Review Article

A narrative review of immune checkpoint mechanisms and current immune checkpoint therapy

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Abstract: In recent decades, the unique advantages of immunotherapy have led to it playing a pivotal role in clinical and scientific research. Immune checkpoints as the key regulatory molecules of immune activation are critical to the function of the immune system. Many foreign invaders, such as cancer cells and other pathogens, overactivated immune checkpoints to escape capture by the immune system. Immune checkpoint therapy has been developed to counter this overactivation, and has achieved considerable clinical success. Cytotoxic T lymphocyte-associated antigen-4 and programmed cell death protein 1 are the two most well-known receptors that downregulate immune responses, and the scientists that discovered these molecules were awarded the 2018 Nobel Prize in Physiology or Medicine. Many other immune checkpoints have been described, such as lymphocyte-activation gene 3, T cell immunoglobulin and mucin domain 3, T-cell immunoreceptor with immunoglobulin and ITIM domain, tumour necrosis factor receptor 2, 4-1BB, CD27 and inducible T-cell co-stimulator (ICOS). Some monoclonal antibodies that target immune checkpoints have led to significant clinical responses, but they have limited monotherapeutic efficacy. Combination therapy may thus provide more effective treatment. In this review, we summarise the mechanisms of the major immune checkpoint molecules and the application of selected immune checkpoint inhibitors in clinical cancer therapy. Finally, we briefly discuss the future application and development of immunotherapies that target immune checkpoints.

Keywords: Immunotherapy; immune checkpoints; immune checkpoint therapy; combination therapy; regulatory mechanism

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Introduction

The immune system of an organism protects it against foreign invaders using three lines of defence. The first line of defence comprises the skin and mucous membrane, which act as a natural barrier; the second comprises macrophages and bactericidal substances, which act as the 'vanguard' of the immune response; and the third comprises the immune organs and cells, which generate specific immune responses. A series of immune checkpoints, with many signalling pathways, control the balance of effective immunity and self-tolerance. Stimulatory checkpoint pathways promote immune responses, while inhibitory checkpoint pathways inhibit immune responses (1).

However, some cancer cells and pathogens use various mechanisms to escape the immune system, such as by overactivating inhibitory immune checkpoints. Therefore, scientists have developed a range of immunotherapy

treatments. Due to the demonstrated efficacy of several of these therapies, such as brake antibodies and genetically modified T cells, *Science* magazine deemed cancer immunotherapy the 'Breakthrough of the Year' in 2013 (2). In addition, the Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo in 2018 for their development of cancer therapy using immune checkpoint inhibitors (ICIs) of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death 1 protein (PD-1) (https://www.nobelprize.org/prizes/medicine/2018/press-release/).

ICIs inhibit the overactivation of immune-checkpoint signalling pathways to a certain extent, thus promoting the immune response. ICIs have achieved prominent success in clinical trials, and the first ICI was approved in 2011 for the treatment of advanced melanoma. Currently, ICIs are also used to treat many other cancers, such as non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (3).

However, the monotherapeutic efficacy of ICIs is limited and resistance that develops after the initial clinical response is a major problem. However, new inhibitory and stimulatory pathways are good targets for immune checkpoint therapy (4,5) and combination therapy for cancer, thus providing patients with more treatment options.

In this review, we briefly summarise the mechanisms of the major immune checkpoint molecules in the immune system and the development of effective ICI drugs for clinical cancer therapy. Finally, we discuss the challenges and future directions of immune checkpoint cancer therapy, based on previous clinical studies. We present the following article in accordance with the Narrative Review reporting checklist (available at https://aob.amegroups.com/article/view/10.21037/aob-21-3/rc).

Summary of immune modulatory mechanisms

The T-cell-mediated immune response involves multiple successive steps, including the positive and negative selection of T cells, which are then activated and proliferate in secondary lymphoid tissues. Subsequently, the T cells migrate to sites containing antigens and exhibiting inflammation, to execute direct effector functions (via cytokines, chemokines and ligands). A balance of the stimulatory and inhibitory signals of these steps is crucial for regulation of the immune response (6).

Co-stimulatory and inhibitory receptors and their ligands that regulate T-cell activation are generally not overexpressed in cancer tissues compared with normal tissues. However, inhibitory receptors and ligands that regulate T-cell effector functions are generally overexpressed in tumour cells or non-transformed cells in the tumour microenvironment. Soluble and membrane-bound receptor-ligand immune checkpoints are good targets for agonist antibodies (to synergistically stimulate pathways) or antagonist antibodies (to inhibit pathways). Therefore, unlike most antibodies that are approved for cancer therapy, these antibodies function by targeting lymphocyte receptors or their ligands to enhance their endogenous anti-tumour activity, rather than by directly targeting tumour cells (7).

Normally, immune checkpoints enable the immune system to respond to infections and malignancies, to protect normal tissues from damage. However, some of the immune checkpoint proteins are expressed in malignant cells, leading to immune dysregulation and the facilitation of tumour growth and expansion (8).

