

REVIEW

Cancer immunotherapy using checkpoint blockade

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The release of negative regulators of immune activation (immune checkpoints) that limit antitumor responses has resulted in unprecedented rates of long-lasting tumor responses in patients with a variety of cancers. This can be achieved by antibodies blocking the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or the programmed cell death 1 (PD-1) pathway, either alone or in combination. The main premise for inducing an immune response is the preexistence of antitumor T cells that were limited by specific immune checkpoints. Most patients who have tumor responses maintain long-lasting disease control, yet one-third of patients relapse. Mechanisms of acquired resistance are currently poorly understood, but evidence points to alterations that converge on the antigen presentation and interferon- γ signaling pathways. New-generation combinatorial therapies may overcome resistance mechanisms to immune checkpoint therapy.

In 2013, *Science* named cancer immunotherapy its Breakthrough of the Year on the basis of therapeutic gains being made in two fields: chimeric antigen receptor (CAR)-modified T cells and immune modulation using antibodies that block immune regulatory checkpoints. It is critical to note that the apparent rapid clinical progress reported in the past few years was the result of decades of investment in basic science in numerous fields. Without basic mechanistic knowledge in molecular biology, virology, immunology, cell biology, and structural biology, clinical advances in cancer immunotherapy never would have been realized. It is also important to consider the long history of efforts to use the potency of the immune system as a therapeutic modality for cancer. The field traces its earliest efforts to the observations of William Coley, a surgeon who correlated the occurrence of postoperative infection with improved clinical outcomes in cancer patients. After a series of fits and starts throughout the ensuing century, several immunotherapeutics were approved for use in cancer, including bacillus Calmette-Guerin, interferon- α , and interleukin-2 (IL-2). The latter is particularly important in that it demonstrated, for the first time, that advanced metastatic cancer, specifically melanoma and renal cell carcinoma, could be durably controlled in a small subset of patients by using a cytokine capable of expanding T cells. The activity of IL-2 substantiated the importance of adaptive immunity in controlling tumors and provided a solid founda-

tion for the incorporation of basic science knowledge of T cell regulation into the development of new immunotherapy strategies.

CTLA-4 as a nonredundant immune checkpoint and clinical activity

A pivotal moment occurred when a protein known as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) was demonstrated to have a potent inhibitory role in regulating T cell responses by two groups, one led by James Allison and the other by Jeffrey Bluestone (1-3). In resting T cells, CTLA-4 is an intracellular protein; however, after T cell receptor (TCR) engagement and a costimulatory signal through CD28, CTLA-4 translocates to the cell surface, where it outcompetes CD28 for binding to critical costimulatory molecules (CD80, CD86) and mediates inhibitory signaling into the T cell, resulting in arrest of both proliferation and activation (Fig. 1) (1). The generation of mouse models lacking CTLA-4 provided additional support of CTLA-4 as a nonredundant coinhibitory pathway, as those animals died of fulminant lymphocytic infiltration of almost all organs (1). While Bluestone went on to apply this critical knowledge to control autoimmune diseases, Allison theorized that if this molecular "brake" could be transiently blocked with an antibody, then this might allow for the T cell repertoire to proliferate and become activated to a higher point than normal physiology would allow (1). After initial preclinical proof-of-principle studies conclusively showed that checkpoint blockade with a CTLA-4-blocking antibody could lead to durable regression of established tumors in syngeneic animal models (1, 2), the strategy moved toward clinical evaluation.

Initially, two fully human CTLA-4-blocking antibodies (ipilimumab and tremelimumab) entered clinical trials in patients with advanced cancer in 2000 (Fig. 2). It quickly became apparent that durable tumor regressions could occur, although these were relatively infrequent and accompanied by a set of mechanism-related

