MINI REVIEW

Development of small molecule drugs targeting immune checkpoints

Luoyi Chen[1]*, Xinchen Zhao[1]*, Xiaowei Liu[2]*, Yujie Ouyang[3], Chuan Xu[1], Ying Shi[1]

[1]Department of Oncology, Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 610054, China; [2]Institute for Breast Health Medicine, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China; [3]Acupuncture and Massage College, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

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

*These authors contributed equally to this work.

Correspondence to: Chuan Xu and Ying Shi

E-mail: xuchuan100@163.com and shiying_uestc@uestc.edu.cn

ORCID ID: https://orcid.org/0000-0002-5320-2277

and https://orcid.org/0000-0001-9852-6067

2

Chen et al. Small molecule inhibitors of immune checkpoint

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
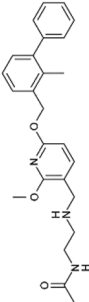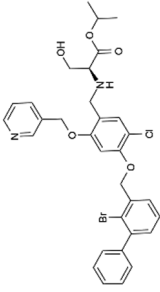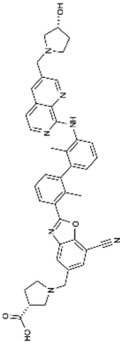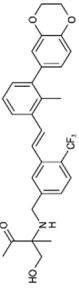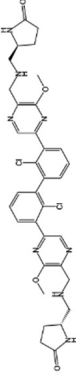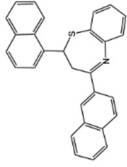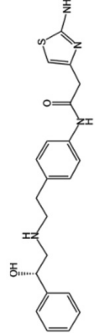
Targets		Compound	Structure	Research stages	Cancer types	References
PD-1/PD-L1	Binding of PD-1 to PD-L1	BSM-202		Preclinical	Melanoma	14
		IMMH-010		Phase I (NCT04343859)	Malignant neoplasms	15
		INCB086550		Phase I (NCT03762447)	Solid tumors	16
		MAX-10181		Phase I (NCT04122339)	Solid tumors	17
		GS-4224		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
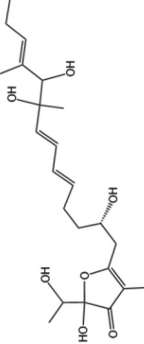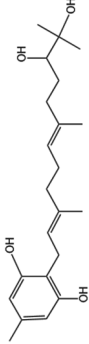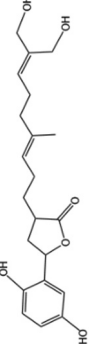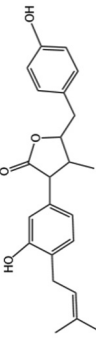
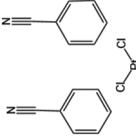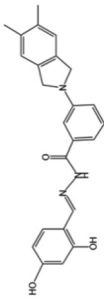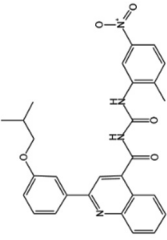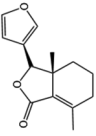| Targets	Compound	Structure	Research stages	Cancer types	References
	Actinofuranone I				
	Cosmosporin A				
	Ganocapenoid A				
	CHEBI:208188				
	Bis(benzonitrile) dichloroplatinum		NA	NA	20
	PubChem CID 135781783		NA	NA	21
	PubChem CID 4096583				
Reduce the expression of PD-1 or PD-L1	Fraxinellone		NA	NA	22,23

Table 1 Continued
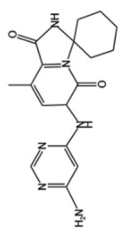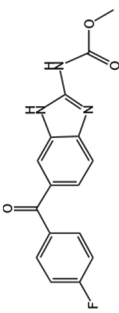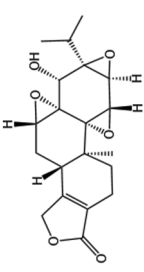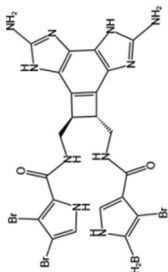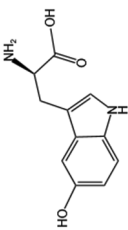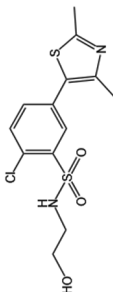
Targets	Compound	Structure	Research stages	Cancer types	References
	eFT508		Preclinical	Triple-negative breast cancer	24
	Flubendazole		Preclinical	Melanoma	25
	Triptolide		Preclinical	NSCLC	26
	Pentamidine		NA	NA	27
	Benzosceptrin C		NA	NA	28
	L-5-hydroxytryptophan		NA	NA	29
	PIK-93		NA	NA	30

6

Chen et al. Small molecule inhibitors of immune checkpoint
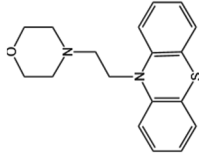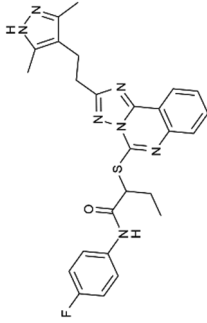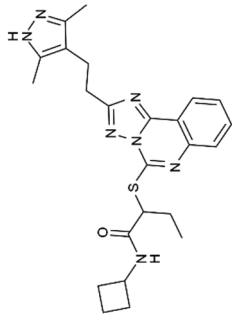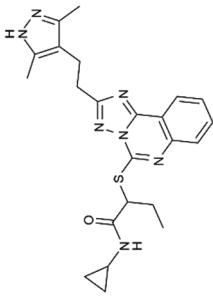
Table 1 Continued

