Inhibitors of the PD-1 Pathway in Tumor Therapy

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Abstract

The programmed death 1 (PD-1) pathway delivers inhibitory signals that function as a brake for immune responses. This pathway limits the initiation and duration of immune responses, thereby protecting tissues from immune-mediated damage and autoimmune diseases. However, the PD-1 pathway also inhibits immune responses to tumors. The critical role of PD-1 in preventing antitumor immunity is demonstrated by the transformative effects of PD-1 pathway blockade in a broad range of cancers with the hallmark of durability of response. Despite this success, most patients do not respond to PD-1 monotherapy, and some patients experience adverse events. In this review, we discuss the functions of the PD-1 pathway and its translation to cancer immunotherapy. We also consider current challenges and opportunities for PD-1 cancer immunotherapy, including mechanisms of response and resistance, identification of biomarkers of response to PD-1 therapy, characterization and treatment of PD-1 therapy–related adverse events, and development of safe and effective combination therapies.

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The immune system can recognize and destroy tumors, but tumors evolve to escape immune attack. The tumor microenvironment (TME) is immunosuppressive and exploits inhibitory checkpoints, which normally promote T cell tolerance and control resolution of immune responses, to limit antitumor immunity. The remarkable success of immune checkpoint blockade, in which Abs are used to block checkpoints that inhibit T cell responses to tumors, illustrates the critical roles of these inhibitory checkpoints in obstructing antitumor immunity. The programmed death 1 (PD-1) pathway is a key target of checkpoint blockade.

The PD-1 inhibitory receptor regulates T cell activation, effector T cell responses, T cell tolerance, and T cell exhaustion (1, 2). PD-1 is rapidly induced on naive T cells following TCR engagement, countering T cell activation, and PD-1 expression decreases when Ag is cleared. When T cells are repetitively stimulated (as in cancer and chronic infection), PD-1 expression is sustained at high levels and T cells enter a dysfunctional state, termed exhaustion (2). Thus, PD-1 is expressed on both activated and exhausted T cells, and PD-1 expression alone does not signify T cell exhaustion. PD-1 is not only expressed on conventional CD4+ and CD8+ T cells, but also on regulatory T cells, B cells, NK cells, and NKT cells (3).

PD-1 has two ligands, programmed death-ligand (PD-L)1 (also called B7-H1; CD274) (4, 5) and PD-L2 (also called B7-DC; CD273) (6, 7). PD-L2 has higher affinity for PD-1, but more restricted expression than PD-L1. PD-L1 is widely expressed on many types of hematopoietic (T, B, macrophages, dendritic cells [DCs]) and nonhematopoietic cells (epithelial, stromal, and endothelial). PD-L2 is expressed mainly on hematopoietic cells (DCs, macrophages, B cells, and Th2 cells), but also on some epithelial cells, especially in the lung. Type 1 and type II IFNs, common γ-chain family cytokines (IL-2, IL-7, IL-15, and IL-21), IL-10, TNF, and VEGF can stimulate PD-L1 expression. IL-4 and GM-CSF are the most common stimuli for PD-L2 expression, but IFNs and common γ-chain family cytokines also can stimulate PD-L2. Upregulation of PD-1 ligands by proinflammatory stimuli may serve as a negative feedback mechanism to attenuate effector T cell responses, protecting tissues from immune-mediated injury or tumors from immune attack; this phenomenon has been termed “adaptive immune resistance” (8).

In addition to binding to PD-1, PD-L1 and PD-L2 each have a second unique binding partner. PD-L1 engagement of B7-1 (CD80) on T cells inhibits T cell responses (9). PD-L2 engagement of repulsive guidance molecule b (RGMb) (10) promotes respiratory tolerance. Further work is needed to understand the functional effects of these interactions and how they are affected by PD-1 pathway blockade.

