

Immune-checkpoint inhibitors for combating T-cell dysfunction in cancer

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Abstract: Under normal conditions, the immune system responds effectively to both external and internal threats without damaging healthy tissues. Cells undergoing a neoplastic transformation are one such threat. An efficient activation of T cells is enabled by T-cell receptor (TCR) interactions with antigen-presenting class I and class II molecules of the major histocompatibility complex (MHC), co-stimulatory molecules, and cytokines. After threatening stimuli are removed from the body, the host's immune response ceases, which prevents tissue damage or chronic inflammation. The recognition of foreign antigens is highly selective, which requires multistep regulation to avoid reactions against the antigens of healthy cells. This multistep regulation includes central and peripheral tolerance toward the body's own antigens. Here, we discuss T-cell dysfunction, which leads to poor effector function against foreign antigens, including cancer. We describe selected cellular receptors implicated in T-cell dysfunction and discuss how immune-checkpoint inhibitors can help overcome T-cell dysfunction in cancer treatment.

Keywords: B- and T-cell lymphocyte attenuator, cytotoxic T-cell antigen 4, lymphocyte-activation gene 3, programmed cell death protein 1, T-cell exhaustion, T-cell immunoglobulin and mucin domain 3, checkpoint inhibitors

Introduction

Complex immune mechanisms enable the differentiation between self and non-self so that the immune response can be effectively directed at foreign antigens, such as cancer cells, and does not damage the body's own healthy tissues. The immune system recognizes certain antigens as own due to both central and peripheral tolerance. Similarly, full activation of the immune system against foreign antigens is precisely regulated and requires several signals.¹ An efficient activation of T cells is enabled by T-cell receptor (TCR) interactions with the antigen-presenting class I and class II molecules of the major histocompatibility complex (MHC) (signal 1), co-stimulatory molecules (signal 2), and cytokines (signal 3).² This multistep regulation enables termination of the immune response when threatening stimuli are removed from the body.

Here, we discuss the role and mechanisms of T-cell dysfunction in cancer, which leads to immune evasion by cancer cells and, thus, to cancer progression.^{3,4} We describe cellular receptors implicated in T-cell dysfunction and discuss how immune-checkpoint inhibitors can help overcome T-cell dysfunction in cancer treatment.

Immune evasion in cancer due to T-cell dysfunction

The complex cross talk between cancer cells, immune cells, and tumor microenvironment involves many mechanisms that lead to an inefficient immune response toward cancer cells. In cancer, T-cell dysfunction may be due to T-cell exhaustion, T-cell anergy,

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decreased phosphorylation of the CD3 ζ chain, and inhibitory signaling within the tumor microenvironment.

Continuous TCR stimulation in effector T cells gradually leads to exhaustion of these cells, which occurs mostly in chronic viral infections and cancer.⁵ Due to T-cell exhaustion, cytotoxic lymphocytes lose their effector function, which leads to an impaired immune response. T-cell exhaustion develops more likely when antigen levels are high or antigen exposure is prolonged.⁶ Lymphocyte exhaustion manifests initially with decreased IL-2 secretion; however, subsequently, other cytokines, including tumor necrosis factor α (TNF α), are secreted in lower amounts.^{7,8} Moreover, T-cell exhaustion impairs antigen-stimulated lymphocyte proliferation, halts lymphocyte renewal (mediated by IL-7 and IL-15), causes abnormal expression and function of transcription factors, decreases cytokine production, and impairs the response of memory T cells.^{9,10} In particular, an accumulation of exhausted cells is observed in the tumor microenvironment, which resembles the microenvironment of chronic inflammation.¹¹ Functionally exhausted T cells have an increased expression of inhibitory molecules.¹² A high expression of inhibitory receptors impairs the effector and proliferative functions of immune cells, and it creates a state of immunosuppression. Thus, the immune response toward cancer cells is insufficient and causes therapeutic failure.¹³

T-cell anergy – that is, tolerance of T cells toward specific antigens – may develop due to TCR stimulation without sufficient co-stimulatory signals or in the presence of inhibitory stimulation. This mechanism of T-cell activation is associated with reduced IL-2 production and a state of hyporesponsiveness of T cells. T-cell anergy may develop in patients with cancer because co-inhibitory signals prevail over co-stimulatory signals in the tumor microenvironment. For example, there is a greater expression of the inhibitory B7 family proteins over B7 stimulatory protein in the tumor microenvironment.¹⁴

The CD3 ζ chain is an intracellular element of the TCR complex. Phosphorylation of the CD3 ζ chain is crucial for antigen-specific T-cell activation, and downregulation of the CD3 ζ chain is associated with a reduced response of T cells. Notably, CD3 ζ downregulation is observed in many cancers, particularly in tumor-infiltrating cells.¹⁵ As the CD3 ζ chain is crucial for T-cell activation, CD3 ζ chain downregulation may be associated with T-cell exhaustion and T-cell apoptosis.¹⁶ Moreover, there is evidence that T-cell activation without CD3 ζ phosphorylation causes T-cell anergy.¹⁷

Finally, inhibitory signaling – due to overexpression of inhibitory molecules in the tumor microenvironment –

is important in the development of T-cell dysfunction in cancer. Below, we describe the most important inhibitory molecules implicated in immune evasion by cancer cells (Table 1).

Inhibitory molecules related to T-cell dysfunction in cancer PD-1

PD-1 mRNA was first detected in the mouse thymus; after treatment with an anti-CD3 antibody, the thymocytes entering the path of the cell's programmed death showed an increase in PD-1 expression.¹⁸ Despite its name, PD-1 does not cause cell death, but it blocks the cell cycle.^{19,20} PD-1 is a transmembrane glycoprotein from the CD28:B7 family. It is mostly expressed on activated T and B cells, but is also expressed on activated monocytes, dendritic cells (DCs), and NK (natural killer) and NKT (natural killer T) cells.⁷ Unlike other molecules from the CD28 superfamily, which are expressed only by T cells, PD-1 is expressed by many cell types. This suggests that PD-1 has a central place in the regulation of immune responses.²¹ PD-1 is a receptor with a length of 288 amino acids, and it is encoded by the *PDCD-1* gene on chromosome 2. PD-1 has an intracellular transmembrane domain and an extracellular immunoglobulin domain, which contains 21%–33% sequences that are identical to the sequences of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), CD28, and the inducible T-cell co-stimulator (ICOS).²² The receptor functions of PD-1 are mediated by its cytoplasmic part, which contains two tyrosine motifs that bind phosphatases responsible for transmitting immunosuppressive signals. The two motifs include the immunoreceptor tyrosine-based inhibitory motif (ITIM), located proximally to the cell membrane, and the immunoreceptor tyrosine-based switch motif (ITSM), which is essential to the inhibitory function of PD-1 (Figure 1).²³ PD-1 expression is induced by the signaling pathways of the TCR and the B-cell receptor (BCR), and it is maintained during antigen stimulation. Moreover, some cytokines (IL-2, IL-7, and IL-15), Toll-like receptors (TLRs; TLR-9), and interferons (IFNs) stimulate the expression of PD-1 in T cells.^{24,25} Moreover, the nuclear factor of activated T cells c1 (NFATc1) is important for PD-1 expression.²⁶

