyrosinase) emulsified with immunoadjuvant (Montanide ISA 51) has been combined with ipilimumab in a dose escalation trial [62]. Additionally, in prostate cancer, ipilimumab has been given in phase I trials in combination with Tricom-PSA (PROSTVAC; Bavarian Nordic Immunotherapeutics, Mountain View, CA), a poxvirus-based vaccine that expresses transgenes for PSA and costimulatory molecules, and GVAX (Aduro Biotech; Berkeley, CA, USA), a GM-CSF-transduced allogenic prostate cancer vaccine [59, 219]. In all of these phase I trials, ipilimumab combined with cancer vaccination was found to elicit a cancer-specific immune response, a low rate of clinical response, and toxicity compared with ipilimumab monotherapy. Further trials will be necessary to prove the efficacy of these combinations and multiple other combinations, which are currently under investigation (NCT01810016, NCT01302496, NCT01838200).

6.8.2 PD-1/PD-L1 and Vaccination

Nivolumab has been tested in combination with ISA 101, a synthetic long-peptide vaccine directed against human papilloma virus (HPV)

16 in patients with incurable oropharyngeal cancer. The phase II trial accrued 22 patients who received 100mcg/peptide ISA 101 on days 1, 22, and 50, plus nivolumab 3 mg/kg IV every 2 weeks for up to 1 year. Eight patients demonstrated a clinical response, with two complete responses and eight partial responses, corresponding to an overall response rate of 36%, greater than the historical nivolumab monotherapy rate of 16% [220]. At a median follow-up of 8.6 months, median progression-free survival was 2.7 months (95% CI, 2.3–8.0). Median overall survival was not reached [221].

Nivolumab has also been tested with or without a peptide vaccine in a phase I study in 90 patients with ipilimumab-naive or refractory unresectable stage III or IV melanoma. Nivolumab was dosed at 1 mg/kg, 3 mg/kg, or 10 mg/kg and was well tolerated at all doses. The median duration of response was 8.1 months, and the overall response rate was 25% [222].

Ongoing studies include PD-1/PD-L1 and vaccination in melanoma (NCT03047928), non-squamous non-small cell lung cancer (NCT03380871), and multiple solid tumors (NCT02897765, NCT02432963).

6.8.3 CTLA-4 Blockade and Cytokine Therapy

Another area of combined immunotherapy undergoing active investigation is combining CTLA-4 blockade with cytokine therapy. IL-2 therapy has been used as adjuvant treatment for melanoma and renal cell carcinoma with benefit in a small subset of patients [223]. IL-2 stimulates T-cell activation, as does CTLA-4 blockade, but through different mechanisms. A phase I/II dose escalation/expansion trial combining ipilimumab with IL-2 was conducted in metastatic melanoma patients. The trial demonstrated a 22% (5/36) tumor response rate and toxicity similar to prior ipilimumab studies [61]. There are multiple ongoing trials examining the combination of ipilimumab and high-dose interferon alpha, the cytokine therapy used most frequently as adjuvant therapy in melanoma (NCT01274338 ongoing, NCT01708941 ongoing). GM-CSF has been used in combination with ipilimumab in a phase I dose escalation trial in CRPC demonstrating an immunologic response to treatment as well as a favorable PSA response in the highest dosing cohort (ipilimumab 3 mg/kg and GM-CSF 250 mg every 4 weeks) with expected toxicities. A recent randomized trial pairing ipilimumab with GM-CSF versus ipilimumab alone in patients with unresectable stage III/IV melanoma demonstrated longer overall survival (17.5 vs. 12.7 months), with no different in progression-free survival [47]. Additional trials of ipilimumab and GM-CSF in CRPC and melanoma are currently underway, NCT01530984).

A recent phase II trial compared talimogene laherparepvec (a genetically modified herpes-simplex virus that expresses GM-CSF) with and without ipilimumab in patients with unresectable stage IIIb and IV melanoma. One hundred ninety-eight patients were randomized, with a 39% objective response rate (ORR) in the combination arm compared to 18% ORR in the ipilimumab monotherapy arm (OR 2.9, 95% CI 1.5–55, $p = 0.002$). Additionally, more patients in the combination arm demonstrated regression of visceral lesions (52% vs. 23%), with severe toxicity comparable between arms (45% vs. 35%) [46].