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
	NCG0053843O		Preclinical		
	NCG0053841 9		Preclinical		

Table 1 Continued
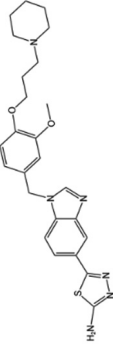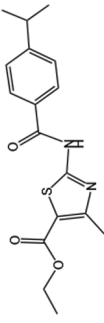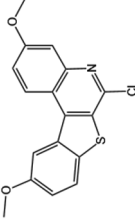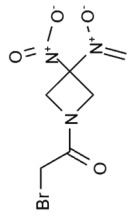
Targets	Compound	Structure	Research stages	Cancer types	References
	IsoQC-IN-1	(structure)	NA	NA	34
	1,2,4-oxadiazole compounds	(structure)	Preclinical	A20 syngeneic model	35
	SWY-AK-309	(structure)	NA	NA	36
	SWY-AM-598	(structure)	NA		
	Pep-20	AWSATWSNYWRH	Preclinical	Solid tumors	15
	RS-17	RRYKQDGGWSHWSPWSS-NH2	Preclinical	NA	37
	D4-2	Ac-yRYSAVYSIHPSWCG-NH2	Preclinical	Solid tumors	38
	SP5	CTQDAWHIC	Preclinical	NA	
Reduce the expression of CD47 or SIRPα	RRx-001	(structure)	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
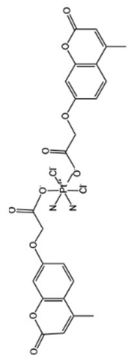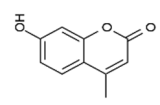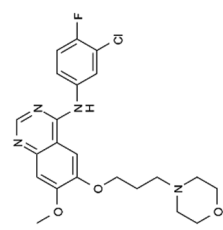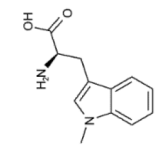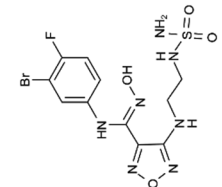
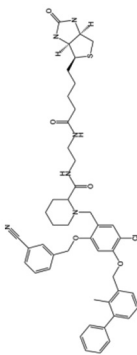| Targets	Compound	Structure	Research stages	Cancer types	References
	DMUP		NA	NA	45
	Docosahexaenoic acid		Preclinical	Colorectal cancer	46
	4-methylumbelliferone		Preclinical	Hepatic cancer	47
	Metformin		Preclinical	Breast cancer	48
	Gefitinib		Preclinical	NSCLC	49
IDO	Indoximod		Phase II (NCT01560923)	Prostate cancer	50
	Epacadostat		Phase III (NCT02752074)	Melanoma	51

Table 1 Continued
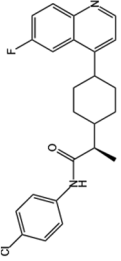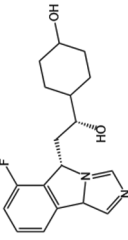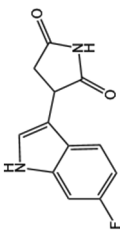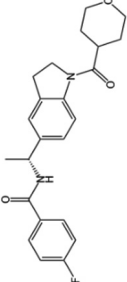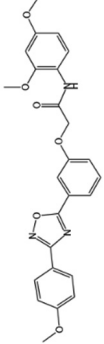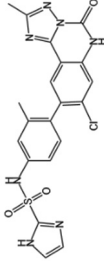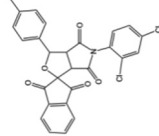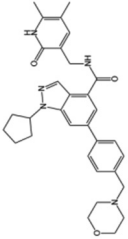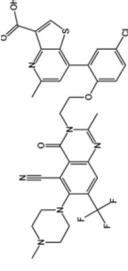
Targets	Compound	Structure	Research stages	Cancer types	References
	Linrodostat		Phase III (NCT03661320)	Muscle-invasive bladder cancer	52
	Navoximod		Phase I (NCT02471846) (NCT02048709)	Advanced solid tumors Recurrent advanced solid tumors	53,54
	PF06840003		Phase I (NCT02764151)	Recurrent malignant 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

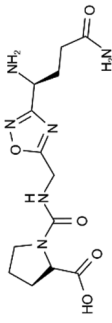| Targets	Compound	Structure	Research stages	Cancer types	References
	eIF4E-IN-1		NA	NA	
Dual targets	CD24/Siglec-10 and PD-1/PD-L1	CSBP	NA	NA	61
	PD-L1 and VISTA	CA-170	Phase II (NCT02812875)	Advanced solid tumors or lymphomas	11,62

