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MINI REVIEW

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Development of small molecule drugs targeting immune checkpoints

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ABSTRACT Immune checkpoint inhibitors (ICIs) are used to relieve and refuel anti-tumor immunity by blocking the interaction, transcription, and translation of co-inhibitory immune checkpoints or degrading co-inhibitory immune checkpoints. Thousands of small molecule drugs or biological materials, especially antibody-based ICIs, are actively being studied and antibodies are currently widely used. Limitations, such as anti-tumor efficacy, poor membrane permeability, and unneglected tolerance issues of antibody-based ICIs, remain evident but are thought to be overcome by small molecule drugs. Recent structural studies have broadened the scope of candidate immune checkpoint molecules, as well as innovative chemical inhibitors. By way of comparison, small molecule drug-based ICIs represent superior oral bioavailability and favorable pharmacokinetic features. Several ongoing clinical trials are exploring the synergetic effect of ICIs and other therapeutic strategies based on multiple ICI functions, including immune regulation, anti-angiogenesis, and cell cycle regulation. In this review we summarized the current progression of small molecule ICIs and the mechanism underlying immune checkpoint proteins, which will lay the foundation for further exploration.

KEYWORDS Immune checkpoints; small molecule drugs; programmed death protein 1; CD47; signal-regulatory protein α

Introduction

The emergence of immunotherapy has shown great potential in cancer treatment, especially the innovative strategy of immune checkpoint blockade (ICB). Immune checkpoints serve to regulate tumoral antigen recognition of T cell receptors (TCRs) during the immune response, which can be grouped into co-stimulatory and -inhibitory immune checkpoints[1-4]. Normally, co-inhibitory immune checkpoints [e.g., programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and V-domain immunoglobulin that inhibits T cell activation (VISTA)] function to protect the body from autoimmune attack, while tumors overtake those pathways to suppress immune activation and provide protection from cytotoxicity[5]. Therefore, blocking inhibitory immune checkpoints is an effective strategy to recover anti-tumor immunity.

Current immune checkpoint inhibitors (ICIs) are mainly comprised of macromolecular monoclonal antibodies and small molecule drugs, including chemical compounds and short peptides[6]. Even though macromolecular ICIs have ushered in a new era of immune therapy with impressive progress observed in numerous clinical trials, poor tissue permeability of macromolecular antibodies greatly limit the efficacy[7,8]. In addition, the off-target effect, risk of side effects, and exorbitant costs are not insignificant[9]. In fact, small molecule drugs comprised of organic compounds retain greater potential in the treatment of cancer. First, small molecule drugs with molecular weights < 1,000 g/mol exhibit better tissue permeability and tissue penetration. A simple structure is also associated with predictable and controllable pharmacokinetics and pharmacodynamics[10]. Second, compared to the high

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specificity of antibodies, small molecule drugs synergistically target and regulate numerous anti-tumor pathways[11]. Third, due to greater oral availability and lack of immunogenicity, most patients are more tolerant to small molecule drugs[12]. Fourth, in consideration of the economy, the cost to manufacture, store, and transport small molecule drugs is generally cheaper because of the stable structure[13]. In this review we summarized the current advances of small molecule drugs targeting immune checkpoints (**Table 1** & **Figure 1**) and highlighted the specific underlying mechanism and interaction residues of small molecule drugs with immune checkpoints.

Small molecule drugs directly targeting immune checkpoints

Unlike antibody-based approaches, most small molecule ICIs are undergoing pre-clinical investigation. The main obstacles impeding current investigation are screening optimal target sites and designation of drugs. Due to the incomplete structural information available for immune checkpoint pairs, research involving small molecule ICIs is proceeding at a slow pace[63]. Protein-protein interactions (PPIs) are hot spots driven by one or a cluster of conserved amino acid (aa) residues locally and intensively arrayed at the interaction interface, while masking the hot spots is expected to effectively block the transmission of immunosuppressive signals[64]. To date hot spots at the PPI interface of a few novel immune checkpoint molecules have been identified, such as PD-1/PD-L1 and CD47/SIRPα[65,66]. However, an inadequate understanding and structural complexity of the PPI interface restrict characterization of the nature, as well as subsequent drug development. In this section we provide a summary of research encountered in drug development of immune checkpoint molecules.

PD-1 and PD-L1

PD-1 (also known as PDCD1 and CD279) is a transmembrane protein belonging to the CD28/CTLA-4 family. PD-1 is mostly expressed on the surface of immune cells (especially T cells). The "brake" function of PD-1 in T cell activation mainly relies on engaging with PD-L1. One ligand of PD-1 is distributed in both immune and tumor cells[67,68]. Once combined, the PD-1 and PD-L1 signal recruits SHP2 to block the activation of various phosphatases (e.g., AKT and ERK)[68], leading to impaired immune signals and the T cell immune response[69]. At present, a number of monoclonal antibodies (mAbs) to PD-1 or PD-L1 drugs are at the clinical investigation stage or have been granted[70]. However, the effect of these mAb drugs remains limited in distinct populations because of low target abundance or insufficient intra-tumoral accessibility[71].