6.8.4 Combination Checkpoint Blockade

There is ample preclinical data supporting dual checkpoint blockade in murine cancer models [215, 224–228]. Based on these principles, investigators have initiated trials of dual checkpoint blockade in humans.

Preliminary phase I results of combination of nivolumab (PD-1 blocking mAb) and ipilimumab (CLTA-4 blocking mAb) in patients with advanced melanoma demonstrated the potential of this combination [229]. This led to a multicenter randomized controlled phase III trial, the CheckMate 067 study. This trial enrolled patients with previously untreated stage III (unresectable) or stage IV melanoma and randomized them (1:1:1) to ipilimumab (3 mg/kg every

3 weeks for four doses) and nivolumab (1 mg/kg every 3 weeks for four doses followed by 3 mg/kg every 2 weeks), nivolumab (3 mg/kg every 2 weeks), or ipilimumab (3 mg/kg every 3 weeks for four doses). The overall survival rate at 36 months was 58% in the nivolumab-ipilimumab combination group, 52% in the nivolumab group, and 34% in the ipilimumab alone group. At 36 months follow-up, the median overall survival had not been reached in the combination group and was 37.6 months in the nivolumab group and 19.9 months in the ipilimumab group, corresponding to a hazard ratio for death with nivolumab plus ipilimumab versus ipilimumab of 0.55 ($p < 0.001$) and 0.65 ($p < 0.001$) for death with nivolumab versus ipilimumab. Treatment-related adverse effects of grades 3 and 4 occurred in 59% of the combination group, 21% receiving nivolumab, and 28% receiving ipilimumab [129].

6.9 Other Checkpoint Pathways Under Development

6.9.1 Lymphocyte Activation Gene-3 (LAG-3)

Lymphocyte activation gene-3 (LAG-3, CD223) is an additional immune coinhibitory checkpoint molecule under investigation for therapeutic purposes in cancer. LAG-3 was first discovered in the 1990s on activated T lymphocytes and NK cells [230]. LAG-3 is structurally similar to CD4, and, like CD4, binds to MHC II complexes on antigen-presenting cells (APCs), but with greater affinity. While some early functional data from experiments is mixed, it appears that LAG-3 plays a predominantly inhibitory role in T-cell activation, while promoting APC activation at the same time [114, 231–235].

LAG-3 is expressed on a subset of Treg cells that secrete immunosuppressive cytokines and are more potent than other LAG-3 negative cells of the Treg phenotype (CD4+, CD25highFoxP3+). They are preferentially expanded in patients with cancer. LAG-3 ligation on $CD8^+$ lymphocytes inhibits lymphocyte function and proliferation, independent of Tregs [18]. Notably, high expression levels of LAG-3 are seen on tumor infiltrating lymphocytes and, like PD-1, appear to represent an anergic phenotype. In contrast to its coinhibitory function on T cells, when soluble LAG-3 binds MHC II complexes on dendritic cells, it promotes activation and maturation [235–238].

Just as with CTLA-4 and PD-1 pathways, tumor cells are able to utilize the LAG-3 pathway to escape host immunity. MHC class II molecule (LAG-3 ligand) expression is sometimes upregulated to varying degrees in a variety of cancers and can be associated with a worse prognosis. Increased expression of LAG-3 on TILs, corresponding with increased $CD8^+$ T-cell anergy, has been noted in Hodgkins lymphoma, melanoma, and ovarian cancer [239, 240]. Additionally, MHC class II expressing melanoma cells (but not MHC class II negative cells) were resistant to FAS-mediated apoptosis when exposed to LAG-3 transfected cells or soluble LAG-3, indicating a bidirectional signaling in the LAG-3 pathway that effects both lymphocytes and tumor cells [114, 239–241].

