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REVIEW

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Immune checkpoint inhibitors: breakthroughs in cancer treatment

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ABSTRACT Over the past two decades, immunotherapies have increasingly been considered as first-line treatments for most cancers. One such treatment is immune checkpoint blockade (ICB), which has demonstrated promising results against various solid tumors in clinical trials. Monoclonal antibodies (mAbs) are currently available as immune checkpoint inhibitors (ICIs). These ICIs target specific immune checkpoints, including cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1). Clinical trial results strongly support the feasibility of this immunotherapeutic approach. However, a substantial proportion of patients with cancer develop resistance or tolerance to treatment, owing to tumor immune evasion mechanisms that counteract the host immune response.

Consequently, substantial research focus has been aimed at identifying additional ICIs or synergistic inhibitory receptors to enhance the effectiveness of anti-PD-1, anti-programmed cell death ligand 1 (anti-PD-L1), and anti-CTLA-4 treatments. Recently, several immune checkpoint molecular targets have been identified, such as T cell immunoreceptor with Ig and ITIM domains (TIGIT), mucin domain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B and T lymphocyte attenuator (BTLA), and signal-regulatory protein α (SIRPα). Functional mAbs targeting these molecules are under development. CTLA-4, PD-1/PD-L1, and other recently discovered immune checkpoint proteins with distinct structures are at the forefront of research. This review discusses these structures, as well as clinical progress in mAbs targeting these immune checkpoint molecules and their potential applications.

KEYWORDS Immunotherapy; cancer; ICIs; PD-1; CTLA-4

Introduction

Cancer is a rapidly progressing disease with a high mortality rate[1]. In recent decades, novel therapeutic modalities, such as targeted therapies and immunotherapies, have emerged as supplements to conventional treatment approaches, such as surgery and radiation therapy. The landscape of cancer management has undergone a paradigm shift with the advent of immunotherapy. The discovery and development of immune checkpoint inhibitors (ICIs) substantially enhanced tumor treatment outcomes. Research in cancer immunology continues to explore innovative agents that target and elicit efficient immune responses. As of March 2023, 11 ICIs had received regulatory approval in the United States[2,3]. In 2018, Ryuji Ohno and James Allison were awarded the Nobel Prize for their contributions to the fields of programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4)[4]. Despite the remarkable therapeutic effects observed for some tumor types, a substantial proportion of patients exhibit inherent or acquired resistance to immune checkpoint interventions[5,6]. Consequently, understanding novel immune checkpoint molecules, such as T cell immunoreceptor with Ig and ITIM domains (TIGIT), mucin domain containing-3 (TIM-3), lymphocyte activation gene-3 (LAG-3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B and T lymphocyte attenuator (BTLA), and signal-regulatory protein α (SIRPα), has emerged as an active area of research.

This review focuses on progress in immune checkpoints in cancer treatment, as well as clinical trials of immune checkpoint combination therapies, to highlight the therapeutic potential of these targets (**Table 1**).

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Table 1 Clinical trials of ICI combination therapies

Combination therapy	Medicine type	Tumor type	Clinical trial ID
Retifanlimab + INCAGN02385 + INCAGN02390	PD-1 mAb + LAG-3 mAb + TIM-3 mAb	HNSCC	NCT04370704
Pembrolizumab + axitinib	PD-1 mAb + anti-angiogenic agent	Untreated advanced RCC	KEYNOTE-426
Sintiliumab + anlotinib	PD-1 mAb + anti-angiogenic agent	Uncommon EGFR-mutated NSCLC	NCT04790409
Toripalimab + SCRT + oxaliplatin + capecitabine	PD-1 mAb + radiotherapy + chemotherapy	MSS LARC	NCT04518280
Nivolumab + XELOX/FOLFOX	PD-1 mAb + chemotherapy	Advanced gastric cancer, GEJ cancer, and esophageal adenocarcinoma	CheckMate-649
Pembrolizumab + pemetrexed/platinum	PD-1 mAb + chemotherapy	Metastatic squamous NSCLC	KEYNOTE-189 KEYNOTE-407
Serplulimab + 5-fluorouracil + cisplatin	PD-1 mAb + chemotherapy	PD-L1-positive ESCC	NCT03958890
iCasp9M28z T cell + pembrolizumab	CAR-T + PD-1 mAb	Breast cancer and metastatic lung cancer	NCT02414269
N-803 + HSC CAR-NK + pembrolizumab	IL-15 superagonist + HSC CAR-NK + PD-1 mAb	GEJ and advanced HNSCC	NCT04847466
NY-ESO-1 TCR-T cell + pembrolizumab	TCR-T + PD-1 mAb	Relapsed/refractory multiple myeloma	NCT03168438
NY-ESO-1 TCR-T cell + DC vaccine + nivolumab	TCR-T + DC vaccine + PD-1 mAb	Sarcoma	NCT02775292
TCR-transduced T cells + CDX-1140 + pembrolizumab	TCR-T + PD-1 mAb + CD40 mAb	Malignant epithelial neoplasms	NCT05349890
FH-MCVA2 TCR-T cell + avelumab/pembrolizumab	TCR-T + PD-L1 mAb/PD-1 mAb	Metastatic or unresectable MCC	NCT03747484
FT500 + nivolumab/pembrolizumab/atezolizumab	iPSC-NK cell + PD-1 mAb/PD-L1 mAb	Advanced solid tumors	NCT03841110
Ipilimumab + nivolumab	CTLA-4 mAb + PD-1 mAb	NSCLC	CheckMate 227
Tremelimumab + durvalumab	CTLA-4 mAb + PD-L1 mAb	Metastatic NSCLC	NCT03164616
Tremelimumab + durvalumab + mFOLFOX6	CTLA-4 mAb + PD-L1 mAb +chemotherapy	Metastatic CRC	NCT03202758
NY-ESO-1 TCR-T cell + DC vaccine + ipilimumab	TCR-T + DC vaccine + CTLA-4 mAb	Advanced sarcoma or melanoma	NCT02070406
Relatlimab + nivolumab	LAG-3 mAb + PD-1 mAb	Unresectable melanoma	RELATIVITY-047
Favezelimab + pembrolizumab	LAG-3 mAb + PD-1 mAb	NSCLC	KEYNOTE-495/KeyImPaCT
Sabatolimab + spartalizumab	TIM-3 mAb + PD-1 mAb	Advanced solid tumors	NCT02608268
LY3300054 + LY3321367	TIM-3 mAb + PD-L1 mAb	MSI-H/dMMR tumors	NCT03099109
Vibostolimab + pembrolizumab	TIGIT mAb + PD-1 mAb	Solid tumors	NCT02964013
Tiragolumab + atezolizumab	TIGIT mAb + PD-L1 mAb	SCLC	SKYSCRAPER-01 and 02
Ociperlimab + tislelizumab	TIGIT mAb + PD-1 mAb	Advanced solid tumors	NCT04047862
Etigilimab + nivolumab	TIGIT mAb + PD-1 mAb	Advanced or metastatic solid tumors	NCT031119428

Table 1 Continued

Combination therapy	Medicine type	Tumor type	Clinical trial ID
HMBD-002 + pembrolizumab	VISTA mAb + PD-1 mAb	Colon cancer	NCT05082610
Tifcemalimab + toripalimab	BTLA mAb + PD-1 mAb	ES-SCLC	NCT05000684
HFB200603 + tislelizumab	BTLA mAb + PD-1 mAb	Advanced solid tumors	NCT05789069
ADU-1805 + pembrolizumab	SIRPα mAb + PD-1 mAb	Advanced solid tumors	NCT05856981

PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; LAG-3, lymphocyte activation gene-3; TIM-3, T cell immunoglobulin and mucin-domain containing-3; TIGIT, T cell immunoglobulin and ITIM domain; VISTA, V-domain immunoglobulin suppressor of T cell activation; BTLA, B and T lymphocyte attenuator; SIRPα, signal-regulatory protein α; HNSCC, head and neck squamous cell carcinoma; RCC, renal cell carcinoma; EGFR, epidermal growth factor receptor; NSCLC, non-small-cell lung cancer; SCRT, short-course radiation; MSS, microsatellite stable; LARC, locally advanced rectal cancer; XELOX, capecitabine + oxaliplatin; FOLFOX, 5-fluorouracil + leucovorin + oxaliplatin; GEJ, gastroesophageal junction; ESCC, esophageal squamous cell carcinoma; CAR-T, antigen receptor T; TCR-T, T cell receptor-engineered T; CAR-NK, chimeric antigen receptor natural killer; HSC, hematopoietic stem cell; iPSC-NK, induced pluripotent stem cell natural killer; DC, dendritic cell; MCC, Merkel cell carcinoma; mFOLFOX6, oxaliplatin + folinic acid + 5-fluorouracil; XELOX, capecitabine + oxaliplatin; CRC, colorectal cancer; MSI-H, microsatellite instability-high; dMMR, mismatch repair-deficient; SCLC, small-cell lung cancer; ES-SCLC, extensive stage small-cell lung cancer.

Traditional ICIs

PD-1/programmed cell death ligand 1 (PD-L1) monoclonal antibodies (mAbs)

Brief description of PD-1/PD-L1

PD-1, also known as CD279, is a member of the CD28 superfamily[7] that is expressed primarily in T cells, B cells, natural killer (NK) cells, and dendritic cells (DCs)[8] (**Table 2**). PD-1 is a protein of approximately 50 kDa comprising 3 parts: an extracellular hydrophobic transmembrane region, IgV-like domains in the N- and C-terminal regions, and 2 intracellular tyrosine residues. The interaction between PD-1 and its ligands, PD-L1 and programmed cell death ligand 2 (PD-L2), inhibits T cell activation and cytokine production. Recent studies[9] have shown that in certain tumors, such as head and neck squamous cell carcinoma (HNSCC) and advanced colorectal cancer (CRC), PD-L2 has a 2–6 times higher affinity for PD-1 than PD-L1, although PD-L1 is more widely expressed. PD-L1 and PD-L2 bind not only to PD-1 but also to their binding partners CD80 and RGMB, respectively, thus forming complexes with distinct roles[10]. The binding of PD-L2 to RGMB inhibits the activity of tumor-infiltrating T cells and cytokine secretion, whereas the binding of PD-L1 to CD80 promotes cytokine production and thus decreases the likelihood of immune evasion. The presence of PD-L2 has dual effects by acting as both an inhibitor and a promoter of T cell activation. Inhibiting the expression of PD-L2 alone might not achieve the desired results. Because of a lack of research on PD-L2 and an absence of consistent outcomes, PD-L2 inhibitors alone cannot be used for immunotherapy, and no PD-L2 inhibitors are available for use in oncology. In contrast, the role of PD-L1 is much better understood.

