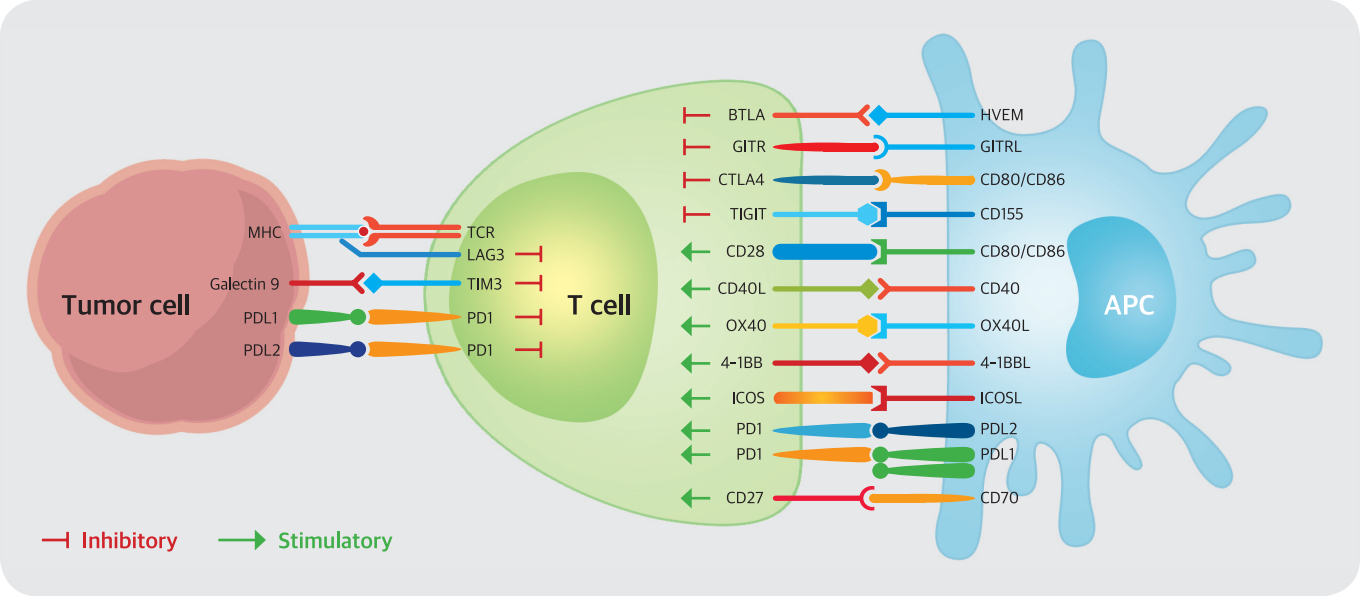
NKMAX

Immune Checkpoint

Tumor cell evades immuno-surveillance and progress through different mechanisms, including activation of immune checkpoint pathways that suppress antitumor immune response. Immune checkpoint inhibitors (ICIs) reinvigorate the antitumor immune response by interrupting co-inhibitory signaling pathways and promote immune-mediated elimination of tumor cells. These molecules are crucial for maintaining self-tolerance and for modulating the length and magnitude of effector immune responses in peripheral tissues to minimize collateral tissue damage. Signalling through these molecules can drive effector immune cells (especially T cells) into a state known as 'exhaustion'. T cell exhaustion is defined by reduced effector function, sustained expression of immune checkpoint molecules and poor recall responses, and a transcriptional state distinct from that of functional effector or memory T cells. There are numerous types of activating and inhibitory interactions that occur between antigen-presenting cells (APCs) and T cells, and these interactions regulate the nature of immune responses.

Interactions of immune checkpoint inhibitors



The most extensive studies about immune checkpoints currently are cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death protein 1, and its ligands (PD-1/PD-L1). Recent studies have identified several new immune checkpoint targets, like killer cell immunoglobulin-like receptors (KIRs), T cell immunoglobulin mucin 3 (TIM-3), natural killer cell group 2 member A (NKG2A), OX40, and 4-1BB, etc.

CTLA-4 (cytotoxic T lymphocyte antigen 4, CD152) is a B7/CD28 family member that is expressed on activated/exhausted $CD4^+$ T cell, regulatory T cell (Treg), activated/exhausted $CD8^+$ T cell, and some tumors. CTLA-4 inhibits T cell activation and proliferation that is mediated by binding to CD80/86 from the cell surface of antigen-presenting cells. This process is an important mechanism by which Treg mediate immune suppression on bystander cells.