toxicities resulting from tissue-specific inflammation (4, 5). The most common of these toxicities included enterocolitis, inflammatory hepatitis, and dermatitis. Algorithmic use of corticosteroids or other forms of immune suppression readily controlled these symptoms without any apparent loss of antitumor activity (6). However, less frequent adverse events also included inflammation of the thyroid, pituitary, and adrenal glands, with the need for lifelong hormone replacement. Clinical activity of CTLA-4 blockade was most apparent in patients with advanced metastatic melanoma, with a 15% rate of objective radiographic response that has been durable in some patients for >10 years since stopping therapy (7, 8). The patterns of clinical response shown by radiographic imaging after ipilimumab were sometimes distinct from those associated with therapies that have more direct antiproliferative mechanisms of action (9). Patients treated with ipilimumab on occasion showed delayed response after initial progression or new tumors appearing and then regressing while baseline tumors decreased in size. This led to challenges in securing regulatory approval on the basis of the commonly used surrogate metrics of objective response rate, or progression-free survival. Instead, it necessitated assessment of overall survival, a much longer-term outcome, as the primary endpoint registration trials. Eventually, two large phase 3 trials showed that ipilimumab was the first treatment to significantly extend survival in metastatic melanoma when compared with a peptide vaccine (10) or with standard dacarbazine chemotherapy (11). Approval from the U.S. Food and Drug Administration (FDA) was granted in 2011. Tremelimumab is still under investigation in clinical trials, and additional CTLA-4-blocking antibodies have recently entered clinical trials (NCT02694822).

Given the relatively low response rate and frequent toxicity associated with CTLA-4 blockade, identification of predictive and pharmacodynamic biomarkers emerged as research priorities. Analysis of tumors from patients with or without a response to anti-CTLA-4 therapy supports that a higher tumor mutational burden is associated with higher likelihood of response (12, 13). On-treatment increases in peripheral blood absolute lymphocyte counts and induction of the inducible costimulator ICOS both correlate with eventual treatment response (14). Despite numerous preclinical mouse studies showing that CTLA-4-blocking antibodies with appropriate Fc domains could mechanistically deplete regulatory T cells (T_{regs}) in regressing tumors, data associating this with clinical response in humans remain scarce. A recently initiated clinical trial (NCT03110307) is being used to investigate a version of ipilimumab with enhanced depleting capability by means of a nonfucosylated Fc domain to test this hypothesis further.

PD-1 as a nonredundant immune checkpoint

The programmed cell death 1 (PD-1) receptor has emerged as a dominant negative regulator of antitumor T cell effector function when engaged by

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its ligand programmed cell death ligand 1 (PD-L1), expressed on the surface of cells within a tumor. PD-1 bears its name from its initial description as a receptor inducing cell death of an activated T cell hybridoma (15). However, further work demonstrated that it is instead an immune checkpoint, with its inhibitory function mediated by the tyrosine phosphatase SHP-2, which dephosphorylates signaling molecules downstream of the TCR (16). PD-1 has two ligands, PD-L1 (also known as CD274 or B7-H1), which is broadly expressed by many somatic cells mainly upon exposure to proinflammatory cytokines (16), and PD-L2 (also known as CD273 or B7-DC), which has more restricted expression in antigen-presenting cells (16). Inflammation-induced PD-L1 expression in the tumor microenvironment results in PD-1-mediated T cell exhaustion, inhibiting the antitumor cytotoxic T cell response (16–18) (Figs. 1 and 3).

Antitumor T cells repeatedly recognize cognate tumor antigen as the cancer advances from

limited phenotype of PD-1-deficient mice compared to that of CTLA-4-deficient mice, as the former are mostly devoid of autoimmune diseases unless these are induced by other means (16). Consequently, PD-1-pathway blockade has a more specific effect on antitumor T cells, perhaps because of their chronically stimulated state, resulting in increased therapeutic activity and more limited toxicity compared to CTLA-4 blockade (22, 23).

Clinical effects of PD-1- and PD-L1-blockade therapies

The underlying biology and durable response rates in patients with multiple types of cancer indicate that therapeutic blockade of the PD-1 pathway is arguably one of the most important advances in the history of cancer treatment. There are currently five anti-PD-1 or anti-PD-L1 antibodies approved by the FDA in 11 cancer indications (Table 1 and Fig. 2). The first evidence of the antitumor activity of PD-1 blockade

encouraging clinical data from nivolumab, pembrolizumab's clinical development focused on patients with metastatic melanoma and NSCLC, resulting in the largest phase 1 trial ever conducted in oncology, eventually enrolling 1235 patients (26, 27).

The first FDA approvals of PD-1-blocking antibodies were through accelerated and breakthrough filing pathways, with pembrolizumab and nivolumab approved for the treatment of patients with refractory melanoma in 2014 and, in 2015, for patients with advanced NSCLC (Fig. 2). The first anti-PD-L1 antibody approved was atezolizumab for urothelial cancers in 2016, followed by avelumab for Merkel cell carcinoma in 2017 (Fig. 2). This class of agents was the first to be granted FDA approval on the basis of a genetic characteristic as opposed to the site of origin of the cancer, with the approval of pembrolizumab and nivolumab for the treatment of microsatellite-unstable cancers of any origin in 2017 (28). This rapid drug development and broad range of approvals are based on a series of characteristics of the clinical activity of PD-1 pathway-blocking antibodies and are outlined below.