PD-L1 and PD-L2

Two PD-1 ligands that induce its inhibitory properties have been identified: PD-L1 (CD274 or B7-H1) and PD-L2 (CD273 or B7-DC). Both these ligands are type I transmembrane glycoproteins.²⁷ The constitutive expression of PD-L1

Table I Immune-checkpoint molecules and their inhibitors

Checkpoint molecules	Cellular expression	Main ligands	Mechanism of action	Inhibitors
CTLA-4	CD4 ⁺ T cells CD8 ⁺ T cells, Tregs	CD80, CD86	Competes with CD28 in binding to CD80 and CD86 Removes CD80 and CD86 from APC surface Treg-mediated immunosuppression Induces T-cell anergy	Ipilimumab – approved for advanced melanoma as monotherapy or combined with nivolumab Tremelimumab – investigated in melanoma, mesothelioma, and NSCLC but not approved due to a lack of significant effect
PD-1	Activated T cells, B cells, APCs, NK cells	PD-L1, PD-L2	Inhibits T-cell proliferation, survival, and effector function through CD3 ζ chain dephosphorylation Decreases expression of survival molecules	Nivolumab – approved for advanced melanoma as monotherapy or in combination with ipilimumab; approved as monotherapy in lung cancer, renal cancer, Hodgkin's lymphoma, urothelial cancer, head and neck cancer, colorectal cancer, and hepatocellular carcinoma Pembrolizumab – approved as monotherapy for advanced melanoma, lung cancer (first-line treatment in tumors with a high PD-L1 expression, after chemotherapy in tumors with a low PD-L1 expression), Hodgkin's lymphoma, large B-cell lymphoma, urothelial head and neck cancer, gastric cancer, cervical cancer, breast cancer
PD-L1	T cells, B cells, DCs, MSCs, cancer cells	PD-1	Same as in PD-1	Atezolizumab – approved as monotherapy for advanced urothelial carcinoma, lung cancer, colorectal cancer, breast cancer, renal cancer Avelumab – approved as monotherapy for metastatic Merkel cell carcinoma Darvolumab – approved as monotherapy for urothelial cancer
LAG-3	Activated T cells, NK cells	MHC class II	Inhibits CD4 ⁺ cell function Directly inhibits cytotoxic function of CD8 ⁺ cells	LAG-3-Ig fusion protein and anti-LAG-3 mAbs – investigated in renal cancer, breast cancer, and pancreatic cancer as monotherapy or combined with other therapies
TIM-3	CD4 ⁺ cells, Th17 cells, CD8 ⁺ cells NK cells	Gal-9	Decreases interferon- γ production Induces T-cell apoptosis Promotes generation of MDSCs	Different anti-TIM-3 mAbs – investigated in many solid tumors and leukemia

Abbreviations: APCs, antigen-presenting cells; DCs, dendritic cells; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; LAG-3, lymphocyte-activation gene 3; mAbs, monoclonal antibodies; MHC, major histocompatibility complex; MDSCs, myeloid-derived stem cells; MSCs, mesenchymal stem cells; NK, natural killer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TIM-3, T-cell immunoglobulin and mucin domain 3; Tregs, T-regulatory cells.

is substantially higher in mice than in humans, particularly in T and B cells, DCs, macrophages, and mesenchymal stem cells (MSCs); moreover, PD-L1 expression increases during activation of these cells.^{28,29} Besides hematopoietic cells, PD-L1 is expressed by other cell types, such as pancreatic cells, epithelial cells, endothelial cells, muscle cells, hepatocytes, astrocytes, spleen cells, kidney cells, and lung cells.^{28–31} PD-L2 is expressed only in the core layer of the thymus and, in lesser amounts, in the fetal myocardium and endothelial cells – particularly within the placenta.^{32,33} PD-L2 expression can be induced on DCs, peritoneal B1 lymphocytes, macrophages, medullary mast cells, and memory B cells.³⁴ Importantly, PD-L1 and PD-L2 are expressed by cancer cells, cancer-associated fibroblasts, and myeloid-derived stem cells. The expression of PD-L2 increases only slightly on stimulated CD8⁺ T cells, but it does not increase at all on

CD4⁺ lymphocytes.³⁵ Binding of PD-1 to PD-L1 or PD-L2 during TCR activation suppresses the proliferation of both B and T cells, decreases cytokine secretion, inhibits cytolysis, and prolongs T-cell survival.³⁶ PD-L1- or PD-L2-mediated prolongation of T-cell survival and impairment of their function may occur both indirectly, through interference with the early activating signals induced by CD28, and directly, through interference with IL-2 secretion.³⁷ Furthermore, PD-L1 is essential for Treg induction by DCs.³⁸

CTLA-4

CTLA-4 is a transmembrane receptor protein that inhibits T-cell function, mostly by competing with the co-stimulatory molecule CD28 for CD80 and CD86 located on antigen-presenting cells (APCs). CTLA-4 is expressed on conventional CD4⁺ and CD8⁺ T cells after TCR stimulation,