Here, we detail some of the most commonly studied immune checkpoint molecules including inhibitory and costimulatory molecules. We also discuss the role of immune checkpoint molecules that carry out opposite functions as membrane proteins or soluble proteins (such as Lymphocyte-activation gene 3, LAG3).

T cell-associated inhibitory molecules

CTLA-4

CTLA-4 is a type 1 transmembrane glycoprotein of the immunoglobulin superfamily and is similar to CD28. CTLA-4 consists of a signal peptide, an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic tail (9,10). CTLA-4 combines with oligomerised CD80 (B7-1) and CD86 (B7-2) ligands to deliver an inhibitory signal (11).

Mechanisms of CTLA-4

The interaction of CTLA-4 with CD80/86 inhibits T-cell activation via antagonism of CD28-mediated co-stimulation and suppresses interleukin-2 (IL-2) secretion and T-cell proliferation, but does not induce apoptosis (12-14). In addition, CTLA-4-expressing cells capture CD80/86, which induces the degradation of these ligands via trans-endocytosis (15).

One of the most significant biological features of CTLA-4 is its intracellular localisation and transport patterns. Most CTLA-4 resides in intracellular vesicles and endosomal

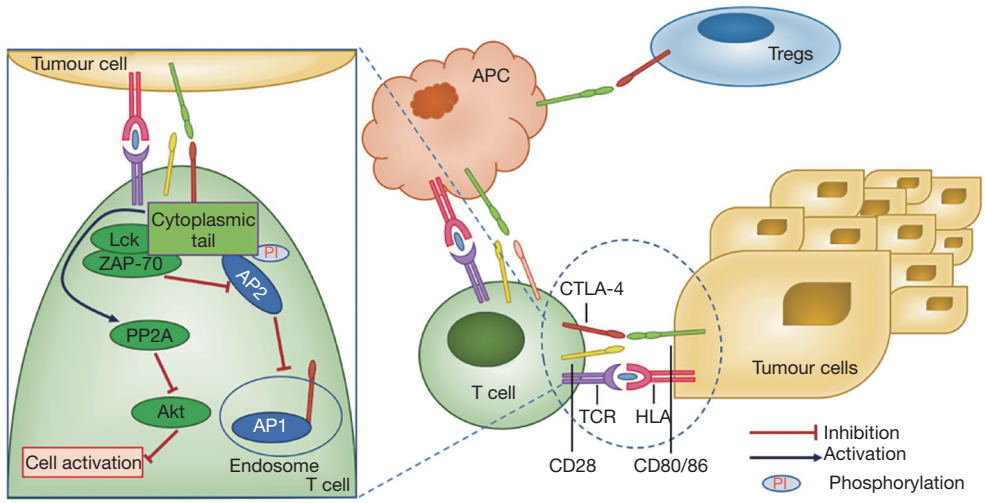

Figure 1 The mechanism of CTLA-4 signalling pathway. When TCR is engaged, the intracellular vesicles containing CTLA-4 relocate to the immune synapse. The cytoplasmic tail of CTLA-4 is phosphorylated by Lck and ZAP-70, which disrupts the intracellular transport of CTLA-4 with the interaction of AP-2. CTLA-4 inhibits T cell activation through activate PP2A to inhibit Akt signalling. The mechanism in the solid blue line box shows a detailed view of the immune synapse in the dotted blue circle. APC, antigen presenting cell; Tregs, regulatory T cells; TCR, T cell receptor; HLA, human leukocyte antigen; mAb, monoclonal antibody; Lck, lymphocyte-specific protein tyrosine kinase; ZAP-70, ζ-chain-associated protein kinase 70; PP2A, protein phosphatase 2A; Pi, phosphorylation; AP2, activator protein 2.

compartments throughout the Golgi apparatus (16-18). In resting T cells, a small amount of CTLA-4 protein continues to circulate from the Golgi apparatus to the cell surface, after which it undergoes rapid endocytosis and lysosomal degradation (18). The cytoplasmic tail of CTLA-4 binds to the clathrin-associated adaptor proteins, activator protein-1 (AP-1) and AP-2/AP-50, to mediate its intracellular transport. In addition, the interaction between CTLA-4 and protein-T cell receptor-interacting molecule (TRIM) is important for the intracellular localisation and transport of CTLA-4 (12).

If T cell receptor (TCR) is engaged, CTLA-4 expression is induced, and intracellular vesicles containing CTLA-4 relocate to the immune synapse (16). The cytoplasmic tail of CTLA-4 is phosphorylated at Y165 by TCR-induced kinases; lymphocyte-specific protein tyrosine kinase (Lck) and f-chain-associated protein kinase 70 (ZAP-70), which disrupts the interaction of CTLA-4 and AP-2, and maintains cell-surface levels of CTLA-4 in the immune synapse (12). Notably, if the TCR signal is strong, more CTLA-4 accumulates in the immune synapse, which provides a dynamic and adjustable inhibitory signal (see *Figure 1*) (19).