Removing or blocking the LAG-3 pathway improves immune-mediated antitumor effects. Blocking LAG-3 with mAbs has been shown to increase CTL expansion and improve $CD4^+$ lymphocyte cytokine production. In melanoma, anti-LAG-3 mAb blockade improved the antitumor function of tolerized $CD8^+$ lymphocytes when coupled with a viral cancer vaccine [242]. In murine cancer models, PD-$1^{-/-}$ LAG-$3^{-/-}$ knockout mice were capable of rejecting tumors that PD-1 or LAG-3 alone knockout mice could not. It is worth noting that LAG-$3^{-/-}$ knockout mice display a very mild phenotype, similar to PD-$1^{-/-}$ knockout mice, while PD-$1^{-/-}$ LAG-$3^{-/-}$ knockout mice develop lethal autoimmunity at about 10 weeks of age, underscoring the potential toxicity of dual blockade therapy [225, 227, 243]. Similar to the knockout mice, dual mAb blockade of PD-1 and LAG-3 was able to cause complete regression in several established tumor models in mice, while blockade of the individual receptors was not [227, 243].

Since LAG-3 binding of MHC II complexes on APC promotes activation and maturation of the

APC, soluble LAG-3 protein has been tested as an immunoadjuvant in cancer. Theoretically, the unbound LAG-3 can promote APC activity while, at the same time, can prevent LAG-3-mediated T-cell inhibition through competitive binding. Supporting this, soluble LAG-3 in the serum of breast cancer patients was associated with improved survival. Based on these findings, a fusion protein of the extracellular portion of LAG-3 and the Fc portion of IgG1 were recognized as IMP321. IMP321 has been tested as a vaccine immunoadjuvant where it was well tolerated and produced encouraging immunologic results. IMP321 has also undergone testing as monotherapy in a phase I dose escalation trial in 21 patients with advanced renal cell carcinoma. The drug produced no significant adverse events and was associated with significantly more disease stability at higher dosing. More recently, IMP321 was tested at two different doses in a phase I trial together with gemcitabine in 12 patients with advanced pancreatic cancer. IMP321 again did not produce significant adverse events but also failed to show any change in immunologic markers after therapy was given [244–248].

LAG-3 has been shown to be synergistic with PD-1/PD-L1. In a murine model, dual anti-LAG-3/anti-PD-1 antibody treatment cured most mice of established tumors that were resistant to single antibody treatment [48] and demonstrated that LAG-3 is required for long-term peripheral CD8 but not CD4 immune tolerance [49]. High level dual LAG-3/PD-1 expression is largely restricted to tumor-infiltrating lymphocytes which are likely advantageous due to focused "attack" instead of nonspecific or self-antigen-specific immune responses.

Ongoing studies of LAG-3/IMP321 are being performed in glioblastoma (NCT02658981), metastatic breast cancer (NCT02614833), and hematologic neoplasms (NCT02061761).

6.9.2 4-1BB

4-1BB (CD137), unlike the inhibitory molecules CTLA-4, PD-1, and LAG-3, is a co-stimulatory molecule. It is a member of the tumor necrosis factor receptor (TNFR) superfamily that is inducibly expressed on activated $CD8^+$ and $CD4^+$ lymphocytes (including Tregs), NK cells, dendritic cells, macrophages, neutrophils, and eosinophils, as well as in some tumor tissue. The 4-1BB receptor is bound by the 4-1BB ligand (4-1BBL) expressed on antigen-presenting cells. 4-1BB functions as a costimulatory signal after a T-cell receptor is bound by an antigen-MHC ligand along with CD28 costimulation to promote $CD4^+$ and $CD8^+$ lymphocyte proliferation, activation, and protection against activation induced cell death. 4-1BB ligation is able to costimulate $CD8^+$ lymphocytes to activation even in the absence of CD28-B7-1/B7-1 signaling and prevent or reverse established anergy in lymphocytes. Additionally, 4-1BB appears to function across both the innate and adaptive immune system as it is able to increase the activity of NK cells which, once activated, are further able to stimulate lymphocyte function. 4-1BB also appears to be functionally important in inhibiting Treg function and promoting antigen priming by dendritic cells. Interestingly, 4-1BB activation via agonistic mAbs is able to prevent or treat antibody-mediated autoimmunity in mouse and primate models by increasing $CD4^+$ (but not $CD8^+$) lymphocyte anergy, a process that is not completely understood [249–258].