**PD-1 signaling and molecular mechanisms**

Most of our knowledge of PD-1 signaling in T cells comes from studies of PD-1 engagement following activation of naive T cells. Upon PD-L1 or PD-L2 engagement, PD-1 becomes phosphorylated on its two tyrosine motifs: an ITIM and an immunoreceptor tyrosine-based switch motif in its cytoplasmic domain (11). This results in recruitment of protein tyrosine phosphatases (particularly SHP2), which dephosphorylate signaling molecules (including Lck and ZAP-70), and oppose positive signals downstream of the TCR.
and CD28 (11–13), leading to reduced T cell activation and effector functions. This leads to diminished signaling through the PI3K-AKT and Ras-MEK-ERK pathways (13), decreased expression of transcription factors important for effector T cell function (Tbet, Gata3, and Eomes), and diminished expression of the prosurvival factor Bcl-xL (14). Inhibition of the PI3K-AKT and Ras-MEK-ERK pathways also blocks cell cycle progression by reducing transcription of SKP2 (a ubiquitin ligase that regulates degradation of the cyclin-dependent kinase inhibitor p27kip1), resulting in p27kip1 accumulation and cell cycle inhibition at the G1 phase (15). PD-1 signaling also reduces production of cytotoxic molecules, thereby decreasing T cell killing capacity (16). PD-1 signaling has further suppressive roles; it can increase expression of the proapoptotic molecule Bim (17) and the transcription factor BATF, which inhibits T cell proliferation and cytokine production (18). Inhibition of T cell function depends on the level of PD-1 expression, with IL-2, TNF, and proliferation being most readily inhibited, followed by both cytotoxicity and IFN-γ and then by MIP-1β (16). In addition, PD-1 alters T cell motility and length of contact between T cells and DCs or target cells (19). PD-1 appears to prevent formation of stable contacts between T cells and DCs during T cell activation, thereby impairing development of effector functions.

PD-1 signaling also modulates T cell metabolism. Upon activation, naive T cells undergo metabolic reprogramming to enable proliferation and differentiation, and glycolysis becomes the dominant energy source. PD-1 signals lead to a metabolic shift in T cells, suppressing glycolysis and enhancing fatty acid oxidation (20). By impairing metabolic reprogramming, PD-1 may affect T cell differentiation and function. Given the central role of AKT in metabolism, PD-1 inhibition of AKT activation likely contributes to this altered metabolic state. The molecular mechanisms by which PD-1 regulates T cells other than during their initial activation are less clear. Further work is needed to understand the effects of PD-1 signals in memory T cells, tolerant T cells, exhausted T cells, and regulatory T cells, as well as in other immune cell (IC) types. T cell differentiation state, Ag, inflammation, metabolic state, and other factors may influence the consequences of PD-1 signaling.

The PD-1 ligands also may regulate immune responses by sending a signal into ligand-expressing cells (21). There is evidence for cell-intrinsic functions of PD-L1 in tumor cells, myeloid cells, and T cells. Culture of tumor cell lines in vitro with anti–PD-L1 directly affected tumor cell metabolism in the absence of PD-1–expressing T cells (21). Expression of glycolytic enzymes, AKT phosphorylation, and glucose uptake were reduced after anti–PD-L1 treatment in vitro (21). Consistent with this, the PD-L1 intracellular domain does not possess canonical conserved signaling motifs, but is highly conserved, suggesting functional significance.

### Inhibitory functions of PD-1 signaling

#### Regulating tolerance and autoimmunity

The PD-1 pathway regulates both central and peripheral tolerance. PD-1 signaling influences positive selection in the thymus, because lack of PD-1 or PD-L1 at this stage increases the number of double positive thymocytes by reducing the TCR signaling threshold during positive selection (22). How PD-1 pathway modulation affects the T cell repertoire remains to be determined.
The PD-1 pathway controls both the induction and the maintenance of peripheral T cell tolerance. PD-1 limits the initial activation, proliferation, and differentiation of self-reactive CD4+ and CD8+ T cells, and constrains potentially pathogenic self-reactive CD4+ and CD8+ effector T cells. PD-1 deficiency or blockade accelerates autoimmunity in several mouse models of autoimmunity including lupus-prone mice (23), experimental autoimmune encephalomyelitis (24), type 1 diabetes in NOD (25), and the rat insulin promoter–membrane-bound OVA model of diabetes (26). The PD-1 pathway restrains priming and differentiation of naive self-reactive T cells in secondary lymphoid organs. PD-L1 is expressed on tolerogenic DCs and helps to control the T cell fate decision between activation and tolerance (27). PD-1/PD-L1 interactions have crucial functions in target organs, controlling self-reactive T cells locally, maintaining tolerance in tissues, and protecting them from autoimmune-mediated damage. For example, in experimental autoimmune encephalomyelitis and diabetes models, PD-1 is highly expressed in the target organ on self-reactive T cells, and inflammation stimulates PD-L1 expression in the target organ, so PD-1/PD-L1 interactions have the potential to counteract stimulatory signals in the target tissues. PD-L1 expression on epithelial cells of organs such as the lungs, liver, and pancreas likely serves as a final barrier to immune destruction when tolerance is lost (1). The functional consequences of PD-L1 expression on other types of non-hematopoietic cells (e.g., endothelial and stromal cells) in tolerance is less clear. In NOD mice, PD-L1 on non-hematopoietic cells, including islet cells, plays an important role in inhibiting effector T cell responses and diabetes onset (25). Thus, the PD-1 pathway can regulate self-reactive T cells at multiple levels: thymic repertoire development, activation and differentiation in secondary lymphoid organs, and effector responses in target organs. Intriguingly, single nucleotide polymorphisms in the PDCD1 gene have been associated with human autoimmune diseases (28), but it is unclear whether any of these SNPs are causative or predictive of immune-related adverse events (IRAEs) in cancer patients treated with PD-1 pathway inhibitors.