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) super-family 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 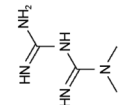clinical trial, exhibiting a higher effect at lower dosage (400 mg) but reduced immunoactivity at a higher dosage (500 mg). However, immune-related adverse events (irAEs) were more frequent in patients taking the 400 mg dose, even though the adverse reaction of CA-170 was restricted and short-term due 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
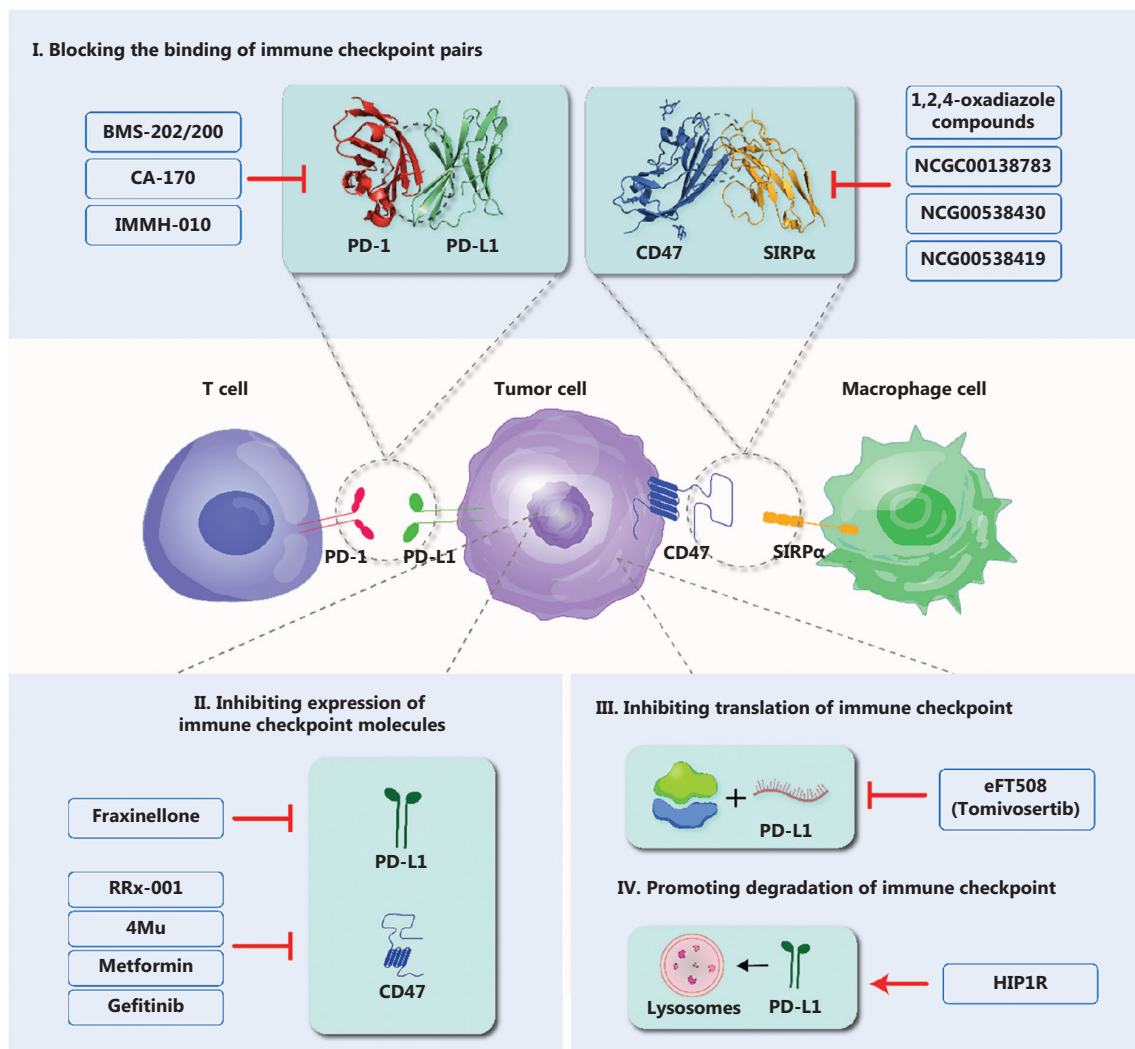
Figure 1 Summary of current small molecular drugs targeting PD-L1 and CD47. Small molecule chemical compounds block the function of major immune checkpoint molecules by **(I)** competitively binding with immune checkpoint molecules to block their combination with paired receptors (BMS202, CA-170, and 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

14

Chen et al. Small molecule inhibitors of immune checkpoint

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.

Grant support

Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Chuan Xu, Ying Shi
Collected the data: Luoyi Chen, Xinchen Zhao, Xiaowei Liu, Yujie Ouyang
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.

References

1. Baumeister SH, Freeman GJ, Dranoff G, Sharpe AH. Coinhibitory pathways in immunotherapy for cancer. Annu Rev Immunol. 2016; 34: 539-73.

2. Sharpe AH. Introduction to checkpoint inhibitors and cancer immunotherapy. Immunol Rev. 2017; 276: 5.

3. Liu X, Song J, Zhang H, Liu X, Zuo F, Zhao Y, et al. Immune checkpoint HLA-E:CD94-NKG2A mediates evasion of circulating tumor cells from NK cell surveillance. Cancer Cell. 2023; 41: 272-87.e9.

4. Zhang T, Lin Y, Gao Q. Bispecific antibodies targeting immunomodulatory checkpoints for cancer therapy. Cancer Biol Med. 2023; 20: 181-95.

5. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res. 2020; 30: 660-9.

6. Sui X, Niu X, Zhou X, Gao Y. Peptide drugs: a new direction in cancer immunotherapy. Cancer Biol Med. 2023; 21: 198-203.

7. Agostinetto E, Eiger D, Lambertini M, Ceppi M, Azambuja ED. Cardiotoxicity of immune checkpoint inhibitors: a systematic

review and meta-analysis of randomised clinical trials. Eur J Cancer. 2021; 148: 76-91.