Preclinical data with agonistic 4-1BB mAbs has demonstrated a robust antitumor effect. In multiple mouse models, mAb treatment has led to increased tumor-specific $CD8^+$ lymphocyte response and substantial tumor regression. Additionally, melanoma cells transfected to express 4-1BB agonist single chain Fv fragments and given to mice as an autologous tumor cell vaccine led to rejection of poorly immunogenic tumors. Treatments were well tolerated in animal models, although polyclonal T lymphocyte accumulation in the liver was noted. Combination of agonist 4-1BB mAb treatment with immunotherapy appears to function synergistically with immunotherapy and chemotherapy. To further test its efficacy and safety, one 4-1BB mAb, BMS 663513, was tested in primates along with a prostate-specific antigen DNA vaccine where it demonstrated encouraging immunologic results [228, 249, 252, 254, 259–266].

Two mAbs have moved into clinical testing in humans. Urelumab (BMS-663513;Bristol Myers-Squibb, New York, NY) is a fully human agonist 4-1BB mAb that was given to advanced cancer patients in a dose escalation trial. Initial results from 83 patients with melanoma (54 patients), renal cell carcinoma (15 patients), ovarian cancer (13 patients), and prostate cancer (1 patient) who were given 0.3–15 mg/kg of the mAb with expansion cohorts at the 1, 3, or 10 mg/kg level of dosing have been reported. Results revealed that there were significant toxicities including grade 3 or 4 transaminitis in 11% and grade 3 or 4 neutropenia in 5% of patients. There were three objective partial responses in melanoma patients and several other patients with stable disease along with increased levels of peripheral activated T lymphocytes and interferon in posttreatment biopsies [267]. A phase II trial in advanced melanoma was conducted; however, as the incidence of grade IV hepatitis was higher than expected, the trial was terminated. Several other trials were terminated at that time. Phase I trials have been performed in which urelumab was given as monotherapy in advanced solid malignancies or non-Hodgkins lymphoma (NCT01775631, completed, results not reported) and in combination with rituximab in non-Hodgkins lymphoma or chronic lymphocytic leukemia (NCT0177563, study withdrawn). A second drug, PF-05082566 (Pfizer, New York, NY), is currently recruiting for a phase I trial as monotherapy in solid tumors or in combination with rituximab in non-Hodgkins lymphoma (NCT01307267).

Multiple studies are in progress evaluating combination therapy with urelumab and nivolumab including urothelial carcinoma (NCT02845323), metastatic melanoma (NCT02652455), and multiple advanced tumor types (NCT02534506). Hepatotoxicity appears to be the limiting factor with 4-1BB monotherapy, but combination therapy is promising.

6.9.3 OX-40

OX-40 (CD134, TNFRSF4) is another member of the TNFR superfamily which is a costimulatory receptor of particular interest in cancer. Like many of the previously described immune checkpoint pathways, OX-40 functions to modulate T-cell activation and proliferation in the setting of inflammation to ensure an adequate immune response, but prevent autoimmunity or unnecessary tissue damage. OX-40 is predominantly expressed on activated $CD4^+$ lymphocytes; however lesser degrees of expression is observed on other cells such as activated $CD8^+$ lymphocytes, Tregs, NK cells, and neutrophils. The only known ligand to OX-40 is the OX-40 ligand (OX-40L), which is primarily expressed on activated APCs. OX-40 stimulates $CD4^+$ lymphocyte clonal expansion, survival, and cytokine production, particularly in late phases of activation. OX-40 is also important in the generation of functional memory T-cell pools. Signaling through the OX-40 pathway does expand Treg populations, but the expanded cells are functionally impaired with an exhausted phenotype. The function of OX-40 was further shown in transgenic mice engineered to have constitutive T-cell expression of OX-40L. These mice developed expansion of $CD4^+$ T-cell (but not $CD8^+$ T cell) pools and an autoimmune phenotype. This is in contrast to OX-$40L^{-/-}$ knockout mice or mice treated with OX-40L blocking mAbs, which demonstrate impaired lymphocyte priming but normal lymphocyte localization and humoral immune responses. While OX-40 appears to function primarily through $CD4^+$ lymphocytes, there is evidence that this ultimately leads to augmented $CD8^+$ lymphocyte function as well [268–283].