The ratio of B7 binding to CD28 or CTLA-4 determines whether T-cell activation is initiated or terminated (20). CTLA-4:B7 binding transmits the inhibitory signal in addition to blocking the stimulatory signal (21,22). Furthermore, there is evidence that CTLA-4 activates inhibitory signals through CD80/CD86 and induces the expression of indoleamine 2,3-dioxygenase (IDO) in antigen presenting cells (APCs), leading to localised tryptophan depletion and effector T cell inhibition, and the induction of regulatory T cells (Tregs) (23,24).

Researchers have demonstrated that CTLA-4 deletion in Tregs leads to spontaneous systemic lymphocyte proliferation, deadly autoimmune diseases and large amounts of immunoglobulin E production in mice with a resultant strong tumour immunity. CTLA-4 deletion in Tregs also impaired the ability of Tregs to inhibit the expression of CD80 and CD86 on dendritic cells (DCs), suggesting that Tregs may need CTLA-4 to inhibit the ability of APCs to recruit other T cells to suppress the immune response (25). In addition to the mechanisms described above, the depletion of Tregs is also considered to be a mechanism by which anti-CTLA-4 treatment functions in mouse tumour models (26).

CTLA-4 blockade appears to inhibit tumours via many different mechanisms. The primary mechanism is believed to involve direct competition of CTLA-4 with CD28 for binding to CD80/86 (27). This mainly occurs in tumour-

draining lymph nodes, as tumour cells do not express B7 ligands, and APCs can cross-present tumour antigens to primary tumour-reactive T cells. In either case, tumour cell death requires the release of tumour cell antigens, such as neoantigens and tumour-associated antigens, which are then processed and presented by APCs. In the case of effective antigen presentation, CTLA-4 blockade enhances the co-stimulation of CD28 and thus activates T cell immune response (27).

The regulation of the TCR repertoire may also contribute to the therapeutic effects of CTLA-4 inhibition. Mechanistically, the absence of CTLA-4 may reduce the threshold of TCR ligation that is required to effectively activate T cells, because CTLA-4 usually weakens the intensity of the TCR signal (27). Thus, the blockade of CTLA-4 increases the mutual stimulation of T cells in a variety of ways, leading to more active tumour-reactive T cells.

Application

A seminal study showing that anti-CTLA-4 antibodies promoted anti-tumour immune responses in mouse tumour models (28) led to the clinical development of the anti-CTLA-4 antibody ipilimumab for cancer therapy, especially for melanoma treatment for Food and Drug Administration (FDA) approval. Significantly, ipilimumab was the first checkpoint inhibitor to gain regulatory approval for therapeutic use in the United States (29). Another antibody, tremelimumab, is a fully humanised IgG2 isotype monoclonal antibody (mAb) against CTLA-4. In a phase 1/2 clinical trial of melanoma patients, tremelimumab demonstrated an objective response rate (ORR) equivalent to standard chemotherapy (30).

Acute myeloid leukemia (AML) patients with the CTLA-4 CT60 AA genotype, which can generate more soluble form of CTLA-4 had increased of recurrence risk after conventional therapy and lower 3-year overall survival (31). 42% AML patients have effective immune responses with ipilimumab at 10 mg/kg dose, among them 3 responses were sustained for over 1 year (32). A phase I clinical study of the combination of ipilimumab and decitabine is in progress, which has achieved early clinical activity, particularly in relapsed/refractory (r/r) AML patients without transplanting (33).

New evidence suggests that anti-CTLA-4 treatment does not have a general effect on all T cells; rather, CTLA-4 blockade leads to the specific amplification of tumour neoantigen-specific CD8 T cells in the tumour microenvironment, rather than in secondary lymphoid organs (34). In fact, anti-CTLA-4 treatment can lead to the expansion of specific tumour-infiltrating T-cell populations, including the phenotypic depletion of CD8 T-cell subsets and PD-1^{+}ICOS^{+}TBET^{+} T helper type 1 (Th1)-like CD4 effector T-cell populations (where ICOS = inducible T-cell co-stimulator) (35). These populations appear to be different from typical Th1 cells, as the co-expression of ICOS and PD-1 are markers of T follicle-helper cells. These findings are supported by clinical observations of ICOS^{+} CD4 effector T-cells amplification after ipilimumab treatment of a variety of tumours (36-39), and after treatment with tremelimumab (40). Therefore, the amplification of ICOS^{+} CD4 effector T-cells may be used as a pharmacodynamic marker for anti-CTLA-4 therapy (41).

PD-1

PD-1, also called CD279, is an inhibitory receptor that belongs to the immunoglobulin superfamily. It is expressed on activated T cells, B cells, natural killer (NK) cells, DCs and activated monocytes (42).

PD-1 binds to two distinct ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), which are members of the B7 protein family (42). In addition, PD-L1 has been reported to bind B7-1 (43). Experimental evidence suggests that the B7-1:PD-L1 interaction inhibits T cell function in a PD-1-independent manner (44). The specific functions of PD-L2 are less clear, as PD-L2-deficient mice have been reported to have increased (45) or decreased T-cell responses (46).

Mechanisms of PD-1

PD-1 are important in the maintenance of peripheral tolerance and the expected physiological response of T cells. Through interactions with PD-L1 and PD-L2, PD-1 regulates T-cell activation (47,48).