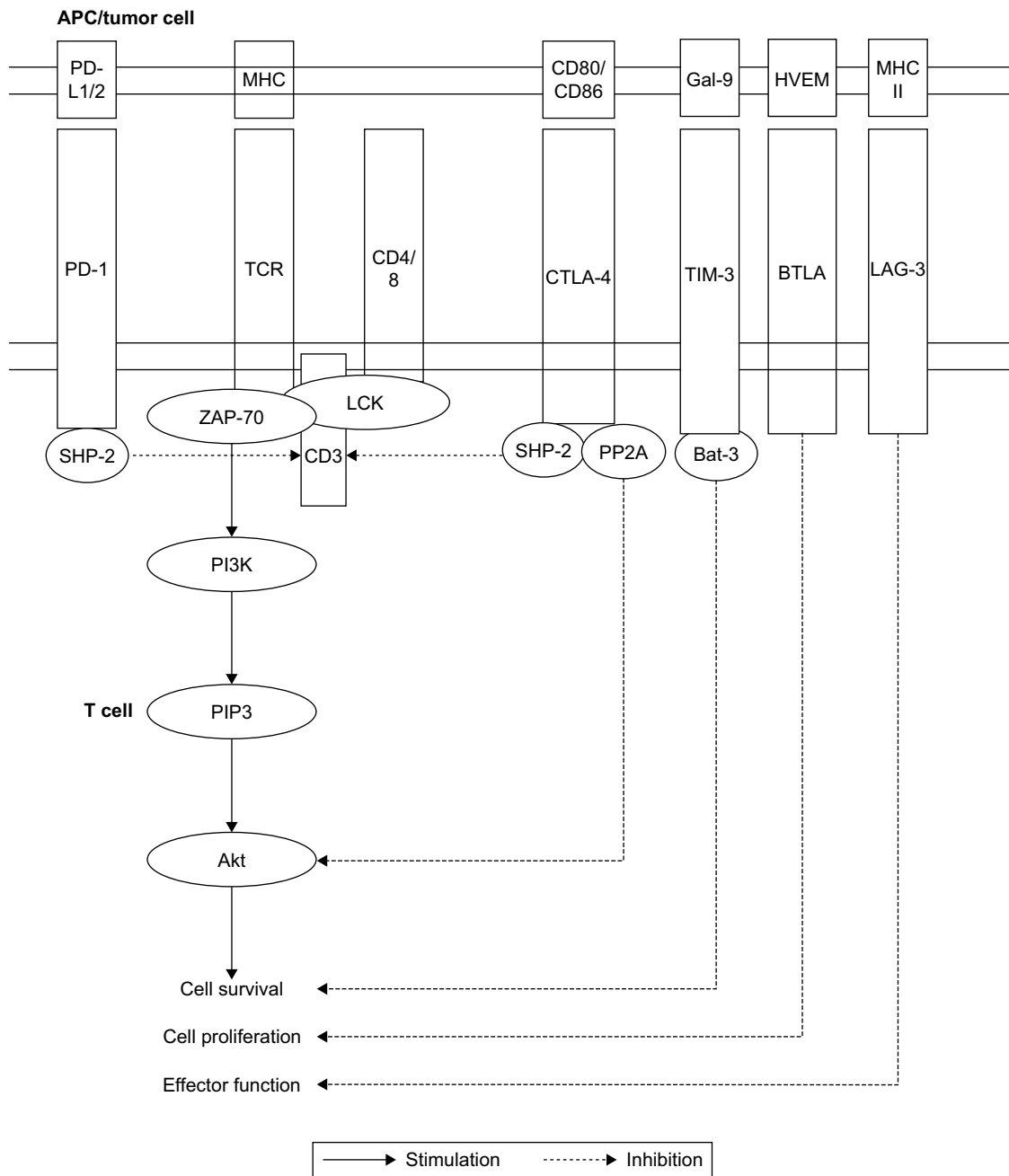


Figure 1 Signaling pathways of immune-checkpoint molecules.

Notes: Binding of PD-L1/L2 to PD-1 recruits SHP-2, which inhibits TCR signaling by CD3 ζ -chain dephosphorylation. Thus, the signaling cascade leading to T-cell survival, proliferation, and effector function is inhibited. The SHP-2 recruitment is dependent on its ITSM, whereas the ITIM is not needed for this action. Binding of CTLA-4 to CD80/86, in addition to SHP-2 recruitment, engages PP2A, which directly dephosphorylates AKT. The signaling pathways of TIM-3, LAG-3, and BTLA are less known. Binding of TIM-3 to galectin-9 phosphorylates the Y265 intracellular TIM-3 domain. This disrupts the interaction between TIM-3 and Bat-3, which otherwise inactivates the inhibitory effects of TIM-3. The inhibitory effects due to the binding of MHC II to LAG-3 are dependent on the intracellular KIEELE domain of LAG-3. It is suspected that the intracellular ITIM domain of BTLA is necessary for its inhibitory effects after binding to HVEM.

Abbreviations: BTLA, B- and T-lymphocyte attenuator; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; HVEM, herpesvirus entry mediator; ITIM, immunoreceptor tyrosine-based inhibition motif; ITSM, immunoreceptor tyrosine-based inhibition motif; LAG-3, lymphocyte-activation gene 3; MHC, major histocompatibility complex; PI3K, phosphoinositide 3-kinase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; PIP3, phosphatidylinositol (3,4,5)-trisphosphat; PP2A, protein phosphatase 2A; TCR, T-cell receptor; TIM-3, T-cell immunoglobulin and mucin domain 3.

which prevents an excessive early immune reaction; moreover, CTLA-4 is essential for the suppressive function of regulatory T cells (Treg).^{39,40} CTLA-4 ligation causes lymphocyte anergy, which reduces the synthesis of IFN γ , IL-2, IL-3, and granulocyte-macrophage colony-stimulating

factor (GM-CSF), and increases the production of transforming growth factor beta (TGF β).⁴¹ The synthesis of CTLA-4 mRNA increases within the first hours of lymphocyte stimulation, and peaks after 48–72 hours.⁴² CTLA-4 stimulation makes lymphocytes more likely to remain in the G₀/G₁

phase of the cell cycle, which is due to a reduced synthesis of cyclin D3 and kinases cdk4/cdk6, degradation of the inhibitory protein p27, and increased expression of cyclin D2.⁴³ Anergic lymphocytes are not activated after antigen recognition even when they receive co-stimulatory signals sufficient to activate a naïve lymphocyte. CD86, like CD80, is a ligand for CD28 and CTLA-4, and it is important in the co-stimulation of T cells during the primary immune response. CD86 belongs to the superfamily of immunoglobulins, and it is expressed on monocytes, DCs, as well as activated T, B, and NK cells. The chromosome region encoding CD86 contains a series of genes involved in carcinogenesis.^{44,45} Blocking CTLA-4 by monoclonal antibodies (mAbs) maintains T cells in an activation state and improves the immune response against cancer cells. Thus, anti-CTLA-4 mAbs are effective in cancer immunotherapy.

LAG-3

LAG-3 (CD223) prevents an excessive immune activation. This receptor is expressed by T and NK cells after MHC class II ligation, and by cytotoxic T cells upon antigen stimulation.^{22,46} LAG-3 inhibits CD4⁺ cell activation and directly decreases the cytotoxic function of CD8⁺ cells.^{46,47} Blocking LAG-3 restores the function of cytotoxic T cells and simultaneously inhibits Tregs.⁴⁸ Preclinical studies showed that LAG-3, fused with immunoglobulin (LAG-3-Ig), binds with a high affinity to MHC II of DCs, which stimulates DC maturation, and that, in turn, potentiates T helper 1 (Th1)-type responses.⁴⁹ In contrast, monomeric LAG-3, shed from the cell surface, does not bind to MHC class II molecules.⁴⁶

TIM-3

Expression of the type I transmembrane protein TIM-3 was shown in many immune cell types, including Th1, Th17, NK, and NKT cells as well as Tregs; on APCs, TIM-3 is co-expressed with PD-1.⁵⁰ TIM-3 binds to galectin-9, which causes apoptosis of CD4⁺ and CD8⁺ cells through the calcium-calpain-caspase-1 pathway.^{51,52} Galectin-9 is expressed on the surface of many cancer cell types, whereas the expression of TIM-3 was observed in tumor-infiltrating T cells in mice. TIM-3 directly inhibits Th1-mediated autoimmunity, and it indirectly promotes immunosuppression by inducing expansion of myeloid-derived suppressor cells (MDSCs), through an unknown mechanism.^{22,53} Blocking TIM-3 increases the production of IFN γ by lymphocytes, but it is unclear as to what forms the molecular basis of this action.⁵⁴ In patients with gastric, colorectal, liver, and pancreatic cancers, TIM-3 tumor expression correlated with tumor

invasion, reduced survival, and metastasis; thus, TIM-3 can be implicated in carcinogenesis.⁵⁵

B- and T-lymphocyte attenuator (BTLA)

BTLA is a glycoprotein containing an immunoglobulin domain, and it is expressed on T cells, resting B cells, macrophages, DCs, and NK cells.⁵⁶ BTLA downregulates the activity of lymphocytes after binding to its ligand – the herpesvirus entry mediator (HVEM) molecule. HVEM belongs to the TNF receptor superfamily, whereas BTLA and CD160 are members of the immunoglobulin superfamily.⁵⁷ The functions and structures of these co-stimulatory molecules are related to positive and negative co-stimulatory pathways.^{57,58} Binding of BTLA to HVEM inhibits the proliferation of CD8⁺ T cells, production of proinflammatory cytokines, and formation of memory T cells; at the same time, it promotes peripheral tolerance.⁵⁹ Studies in the HVEM^{-/-} knockout mouse have shown, however, that immunosuppressive function is preserved in this animal model.⁶⁰