In cancer, agonistic therapies to the OX-40 pathway have proved successful in overcoming cancer immune tolerance. In mouse models, agonist OX-40 mAbs have led to complete regression of established tumors and protective immunity against repeat inoculation. The antitumor effect was dependent on both $CD4^+$ and $CD8^+$ lymphocytes. Treatment with agonistic OX-40 mAbs was more effective than blocking CTLA-4 mAbs in generating antigen-specific memory T-cell pools after antigen inoculation. Finally, OX-40 mAbs have been shown to function synergistically with other cancer immunotherapies, surgery, and radiation in murine models. These findings along with observations

that OX-40 has been noted to be relatively overexpressed in tumor-infiltrating lymphocytes and lymphocytes from draining lymph nodes from human melanoma, head and neck, and breast cancers led to trials in primates and then humans [273, 284–291].

A mouse agonist OX-40 mAb was used to treat 30 patients with advanced solid tumors in a dose escalation phase I trial that completed enrollment in 2009. The mAb was given as three doses over 5 days along with tetanus toxin and keyhole limpet hemocyanin. Initial results indicate that the treatment was well tolerated with evidence of clinical response in heavily pretreated patients. A humanized agonist OX-40 mAb has been developed and is currently undergoing trials combined with stereotactic radiation therapy in metastatic breast cancer (NCT01642290 in progress), combined with low-dose cyclophosphamide and radiation in metastatic CRPC (NCT01303705, in progress) renal cell carcinoma (NCT03092856), metastatic colorectal cancer (NCT02559024), and head and neck SCC or melanoma (NCT03336606) [54].

A recent study investigating combination therapy of OX-40 agonist alone or in combination with ipilimumab, durvalumab (anti-PD-L1), and rituximab was terminated at the sponsor's discretion (NCT02205333); however, ongoing studies of combination therapy include OX-40 agonists and atezolizumab (NCT02410512) and durvalumab (NCT02221960) in solid tumors [55].

6.9.4 Glucocorticoid-Induced TNFR-Related Protein (GITR)

Glucocorticoid-induced TNFR-related protein (GITR) is a third member of the TNFR superfamily with costimulatory properties. Like OX40 and 4-1BB, it has a low basal expression level on naïve T-lymphocytes, but is significantly upregulated upon activation. It is also expressed constitutively on Tregs and to a lesser degree on NK cells and mast cells, but expression is increased with activation in all cases. Also like OX40 and 4-1BB, GITR is instrumental in modulation of T-cell responses to infection and cancer; however, it operates through non-redundant pathways. GITR is bound by GITR ligand (GITR-L), which is expressed predominantly on APCs after activation, but also at lower levels on endothelial tissue and activated T cells. GITR ligation enhances T-lymphocyte activation, proliferation, resistance to activation-induced cell death, and resistance to Treg-mediated suppression. However, the in vivo effect in immunomodulation may be subtle as $GITR^{-/-}$ knockout mice demonstrate a mild phenotype with differences in response to certain infection and severe inflammatory conditions [292–304].

In preclinical studies, agonistic GITR mAbs were shown to stimulate T lymphocytes and overcome Treg-mediated tolerance. This finding led to a series of experiments in mice that demonstrated agonist GITR mAbs enhance antitumor immunity [107, 290, 305–307]. Agonistic GITR mAbs have also shown to improve the effectiveness of cancer vaccines in animal models. Based on these results, a humanized agonist GITR mAb, TRX518, is being tested in phase I trials in metastatic melanoma and other advanced solid tumors (NCT01239134, still recruiting). Multiple other studies using GITR agonists are in progress in solid tumors (NCT02628574), in combination with checkpoint inhibitors (NCT02553499, NCT02132754, NCT02598960), and using GITRL proteins (NCT02583165).