After stimulation of TCR, PD-1 is phosphorylated at tyrosine residues between the immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic tail, resulting in the recruitment of phosphatases SHP-1 and SHP-2, which further dephosphorylate proximal signalling molecules downstream of TCR and CD28 (47-49). Point mutation studies indicate that the ITSM motif is necessary for inhibition by PD-1 (50,51). In addition, PD-1 ligation and recruitment to immune synapses appears necessary to mediate the inhibition of proximal TCR signals (51,52).

CTLA-4 and PD-1 inhibit Akt-induced T cell activation, thereby inhibiting the CD28-mediated induction of glucose

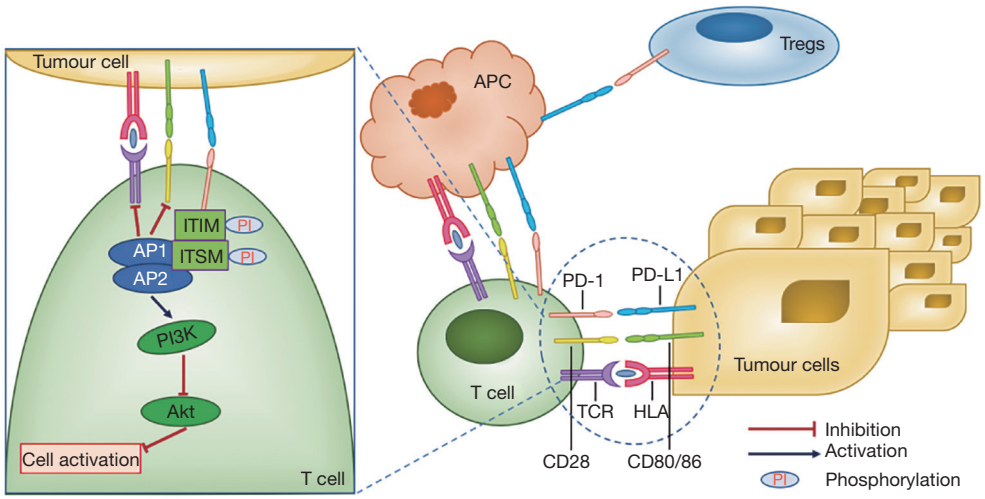

Figure 2 The mechanism of PD-1 signalling pathway. After stimulation of TCR, PD-1 is phosphorylated at tyrosine residues between the ITIM and ITSM in the cytoplasmic tail, then recruits phosphatases SHP-1 and SHP-2, which further dephosphorylate proximal signalling molecules downstream of TCR and CD28. PD-1 inhibits T cell activation through activate PI3K by SHP-2 to inhibit Akt signalling. The mechanism in the solid blue line box shows a detailed view of the immune synapse in the dotted blue circle. ITIM, immunoreceptor tyrosine-based inhibition motif; ITSM, immunoreceptor tyrosine-based switch motif; PI3K, phosphoinositide 3-kinase.

uptake. However, their levels of inhibition are different (49). PD-1 inhibits Akt activation through the activation of proximal phosphoinositide 3-kinase (PI3K) by SHP-2, but CTLA-4 inhibits Akt activation through the activity of protein phosphatase 2A (PP2A) (see *Figure 2*) (49,53). Therefore, the combination of CTLA-4 and PD-1 results in the activation of partially overlapping yet distinct intracellular signalling pathways.

To maintain the immune homeostasis, Tregs induced by the PD-1 pathway maintains a high threshold for T cells activation to prevent autoimmunity. PD-L1 has the ability to promote the development and functions of Tregs in lymphoid system to avoid autoimmune responses, as it is expressed on both non-hematopoietic cells and hematopoietic cells. PD-L1 may also promote the de novo development of Tregs in tissues expressing transforming growth factor-β (54).

PD-1 blockade may induce tumour rejection by reactivating CD8 T cells, and increasing their functional activity and number. In addition, blocking the PD-1 signalling axis can prevent the attenuation of proximal TCR signalling, which is mediated by PD-1, to restore the activity of exhausted CD8 effectors. Therefore, although PD-L1 is continuously expressed in the tumour microenvironment, exhausted T cells can still be reactivated to produce an effective immune response (27).

In addition to restoring T-cell activity by regulating TCR signalling and gene expression, blocking PD-1 signalling can partially reverse relevant metabolic reprogramming, which mediates T-cell reactivation (55). In addition to directly blocking PD-1, immune-based tumour rejection can also be induced by antibodies targeting PD-L1. Due to the dominant expression of PD-L1, its blockade is considered to largely phenocopy the effect of PD-1 blockade. PD-L1 is induced by Th1 cytokines, such as interferon gamma (IFN-γ), while PD-L2 is induced by Th2 cytokines (56).

PD-1 signalling also plays a role in haematological neoplasia. AML blasts can down-regulate the expression of human leukocyte antigen, while promoting the overexpression of PD-L1 and other inhibitory T cell ligands. It can also promote the release of reactive oxygen species, IDO, arginase, and extracellular vesicles, which not only inhibit T and NK cells activities, but also mobilize Tregs and myeloid-derived suppressor cells (MDSCs), and promote macrophages transformation from M1 to M2 phenotype (57).