Antitumor activity of PD-1-pathway blockade has been observed in a subset of patients within a broad range of cancers, particularly in carcinogen-induced cancers or cancers driven by viral infections (Table 1). The highest antitumor activities of single-agent PD-1-blockade therapy are in Hodgkin's lymphoma, in which there is constitutive expression of PD-L1 through a common amplification of the PD-L1-encoding locus together with PD-L2 and Janus kinase 2 (JAK2) (termed PDJ amplicon) (29); the virally induced

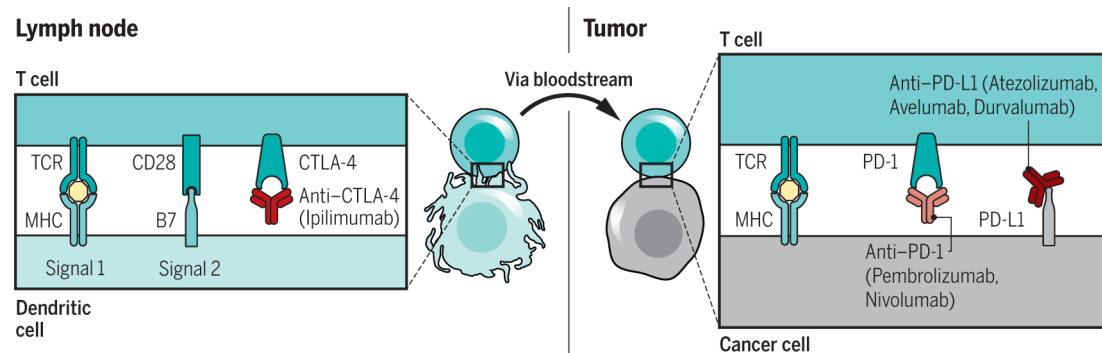


Fig. 1. Blockade of CTLA-4 and of PD-1 and PD-L1 to induce antitumor responses. (Left) CTLA-4 is a negative regulator of costimulation that is required for initial activation of an antitumor T cell in a lymph node upon recognition of its specific tumor antigen, which is presented by an antigen-presenting cell. The activation of CTLA-4 can be blocked with anti-CTLA-4 antibodies. (Right) Once the T cells are activated, they circulate throughout the body to find their cognate antigen presented by cancer cells. Upon recognition, the triggering of the TCR leads to the expression of the negative regulatory receptor PD-1, and the production of IFN- γ results in the reactive expression of PD-L1, turning off the antitumor T cell responses. This negative interaction can be blocked by anti-PD-1 or anti-PD-L1 antibodies.

primary to metastatic lesions over time. Triggering of the TCR results in the production of proinflammatory cytokines, including interferon- γ (IFN- γ), which is the strongest stimulator of reactive PD-L1 expression (16, 19). Chronic exposure of T cells to cognate antigen results in reactive PD-L1 expression by target cells, and continuous PD-1 signaling in T cells induces an epigenetic program of T cell exhaustion (20, 21). Several other interactions in the PD-1 pathway have a less clear functional meaning. PD-L1 has been shown to bind the costimulatory molecule CD80 (B71) expressed on T cells, delivering an inhibitory signal (16). Repulsive guidance molecule b (RGMb) binds to PD-L2, but not PD-L1, and seems to be relevant for pulmonary tolerance (16). PD-1 is therefore a negative regulator of pre-existing immune responses, which becomes relevant to cancer because its blockade results in preferential stimulation of antitumor T cells (Fig. 3). The restricted effect of PD-1 is highlighted by the

was with the fully human monoclonal antibody nivolumab (previously known as MDX-1106/BMS936558). Nivolumab was first administered to a patient in October 2006 in a phase 1 single-infusion dose-escalation trial and represents the first instance of PD-1 blockade in humans (Fig. 2). Among the 16 initial patients who received nivolumab every 2 weeks, six (37.5%) had objective tumor responses, including patients with melanoma, renal cell carcinoma, and non-small cell lung cancer (NSCLC) (24). The notable early evidence of antitumor activity in this phase 1 trial was accompanied by limited toxicity, although the rare development of pneumonitis was an indicator of occasional serious toxicities (24, 25). The presentation of the phase 1 data with nivolumab triggered rapid acceleration of clinical trial plans with this and other anti-PD-1 and anti-PD-L1 antibodies (Fig. 2). The anti-PD-1 antibody pembrolizumab entered clinical testing in April 2011. With the