The cytoplasmic tail of PD-1 is composed of 2 tyrosine-based structural motifs: the immunoreceptor tyrosine-based inhibitory motif (ITIM) and the immunoreceptor tyrosine-based switch motif (ITSM)[11]. The core of PD-1's inhibitory function lies in the ITSM-Y248 residue rather than the ITIM-Y223 residue[12]. When PD-L1/PD-L2 binds PD-1, ITSM is phosphorylated by the T cell receptor (TCR) proximal Src family kinase. This phosphorylation triggers the recruitment of Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2). SHP2, an important dephosphorylase, plays a key role in the PD-1 signaling pathway: it regulates the activity of downstream signal transduction by dephosphorylating CD3ζ-chain-associated

Table 2 Immune checkpoints and their expression in cells

Checkpoint	Cells	Ligand
PD-1	T cells, DCs, NK cells, and B cells	PD-L1, PD-L2
CTLA-4	$CD4^+$ T cells, $CD8^+$ T cells, and Tregs	CD86, CD80
LAG-3	$CD4^+$ T cells, $CD8^+$ T cells, Tregs, NK cells, B cells, and DCs	MHC II, FGL-1, Gal-3, LSECtin
TIM-3	NK cells, monocytes, macrophages, DCs, and $CD4^+$ and $CD8^+$ T cells	Gal-9, CEACAM-1, PtdSer, HMGB-1
TIGIT	$CD4^+$ and $CD8^+$ T cells, NK cells, and DCs	CD155, CD112
VISTA	Basophils, monocytes, resting T cells, memory T cells, and $CD68^+$ tumor-associated macrophages	VSIG-3, PSGL-1
BTLA	$CD4^+$ and $CD8^+$ T cells, DCs, NK cells and macrophages	HVEM
SIRPα	DCs, macrophages, and neutrophils	CD47

PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PD-L2, programmed cell death ligand 2; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; LAG-3, lymphocyte activation Gene-3; TIM-3, T cell immunoglobulin and mucin-domain containing-3; TIGIT, T cell immunoglobulin and ITIM domain; VISTA, V-domain immunoglobulin suppressor of T cell activation; BTLA, B and T lymphocyte attenuator; SIRPα, signal-regulatory protein α; DCs, dendritic cells; NK cells, natural killer cells; MHC II, major histocompatibility complex II; FGL-1, fibrinogen-like protein-1; Gal-3, galectin-3; LSECtin, liver and lymph node sinusoidal endothelial cell C-type lectin; Gal-9, galectin-9; CEACAM-1, carcinoembryonic antigen-related cell adhesion molecule-1; PtdSer, phosphatidylserine; HMGB-1, high-mobility group box-1; VSIG-3, V-Set and immunoglobulin domain containing-3; PSGL-1, P-selectin glycoprotein ligand-1; HVEM, herpes virus entry mediator.

protein of 70 kDa (ZAP70) and Phospholipase Cγ1 (PLCγ1)[3]. The interaction between SHP2 and the ITSM-Y248 residue of PD-1 has been verified through live cell imaging experiments[13]. SHP2 also inhibits the function of lymphocyte-specific protein tyrosine kinase (Lck), thereby inhibiting the phosphorylation state of the downstream molecule ZAP70 of Lck[14]. This process directly affects the PI3K/AKT signaling pathway and decreases activation of T cells[15].

Furthermore, SHP2 also blocks the RAS/MEK/ERK signaling pathway by inhibiting the activation of PLCγ1[16]. Alterations in this signaling pathway further affect T cell activation and function. SHP2 interacts with casein kinase (CK2), which in turn regulates the phosphorylation status of phosphatase and tensin homolog (PTEN). PTEN, a tumor suppressor gene, functions in normal cells by inhibiting cell proliferation and promoting cell differentiation through its phosphatase activity. Additionally, the PI3K/AKT signaling pathway, which is crucial for maintaining cellular homeostasis, is negatively regulated by PTEN. When SHP2 inhibits the activity of CK2, PTEN phosphorylation is suppressed, thereby maintaining its active state[3]. This process effectively blocks the downstream transmission of PI3K signals and consequently inhibits T cell activation[17] (**Figure 1A**).

Signal transducer and activator of transcription 3 (STAT3) as a transcription factor plays an important role in cancer[18]. Its rapid and transient activation is achieved through tyrosine phosphorylation within a series of complex signaling pathways. This process involves factors including the cytokine IL-6, which activates STAT3 phosphorylation, promotes tumor growth and survival, and suppresses T cell function[19]. Targeting the PD-L1/PD-1 pathway through the specific binding of PD-1 mAb or PD-L1 to PD-1 or PD-L1, respectively, has become an effective cancer treatment strategy. This approach blocks the interaction between these proteins, thereby disrupting the PD-L1/PD-1 signaling pathway and restoring T cell immune function to achieve cancer treatment (**Figure 1B**).

A considerable number of mAbs licensed by the Food and Drug Administration (FDA) in the United States are PD-1/PD-L1 mAbs, which are also the most commonly used mAbs in tumour immunotherapy.

PD-1/PD-L1 mAb monotherapy

In 2014, the FDA approved 2 PD-1 mAbs, nivolumab and pembrolizumab, for the treatment of advanced melanoma. Although both mAbs can be used in various cancer therapies to increase overall survival (OS), the efficacy of monotherapies might not benefit most patients, given the limited available clinical data, and the potential for inherent or acquired patient tolerance[20]. Combined therapy or switching to other treatments should be considered in a timely manner[21].

Notably, on March 22, 2023, Incyte's retifanlimab (PD-1 mAb) was approved by the FDA, representing a major advancement in the field of oncology treatment with ICIs. This therapy is the first

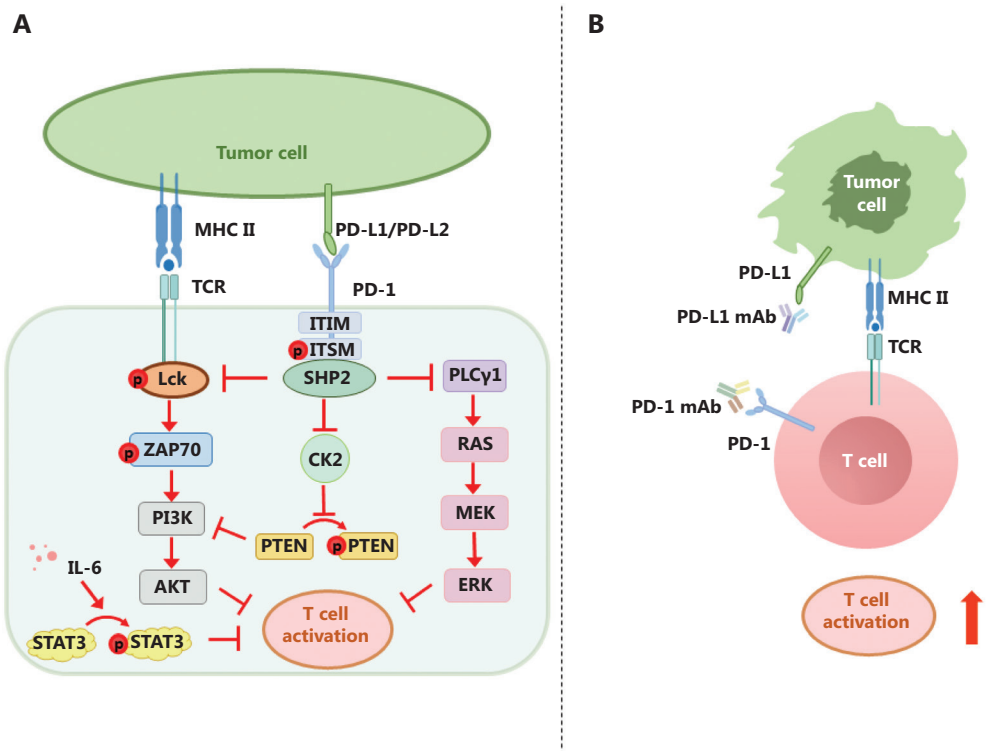

Figure 1 Inhibition of T cell activation by the PD-L1/PD-1 signaling pathway: (A) When PD-L1/PD-L2 binds PD-1, ITSM is phosphorylated by the Src family of kinases and recruits SHP2. SHP2 regulates the PD-1 signaling pathway by inhibiting key molecules such as ZAP70 and PLCγ1 through its phosphatase activity; it also modulates downstream signaling activity and inhibits the function of Lck and phosphorylation of its downstream molecule, ZAP70. This process directly inhibits the TCR-activated signaling pathway and decreases T cell activation. Furthermore, SHP2 inhibits the RAS/MEK/ERK signaling pathways by blocking the activity of PLCγ1. Additionally, SHP2 inhibits T cell activation by suppressing the activity of CK2, which in turn prevents the phosphorylation of PTEN and blocks downstream PI3K signaling. Furthermore, STAT3, a common transcription factor in cancer, is overactivated by IL-6 through phosphorylation, thus promoting tumor growth. These intricate regulatory mechanisms collectively impede the activation of T cells. (B) Targeting the PD-L1/PD-1 pathway through the specific binding of PD-1 mAb or PD-L1 to PD-1 or PD-L1, respectively, has become an effective cancer treatment strategy. This approach blocks the interaction between these two proteins and restores the immune function of T cells, thereby treating cancer by disrupting the PD-L1/PD-1 signaling pathway. PD-1, programmed cell death protein; PD-L1, programmed cell death ligand 1; PD-L2, programmed cell death ligand 2; ITSM, immunoreceptor tyrosine-based switch motif; SHP2, Src homology region 2-containing protein tyrosine phosphatase 2; ZAP70, CD3ζ-chain-associated protein of 70 kDa; PLCγ1, phospholipase Cγ1; CK2, casein kinase; PTEN, phosphatase and tensin homolog; Lck, lymphocyte-specific protein tyrosine kinase; TCR, T cell receptor; STAT3, signal transducer and activator of transcription 3.

