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REVIEW

Insights into therapeutic peptides in the cancer-immunity cycle: Update and challenges

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Peptide-major histocompatibility complex

Abstract Immunotherapies hold immense potential for achieving durable potency and long-term survival opportunities in cancer therapy. As vital biological mediators, peptides with high tissue penetration and superior selectivity offer significant promise for enhancing cancer immunotherapies (CITs). However, physicochemical peptide features such as conformation and stability pose challenges to their on-target efficacy. This review provides a comprehensive overview of recent advancements in therapeutic peptides targeting key steps of the cancer-immunity cycle (CIC), including tumor antigen presentation, immune cell regulation, and immune checkpoint signaling. Particular attention is given to the opportunities and challenges associated with these peptides in boosting CIC within the context of clinical progress. Furthermore, possible future developments in this field are also discussed to provide insights into emerging CITs with robust efficacy and safety profiles.

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1. Introduction

Cancer immunotherapies (CITs) have revolutionized cancer treatments by providing potential for systemic therapies with high specificity, lasting efficacy, and long-term survival opportunities[1]. The current landscape of CITs is dominated by several modalities, including adoptive cell transfer, immune checkpoint inhibitors (ICIs), oncolytic viruses, cancer vaccines, and cytokine therapies. These approaches represent significant milestones in the field of anticancer treatment[2]. Despite these notable advances, most currently available CITs still have limitations, such as low objective response rates, inevitable side effects, time-consuming and technically challenging production processes, and high costs[3]. Therefore, novel agents that are efficient and safe could significantly improve cancer treatment efficacy and become an emerging alternative to current drugs.

Peptides serve as natural ligands for numerous cell surface receptors and play critical physiological roles by orchestrating intracellular signal transduction pathways[4]. Thus, peptides, particularly endogenous ones, are frequently employed as viable and efficacious lead compounds in the pursuit of new drug discovery[5]. Currently, there are approximately 100 approved peptide drugs on the global market alongside hundreds of peptides undergoing clinical trials or preclinical studies[6,7]. It is estimated that the peptide pharmaceutical market will reach a value of around USD 48 billion by 2025, exhibiting a compound annual growth rate of 9.4[7]. Compared to biomacromolecules such as recombinant proteins or antibodies commonly employed in CITs, peptides exhibit reduced immunogenicity, enhanced tissue penetration, and facile modification. As crucial biological mediators, peptides also demonstrate superior selectivity and specificity in inhibiting protein−protein interactions compared to small molecule drugs. Consequently, peptides possess immense potential for augmenting the efficacy of CITs by precisely activating and sustaining the so-called cancer-immunity cycle (CIC), which encompasses a series of functional sequential events describing how the immune system activates and eliminates cancer cells (Fig. 1)[8−10]. Additionally, low-molecular-weight peptides are generally easy to prepare, modify, store, and utilize at reduced costs, thereby facilitating their sustainable development. This review presents the roles of the peptides in CIC and various peptide-based strategies developed for achieving tumor-specific immunotherapy. Particular emphasis is placed on recent preclinical and clinical advancements related to these peptides that enhance CIC, as well as the opportunities and challenges in this field.

2. Peptides as immunogenic cell death (ICD) inducers

2.1. *Effect of ICD in the CIC*

The emission of five key signaling hallmarks characterizes ICD, which is triggered by tumor-associated antigens (TAAs) and danger-associated molecular pattern molecules (DAMPs) released from dying tumor cells. During ICD, secreted ATP acts as a "come-to-me signal" to recruit and activate immature dendritic cells (DCs); Annexin A1 guides the migration of immature DCs toward dying cells by a "find me" signal. Exposed CRT functions as an "eat-me" signal for antigen uptake by immature DCs associated with dead cells. HMGB1 release promotes DC maturation, while interferon I secretion stimulates immune cell activation through binding to surface receptors, thereby initiating an adaptive immune response[11].

Although traditional chemotherapeutics, radiotherapy, and phototherapy have been shown to induce ICD, their effectiveness is limited by nonspecific cytotoxicity, scarce tumor antigens, inferior tumor penetration ability, or hypoxia[12]. In recent years, oncolytic viruses have generated tremendous excitement in the field of ICD-induced immunotherapy since talimogene laherparepvec was approved for advanced melanoma therapy by the FDA in 2015[13]. However, oncolytic viruses inevitably face multiple concerns in terms of safety, storage, and administration[14].

2.2. *Oncolytic peptide-induced ICD*

Oncolytic peptides, a class of emerging oncolytic alternatives derived from α-helical natural antimicrobial peptides, exhibit lower immunogenicity and production costs than oncolytic viruses. They possess distinct advantages based on the relatively homogeneous surface properties of tumor cells despite intratumoral heterogeneity[15]. Cationic charges and hydrophobic networks are two critical attributes that enable oncolytic peptides to selectively bind to anionic components (such as phosphatidylserine, *O*-glycosylated mucin, sialoganglioside, and heparin) expressed on tumor cell membranes through electrostatic adsorption. This binding interaction provides a foundation for the selective action of oncolytic peptides against tumor cells. The mechanisms underlying membrane-cavity formation can be categorized into three major models: barrel-stave, toroidal, and carpet models[16]. For instance, in the "barrel-stave" mechanism, peptides, such as melittin, form a bundle that inserts into the cell membrane once it reaches a certain concentration[17]. In the "toroidal" mechanism, peptides, such as maganins, induce the formation of various pores that cause thinning and curvature of the membrane[18]. In the "carpet" mechanism, peptides, including LL-37, cover the surface and disrupt the membrane structure like surfactants[19]. Oligomerization or structural rearrangement of tumor cell membranes can lead to rapid membrane lysis and accidental cell death, resulting in the release of abundant TAAs and DAMPs independent of intratumoral heterogeneity[20,21]. Subsequently, mature DCs present captured TAAs to naïve T cells, leading to their differentiation. In addition to disrupting the cell membrane, oncolytic peptides can also translocate into intracellular compartments, including the mitochondrial outer membrane, endoplasmic reticulum (ER), and Golgi apparatus, thereby triggering accidental cell death. For instance, LL-37 activated the intrinsic apoptotic pathway by inducing the release of apoptosis-inducing factor from the mitochondrial to the cytosol, resulting in mitochondrial outer-membrane permeabilization[22]. The peptide PFR derived from lactotransferrin targeted the ER and induced ER stress, elevation of cytoplasmic calcium levels, and the production of mitochondrial reactive oxygen species that ultimately lead to tumor cell death[23]. LTX-401 is an oncolytic peptidomimetic derivative that selectively targets the Golgi apparatus causing prominent cytoplasmic vacuolization and ICD[24].