Application

In 2015, nivolumab and pembrolizumab gained regulatory approval for use as monotherapy for advanced recurrent NSCLC. Nivolumab, which was the first anti-PD-1 antibody to be approved, demonstrated promising

therapeutic potential in phase I clinical trials, particularly for metastatic melanoma patients, NSCLC, and renal cell cancer (58). Pembrolizumab is a highly selective humanised IgG4 mAb against PD-1 (59). The success of pembrolizumab led to the rapid development of several other antibodies that block the PD-1/PD-L1 pathway for cancer therapy, such as pidilizumab (CureTech), MPDL3280A (Genentech), BMS-936559 (Bristol-Myers Squibb), and MEDI4736 (MedImmune/AstraZeneca).

Nivolumab and pembrolizumab have achieved an exciting overall response rate in r/r classical Hodgkin lymphoma (cHL) of 65–87% in phase I/II studies. Pembrolizumab has get shown an overall response rate in primary mediastinal large B-cell lymphoma (PMBL) of 48%. Nivolumab also acquired 36% and 40% ORR in diffused large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) patients, respectively. A phase II trial is ongoing with pembrolizumab in the treatment of DLBCL patients (60). For AML patients, nivolumab combined with azacitidine have achieved 33% ORR (57).

The combination of anti-CTLA-4 and anti-PD-1/PD-L1 mAbs in checkpoint-blockade cancer treatment can be both an opportunity and a challenge for immune therapy. However, progress has been made in combination therapy strategies in recent studies. Interim phase I results have been reported for nivolumab and ipilimumab as first-line therapy in patients with advanced NSCLC (NCT01454102), showing that this combination treatment had acceptable toxicity and activity in both PD-L1$^+$ and PD-L1$^-$ patients (61). The combination of pembrolizumab and ipilimumab as second-line therapy in stage IIIB/IV NSCLC is currently being studied in the KEYNOTE-021 trial (NCT02039674), and has shown complete responses of 9% and partial responses of 45% for certain clinical aspects (61).

Lymphocyte activation gene-3 (LAG-3)

LAG-3 is the ligand of major histocompatibility complex (MHC) class II molecules and is thus a member of the immunoglobulin superfamily (62,63). It is expressed on activated NK cells and T cells, but not on resting T cells (62,64). MHC II, fibrinogen-like protein 1 (FGL1), galectin-3, LSECtin and α-synuclein are all ligands for LAG-3. FGL1 is an inhibitory ligand of LAG-3, independent of MHC II (65).

Mechanisms of LAG-3

LAG-3 is a negative regulator of T-cell activation. It is expressed as a co-receptor on T cells and modulates effector T-cell activity and Tregs suppressor activity (66).

The combination of LAG-3 and MHC class II, together with the CD40 and CD40L (the ligand of CD40) can affect the secretion of IL-12 and IFN-γ by APCs *in vitro* (67). Soluble LAG-3 can directly induce the production of Th1 cytokines or chemokines, such as macrophage-derived chemokine and thymus activation-regulated chemokine, by DCs. In response to these signals, mature DCs migrate to lymph nodes (68,69).

LAG-3 has also been found to be selectively upregulated on CD4$^+$ Tregs (70). More recently, LAG-3 blockade (or genetic knockout) has been shown to affect the ability of conventional T cells to be suppressed by Tregs (71,72). Additionally, LAG-3 can maintain a tolerogenic state in CD8 cells, thus LAG-3 blocking antibodies augment CD8 T cell function *in vivo*, in the absence of CD4 T cells (73).

Application

The above preclinical studies have led to the development of two anti-LAG-3 molecules, BMS-986016 and LAG525, which are in clinical trials. BMS-986016 is an mAb antagonist of LAG-3 and is currently being assessed in five active clinical trials. These are phase I or II trials for the treatment of a variety of advanced solid tumours and haematological diseases. Most trials of BMS-986016 are being conducted in combination with a PD-1 inhibitor. LAG525 is being studied in a phase I/II clinical trial (NCT02460224), in combination with a PD-1 inhibitor, for the treatment of patients with advanced solid tumours (74).

IMP321 is a soluble form of LAG-3 that contains the first four extracellular domains of LAG-3, but lacks the transmembrane domain and intracellular region. It enhances tumour immune responses by upregulating co-stimulatory molecules and increasing IL-12 production. IMP321 has been assessed in two phase I clinical trials for the treatment of advanced renal cell carcinoma and pancreatic adenocarcinoma. It was shown to increase the number of tumour-reactive T cells, but no significant objective response (OR) was observed (75,76). IMP321 combined with paclitaxel for metastatic breast cancer treatment has also been assessed in a phase I clinical trial, in which an OR rate of 50% was observed (77). With this promising result, this study is currently recruiting metastatic breast cancer patients for a phase IIb clinical trial (NCT02614833) (78).

REGN3767, a fully human IgG4 antibody that in combination with a similar antibody, REGN2810, blocks the interaction of LAG3 and MHC II, has exhibited greater antitumour efficacy in preclinical tumour models than

either antibody alone (79).