6.9.5 CD40

CD40 is another costimulatory molecule of interest in cancer immunotherapy. Like OX-40, it is a member of the TNFR superfamily. CD40 is expressed and functionally important on APCs, but it is also found on a broad range of normal and tumor tissue. On cells such as monocytes and dendritic cells, ligation of the CD40 receptor acts to license the cells into mature, active APCs. For example, ligation of CD40 on monocytes and dendritic cells leads to increased survival, increased expression of MHC complexes and costimulatory molecules, and increased cytokine production. In other tissues, CD40 appears to primarily play a role in modulating local inflammation. It is bound

primarily by CD40 ligand (CD40L); however, binding by mycobacterial heat shock protein 70 and C4b binding protein has also been identified. CD40L is expressed primarily on active (but not resting) T lymphocytes, in particular, $CD4^+$ lymphocytes, although some level of expression has been identified on other cell types. By playing a role in APC maturation, CD40 is also integrally important to lymphocyte priming and activation. Activated $CD4^+$ lymphocytes express CD40L which bind to CD40 on APCs, allowing the APCs to mature and effectively cross prime $CD8^+$ lymphocytes. The central role of the CD40 pathway in immunity is revealed by X-linked hyper IgM syndrome, a severe immune deficiency characterized by neutropenia, susceptibility to opportunistic infection, and autoimmunity, which is due to genetic mutations in the CD40L gene [308–318].

Interest in the CD40 pathway in cancer has come from observations that CD40 ligation is necessary for immune-mediated destruction of cancer cells and that CD40 is expressed on a variety of malignant tissues and from preclinical trials with CD40 mAbs. Treatment of established tumors in mice with agonistic CD40 mAbs has resulted in impressive immune-mediated tumor regression and protective immunity, while treatment with CD40L blocking mAbs results in abrogation of the antitumor immune response. The mechanism of action for agonistic CD40 mAbs is likely twofold and dependent on tumor CD40 expression level and antibody subtype used. In CD40 expressing tumors, anti-CD-40 IgG1 mAbs are able to bind and induce antibody-dependent cytotoxicity (ADCC) of the tumor cells. There is also evidence that high level of ligation of CD40 in certain cancers, particularly multiple myeloma and high-grade B-cell lymphoma, can inhibit cancer growth. The second mechanism of tumor inhibition, which is independent of CD40 expression on tumor cells, is through the immunostimulatory effects of CD40 ligation [319–329].

Multiple strategies have been investigated to therapeutically target CD40 in human malignancy. The first human trials involved treating advanced solid tumors and non-Hodgkins lymphoma with recombinant human CD40L (Avrend; Immunex Corp, Seattle, WA). Treatment was given to 32 patients with dose-limiting toxicity of grade 3 and 4 transaminitis seen with higher dosing. There was evidence of clinical activity with partial responses seen in patients with laryngeal carcinoma and non-Hodgkins lymphoma [330]. More recent efforts have focused on targeted mAb blockade of CD40, with multiple drugs currently under investigation in clinical trials.

CP870,893 (now RO7009789, Selicrelumab) (Pfizer, New York, NY is a fully humanized anti-CD40 IgG2 mAb with strong agonistic properties that has been tested in several clinical trials. Interestingly, CP870,893 with its IgG2 Fc domain has a relatively low binding affinity to human FcgRs when compared to second generation drugs, and may function by binding to a unique epitope on human CD40. It was first given as a single dose, dose escalation phase I trial to 29 patients with advanced malignancy where partial objective responses were noted in 27% (4/15) of melanoma patients but not in other tumor types. A second phase I trial evaluated weekly dosing of CP870,893 in 27 patients with advanced malignancies. Less evidence of clinical benefit was seen with no objective responses observed. CP870,893 was tested in combination with chemotherapy in two trials; in combination with gemcitabine in pancreatic carcinoma and in combination with carboplatin and paclitaxel in a variety of advanced malignancies. In these trials partial objective responses were seen in 19% (4/21) and 20% (6/30) of patients, respectively. [327, 331–334].