LTX-315, which is derived from the host defense peptide bovine lactoferricin, has been extensively studied and is considered a representative oncolytic peptide due to its ability to induce complete tumor regression and systemic immune responses in various carcinomas[25]. This effect was achieved through selective

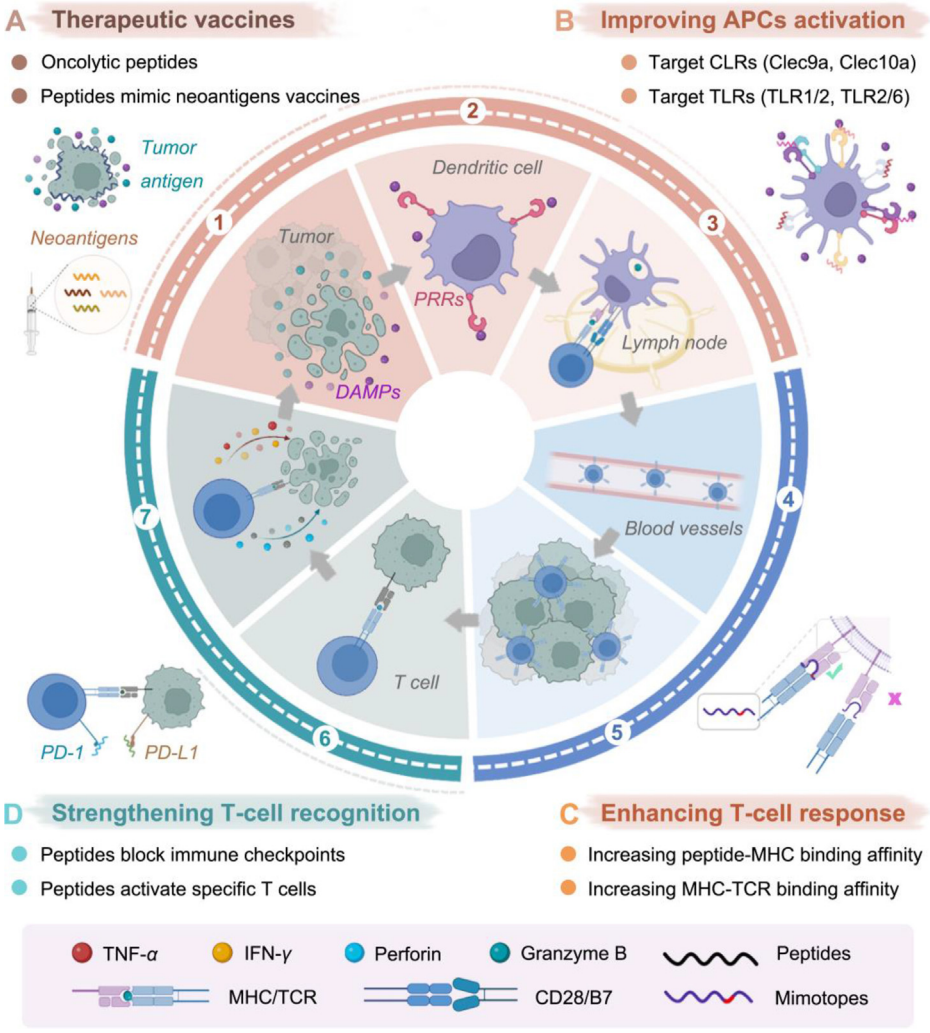

Figure 1 Schematic symphony of peptides in cancer-immunity cycle (CIC). CIC can be divided into seven steps (numbered 1−7): 1) antigens release from dying tumor cells, 2) APCs uptake tumor antigens, 3) T-cell priming and activation in local lymph nodes, 4) trafficking of T cells to tumors *via* blood vessels, 5) infiltration of T cells into tumor tissues, 6) recognition of tumor cells by T cells, and 7) T-cell-mediated killing of tumor cells in the tumor microenvironment; then, dying tumor cells further contribute to a new round of CIC. Among them, peptides are mainly involved in four steps of the above cycle: (A) therapeutic vaccines, (B) improving APC activation, (C) enhancing T-cell response, and (D) strengthening T-cell recognition.

rapid membrane disruption and subsequent stimulation of ICD[25]. In addition, recent findings have identified ATP11B as a novel potential target of LTX-315 for inhibiting programmed cell death 1 ligand 1 (PD-L1) expression in pancreatic tumor cells[25]. Clinical evidence also demonstrated that LTX-315 can remodel the tumor microenvironment (TME) and increase intralesional $CD8^+$ T cells in 86% of evaluated patients (12 out of 14), resulting in reduced a volume of injected tumors in 29% of patients[26]. Although intratumoral injection of LTX-315 caused vascular disorders and hypersensitivity/anaphylaxis in patients with advanced solid tumors, all treatment-related adverse events and toxicities were manageable and reversible[26].

Additionally, ongoing clinical trials of several other oncolytic peptides for advanced solid tumors have shown promising results (Table 1). The preference of oncolytic peptides for targeting tumor cells minimizes nonspecific toxicity to normal tissues and holds the potential for overcoming drug resistance. However, the limited applicability of intratumoral administration restricts the use of oncolytic peptides for inaccessible or metastatic cancers.

3. Peptide-based neoantigen vaccines

In the past decade, active immunotherapy utilizing *de novo* mutated tumor neoantigens has emerged as a personalized therapeutic strategy for cancer patients, which is distinct from passive immunotherapy induced by oncolytic peptides[27].

3.1. *Tumor specific antigens/neoantigens*

The selection of antigens plays a crucial role in determining the efficacy of therapeutic vaccines. Early therapeutic vaccines primarily composed of TAAs have shown immune tolerance due to their expression in normal tissues, leading to unsatisfactory results in clinical trials[28,29]. In contrast, tumor-specific antigens/neoantigens, derived from nonsynonymous mutations, gene

alterations, and genetic information are exclusively expressed in cancerous tissues[30,31]. Stimulation of antigen-specific T-cell responses by neoantigens prevents "off-target" damage to nonmalignant tissues and central tolerance of T cells toward self-epitopes. Consequently, neoantigens hold promise as cancer vaccines capable of eliciting a more specific and durable T-cell immune response[32,33].

3.2. *Synthetic long peptide (SLP) vaccines*

Peptide vaccines, nucleic acid vaccines, and neoantigen-pulsed DC vaccines represent the three platforms of personalized neoantigen vaccines[34,35]. Among these platforms, neoantigen-derived peptides offer distinct advantages in terms of their explicit sequence, ease of preparation, and stable chemical properties compared to other vaccine forms. Peptide vaccines are typically designed as SLPs containing multiple sequences (25−35 amino acids)[36,37], which can be more efficiently endocytosed and processed by antigen-presenting cells (APCs) within lysosomes. Subsequently, cleaved short peptides (9−11 residues) and long peptides (14−16 residues) are loaded onto major histocompatibility complex (MHC)-I and MHC-II molecules of APCs, respectively. These complexes then present the antigens to $CD8^+$ or $CD4^+$ T cells in a double-signal manner, thereby eliciting a potent T-cell response[38]. However, MHC-I-restricted short peptide-based vaccines alone may not be sufficient to induce robust $CD8^+$ T-cell responses due to the relatively weak efficacy of MHC-I peptides[39].

Based on whole-exome DNA sequencing of matched tumor and normal cells, NeoVax (Table 1), a personalized SLP vaccine targeting up to 20 individualized tumor neoantigens, was synthesized by predicting mutated peptides with high-affinity for binding to autologous human leukocyte antigen (HLA) molecules. In a phase I clinical study, four of six patients with high-mutation-rate melanoma showed no disease recurrence at the two-year follow-up after NeoVax vaccination. Additionally, two other patients with recurrent melanoma achieved complete tumor regression following treatment with a programmed cell death 1 (PD-1) antibody[40,41]. Compared to previous TAA vaccines, NeoVax demonstrated enhanced tumor specificity and immunogenicity[32]. Furthermore, when NeoVax was administered as an immunotherapy for glioblastoma, an immunologically "cold" tumor type characterized by insufficient mutation burden (Table 1), neoantigen-specific T-cell responses were induced within intracranial glioblastoma tumors[42]. Unfortunately, all glioblastoma patients experienced subsequent tumor recurrence and ultimately succumbed to progressive disease, indicating considerable challenges for the CITs of glioblastoma and the limitations of NeoVax immunotherapy[43].