Exploiting PD-1 inhibitory signals during chronic viral infection

The importance of the PD-1 pathway in regulating chronic viral infection was first described using the chronic lymphocytic choriomeningitis (LCMV) infection model in mice (2), and it rapidly extended to human chronic viral infections (HIV, hepatitis C virus, hepatitis B virus) (18, 29). During chronic viral infections, cytotoxic T cells progressively lose the ability to produce IL-2, TNF-α, and IFN-γ and enter a dysfunctional state termed exhaustion (30). Persistent Ag encounter and TCR signaling stimulate high and sustained PD-1 expression (2), whereas inflammatory stimuli upregulate and sustain PD-L1 expression. The PD-1 pathway plays a major role in regulating T cell exhaustion, because its blockade during chronic viral infection can enhance CD8+ T cell responses and reduce viral burden (2).

PD-1 is not absolutely required for induction of the T cell exhaustion program; PD-1 prevents early overstimulation of T cells and excessive T cell death during chronic LCMV infection. In contrast, PD-1 plays a crucial role in the maintenance stage of the exhaustion program; the absence of PD-1 signaling results in accumulation of terminally exhausted T cells (31). Thus, PD-1 preserves exhausted T cells from terminal exhaustion and instead maintains partially exhausted T cells in a dysfunctional state from which they can be reinvigorated.

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PD-1 blockade after the onset of LCMV-induced exhaustion enhances CD8+ T cell effector functions, due to response of a subset of cells to PD-1 blockade (32–34). High PD-1 expression on HIV-specific CD8+ T cells isolated from PBMCs of viremic individuals was associated with impaired cytokine production, proliferation, and survival. Further work is needed to understand relationships between populations of HIV-specific CD8+ T cells that express different PD-1 levels and their responses to PD-1 blockade during chronic human viral infections (35). During chronic LCMV infection, two distinct populations of Ag-specific dysfunctional T cells can be distinguished based on their PD-1 expression levels and responses to PD-1 blockade (36): T cells with intermediate levels of PD-1 (PD-1int) are dysfunctional but can be reinvigorated, whereas T cells with high PD-1 expression (PD-1hi) are terminally exhausted and cannot be reinvigorated (36). PD-1int are found primarily in secondary lymphoid organs, whereas the PD-1hi predominate in nonlymphoid tissues. PD-1int have a better ability to proliferate and produce effector cytokines compared with PD-1hi, and they can convert into PD-1hi, which have higher cytotoxic function. Exhausted CD8+ T cells from mice and humans have a distinct epigenetic landscape compared with naive, effector, and memory cells (37), and PD-1 pathway blockade does not durably reprogram exhausted T cells epigenetically (38). Combination therapy with epigenetic-modifying agents may provide a means to reverse epigenetic changes in exhausted T cells.

Inhibiting antitumor immunity in preclinical models and translation to cancer

The PD-1/PD-L1 axis is of vital importance for restraining the antitumor T cell response (8). In preclinical models, PD-1/PD-L1 blockade results in enhanced antitumor T cell cytotoxicity, proinflammatory cytokine production, and proliferation. In addition, PD-1 blockade increased concentrations of glucose in the TME and rates of glycolysis (21). Thus, PD-1 pathway blockade may also function by altering metabolism in the TME (39).