The crystal structure of the PD-1/PD-L1 binding interaction was decoded in 2015 and is mediated by the GFCC 'β folding structure in the PPI region[65]. The CC' ring at Met70-Asp77 rotates 90 degrees after combination, switching from the "open" state to the "closed" state to form four hydrogen bonds between PD-1 and PD-L1. There are three major hot spots on the interaction surface between PD-1 and PD-L1. The first hot spot is defined as the Ile134 pocket, which can accommodate a six-membered aromatic ring with the hydrophobic character. The second hot spot has been tested to be efficiently filled by branched aliphatic moieties, while the third hot spot is an extended groove that is relatively shallow and difficult to target[65]. At present, several small molecule drugs directly targeting PD-1-PD-L1 are being investigated. Based on the structural features, the drugs in development can be classified into biphenyls, sulfonamides, and oxadiazoles, some of which have been investigated in preclinical or clinical trials.

BMS-202 and BMS-200

BMS-200 and BMS-202 were screened with potent inhibitory activity from a batch of small molecule drugs with a core biphenyl structure. The structure of BMS-200 is based on a 3-(2,3-dihydro-1,4-benzodioxan-6-yl)-2-methylphenyl methanol skeleton, while BMS-202 is based on a 2-methyl-3-biphenyl methanol skeleton. BMS-202 can bind to the two hot spots on PD-L1, inducing dimerization of PD-L1. BMS-202 can insert into the cylindrical hydrophobic pocket formed within the dimer, eventually dissociating the PD-1/PD-L1 complex. It has been observed that BMS-202-induced dimerized PD-L1 completely masks the surface of the dimer, which interacts with PD-1 and interferes with the PD-1 and PD-L1[14] interaction. The antitumor effects of BMS-202 have been verified in melanoma and mouse models, while inhibition of PD-1/PD-L1 binding by BMS-202 successfully rescues the suppressed IFN-γ production in T cells[72]. In addition, BMS-202 has the advantages of high bioavailability and low toxicity[73-75]. In addition, a series of derivatives of benzo[d] isothiazole have been developed using BMS-202 as a starting point, exhibiting a superior effect in inhibiting the PD-1/PD-L1 interaction and lower cytotoxicity to T cells compared to BMS-202[76,77].

Table 1 Small molecule anti-cancer drugs directly targeting immune checkpoints

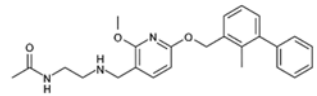
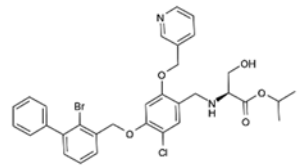
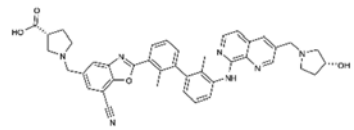
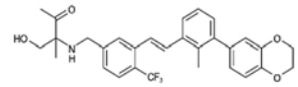
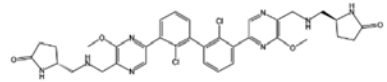
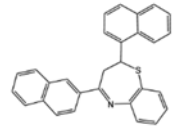
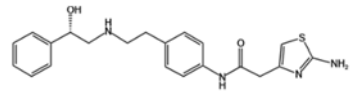
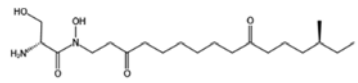
Targets		Compound	Structure	Research stages	Cancer types	References
PD-1/PD-L1	Binding of PD-1 to PD-L1	BSM-202		Preclinical	Melanoma	14
		IMMH-010		Phase I (NCT04343859)	Malignant neoplasms	15
		INCB086550		Phase I (NCT03762447)	Solid tumors	16
		MAX-10181		Phase I (NCT04122339)	Solid tumors	17
		GS-4224		Phase I (NCT04049617)	Advanced solid tumors	NA
		BPI-371153	NA	Phase I (NCT05341557)	Advanced solid tumors or relapsed/refractory lymphoma	
		ASC61	NA	Phase I (NCT05287399)	Advanced solid tumors	
		NSC631535		NA	NA	18
		Mirabegron		NA	NA	19
		Neoenactin B1		NA	NA	20

Table 1 Continued

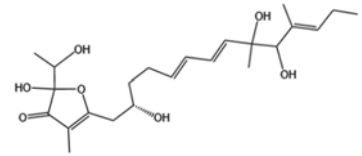
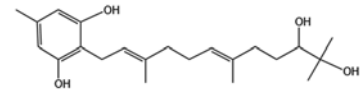
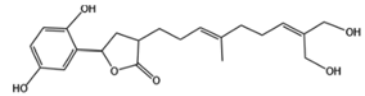
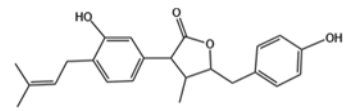
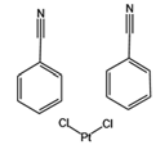
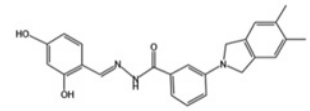
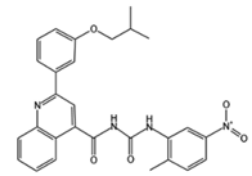
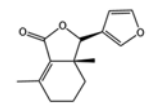
Targets	Compound	Structure	Research stages	Cancer types	References
	Actinofuranone I				
	Cosmosporin A				
	Ganocapenoid A				
	CHEBI:208188				
	Bis(benzonitrile) dichloroplatinum		NA	NA	20
	PubChem CID 135781783		NA	NA	21
	PubChem CID 4096583				
Reduce the expression of PD-1 or PD-L1	Fraxinellone		NA	NA	22,23