4. Immunomodulatory adjuvants based on peptides

4.1. *The role of DCs in the CIC*

Successful CITs entail activation of innate immune responses, leading to DC maturation, tumor antigen presentation, and subsequent activation of a cascade of adaptive immunity, ultimately leading to activation of antigen-specific T cells[44]. As professional APCs, DCs possess the ability to process endogenous proteins *via* the proteasome and present antigens to cytotoxic T cells in an MHC class I-restricted manner, while exogenous proteins within late endosomes undergo processing through phagocytosis and endocytosis, delivering antigens to helper T cells in an MHC class II-restricted way[45−47]. However, immature DCs can induce T-cell conversion into anergic and regulatory/inhibitory T cells when exposed to antigens[48]. In contrast, only mature DCs with costimulatory signals facilitate the differentiation of specific effector T cells, highlighting the significance of DC maturation in immune therapy[49]. Thus, the magnitude of the T-cell immune response primarily relies on the use of vaccine adjuvants; however, due to the potential risks associated with induced systemic inflammation, only a limited number of FDA-approved adjuvants have been used in humans since 1939[50]. In terms of the underlying mechanism, in addition to self-derived DAMPs, vaccine adjuvants can also mimic pathogen-associated molecular patterns (PAMPs) and transmit danger signals to DCs through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), C-type lectin receptors, and NOD-like receptors[51]. Consequently, PRR agonists have been extensively employed as potent vaccine adjuvants for inducing functional maturation of DCs[49].

4.2. *Peptide-mediated DC maturation*

TLRs are the most extensively studied family of PRRs that specifically recognize PAMPs in microbial species[52]. Among the TLR family members, TLR2 has garnered significant attention as a potential therapeutic target for immune adjuvants due to its ability to sensitively detect diverse PAMPs derived from various pathogens on both mouse and human conventional DCs (cDCs)[53]. By forming heterodimers with either TLR1 or TLR6, TLR2 expands the repertoire of ligands against pathogens by recognizing lipopeptides or lipoproteins produced by microorganisms[54] (Figs. 2 and 3). Consequently, lipopeptide agonists, represented by MALP-2, Pam_2CSK_4, and Pam_3CSK_4, were consecutively reported and applied to enhance the efficacy of cancer vaccination and tumor rejection[55]. However, two major intrinsic challenges significantly hinder the utilization of these lipopeptides under physiological conditions: poor esterase stability and susceptibility to oxidation[56,57]. Increasing attention has been given to the development of chemically and metabolically stable TLR2 agonists as immune adjuvants. Wu group[56] reported a newly designed Pam_3CSK_4 derivative SUP3 by replacing two ester groups with carbamates, substituting serine with glycine, and eliminating the fourth lysine residue (Fig. 2). SUP3 maintained its specificity and affinity for TLR2 while demonstrating remarkable improvements in stability and eliciting a more robust antitumor response in mice without inducing excessive inflammation compared to Pam_3CSK_4[56]. Moreover, a novel Pam_3Cys derivative called XS15 (Table 1) was evaluated as an effective adjuvant in a healthy human volunteer. The combination of XS15 and multipeptide vaccination in a water-in-oil emulsion elicited long-lasting antigen-specific T-cell responses in circulation for more than one year following a single vaccination[58]. These results highlight the potential of XS15 as an adjuvant for cancer vaccination, leading to the ongoing phase I clinical trial recruiting patients with newly diagnosed HLA-A2-positive MGMT-methylated glioblastoma (NCT04842513). Of note, XS15 has also been employed in COVID-19 vaccine development to induce SARS-CoV-2 T-cell immunity[59].

Among all subsets of DCs, cDCs have demonstrated superiority in antigen presentation and immune enhancement compared to other DC subsets[60,61]. Due to the remarkable impact of cDCs on

Table 1 Clinical trials of representative peptides participated in CIC.

Sort	Agent	Conditions	Phase	Status	Combination	NCT number[b]
Oncolytic peptide	CyPep-1	Advanced solid tumor malignancy	I/II	Recruiting	Use alone or combined with pembrolizumab	NCT04260529
		Advanced head and neck squamous cell, carcinoma, advanced breast cancer, and advanced melanoma	I/II	Recruiting	Use alone or combined with pembrolizumab	NCT05383170
	EP-100	Ovarian cancer	II	Completed	Use alone or combined with paclitaxel	NCT01485848
		Advanced solid tumors	I	Completed	Use alone	NCT00949559
	LL-37	Melanoma		Completed	Use alone	NCT02225366
	LTX-315	Transdermal accessible tumors	I	Completed	Use alone	NCT01058616
		Melanoma, breast cancer, head and neck cancer, lymphoma, and triple-negative breast cancer	I	Completed	Use alone or combined with ipilimumab or pembrolizumab	NCT01986426
		Carcinoma	I	Completed	Combine with a cancer vaccine (GV1001)	NCT01223209
		Advanced solid tumor	II	Recruiting	Combine with pembrolizumab	NCT04796194
		Cancer of the skin and basal cell	II	Recruiting	Use alone	NCT05188729
		Soft tissue sarcoma	II	Active, not recruiting	Combined with TILs	NCT03725605
Synthetic long peptide vaccines	NeoVax	Melanoma	I	Completed	Combined only with poly-ICLC	NCT01970358
		Glioblastoma	I	Recruiting	Combined with radiation therapy, Pembrolizumab and Temozolomide	NCT02287428
		Follicular lymphoma	I	Recruiting	Combined with Rituximab and Pembrolizumab	NCT03361852
		Lymphocytic leukemia	I	Recruiting	Combined with Cyclophosphamide and Pembrolizumab	NCT03219450
		Melanoma	I	Recruiting	Combined with Nivolumab Ipilimumab and Montanide	NCT03929029
		Ovarian cancer	I	Recruiting	Combined with Nivolumab	NCT04024878
		Kidney cancer	I	Recruiting	Combined with Ipilimumab	NCT02950766
		Melanoma and metastatic melanoma	I	Recruiting	Combined with CDX-301 and Nivolumab	NCT04930783
	GAPVAC	Glioblastoma	I	Completed	Combined with Poly-ICLC and GM-CSF	NCT02149225
		Melanoma	I	Completed	Combined with poly-ICLC	NCT01970358
Immunomodulator peptide	Pam_3Cys-GDPKHPKSF (XS15)	Glioblastoma Multiforme of brain	I	Recruiting	Use alone	NCT04842513
Point mutation antigenic peptide (In the past ten years)	MART-$1_{26-35(27L)}$[a] $gp100_{209-217(210M)}$[a]	Melanoma (Skin)	I	Completed	Combined with BMS-936558 and Montanide ISA 51 VG	NCT01176461
	$gp100_{209-217(210M)}$[a]	Recurrent melanoma	II	Completed	Combined with HPV 16 E7:12-20	NCT00003895
		Recurrent melanoma	III	Completed	Combined with Aldesleuk and Montanide ISA 51 VG	NCT00019682
		Melanoma	II	Completed	Combined with Leuprolide and MAGE-3 Peptide	NCT00254397