Merkel cell carcinoma of the skin (30); microsatellite-instability cancers with high mutational load from mismatch-repair deficiency, leading to a high frequency of insertions and/or deletions (indels) (28); and desmoplastic melanoma, a rare subtype of melanoma that has a very high mutational load arising from chronic ultraviolet light-induced point mutations (31). In these cases, response rates are now 50 to 90%. A second group of cancers with relatively high response rates are carcinogen-induced cancers, such as the more common variants of melanoma arising from intermittently exposed skin, where upfront response rates are presently in the range of 35 to 40%, and a series of cancers associated with the carcinogenic effects of cigarette smoking, such as NSCLC and head and neck, gastroesophageal, and bladder and urothelial cancers, with response rates in the range of 15 to 25% (26, 32–34). The other two approvals of single-agent anti-PD-1 therapies are in hepatocellular

carcinoma, with its known relationship to hepatitis virus infection (35), and renal cell carcinoma (36), which has a low single-nucleotide mutational load but a higher frequency of indels than other common cancers, resulting in increased immunogenicity (37).

Once an objective tumor response has been achieved, most remain durable. As opposed to targeted oncogene therapies, in which most tumor responses last until the cancer develops a way to reactivate the pathway or alternate oncogene signaling to bypass the blocked oncogene, in cancer immunotherapies, the rate of relapse is lower. It was hoped that immunotherapy could induce long-lasting responses, because of the ability of T cells to maintain memory to their target, and a polyclonal response that the cancer should have trouble escaping. However, primary refractoriness and acquired resistance after a period of response are major problems with checkpoint blockade therapy [reviewed in (38)].

Single-agent PD-1-pathway blockade has a relatively favorable toxicity profile, with toxicities requiring medical intervention (grades 3 to 4) in the range of 10 to 15% in most series (22, 26, 27, 33, 39). Most patients treated with single-agent anti-PD-1 or anti-PD-L1 antibodies have no toxicities above what would be expected from placebo, and treatment-related deaths are very uncommon. Very few patients (~5%) discontinue therapy because of toxicities. The most common treatment-related adverse events of any grade are fatigue, diarrhea, rash, and pruritus in

15 to 20% of patients (22, 26, 27, 33, 39). In a smaller percentage of patients, toxicities are more serious and include several endocrinopathies, in which the immune system infiltrates a hormone-producing gland, leading to permanent dysfunction that requires lifelong substitutive hormonal therapy, such as thyroid disorders (10 to 15%), hypophysitis, adrenal gland disorders (1 to 3%), and type 1 diabetes (1%). Serious visceral organ inflammatory toxicities are uncommon (~1%) but can affect any organ, including the brain (encephalopathy), meninges (meningitis), lung (pneumonitis), heart (myocarditis), gastrointestinal tract (esophagitis, colitis), liver (hepatitis), and kidney (nephritis), in addition to muscles (myositis) and joints (arthritis). These can be life-threatening. The cornerstone of treatment for clinically relevant toxicities with both PD-1- and CTLA-4-blockade therapies is immune suppressive therapy, with high doses of corticosteroids, and sometimes tumor necrosis factor antagonists (which are counter-indicated in patients with hepatitis) and mycophenolate mofetil (6).

Mechanisms of response and resistance to single-agent PD-1 therapy

Most of the data support a model in which patients respond to single-agent anti-PD-1 or anti-PD-L1 therapy because of a preexisting antitumor T cell response. Such a response retains therapeutic potential until the infiltrating T cells engage their TCR through recognition of a tumor antigen, triggering expression of PD-1 on T cells and release of IFN- γ , resulting in reactive expression of

PD-L1 by cancer-resident cells (16-18, 31, 40, 41) (Fig. 3). This process, termed adaptive immune resistance, occurs when tumor cells disarm specific T cells through PD-L1 expression (17, 18). It results in a specific state of immune privilege that does not require a systemic immune deficiency and is reversible simply by blocking the PD-1-PD-L1 interaction (41) (Fig. 3).