Table 1 Continued

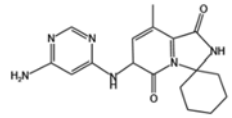
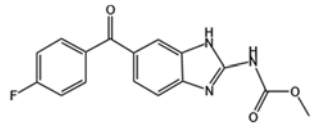
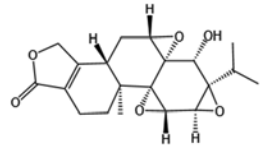
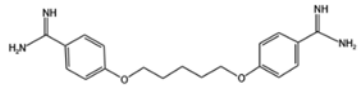
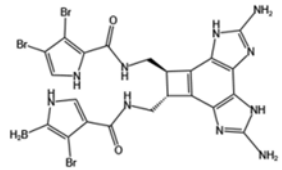
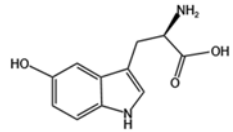
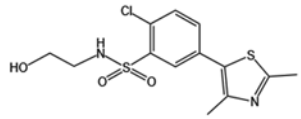
Targets	Compound	Structure	Research stages	Cancer types	References
	eFT508		Preclinical	Triple-negative breast cancer	[24]
	Flubendazole		Preclinical	Melanoma	[25]
	Triptolide		Preclinical	NSCLC	[26]
	Pentamidine		NA	NA	[27]
	Benzosceptrin C		NA	NA	[28]
	L-5-hydroxytryptophan		NA	NA	[29]
	PIK-93		NA	NA	[30]

Table 1 Continued

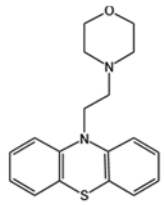
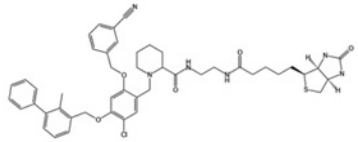
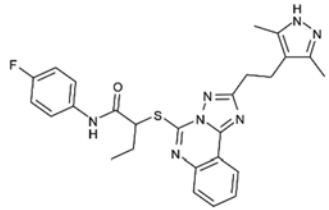
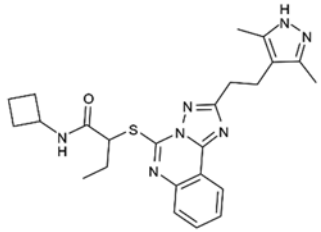
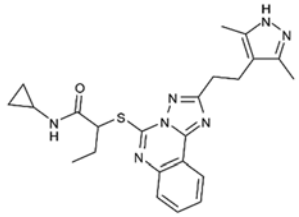
Targets		Compound	Structure	Research stages	Cancer types	References
		LSD1-IN-24		NA	NA	[31]
		SWS1		NA	NA	[32]
CD47/SIRPα	Binding of CD47 to SIRPα	NCGC00138783		Preclinical	Leukemia	[33]
		NCG00538430		Preclinical		
		NCG00538419		Preclinical		

Table 1 Continued

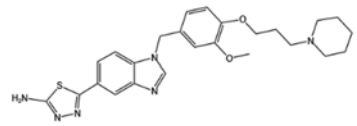
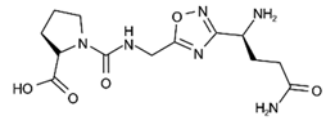
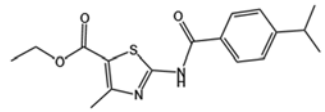
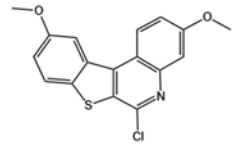
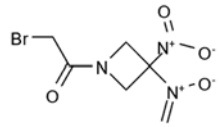
Targets	Compound	Structure	Research stages	Cancer types	References
	IsoQC-IN-1		NA	NA	[34]
	1,2,4-oxadiazole compounds		Preclinical	A20 syngeneic model	[35]
	SWY-AK-309		NA	NA	[36]
	SWY-AM-598		NA		
	Pep-20	AWSATWSNYWRH	Preclinical	Solid tumors	[15]
	RS-17	RRYKQDGGWSHWSPWSS-NH2	Preclinical	NA	[37]
	D4-2	Ac-yRYSAVYSIHPSWC G-NH2	Preclinical	Solid tumors	[38]
	SP5	CTQDAWHIC	Preclinical	NA	
Reduce the expression of CD47 or SIRPα	RRx-001		Phase Ⅰ (NCT02215512) (NCT02096341) (NCT02801097) (NCT02518958) (NCT04525014) Phase Ⅱ (NCT02452970) (NCT02096354) (NCT03515538) (NCT02489903) Phase Ⅲ (NCT03699956) (NCT05566041)	Malignant solid tumor Advanced cancer Lymphomas Brain tumor Small cell carcinoma Neuroendocrine tumors Ovarian epithelial cancer Oral mucositis Colorectal neoplasms NSCLC CHOL SCLC	[39-44]