	NY-ESO-$1_{157-165(165V)}$[a]	Melanoma (Skin)	I	Active, not recruiting	Combined with Nivolumab, gp100:280-288 (288V), Montanide ISA 51 vegetable grade (VG), and Ipilimumab	NCT01176474
Target immune checkpoint peptide	IO103	Multiple myeloma	I	Completed	Combined with Montanide	NCT03042793
		Metastatic melanoma	I/II	Recruiting	PD-L1(IO103)/IDO(IO102) peptide vaccine combined with Nivolumab	NCT03047928
		Chronic lymphocytic leukemia	II	Completed	PD-L1(IO103), PD-L2(IO120) peptides with Montanide ISA51	NCT03939234
		Oropharynx/larynx/hypopharynx/ oral cavity squamous cell carcinoma	II	Recruiting	PD-L1(IO103)/IDO(IO102) peptide vaccine	NCT04445064
		Basal cell carcinoma	II	Completed	Use alone	NCT03714529
		Lung cancer non-small cell, head and neck squamous cell, carcinoma, urothelial carcinoma bladder	II	Recruiting	PD-L1(IO103)/IDO(IO102) peptide vaccine combined with pembrolizumab	NCT05077709
		Melanoma, squamous cell carcinoma of the head and neck	II	Not yet recruiting	PD-L1(IO103)/IDO(IO102) peptide vaccine combined with pembrolizumab	NCT05280314
		Metastatic melanoma, unresectable melanoma	III	Recruiting	PD-L1(IO103)/IDO(IO102) peptide vaccine combined with pembrolizumab	NCT05155254

[a]Sequence: MART-$1_{26-35\ (27L)}$: ELAGIGILTV, $gp100_{209-217\ (210M)}$: IMDQVPFSV, NY-ESO-$1_{157-165\ (165V)}$: SLLMWITQV.
[b]Clinical trials registered with the US National Library of Medicine at clinicaltrials.gov.

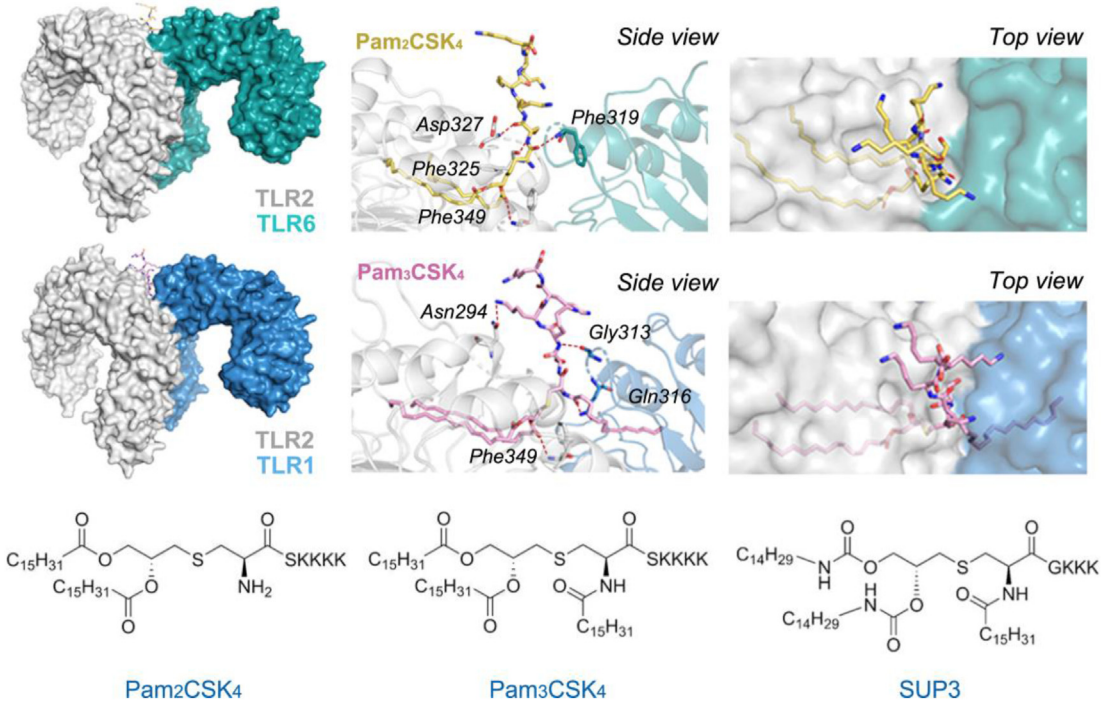

Figure 2 Structures of the human TLR2/TLR6-Pam_2CSK_4 complex (PDB: 3A79), human TLR2/TLR1-Pam_3CSK_4 complex (PDB: 2z7x), and SUP3. TLR1, TLR2, and TLR6 are depicted in blue, grey, and green respectively. Additionally, the Pam_2CSK_4 and Pam_3CSK_4 are shown by sticks in yellow and pink. The hydrogen bonds are marked by red dotted lines and their interaction residues are labeled accordingly.

antigen presentation and immunopotentiation[61], C-type lectin-like receptor 9A (Clec9a) has emerged as a crucial target for delivering cancer antigens to DCs. Clec9a is highly expressed on cDCs in lymphoid organs, blood, and peripheral tissues such as tumors. It specifically recognizes and phagocytoses fragments from dead cells, leading to the subsequent routing of dead cell-associated antigens into the cross-priming pathway (Fig. 3)[62,63]. Therefore, Clec9a-based peptides offer potential tools for the direct delivery of cancer antigens without the need for adjuvants, thereby preventing the inflammatory side effects associated with adjuvants. CBP-12 was developed as a 12-mer peptide carrier with high affinity for the Clec9a (K_D = 0.61 ± 0.09 μmol/L) *via* an *in silico*-aided method. CBP-12-conjugated peptide vaccines enhanced uptake and cross-presentation of antigens, leading to stimulation of specific $CD8^+$ T-cell responses and inhibition of tumor growth, even under adjuvant-free conditions[64]. Ca^{2+}-dependent lectin-type receptor family member 10A (Clec10a) is another essential pathogen-recognition receptor for DC maturation and is characterized by high recognition for those structures containing terminal *N*-acetyl galactosamine (GalNAc)[65]. Generally, glycans containing terminal GalNAc residues are referred to as Tn antigens (GalNAc-Ser/Thr) and are highly expressed in nearly all cancer cells (Fig. 3). These antigens can trigger a cascade of immune events, including DC maturation and T-cell activation[66]. Using a phage display library with GalNAc-specific lectin receptor analogs, researchers developed sv6D, a tetravalent compound, with a tri-lysine core based on a 6-mer peptide. At a concentration of 10 nmol/L, sv6D demonstrated a significant cellular response. Subcutaneous injection of Sv6D led to substantial expansion of mature DCs in the peritoneal cavity and prolonged survival time in mice bearing ovarian tumors without any observed toxicity[67].

5. Peptide mimotopes for enhanced T-cell response

Peptides function as epitopes and elicit a robust adaptive response in the antigen processing and presentation pathway[68]. The processed antigens are displayed on the surface of target cells or APC as peptide−MHC complex, which interacts with and binds to T cells, initiating T-cell recognition. Subsequently, the antigen recognition signal is transduced into T cells through CD3. Upon receiving additional synergistic stimulation signals, T cells can be activated and differentiate into effector T cells and memory T cells. $CD8^+$ cytotoxic T cells then exert their primary antitumor immune effect by recognizing antigenic peptides presented by MHC I molecules and eliminating cancer cells. Furthermore, accumulating evidence has demonstrated that $CD4^+$ T cells can also eliminate autologous tumors through the MHC II pathway while impeding cancer cell cycle progression to play an antiproliferative role[69,70]. Simultaneously, $CD4^+$ T cells can secrete cytokines such as tumor necrosis factor and IFN-γ to facilitate the activation, differentiation, and proliferation of cytotoxic T lymphocytes, thereby augmenting their killing capacity[71−73]. However, previous clinical trials using peptide antigens have encountered setbacks due to their limited efficacy and affinity[74], as T-cell responses are typically influenced by the affinities between antigenic peptides and MHC molecules. Therefore, peptide mimotopes that function as epitope analogs have been developed to enhance the effectiveness of epitope-based vaccines and elicit a robust antigen-specific T-cell response[73].