Tumor cells can express PD-L1 and/or PD-L2, which can be induced by multiple pathways, including upregulation by cytokines, chromosomal copy gain, disruptions of the PD-L1 3' untranslated region, mutations of the genes encoding PI3K AKT, EGFR, and CDK5, MYC overexpression, and viral proteins (EBV LMP1) (40). PD-1 may restrain T cells that have been activated by tumor Ag-bearing APCs in lymph nodes, T cells trafficking to the tumor, and effector T cells within the tumor. In the TME, PD-L1 can be expressed on tumor cells, as well as endothelial cells, stromal cells, APC/myeloid subsets, and T cells. All can contribute to the immunosuppressive environment. Further studies are needed to address their specific contributions during antitumor immunity and the response to PD-1 checkpoint blockade.

In animal models, the effects of PD-1 pathway blockade vary with tumor type; some tumors are resistant to PD-1 blockade or clearance in PD-1−/− mice, whereas others are very susceptible to loss of PD-1 signaling (41, 42). The cellular PD-1/PD-L1 interaction may also vary; tumor-derived PD-L1 was required for blocking CTL-mediated killing in the highly immunogenic MC38 colorectal carcinoma, yet host-derived PD-L1 was more important for the less immunogenic B16 and D4m melanoma lines (41). Differences in tumor
immunogenicity may impact the relative contributions of tumor versus host-derived PD-L1. Moreover, studies of methylcholanthrene-induced sarcomas showed that the level of tumor-derived PD-L1 needed for immune escape was inversely proportional to the antigenicity of the tumor cell (42). More work is needed to understand differential sensitivity of PD-1 blockade in different tumor models (MC38, B16), as well as different TMEs, which have been broadly classified into: 1) inflamed or “hot” tumors with robust immune infiltration; 2) immune-excluded in which inflammatory cells are present at the tumor margin but do not enter the tumor parenchyma; and 3) “cold” tumors, which are devoid of inflammatory cells, that is, an “immune desert.”

The effects of PD-1 blockade depend on the presence of T cells, BATF3+ cross-presenting DCs, and the Sec22b cross-presentation pathway (39, 43). Priming of the antitumor T cell response also depends on activation of the STING pathway in these DCs, which produce IFN-β to initiate this response (44, 45). Furthermore, the microbiome plays a role because Bifidobacterium is important for promoting initial T cell priming during PD-1 blockade by enhancing the priming potential of DCs via a yet unknown mechanism (46). Given its broad expression, understanding differential roles of PD-1 signaling in CD4+ and CD8+ effector cells and regulatory T cells will be important for optimally targeting this pathway for cancer therapy. Fc Ab design will be important as well, given that Fc receptors on macrophages can affect the potency of PD-1 blockade (47, 48).

Most tumors require combination therapies for optimal clinical efficacy, and PD-1 has become a foundational building block for combination therapies primarily at the priming and effector steps (8, 49, 50). In animal models, enhancing T cell priming using combinations with TLR ligands such as CpG, vaccines such as GVAX, or oncolytic viruses have shown additive or synergistic effects with PD-1 blockade. At the tumor site, dysfunctional T cells express multiple coinhibitory receptors such as TIM-3, LAG-3, TIGIT, and VISTA, and their coblockade with PD-1 is generally at least additive. Dysfunctional T cells can also express costimulatory receptors, and stimulation of these pathways (OX-40, CD137, ICOS, and GITR) using agonist Abs also provides an additive effect with PD-1 blockade. Lastly, current cancer therapies such as BRAF inhibitors, angiogenesis blockade, radiation, chemotherapies that induce immunogenic cell death (doxorubicin, oxaliplatin, and cyclophosphamide), and HDAC inhibitors also are additive with PD-1 blockade. Given the wide range of potential combinations, a mechanistic understanding of synergies is needed to rationally develop effective combination therapies.