8. Johnson DB, Mcdonnell WJ, Gonzalez-Ericsson PI, Al-Rohil RN, Balko JM. A case report of clonal EBV-like memory CD4+ T cell activation in fatal checkpoint inhibitor-induced encephalitis. Nat Med. 2019; 25: 1243-50.

9. Liu X, Feng Y, Xu J, Shi Y, Yang J, Zhang R, et al. Combination of MAPK inhibition with photothermal therapy synergistically augments the anti-tumor efficacy of immune checkpoint blockade. J Control Release. 2021; 332: 194-209.

10. Thierauch K-H. Small molecule drugs. In: Schwab, editor. Encyclopedia of cancer. Berlin, Heidelberg: Springer Berlin Heidelberg, 2011. p. 3448-51.

11. Sasikumar PG, Sudarshan NS, Adurthi S, Ramachandra RK, Samiulla DS, Lakshminarasimhan A, et al. PD-1 derived CA-170 is an oral immune checkpoint inhibitor that exhibits preclinical anti-tumor efficacy. Commun Biol. 2021; 4: 1-12.

12. Imai K, Takaoka A. Comparing antibody and small-molecule therapies for cancer. Nat Rev Cancer. 2006; 6: 714-27.

13. Hoelder S, Clarke PA, Workman P. Discovery of small molecule cancer drugs: successes, challenges and opportunities. Mol Oncol. 2012; 6: 155-76.

14. Zak KM, Grudnik P, Guzik K, Zieba BJ, Musielak B, Dömling A, et al. Structural basis for small molecule targeting of the programmed death ligand 1 (PD-L1). Oncotarget. 2016; 7: 30323-35.

15. Wang H, Sun Y, Zhou X, Chen C, Gao Y. CD47/SIRPα blocking peptide identification and synergistic effect with irradiation for cancer immunotherapy. J Immunother Cancer. 2020; 8: e000905.

16. Koblish HK, Wu L, Wang L, Liu PC, Wynn R, Rios-Doria J, et al. Characterization of INCB086550, a potent and novel small-molecule PD-L1 inhibitor. Cancer Discov. 2022; 12: 1482-99.

17. Wang Y, Zhang N, Wang F, Zhao Q, Li Z, editors. Abstract LB-018: orally active small molecule PD-L1 inhibitor demonstrating similar efficacy as durvalumab in human knock-in MC38 model. Proceedings of the AACR Annual Meeting 2019 March 29-April 3; Atlanta, GA; 2019. p. GA2019.

18. DiFrancesco M, Hofer J, Aradhya A, Rufinus J, Stoddart J, Finocchiaro S, et al. Discovery of small-molecule PD-1/PD-L1 antagonists through combined virtual screening and experimental validation. Comput Biol Chem. 2023; 102: 107804.

19. Chandrasekaran J, Elumalai S, Murugesan V, Kunjiappan S, Pavadai P, Theivendren P. Computational design of PD-L1 small molecule inhibitors for cancer therapy. Mol Diver. 2023; 27: 1633-44.

20. Wang R, Yu Q, Wang X, Zhu D, Li G, Li Z, et al. Bis(benzonitrile) dichloroplatinum (II) interrupts PD-1/PD-L1 interaction by binding to PD-1. Acta Pharmacol Sin. 2023; 44: 2103-12.

21. Kamal MA, Badary HA, Omran D, Shousha HI, Abdelaziz AO, El Tayebi HM, et al. Virtual screening and biological evaluation of potential PD-1/PD-L1 immune checkpoint inhibitors as anti-hepatocellular carcinoma agents. ACS Omega. 2023; 8: 33242-54.

22. Bailly C, Vergoten G. Fraxinellone: from pesticidal control to cancer treatment. Pestic Biochem Physiol. 2020; 168: 104624.

23. Xing Y, Mi C, Wang Z, Zhang Z, Li M, Zuo H, et al. Fraxinellone has anticancer activity in vivo by inhibiting programmed cell death-ligand 1 expression by reducing hypoxia-inducible factor-1α and STAT3. Pharmacol Res. 2018; 135: 166-80.

24. Xu Y, Poggio M, Jin HY, Shi Z, Forester CM, Wang Y, et al. Translation control of the immune checkpoint in cancer and its therapeutic targeting. Nature Med. 2019; 25: 301-11.

25. Li Y, Wu B, Hossain MJ, Quagliata L, O'Meara C, Wilkins MR, et al. Flubendazole inhibits PD-1 and suppresses melanoma growth in immunocompetent mice. J Transl Med. 2023; 21: 467.

26. Xie Y, Ding J, Gao J, Zhang J, Cen S, Zhou J. Triptolide reduces PD-L1 through the EGFR and IFN-γ/IRF1 dual signaling pathways. Int Immunopharmacol. 2023; 118: 109993.

27. Gu T, Tian X, Wang Y, Yang W, Li W, Song M, et al. Repurposing pentamidine for cancer immunotherapy by targeting the PD1/PD-L1 immune checkpoint. Front Immunol. 2023; 14: 1145028.

28. Wang Q, Wang J, Yu D, Zhang Q, Hu H, Xu M, et al. Benzosceptrin C induces lysosomal degradation of PD-L1 and promotes antitumor immunity by targeting DHHC3. Cell Rep Med. 2024; 5: 101357.

29. Huang J, Wang X, Li B, Shen S, Wang R, Tao H, et al. L-5-hydroxytryptophan promotes antitumor immunity by inhibiting PD-L1 inducible expression. J Immunother Cancer. 2022; 10: e003957.