PD-1 (programmed death protein 1, CD279) is a member of the B7/CD28 family of co-stimulatory receptors. PD-1 is primarily expressed on activated T cells, natural killer (NK) cells, B cells, and certain myeloid cells that acts as a negative regulator of apoptosis and is instrumental in maintaining a T cell immune response. PD-1 has two ligands: PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273). Both can be found on antigen-presenting cells such as dendritic cell, macrophage, and monocyte, but are otherwise differentially expressed on various non-lymphoid tissues. Interferon (IFN)-γ is the main trigger known to cause PD-L1 and PD-L2 upregulation.

Table 1 Key immune checkpoint receptor-ligand pairs

Receptor	Expressing cell	Ligand	Ligand-expressing cell
Suppressive (negative) immune checkpoint			
CTLA-4	Activated T cell, Treg	B7 molecules (CD80 or CD86)	Antigen-presenting cell
PD-1	Activated B and T cell, Antigen-presenting cell, NK cell	PD-L1 (CD274) or PD-L2 (CD273)	DC, macrophages, peripheral non-lymphoid tissue
LAG-3	Activated T cell, Treg, NK cell, B cell, DC	MHC class II/Lectins	Antigen-presenting cell
KIRs	Activated T and NK cell	MHC class I	Antigen-presenting cell
TIGIT	Activated T cell, Treg, NK cell	PVR (CD155)/Nectine-2 (CD112)	Normal epithelial, endothelial, neuronal, fibroblastic cell
TIM-3	Activated T cell	Galectin-9/HMGB-1	Multiple tissues
VISTA	Naïve and activated T cell	VSIG-3	Neuron, glial cell
CEACAM-1	Activated T and NK cell	CEACAM-1	T cell, NK cell
NKG2A	Activated T and NK cell	MHC class I	Antigen-presenting cell
Stimulatory (positive) immune checkpoint			
CD28	T cell	B7 molecules (CD80 or CD86)	Antigen-presenting cell
OX40	Activated T cell, Treg, NK cell, neutrophils	OX40L	DC, macrophage, B cell, endothelial cell, smooth muscle cell
CD137 (4-1BB)	Activated T cell, NK cell, B cell, DC, endothelial cell	CD137L	Antigen-presenting cell
GITR	T cell, NK cell, Treg	GITRL	Antigen-presenting cell, endothelium
ICOS	Naïve and activated T cell	ICOSLG	Antigen-presenting cell, B cell, DC, macrophage
CD27	Activated T and NK cell	CD70	Activated lymphocyte

Abbreviations: CTLA-4, cytotoxic T lymphocyte antigen 4; Treg, regulatory T cell; PD-1, programmed death protein 1; NK cell, natural killer cell; PD-L1/2, programmed death protein ligand 1/2; DC, dendritic cell; LAG-3, lymphocyte-activation gene 3; KIRs, killer cell immunoglobulin-like receptors; TIGIT, T cell immunoreceptor with Ig and ITIM domains; PVR, poliovirus receptor; TIM-3, T cell immunoglobulin mucin 3; HMGB-1, high mobility group box 1; VISTA, V-domain Ig suppressor of T cell activation; VSIG-3, V-set and Ig domain-containing 3; CEACAM-1, carcinoembryonic antigen-related cell adhesion molecule 1; NKG2A, natural killer cell group 2 member A; GITR, glucocorticoid-induced TNFR-related protein; GITRL, glucocorticoid-induced TNFR-related protein ligand; ICOS, Inducible T-cell co-stimulator; ICOSLG, Inducible T cell co-stimulator ligand.

KIRs (killer cell immunoglobulin-like receptor, CD158) are mainly expressed on NK cells and they bind to major histocompatibility complex (MHC) class I allotype (HLA-A, -B, or -C) molecules on the cell surface, which results in the negative regulation of the NK cell function, reducing NK cell-mediated lysis. In addition to NK cells, T cell subsets and iNKT (invariant natural killer T cells) also expressed KIR. Antibodies generated against KIR have been shown to induce NK cell-mediated lysis and therefore they make an ideal target for immunotherapy.