PD-1 mAb developed to treat Merkel cell carcinoma (MCC)[2,22]. A phase II clinical trial (NCT03679767)[23] has recently published findings on the efficacy of retifanlimab in the treatment of solid tumors. The results have indicated significant anti-tumor activity in melanoma, non-small-cell lung cancer (NSCLC), and renal cell carcinoma (RCC). In addition, according to clinicaltrials.gov, Incyte is currently conducting a trial (NCT04370704) to assess the safety and efficacy of a combination therapy comprising retifanlimab, INCAGN02385 (LAG-3 mAb), and INCAGN02390 (TIM-3 mAb) as a first-line treatment for HNSCC. The findings of that trial remain to be published.

PD-1/PD-L1 mAb combination therapies

Combination therapy is currently considered an optimal approach to enhance the feasibility of cancer treatment by increasing the number of antigen-presenting cells (APCs) and released tumor antigens. Multiple clinical trials have demonstrated that PD-1/PD-L1 mAbs can be a part of highly effective therapeutic combination regimens, and have evaluated their clinical efficacy alongside other inhibitors in treating malignancies[24,25].

PD-1/PD-L1 mAbs plus anti-angiogenic agents Proliferative tumors may show altered ratios of pro-angiogenic to

anti-angiogenic factors in a malignant environment, thereby modifying the balance in favor of pro-angiogenic factors and activating angiogenesis. This phenomenon, also known as the "angiogenic switch," is crucial for tumor growth, proliferation, and metastasis[26]. Major anti-angiogenic agents include axitinib and sunitinib.

Blockade of immune checkpoints in conjunction with anti-angiogenic drugs may facilitate vascular normalization and increase the immune cell response to tumor regression[27]. Researchers have examined data from early stages of the KEYNOTE-426[28] clinical study to determine how pembrolizumab, an mAb against PD-1, and anti-angiogenesis medications (axitinib and sunitinib) might confer clinical advantages in patients with advanced RCC. After a long follow-up period, the KEYNOTE-426 clinical study has demonstrated that treating untreated advanced RCC with a combination of pembrolizumab and axitinib is beneficial for patients. In the recently conducted clinical trial NCT0479040926[29], sintiliumab, the second domestically developed PD-1 inhibitor approved in China, in combination with the anti-angiogenesis agent anlotinib, has shown remarkable results. The combination therapy achieved a superior overall response rate to those of chemotherapy and monotherapy with either agent alone. Therefore, sintiliumab and anlotinib have the potential to serve as new treatment options for patients with advanced stages of NSCLC with rare epidermal growth factor receptor (EGFR) mutations.

PD-1/PD-L1 mAbs plus radiotherapy Radiotherapy, which is essential for the eradication of cancer, is administered to 40% of patients with cancer and can be divided into 2 types: stereotactic radiotherapy and stereotactic body radiotherapy. The duration of radiotherapy treatment determines the classification into short-course radiation (SCRT) or long-course radiation. Radiotherapy, which serves as a coadjuvant in numerous combination regimens, is frequently used to synergistically augment the therapeutic potency of tumor immunotherapy. This modality provides a new trategy on anti-tumor immunity, encompassing the elimination of tumor cells and the stimulation of T cell immunological activity. Consequently, radiotherapy serves as a potent tool to fortify the host immune response[30-32].

The abscopal effect is the most notable example of how radiation at one location can decrease tumor sizes at nearby and distant non-irradiated sites[33,34]. Beyond removing local lesions, radiotherapy activates the body's natural immunological defenses against tumors[35]. Although abscopal effects have been well known since their identification, how radiotherapy influences immune cell functioning, and how to elicit the regression of distant unirradiated tumors through abscopal effects remain unkown[36].

A pre-clinical study[37] published in May 2023 has demonstrated that radiotherapy enhances the body's immune response, thus enabling tumor killing and potentially increasing the sensitivity of microsatellite stable (MSS) rectal cancer to immunotherapy. In a trial conducted by the Fudan University Cancer Center in China, patients with locally advanced rectal cancer (LARC) were treated with SCRT as the basic adjuvant treatment and immunotherapy to kill tumors, to determine whether the treatment might decrease tumor size or cause regression. The trial, called the TORCH study, has progressed to phase II, and its clinical trial registration number is NCT04518280. The results of the trial have indicated a strong complete response after SCRT combined with chemotherapy (oxaliplatin plus capecitabine) plus the PD-1 mAb toripalimab. The preliminary results suggest that this treatment is better tolerated and achieves clearer tumor regression than other treatments. However, these results are preliminary, and long-term follow-up is necessary to confirm the benefits to patients.

Nevertheless, because of poor response to PD-1/PD-L1 mAbs among patients with MSS LARC, more optimized methods are necessary to treat this disease; developing such methods is currently a research hotspot. In the above TORCH trial, radiotherapy as an adjuvant treatment, together with the immune mAbs, has been found to increase the sensitivity of MSS LARC and promote the effects of tumor immunotherapy[37].

PD-1/PD-L1 mAbs plus chemotherapy Clinically, chemotherapeutic agents are used to kill cancer cells to achieve therapeutic goals. Most agents were developed to inhibit tumor growth by blocking cell growth cycles, interfering directly with cell metabolism, and exerting cytotoxic effects. Tumors become more responsive to immunotherapy when their expression of tumor antigens increases, immune cell suppression decreases, and the tumour microenvironment (TME) is remodeled to promote immune infiltration of T cells and activation of DCs. However, some cytotoxic chemotherapeutic agents, such as paclitaxel, oxaliplatin, and anthracyclines, can potentially lead to lymphocyte exhaustion, thereby suppressing immune responses.

Preliminary studies in mouse models have demonstrated that enhancing immune function decreases the immune rejection of tumors to anthracyclines, thus highlighting the benefits of combining chemotherapeutic and immunotherapeutic agents[38]. The effects of chemotherapy on the immunotherapy process have been well demonstrated, and such treatments are being used alongside a variety of combination regimens. Numerous chemotherapeutic mixed regimens have now received FDA approval.

The phase III trial CheckMate 649[39] has demonstrated that FOLFOX (comprising 5-fluorouracil plus leucovorin plus oxaliplatin)/XELOX (comprising capecitabine plus oxaliplatin) chemotherapy combined with nivolumab increases OS and confers therapeutic advantages in patients with advanced gastric cancer, gastroesophageal junction (GEJ) cancer, or esophageal adenocarcinoma. In the KEYNOTE-189[40,41] and KEYNOTE-407[42] clinical trials, the participants were primarily treatment-naïve patients with metastatic squamous NSCLC. The experimental group (receiving pembrolizumab plus pemetrexed/platinum chemotherapy), as compared with the control group (receiving chemotherapy plus a placebo combination regimen), showed a significant doubling of the OS rate, an improvement in progression-free-survival (PFS), manageable toxicity, and a durable response after 2 consecutive years of treatment with pembrolizumab. Pembrolizumab plus chemotherapy is currently the standard treatment of choice for metastatic squamous NSCLC.

Serplulimab, an intravenously administered PD-1 mAb, was approved in China in 2022 for the treatment of advanced unresectable or metastatic microsatellite instability-high (MSI-H) solid tumors[43]. A study (NCT03958890)[44] on PD-L1-positive esophageal squamous cell carcinoma (ESCC) has shown that serplulimab plus chemotherapy (5-fluorouracil plus cisplatin), compared with placebo plus chemotherapy, improves PFS and prolongs OS.

PD-1 mAbs plus cell therapies Cell therapies can be broadly categorized into 2 main types: (1) adoptive cell transfer (ACT) therapies and (2) stem cell therapies, which further encompass a variety of therapeutic modalities such as T cell receptor-engineered T (TCR-T) cell therapy, chimeric antigen receptor T (CAR-T) cell therapy, and chimeric antigen receptor natural killer (CAR-NK) cell therapy[45]. Among these therapies, CAR-T and TCR-T cell therapies notably exhibit highly specific recognition of tumor cells and have potent killing efficacy and therefore have become critical in the field of tumor therapy, thus bringing new therapeutic hope to researchers and patients.

(1) PD-1 mAbs plus ACT therapy CAR-T cell therapy is an emerging technology that precisely targets tumor cells for treatment. This therapy involves the isolation and extraction of T-lymphocytes from patients with cancer. The cells are subsequently genetically engineered *in vitro* to express a chimeric antigen receptor (CAR). The modified CAR-T cells specifically recognize antigens from tumor cells, thereby enabling targeted treatment. The genetically engineered and edited CAR-T cells are expanded *in vitro* to a specific number and are subsequently reinfused into the patient's body, where they specifically recognize tumor antigens and kill tumor cells[46].