5.1. *Improvement of peptide-MHC binding affinity*

Based on the crystal structures of MHC molecules and epitope peptides, which elucidate their critical protein−protein interaction

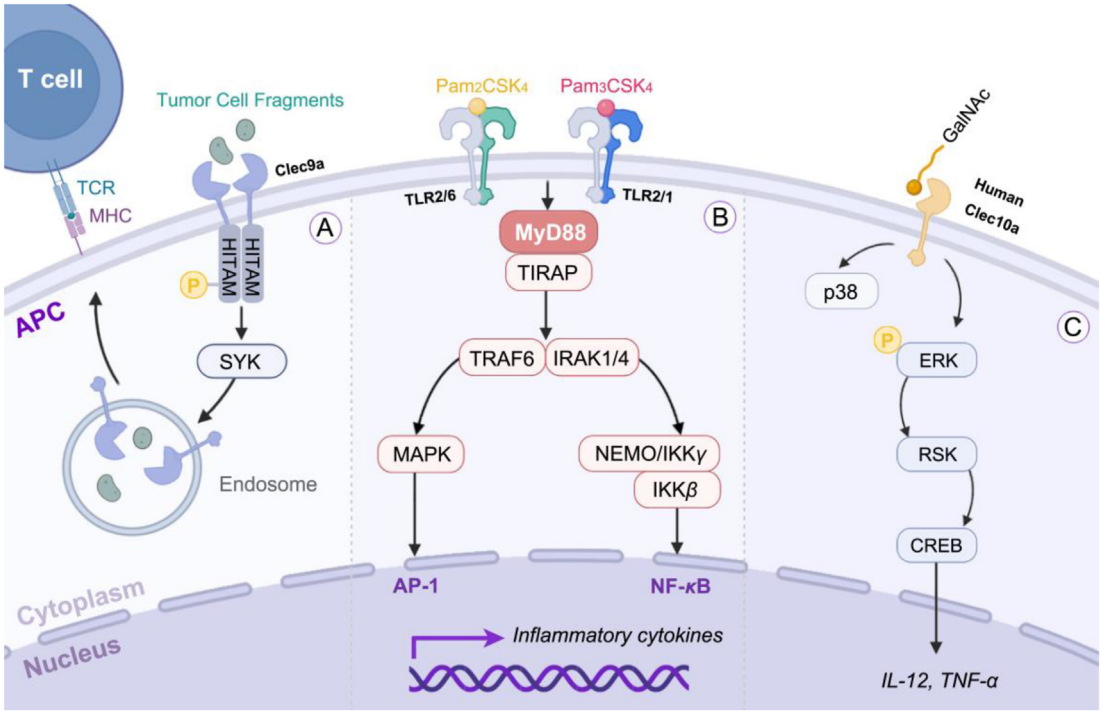

Figure 3 Signaling pathways induced by Clec9a, Clec10a, TLR2/TLR1, and TLR2/TLR6. (A) Clec9a possesses a hemi-immunoreceptor tyrosine-based activation motif (HITAM) cytoplasmic tail in the signaling pathway that contains a highly conserved tyrosine. Upon phosphorylation of this tyrosine, it facilitates the recruitment and binding of spleen tyrosine kinase (SYK). Consequently, the phagocytosed dead cell fragments are transported to a recycling endosome compartment. (B) TLR2 specifically triggers the MyD88-dependent pathway, recruiting interleukin-1 receptor-associated kinases (IRAKs) to the TLR1/TLR2 and TLR2/TLR6 complexes. Subsequently, phosphorylated and activated IRAK binds to tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), thereby activating the IκB kinase (IKK) complex for promoting NF-κB translocation into the cell nucleus. Additionally, activation of the mitogen-activated protein kinase (MAPK) pathway can also occur, facilitating AP-1 activation. Ultimately, these aforementioned signaling molecules induce inflammatory cytokines and chemokines in a concerted manner. (C) When combined with GalNAc ligands, human Clec10a can trigger extracellular regulated protein kinase (ERK)-dependent pathways, leading to the phosphorylation of ERK and subsequent activation of p90-RSK, cAMP, and response-element binding protein (CREB). This cascade ultimately enhances the secretion of IL-12 and TNF-α.

modes (Fig. 4), anchor residues of epitope peptides can be further modified using artificial or natural amino acids to generate mimotopes, thereby improving antigenicity and binding affinity[73]. The peptide binding grooves of both the MHC-I and MHC-II molecules are flanked by two α-helices and one beta-pleated sheet. Epitope peptides, in an extended conformation, interact with six pockets (from A to F) within the grooves. In MHC-I molecules, the last residue of the epitope peptide is buried within pocket F, whereas in MHC-II molecules, the epitope peptide extends beyond the F pocket. Through comprehensive analysis of MHC molecules, consensus peptide binding motifs have been identified for numerous alleles and utilized to predict high-affinity peptides through continuously improved algorithms. One such instance was the MUC1 epitope, an ideal tumor antigen for various tumor types, but MUC1 epitopes have not yet been approved for phase III clinical trials. A possible explanation lies in the low affinities of wild-type MUC1 epitopes for MHC molecules. Through computational prediction algorithms, three modified MUC1 peptides were generated by introducing point mutations at position 1 (P1) and position 2 (P2) residues of the MUC1 wild-type epitopes. These altered MUC1 mimotopes exhibited enhanced affinities for MHC-I HLA-A0201 and demonstrated stronger immunogenicity compared to their respective wild-type MUC1 epitopes, suggesting their potential utility as antitumor peptide vaccines[75]. However, modifications to the anchor residues of the typical melanoma-associated MHC-I-restricted epitopes $gp100_{209-217}$[76] (Table 1) and Melan-A/MART-1_{26-35}[77] (Table 1) did not generate T cells with improved functionality or cross-reactivity compared to wild-type epitopes. This finding suggested that mutagenesis may sometimes be limited by a loss of peptide specificity, highlighting the importance of precise T-cell receptor (TCR)-pMHC interactions for generating a specific T-cell repertoire[78,79].

5.2. *Improvement of TCR-pMHC binding affinity*

The TCR-pMHC interaction plays a crucial role in antigen-specific adaptive immunity, thus another avenue for mimotope development is to increase the binding affinity between pMHC molecules and TCRs[80]. One extensively studied tumor antigen is NY-ESO-1, which is presented by HLA-A*0201 in complex with the TCR. By analyzing the structure of TCR-like antibodies bound to the NY-ESO-$1_{157-165}$ peptide (SLLMWITQC), noncritical residues at position 9 (P9) of NY-ESO-$1_{157-165}$ were mutated, resulting in an analog called NY-ESO-$1_{157-165V}$ (SLLMWITQV) (Table 1). This analog exhibited a 20-fold improvement in binding affinity[81] and a 100-fold increase in potency for activating both $CD4^+$ and $CD8^+$ T cells[82]. Wei et al.[83] screened peptide libraries to identify residue substitutions in $GP70_{423-431}$ (SPSYVYHQF),