**PD-1/PD-L1 blockade in the clinic**

A number of anti–PD-1 and anti–PD-L1 Abs have been approved by the U.S. Food and Drug Administration (FDA) for cancer treatment (Fig. 1, Table I) (51–76). The first approval was for the anti–PD-1 Ab pembrolizumab for melanoma in 2014, followed closely by a second anti–PD-1 Ab (nivolumab) (51–53). In 2015, a number of additional approvals followed, notably in non–small cell lung cancer (NSCLC) and in renal cell carcinoma (RCC) (56–58). The year 2015 also marked the first approval of combined immune checkpoint blockade; the combination of anti–PD-1 (nivolumab) and anti–CTLA-4 (ipilimumab) was approved for the treatment of melanoma (54). Subsequent approvals demonstrated the truly
broad-spectrum activity of these agents in cancer, with approvals in urothelial cancer (67–72), head and neck cancer (73, 74), and Hodgkin’s lymphoma, where the objective response rate approaches 55–75% (64–66). In a recent landmark trial, the anti–PD-1 agent pembrolizumab was granted cross-cancer approval for tumors with microsatellite instability (MSI) (76). In colorectal carcinoma, MSI is relatively common (~15% of cases) (77), but there are significant numbers of MSI+ cases among nearly all cancer types, including tumor types considered refractory to immunotherapy, such as prostate cancer. This approval marked the first time an agent has been approved across multiple tumor types, based on a predictive genetic biomarker.

**Mechanism of action of PD-1/PD-L1 blockade in humans**

As described earlier, studies in animal models show that PD-1 blockade augments a CD8+ T cell antitumor response. Similar data have been generated in patients; for example, the presence of proliferating (Ki67+) T cells at the invasive tumor margin correlated with response in melanoma patients treated with anti–PD-1 (78). Similarly, a recent study showed that CD8+ T cell expansion mediated by PD-1 blockade can also be detected in the peripheral blood, where the ratio of reinvigorated circulating (Ki67+) exhausted CD8+ T cells to tumor burden appears to correlate with response (79). In addition, this reinvigoration was indicative of a clonal response because several of the T cell clones that were Ki67+ were also found in the tumor. As is the case in animal models, one open question is whether PD-1 immunotherapy blocks PD-1 interaction with PD-L1 on tumor cells or PD-L1 on APCs in the tumor or in the tumor-draining lymph node. Two recent studies showed CD28/B7 interactions are required for PD-1/PD-L1 blockade to function, implying that the primary interaction involves APCs (80, 81). Even though elegant knockout and blocking studies like this are impossible in patients, the observation that PD-L1 staining on tumor-infiltrating ICs correlates with response to PD-L1 blockade (with atezolizumab) (82) provides some support for this hypothesis. However, in other studies, responses to PD-1/PD-L1 blockade seem to correlate more strongly with PD-L1 expression on tumor cells (83); one factor that may explain these opposing findings is the use of different metrics/reagents for scoring PD-L1 positivity in the tumor. Similar to animal models, the role of PD-L1 on tumor cells, hematopoietic cells, and nonhematopoietic cells may differ with differ types of tumors.

**Adverse events: mechanism, timing, and treatment**

As discussed earlier, the PD-1/PD-L1 axis protects normal tissues from immune attack during episodes of local inflammation by promoting T cell tolerance. These PD-1 immunoregulatory functions likely explain why PD-1/PD-L1 blockade is associated with IRAEs. IRAEs can affect most major organs and include fever, rash, diarrhea, colitis, pneumonitis, myocarditis, hepatitis, elevated AST/ALT, endocrinopathies (hypothyroidism/hypophysitis), and pancreatitis/diabetes. Across multiple trials with multiple agents in multiple cancer types, the rate of grade III/IV adverse events (ones that must be treated) is remarkably consistent in the 15–20% range (84). The organ most commonly affected by IRAEs is the skin, with toxicity ranging from mild rash to more severe skin involvement requiring treatment with either topical or systemic corticosteroids. The two other most common IRAEs are fatigue and diarrhea, whereas pneumonitis, colitis, pancreatitis,
myocarditis, hepatitis, and endocrinopathies are rarer. Whereas IRAEs are often mild, the involvement of certain organ systems is more worrisome and requires aggressive treatment. In the phase Ib trial of the anti–PD-1 Ab nivolumab, several cases of severe lung inflammation (pneumonitis) occurred, some of which were fatal (85). More recently, prompt intervention with immunosuppressive corticosteroids and TNF-α blockade has improved treatment safety (86). Another worrisome IRAE is inflammation of the cardiac muscle (myocarditis), which can also prove deadly (87). As described earlier, colitis may occur but is less common than that seen with CTLA-4 blockade. Interestingly, IRAEs involving various organ systems occur at different points in the treatment course, with skin-related events occurring early (generally 2–6 wk) and other IRAEs appearing later in the treatment course (within 12 wk) (84). There appear to be very few new events that occur after the first 6–9 mo of treatment (88–90). When tissue is available, pathological examination generally reveals a T cell infiltrate, consistent with the mechanism of action of PD-1/PD-L1 blocking agents. Immunologically, a deeper understanding of the immunological mechanisms involved in IRAEs is likely to provide additional insights regarding the pathogenesis of autoimmunity.