Table 1 Continued

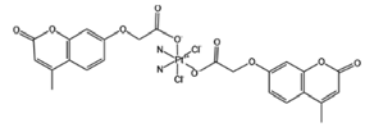
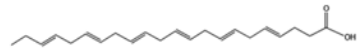
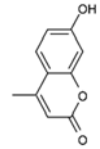
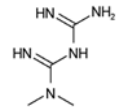
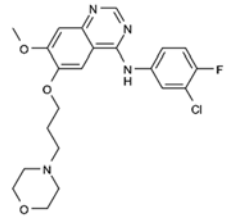
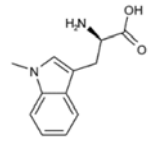
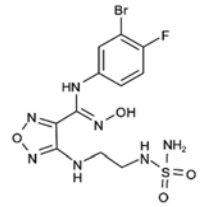
Targets	Compound	Structure	Research stages	Cancer types	References
	DMUP		NA	NA	[45]
	Docosahexaenoic acid		Preclinical	Colorectal cancer	[46]
	4-methylumbelliferone		Preclinical	Hepatic cancer	[47]
	Metformin		Preclinical	Breast cancer	[48]
	Gefitinib		Preclinical	NSCLC	[49]
IDO	Indoximod		Phase Ⅱ (NCT01560923)	Prostate cancer	[50]
	Epacadostat		Phase Ⅲ (NCT02752074)	Melanoma	[51]

Table 1 Continued

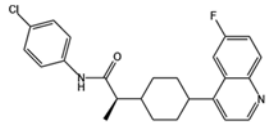
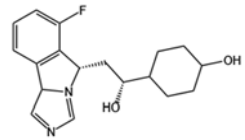
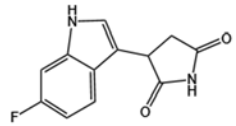
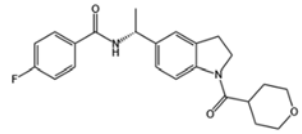
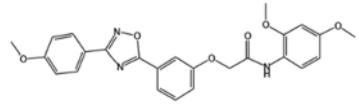
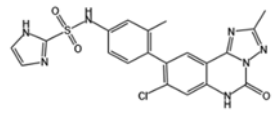
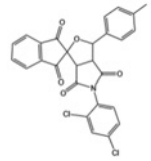
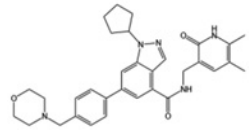
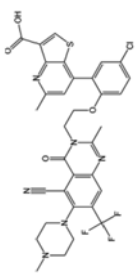
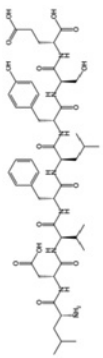
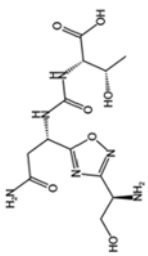
Targets		Compound	Structure	Research stages	Cancer types	References
		Linrodostat		Phase III (NCT03661320)	Muscle-invasive bladder cancer	52
		Navoximod		Phase I (NCT02471846) (NCT02048709)	Advanced solid tumors Recurrent advanced solid tumors	53,54
		PF06840003		Phase I (NCT02764151)	Recurrent malignant glioma	55
		LY-3381916		Phase I (NCT03343613)	Advanced cancer	56
TIM-3	Binding of TIM-3	TIM-3-IN-2		Preclinical	Acute myeloid leukemia	57
		TIM-3-IN-1		NA	NA	58
		ML-T7		NA	NA	59
	Reduce the expression of TIM-3	EPZ005687		NA	NA	60

Table 1 Continued

Targets	Compound	Structure	Research stages	Cancer types	References
	eIF4E-IN-1		NA	NA	
Dual targets	CD24/Siglec-10 and PD-1/PD-L1	CSBP		NA	NA	61
	PD-L1 and VISTA	CA-170		Phase II (NCT02812875)	Advanced solid tumors or lymphomas	11,62

NSCLC, non-small cell lung cancer; CHOL, Cholangiocarcinoma; SCLC, small cell lung cancer.

CA-170

CA-170 is a small molecule with dual targets (PD-L1 and VISTA), which belongs to the immunoglobulin (Ig) superfamily inhibitory ligand and is enriched in hematopoietic cells. Because the development of CA-170 predated the publication of interpreting the complete structure of hPD-1/PD-L1, whether CA-170 acts on the checkpoint itself has been disputed[78]. A study conducted in 2019 showed that CA-170 failed to bind to hPD-L1 or dissociate the human PD-1/PD-L1[79] complex. The pharmacodynamic action and mechanism underlying CA-170 were clarified by Sasikumar et al. in 2021 based on the accurate structure of CA-170, which is an AA-fused heterocyclic with a 1,2,4-oxadizole template. Sasikumar et al. suggested that CA-170 interacts at relatively small hydrophilic solvent-exposed residues on PD-L1 to form a ternary complex, hindering the transduction of inhibitory signals by inactivating the ternary complex rather than disrupting the hPD-1/hPD-L1 interaction[11]. However, the critical residues or hotspots have not been identified.