CAR-T cell therapy has broad clinical application prospects. The results of a phase I clinical trial (NCT02414269)[47] support the combination of iCasp9M28z T cells with pembrolizumab (PD-1 mAb) for the treatment of malignant pleural mesothelioma (including breast cancer and metastatic lung cancer). In that study[48], the combination of CAR-T cells and PD-1 inhibitors has been shown to amplify anti-tumor immune effects. A lentiviral vector has been developed to target dual shRNA CAR: PD-1/TIGIT for infecting xenografts in a mouse model of disseminated human blood cancer. Moreover, PD-1/TIGIT downregulation has been found to enhance the anti-tumor activity of CAR-T cells targeting CD19. This finding provides the first conclusive evidence that the blockade of 2 immune checkpoints synergistically augments the anti-tumor activity of CAR-T cells, thereby offering novel strategies and insights for future immunotherapies against malignancies.

However, most regimens for the treatment of solid tumors consist of PD-1 mAbs with CAR-T cells. Therefore, other ICIs can be targeted for combination therapy with CAR-T cells to provide more therapeutic options for patients with cancer.

CAR-T cell therapy has gained widespread attention for its efficacy as a tumor immunotherapy. CAR-NK cell therapy is a therapeutic means that uses the anti-tumor abilities of NK cells and genetic engineering technology[49]. Compared with CAT-T cell therapy. In research using a mouse tumor model, hematopoietic stem cell (HSC)-derived CAR-NK cells have shown exceptional anti-tumor efficacy in combination with nivolumab[50]. N-803, an IL-15 superagonist, has been shown to expand NK cells in humans after injection and to be well

tolerated[51]. An ongoing clinical trial (NCT04847466)[52] is investigating the combination of N-803, pembrolizumab, and HSC CAR-NK for the treatment of GEJ and advanced HNSCC; trial completion is expected by the end of 2025.

NY-ESO-1, a cancer-testis antigen, is a tumor-associated antigen that is specifically expressed in cancerous tissues and therefore is a target for cancer therapies. In TCR-T cell therapy, the modifier genes for the NY-ESO-1 TCR are usually introduced into the patient's T cells and then reinfused into the patient, thus potentially helping the body to mount an immune response to kill tumor cells that express NY-ESO-1. Modest anti-tumor activity has been observed in a clinical trial (NCT03168438)[53] targeting NY-ESO-1 specific TCR-T cells alone or in combination with pembrolizumab for the treatment of relapsed/refractory multiple myeloma. In another clinical trial, NCT02775292[54], the combination of NY-ESO-1 TCR-T cells with the DC vaccine and nivolumab (PD-1 mAb) has been shown to block sarcoma progression. A search of ClinicalTrials.gov identified the clinical trial NCT05349890, started in March 2023, which is combining TCR-transduced T cells with CDX-1140 (CD40 mAb) and pembrolizumab (PD-1 mAb) for the treatment of malignant epithelial neoplasms.

NCT03747484 in ClinicalTrials.gov is an ongoing clinical trial evaluating the safety and overall therapeutic efficacy of injecting FH-MCVA2 TCR-T cells in combination with avelumab (PD-L1 mAb) or pembrolizumab (PD-1 mAb) in patients with metastatic or unresectable MCC.

Currently, most TCR-T cell therapies use autologous T cells derived from individual patients. Nevertheless, as research deepens and technological advancements accelerate, the use of allogeneic T cells and those differentiated from induced pluripotent stem cells (iPSCs) as alternative cell sources for TCR-T therapies has emerged as a major area of investigation[55]. The ongoing exploration and refinement of these novel methods are anticipated to broaden the potential applications of TCR-T therapies in the future and to enable breakthroughs in the field through the use of next-generation technological innovations[56].

(2) PD-1 mAbs plus stem cell therapy Stem cell therapy is a therapeutic approach that harnesses the inherent differentiation capabilities of stem cells. Typically, this process begins with the isolation and extraction of stem cells from the patient's body. Subsequently, these cells are cultured and expanded *in vitro*, to promote their differentiation into diverse cell types, including NK cells and T cells. Finally, these regenerated and healthy stem cells are reintroduced into the patient to achieve therapeutic benefits for the treatment of various diseases[57].

FT500, an induced pluripotent stem-cell-derived NK (iPSC-NK) product, has been investigated in the clinical trial NCT03841110 for the treatment of advanced solid tumors, either as a monotherapy or in combination with ICIs, such as nivolumab, pembrolizumab, and atezolizumab. However, no studies associated with this trial have been published to date[58].

Currently, no clinical trials have explored the combination of iPSC-derived T (iPSC-T) cell therapy with ICIs; instead most trials have focused on iPSC-NK cell therapy. Stem cell therapy has the potential to enhance the immune system's ability to recognize a wide range of non-mutated tumor antigens. Additionally, stem cells can be genetically edited and modified *in vitro* to create cells with specific anti-tumor functions[59]. Although many studies have demonstrated the safety of iPSCs, potential risks remain, such as the possibility of teratoma formation in undifferentiated iPSCs[60]. Additionally, the administration of differentiated stem cells does not expedite patient recovery time. Furthermore, the high cost and prolonged production process associated with iPSC-T cell therapies remain substantial obstacles for both patients and researchers[57].

CTLA-4 mAbs

Brief description of CTLA-4

CTLA-4 is a leukocyte differentiation antigen and a co-stimulatory signaling molecule that decreases T cell activity in specific environments, such as the TME, thereby enabling immune escape. This antigen is found primarily on the surfaces of $CD4^+$ T cells, $CD8^+$ T cells, and Tregs (**Table 2**).

Both CD86 and CD80 are ligands for CTLA-4 and are located on APCs. CD80 has a higher affinity for CD28 and CTLA-4 than for CD86[61]. Because CTLA-4 and CD28 not only have similar functional properties but also share the same ligand, CTLA-4 and CD28 compete with each other[62]. However, in the TME, CD28 has a lower affinity for the ligand than CTLA-4, thus hindering the positive regulatory effect of CD28[63]. In contrast, CTLA-4 inhibits the activating effect of T cells by forming a complex with the ligand, thereby limiting the normal anti-tumor immune response[64] (**Figure 2**). Theoretically, CTLA-4 mAbs bind CTLA-4 molecules expressed on tumor cells and subsequently trigger a signaling cascade that leads to engagement of the ligand CD80/86 with CD28. This interaction restores T cell activity and effectively

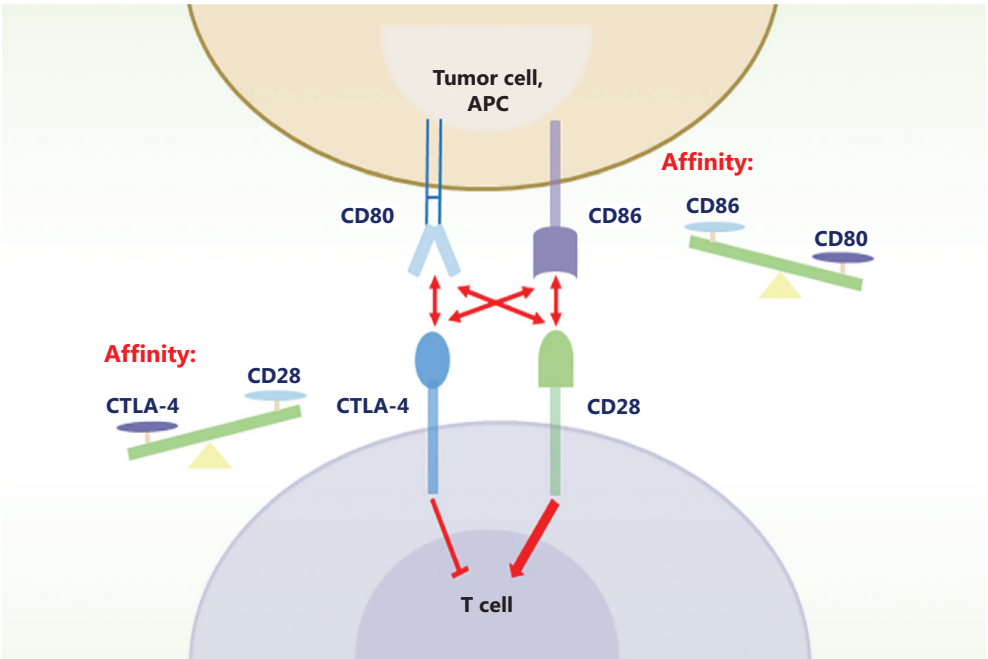

Figure 2 CTLA-4 and CD28 with their ligand-binding activities: On the surfaces of T cells, CTLA-4 and CD28 are co-inhibitory and co-stimulatory receptors, respectively. CD80 and CD86 are both ligands for CTLA-4 and CD28, but CD80 has a higher affinity for both receptors. Both ligands have high affinity for CTLA-4, which sends inhibitory signals to T cells and leads to shutdown of the T cell pathway. CTLA-4, cytotoxic T-lymphocyte-associated antigen-4.

transforms the initial suppression of anti-tumor immunity into promotion of tumor immunity.

CTLA-4 mAb monotherapy

Tremelimumab, also known as IMJUDO, is a CTLA-4 mAb produced by AstraZeneca that is used to cure many types of malignant cancers[65]. Another CTLA-4 mAb, ipilimumab, received FDA approval in 2011 and has been prescribed primarily for treating melanoma. This antibody is the first clinical CTLA-4 mAb[65]. However, like other ICIs, it may have severe adverse effects, such as acute liver and cholestatic damage, which can be fatal in extreme situations. Because of these adverse effects, the drug is rarely used clinically on its own; instead, it is usually paired with other immune mAbs or radiotherapy.

CTLA-4 mAb combination therapies

CTLA-4 mAbs plus PD-1/PD-L1 mAbs The CheckMate 227[66] clinical trial focused on drug efficacy in patients with NSCLC in various experimental arms, with OS as the primary endpoint. Treatment with nivolumab plus ipilimumab was more effective than chemotherapy. At the final follow-up time point of 61.3 months, patients receiving nivolumab plus ipilimumab had a better OS rate than those treated with chemotherapy, and showed long-term benefits and a manageable safety profile. On the basis of these data, nivolumab plus ipilimumab is a reasonable choice as a first-line agent for the treatment of patients with NSCLC, regardless of PD-L1 expression.