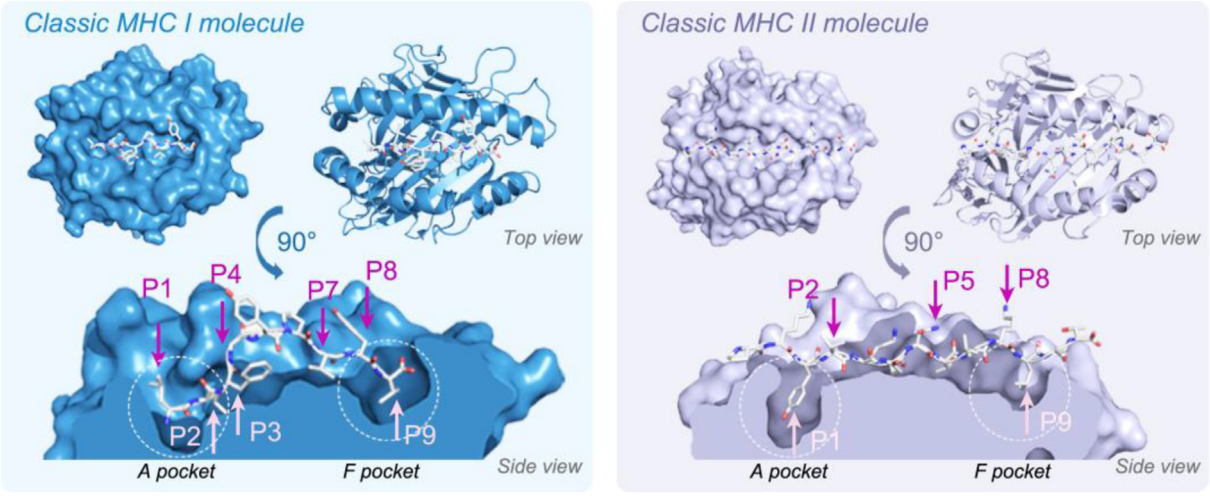

Figure 4 Binding of epitope peptides to the classic MHC I and MHC II molecules. The human MHC I class molecule (HLA-A*0201, blue) and MHC II class molecule (HLR-DR1, purple) are bound to the peptide LLFGYPVYV (left, PDB ID:1HHK) and the peptide PKYVKQNTLKLAT (right, PDB ID:1FYT), respectively. Stick views of peptides are used to display their binding grooves with MHC I and MHC II molecules. The arrows indicate the critical positions for binding with MHC molecules (in pink) and binding to TCRs (in rose red), numbered P1−P9. The function of the unnumbered pockets depends on the specific MHC molecules. Figures were generated by PyMOL.

which were derived from an endogenous retrovirus known as MuLV and restricted by the MHC-I molecule. They discovered that alanine substitution at position 5 within $GP70_{423-431}$ induced significant conformational changes during TCR engagement with the wide-type peptide, leading to enhanced stability of the pMHC−TCR complex. These findings elucidate the efficacy of modified $GP70_{423-431}$ as a vaccine candidate[83].

Collectively, these findings suggest that ideal peptide mimotopes should be efficiently presented by MHC molecules, and accurately recognized by TCRs. Although the mutation of weak epitope peptides to generate high-affinity and effective mimotopes remains challenging, methods for predicting and enhancing the binding of peptide mimotopes to MHC or TCR are currently under development[80,84].

6. Peptide-mediated immune checkpoint blockade (ICB)

The amplitude and quality of the antitumor immune response rely on the balance between inhibitory signals and costimulatory signals. In CIC, dysregulation of effector immune cells caused by immune checkpoint activation leads to the evasion of cancer cells from immune surveillance[85]. To counteract this negative feedback, immunotherapeutic antibodies have been developed to block various immune checkpoints, including the well-studied cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), PD-1, and its ligand PD-L1. However, a drawback of these antibodies is the emergence of severe immune-related adverse events (irAEs) in patients[86]. In contrast to therapeutic antibodies, peptides offer promising alternatives with enhanced tumor penetration and reduced systemic retention and irAEs. Of note, numerous peptide-based ICIs have been extensively employed in nanomedicines and combination therapies for CITs (Fig. 5)[87].

6.1. *Design strategies of peptide ICIs*

Peptide ICIs are specifically designed to target critical protein−protein interaction interfaces known as “hot spots” or epitopes, with the aim of blocking immune checkpoints. These essential motifs can be categorized into three types: (a) short-chain sequences comprising consecutive peptide epitopes; (b) secondary structural epitopes, such as a side of the face from the α-helix that can bind to the hydrophobic groove in complementary residues; (c) tertiary structural epitopes targeting both sides of the protein−protein interaction interface[88]. Based on these structural features, the rational design principles for peptide ICIs can be summarized as follows. (a) If the binding motif between receptors and ligands is known, specific fragments from the interaction interface can be utilized to obtain the desired peptide. (b) In cases where only one of these proteins is known, a “Peptide Walking” strategy could be employed to develop a self-inhibitory peptide. (c) When the motif is unknown, ICI peptides can be generated through computational *de novo* sequencing methods or screening phage-display libraries for potential targets[8].

6.2. *Peptides targeting classic immune checkpoints*

The PD-1/PD-L1 signaling pathway plays an essential role in maintaining immune homeostasis. Engagement of PD-L1/PD-L2 and PD-1 triggers phosphorylation of the ITIM and ITSM motifs on PD-1. Subsequently, SH1 and SH2 containing tyrosine phosphatase 1/2 (SHP-1, SHP-2) are recruited by PD-1 to inhibit the downstream PI3K/AKT pathway. This leads to the suppression of T-cell proliferation, survival, as well as protein synthesis and IL-2 production. In addition, the interaction between PD-1 and PD-L1 also enhances PTEN activity to further restrain the PI3K pathway (Fig. 5). Tumor cells often exploit the negative feedback mechanism mediated by PD-1/PD-L1 to evade immune surveillance and achieve immune escape. On the other hand, IFN-γ secreted by tumor-infiltrating lymphocytes could induce the upregulation of PD-L1 expression on tumor cells through the IFNGR-1/2- JAK1/2-STAT1/3-IRF-1 axis. Thus, blockade of either PD-1 or PD-L1 could augment CIT efficacy while prolonging the duration of the immune response[89−91]. To mitigate irAEs, the peptide NP-12 (Table 2) was developed by utilizing critical binding epitopes as the first PD-1-targeting peptide, demonstrating remarkable anti-tumor activity *in vivo*[92]. CLP002 (Table 2), a 12-residue anti-PD-