CA-170 is the first small molecule oral ICI to enter clinical trials. As reported in a phase I trial in 2016, CA-170 activates peripheral blood T cells in solid tumors and lymphomas, and was favorably tolerated to the maximum dose up to 1,200 mg twice daily[80]. Similar to PD-1/PD-L1 mAbs, a bell-shaped curve of immune-activated CA-170 was observed in a phase II clinical trial, exhibiting a higher effect at lower dosage (400 mg) but reduced immunoactivity at a higher dosage (500 mg). However, immune-related adverse events (irAEs) were more frequent in patients taking the 400 mg dose, even though the adverse reaction of CA-170 was restricted and short-term due to the short half-life[62]. In view of this evidence, a more potent and safer dose of CA-170 is anticipated.

YPD-30 and YPD-29B

YPD-29B is an oral inhibitor of PD-1 and PD-L1, which is [4-(2-bromo-biphenyl-3-ylmethoxy)-5-chloro-2-(pyridin-3-ylmethoxy)-benzylamino]-3-hydroxy-propionic acid. As disclosed in 2022, YPD-29B interacts with dimerized PD-L1 through solid π-π stacking, a weak cation-π interaction, a salt bridge, and the phenyl ring of YPD-29B surrounded by a hydrophobic cavity composed of ILE54, TYR56, MET115, and ALA121 on chain B and ALA121, TYR123, MET115, and ILE54 on chain A of PD-L1[81]. This combination effectively induced internalization and lysosome-mediated degradation of PD-L1, avoiding an interaction with PD-1 and inactivating T lymphocytes. YPD-29B has also shown superior activity in