Tremelimumab is rarely used as a monotherapy and instead is usually administered in combination with durvalumab. Both agents were first approved in the USA in October 2022[66,67]. The POSEIDON phase III study (NCT03164616)[68] has explored the clinical efficacy of tremelimumab and durvalumab treatment regimens in patients with EGFR/anaplastic lymphoma kinase (ALK) wild-type metastatic NSCLC (mNSCLC). The use of durvalumab plus chemotherapy was associated with longer PFS than chemotherapy alone.

The phase 1b/2 clinical trial NCT03202758[69] has investigated the safety and efficacy of durvalumab plus tremelimumab plus chemotherapy (oxaliplatin plus folinic acid plus 5-fluorouracil, mFOLFOX6) in patients with metastatic CRC with MSS and a RAS mutated status[69]. The main objective of the study was to evaluate safety, which was achieved with no issues during the phase II study. The mFOLFOX6 regimen

achieved the best overall results, with a PFS of 90.7% at 3 months and 60% at 6 months. In contrast, the use of chemotherapeutic agents alone resulted in significantly less favorable outcomes. Therefore, combination therapies are useful and clinically promising.

CTLA-4 mAbs plus cell therapy In the NCT02070406 clinical trial[70], the therapeutic efficacy of NY-ESO-1-specific TCR transgenic lymphocytes, in combination with DC vaccine and ipilimumab, has been evaluated in patients with advanced sarcoma or melanoma. The aim of the study was to assess the effects of TCR transgenic cell dosing on the treatment outcomes. After the conclusion of the experiment, the therapeutic effects remained unaffected, regardless of the inclusion of ipilimumab in the treatment regimen.

Emerging immune checkpoint inhibitors

Similarly to how PD-1/PD-L1 mAbs and CTLA-4 mAbs ushered in a new era of immunotherapies, the emergence of novel immune checkpoints, such as LAG-3, TIM-3, TIGIT, VISTA, BTLA, and SIRPα, has opened a new frontier in cancer treatment. Researchers have devoted substantial resources to the study of these mAbs and their combination therapeutic regimens, thus reinvigorating immunotherapy treatment[8].

Next, we focus on novel ICI antibodies investigated in recent clinical trials, to demonstrate their in the treatment of certain solid tumors.

LAG-3 mAbs

Brief description of LAG-3

LAG-3, a transmembrane protein, is an immunological marker closely associated with CD4, according to RNA sequencing and localization experiments[71]. This protein is expressed primarily in $CD4^+$ T cells, $CD8^+$ T cells, Tregs, NK cells, B cells, and DCs[72] (**Table 2**). The protein is composed of three parts—extracellular, transmembrane, and intracellular regions—which together comprise 4 structural domains, D1–D4[73,74]. The intracellular tail contains 3 comparatively conserved motifs: the EP motif, the Kieele motif, and the serine phosphorylation site. The remaining 2 motifs contribute to the immunosuppressive effect of LAG-3. However, the question of whether the serine phosphorylation site influences LAG-3 function remains under debate[71].

Major histocompatibility complex II (MHCII) is a abundant ligand for LAG-3 and CD4[75]. In contrast, LAG-3 binds MHC II with higher affinity, competes with CD4 for the binding of MHC II, and downregulates cytokine secretion and the proliferative capacity of $CD4^+$ T cells[76]. LAG-3 additionally binds other ligands, such as fibrinogen-like protein-1 (FGL-1), galectin-3 (Gal-3), and liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin), all of which affect $CD8^+$ T cells (**Figure 3**). LSECtin protein is highly expressed in the liver[77]. According to a pre-clinical study, the binding of LSECtin to LAG-3 in a B16 melanoma model downregulates IFN-γ secretion, thereby blocking T cell immunity. Furthermore, LSECtin plays a role in the invasion and growth of gastric cancer cells, and eventually results in the development of gastric cancer[78]. FGL-1 directly downregulates the secretion of IL-2, thus conveying a negative regulatory signal[79], whereas Gal-3 binds oncoproteins and elicits tumor cell proliferative effects. For example, binding of N-Ras to Gal-3 leads to transformation of the former into K-Ras, which in turn increases the number of breast cancer cells[80].

Another concern pertains to one of the components of LAG-3, the Kieele motif. When the Kieele motif is removed from LAG-3, the protein is completely inactivated[8], thus suggesting that the Kieele motif acts as an "initiator" that triggers the downstream signaling pathway in $CD4^+$ T cells[73,81] (**Figure 3**). LAG-3 is also an immune checkpoint protein with inhibitory properties that promotes the anti-tumor inhibitory function of Tregs.

LAG-3 mAb combination therapies

LAG-3 mAbs plus PD-1 mAbs The interaction between LAG-3 and PD-1 has been extensively studied in clinical practice. These 2 receptors have synergistic effects resulting in the dual inhibition of tumor activity and the control of immune homeostasis[82], thereby aiding in avoidance of autoimmunity and improving tumor immune-mediated tolerance. Studies in mouse models have shown that the blockade of both receptors with antibodies is much more effective than blockade of either receptor alone[82-84]. Antibodies have been applied in the treatment of tumors to demonstrate the effects of blocking LAG-3 and PD-1. Relatlimab, often in combination with nivolumab[85,86], is the most frequently used LAG-3 inhibitor.

Blocking the LAG-3 pathway significantly improves the inhibitory effects of PD-1 on tumor immunity in a variety of illnesses, including NSCLC[87], gastric cancer[88], triple-negative

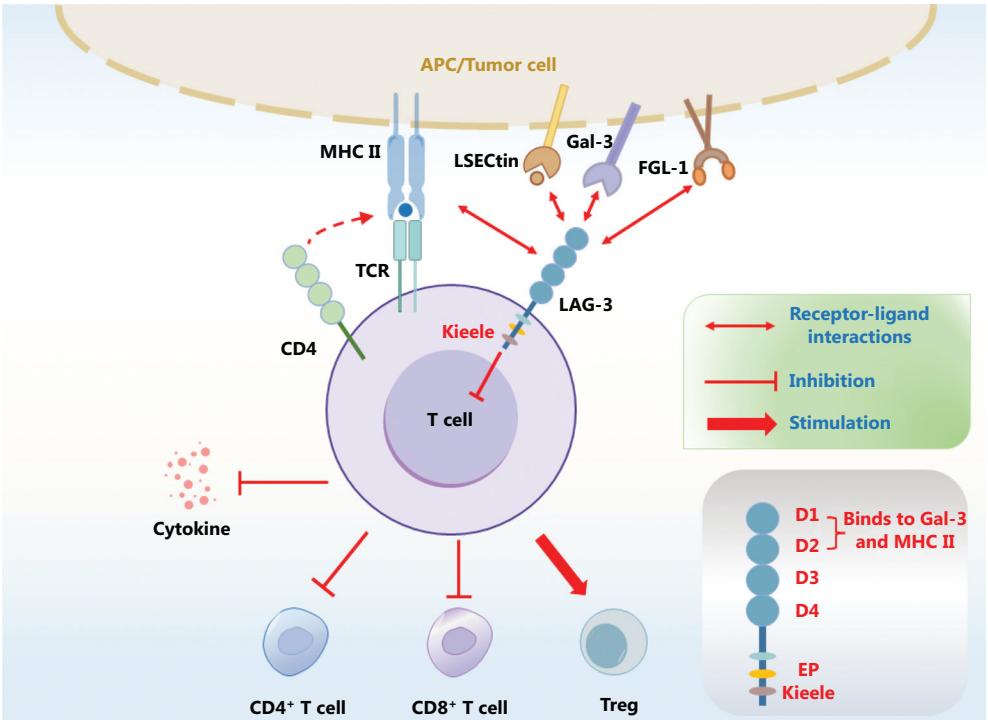

Figure 3 Functions of LAG-3 and ligands: FGL-1, Gal-3, LSECtin, and MHC II are all LAG-3 ligands. The Kieele structure of LAG-3 triggers downstream pathways and inhibits T cells. CD4, the homologue of LAG-3, competes with LAG-3 for MHC II binding. The binding of LAG-3 to the ligand MHC II downregulates CD4$^+$ T cell activity and decreases cytokine secretion. Additionally, LAG-3 inhibits the activity of CD8$^+$ T cells. The activity of CD8$^+$ T cells is inhibited by FGL-1, Gal-3, and LSECtin. LAG-3, lymphocyte activation gene-3; MHC II, major histocompatibility complex II; FGL-1, fibrinogen-like protein-1; Gal-3, galectin-3; LSECtin, liver and lymph node sinusoidal endothelial cell C-type lectin.

breast cancer[89], and ovarian cancer[74]. Currently, 10 humanized IgG4 LAG-3 mAbs are currently under investigation in a total of 78 clinical trials[72]. On March 18, 2022, the FDA authorized a combination treatment using relatlimab and nivolumab[90], known as opdualag, which can be prescribed for adults and children older than 12 years to treat metastatic or incurable melanoma[91,92].

A phase II/III study, RELATIVITY-047[86], has investigated the effects of combining the medications relatlimab and nivolumab in patients with unresectable melanoma. The combination was found to be superior to nivolumab monotherapy with respect to mPFS, thus suggesting that the targeting of both immune checkpoints, PD-1 and LAG-3, might be more effective than targeting either checkpoint alone and might provide greater survival benefits.

In patients with advanced MSS CRC, favezelimab, a LAG-3 mAb, has shown preliminary anti-tumor activity, both in combination with pembrolizumab and as a monotherapy[93]. However, in a clinical trial (KEYNOTE-495/KeyImPaCT)[94] for the treatment of NSCLC, the combination regimen was not available, because a lower overall response rate was observed among patients treated with pembrolizumab plus favezelimab than in the rest of the experimental group. Therefore, this therapy was replaced with another combination regimen of pembrolizumab plus lenvatinib, which met or exceeded the desired efficacy criteria.