TIM-3 (T cell immunoglobulin mucin 3, as known as hepatitis A virus cellular receptor 2, HAVCR2) also contributes to immune tolerance by providing negative regulation of lymphocytes activation. It is expressed on multiple immune cells, including conventional T cells (activated, memory, and exhausted), Tregs, and innate immune cells. In cancer, chronic stimulation induces TIM-3 upregulation in tumor antigen-specific T lymphocytes, especially in $CD8^+$ TIL (tumor infiltrating lymphocyte), and, at the same time, peripheral T cells show minimal TIM-3 expression. Similar to the PD-1/PD-L1 axis, TIM-3 plays a role in T cell exhaustion during chronic immune stimulation, and especially in trimming the Th1-type immune responses.

NKG2A/CD94 (natural killer cell group 2 member A, CD159) is a C-type lectin family and recognizes a non-classical MHC I molecule, HLA-E, as ligand. Almost 50% of the NK cells in the peripheral blood express NKG2A/CD94, primarily those that do not express inhibitory KIR. In addition, γδ and $CD8^+$ T cells also express NKG2A/CD94. Ligation of NKG2A and CD94 to HLA-E expressed on normal cells suppresses signaling activation, thereby avoiding the destruction of normal bystander cells.

OX40 (CD134, TNFRSF4) belongs to the superfamily of TNFR (tumor necrosis factor receptor) and can be detected on the surface of activated $CD4^+$ and $CD8^+$ T cells, and also on Tregs, NK cells, and neutrophils. The expression of its natural ligand, OX40L (CD252), can be induced by pro-inflammatory cytokines on dendritic cells, macrophages, B cells, and endothelial or smooth muscle cells. Stimulation via OX40 has been shown to overcome the negative effects induced by CTLA-4 in T cells and to antagonize the suppressive effects of Tregs on the activation of the effector cells.

4-1BB (CD137, TNFRSF9) can be primarily detected on activated $CD8^+$ and $CD4^+$ T cells but following induction with pro-inflammatory stimuli also appears on other cell types, including NK cells, B cells and dendritic cells, or endothelial cells following induction with pro-inflammatory stimuli. CD137 ligand (4-1BBL, CD137) is expressed on various antigen-presenting cells. Ligation of CD137 results in a pro-stimulatory signal, enhancing among others the tumor-selective cytotoxicity of $CD8^+$ T cells and NK cells and secretion of IFN-γ.

Currently, the FDA (food and drug administration) has approved seven monoclonal antibodies targeting classical inhibitory immune checkpoints for the clinical treatment of patients with numerous cancer types: ipilimumab targeting CTLA-4 pathway, and six antibodies targeting PD-1/PD-L1 axis, including atezolizumab, avelumab, durvalumab, nivolumab, cemiplimab, and pembrolizumab. The FDA approval status for each of these antibodies in various cancer types in summarized as below.

Table 2 The list of FDA-approved monoclonal antibodies acting as inhibitors of negative checkpoints in human cancer

Checkpoint Inhibitor	Antibody Format	Examples of Types of Cancers with FDA-Approved Use	Year of First Approval
Ipilimumab	Human anti-CTLA4 IgG1	Melanoma, renal cell carcinoma (RCC), metastatic colorectal cancer	2011
Pembrolizumab	Humanized anti-PD-1 IgG4	Melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), urothelial bladder cancer, Hodgkin's lymphoma, head and neck cancer, Merkel cell carcinoma, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer, gastric cancer, hepatocellular carcinoma (HCC), cervical cancer, primary mediastinal large B-cell lymphoma (PMBCL)	2014
Nivolumab	Human anti-PD-1 IgG4	Melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), urothelial bladder cancer, Hodgkin's lymphoma, head and neck cancer, colorectal cancer, hepatocellular carcinoma (HCC), small cell lung cancer (SCLC)	2014
Atezolizumab	Humanized anti-PD-L1 IgG1	Non-small cell lung cancer (NSCLC), urothelial bladder cancer, small cell lung cancer (SCLC), triple-negative breast cancer	2016
Avelumab	Human anti-PD-L1 IgG1	Merkel cell carcinoma, urothelial bladder cancer, renal cell carcinoma (RCC)	2017
Durvalumab	Human anti-PD-L1 IgG1	Non-small cell lung cancer (NSCLC), urothelial bladder cancer	2017
Cemiplimab	Human anti-PD-L1 IgG4	Cutaneous squamous-cell carcinoma (cSCC)	2018

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