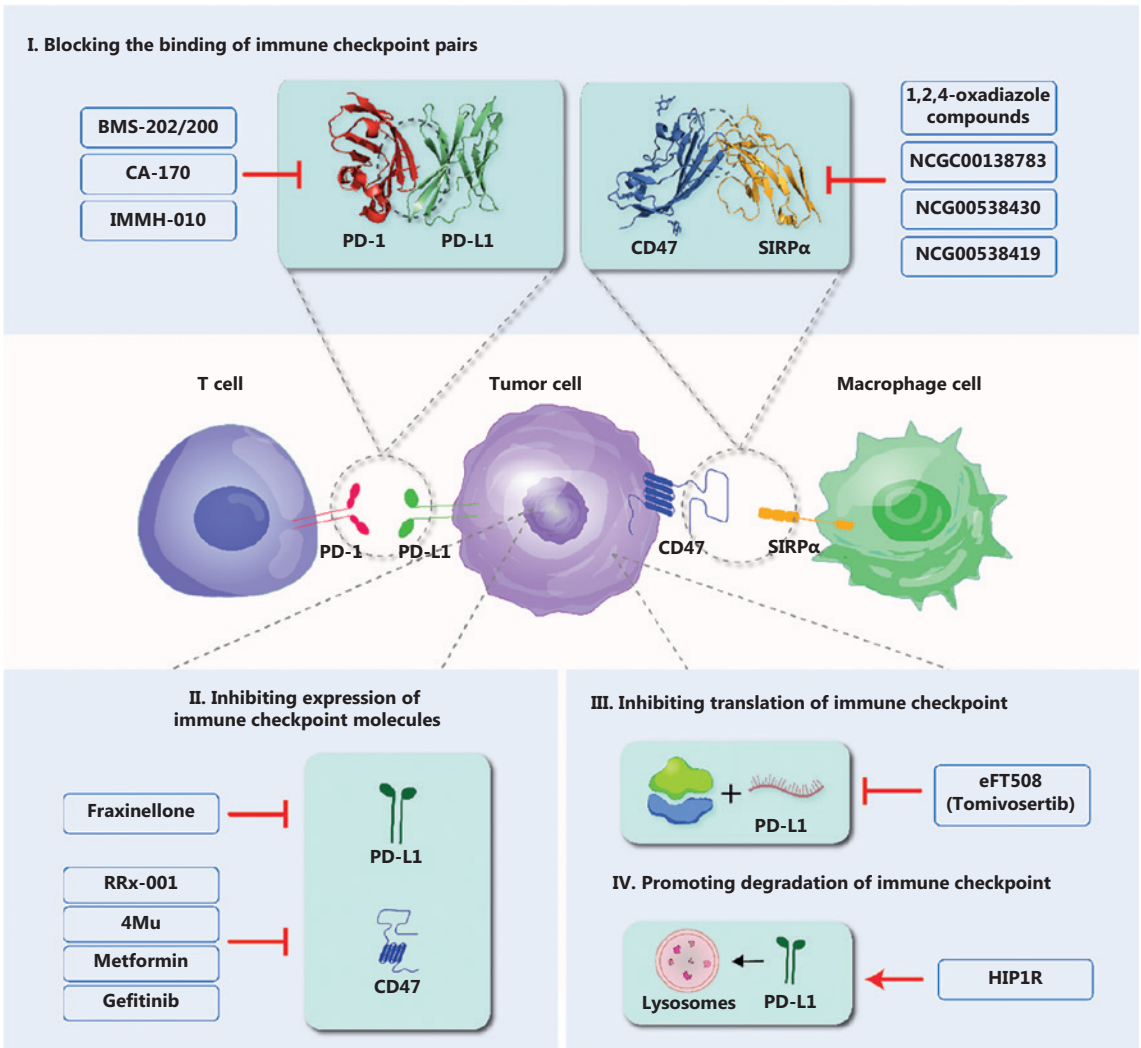


Figure 1 Summary of current small molecular drugs targeting PD-L1 and CD47. Small molecule chemical compounds block the function of major immune checkpoint molecules by **(I)** competitively binding with immune checkpoint molecules to block their combination with paired receptors (BMS202, CA-170, and IMMH-010 targeting PD-L1; NCGC00138783 and 1,2,4-oxadiazole compounds targeting CD47); **(II)** inhibiting the level of PD-L1 and CD47 expression (e.g., fraxinellone, RRx-001, and metformin); **(III)** inhibiting the translation of PD-L1 (e.g., eFT508); **(IV)** promoting degradation of PD-L1 (e.g., HIP1R).

promoting dimerization of PD-L1 and a high safety profile with rare toxic effects on normal human or cancer cells compared to BSM-202[82]. Moreover, YPD-29B had a longer elimination half-life ($t_{1/2}$) and a higher concentration in tumor tissue than plasma for insufficient drug-metabolizing enzymes in the tumor and high affinity with PD-L1[83]. YPD-29B is marked by better safety and efficacy in blocking the PD-1 and PD-L1 combination than BMS-202.

To improve the drug ability, an esterified product of original hygroscopic YPD-29B [YPD-30 (IMMH-010)] was synthesized. YPD-30 is extensively catalyzed to YPD-29B in the liver by carboxylesterase 1 (CES1) 1 h after oral administration, with significant antitumor activity and PK characteristics. Considering the prominent safety and tolerability (single dose or multiple doses), YPD-30 (IMMH-010) has entered phase I clinical trials in China[81].

INCB086550

As an oral PD-1/PD-L1 interaction inhibitor, INCB086550 selectively binds to PD-L1 at a site similar to anti-PD-L1 mAbs, impeding the downstream pathway[84]. Apart from directly blocking the binding site of PD-1 on PD-L1, INCB086550

mediates the dimerization and internalization of PD-L1. Administration of INCB086550 also augments the production of IFN-γ in a concentration-dependent manner, with a maximal level comparable to atezolizumab (425% *vs.* 433%)[16]. In addition, molecular pharmacology studies have shown that INCB086550 abolishes recruitment of ITIM to SHP in PD-1, then restores activation of the nuclear factor of activated T cells (NFAT) pathway and infiltration of immune cells into the tumor microenvironment. The INCB086550 regulation pattern on the transcription profile and immunity is similar to other therapeutic antibodies, such as atezolizumab, with comparable cytotoxicity[16,85]. Data from a phase I study of advanced solid tumors revealed that patients receiving INCB086650 had an objective response rate of 11.8% and a disease control rate of 19.1%. INCB086650 demonstrated safety and tolerability in other preclinical studies, while the irAEs were similar to mAb-based ICI therapy, except for peripheral neuropathy[16,84,86].

MAX-10181

MAX-10181 is an oral PD-L1 inhibitor that has also entered a phase I clinical trial (NCT04122339) in Australia. The tumor growth inhibition of MAX-10181 in MC38 cells is comparable to durvalumab[17]. In a mouse model without adequate PD-L1 expression, MAX-10181 also showed superior efficacy, laying the foundation for combined therapy with other PD-1 or PD-L1 inhibitors.

Others

In addition to interfering with PD-1 and PD-L1 binding, there are some small molecules that also inhibit the expression and translation of PD-1/PD-L1 or degrade PD-1/PD-L1. For example, fraxinellone, which is isolated from the antitumor herb, *Dictamnus dasycarpus*, suppresses the synthesis of HIF-1α and STAT3 to reduce the expression of PD-L1, thereby restricting proliferation and angiogenesis in tumor cells[22,23]. In addition, translation of PD-L1 has been reported to be inhibited by eFT508 (tomivosertib). Working as a kinome selective inhibitor, eFT508 suppresses phosphorylation of eukaryotic initiation factor 4E (eIF4E) to restrain mRNA translation, preventing progression and metastasis of liver cancer[24]. With respect to PD-L1 degradation, Huntingtin-interacting protein 1-related (HIP1R) has been shown to directly interact with PD-L1 at the C-terminal tail (771–867), transporting PD-L1 to lysosomes through the carboxy-terminal lysosomal sorting sequence (966–979) containing a di-leucine sorting signal. Based on the motifs of HIP1R, a chimeric peptide (PD-LYSO) was designed to mediate lysosomal degradation of PD-L1 in tumor cells[87]. Furthermore, several conventional small molecule drugs that have been on the market for some time have recently demonstrated the potential to reduce PD-1 expression[25-27].