**Biomarkers to predict anti–PD-1/anti–PD-L1 activity**

Despite its remarkable success in some patients, objective tumor responses occur only in a minority of patients treated. This, coupled with the possibility of IRAEs, has led to the search for tissue or serum biomarkers that might predict which patients respond to anti–PD-1/PD-L1 therapy. Based on the notion that PD-1 pathway blockade inhibits interactions between tumor cell PD-L1 and PD-1 on T cells, much work has focused on investigating PD-L1 expression as a biomarker to predict response using immunohistochemical quantification of PD-L1 in pretreatment and/or archival tumor tissue samples. These studies indicate that PD-L1 expression on either tumor or tumor-infiltrating ICs is at best an imperfect tool by which to enrich patients for treatment (83, 85, 91). One key challenge has been that clinical agents have each been paired with a different companion diagnostic (92). A second related challenge is that the various assays chose disparate cut points for PD-L1 positivity. In some instances, such as RCC and first-line bladder cancer (63, 67), there appears to be no correlation between PD-L1 expression and the likelihood of clinical response. Even when PD-L1 expression is predictive, there are always PD-L1–“negative” patients who respond, although this perhaps reflects sampling error (93), it makes it challenging for clinicians to withhold a potentially beneficial drug from patients with few or no other treatment options. The use of multiple biomarkers is likely to be more effective for predicting responders than PD-L1 expression alone. Nevertheless, in certain clinical scenarios, that is, first-line treatment of patients with lung cancer where chemotherapy is effective, PD-1 blockade with pembrolizumab is FDA-approved only for patients whose tumors are positive for PD-L1 expression (61).

Based on the notion that nonsynonymous mutations in cancer may give rise to novel class I–restricted epitopes (neoantigens), another potential biomarker under study is tumor mutational burden. Indeed, in melanoma (94), NSCLC (95, 96), and urothelial bladder cancer (97), tumor mutational burden correlates with the likelihood of response, although an optimal cut point for patient selection has yet to be defined prospectively for any of these
cancer types. In addition, transcriptional signatures, including metabolic and immunological signatures, are actively being investigated (98, 99). A molecular signature of IFN signaling (100) is under development, which will quantify a set of 16 target gene expression levels, and is being prospectively analyzed in three ongoing randomized phase III trials. The use of PD-1 signaling and expression of downstream signaling molecules as potential biomarkers are also being investigated for identifying potential responders to PD-1 blockade (101).

**Mechanisms of resistance to PD-1/PD-L1 blockade**

Despite impressive clinical activity, most patients treated with PD-1/PD-L1 blocking agents either fail to respond or eventually develop resistance. Three interesting studies highlighted mechanisms of resistance to immune checkpoint blockade (102). Defects in class I Ag presentation (β2-microglobulin mutations) were associated with nonresponse; these data are consistent with the notion that PD-1/PD-L1 blockade requires tumor Ag presentation to specific CD8+ T cells to be successful (103). A second study indicated the potential importance of the Wnt/β-catenin pathway, which had previously been shown to be immunosuppressive (104). Tumors in which this pathway was active were relatively noninflamed, that is, cold, and hence far less likely to respond to PD-1/PD-L1 blockade (105). A third study, in which patients were treated with anti–CTLA-4, identified defects in IFN (IFNGR1, IFNGR2, and JAK2) signaling in resistance (106). Additional work is needed to identify additional resistance mechanisms, determine the extent to which individual mechanisms are involved, and stratify which mechanisms are applicable to specific tumor types. Clinically, combination treatment regimens also highlight potential mechanisms of resistance; for example, the activity of combined PD-1/CTLA-4 blockade in melanoma (54) and in RCC (107) strongly implicate CTLA-4 activity as a factor limiting the efficacy of PD-1 blockade in patients. Other clinically apparent mechanisms of resistance include LAG-3 expression (108), IDO expression (109–111), as well as many others (112).