In all trials, the immunomodulatory properties of the mAb were evident with transient elevation in IL-6 and TNF-α, as well as depletion and stimulation of B lymphocytes. The most common toxicities were cytokine release syndrome (typically grade 1 and 2) and transient elevation of transaminases. Ongoing studies with CP870,893 include additional trials in combination with gemcitabine in advanced pancreatic cancer, and combination trials with peptide vaccines and CTLA-4 blocking tremelimumab in metastatic melanoma (NCT01456585 completed without reported results, NCT01008527 completed without reported results, NCT01103635 ongoing). Current studies

investigating CD40 combinations include combining anti-PD-L1 in solid tumors (NCT02304393), anti-Ang2/VEGF in solid tumors (NCT02665416), anti-CSF1 R in solid tumors (NCT02760797), and gemcitabine/nab-Paclitaxel in pancreatic carcinoma (NCT02588443).

APX005M is a humanized rabbit IgG1 CD40 agonist being tested in multiple trials, in combination with anti-PD-1 (NCT02706353, NCT03123783) and CD40 alone (NCT02482168).

ADC-1013 is a fully human IgG1 CD40 agonist being studied as monotherapy in multiple studies (NCT02379741, completed without reported results, NCT02829099).

SEA-CD40: non-fucosylated humanized IgG1 agonist, CD40 alone (NCT02376699, recruiting).

Dacetuzumab is a humanized anti-CD40 IgG2 mAb that has been tested in B-cell hematologic malignancies, which have high constitutive expression of CD40. Dacetuzumab was first given as a phase I dose escalation trial in 44 multiple myeloma patients where the addition of steroid premedication was found to increase the tolerated dose; however, it demonstrated no objective clinical response. Similarly, it was tested in a phase I dose escalation trial in 12 patients with chronic lymphocytic leukemia, and again, no objective responses were seen. Based on preclinical data suggesting synergy with rituximab (anti-CD20 mAb), dacetuzumab was tested along with rituximab (and gemcitabine) in 33 patients with refractory diffuse large B-cell lymphoma (DLBCL). In this trial, the combination generated six (20%) complete responses and eight (27%) partial responses. However, a randomized phase II trial comparing this combination with chemotherapy alone in DLBCL was terminated early based on perceived futility. In these trials, dacetuzumab therapy also caused cytokine release syndrome in a minority of patients, but was generally well tolerated. There are no ongoing trials registered for dacetuzumab [326, 335–338].

A third agonistic anti-CD40 mAb being tested is Chi Lob 7/4. This chimeric IgG1 mAb has undergone phase I testing in patients with $CD40^+$ advanced solid malignancies or DLBCL. 15/29 treatments were accompanied by disease stabilization for a median of 6 months with acceptable toxicities when single-dose corticosteroids were administered [339]. No further studies are registered.

The fourth anti-CD40 mAb under investigation is lucatumumab, a fully humanized IgG1mAb, which, unlike the previously described CD40-targeted therapies, is antagonistic. As previously discussed, there is evidence that CD40 ligation can promote proliferation and cell growth in low grade B-cell malignancies as in normal B lymphocytes, although the data is mixed. Thus, the proposed mechanisms of action for lucatumumab include blocking of CD40 ligation on malignant cells and ADCC, but not immunostimulation. Lucatumumab has been tested in two dose escalation phase I trials in chronic lymphocytic leukemia and in multiple myeloma with minimal toxicity but only modest clinical responses. No further studies are currently registered [328, 329, 340–342].

There is currently one actively recruiting study evaluating CDX-1140, a fully human monoclonal anti-CD40 antibody (NCT03329950). No results have been reported.