The first step in this mechanism is the differential recognition of cancer cells from normal cells by the immune system, in a situation in which the cancer cells had autovaccinated the patient to induce a specific T cell response. The most common mechanism for this differential recognition is related to the increased mutational load in cancers (41, 42). However, not all mutations seem to have the necessary qualities to give rise to robust targets of an antitumor immune response. Mutations that appear in the founder cancer cell and are carried on by most of the progeny cells (clonal mutations) are favorable, whereas mutations that appear later in the course of the cancer and may vary among different cancer cells (subclonal mutations) are not sensitive to PD-1 blockade (43). The processing and presentation by major histocompatibility complex (MHC) molecules of neopeptides that result from mutations further shapes the landscape of neoantigens recognized by antitumor T cells (44, 45).

The most common reason why a cancer would not have preexisting T cell infiltration is likely a state of low immunogenicity resulting from a lack of mutations that become recognized neoantigens

Table 1. Major indications approved for the use of anti-PD-1 and anti-PD-L1 therapies and the suspected mechanism of action of the antitumor response.

Group	Indication	Objective response rate (%)	Agents approved*	Main driver of response
High response rate	Hodgkin's disease	87	nivolumab pembrolizumab	PDJ amplicon
	Desmoplastic melanoma	70	nivolumab pembrolizumab	Mutations from chronic sun exposure
	Merkel cell	56	avelumab pembrolizumab	Merkel cell virus
	MSI-h cancers	53	nivolumab pembrolizumab	Mutations from mismatch-repair deficiency
Intermediate response rate	Skin melanoma	35 to 40	nivolumab pembrolizumab	Mutations from intermittent sun exposure
	NSCLC	20	atezolizumab	Mutations from cigarette smoking
			nivolumab pembrolizumab	
	Head and neck	15	nivolumab pembrolizumab	Mutations from cigarette smoking
	Gastroesophageal	15	pembrolizumab	Mutations from cigarette smoking
	Bladder and urinary tract	15	atezolizumab	Mutations from cigarette smoking
			avelumab	
			durvalumab nivolumab pembrolizumab	
Renal cell carcinoma	25	nivolumab pembrolizumab	Insertions and deletions (indels)	
Hepatocellular carcinoma	20	nivolumab	Hepatitis virus	

*In alphabetical order

(42), or an active means of T cell exclusion (38). Certain cancer phenotypes resulting from expression of specific transcriptomic programs may contribute to the lack of T cell recognition, such as expression of genes of the Wnt pathway (46) or a series of partially overlapping gene sets that are related to stemness, mesenchymal transition, and wound healing, collectively termed IPRES (for innate anti-PD-1 resistance) because they are enriched in biopsies of patients with melanoma that does not respond to anti-PD-1 therapy (47). It is also possible that antitumor T cells are impaired by earlier checkpoints, such as CTLA-4, or immune suppressive cells in the tumor microenvironment, such as myeloid lineage cells or T_{regs} (38).

The expression of PD-L1 by cells within a cancer was explored as a biomarker to identify patients who may be more likely to respond to PD-1-blockade therapies (25, 26, 48). PD-L1 is most frequently expressed reactively upon T cell infiltration and sensing of IFN- γ production, in which case it could be considered a “canary in a coal mine,” where its presence is a surrogate for a preexisting T cell response (Fig. 3). In this setting, colocalization of PD-L1, PD-1, and CD8⁺ T cells in an area of the tumor termed the invasive margin is associated with response to PD-1 blockade (31, 49). PD-L1 can also be expressed constitutively through a series of processes, and it is currently unclear if the mere presence of PD-L1 without detecting a T cell infiltrate is a favorable or detrimental event for PD-1-blockade therapy. Therefore, tumors that may be strongly

positive for PD-L1, but do not contain a preexisting cytotoxic CD8⁺ T cell response, would be unlikely to respond to therapy. The notable exception is Hodgkin’s lymphoma, in which the Reed-Stenberg cells have the PD-1 amplicon, resulting in constitutive PD-L1 expression (29). Of note, this is a cancer that is notorious for both a reactive T cell infiltrate mostly composed of CD4 T helper cells and Reed-Stenberg cells that are frequently deficient in β_2 -microglobulin (β_2 M), the required subunit for surface expression of MHC class I (50). These facts are at odds with the notion that PD-1-blockade therapy mainly reactivates preexisting intratumoral MHC class I-restricted CD8⁺ T cells.