**Combination therapies with PD-1**

Mirroring animal studies (113, 114), combined checkpoint blockade has already enjoyed clinical success, with PD-1/CTLA-4 blockade FDA-approved in melanoma and the results of pivotal phase III trials eagerly awaited in multiple other tumor types including RCC (NCT02231749), NSCLC (NCT01454102, NCT02453282), and others. Building further upon preclinical studies, clinical trials that coordinately block other inhibitory checkpoints, including LAG-3 (115), TIM-3 (50), TIGIT (50, 116), and VISTA (117), are under way. Additional clinical trials combine PD-1 blockade with agents that address other aspects of the immunosuppressive tumor microenvironment, that is, inhibitors of the IDO pathway (118), adenosine signaling (119), and agents that affect CSF-1R signaling (120). There are also multiple “vaccine” approaches under study; these seek to turn cold tumors hot, hence enabling PD-1/PD-L1 blockade efficacy. Perhaps most exciting among the various vaccine approaches are personalized cancer vaccines, which seek to target a patient’s individual mutated peptides (121). Two very recent publications highlight the feasibility and potential activity of this approach (122, 123), yet the complexity and time frame involved in generating patient-specific vaccines still presents a clinical challenge. It is further worth noting that conventional cancer treatments such as radiation therapy, chemotherapy, and
targeted agents can induce immunogenic cell death and might thus also serve in a way as cancer vaccines to prime an antitumor response (124).

**Future directions**

The impressive clinical success of PD-1/PD-L1 blockade is a great example of translation of basic immunology to patient care. To build on this success and develop effective combination therapies, a better mechanistic understanding of the efficacy of PD-1 pathway blockade is needed. More work is needed to understand mechanisms of response and resistance, and to develop biomarkers to predict response and IRAsEs. In addition, further investigations are needed to rationally develop effective combinations with PD-1 pathway inhibitors. For example, understanding the unique and overlapping functions and signaling pathways of PD-1 with other inhibitory receptors is needed to optimally combine them. In addition to increasing the number of patients who do respond, there is a need to develop predictive biomarkers and understand the immunological mechanisms underlying durability. Tying into this, increased understanding of the role that PD-1 blockade plays in the formation and maintenance of memory T cells will be important. Lastly, by utilizing the newest genome-editing approaches, it may be possible to target coinhibitory molecules more specifically by modulating context-specific enhancers as opposed to the genes themselves. In summary, a deep understanding of the antitumor immune response generated by basic science studies will continue to drive the field forward, with favorable results for patients.

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**Abbreviations**

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>DC</td>
<td>dendritic cell</td>
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<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
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<tr>
<td>IC</td>
<td>immune cell</td>
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<tr>
<td>IRAE</td>
<td>immune-related adverse event</td>
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<tr>
<td>LCMV</td>
<td>lymphocytic choriomeningitis</td>
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<td>MSI</td>
<td>microsatellite instability</td>
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<td>NSCLC</td>
<td>non–small cell lung cancer</td>
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<td>PD-1</td>
<td>programmed death 1</td>
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<td>T cell with high PD-1 expression</td>
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<td>PD-L</td>
<td>programmed death-ligand</td>
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RCC renal cell carcinoma

References


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FIGURE 1.
Timeline for basic and clinical development of PD-1/PD-L1 targeted cancer immunotherapy.
Upper timeline, Preclinical studies. Lower/magnified timeline, FDA approvals.
Table I

FDA-approved anti–PD-1/PD-L1 agents

<table>
<thead>
<tr>
<th>Target</th>
<th>Generic Name</th>
<th>Isotype</th>
<th>Tumor Types</th>
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<td>PD-1</td>
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<td>Humanized IgG4 (hinge region modified)</td>
<td>Melanoma, NSCLC, Hodgkin’s lymphoma, urothelial carcinoma, HNSCC, MSI-H, dMMR</td>
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<tr>
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<tr>
<td>PD-L1</td>
<td>Atezolizumab</td>
<td>Humanized IgG1 (FcR-binding deficient)</td>
<td>NSCLC, urothelial carcinoma</td>
</tr>
<tr>
<td></td>
<td>Durvalumab</td>
<td>Humanized IgG1 (FcR-binding deficient)</td>
<td>Urothelial carcinoma</td>
</tr>
<tr>
<td></td>
<td>Avelumab</td>
<td>Fully human IgG1 λ</td>
<td>Urothelial carcinoma, Merkel cell carcinoma</td>
</tr>
</tbody>
</table>

dMMR, mismatch repair deficient; HNSCC, head and neck squamous cell carcinoma; MSI-H, MSI-high.