TIM-3 mAbs

Brief description of TIM-3

TIM-3, also called CD366, belongs to the TIM family. The variable immunoglobulin domain (IgV), transmembrane region, mucin domain (including an O-linked glycosylation site), and the C-terminal cytoplasmic tail are the 4 unique parts of TIM-3. In addition, an N-linked glycosylation site connects mucin and the transmembrane region[8]. TIM-3 exhibits a distinct structural profile from those of other immunosuppressive molecules, and is characterized by the presence of 5 conserved tyrosine residues[95,96]. According to previous research, human monocytes, macrophages, NK cells, DCs, and CD4$^+$

and CD8+ T cells (**Table 2**) produce TIM-3[97,98]. Galectin-9 (Gal-9), carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM-1), phosphatidylserine (PtdSer), and high-mobility group box-1 (HMGB-1) are its ligands[97,99]. These ligands bind the target protein TIM-3, which is present in many types of cancer cells, including those causing colorectal cancer[100], cervical cancer[101], ovarian cancer[74], gastric cancer[102], and other cancers[103].

The interaction of HLA-B-associated transcript 3 (Bat-3) with TIM-3 plays a key role in the activation or inhibition of T cells[96,104]. Two tyrosine residues (Y256 and Y263) in the cytoplasmic tail region of TIM-3 are particularly important in Bat-3's physiological functions. When TIM-3 ligand is absent, Bat-3 interacts with the Y256/Y263 residues in the cytoplasmic tail of TIM-3. This promotes the activity of the Src kinase Lck, which in turn facilitates the recruitment of ZAP70[105]. Subsequently, T cell activation and suppression of the negative regulation of TIM-3 lead to enhanced effector activity of T cells (**Figure 4A**). However, when TIM-3 binds its ligand, Bat-3 dissociates after phosphorylation of Y256/Y263[106], thus allowing Fyn, another Src kinase, to bind TIM-3. Lck is inactivated, and ZAP70 function is downregulated, thereby ultimately inducing T cell exhaustion[107]. Therefore, the interaction between TIM-3 and its associated molecules is crucial in regulating the balance between T cell activation and exhaustion (**Figure 4B**).

TIM-3 mAb combination therapies

NK cells, CD4+ T cells, and CD8+ T cells express TIM-3, and the percentage of TIM-3 in tumor-infiltrating CD4+/CD8+ T cells is closely associated with the prognosis of patients with cancer[74,99]. High expression of TIM-3 and PD-1 in acral melanoma, a subtype of melanoma, has been found to substantially deplete CD8+ T cells[108]. In addition, a study of bone marrow cells extracted from patients with colorectal cancer and then tested for co-expression with T cells has shown high TIM-3 expression on the surfaces of T cells, mononuclear myeloid cells, and APCs in tumor tissues[109].

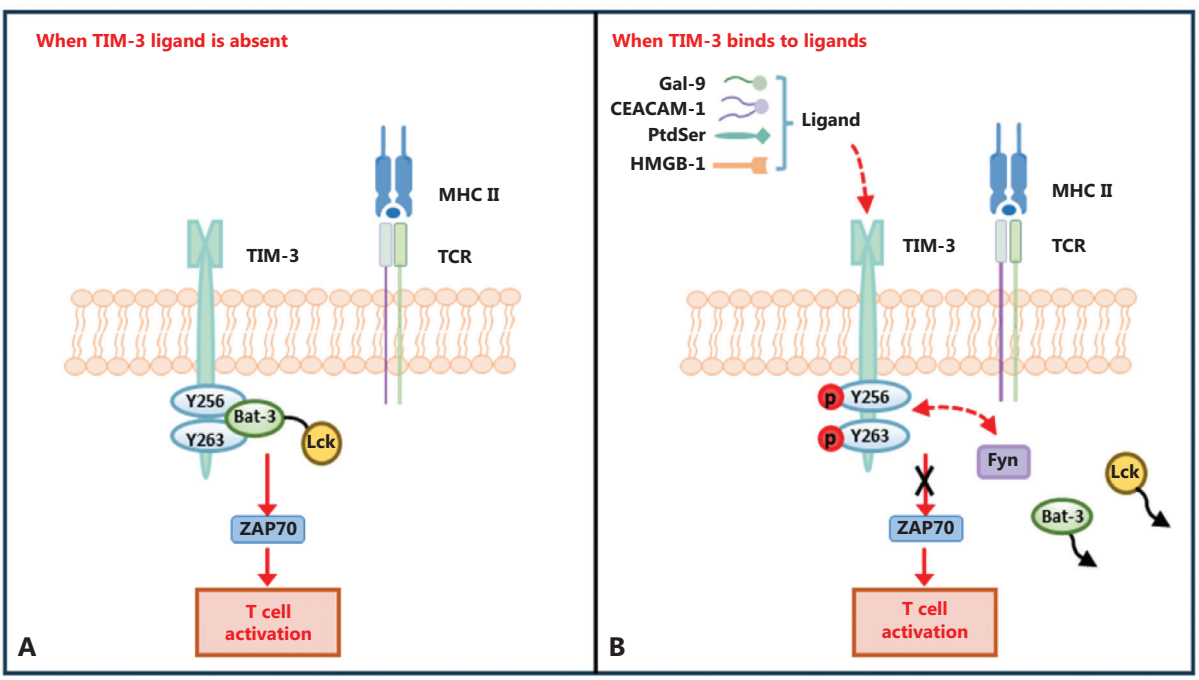

Figure 4 Mechanisms of TIM-3-mediated T cell activation and suppression: (A) In the absence of the TIM-3 ligand, Bat-3 interacts with the Y256/Y263 residues located in the cytoplasmic tail of TIM-3, promoting the activity of Lck. Subsequently, this process promotes the recruitment of ZAP70 and facilitates T cell activation while suppressing the negative regulation of TIM-3. (B) After binding of TIM-3 to its ligand, phosphorylation of Y256/Y263 triggers the dissociation of Bat-3, thus enabling the binding of another Src kinase, Fyn, to TIM-3. Subsequently, inactivation of Lck and downregulation of ZAP70 function ultimately induce T cell exhaustion. TIM-3, mucin domain containing-3; Gal-9, galectin-9; CEACAM-1, carcinoembryonic antigen-related cell adhesion molecule-1; PtdSer, phosphatidylserine; HMGB-1, high-mobility group box-1; Bat-3, HLA-B-associated transcript 3; Lck, lymphocyte-specific protein tyrosine kinase.

In another study, mice with ID8 tumors received intraperitoneal injections of either TIM-3 mAbs or CD137 mAbs to treat ovarian cancer[110,111]. The makeup and gene expression of immune cells infiltrating the tumors were analyzed, and mouse survival was tracked. After 3 days, the mouse tumor model responded favorably to either CD137 mAbs or TIM-3 mAbs alone. However, 10-day-old tumors showed promotion of tumor growth after the injection of TIM-3 mAb or CD137 mAb. According to the results, $CD4^+$ T cells and $CD8^+$ T cells are key to treatment with TIM-3 mAb and CD137 mAb. A significant increase in the number of $CD4^+$ T cells was observed with treatment with TIM-3 mAb alone, whereas treatment with CD137 mAb alone significantly increased the number of $CD8^+$ T cells. Therefore, the use of TIM-3 mAb or CD137 mAb alone is not effective for treating ID8 ovarian cancer when $CD4^+$ T cells or $CD8^+$ T cells are deficient. Notably, in the peritoneal fluid of 60% of the mice after 90 days of treatment with a combination of TIM-3 mAb and CD137 mAb, elevated $CD8^+$ and $CD4^+$ T-infiltrating cells and tumor regression were observed (**Figure 5**). These findings indicated a shift from "cold tumors" to "hot tumors" with tumor regression. The combined treatment significantly delayed ovarian cancer growth. Therefore, a potential immunotherapy strategy may involve the inhibition of TIM-3 and the activation of CD137.

A clinical study (NCT02608268)[112] has been conducted to investigate the effects of sabatolimab, administered alone or in combination with spartalizumab (PD-1 mAb), in the management of advanced solid tumors. Fatigue was the most prevalent treatment-related adverse event (TRAE), and the maximum tolerated dose was not reached. The combination of the 2 medications was well-tolerated and showed early beneficial effects against cancer.

Another phase I trial, clinical ID NCT03099109[113], was aimed at performing the first evaluation of the safety of the novel TIM-3 mAb on humans. In that trial, researchers validated the effectiveness of 2 novel mAbs, LY3321367(PD-L1 mAb) and LY3300054(TIM-3 mAb), as a combination therapy or alone. According to the experimental results, LY3321367 has a safety profile in advanced solid tumour patients and achieves a general level of anti-tumor activity. The use of LY3321367 and LY3300054 to treat MSI-H/mismatch repair-deficient (dMMR) tumors was further investigated in light of these experimental findings; however, the tumor samples did not show statistically significant results, and the experiment was paused.

TIGIT mAbs

Brief description of TIGIT

TIGIT is an innovative inhibitory ICI. The TIGIT proteins VSTM3, VSIG9, and WUCAM[114] were first reported in 2009[115]. The general TIGIT structure includes 2 tyrosine bases in the cytoplasm: ITIM and the Ig tail-tyrosine (ITT)-like motif. These tyrosine residues are crucial for TIGIT's inhibitory function after phosphorylation, because mutation of these residues leads to dysregulated inhibitory function[116].