Once a tumor is immunogenic enough to trigger a specific T cell response, the cancer cells may undergo a series of genetic and nongenetic processes to avoid being eliminated by the immune system, termed cancer immunoediting (51). Cancer immunoediting may result in the loss of mutations that are most immunogenic or the mutation or decreased expression of genes involved in the antigen-presentation pathway. Any of these events would be expected to result in primary resistance to PD-1 blockade or lead to acquired resistance, if they developed during therapy. Strong immune selective pressure can lead to a shaping of the mutational landscape of cancer (44, 45, 52), specific deletion of human leukocyte antigen (HLA) class I alleles that putatively present strong neoantigens (45), or loss of β_2 M (53–55). Genetic immunoediting events that can be found at baseline, and, in particular,

homozygous loss-of-function mutations in the gene encoding β_2 M, have been reported to be associated with both primary and acquired resistance to PD-1 blockade (53–55).

The process that leads to the reactive expression of PD-L1 upon T cell attack of cancer is mediated by IFN- γ pathway signaling (16, 19, 21) (Fig. 3). If the cancer cell is unable to sense IFN- γ and signal through the pathway, then PD-L1 will not be reactively expressed. In this setting, it could be futile to give antibodies blocking the PD-1–PD-L1 interaction (19, 21, 56). Within the IFN- γ receptor pathway, the bottleneck for signaling seems to be JAK1 and JAK2, as absence of either one results in complete lack of signaling (19, 21). Homozygous loss-of-function mutations in the *JAK1/2* genes are rare baseline events but are more frequent than would be expected randomly, suggesting an active immunoediting process to delete them (21, 57). In the setting of fully inactivating *JAK1/2* mutations, patients do not respond to anti-PD-1 therapy (21, 57). Mutating *JAK1/2* provides an advantage to the cancer cells, as it limits favorable effects of IFN- γ , such as increased expression of antigen-presenting machinery molecules, production of chemokines that potentially attract other T cells to that area and amplify the immune response, or avoiding the direct antiproliferative effects of interferon (56). In some cases of acquired resistance to anti-PD-1 therapy, homozygous loss of *JAK1* or *JAK2* has been documented (54, 57). These are rare genetic events that could explain a minority of cases