Novel immune-checkpoint molecules

Novel immune-checkpoint molecules that could be future targets for cancer treatments are being investigated. They include, for example, HHLA2, TMIGD2, B7x, B7 homologue 3 (B7-H3), T-cell immunoglobulin and ITIM domain (TIGIT), CD96, 2B4, and adenosine A2a receptor (A2aR).^{22,61} Moreover, blockade of the V-domain Ig Suppressor of T cell Activation (VISTA) protein is a promising add-on therapy to PD-1 inhibitors because it inhibits T-cell activation via different pathways than does PD-1.⁶² In addition to immune-checkpoint inhibition, enhancement of immune-stimulatory pathways (OX40, GITR, and CD40) is considered in cancer treatment. Future studies will show which new molecule will be used to treat cancer along the currently approved CTLA-4 and PHD-1 inhibitors. In our opinion, because of an advanced program of clinical trials, LAG-3 will be the third approved target for immune-checkpoint inhibition.⁴⁶

Blockade of T-cell dysfunction as a new method of cancer immunotherapy

TCR-mediated antigen recognition is the most important signal for T-cell activation. In addition, there are co-stimulatory and co-inhibitory molecules on the surface of effector T cells that take part in the immune response against tumor cells. Ligands for these molecules are found on the surface of APCs and tumor cells (Figure 1).⁶³ The interaction between specific cell-surface molecules and their ligands directs lymphocyte response. PD-L1 and

PD-L2, both located on the surface of APCs and tumor cells, inhibit lymphocyte activity by binding to PD-1 on the lymphocyte surface.⁶⁴ The interaction between CD28 on the T-cell surface and CD80 (B7/B7.1) and CD86 (B70/B7-2) on the surface of APCs or tumor cells is crucial for the activation of effector T cells. However, when CD80 and CD86 bind to the cytotoxic T-cell antigen 4 (CTLA-4) instead of CD28, lymphocyte anergy and apoptosis occur.⁶⁵ In the tumor microenvironment, wherein signals that inhibit effector T cells predominate, tumor cells may avoid immune response.⁶⁶ Because mAbs against the molecules implicated in T-cell dysfunction may boost the immune response in the tumor microenvironment, these antibodies have a therapeutic potential in certain cancers. Currently, mAbs against the PD-1/PD-L1 pathway, CTLA-4, lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain 3 (TIM-3), and BTLA are either an approved treatment or are undergoing phase III clinical trials in patients with different cancers; some of these treatments are investigated in preclinical studies.⁶³ Table 2 presents a list of completed clinical trials of immune-checkpoint inhibitors in patients with cancer.⁶⁷

Blockade of PD-1 and its ligands

PD-1 and PD-L1 are expressed both on tumor cells and on tumor-specific immune cells.^{22,23,29} In humans, PD-L1 is expressed by different tumors, and it is a negative prognostic factor in some of them. In cervical cancer, however, PD-L1 expression was associated with a longer overall survival.⁶⁸ The expression of PD-L1 on tumor cells may be associated with a decreased number of tumor-infiltrating lymphocytes.⁶⁹ PD-L2 is expressed by colorectal cancer, non-small-cell lung cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, cervical cancer, and some B-cell leukemias.⁷⁰ Moreover, PD-1 and its ligands are expressed by immune cells in the tumor microenvironment. In breast cancer, Hodgkin's lymphoma, and head and neck cancer, PD-1 expression by tumor-infiltrating lymphocytes correlated with tumor size and a lower overall survival.⁷¹ Further, increased PD-1 expression was observed on DCs in the tumor microenvironment, which reduced the DC-mediated activation of T cells.⁷² PD-L1 expression on tumor cells is induced mainly by IFN γ , which is produced by tumor-infiltrating lymphocytes.⁷³ Thus, tumor cells can protect themselves from lymphocytes by expressing PD-L1, which inhibits lymphocyte activation and considerably reduces their efficacy.⁷⁴ Tumor-associated DCs expressing PD-L1 produce suppressive IL-10.⁷⁵ In animal studies, the PD-1:PD-L1

interaction enables Treg-mediated suppression of CD8⁺ T cells in the tumor microenvironment.⁷⁶ Blockade of the PD-1:PD-L1/2 pathway may increase the therapeutic effectiveness in patients with cancer by reducing the exhaustion of effector T cells.

In 2002, the therapeutic effects of anti-PD-1 antibodies were first observed in mice with PD-L1-positive tumors.⁷⁷ These and other preclinical findings encouraged phase I clinical trials in patients with cancer.⁶⁶ In the first clinical trials of an anti-PD-1 mAb (MDX-1106, nivolumab), an objective response was observed in multiple cancer types, including melanoma, non-small-cell lung cancer, and renal cell cancer.⁷⁸ The drug-related toxicity was acceptable, and the anticancer effect was long term.⁵⁶ Nivolumab proved effective not only in immunogenic tumors like melanoma and renal cell cancer, but also in non-small-cell lung cancer (considered insensitive to immunotherapy), hepatocellular cancer, metastatic colorectal cancer, squamous cell carcinoma of the head and neck, and urothelial carcinoma.^{56,79,80} In addition, nivolumab was investigated in patients with hematological malignancies (Hodgkin's lymphoma) because PD-1 ligands are expressed in these cancers.⁸¹ Currently, nivolumab and nivolumab-combined therapies are approved by the US Food and Drug Administration (FDA) for the treatment of melanoma, lung cancer, advanced and metastatic renal cell carcinoma, Hodgkin's lymphoma, head and neck cancers, urothelial carcinoma, colorectal cancer, and hepatocellular carcinoma (Table 1).⁸²

In phase I clinical trials, treatment with pembrolizumab – an anti-PD-1 mAb – was associated with a favorable objective response and a high survival rate in patients with advanced melanoma.⁸³ Further studies showed that pembrolizumab was effective in patients with advanced urothelial carcinoma, gastric cancer, non-small-cell lung cancer, and squamous cell carcinoma of the head and neck.⁵⁶ Similarly to nivolumab, the effects of pembrolizumab are durable, and the frequency of third- or fourth-level adverse effects related to drug administration is relatively low.⁸⁴ In a phase III clinical trial, in patients with advanced melanoma, pembrolizumab was associated with a higher survival rate and a higher percentage of objective responses as compared with ipilimumab – an anti-CTLA-4 mAb.⁸⁵ Currently, pembrolizumab is approved for the treatment of advanced melanoma, advanced or metastatic non-small-cell lung cancer, recurrent or metastatic head and neck squamous cell carcinoma, Hodgkin's lymphoma, advanced or metastatic urothelial carcinoma, and recurrent locally advanced or metastatic gastric or gastroesophageal junction cancers.⁸² Treatment with another anti-PD-1 mAb –

Table 2 Completed clinical trials of molecules used to overcome T-cell exhaustion in patients with cancer

