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REVIEW

Emerging immunological strategies: recent advances and future directions

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Abstract **Immunotherapy plays a compelling role in cancer treatment and has already made remarkable progress. However, many patients receiving immune checkpoint inhibitors fail to achieve clinical benefits, and the response rates vary among tumor types. New approaches that promote anti-tumor immunity have recently been developed, such as small molecules, bispecific antibodies, chimeric antigen receptor T cell products, and cancer vaccines. Small molecule drugs include agonists and inhibitors that can reach the intracellular or extracellular targets of immune cells participating in innate or adaptive immune pathways. Bispecific antibodies, which bind two different antigens or one antigen with two different epitopes, are of great interest. Chimeric antigen receptor T cell products and cancer vaccines have also been investigated. This review explores the recent progress and challenges of different forms of immunotherapy agents and provides an insight into future immunotherapeutic strategies.**

Keywords cancer immunotherapy; bispecific antibodies; small molecules; chimeric antigen receptor T therapy; cancer vaccines

Introduction

Immunotherapy has brought tumor therapy into a new era. From surgery, to radiotherapy, chemotherapy, and targeted therapy, immuno-oncology therapy is almost within reach. However, many obstacles remain for this treatment. Although immune checkpoint inhibitors (ICIs) are now widely studied and have shown promising clinical data, many patients receiving ICIs fail to achieve clinical benefits, show varying response rates among different tumor types [1,2], and suffer from risk of immune-related adverse events (irAEs) [3].

New approaches that promote anti-tumor immunity have recently been developed, such as small molecules, bispecific antibodies (bsAbs), chimeric antigen receptor (CAR) T cell products, and even cancer vaccines. These new drugs can be used alone or in conjunction with existing biological antibodies and traditional therapies (radiotherapy or chemotherapy) to affect various members of the immune system and microenvironment, promote antitumor effectiveness, and benefit many patients.

This review explores the mechanisms and recent advances of small molecule drugs, bsAbs, cancer vaccines, and CAR T cell therapy. Challenges and future directions of these novel immunotherapy strategies are also discussed.

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Small molecules in immunotherapy

An overview

With deepened understanding of innate immunity and tumor microenvironment (TME), many small molecules and their importance in cancer immunity have been discovered. Small molecule drugs include agonists and inhibitors that can reach the intracellular or extracellular targets of immune cells participating in specific immune pathways, enhancing anti-tumor immunity, or reducing

immune suppression. These substances also have potential complementary or synergistic effects with existing immunotherapy. Compared with therapeutic antibodies, small molecule drugs are more permeable to tissues and the TME, and can cross the blood–brain barrier and other physiologic barriers, thus providing new options for the treatment of brain tumors and brain metastases. By adjusting the pharmacokinetic and pharmacodynamic parameters, small molecule drugs may provide the best bioavailability and avoid some of the irAEs associated with long-lasting antibody therapies. These medications also have relatively low production costs and are usually taken orally which enables easy administration.

Although a growing number of small molecules have entered early phase clinical trials, many challenges remain to be solved. Specific issues relate to understanding their mechanisms of action in the immune system and the theoretical basis for further clinical applications, as well as, the need for more safety and efficacy evaluations.

Mechanisms of small molecule drugs

Over the past decade, more than 50 small molecule drugs have been produced as single agents or in combination with monoclonal antibodies for tumor immunotherapy [4], and over 100 clinical trials are currently underway (Table 1 and Fig. S1). Small molecule agonists and inhibitors target specific pathways participating in innate or adaptive immunity through different mechanisms (Fig. 1). Understanding the mechanism of small molecule drugs and their current clinical research progress will aid in exploring their role in immunotherapy.