6.9.6 TIM-3

The function of T-cell immunoglobulin and mucin domain 3 (TIM-3) is becoming better understood. TIM-3 is expressed on multiple cell types including IFN-gamma secreting $CD8^+$ T-cells, Treg cells, and cells of the innate immune system (macrophages, dendritic cells), affecting both adaptive and innate immune responses. TIM-3 is expressed on Th1 cells and generates an inhibitory signal-inducing apoptosis of Th1 cells. It is also expressed on some dendritic cells leading to apoptotic cell phagocytosis and disruption of cross-antigen presentation. TIM-3 is upregulated in tumor-specific $CD8^+$ T cells and $CD8^+$ TILs, while administration of TIM-3 increases proliferation and activity of antigen-specific T cells. In multiple cancers, TIM-3 expression has been associated with tumor progression and shorter survival. Preclinical data suggests that TIM-3 blockade may be most

effective when given in combination with PD-1 mAbs. In addition, since TIM-3 is expressed on non-T cells, a possible mechanism for penetration of the tumor microenvironment is theorized. In general, TIM-3 is seen as a negative regulator of antitumor immunity. Its selective expression on intratumoral T cells may reduce nonspecific toxicity and even offers theoretical synergy with checkpoint inhibitors [343–349].

There are two TIM-3 monoclonal antibodies in development. MBG 453 (Novartis, Basel, Switzerland) is being studied in a phase Ib/II open-label trial comparing single-agent therapy to combination therapy with PD-1 antibodies in adults with advanced malignancies (NCT02608268 recruiting, NCT03066648 recruiting).
TSR-022 (TESARO, Waltham, USA) is being evaluated in a phase 1 study (NCT02817633, recruiting) as a single agent in adults with advanced solid malignancies. Some select patients will receive combination therapy with anti-PD-1 antibodies.

6.9.7 TGN1421: A Cautionary Tale

A word of caution is warranted about trying new individual or combination immune checkpoint therapies. While some immunomodulatory therapies have been well tolerated, it is clear that they have the potential for severe, lasting, and sometimes fatal toxicities. Just as animal models have proven inadequate for reliable prediction of human cancer responses to therapy, they are also inconsistent predictors of treatment toxicity. The most notable example of this is experience with TGN1412 (TeGenero). TGN1412 is a novel agonist anti-CD28 mAb, which was under development for treatment of chronic lymphocytic leukemia. In animal models, the drugs showed encouraging immunologic results without detectable toxicities. Thus, the drug was given as a single infusion to six healthy volunteers. Within 90 min, all displayed signs of cytokine release syndrome, and within 16 h all were critically ill. All patients suffered from multisystem organ failure including acute lung injury, renal failure, and disseminated intravascular coagulation. Fortunately, all six survived and recovered [350]. This example underscores the care that is necessary when designing and conducting clinical trials in order to maximize patient safety.

6.10 Conclusion

If decades of cancer research and, in particular, cancer immunotherapy research have taught us anything, it is that cancer is a resilient and adaptable foe. For now, checkpoint inhibition has added another weapon to our arsenal in the battle against cancer. As its current indications are expanding, it serves as proof of principle that immune checkpoint blockade can overcome cancer immune tolerance and escape in a clinically meaningful way. It has also reinvigorated research in cancer immunology and spurred the search for new immune coinhibitory and costimulatory checkpoints to target. While the initial work in new targets is encouraging, many large trials, at the cost of millions of dollars, are needed before its full potential is established. As we further elucidate the mechanisms by which cancer evades immune detection and destruction and learn to counter them, more effective and better-tolerated therapies are sure to emerge. Additionally, further characterization of the interactions between cancer and host immune system and how this changes with checkpoint blockade may help us understand and discover biomarkers for predicting which patients will respond, allowing treatment to be tailored and toxicity to be minimized.

Perhaps the greatest potential for improving outcomes and achieving broader applicability lies in using immune checkpoint blockade as combination therapy, by using blocking antibodies on coinhibitory receptors and agonist antibodies on costimulatory receptors. By combining checkpoint blockade therapy with conventional therapies such as chemotherapy and radiation, the destructive power of these therapies can be parlayed into a purposeful, long-lasting, cancer-specific immune response. Similarly, checkpoint blockade may help break down the barriers that have prevented most cancer vaccines from working and thus fulfill the long sought-after promise of active immunotherapy—a

stimulated, long-lasting, cancer-specific immune response that eliminates established tumors or prevents their recurrence.

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