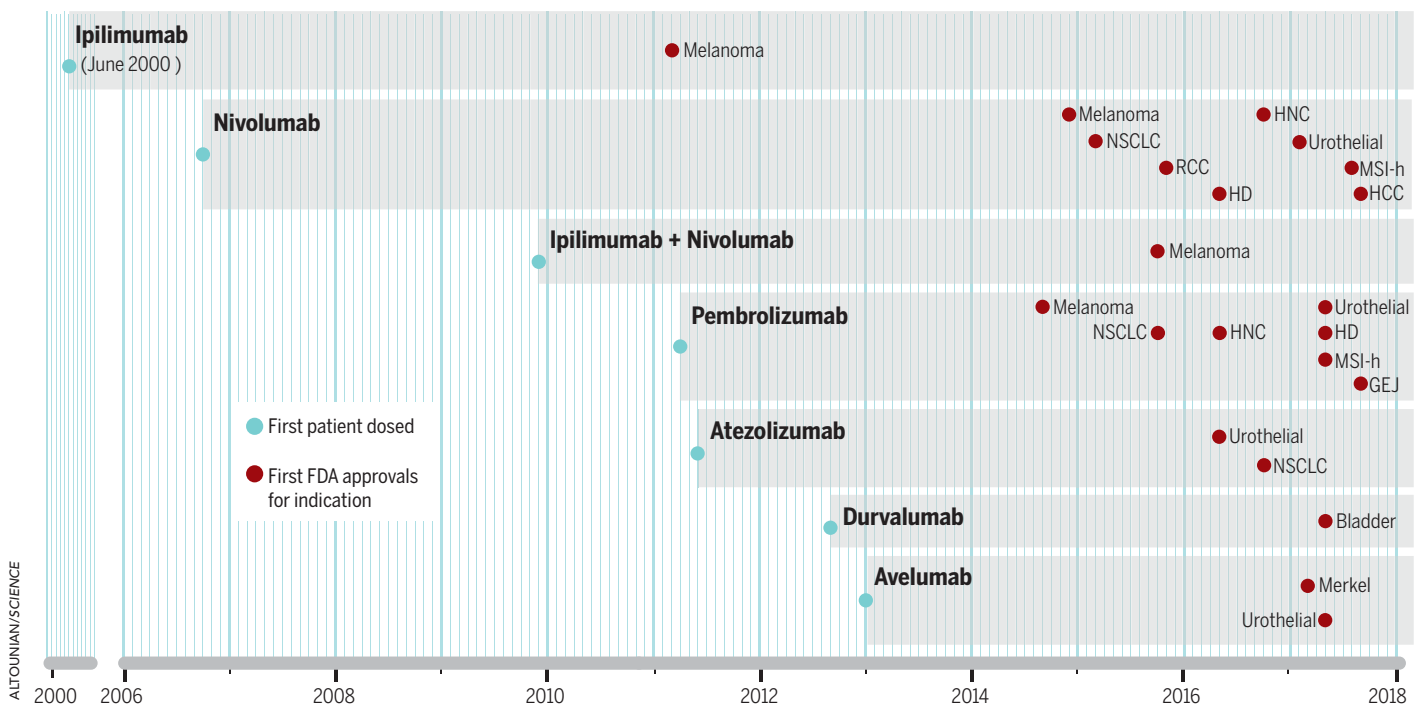
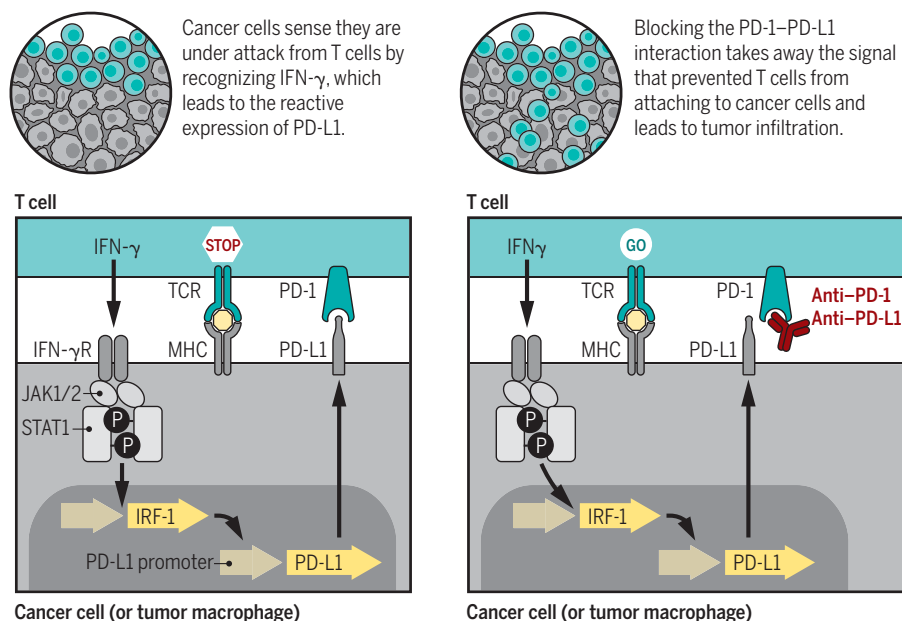


Fig. 2. Timing of clinical development of anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies, from first administration to humans to FDA approval.

Thus far, there has been drug regulatory approval for six antibodies that block immune checkpoints and a combination of two immune checkpoint-blocking antibodies. The gray shading represents the period of clinical development for each of these antibodies, from the dosing of the first patient until regulatory approval (red circles) in different indications. HNC, head and neck cancer; RCC, renal cell carcinoma; MSI-h, high microsatellite instability; HD, Hodgkin’s disease; HCC, hepatocellular carcinoma; GEJ, gastroesophageal junction.

Fig. 3. Mechanism of action of PD-1–blockade therapy.

(Left) TCR recognition of the cognate antigen presented by MHC molecules on the surface of cancer cells results in T cell activation. T cells then produce IFN- γ and other cytokines. Cancer cells and other cells in the tumor microenvironment have IFN- γ receptors (IFN- γ R) that signal through JAK1/2, which phosphorylate (P) and activate signal transducers and activators of transcription (STAT) proteins that dimerize and turn on a series of interferon-response genes, including interferon regulatory factor 1 (IRF-1), which binds to the promoter of PD-L1, leading to its surface expression. The reactive expression of PD-L1 turns off the T cells that are trying to attack the tumor, and these T cells remain in the margin of the cancer. **(Right)** Blockade of the PD-1–PD-L1 interaction with therapeutic antibodies results in T cell proliferation and infiltration into the tumor, inducing a cytotoxic T cell response that leads to an objective tumor response.



with primary or acquired resistance to PD-1 blockade, and they highlight the ability to mechanistically understand these processes. This body of data suggests that molecular mechanisms of resistance to anti-PD-1 therapy converge in alterations in the antigen-presentation machinery and the IFN- γ receptor pathway, an observation recently confirmed in unbiased CRISPR-Cas9 screens in preclinical models (58, 59).