30. Lin C, Huang K, Kao S, Lin M, Lin C, Yang S, et al. Small-molecule PIK-93 modulates the tumor microenvironment to improve immune checkpoint blockade response. Sci Adv. 2023; 9: eade9944.

31. Dai X, Zhao L, Yang L, Guo T, Xue L, Ren H, et al. Phenothiazine-based LSD1 inhibitor promotes T-cell killing response of gastric cancer cells. J Med Chem. 2023; 66: 3896-916.

32. Ding Z, Wang S, Shi Y, Fei X, Cheng B, Lu Y, et al. Discovery of novel d-(+)-biotin-conjugated resorcinol dibenzyl ether-based PD-L1 inhibitors for targeted cancer immunotherapy. J Med Chem. 2023; 66: 10364-80.

33. Burgess TL, Amason JD, Rubin JS, Duveau DY, Lamy L, Roberts DD, et al. A homogeneous SIRPα-CD47 cell-based, ligand-binding assay: utility for small molecule drug development in immuno-oncology. PLoS One. 2020; 15: e0226661.

34. Park E, Song KH, Kim D, Lee M, Van Manh N, Kim H, et al. 2-Amino-1,3,4-thiadiazoles as glutaminyl cyclases inhibitors increase phagocytosis through modification of CD47-SIRPα checkpoint. ACS Med Chem Lett. 2022; 13: 1459-67.

35. Sasikumar PGN, Ramachandra M, Naremaddepalli SSS, Gundala C. 1,2,4-Oxadiazole compounds as inhibitors of CD47 signalling. 20190218252; 2019 July. Available from: https://www.freepatentsonline.com/y2019/0218252.html.

36. Shan W, Chen L, Xu H, Zhong Q, Xu Y, Yao H, et al. GcForest-based compound-protein interaction prediction model and its application in discovering small-molecule drugs targeting CD47. Front Chem. 2023; 11: 1292869.

37. Hatherley D, Lea SM, Johnson S, Barclay AN. Polymorphisms in the human inhibitory signal-regulatory protein α do not affect binding to its ligand CD47. J Biol Chem. 2014; 289: 10024-8.

38. Hazama D, Yin Y, Murata Y, Matsuda M, Matozaki T. Macrocyclic peptide-mediated blockade of the CD47-SIRPα interaction as a

potential cancer immunotherapy. Cell Chem Biol. 2020; 27: 1181-91.e7.

39. Cabrales P. RRx-001 is a phase III aerospace-derived small molecule that immunonormalizes the tumor microenvironment. J Clin Oncol. 2019; 37: 156.

40. Brzezniak C, Quinn M, Zeman K, Oronsky B, Scicinski J, Caroen S, et al. P2.06-031 QUADRUPLE THREAT: a pilot phase 2 study of RRx-001 in advanced lung cancer prior to re-administration of platinum doublets. J Thorac Oncol. 2017; 12: S1090-S1.

41. Oronsky B, Scicinski J, Ning S, Peehl D, Oronsky A, Cabrales P, et al. RRx-001, a novel dinitroazetidine radiosensitizer. Invest New Drugs. 2016; 34: 371-7.

42. Reid T, Oronsky B, Caroen S, Quinn M, Williams J, Cabrales P, et al. Phase 1 pilot study of RRx-001 + nivolumab in patients with advanced metastatic cancer (PRIMETIME). Front Immunol. 2023; 14: 1104753.

43. Guo Y, Bao Q, Hu P, Shi J. Nanomedicine-based co-delivery of a calcium channel inhibitor and a small molecule targeting CD47 for lung cancer immunotherapy. Nat Commun. 2023; 14: 7306.

44. Bonomi M, Blakaj DM, Kabarriti R, Colvett K, Takiar V, Biagioli M, et al. PREVLAR: phase 2a randomized trial to assess the safety and efficacy of RRx-001 in the attenuation of oral mucositis in patients receiving head and neck chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2023; 116: 551-9.

45. Tan Y, Chen H, Zhang J, Cai L, Jin S, Song D, et al. Platinum(IV) complexes as inhibitors of CD47-SIRPα axis for chemoimmunotherapy of cancer. Eur J Med Chem. 2022; 229: 114047.

46. Fadaee M, Abbasi H, Maralbashi S, Baradaran B, Shanehbandi D, Dinevari MF, et al. Docosahexaenoic acid may inhibit immune evasion of colorectal cancer cells through targeting immune checkpoint and immunomodulator genes and their controlling microRNAs. Biofactors. 2022; 48: 1137-44.

47. Rodríguez MM, Fiore E, Bayo J, Atorrasagasti C, García M, Onorato A, et al. 4Mu decreases CD47 expression on hepatic cancer stem cells and primes a potent antitumor T cell response induced by interleukin-12. Mol Ther. 2018; 26: 2738-50.

48. Tan W, Tang H, Jiang X, Ye F, Huang L, Shi D, et al. Metformin mediates induction of miR-708 to inhibit self-renewal and chemoresistance of breast cancer stem cells through targeting CD47. J Cell Mol Med. 2019; 23: 5994-6004.

49. Nigro A, Ricciardi L, Salvato I, Sabbatino F, Vitale M, Crescenzi MA, et al. Enhanced expression of CD47 is associated with off-target resistance to tyrosine kinase inhibitor gefitinib in NSCLC. Front Immunol. 2019; 10: 3135.

50. Fox E, Oliver T, Rowe M, Thomas S, Zakharia Y, Gilman PB, et al. Indoximod: an immunometabolic adjuvant that empowers T cell activity in cancer. Front Oncol. 2018; 8: 370.