T cell immunoglobulin and mucin domain 3

T cell immunoglobulin and mucin domain 3 (TIM-3) is expressed on the surface of activated T cells, NK cells, and monocytes. Galectin-9 and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) are the main ligands of TIM-3, among others (80,81).

Mechanisms of TIM-3

Upon binding to galectin-9 and several other ligands, TIM-3 facilitates peripheral immune tolerance by inducing Th1 cell death (82). TIM-3 is thought to be an important regulator of $CD8^+$ T cell exhaustion in cancer (83). Recent studies have shown that TIM-3 is co-expressed with and interacts with CECAM1. This interaction is crucial to the regulatory function of TIM-3 (84).

Studies have shown that PD-1 is co-expressed with TIM-3 in tumour-infiltrating lymphocytes, suggesting a potential synergistic effect between these two checkpoint co-inhibitors. $TIM\text{-}3^+$ $PD\text{-}1^+$ tumour-infiltrating lymphocytes have an exhausted phenotype and secrete less IFN-γ, IL-2, and tumour necrosis factor alpha (TNF-α) (81,85).

Application

Preclinical studies indicate that TIM-3 inhibition enhances the function of effector T cells in the tumour microenvironment and increases their anti-tumour effect. This is especially true when TIM-3 blockage is combined with PD-1 inhibition. In mouse colorectal cancer models, the dual blockade of PD-1 and TIM-3 was more effective than monotherapies at inhibiting tumour growth (85). In addition, in a mouse model of head and neck squamous cell carcinoma, treatment with an anti-TIM-3 mAb resulted in decreased tumour growth, through the recovery of effector T-cell function (86).

A preclinical study of mice with lung adenocarcinoma found that TIM-3 expression increased in the tumours in the absence of PD-1 blockade. Subsequent TIM-3 blockade resulted in a significant survival advantage. The researchers then analysed two patients treated with PD-1 blockade and found that, with the increase in TIM-3 expression, a similar adaptive resistance pattern was observed (87). These promising data from preclinical studies have resulted in the development of two anti-TIM-3 mAbs, TSR-022 and MGB-453, which are currently being assessed in phase I clinical trials, in combination with PD-1 inhibitors, for treatment of patients with advanced solid tumours (NCT02817633, NCT02608268) (74).

T-cell immunoglobulin and ITIM domain

The T-cell immunoglobulin and ITIM domain (TIGIT) is part of the CD28-like family of receptors and is expressed in NK and T cells. TIGIT is composed of an extracellular IgV domain, a type 1 transmembrane region and a cytoplasmic tail. The cytoplasmic tail contains an ITIM domain and an immunoglobulin tail tyrosine (ITT)-like motif (88).

Mechanisms of TIGIT

TIGIT binds to CD155 to induce the phosphorylation of its cytoplasmic tail by Fyn and Lck. The phosphorylation of TIGIT causes its ITT-like motif to bind β-arrestin 2, which leads to the recruitment of SH2 domain containing inositol-5-phosphatase 1 (SHIP1) via the cytosolic adaptor growth-factor-receptor-bound protein 2 (Grb2). SHIP1 then blocks specific pathways, including the PI3K, mitogen-activated protein kinase (MAPK) and nuclear factor-κB signalling (NF-κB) pathways. The combined effects of TIGIT on these signalling pathways strongly inhibits the function of NK cells (89,90).

TIGIT has been shown to directly inhibit T cells in a cell-intrinsic manner, by targeting molecules in the TCR signalling pathway (91). As a result, TIGIT inhibits the activation, amplification and effector functions of T cells. In addition, TIGIT indirectly suppresses T-cell responses by interacting with CD155 on DCs (92).

TIGIT also contributes to immune suppression by promoting the function of Tregs. *TIGIT* is a direct target gene of forkhead box protein P3 (Foxp3), the master transcription factor in Tregs (93). Moreover, TIGIT expression levels correlate with markers of natural Tregs, rather than those of peripherally induced Tregs. $TIGIT^+$ Tregs show enhanced demethylation in Treg-specific demethylation regions, compared to their $TIGIT^-$ counterparts, which leads to greater lineage stability. Further, $TIGIT^+$ Tregs express higher levels of Treg markers, such as Foxp3, CD25 and CTLA-4, and the expression of TIGIT on Treg cells results in the upregulation of the inhibitory mediator, fibrinogen-like protein 2 (Fgl2), which enhances the suppressive function of $TIGIT^+$ Tregs. Importantly, the TIGIT-dependent expression of Fgl2 results in the selective sparing of Th2 cell responses, while potently suppressing the responses of Th1 and Th17 cells (94).

CD226 (DNAM-1) and CD96 (Tactile) are known to

bind TIGIT. CD226 transmits a positive co-stimulatory signal (95), whereas CD96 transmits an inhibitory signal (96). Similar to CTLA-4, TIGIT has a much higher affinity for ligands than CD226. Therefore, TIGIT blocks the interaction between CD226 and CD155, to inhibit co-stimulatory signals (92). However, TIGIT directly binds CD226 in cis, which disrupts the homodimerization of CD226 and inhibits its co-stimulatory function (97).