Target molecule related to T-cell exhaustion	Study title	Conditions	Treatment	Phase	Number enrolled	Study start	Study completion
PD-1	ONO-4538 Phase I Study in Patients With Advanced Malignant Solid Tumors in Japan	Malignant solid tumor	ONO-4538	I	17	January 2009	September 2010
PD-1	Safety Study of IL-21/Anti-PD-1 Combination in the Treatment of Solid Tumors	Neoplasms by site	Denenicolin, nivolumab	I	33	June 2012	December 2014
PD-1	Study of Pembrolizumab (MK-3475) in Participants With Advanced Non-Small Cell Lung Cancer (MK-3475-025/KEYNOTE-025)	Non-small-cell lung cancer	Pembrolizumab	I	38	February 2014	March 2017
PD-1	Study of Pembrolizumab (MK-3475) in Participants With Advanced Melanoma (MK-3475-041/KEYNOTE-041)	Melanoma	Pembrolizumab	I	42	July 2014	August 2017
PD-1	A Study Of 4-1BB Agonist PF-05082566 Plus PD-1 Inhibitor MK-3475 In Patients With Solid Tumors (B1641003/KEYNOTE-0036)	Advanced solid tumors	PF-05082566; MK-3475	I	23	August 2014	February 2017
PD-1	Study of Pembrolizumab (MK-3475) Compared to Platinum-Based Chemotherapies in Participants With Metastatic Non-Small Cell Lung Cancer (MK-3475-024/KEYNOTE-024)	Non-small-cell lung carcinoma	Pembrolizumab, paclitaxel, carboplatin, pemetrexed, cisplatin, gemcitabine	3	305	August 2014	May 2016
PD-1	AMP-224, a PD-1 Inhibitor, in Combination With Stereotactic Body Radiation Therapy in People With Metastatic Colorectal Cancer	Colorectal cancer, colorectal neoplasms, colorectal carcinoma	AMP-224, stereotactic body radiation therapy (SBRT), cyclophosphamide	I	17	November 2014	March 2017
PD-1	Neoadjuvant Nivolumab in Glioblastoma	Glioblastoma multiforme	Nivolumab	2	29	June 2015	March 2017
PD-1	Nivolumab in Patients With Recurrent Malignant Mesothelioma	Malignant pleural mesothelioma	Nivolumab	2	33	July 2015	July 2017
PD-1	Pilot Study of Stereotactic Ablation for Oligometastatic Breast Neoplasia in Combination With the Anti-PD-1 Antibody MK-3475	Breast neoplasms; Bone neoplasms	Stereotactic ablative body radiosurgery (SABR); MK-3475	I	15	September 2015	April 2017

(Continued)

Table 2 (Continued)

Target molecule related to T-cell exhaustion	Study title	Conditions	Treatment	Phase	Number enrolled	Study start	Study completion
PD-1	Pilot Study of Vigil™ + Pembrolizumab for Advanced Melanoma	Melanoma recurrent, melanoma, malignant melanoma	Vigil, pembrolizumab	I	2	October 2015	September 2017
PD-1	Phase I Study of PDR001 in Patients With Advanced Malignancies	Advanced malignancies	PDR001	I	18	February 2016	September 2017
PD-L1	Multiple Ascending Dose (MDX1105-01)	Cancer, multiple indications	Anti-PD-L1 mAb	I	281	February 2009	July 2015
PD-L1	A Study of Atezolizumab in Participants With Programmed Death-Ligand 1 (PD-L1) Positive Locally Advanced or Metastatic Non-Small Cell Lung Cancer (NSCLC)	Non-small-cell lung cancer	Atezolizumab (MPDL3280A) [TECENTRIQ]	2	138	May 2013	December 2017
PD-L1	A Multi-Center Study of Ibrutinib in Combination With MEDI4736 in Subjects With Relapsed or Refractory Solid Tumors	Non-small-cell lung cancer, breast cancer, pancreatic cancer	Ibrutinib, durvalumab (MEDI4736)	I; 2	124	March 2015	August 2017
PD-L1	A Study of Atezolizumab Compared With Gemcitabine Plus (+) Cisplatin or Carboplatin in Participants With Stage IV Squamous Non-Small Cell Lung Cancer (NSCLC)	Non-small-cell lung cancer	Atezolizumab, carboplatin, cisplatin, gemcitabine	3	8	May 2015	December 2017
PD-L2	Monoclonal Antibody Therapy in Treating Patients With Stage IV Melanoma	Melanoma (skin)	B7-DC cross-linking antibody rHlgM12B7	I	7	April 2008	May 2012
PD-L2	Study to Assess the Safety, Tolerability, and Pharmacokinetics of AMP-224 in Patients With Advanced Cancer	Cancer	AMP-224	I	44	March 2011	January 2014
CTLA-4	Novel Adjuvants for Peptide-Based Melanoma Vaccines	Melanoma	MDX-CTLA-4 antibody, tyrosinase/gp100/MART-1 peptides melanoma vaccine	I	19	August 2001	June 2005
CTLA-4	Monoclonal Antibody and Vaccine Therapy in Treating Patients With Stage III or Stage IV Melanoma That Has Been Removed During Surgery	Intraocular melanoma, melanoma (skin)	MART-1 antigen; gp100 antigen, incomplete Freund's adjuvant, ipilimumab, tyrosinase peptide	I	19	October 2001	June 2005

CTLA-4	Monoclonal Antibody Therapy in Treating Patients With Lymphoma or Colon Cancer That Has Not Responded to Vaccine Therapy	Lymphoma	Ipilimumab	I	89	September 2002	November 2010
CTLA-4	Comparison Study of MDX-010 (CTLA-4) Alone and Combined With DTIC in the Treatment of Metastatic Melanoma	Melanoma	MDX-010 (CTLA-4); dacarbazine (DTIC)	2	72	October 2002	February 2004
CTLA-4	Comparison Study of MDX-010 (CTLA-4) Alone and Combined With Docetaxel in the Treatment of Patients With Hormone Refractory Prostate Cancer	Prostate cancer	MDX-010, MDX-010 + docetaxel	2	43	November 2002	November 2004
CTLA-4	Monoclonal Antibody Therapy and Interleukin-2 in Treating Patients With Metastatic Melanoma	Intraocular melanoma, melanoma (skin)	Aldesleukin, ipilimumab	1; 2	36	February 2003	August 2006
CTLA-4	Ipilimumab After Allogeneic Stem Cell Transplant in Treating Patients With Persistent or Progressive Cancer	Adult acute myeloid leukemia, atypical chronic myeloid leukemia, childhood leukemia, myelodysplastic syndromes, chronic myelogenous leukemia, neuroblastoma, ovarian cancers, testicular cancers, recurrent leukemia and lymphomas, multiple myeloma, Stage IIIA/IIIB/IIIC/IV breast cancer	Ipilimumab, therapeutic allogeneic lymphocytes	I	21	April 2003	April 2008
CTLA-4	Ipilimumab and Sargramostim in Treating Patients With Metastatic Prostate Cancer	Recurrent prostate carcinoma, Stage IV prostate cancer	Ipilimumab, sargramostim	I	42	May 2003	February 2011
CTLA-4	CP-675,206 (CTLA-4-Blocking Monoclonal Antibody) Combined With Dendritic Cell Vaccine Therapy in Treating Patients With Stage III or Stage IV Melanoma That Cannot Be Removed With Surgery	Melanoma (skin)	Maximum tolerated dose of anti-CTLA-4 mAb	I	18	April 2004	October 2009

(Continued)

Table 2 (Continued)