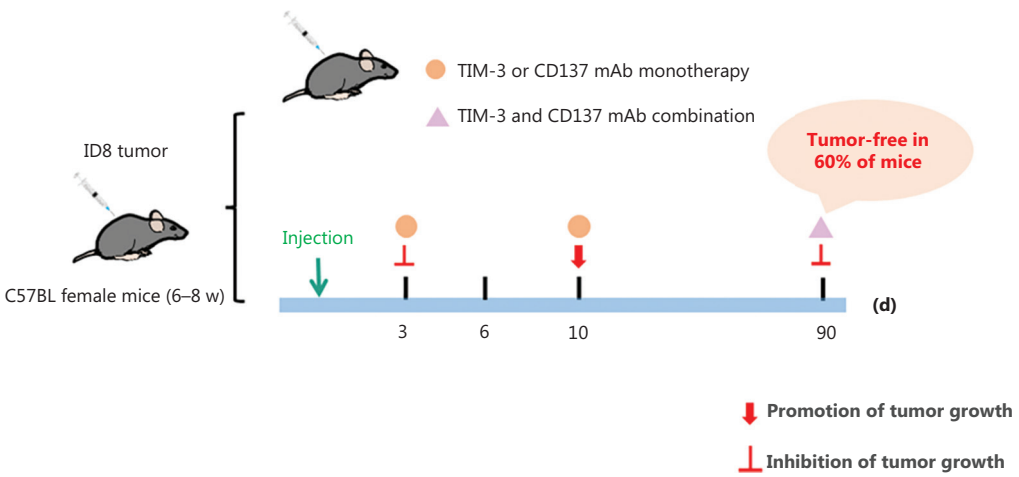

Figure 5 TIM-3 mAbs and CD137 mAbs for treatment in a mouse ID8 ovarian cancer model: In a mouse model of ovarian cancer, mice were treated with TIM-3/CD137 alone or TIM-3 in combination with CD137. By day 3, monotherapy effectively regressed the tumors, but by day 10, the tumors had become larger. In contrast, the combination treatment regressed tumors in 60% of the mice by day 90.

A trial examining cutaneous melanoma with TIGIT infiltration of human skin melanoma tissue[117] has shown differences in gene expression across various environments, in the presence or absence of tumor-infiltrating lymphocytes. Differential expression of multiple genes was observed in the presence of tumor-infiltrating lymphocytes, including the co-expression of TIGIT, LAG-3, and PD-1. According to multiplex immunofluorescence staining, TIGIT is expressed primarily in $CD8^+$ T cells, $CD4^+$ T cells, and DCs, and is less frequently expressed in NK cells (**Table 2**); however, TIGIT signaling is dependent primarily on NK cells[116].

CD155 and CD112 are ligands for TIGIT, and CD226 and CD96 can also bind CD155[118]. However, the binding mechanism is similar to that of CTLA-4: because CD112 has a lower affinity than CD155 for TIGIT, TIGIT tends to bind CD155 and form a complex[114]. In contrast, CD96 and CD226 play different roles in the TME: the former is a co-inhibitory receptor, whereas the latter is a co-stimulatory receptor (**Figure 6**). A study of the anti-tumor immune response of $CD8^+$ T cells by TIGIT and PD-1[119] has focused on the CD226 signaling pathway. Mechanistically, PD-1 and TIGIT "encircles" CD226 intracellularly and extracellularly, respectively: the ITIM structural domain of PD-1 inhibits the phosphorylation of CD226 and consequently prevents CD226 from binding CD155. TIGIT inhibits CD226 binding to CD155, thus restraining the co-stimulatory activity of CD226 and decreasing $CD8^+$ T cell infiltration. If both PD-1 and TIGIT inhibitors are combined, the "lock" on CD226 signaling from both intracellular and extracellular sources is opened, the CD226 signaling pathway is restored to its original state, and the immune function of $CD8^+$ T cells is enhanced. The simultaneous inhibition of TIGIT and PD-L1 restores the anti-tumor capacity of NK cells[120].

TIGIT combination therapies

A substantial TRAE frequency may be elicited by a combination of mAbs[121]. To mitigate these effects, the co-administration of other less harmful agents is recommended. TIGIT mAbs may be a preferable alternative to CTLA-4 mAbs, because TIGIT knockout animals do not develop autoimmune

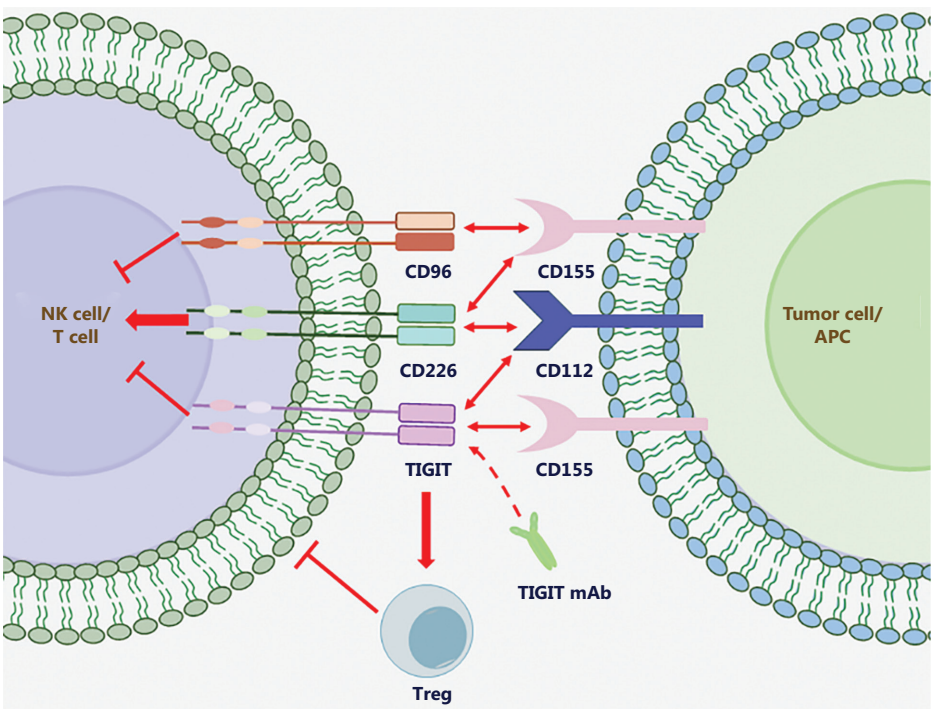

Figure 6 CD226, TIGIT, and CD96 with their ligand-binding activities: CD155 and CD112 are the ligands of TIGIT and CD226. CD96 also binds CD155. TIGIT and CD96 are co-inhibitory receptors that promote the infiltration ability of Tregs after binding ligands. They also transmit inhibitory signals to NK cells and T cells. CD226 is a co-stimulatory receptor responsible for activating NK cells and T cells. TIGIT mAbs bind TIGIT on the surfaces of NK cells and T cells, thus causing TIGIT to bind CD155 and CD112, and restoring the activity of immune cells. TIGIT, T cell immunoreceptor with Ig and ITIM domains; APC, antigen-presenting cell; NK cells, natural killer cells.

diseases, whereas CTLA-4 knockout mice quickly succumb to severe autoimmunity[114]. The integration of TIGIT and PD-1 mAbs has been found to double the inhibition efficiency. Simultaneously, the combination of both mAbs accelerates the infiltration of T cells into the TME and enhances the anti-tumor activity of NK cells. Furthermore, greater production of effector T cells enables better tumor killing ability, although the therapeutic efficacy is low, and the prognosis is poor in subcutaneous tumors[122].

Pharmaceutical companies have recently devoted substantial attention to the functionality of TIGIT mAbs. Six TIGIT mAbs have entered clinical phase I–III trials for the treatment of advanced solid tumors, all of which are being used in combination with chemotherapy or PD-1/PD-L1 mAbs[114].

In the NCT02964013 phase I clinical trial[123], the combination of vibostolimab (TIGIT mAb) and pembrolizumab was well-tolerated in patients with solid tumors, showing modest anti-tumor activity. However, the results for the other TIGIT mAb treatments were less promising: 2 clinical trials, SKYSCRAPER-01[124] and -02[125], exploring whether atezolizumab (PD-L1 mAb) in combination with tiragolumab is beneficial for the treatment of small-cell lung cancer, have indicated unsatisfactory final results, in which OS and PFS were not achieved. No recent research updates for tiragolumab have been reported.

Another novel TIGIT mAb, ociperlimab, developed by Beigene, Ltd., is currently recruiting volunteers worldwide for a new round of clinical trials. In the NCT 04047862[126] drug dose-escalation trial in advanced solid tumors, the pharmacokinetics and safety of treatment with ociperlimab plus tislelizumab, a novel PD-1 mAb, were examined, and the combination treatment showed preliminary anti-tumor activity in a phase I trial. The symptoms of the adverse reactions occurring were the same as those of the other TIGIT mAbs. The treatment is currently undergoing phase II and phase III trials.

In NCT031119428[127], etigilimab (TIGIT mAb) has been shown to be effective alone or in combination with nivolumab for the treatment of locally advanced or metastatic solid tumors; however, further clinical trials are warranted to confirm its therapeutic potential.

VISTA, BTLA, and SIRPα mAbs

VISTA is an B7 immunoglobulin superfamily molecule. V-Set and immunoglobulin domain containing-3 (VSIG-3) and P-selectin glycoprotein ligand-1 (PSGL-1) bind VISTA as ligands. Unlike other co-suppressive immune checkpoints, VISTA is expressed on both tumor cells and immune cells, and has significant effects on anti-tumor immunity[128] (**Table 2**). VISTA is expressed predominantly on basophils, monocytes, resting T cells, memory T cells, and $CD68^+$ tumor-associated macrophages[129]. Currently, no inhibitors of VISTA mAbs are approved by the FDA, but several investigational drugs have emerged, such as CI-8993, HMBD-002, and KVA12123, all of which are VISTA mAbs. A dose-escalation trial (NCT05082610) of HMBD-002 in combination with pembrolizumab for the treatment of colon cancer is currently being conducted[130]. However, because VISTA can act as both a receptor and a ligand, uncertainty persists regarding its clinical applications, and further study is therefore warranted[131].