The current understanding of response and resistance to PD-1–blockade therapy suggests that there cannot be a single biomarker to select patients. Therefore, selection of patients who are highly likely to respond to single-agent anti-PD-1 therapy (as opposed to being exposed to the greater toxicity and expense of combined therapy) would require a combination of studies in baseline tumor biopsies with sufficient tissue to include: (i) DNA analyses for tumor mutational load and absence of deleterious mutations in key immune signaling pathways, (ii) RNA analyses to detect the presence or absence of IFN- γ signaling and a favorable tumor phenotype, and (iii) morphological analyses documenting the colocalization of CD8⁺ T cells expressing PD-1 and interacting with reactively expressed PD-L1 in the tumor microenvironment. However, such extensive testing is currently not done routinely and in a timely enough manner to inform therapeutic decisions in patients with advanced cancer.

Combination CTLA-4 and PD-1–blockade therapy

In December 2009, the first patient was treated with combination checkpoint blockade by using ipilimumab to block CTLA-4 and concurrent nivolumab to block PD-1 (Fig. 2). This was designed on the basis of the nonredundant coinhibitory roles of the two pathways, after preclinical studies showed evidence of synergy in syngeneic mouse models (60). Further, the distinct immune microenvironments in which CTLA-4 and PD-1–pathway blockade could act provided an additional mech-

anistic rationale (Fig. 1). CTLA-4 is mainly associated with affecting inhibitory cross-talk in the draining lymph node. Although PD-1 blockade may also have activity in that immunologic space, the presence of PD-L1 on tumor and immune cells in the immediate tumor microenvironment provides an additional anatomic venue for activity (Fig. 1). Most recently, by using mass cytometry (or CyTOF), the Allison lab has shown that CTLA-4 and PD-1 blockade results in distinct phenotypic signatures in T cell subsets (61). The initial phase 1 dose-ranging trial of ipilimumab plus nivolumab was conducted in patients with metastatic melanoma and demonstrated a >50% objective response rate in the dose level that was chosen to move to phase 2 and 3 trials (60). Importantly, this was associated with a higher frequency of high-grade immune-related toxicities (up to 60%) in comparison to data from monotherapy trials. Phase 2 and 3 studies of the combination of ipilimumab plus nivolumab confirmed a response rate of approximately 60%, and the most recent analysis showed that patients initially randomized to the combination had a slightly higher 3-year survival than patients initially receiving nivolumab alone (58 versus 52%), yet with higher frequency of toxicity (23). Initial attempts to identify which patients require the combination have focused on tumor expression of PD-L1 and do suggest that patients with tumors that have little or no PD-L1 expression (<1% of tumor cells with surface staining) have improved survival with combination therapy compared to that with nivolumab alone. Ongoing trials are examining an adaptive dosing regimen with early assessment for response in an attempt to minimize the dosage of the combination and reduce toxicity (NCT03122522).

Other combination therapies and conclusions

Immune checkpoint–blocking antibodies are actively being investigated in combination with

an ever-widening spectrum of agents. Although the goal of such investigations—to increase the number of patients who may benefit from this type of therapy—is laudable, the sometimes empiric manner of how agents are brought together is leading to an unrealistic number of trials and expected volunteers, making it unlikely that all hypotheses will be robustly answered. Yet, there are some combination strategies that are in late-stage development and are mechanism based. The description of cellular and molecular mechanisms of primary and acquired resistance to checkpoint blockade therapy allows for designing combination immunotherapy approaches to overcome these resistance mechanisms. In the setting of low preexisting levels of T cells in the tumor, besides the combination of anti-CTLA-4 and anti-PD-1 therapies, other potential approaches include changing the tumor microenvironment by direct injection of interferon-inducing molecules such as toll-like receptor agonists or oncolytic viruses, blocking T cell–excluding proteins like indoleamine 2,3-dioxygenase or arginase, or inhibiting immune suppressive cells like T_{regs} or macrophages [reviewed in (60)]. Furthermore, other modes of cancer therapy, such as radiotherapy, chemotherapy, or oncoenzyme-targeted therapies, have been shown to change the immune suppressive tumor microenvironment and potentially synergize with immune checkpoint blockade therapy [reviewed in (60)]. Building on recent success in this field is important, but continuing to incorporate the emerging knowledge from mechanistic basic-science studies is critical to achieve greater therapeutic success.

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