Target molecule related to T-cell exhaustion	Study title	Conditions	Treatment	Phase	Number enrolled	Study start	Study completion
CTLA-4	Hormone Therapy and Ipilimumab in Treating Patients With Advanced Prostate Cancer	Prostate adenocarcinoma, prostate carcinoma, recurrent prostate carcinoma, Stage III/IV prostate cancer	Bicalutamide, flutamide, goserelin acetate, ipilimumab, leuprolide acetate	2	112	June 2004	June 2013
CTLA-4	MDX-010 Antibody, MDX-1379 Melanoma Vaccine, or MDX-010/MDX-1379 Combination Treatment for Patients With Unresectable or Metastatic Melanoma	Melanoma, metastatic melanoma	MDX-010 mAb, MDX-1379 (gp100) melanoma peptide vaccine	3	1,783	September 2004	October 2009
CTLA-4	Vaccine and Antibody Treatment of Prostate Cancer	Prostatic neoplasms	PROSTVAC-V/ TRICOM, PROSTVAC-F/ TRICOM, MDX-010, sargramostim	1	30	June 2005	December 2011
CTLA-4	MDX-010 in Treating Patients With Stage IV Pancreatic Cancer That Cannot Be Removed By Surgery	Pancreatic cancer	Ipilimumab	2	82	July 2005	June 2009
CTLA-4	Study of CP-675,206 in Refractory Melanoma	Refractory melanoma	CP-675,206	2	251	December 2005	December 2009
CTLA-4	CP-675,206 vs Either Dacarbazine Or Temozolomide In Patients Without Prior Therapy	Melanoma	Dacarbazine, CP-675,206, temozolomide	3	655	March 2006	August 2010
CTLA-4	Study of Tivolumab in Patients With Metastatic Colorectal Cancer Whose Disease Had Progressed After Treatment	Colorectal neoplasms	CP-675,206	2	49	May 2006	June 2008
CTLA-4	Study to Compare Two Formulations of CP-675,206 Monoclonal Antibody	Melanoma	CP-675,206	1	85	June 2006	February 2008
CTLA-4	Safety and Efficacy of Combination Biotherapy With High-dose Interferon alpha-2b and Anti-CTLA-4 Monoclonal Antibody for Recurrent Inoperable Stage III or Stage IV Melanoma	Melanoma	Anti-CTLA-4 mAb, interferon alpha-2b	2	37	November 2006	January 2015

CTLA-4	Phase I Study of Ipilimumab (Anti-CTLA-4) in Children and Adolescents With Treatment-Resistant Cancer	Sarcoma, Wilim's tumor, lymphoma, neuroblastoma	Ipilimumab	1	33	October 2007	November 2015
CTLA-4	Anti-CTLA-4 Human Monoclonal Antibody CP-675,206 in Patients With Advanced Hepatocellular Carcinoma	Hepatocellular carcinoma	CP 675,206	2	20	December 2008	May 2012
CTLA-4	Ipilimumab±Vaccine Therapy in Treating Patients With Locally Advanced, Unresectable or Metastatic Pancreatic Cancer	Pancreatic cancer	Ipilimumab, pancreatic cancer vaccine	1	30	February 2009	July 2012
CTLA-4	Laboratory-Treated T Cells and Ipilimumab in Treating Patients With Metastatic Melanoma	Recurrent melanoma, stage IV melanoma	Ipilimumab, cyclophosphamide	1; 2	10	February 2009	October 2013
CTLA-4	Immunogenicity and Biomarker Analysis of Neoadjuvant Ipilimumab for Melanoma	Melanoma	Ipilimumab	Early 1	59	December 2009	September 2017
CTLA-4	Ipilimumab in Patients With Advanced Melanoma and Spontaneous Preexisting Immune Response to NY-ESO-1	Metastatic melanoma	Ipilimumab	2	25	November 2010	August 2016
CTLA-4	Autologous TriMix-DC Therapeutic Vaccine in Combination With Ipilimumab in Patients With Previously Treated Unresectable Stage III or IV Melanoma	Malignant melanoma stage III/IV	TriMix-DC, ipilimumab	2	39	February 2011	January 2017
CTLA-4	Addition of Ipilimumab (MDX-010) To Isolated Limb Infusion (ILI) With Standard Melphalan and Dactinomycin In The Treatment of Advanced Unresectable Melanoma of The Extremity	Melanoma	Ipilimumab, melphalan, dactinomycin	2	26	February 2011	August 2017
CTLA-4	The Addition of Ipilimumab to Carboplatin and Etoposide Chemotherapy for Extensive Stage Small Cell Lung Cancer	Extensive-stage small-cell lung cancer	Ipilimumab	2	42	June 2011	June 2015
CTLA-4	Pre-Operative, Single-Dose Ipilimumab and/or Cryoablation in Early Stage/ Resectable Breast Cancer	Breast cancer, invasive adenocarcinoma of the breast	Cryoablation, ipilimumab	1	19	December 2011	December 2014
CTLA-4	Dasatinib and Ipilimumab in Treating Patients With Gastrointestinal Stromal Tumors or Other Sarcomas That Cannot Be Removed by Surgery or Are Metastatic	Gastrointestinal stromal tumor, Stage III/IV soft-tissue sarcoma	Dasatinib, ipilimumab	1	29	July 2012	June 2016

(Continued)

Table 2 (Continued)

Target molecule related to T-cell exhaustion	Study title	Conditions	Treatment	Phase	Number enrolled	Study start	Study completion
CTLA-4	Ipilimumab and Rituximab in Treating Patients With Relapsed or Refractory B-cell Lymphoma	CD20-positive, recurrent B-cell non-Hodgkin lymphoma, refractory B-cell non-Hodgkin lymphoma	Ipilimumab, rituximab	I	32	November 2012	March 2018
CTLA-4	Safety Study of BMS-986015 (Anti-KIR) in Combination With Ipilimumab in Subjects With Selected Advanced Tumor	Advanced (metastatic and/or unresectable) solid tumors	Lirilumab, ipilimumab	I	22	December 2012	April 2015
CTLA-4	Ipilimumab in Treating Patients With Relapsed or Refractory High-Risk Myelodysplastic Syndrome or Acute Myeloid Leukemia	Acute myeloid leukemia arising from previous myelodysplastic syndrome, chronic myelomonocytic leukemia, myelodysplastic syndrome, previously treated myelodysplastic syndrome, recurrent adult acute myeloid leukemia, secondary myelodysplastic syndrome	Ipilimumab	I	42	December 2012	December 2016
CTLA-4	Tolerability and Efficacy of Tremelimumab in Combination With Gefitinib in NSCLC Patients	Non-small-cell lung cancer	Gefitinib, tremelimumab	I	27	January 2014	March 2016
CTLA-4	Study of Combined Ionizing Radiation and Ipilimumab in Metastatic Non-Small-Cell Lung Cancer (NSCLC)	Non-small-cell lung cancer	Ipilimumab, radiotherapy	2	39	June 2014	October 2016

CTLA-4	A Phase I/II Study of Intratumoral Injection of SD-101	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue, recurrent grade 1/2 follicular lymphoma, nodal marginal zone B-cell lymphoma, recurrent marginal zone lymphoma, recurrent small lymphocytic lymphoma, splenic marginal zone lymphoma	Ipiilimumab, SD-101, radiation therapy	I; 2	9	September 2014	January 2017
CTLA-4	Trial of SBRT With Concurrent Ipiilimumab in Metastatic Melanoma	Melanoma, effects of immunotherapy, adverse effect of radiation therapy	Stereotactic body radiotherapy, ipilimumab	I	13	March 2015	August 2016
CTLA-4 and PD-1	Combination Checkpoint Inhibitor Plus Erlotinib or Crizotinib for EGFR or ALK Mutated Stage IV Non-small-cell lung cancer	Non-small-cell lung cancer	Ipiilimumab, erlotinib, crizotinib, nivolumab	I	14	December 2013	March 2018
LAG-3	IMP321 Phase I Trial in Metastatic Renal Cell Carcinoma (MRCO)	Stage IV renal cell carcinoma	IMP321	I	24	September 2005	October 2008
LAG-3	IMP321 Plus First-line Paclitaxel in Metastatic Breast Carcinoma	Metastatic breast cancer	IMP321	I	33	July 2006	January 2010
LAG-3	Lag-3 and Gemcitabine for Treatment of Advanced Pancreas Cancer	Pancreatic neoplasms	Gemcitabine, LAG-3	I	18	February 2009	September 2012

Note: Data from <https://ClinicalTrials.gov> website; accessed April 29, 2018.