Table 1 Small molecules under global clinical development as of August 2020

Small molecule	Target	Clinical studies	Phase	Cancer type
CA-170	PD-L1/VISTA	NCT02812875	Phase 1	Advanced solid tumors or lymphomas
Imiquimod	TLR7		Approved	
Motolimod	TLR78	NCT02431559	Phase 1/2	Ovarian cancer
		NCT03906526	Phase 1	Head and neck cancer
		NCT04272333	Early phase 1	Head and neck squamous cell carcinoma
		NCT02650635	Phase 1	Metastatic, persistent, recurrent, or progressive solid tumors
		NCT02124850	Phase 1	Head and neck squamous cell carcinoma
Resiquimod	TLR7/8	NCT00821652	Phase 1	Tumors
		NCT00948961	Phase 1/2	Advanced malignancies
		NCT00960752	Phase 2	Melanoma
		NCT01204684	Phase 2	Brain tumors
		NCT01808950	Phase 1/2	Nodular basal cell carcinoma
		NCT00470379	Phase 1	Melanoma (skin)
		NCT01748747	Phase 1	Melanoma
		NCT02126579	Phase 1/2	Melanoma
		NCT01676831	Phase 1/2	Cutaneous T cell lymphoma
VTX-2337	TLR8	NCT01666444	Phase 1/2	Epithelial ovarian cancer, fallopian tube cancer, primary peritoneal cancer
		NCT01334177	Phase 1	Locally advanced, recurrent, or metastatic squamous cell cancer of head and neck
		NCT03906526	Phase 1	Head and neck cancer
		NCT01836029	Phase 2	Head and neck squamous cell carcinoma
		NCT02124850	Phase 1	Head and neck squamous cell carcinoma
Epacadostat	IDO1	NCT03322540	Phase 2	Metastatic non-small cell lung cancer
		NCT03348904	Phase 3	Non-small cell lung cancer
		NCT03322566	Phase 2	Metastatic non-small cell lung cancer
		NCT02959437	Phase 1/2	Advanced solid tumors
		NCT03085914	Phase 1/2	Advanced or metastatic solid tumors
		NCT02318277	Phase 1/2	Advanced solid tumors
		NCT03347123	Phase 1/2	Advanced or metastatic malignancies
		NCT03006302	Phase 2	Metastatic pancreas cancer
		NCT03361865	Phase 3	Urothelial carcinoma
		NCT02327078	Phase 1/2	B cell malignancies, colorectal cancer, head and neck cancer, lung cancer, lymphoma, melanoma, ovarian cancer, glioblastoma

(*Continued*)