Application

In vitro studies and *in vivo* studies in mice have shown that dual blockade of TIGIT and PD-1 or TIM-3 has a synergistic effect on immune cell amplification, cytokine release, threshing, and the reversal of T cell exhaustion, resulting in tumour rejection and the induction of protective memory responses (98,99). Significantly, the expression levels of TIGIT in cells in the tumour microenvironment appear to be higher than the levels in peripheral cells, which should theoretically provide a more targeted, less toxic treatment method. In addition, TIGIT mainly appears to limit the competency of cytokines and the function of CD8 T cells, which accounts for its complementing the activity of other types of ICIs (82).

A phase I clinical trial (NCT03119428) is currently recruiting patients to assess the safety and potency of the anti-TIGIT mAb, OMP-31M32. However, the results of this trial are not yet available (8).

Tumour necrosis factor receptor 2 (TNFR2)

TNFR2 is a ligand of tumour necrosis factor alpha (TNFα), which has another ligand TNFR1. TNFα is an inflammatory cytokine with dual function. While it interacts with the widely expressed TNFR1 causing pro-inflammatory role and cell death, it also interacts with the limitedly expressed TNFR2 causing anti-inflammatory role and cell survival (100).

Mechanisms of TNFR2

The binding of TNF to TNFR2 induces the activation of NF-κB through NF-κB-inducible kinase (NIK), which further leads to phosphorylation of IKKα and p100 processing. TNFR2 also recruits the TRAF2-cIAP1-cIAP2 complex. cIAP has ubiquitin ligase activity, which can inhibit caspases and other apoptosis-inducing factors, thereby activating NF-κB/Rel and MAPK signalling pathways. TNFR2 signal transduction is mediated through RIPK1 and Etk respectively. RIPK1 triggers NF-κB through the IkB kinase (IKK) complex, which activates the expression of IL-2 and increases the transcription of several genes that are positively related to cell survival and proliferation. TNFR2-mediated phosphorylation of Etk can partially activate vascular endothelial growth factor receptor 2 (VEGFR2), then activates the PI3K/Akt signalling pathway to maintain cell survival and proliferation. It can also form a TNFR2-Etk-VEGFR2 complex by recruiting Etk to promote cell activation. In addition, it also enhances the phosphorylation of STAT5, which plays a key role in immunosuppression (101,102).

The stability, response to TCR stimulation, amplification and function of Tregs is enhanced when TNFR2 signal is activated. Researchers have demonstrated that blocking intrinsic membrane-bound TNF/TNFR2 signalling in CD4+ T-cells reduces IL-2 production and elevates Th17 differentiation which also correlated with enhanced STAT3 activity, increased ROR-γt level, and decreased STAT5 activity. The membrane-bound form mTNFR2 may change into the soluble form sTNFR2, which then binds TNF to inhibit the expression of IL-6 in inflammatory conditions (101).

Application

TNFR2 is expressed as an oncogene on many tumours. At present, the expression of TNFR2 has been determined in at least 25 tumours, such as ovarian cancer, colon cancer, multiple myeloma, cutaneous T-cell lymphoma (CTCL), *et al.* The tumour microenvironment cunningly recruits highly immunosuppressive TNFR2$^+$ Tregs, thereby promoting tumour immune escape. The increased expression of TNFR2 gene in Tregs in patients with metastatic melanoma is related to the depletion of CD8$^+$ T cells. TNFR2 knockout mice showed an enhanced immune response to tumours, and impaired tumour growth. This may be due to the lack of TNFR2$^+$ Tregs, or the failure to develop systemic autoimmunity, or the decrease in the number of MDSCs and impaired function (103).

In vitro studies have shown that TNFR2 antagonist antibodies can inhibit the proliferation of ovarian cancer cells and tumour-related Tregs (104). Tumour cells expressing TNFR2 in advanced Sézary syndrome can be eliminated by TNFR2 antagonist antibodies, and TNFR2 antagonist antibodies can also kill TNFR2$^+$ Tregs, adjust Treg/Teff ratio to normal level by inhibiting Tregs and Teff (103).

T cell-associated costimulatory molecules

4-1BB

4-1BB as a member of the TNFR superfamily 9, is a costimulatory molecule interaction with 4-1BBL, which is

expressed in the surface of activated T cells and NK cells (105).

Mechanisms of 4-1BB

4-1BB recruits TNFR-associated factors (TRAF), TRAF1 and TRAF2 to form heterotrimers, through extracellular signal-regulated kinase (ERK) pathway, β-catenin and AKT pathways to enhance signal transduction. The main transcription factor NF-κB and MAPKs regulates 4-1BB signal to promote the production and secretion of IL-2 and IFN-γ, while promoting the survival and activation T cells by increasing the expression of antiapoptotic genes Bcl-xL and Bfl-1 (105,106).

However, the effect of 4-1BB on Tregs is still very controversial: 4-1BB agonist treatment can either inhibit the differentiation of conventional effector cells into Treg, while impacting the inhibitory effect of Tregs, or maintain Tregs amplification and inhibition ability (107,108).