Abbreviations: CTLA-4, cytotoxic T-cell antigen 4; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2.

pidilizumab – showed an encouraging effect in patients with diffuse large B-cell lymphoma (DLBCL) after autologous hematopoietic stem-cell transplantation and with relapsed follicular lymphoma.^{86,87}

The effects of anti-PD-L1 mAbs can differ from those of the anti-PD-1 mAbs because PD-L1 and PD-1 have different ligands.⁶⁶ BMS-936559 – an anti-PD-L1 mAb – caused an objective and durable response in patients with melanoma, lung cancer, kidney cancer, and ovarian cancer.⁸⁸ MPDL3280A (atezolizumab), another anti-PD-L1 mAb, proved effective in patients with metastatic urinary bladder cancer.⁸⁹ Based on favorable outcomes of clinical trials, the FDA approved atezolizumab for the treatment of urothelial carcinoma, certain types of metastatic lung cancer, and bladder cancer.⁸² In 2017, the FDA approved avelumab – another anti-PD-L1 mAb – for the treatment of metastatic Merkel cell carcinoma and urothelial carcinoma.⁸² The effectiveness and safety of avelumab are currently under investigation in ovarian cancer, non-small-cell lung cancer, gastric cancer, and mesothelioma.⁹⁰ Darvulumab, an anti-PD-L1 mAb, was recently approved for the treatment of patients with advanced bladder cancer and for unresectable stage III non-small-cell lung cancer.⁸² Currently, the effectiveness of mAbs against the PD-1:PD-L1/2 pathway is being investigated in patients with nearly 250 different neoplastic disorders.⁶⁷ Although several anti-PD-1 and anti-PD-L1 mAbs are approved, they have similar efficacy and toxicity profiles.^{91,92}

Blockade of CTLA-4

At the end of the 20th century, studies showed that removing signals that blocked co-stimulation led to a stronger anticancer response. In mice with immunogenic colorectal cancer, treatment with anti-CTLA-4 mAbs before the transfer of tumor cells prevented disease development, mostly due to the activation of CD8⁺ T cells. Moreover, anti-CTLA-4 mAbs caused cancer regression in mice with developed tumors, including weakly immunogenic tumors. This treatment led to the formation of immunological memory against tumor cells.^{93,94} Such encouraging preclinical findings prompted clinical trials with anti-CTLA-4 mAbs in patients with various neoplastic diseases.⁹⁵ The effectiveness of ipilimumab, an anti-CTLA-4 mAb, was investigated both as a standalone treatment and in combination with other treatments (IL-2, melanoma vaccine, and chemotherapy).^{96,97} In 2011, ipilimumab was approved by the FDA for the treatment of patients with advanced melanoma. Subsequently, in 2015, ipilimumab was approved as an adjuvant treatment in patients with melanoma

after surgery and, in 2017, as a treatment for children with advanced melanoma.^{82,99} Ipilimumab is a promising treatment for relapsed and refractory B-cell non-Hodgkin lymphomas, metastatic renal cell carcinoma, small-cell and non-small-cell lung cancer, prostate cancer, urothelial carcinoma, and ovarian cancer.¹⁰⁰ In phase I and phase II clinical trials in patients with metastatic melanoma, tremelimumab (ticilimumab), also an anti-CTLA-4 antibody, was associated with a durable tumor regression.¹⁰¹ Subsequently, the effect of tremelimumab was shown in patients with advanced gastric and esophageal adenocarcinoma, colorectal carcinoma, non-small-cell lung cancer, and malignant mesothelioma.⁶³ Treatment with anti-CTLA-4 mAbs such as ipilimumab and tremelimumab is associated with significant immune-related adverse effects.⁸⁴ A high incidence of immune-related adverse events of anti-CTLA-4 treatments is likely due to the depletion of Treg cells and a systemic activation of autoimmune T cells in the lymphoid tissue.⁵⁶ Currently, approximately 300 clinical trials are investigating the effectiveness of ipilimumab and ipilimumab-combined therapies, and 100 clinical trials are investigating the effectiveness of tremelimumab and tremelimumab-combined therapies.⁶⁷

Blockade of LAG-3

In ovarian and prostate cancers, LAG-3 is expressed by CD8⁺ tumor-specific T cells that co-express PD-1, which suggests that LAG-3 might be implicated in the formation of an immunosuppressive tumor microenvironment.¹⁰² In pre-clinical trials, LAG-3 blockade with mAbs was investigated as a standalone therapy and combined with anti-PD-1 and anti-CTLA-4 mAbs.¹⁰³ In mice, the blockade of either PD-1 or LAG-3 did not effectively inhibit tumor development after transfer of cancer cells, but a dual blockade was more effective and was associated with higher percentages of T CD8⁺/IFN γ ⁺ and CD4⁺ T cells.¹⁰⁴ In another study, dual PD-1 and LAG-3 blockade caused a considerable tumor regression in all treated mice.¹⁰⁵ A triple blockade of PD-1, CTLA-4, and LAG-3 significantly increased the effectiveness of cytotoxic T lymphocytes injected to mice with leukemia.¹⁰⁶ However, targeting of multiple T-cell inhibitory molecules might be associated with an increased incidence of autoimmune adverse events.¹⁰⁷ To date, two approaches to inhibit LAG-3 signaling have been developed: a LAG-3-Ig fusion protein and anti-LAG-3 mAbs (IMP321, LAG525, IMP701, TSR-033, REGN3767, and BMS-986016).^{22,108} Inhibition of LAG-3 may be effective not only due to the enhancement of Th1 responses, but also due to the stimulation of DC maturation, in which IL-12 is implicated.²² The effectiveness

of IMP321 was shown in phase I clinical trials in patients with breast cancer, renal cell carcinoma, and pancreatic cancer.^{109–111} Currently, approximately 20 clinical trials are investigating anti-LAG-3 mAbs as a standalone treatment or combined with other therapies; bispecific proteins binding to PD-1 and LAG-3 are investigated in different metastatic cancers, small-cell lung cancer, gastrointestinal cancers, virus-associated tumors, hematologic neoplasms, brain tumors, and melanoma.⁶⁷