51. Long GV, Dummer R, Hamid O, Gajewski TF, Mitchell TC. Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): a phase 3, randomised, double-blind study. Lancet Oncol. 2019; 20: 1083-97.

52. Sonpavde G, Necchi A, Gupta S, Steinberg GD, Gschwend JE, Van Der Heijden MS, et al. ENERGIZE: a Phase III study of neoadjuvant chemotherapy alone or with nivolumab with/without linrodostat mesylate for muscle-invasive bladder cancer. Future Oncol. 2020; 16: 4359-68.

53. Jung KH, LoRusso P, Burris H, Gordon M, Bang YJ, Hellmann MD, et al. Phase I study of the indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor navoximod (GDC-0919) administered with PD-L1 inhibitor (atezolizumab) in advanced solid tumors. Clin Cancer Res. 2019; 25: 3220-8.

54. Nayak-Kapoor A, Hao Z, Sadek R, Dobbins R, Marshall L, Vahanian NN, et al. Phase Ia study of the indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor navoximod (GDC-0919) in patients with recurrent advanced solid tumors. J Immunother Cancer. 2018; 6: 61.

55. Reardon DA, Desjardins A, Rixe O, Cloughesy T, Alekar S, Williams JH, et al. A phase 1 study of PF-06840003, an oral indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor in patients with recurrent malignant glioma. Invest New Drugs. 2020; 38: 1784-95.

56. Kotecki N, Vuagnat P, O'Neil BH, Jalal S, Rottey S, Prenen H, et al. A phase I study of an IDO-1 inhibitor (LY3381916) as monotherapy and in combination with an anti-PD-L1 antibody (LY3300054) in patients with advanced cancer. J Immunother. 2021; 44: 264-75.

57. Abdel-Rahman SA, Talagayev V, Pach S, Wolber G, Gabr MT. Discovery of small-molecule TIM-3 inhibitors for acute myeloid leukemia using pharmacophore-based virtual screening. J Med Chem. 2023; 66: 11464-75.

58. Rietz TA, Teuscher KB, Mills JJ, Gogliotti RD, Lepovitz LT, Scaggs WR, et al. Fragment-based discovery of small molecules bound to T-cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3). J Med Chem. 2021; 64: 14757-72.

59. Ma S, Tian Y, Peng J, Chen C, Peng X, Zhao F, et al. Identification of a small-molecule Tim-3 inhibitor to potentiate T cell-mediated antitumor immunotherapy in preclinical mouse models. Sci Transl Med. 2023; 15: eadg6752.

60. Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med. 2016; 22: 128-34.

61. Shen W, Shi P, Dong Q, Zhou X, Chen C, Sui X, et al. Discovery of a novel dual-targeting D-peptide to block CD24/Siglec-10 and PD-1/PD-L1 interaction and synergize with radiotherapy for cancer immunotherapy. J Immunother Cancer. 2023; 11: e007068.

62. Radhakrishnan V, Bakhshi S, Prabhash K, Deshmukh C, Nag S, Lakshmaiah K. Phase 2 trial of CA-170, a novel oral small molecule dual inhibitor of immune checkpoints VISTA and PD-1, in patients (pts) with advanced solid tumor and Hodgkin lymphoma. J Immunother Cancer. 2018; 6: 714.

63. Wu Q, Jiang L, Li S, He Q, Yang B, Cao J. Small molecule inhibitors targeting the PD-1/PD-L1 signaling pathway. Acta Pharmacol Sin. 2021; 42: 1-9.

64. Scott DE, Bayly AR, Abell C, Skidmore J. Small molecules, big targets: drug discovery faces the protein–protein interaction challenge. Nat Rev Drug Discov. 2016; 15: 533-50.

65. Zak K, Kitel R, Przetocka S, Golik P, Guzik K, Musielak B, et al. Structure of the complex of human programmed death 1, PD-1, and its ligand PD-L1. Structure. 2015; 23: 2341-8.

66. Huang B, Bai Z, Ye X, Zhou C, Xie X, Zhong Y, et al. Structural analysis and binding sites of inhibitors targeting the CD47/SIRPα interaction in anticancer therapy. Comput Struct Biotechnol J. 2021; 19: 5494-503.

67. Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. Immunity. 2018; 48: 434-52.

68. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol. 2007; 19: 813-24.

69. Blank C, Gajewski TF, Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. Cancer Immunol Immunother. 2005; 54: 307-14.

70. Ming Y, Xiaoli Z, Mengke N, Shuangli Z, Hong G, Kongming W. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. Mol Cancer. 2022; 21: 1-27.

71. Bagchi S, Yuan R, Engleman EG. Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annu Rev Pathol. 2021; 16: 223-49.

72. Wang H, He Z, Gao Y, Feng D, Wei X, Huang Y, et al. Dual-pronged attack: pH-driven membrane-anchored NIR dual-type nano-photosensitizer excites immunogenic pyroptosis and sequester immune checkpoint for enhanced prostate cancer photo-immunotherapy. Adv Sci (Weinh). 2023; 10: e2302422.

73. Ashizawa T, Iizuka A, Tanaka E, Kondou R, Akiyama Y. Antitumor activity of the PD-1/PD-L1 binding inhibitor BMS-202 in the humanized MHC-double knockout NOG mouse. Biomed Res. 2019; 40: 243-50.

74. Hu Z, Yu P, Du G, Wang W, Zhu H, Li N, et al. PCC0208025 (BMS202), a small molecule inhibitor of PD-L1, produces an antitumor effect in B16-F10 melanoma-bearing mice. PLoS One. 2020; 15: e0228339.