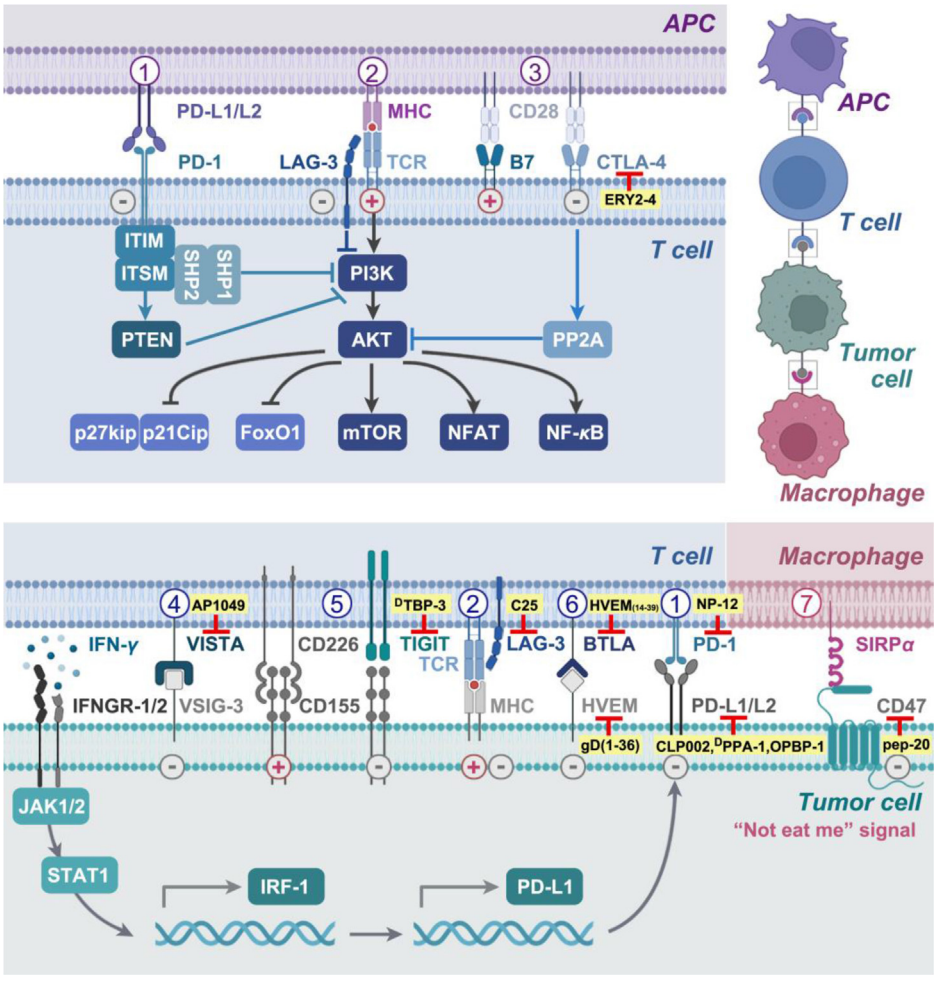

Figure 5 Peptides targeting immune checkpoints. There are three pairs of immune checkpoints in the interaction between APCs and T cells: 1) PD-L1 or PD-L2/PD-1, 2) LAG-3/MHC, and 3) CD28/CTLA-4. Regarding the interaction between T cells and tumor cells, five pairs of immune checkpoints associated with peptides are shown. Among them, 4) VISTA/VSIG-3, 5) TIGIT/CD155, and 6) BTLA/HVEM are new immune checkpoints. In addition, 7) CD47/SIRPα represents a pair of immune checkpoints in the interaction between macrophages and tumor cells. The plus sign (pink) indicates a positive effect on the antitumor response, while the minus sign (gray) indicates an inhibitory effect. The peptides targeting these immune checkpoints are highlighted in yellow.

L1 peptide inhibitor, was developed through phage display biopanning and exhibited superior tumor penetration compared to that of the PD-L1 antibody[93]. To enhance the proteolytic resistance of the peptide, ^{D}PPA-1 (Table 2), the first D-enantiomeric peptide antagonist, was designed by combining mirror-image phage display and chemical protein synthesis techniques[94] and subsequently was optimized to yield OPBP-1 (Table 2) with improved antitumor efficacy *via* point mutation and molecular dynamics simulation approaches[95]. Notably, due to the potent antitumor activities and enzymatic resistance of OPBP-1 *in vivo*, N,N,N-trimethyl chitosan was used to fabricate hydrogel for the oral delivery of OPBP-1. The OPBP-1-based hydrogel exhibited high bioavailability and significant antitumor efficacy with negligible toxicity, opening a new prospect for the oral administration of ICIs in cancer immunotherapy[95].

CD28 binds to its ligand CD80 (B7-1)/CD86 (B7-2) to amplify TCR signaling and subsequently activate T cells. CTLA-4, derived from naïve T cells, inhibits AKT phosphorylation by activating PP2A and competitively binds to CD80/CD86, resulting in the suppression of T-cell function[96]. Although anti-CTLA-4 antibodies have been proven to increase the survival of patients with melanoma, head and neck squamous cell cancer, renal cell carcinoma, and non-small cell lung cancer[97], antibody-dependent cellular toxicity-mediated natural killer (NK) cells may also eliminate CTLA-4 expressing regulatory T cells (Tregs) and cytotoxic T lymphocytes[98]. Under these circumstances, ERY2-4 (Table 2) was identified through yeast-displayed libraries with a K_D value of 196.8 ± 2.3 nmol/L for CTLA-4; it effectively disrupted the interaction between CTLA-4 and B7 without the limitations of antibody-dependent cellular toxicity or cross-reactivity with CD28[99].

6.3. *Peptides targeting new immune checkpoints*

Due to insufficient antigens and extraordinarily high/low PD-L1 expression, the majority of tumors are unresponsive to PD-1/PD-L1 blockade therapy, with a response rate below 30%[100]. The limited therapeutic efficacy and emergence of acquired drug resistance have prompted a gradual shift in research focus toward alternative ICB agents[101,102], such as T-cell immunoglobulin and

Table 2 Targets, names, and sequences of representative peptides targeting immune checkpoints.

Target	Peptide name	Sequence	Ref.
PD-1	NP-12	H-SNTSESFK(H-SNTSESF)FRVTQLAPKAQIKE-NH_2	92
PD-L1	CLP002	WHRSYYTWNLNT	93
	^{D}PPA-1	H-nyskptdrqyhf-NH_2[a]	94
	OPBP-1	gqsehhmrvysf[a]	95
CTLA-4	ERY2-4	Ac-C_1AWGQAILEGELAWLEGGGGGAGQLADLKRQLAWWKQAC$_1$-NH_2[b]	99
TIGIT	^{D}TBP-3	H-ggytfhwhrlnp-NH_2[a]	106
BTLA	$HVEM_{(14-39)}$	Ac-ESC$_1$PKC$_2$SPGYRVKEAC$_1$GELTGTVC$_2$EP-NH_2[b]	108
HVEM	gD $(1-36)_{(K10C-T29C)}$	Ac-KYALVDASLC$_1$MADPNRFRGKDLPVLDQLC$_1$DPPGVRR-NH_2[b]	110
VISTA	AP1049	H-SSAC$_1$DWIKRSC$_1$H-NH_2[b]	115
LAG-3	C25	H-C$_1$VPMTYRAC$_1$[b]-OH	118
CD47	Pep-20	AWSATWSNYWRH	122

[a]Lowercase letters indicate D-amino acids.
[b]C_n: two cysteines with the same number form a corresponding disulfide bond.

the ITIM domain (TIGIT), B and T cell lymphocyte attenuator (BTLA), V-domain Ig suppressor of T cell activation (VISTA), and lymphocyte activation gene-3 (LAG-3) (Fig. 5).

TIGIT, which is expressed on Tregs, activated T cells, and NK cells, competes with CD226 for binding to their ligand PVR (CD-155 or Nectin-2) to transmit inhibitory signals[103−105]. The identification of ^{D}PPA-1 facilitated the discovery of ^{D}TBP-3 (Table 2), the first peptide capable of blocking TIGIT, offering a potential candidate for overcoming anti-PD-1 resistance *in vivo*[106].

BTLA, expressed on human $CD8^+$ T lymphocytes, interacts with herpes virus entry mediator (HVEM) to form an immunosuppressing TME, in which dysfunctional T cells continuously express BTLA[107,108]. Based on the BTLA/HVEM complex, the disulfide-linked peptides $HVEM_{(14-39)}$[109] and gD $(1-36)_{(K10C-T29C)}$[110] were designed as inhibitors of BTLA/HVEM interactions with a K_D = 250 nmol/L and demonstrated the ability to effectively block the formation of the BTLA/HVEM complex in 293T cells (Table 2).

VISTA functions as a stimulatory ligand for APCs, promoting immune activation, while also acting as a negative ligand for T cells, suppressing their proliferation, activation, and cytokine production[111]. It is expressed mainly on myeloid cells and T lymphocytes, particularly on naïve $Foxp3^+$ Tregs and $CD4^+$ T cells[112]. Studies have indicated that VISTA can potentially interact with inhibitory proteins such as VSIG3 but is also regarded as a self-signaling receptor[113,114]. The peptide AP1049 (Table 2) was designed to target the critical regions called "hot spots" of VISTA and exhibited superior efficacy in driving T-cell proliferation compared to anti-PD-L1 antibodies and anti-VISTA antibodies[115].