Blockade of TIM-3

TIM-3-blocking mAbs enhance T-cell proliferation and increase cytokine production, which explains their antitumor activity.²² Tim-3⁺ tumor-infiltrating Tregs can greatly inhibit the proliferation of naïve T cells.¹¹² In mice, anti-TIM-3 mAbs trigger an anticancer response, which is dependent mostly on CD8⁺ T cells that secrete IFN γ and on CD4⁺ T cells. Although a substantial proportion of tumor-infiltrating CD4⁺TIM-3⁺ cells co-express Foxp3, the role of TIM-3 in Treg signaling remains unknown.¹¹³ In a mouse model of hepatitis B, TIM-3 blockade was associated with increased production of IFN γ by CD8⁺ cells.¹¹⁴ Anti-TIM-3 antibodies slowed tumor growth in mice, which was associated with a decreased percentage of exhausted TIM-3⁺ lymphocytes.¹¹⁵ A more potent anticancer response was observed when anti-TIM-3 mAbs were given in combination with anti-PD-1 or anti-CTLA-4 mAbs, when compared with the individual effects of these antibodies.¹¹⁶ The presence of TIM-3⁺ T cells correlates with disease severity and poor prognosis in patients with non–small-cell lung carcinoma and follicular lymphoma.^{22,117} In contrast, expression of galectin-9 – the main TIM-3 ligand – is associated with a favorable outcome in many solid tumors, which suggests that galectin-9 may have other effects in cancer than those associated with TIM-3 signaling.¹¹⁸ Currently, anti-TIM-3 mAbs (MBG453, Sym023, TSR-022, and LY3321367) are being investigated in phase I and II clinical trials in patients with advanced malignancies, including leukemia; these treatments will be investigated in patients with solid tumors and lymphomas from June 2018 (six clinical trials).^{22,67}

Blockade of BTLA

Tumor cells change the BTLA/HVEM signaling by either promoting the development of dysfunctional T cells with persistent BTLA expression (cells susceptible to inactivation) or by expressing HVEM – for example, in melanoma.^{22,119} In patients with advanced melanoma, BTLA is expressed by tumor-specific CD8⁺ T cells; moreover, an *in vitro* BTLA

blockade of melanoma-specific CD8⁺ T cells increased their proliferation and secretion of IL-2, IFN γ , and TNF α ; these effects were even greater with a triple blockade (anti-BTLA, anti-PD-1, and anti-TIM-3).⁵⁹ Both BTLA and HVEM are expressed by tumor cells and T-follicular helper cells in patients with chronic lymphocytic leukemia. These findings might direct the development of future immunotherapies.¹²⁰ BTLA and HVEM are investigated as treatment targets in preclinical studies.⁵⁶

The place of immune-checkpoint inhibitors in cancer treatment

Although several immune-checkpoint inhibitors are now available in clinical practice, the place of cancer immunotherapy is unclear. It is not often evident which patients will benefit from immune treatments more than from standard therapies. The choice between the immune and standard cancer treatments is even more difficult because the immune treatments are associated with a new class of adverse effects.

Based on the available data, a significant proportion of patients do not respond to treatment with immune-checkpoint inhibitors. Among patients with advanced melanoma, less than 20% respond to ipilimumab, approximately one third respond to pembrolizumab, and less than a half respond to nivolumab.^{121–123} When anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) treatments are given in combination, the response rate increases to approximately 60%.¹²⁴ The same treatment combination is associated with a response rate of approximately 40%–50% among patients with lung cancer.¹²⁵ These observations suggest non-redundant effects of CTLA-4 and PD-1 blockade.¹²³ For instance, T cells expressing CTLA-4 are found predominantly in secondary lymphoid organs, whereas PD-1 expression is characteristic for T cells in the tumor microenvironment.¹²⁶ Moreover, CTLA-4 targets mostly recently primed cells, and PD-1 targets primarily effector T cells.¹²⁶ Furthermore, CTLA-4 and PD-1 have different intracellular signaling pathways (Figure 1).

It is important to establish predictive factors for selecting patients who will most likely benefit from immunotherapy. In general, it is believed that patients with tumors that are well infiltrated by immune cells (hot tumors) respond to immunotherapy better than patients with tumors that display scarce immune infiltration (cold tumors). Infiltration of tumors by immune cells depends both on tumor immunogenicity and on host immune function. For example, immunogenic tumors – that is, those characterized by a high mutational load and, thus, a high neoantigen load – respond well to

immune-checkpoint inhibitors.¹²⁶ Similarly, high counts of circulating immune cells with proliferative potential (CD8⁺ Ki67+), relative to tumor burden, are associated with a favorable response to immune-checkpoint inhibition.¹²⁷ Moreover, expression of immune-checkpoint ligands by tumors seems important, because immune-checkpoint inhibitors are thought to act by competing with its ligands. Indeed, high concentrations of PD-L1 are associated with a favorable clinical response to PD-1 blockade.^{128,129} In line with this observation, in patients with urothelial cancer who have a low PD-L1 status, the effects of PD-1 blockade are worse than those of chemotherapy.¹³⁰ Other predictors of clinical response to immune-checkpoint inhibitors are being investigated. For example, a recent study showed that patients with melanoma who responded to anti-PD-1 immunotherapy had different gut microbiome than did non-responders.¹³¹

Immune-checkpoint inhibitors are associated with a new class of immune-mediated adverse events. In general, immune-related adverse effects occur more commonly with anti-CTLA-4 blockade (~50%) than with anti-PD-1 blockade (~25%).¹³² Moreover, the frequency of immune-related adverse effects is higher with a combination of CTLA-4 and PD-1 blockade. However, patients with advanced cancer seem to better tolerate PD-1/PD-L1 blockade than chemotherapy.¹³³ The immune-related adverse effects are due to immune overactivation, and they include skin changes, diarrhea related to colitis, hepatotoxicity, pneumonitis, and different endocrinopathies such as autoimmune thyroid disease (hypothyroidism and hyperthyroidism), hypophysitis, adrenal insufficiency, and type 1 diabetes mellitus. These adverse effects are usually managed with glucocorticoids, which, in turn, may cause infections such as tuberculosis.¹³⁴ The adverse effects of immune-checkpoint inhibitors are often severe and lead to treatment discontinuation. For example, approximately half the patients who received adjuvant ipilimumab after surgery for melanoma discontinued treatment due to adverse effects.¹³⁵ Thus, the adverse effects of immune-checkpoint inhibitors should be weighed against their expected benefit, particularly when considering combined CTLA-4 and PD-1 blockade. Although this combined treatment is more effective than its individual components, it is associated with the highest risk of immune-related adverse effects.

Conclusion

In most advanced cancers, chemotherapy is possibly approaching or has already reached the greatest possible

therapeutic effect. The different methods used to overcome T-cell dysfunction have proved effective in some cancers, and this approach might replace chemotherapy in the future. Treatments aimed at boosting immune function have several advantages over other treatments, such as a relatively short treatment period (several weeks). Moreover, they do not need to be specifically prepared for each individual patient (like DC vaccines). Some of these treatments are already approved on the basis of encouraging outcomes of clinical trials. Cancer immunotherapy has lower toxicity compared with chemotherapy; in some cancers, it may achieve a long-term disease control. Currently, finding reliable response factors to immunotherapy is crucial to properly select the best treatment for each patient. Treatment with mAbs that boost immune function, particularly a simultaneous use of antibodies that target different mechanisms of T-cell exhaustion, in combination with other treatments, shows the greatest promise for patients with cancer, including those with cancer resistant to standard therapies.

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Disclosure

The authors report no conflicts of interest in this work.

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