75. Jiang Y, Zhang J, Qiu J, Cui S. The PD-1/PD-L1 binding inhibitor BMS-202 suppresses the synthesis and secretion of gonadotropins and enhances apoptosis via p38 MAPK signaling pathway. Drug Dev Res. 2022; 83: 176-83.

76. Gao Y, Wang H, Shen L, Xu H, Deng M, Cheng M, et al. Discovery of benzo[d]isothiazole derivatives as novel scaffold inhibitors targeting the programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) interaction through "ring fusion" strategy. Bioorg Chem. 2022; 123: 105769.

77. Jing T, Zhang Z, Kang Z, Mo J, Yue X, Lin Z, et al. Discovery and optimization of novel biphenyl derivatives bearing cyclopropyl linkage as potent programmed cell death-1/programmed cell death-ligand 1 inhibitors. J Med Chem. 2023; 66: 6811-35.

78. Ganesan A, Ahmed M, Okoye I, Arutyunova E, Babu D, Turnbull WL, et al. Comprehensive in vitro characterization of PD-L1 small molecule inhibitors. Sci Rep. 2019; 9: 1-19.

79. Musielak B, Kocik J, Skalniak L, Magiera-Mularz K, Sala D, Czub M, et al. CA-170–a potent small-molecule PD-L1 inhibitor or not? Molecules. 2019; 24: 2804.

80. Lee JJ, Powderly JD, Patel MR, Brody J, Hamilton EP, Infante JR, et al. Phase 1 trial of CA-170, a novel oral small molecule dual inhibitor of immune checkpoints PD-1 and VISTA, in patients (pts) with advanced solid tumor or lymphomas. Am Soc Clin Oncol. 2017; 35: TPS3099.

81. Lai F, Ji M, Huang L, Wang Y, Xue N, Du T, et al. YPD-30, a prodrug of YPD-29B, is an oral small-molecule inhibitor targeting PD-L1 for the treatment of human cancer. Acta Pharm Sin B. 2022; 12: 2845-58.

82. Jiang J, Zou X, Liu Y, Liu X, Dong K, Yao X, et al. Simultaneous determination of a novel PD-L1 inhibitor, IMMH-010, and its active metabolite, YPD-29B, in rat biological matrices by polarity-switching liquid chromatography-tandem mass spectrometry: application to ADME studies. Front Pharmacol. 2021; 12: 1549.

83. Wang Y, Liu X, Zou X, Wang S, Luo L, Liu Y, et al. Metabolism and interspecies variation of IMMH-010, a programmed cell death ligand 1 inhibitor prodrug. Pharmaceutics. 2021; 13: 598.

84. Park JJ, Thi EP, Carpio VH, Bi Y, Cole AG, Dorsey BD, et al. Checkpoint inhibition through small molecule-induced internalization of programmed death-ligand 1. Nat Commun. 2021; 12: 1222.

85. Tourneau CL, Piha-Paul S, Prenen H, Delafontaine B, Pinato D, Santoro A, et al. 774 A phase 1 study exploring the safety and tolerability of the small molecule PD-L1 inhibitor, INCB086550, in patients with select advanced tumors. J Immunother Cancer. 2022; 10: A804.

86. Cutsem EV, Prenen H, Delafontaine B, Spencer K, Mitchell T, Burris H, et al. 529 Phase 1 study of INCB086550, an oral PD-L1 inhibitor, in immune-checkpoint naive patients with advanced solid tumors. BMJ Spec J. 2021; 9: A559-60.

87. Wang H, Yao H, Li C, Shi H, Lan J, Li Z, et al. HIP1R targets PD-L1 to lysosomal degradation to alter T cell–mediated cytotoxicity. Nat Chem Biol. 2019; 15: 42-50.

88. Logtenberg ME, Scheeren FA, Schumacher TN. The CD47-SIRPα immune checkpoint. Immunity. 2020; 52: 742-52.

89. Fenalti G, Villanueva N, Griffith M, Pagarigan B, Lakkaraju SK, Huang RY-C, et al. Structure of the human marker of self 5-transmembrane receptor CD47. Nature Commun. 2021; 12: 1-14.

90. Veillette A, Chen J. SIRPα–CD47 immune checkpoint blockade in anticancer therapy. Trends Immunol. 2018; 39: 173-84.

91. Sikic BI, Lakhani N, Patnaik A, Shah SA, Chandana SR, Rasco D, et al. First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. J Clin Oncol. 2019; 37: 946-53.

92. Oldenborg PA, Gresham HD, Lindberg FP. CD47-signal regulatory protein alpha (SIRPalpha) regulates Fcgamma and complement receptor-mediated phagocytosis. J Exp Med. 2019; 193: 855-62.

93. Yu W-B, Ye Z-H, Chen X, Shi J-J, Lu J-J. The development of small-molecule inhibitors targeting CD47. Drug Discov Today. 2021; 26: 561-8.

94. Miller TW, Amason JD, Garcin ED, Lamy L, Dranchak PK, Macarthur R, et al. Quantitative high-throughput screening assays

for the discovery and development of SIRPα-CD47 interaction inhibitors. PLoS One. 2019; 14: e0218897.

95. Cabrales P. RRx-001 acts as a dual small molecule checkpoint inhibitor by downregulating CD47 on cancer cells and SIRP-α on monocytes/macrophages. Transl Oncol. 2019; 12: 626-32.