4-1BB is also expressed in many non-T cells, such as DCs, monocytes, B cells, mast cells, NK cells and neutrophils. 4-1BB up-regulates B7-1 and B7-2, and increases the secretion of IL-6 and IL-12 in DC cells. The agonistic anti-4-1BB monoclonal antibody enhances the ability of DCs to stimulate T cell amplification and promotes the phosphorylation of STAT3 to enhance the $CD8^+$ T cells response (109). After 4-1BB is triggered, NK cells upregulate 4-1BB and increase cytotoxic function, but 4-1BB activation on resting NK cells will reduce NK cells and impair the cytotoxic function of NK cells (105,110).

Application

CTL019 was the first CAR therapy to be approved by US Food and Drug Administration, which contains 4-1BB as an intracellular domain. Urelumab (BMS 663513) is a fully human IgG4 mAb, which is the first 4-1BB agonist antibody in clinical treatment. Utomilumab is a fully human IgG2 mAb, which has shown promising signs in patients with advanced solid tumours treated with monotherapy. The combination of utomilumab and pembrolizumab have shown 26% complete or partial responses (NCT02179918) (111).

CD27

CD27 is a member of TNFR superfamily, which is expressed in the early thymus of naïve $CD4^+$ and $CD8^+$ T cells (112).

Mechanisms of CD27

In the process of T cell activation, CD27 expression increased transiently, but after several rounds of T cells differentiation, the expression decreased (112). CD27 is also a marker of memory B cells, and expresses on NK cells (113,114). CD27 interacts with TRAF2 and TRAF5, while inducing the activation of NF-κB and MAPK8/JNK signalling pathway (115).

Application

Varlilumab is a CD27 agonist, which increases the production of chemokines, promotes T cells activation, and Tregs downregulation. It has shown 26% stable disease (SD) at 3 months in melanoma/renal cell carcinoma patients, and 1 partial response, 3 SD in 15 lymphoma patients. MDX-1203 is a CD70 mAb, which has shown 69% SD in patients (NCT00944905). SGN-75 is an antibody-drug conjugate of CD70 and monomethyl auristatin F, which also induces immune responses in renal cell carcinomas and lymphomas (NCT01015911) (116).

Inducible T-cell Co-stimulator (ICOS)

ICOS is a CD28 family protein, which is also called CD278 or AILIM, and mainly expressed in activated T cells. ICOS binding with a B7 family protein ICOSL plays the role of activating T cells (117,118).

Mechanisms of ICOS

ICOS binding with ICOSL, which is mainly expressed on APCs, through phosphoinositide 3-kinase (PI3K) signal pathway to enhance the function of Th1 and Th2 mainly by increasing the production of effector cytokines such as IL-4, IL-5, IL-10, IL-21, IFN γ and TNFα (119,120).

Application

T cells with high expression of ICOS had stronger immune response, upon using anti-CTLA-4 or anti-PD-1 mAbs to treat tumour of mice and patients, suggesting that ICOS maybe a useful target of tumour treatment (121-123). ICOS agonists are unlikely to be used for cancer monotherapy because they cannot directly induce a cytotoxic immune reaction independently. JTX-2011 is an ICOS agonist, which is used in combination with nivolumab in the phase 1 ICONIC clinical trial (NCT02904226). GSK3359609 is also a ICOS agonist, which is used in combination with pembrolizumab in phase I trials (NCT02723955) (116).

Methods

In this review, we mainly use PubMed (https://pubmed.ncbi.

nlm.nih.gov/) to search literatures for two different parts: mechanisms and current therapy. Because the mechanisms are well-documented in early published articles, the literature year for this part is earlier, whereas the literature year for current therapy is chosen for recent 5 years mostly with the newer clinical outcome. Studies published in higher impact factor journals were prioritised.

The future of immune checkpoint therapy

Immune checkpoint therapy is an important component of immune therapy, and has enabled significant progress in cancer treatment. However, the use of immune checkpoint therapy alone may have limitations and induce side effects, such as autoimmune conditions. The combination of immune checkpoint therapy with other forms of immune therapy, such as chimeric antigen receptor-T cells (CAR-T), TCR-T cells or vaccines, or in combination therapy with multiple ICIs, are therefore promising approaches for cancer treatment, and several clinical studies are already underway to assess these strategies. Nevertheless, further research is needed to determine the anti-cancer mechanisms of ICIs, to enable the development of effective combination therapies. The similarities and differences in the mechanisms of different immune checkpoints must be established, as this will maximise the benefits of combination therapy, while minimising adverse effects.

In this review, we discuss the mechanisms and summarize current therapies of main immune checkpoint. Some mAbs of immune checkpoint are already applied in clinical trials or preclinical studies, which can provide some ideas to other scientists. A better understanding of the mechanisms of immune checkpoint will benefit the design of protein drugs, or combination therapy with immune checkpoint-blockade. Immune checkpoint therapy is considered as both an opportunity and a challenge for researchers. With the continued joint efforts of scientists and clinicians, further progress will be made in the future.

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