BTLA, also referred to as CD272, is a member of the CD28 immunoglobulin superfamily expressed primarily on $CD4^+$ and $CD8^+$ T cells, as well as DCs, NK cells, and macrophages[132] (**Table 2**). BTLA binds the ligand HVEM, thereby inhibiting T and B cell activation and proliferation, and promoting the immune escape of tumors; consequently, BTLA-HVEM is a potential target for tumor immunotherapies[133]. In a phase I/II clinical trial (NCT05000684)[134], tifcemalimab, the first BTLA mAb, demonstrated preliminary anti-tumor activity in combination with toripalimab (PD-1 mAb) for the treatment of refractory extensive stage small-cell lung cancer (ES-SCLC). However, further clinical evaluation is required. A search of ClinicalTrials.gov identified another clinical trial (NCT05789069) that is recruiting volunteers to study the novel BTLA mAb HFB200603 as a single agent or for use in combination with tislelizumab for advanced solid tumors.

SIRPα is an inhibitory receptor expressed on DCs, macrophages, and neutrophils (**Table 2**); its typical ligand is CD47, and it is expressed by both normal and tumor cells. Because of the ubiquitous expression of CD47, targeting of CD47 causes anemia and thrombocytopenia[135]. The structure of SIRPα contains the ITIM motif. When SIRPα binds CD47, SIRPα triggers ITIM phosphorylation and recruits tyrosine phosphatase (SHP)-1 and SHP-2. Subsequent activation of SHP-1 and SHP-2 leads to dephosphorylation of intracellularly associated proteins, thus resulting in a loss of the biological functions of the proteins and, ultimately, the inhibition of phagocytosis by macrophages[136]. In addition, the SIRPα-CD47 axis inhibits the antigen uptake and presentation ability of DCs and the killing ability of NK cells[137]. An experimental study has indicated that treatment of KWAR23 (SIRPα mAb) with vorsetuzumab (CD70 mAb) greatly enhances macrophage phagocytosis in

renal carcinoma cells and prevents tumor growth in mice[138]. BR105 is another novel SIRPα-targeted mAb that effectively inhibits tumor growth in mice. Its good safety profile both *in vivo* and *in vitro* supports the use of other ICIs or radiotherapy in combination therapies[139]. In an experimental study, ADU-1805, another SIRPα mAb, has been demonstrated to promote phagocytosis by macrophages without interfering with T cell activation[140]. To further advance the clinical development of ADU-1805, a study currently underway is evaluating its safety and pharmacokinetics, both as a monotherapy and in combination with pembrolizumab (PD-1 mAb), for the treatment of advanced solid tumors in NCT05856981.

Conclusions

Several clinical trials have established the efficacy of traditional ICIs for the treatment of patients with cancer. Recently, interest has grown in targeting novel immune checkpoints for immunotherapies. Pharmaceutical companies have extensively studied these checkpoints and conducted numerous clinical trials. However, most of those trials have been limited to animal models. Therefore, clinical trials are necessary to validate the safety and adverse effects of combination therapies, to ensure their success. Furthermore, additional research is required to clarify the mechanisms of immune regulation

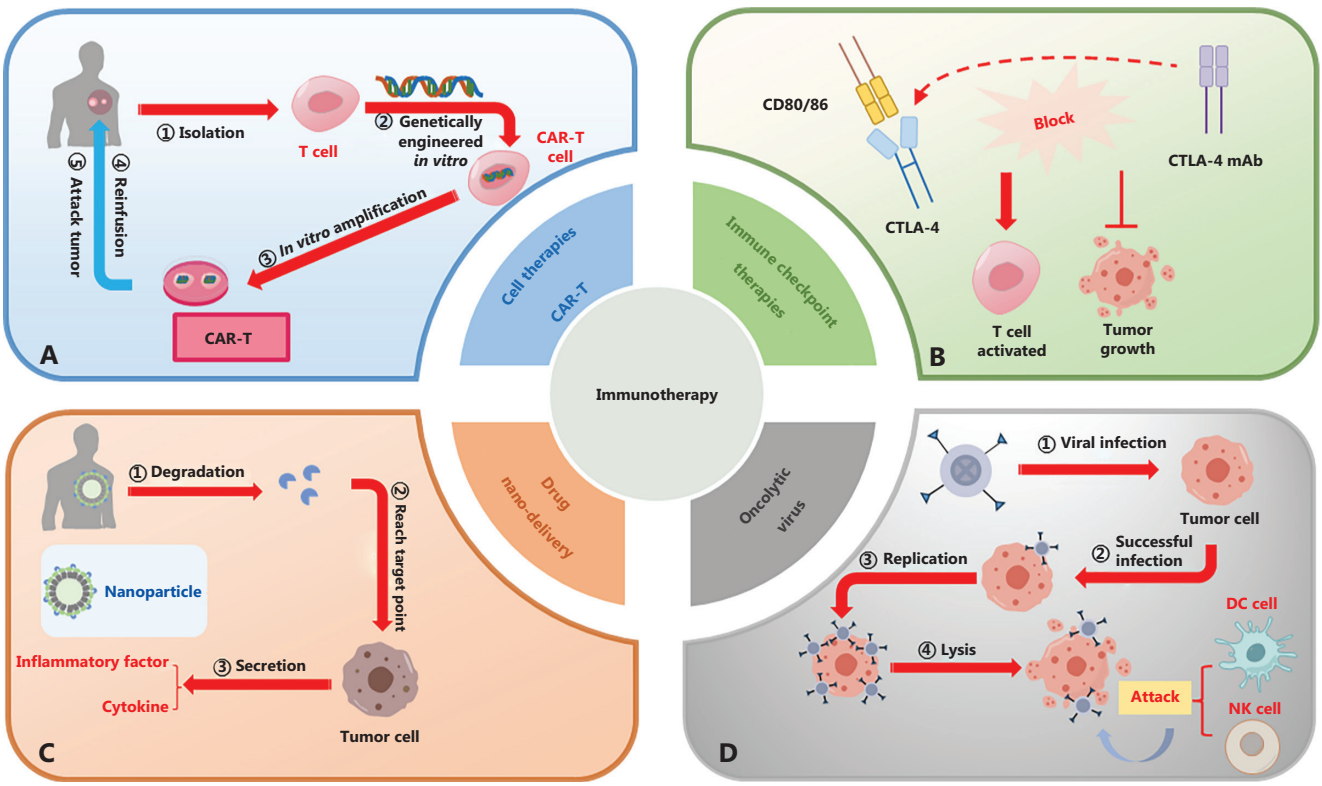

Figure 7 Types of immunotherapeutic treatments. Tumor immunotherapy approaches can be broadly categorized as (A) cell therapies (CAR-T), (B) immune checkpoint inhibitor therapies, (C) drug nano-delivery, and (D) oncolytic virus therapies. (A) In CAR-T, for example, T cells are isolated from the human body and genetically engineered *in vitro* to express CAR and form CAR-T cells, which are then massively expanded *in vitro* and reinfused into the patient's body. These CAR-T cells specifically recognize target antigens, proliferate rapidly, and exert anti-tumor effects *in vivo*. (B) CTLA-4 mAbs preferentially bind CTLA-4, and the ligand CD80/86, which has a stronger affinity for CTLA-4, binds CD80, thus restoring the normal function of T cells and leading to a transition from suppression of anti-tumor immunity to promotion of tumor immunity. (C) Nano-delivered drugs are degraded after being injected into the body and subsequently reach specific target sites, thereby stimulating the secretion of inflammatory factors and cytokines. This nano-delivery system improves tumor immunity efficacy. (D) After infection of tumor cells and normal cells with natural or genetically engineered oncolytic viruses (OVs), normal cells are not affected, whereas OV specifically targets tumor cells and proliferates in them, thus causing tumor cells to undergo lysis, apoptosis, and activating DC cells, NK cells, and cytotoxic T lymphocytes (CTLs) for further attack on tumor cells.

and tumorigenesis in humans. ICIs also encounter several challenges, and TRAEs remain a major concern. At present, tumor immunotherapies can be broadly categorized into 4 main types: ICIs, drug nano-delivery therapies, cell therapies, and oncolytic virus therapies[141] (**Figure 7**). Among these, drug nano-delivery therapies[62,142,143] and oncolytic virus therapies[144] exhibit high specificity in terms of reaching targets, thus enabling drug accumulation in tumor tissue while minimizing off-target effects. These 2 approaches can be combined with ICIs to achieve significantly lower toxicity than that with systemic administration.

Furthermore, both ACT and stem cell therapy, as described earlier, provide distinct advantages and disadvantages. In practical applications, selecting the most suitable treatment modality according to the patient's physical condition and the specific tumor type is imperative. Additionally, with the continuing advancement of technology and the deepening of research endeavors, these 2 therapeutic approaches are expected to undergo further optimization and find broader applications in forthcoming years.

Despite notable successes in clinical applications, the currently available ICIs, such as PD-1/PD-L1 mAb, continue to face challenges including drug resistance and off-target toxicity. Consequently, combination therapies have emerged as a critical future research focus to integrate ICIs with other immunotherapeutic strategies, such as ACT therapies and tumor vaccines, or with conventional treatments such as chemotherapy and radiotherapy. This integrated approach holds promise for achieving synergistic enhancement of therapeutic efficacy while minimizing adverse effects.

As tumor immunology research continues to advance, additional immune checkpoint targets may be identified in the future, such as LAG-3, TIM-3, and BTLA. The exploration of these novel targets is expected to provide new opportunities for the development of ICIs.

With the increasing elucidation of tumor immune mechanisms and the emergence of innovative ICIs, clinical indications are expected to undergo more extensive expansion. We eagerly anticipate further innovative research breakthroughs and advancements in clinical applications in this field, which will ultimately benefit a vast population of patients with cancer.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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