CD47-SIRPα

The CD47-SIRPα axis bears a resemblance to PD1-PD-L1, governing myeloid cell-mediated cytotoxicity, especially macrophages. CD47 belongs to the immunoglobulin superfamily and is an integrin-associated protein (IAP) overexpressed on tumor cells[88,89]. SIRPα is mainly expressed on myeloid cells with two immunoreceptor tyrosine-based inhibition motifs (ITIMs). The combination of CD47 and SIRPα results in phosphorylation of ITIM, which in turn inhibits the role of non-muscle myosin IIA and eventually prevents phagocytosis of macrophages[90].

Like PD-1 and PD-L1, numerous antibodies blocking the CD47 and SIRPα axis have shown encouraging advances in clinical evaluation and treatment (mAbs, fusion proteins, and bispecific antibodies). However, due to an abundance of CD47 on healthy cells, antibodies targeting CD47 may cause blood toxicity, such as anemia, neutropenia, and a low platelet count[90-92]. Therefore, small molecule inhibitors (e.g., NCGC00138783) that target PPI sites or expression of CD47 and SIRPα, are expected to avoid this issue[66,93].

NCGC00138783, NCG00538430, and NCG00538419

NCGC00138783 was screened from a small molecule library by Miller et al.[94] through quantitative high-throughput screening (qHTS) assays. By means of a library possessing 90,000 compounds, NCGC00138783, NCG00538430, and NCG00538419 were screened with antagonistic activity[33]. Based on prediction results, NCGC00138783 forms hydrogen bonds and T-stacking interactions with key residues on SIRPα, including Leu30, Gly34, Pro35, Gln52, Lys53, and Lys93, and inserts into the hydrophobic cavity by forming π–π stacking with Phe74 and hydrogen bonding with Gly34. These interactions occupy the key binding position, resulting in failure of the combination between CD47 and SIRPα[66].

1,2,4-oxadiazole compounds

Sasikumar et al. synthesized a batch of compounds with oxadiazole scaffolds. Compound #6 demonstrated the best efficacy on recovering macrophage-mediated phagocytosis, with high normalized phagocytosis rates of 66% and 74%, as detected by luciferase assay and fluorescence-activated cell sorting (FACS),

respectively. Using the A20 syngeneic lymphoma model, compound #6 exhibited no clinical signs of toxicity and an outstanding TGI value (53%) at an oral dose of 3 mg/kg, suggesting that compound #6 is well-tolerant and has good antitumor efficacy[35]. 1, 2, 4-oxadiazole was inserted into the hydrophobic pocket of CD47, masking the key residues of the CD47/SIRPα interaction (Trp40, Thr107, and Lys6) and forming hydrogen bonds with Thr7 and Thr107 near the core interaction area[66]. Based on existing research, the hot spots on CD47 were shown to be Glu104 and Glu106, while the hot spots on SIRPα were Gln52, Lys53, and Phe56[66].

RRx-001

As the first inhibitor of CD47 entering clinical studies, RRx-001 acts to inhibit the expression of CD47 and SIRPα rather than blocking PPIs[95]. The reduction in CD47 expression induced by RRx-001 was mediated by inhibiting myelocytomatosis viral oncogene homolog (MYC) *via* binding to peroxisome proliferator activated receptor gamma (PPAR-γ), which forms a heterodimer with retinoid X receptor (RXR)[39]. As a pleiotropic anticancer agent, RRx-001 was shown to be involved in polarization of tumor-associated macrophages (TAMs). After binding with RRx-001, the amounts of iron, heme, and free radicals in the form of oxidized lipids carried by erythrocytes were increased. When these erythrocytes are phagocytosed by TAMs, TAMs are easily transformed into the pro-inflammatory, high-phagocytic M1 phenotype[96]. In this situation, the phagocytic activity of M1 TAMs towards tumors is reinforced[97]. Macrophage depletion attenuates the anti-tumor ability of RRx-001 *in vivo*, validating that the presence of tumoral-infiltrated macrophages is an indispensable condition for the antitumor activity of RRx-001[95,98]. Additionally, compared to other ICIs, RRx-001 exerts more antitumor effects, either alone or in combination, for its multiple roles in anti-angiogenesis, sensitization to chemotherapeutic agents, and immune sensitization[39-42]. In addition to RRx-001, some traditional anti-tumor small molecule drugs, including 4-methylumbelliferone (4Mu), metformin, and gefitinib, have been shown to reduce the expression of CD47 protein and enhance the phagocytic activity of TAMs[99]. Relative research remains at the stage of preliminary discovery, which warrants further specific research.