LAG-3, also known as CD223, is a transmembrane protein primarily expressed in T cells, NK cells, and Tregs[116]. It predominantly attenuates the effector function of CIT, while enhancing the inhibitory function of Tregs[117]. As a promising complement to the LAG-3 antibody, the cyclic peptide C25 (Table 2) was developed through phase display to specifically bind with high affinity to LAG-3 and elicit potent antitumor effects by $CD8^+$ T cells in mice bearing CT26, B16, and B16-OVA cells. Simultaneously, the infiltration of Tregs into the tumor significantly decreased[118].

In addition to DCs and T cells, macrophages are also involved in CIC by facilitating the phagocytosis of tumor cells through the blockade of cluster of differentiation 47 (CD47). This mechanism enhances the cross-presentation of tumor antigens and promotes antigen-specific $CD8^+$ T-cell responses (Fig. 5)[119]. CD47, which acts as a "don't eat me" signal, is often overexpressed in tumor cells to evade immune surveillance[120]. However, CD47 antibodies used in clinical trials have shown potential side effects such as anemia[121]. Thus, a CD47-targeting peptide with a low molecular weight, Pep-20 (Table 2), was screened for specific binding to the human CD47-IgV domain and disrupting the interaction between CD47 and SIRPα by effectively interfering with tyrosine phosphorylation of SIRPα. Importantly, blocking CD47 with Pep-20 initiated an antitumor T-cell immune response without significant blood toxicity compared to blocking CD47 with an anti-CD47 antibody[122].

7. Peptide vaccines targeting immunosuppressive cells

Treating cancer patients with immune-modulatory vaccines targeting tumor immune escape mechanisms is a novel, generalizable strategy. For example, PD-L1- and IDO-specific T cells are easily and rapidly activated to kill $PD\text{-}L1^+$ and/or IDO^+ tumor/immunosuppressive cells, yet they exhibit strict regulation without inducing autoimmune reactions[123]. IO103, a PD-L1-derived peptide epitope with 19 amino acids, was developed to effectively stimulate the cytotoxicity of PD-L1-specific T cells against both PD-L1-positive cancer cells and immune-suppressive cells[124]. In clinical trials, IO103 demonstrated significant immune modulatory effects in multiple myeloma[124] and basal cell carcinoma[125], leading to complete regression in certain cancer patients with low-level and reversible adverse reactions. Moreover, the combination of the PD-L1/IDO (IO103/IO102) peptide vaccine with an anti-PD-1 antibody and Montanide adjuvant achieved an unprecedentedly high objective response rate of 80%, including 43% complete responses, along with a remarkable median progression-free survival of 26 months in metastatic melanoma patients[123] (Table 1).

8. Current challenges and future opportunities for peptides targeting CIC

Although much progress has been achieved, current peptides targeting CIC in clinical trials have limitations such as conformational flexibility and susceptibility to proteolysis, which results in a restricted application of local administration. To address these challenges, various modification methods for peptide stabilization, including stapling chemistry[126], D-amino acid substitution[106], PEGylation[127], and self-assembly[128], can be employed. We developed oncolytic peptides derived from wasp venom (StAno)

with significantly enhanced structural stability and antitumor activity through a side-chain-retention stapling modification. Strikingly, the leading StAno exhibited superior oncolytic potency compared to that of LTX-315, resulting in a 50% long-term survival rate in mice bearing B16F10 tumors[129]. To improve the circulation half-life and tumor targeting of oncolytic peptides, we further conjugated a peptide chimera by coupling a D-type PD-L1 targeting peptide with a PEG-functionalized oncolytic peptide *via* a matrix metalloproteinase-2-sensitive linker[130]. This all-in-one polymer chimera offers a promising application for tumor targeting and optimizing the pharmacokinetic properties of both oncolytic peptides and peptide ICIs. In addition to improving the bioavailability of therapeutic vaccines, such as oncolytic peptides, this innovative design effectively connected two critical steps (tumor antigen release and recognition of cancer cells by T cells) in CIC, thereby overcoming the immune evasion mechanisms that develop in the TME. Given the fact that numerous vaccine-based peptides have undergone extensive preclinical investigations but ultimately proven ineffective during clinical trials, this design may provide a paradigm for enhancing the efficacy of current therapeutic vaccines. Moreover, despite advancements in preclinical and clinical studies involving immune agonists, these agents have demonstrated modest therapeutic efficacy due to issues related to size constraints, epitope orientation binding affinity low stability, and undesired profile when administered *in vivo*[131]. Due to the same backbone and noncovalent interactions between residues, peptides possess the ability to self-assemble into nanomaterials that exhibit exceptional resistance against proteases while demonstrating polyvalent efficacy toward specific targets[132]. Wang's group[133] reported the development of a polyvalent peptide CD40 nanoagonist (PVA-CD40) with a preorganized self-assembly structure, which effectively activates DCs for an amplified and durable antitumor immune response. When combined with an anti-PD-1 antibody, PVA-CD40 demonstrated significant inhibition of tumor growth in 4T1 mice, resulting in an optimal survival rate of up to 37%. In contrast, none of the mice survived when treated with clinically relevant combination therapy involving CD40 antibody and anti-PD-1 treatment. This proof-of-concept study highlights the potential clinical translation of self-assembled peptides in CITs. Furthermore, rapid advancements in artificial intelligence and machine learning algorithms also facilitate the discovery and optimization of immunoregulatory peptides[134]. We anticipate that these novel approaches can further enhance peptide functionality and therapeutic efficacy by specifically targeting CIC.

9. Conclusions

Since CIT was listed as a breakthrough in 2013 by the journal Science and subsequent acknowledgment through the 2018 Nobel Prize in Physiology or Medicine, it has garnered significant attention from both academic and industry scientists who have made notable advancements in immunomodulators, cell therapies, cancer vaccines, oncolytic viruses, and CD3-targeted bispecific antibodies[135]. Building upon these milestones, CITs hold immense potential as promising cancer treatment modalities and have demonstrated success in clinical applications[136]. Notably, CIC is a crucial aspect of CITs that drives the development of diverse strategies to regulate stimulatory and inhibitory factors within tumors. Here, we focused on elucidating how peptides exert their prominent roles in regulating CIC as an emerging form of cancer immunotherapy. Despite the inherent limitations of peptides, including poor stability, extensive chemical and pharmaceutical investigations have been undertaken to propose promising strategies for overcoming these challenges. Furthermore, the implementation of novel therapeutic approaches serves as a viable strategy to enhance the effectiveness of peptides. For instance, targeted protein degradation technology has been widely utilized to degrade pathogenic proteins across different tumor types while exhibiting favorable attributes in clinical settings[137−140]. Peptide-based protein degraders targeting immune checkpoints (*e.g.*, PD-L1, CD155, and CD47) may serve as future directions for better CITs while overcoming drug resistance caused by inhibitors.

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Author contributions

Xiaokun Zhang: Conceptualization, Writing − original draft. Ye Wu: Conceptualization, Writing − original draft. Jiayi Lin: Resources. Shengxin Lu: Resources. Xinchen Lu: Resources. Aoyu Cheng: Resources. Hongzhuan Chen: Writing − review & editing, Funding acquisition. Weidong Zhang: Writing − review & editing, Funding acquisition. Xin Luan: Funding acquisition, Writing − review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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