96. Oronsky B, Paulmurugan R, Foygel K, Scicinski J, Knox SJ, Peehl D, et al. RRx-001: a systemically non-toxic M2-to-M1 macrophage stimulating and prosensitizing agent in Phase II clinical trials. Expert Opin Investig Drugs. 2017; 26: 109.

97. Wu M, Shi Y, Zhu L, Chen L, Zhao X, Xu C. Macrophages in glioblastoma development and therapy: a double-edged sword. Life (Basel). 2022; 12: 1225.

98. Feng M, Wang F, Liu X, Hao T, Zhang N, Deng M, et al. Neutrophils as key regulators of tumor immunity that restrict immune checkpoint blockade in liver cancer. Cancer Biol Med. 2023; 20: 421-37.

99. Lu J, Li J, Lin Z, Li H, Lou L, Ding W, et al. Reprogramming of TAMs via the STAT3/CD47-SIRPα axis promotes acquired resistance to EGFR-TKIs in lung cancer. Cancer Lett. 2023; 564: 216205.

100. Wang L, Rubinstein R, Lines JL, Wasiuk A, Ahonen C, Guo Y, et al. VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. J Exp Med. 2011; 208: 577-92.

101. Flies DB, Han X, Higuchi T, Zheng L, Sun J, Ye J, et al. Coinhibitory receptor PD-1H preferentially suppresses CD4+ T cell–mediated immunity. J Clin Invest. 2014; 124: 1966-75.

102. Villarroel F. Spatially resolved and quantitative analysis of VISTA/PD-1H as a novel immunotherapy target in human non-small cell lung cancer. Clin Cancer Res. 2018; 24: 1562-73.

103. Gao J, Ward JF, Pettaway CA, Shi LZ, Subudhi SK, Vence LM, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat Med. 2017; 23: 551-5.

104. Gabr MT, Gambhir SS. Discovery and optimization of small-molecule ligands for V-domain Ig suppressor of T-cell activation (VISTA). J Am Chem Soc. 2020; 142: 16194-8.

105. Huang X, Zhang X, Li E, Zhang G, Wang X, Tang T, et al. VISTA: an immune regulatory protein checking tumor and immune cells in cancer immunotherapy. J Hematol Oncol. 2020; 13: 83.

106. Kakavand H, Jackett LA, Menzies AM, Gide TN, Carlino MS, Saw RPM, et al. Negative immune checkpoint regulation by VISTA: a mechanism of acquired resistance to anti-PD-1 therapy in metastatic melanoma patients. Mod Pathol. 2017; 30: 1666-76.

107. Blando J, Sharma A, Higa MG, Zhao H, Vence L, Yadav SS, et al. Comparison of immune infiltrates in melanoma and pancreatic cancer highlights VISTA as a potential target in pancreatic cancer. Proc Natl Acad Sci U S A. 2019; 116: 1692-7.

108. Liu J, Yuan Y, Chen W, Putra J, Suriawinata AA, Schenk AD, et al. Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses. Proc Natl Acad Sci U S A. 2015; 112: 6682-7.

109. Kondo Y, Ohno T, Nishii N, Harada K, Yagita H, Azuma M. Differential contribution of three immune checkpoint (VISTA, CTLA-4, PD-1) pathways to antitumor responses against squamous cell carcinoma. Oral Oncol. 2016; 57: 54-60.

110. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity. 2016; 44: 989-1004.

111. Knutson SK, Wigle TJ, Warholic NM, Sneeringer CJ, Allain CJ, Klaus CR, et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. Nat Chem Biol. 2012; 8: 890-6.

112. Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, et al. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood. 2010; 115: 3520-30.

113. Blay V, Tolani B, Ho SP, Arkin MR. High-throughput screening: today's biochemical and cell-based approaches. Drug Discov Today. 2020; 25: 1807-21.

114. Chen L, Fan Z, Chang J, Yang R, Hou H, Guo H, et al. Sequence-based drug design as a concept in computational drug design. Nat Commun. 2023; 14: 4217.

115. Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, et al. Applications of machine learning in drug discovery and development. Nat Rev Drug Discov. 2019; 18: 463-77.

116. Mullowney MW, Duncan KR, Elsayed SS, Garg N, van der Hooft JJJ, Martin NI, et al. Artificial intelligence for natural product drug discovery. Nat Rev Drug Discov. 2023; 22: 895-916.

117. Chen W, Liu X, Zhang S, Chen S. Artificial intelligence for drug discovery: resources, methods, and applications. Mol Ther Nucleic Acids. 2023; 31: 691-702.

118. Lv Q, Zhou F, Liu X, Zhi L. Artificial intelligence in small molecule drug discovery from 2018 to 2023: does it really work? Bioorg Chem. 2023; 141: 106894.

119. Zhang C, Xu M, He S, Huang J, Xu C, Pu K. Checkpoint nano-PROTACs for activatable cancer photo-immunotherapy. Adv Mater. 2023; 35: e2208553.

120. Wang Y, Zhou Y, Cao S, Sun Y, Dong Z, Li C, et al. In vitro and in vivo degradation of programmed cell death ligand 1 (PD-L1) by a proteolysis targeting chimera (PROTAC). Bioorg Chem. 2021; 111: 104833.

121. Dale B, Cheng M, Park KS, Kaniskan HÜ, Xiong Y, Jin J. Advancing targeted protein degradation for cancer therapy. Nat Rev Cancer. 2021; 21: 638-54.

122. Domostegui A, Nieto-Barrado L, Perez-Lopez C, Mayor-Ruiz C. Chasing molecular glue degraders: screening approaches. Chem Soc Rev. 2022; 51: 5498-517.