VISTA

VISTA (PD-1H and B7-H5) also belongs to the B7 family, carrying an extracellular domain bearing homology to PD-L1. VISTA is a type I transmembrane protein consisting of a single N-terminal Ig V domain, an approximately 30-aa stalk, a transmembrane domain, and a 95-aa cytoplasmic tail[100]. VISTA, which is predominantly enriched in hematopoietic cells or intra-tumoral leukocytes, is thought to overcome immune resistance induced by myeloid-derived suppressor cells (MDSCs)[11]. Importantly, VISTA is effective in maintaining T cell and myeloid quiescence. As a ligand distributed in antigen-presenting cells (APCs), VISTA-Ig fusion protein significantly inhibits the proliferation of T cells (both $CD4^+$ and $CD8^+$) and the production of cytokines[100,101]. In addition, as a V-set receptor, T cell-associated responses are clearly suppressed by VISTA, inducing immunosuppressive environments in multiple human cancers[102,103].

In addition to CA-170, as mentioned above[11], another small-molecule ligand of VISTA (NSC622608) was identified based on FRET-based high-throughput screening. Structural optimized NSC622608 effectively blocks VISTA signal transduction, relieves inhibition of VISTA in proliferation of Jurkat T cells, and promotes secretion of IFN-γ and TNF-α in T cells[104]. In addition to appearing in a variety of cancers as a suppressive immune checkpoint, VISTA may have a stimulating immune checkpoint function[105]. Therefore, dual roles of VISTA allow VISTA to overcome the shortcomings of current anti-cancer therapies. For example, acquired resistance is frequent when treated with anti-PD-1 inhibitors, along with the increased expression of $VISTA^+$ lymphocytes in these resistant tumors[106,107]. For this reason, combining anti-VISTA therapy with other therapeutic options is superior to anti-VISTA alone[108,109].

Others

In addition to the above immune checkpoint factors, exploration of other unearthed immune checkpoints that have not been described is ongoing. T cell Ig domain and mucin domain-3 (TIM-3), which is principally expressed on activated human $CD4^+$ T cells, has been shown to regulate cytokines secreted by Th1 and Th17 and trigger cell death by interacting with the ligand of galectin-9. Lymphocyte activation gene-3 (LAG3) is mainly enriched in activated T lymphocytes, B lymphocytes, natural killer (NK) cells, and plasmacytoid dendritic cells, which can selectively upregulate the expression of CD4 on the Tregs, restraining T cell-related functions[110]. Some rationally designed small-molecule drugs, such as ML-T7, TIM-3-IN-1, and TIM-3-IN-2, have the ability to bind TIM-3, showing

encouraging value in tumor treatment[57-59]. Other small molecule drugs, like EPZ005687 and eIF4E-IN-1, have been shown to reduce the expression of TIM-3[111]. Furthermore, indoleamine-2,3-dioxygenase (IDO) participates in the metabolism of tryptophan, thus inhibiting the function of T cells. Upregulation of IDO also negatively regulates multiple immune effector cells, such as cytotoxic T lymphocyte cells, helper T cells, and NK cells. Many small molecule inhibitors of IDO have been successfully used in clinical trials, such as 1-MT, INCB024360, and NLG-919, which have an important role in the treatment of rectal, head and neck, and lung cancers[112].

Prospect

As illustrated above, on account of properties of safety and pharmacologic accessibility, small molecule drugs are intended to overcome the limitations of existing antibody-based ICIs, which are still the focus of innovative drug development. For the past few years, dual-target inhibitors of immune checkpoints have been innovatively designed, which improve the therapeutic effect and reduce drug toxicity, including CSBP simultaneously targeting CD24/Siglec-10 and PD-1/PD-L1[61], and CA-170 targeting PD-L1 and VISTA[11]. However, notwithstanding the advantages mentioned above, limited screening efficiency, production costs, and technical requirements remain hindrances to the research of small molecule compounds. In particular, common drug screening technologies (e.g., high-throughput screening, structure-based drug discovery, and fragment-based drug discovery), are not conducive to improving drug discovery efficiency[113,114].

With the establishment of some libraries containing structurally diverse compounds and the extraordinary advances of machine learning and deep learning, artificial intelligence (AI) has begun to participate in the screening of small molecule drugs[115,116], including protein structural prediction, molecular virtual screening, molecular design, and drug pharmacokinetic prediction[117,118]. At present, novel small molecule inhibitors of CD47 have been predicted and designed by AI technology[36]. The involvement of AI technology substantially reduces the cycle and cost of drug research and development, and raises drug discovery efficiency, which represents promising prospects for discovering small molecule inhibitors of immune checkpoints. Beyond that, proteolysis-targeting chimaeras (PROTA) and molecular glues, which are emerging small molecule drug research strategies, are also expected to provide an attractive approach for advancing the development of immune checkpoint small molecule drugs[119-122]. Along with the continuously updated exploration of binding sites and chemical bonds, more small molecule drugs are under development and optimization.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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Contributed data or analysis tools: Luoyi Chen, Xiaowei Liu, Ying Shi
Performed the analysis: Luoyi Chen, Xinchen Zhao, Ying Shi
Wrote the paper: Luoyi Chen, Xinchen Zhao.

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