Small molecule	Target	Clinical studies	Phase	Cancer type
		NCT03374488	Phase 3	Recurrent or progressive metastatic urothelial carcinoma
		NCT03196232	Phase 2	Metastatic or unresectable gastresophageal junction or gastric cancer
		NCT03358472	Phase 3	Recurrent or metastatic head and neck squamous cell carcinoma
		NCT02364076	Phase 2	Thymic carcinoma
		NCT03493945	Phase 1/2	Metastatic prostate cancer, prostate cancer, prostate neoplasm, advanced solid tumors, solid tumor
		NCT03823131	Phase 2	Unresectable head and neck cancer
		NCT03414229	Phase 2	Advanced sarcoma
		NCT03260894	Phase 3	Renal cell carcinoma
		NCT02752074	Phase 3	Melanoma
		NCT03532295	Phase 2	Recurrent gliomas
Navoximod (GDC-0919)	IDO1	NCT02048709	Phase 1	Advanced solid tumors
		NCT02471846	Phase 1	Locally advanced or metastatic solid tumors
BMS-986205	IDO1	NCT03519256	Phase 2	Bladder cancer
		NCT03792750	Phase 1/2	Advanced malignant solid tumors
		NCT03192943	Phase 1	Advanced cancer
		NCT03661320	Phase 3	Muscle-invasive bladder cancer
		NCT02658890	Phase 1/2	Advanced cancer
		NCT04106414	Phase 2	Endometrial cancer or endometrial carcinosarcoma
		NCT03329846	Phase 3	Advanced melanoma
		NCT03854032	Phase 2	Head and neck squamous cell carcinoma
		NCT03695250	Phase 1/2	Liver cancer
		NCT04047706	Phase 1	Glioblastoma
PF-06840003	IDO1	NCT02764151	Phase 1	Malignant gliomas
CB-1158 (INCB001158)	ARG	NCT03910530	Phase 1	Advanced solid tumors
		NCT02903914	Phase 1/2	Advanced/metastatic solid tumors
		NCT03837509	Phase 1/2	Multiple myeloma
AT-38	ARG	NCT01109004	Phase 3	Multiple myeloma
CB-839	Glutaminase 1	NCT03263429	Phase 1/2	Ras wildtype colorectal cancer
		NCT02771626	Phase 1/2	Clear cell renal cell carcinoma, melanoma, non-small cell lung cancer
CPI-444 (V81444; ciforadenant)	A2A receptor	NCT02655822	Phase 1	Renal cell cancer, metastatic castration resistant prostate cancer
		NCT04280328	Phase 1	Multiple myeloma
		NCT03454451	Phase 1	Advanced cancers
Preladenant	A2A receptor	NCT03099161	Phase 1	Advanced solid tumors
PBF 509	A2A receptor	NCT02403193	Phase 1/2	Non-small cell lung cancer
AZD4635	A2A receptor	NCT04089553	Phase 2	Prostate cancer
		NCT04495179	Phase 2	Prostate cancer
		NCT03980821	Phase 1	Advanced solid malignancies
		NCT02740985	Phase 1	Advanced solid malignancies
		NCT03381274	Phase 1/2	Non-small cell lung cancer
ADU-S100	STING	NCT03937141	Phase 2	Head and neck cancer
		NCT02675439	Phase 1	Advanced/metastatic solid tumors or lymphomas
		NCT03172936	Phase 1	Solid tumors and lymphomas
MK1454	STING	NCT03010176	Phase 1	Solid tumors, lymphoma
		NCT04220866	Phase 2	Head and neck squamous cell carcinoma
Turalio (pexidartinib) (PLX3397)	CSF1R	NCT02777710	Phase 1	Metastatic/advanced pancreatic or colorectal cancers
		NCT02734433	Phase 1	Advanced solid tumors
		NCT01525602	Phase 1	Advanced solid tumors
		NCT02452424	Phase 1/2	Advanced melanoma and other solid tumors
		NCT01349036	Phase 2	Recurrent glioblastoma
		NCT02975700	Not applicable	Melanoma

(*Continued*)

Small molecule	Target	Clinical studies	Phase	Cancer type
		NCT01790503	Phase 1/2	Glioblastoma
LYC-55716	RORγt	NCT02929862	Phase 1/2	Advanced or metastatic cancer
		NCT03396497	Phase 1	Non-small cell lung cancer
TNO155	SHP2	NCT04000529	Phase 1	Non-small cell lung carcinoma, head and neck squamous cell carcinoma, esophageal SCC, gastrointestinal stromal tumors, colorectal cancer
		NCT03114319	Phase 1	Advanced solid tumors
RMC-4630 (SAR442720)	SHP2	NCT03989115	Phase 1/2	Solid tumor
		NCT03634982	Phase 1	Relapsed/refractory solid tumors
		NCT04418661	Phase 1	Metastatic neoplasm
JAB-3068	SHP2	NCT03518554	Phase 1	Advanced solid tumors
		NCT03565003	Phase 1/2a	Advanced solid tumors
JAB-3312	SHP2	NCT04121286	Phase 1	Advanced solid tumors
		NCT04045496	Phase 1	Advanced solid tumors
Idelalisib	PI3K-δ		Approved	
IPI-549	PI3K-γ	NCT03961698	Phase 2	Breast cancer, renal cell carcinoma
		NCT03719326	Phase 1	Triple-negative breast cancer, ovarian cancer
		NCT02637531	Phase 1	Advanced solid tumors
		NCT03980041	Phase 2	Advanced urothelial carcinoma
		NCT03795610	Phase 2	Head and neck squamous cell carcinoma
Ibrutinib	BTK		Approved	
Plerixafor (AMD3100)	CXCR4		Approved	
SX-682	Dual CXCR1/2	NCT04599140	Phase 1/2	Metastatic colorectal cancer
		NCT04574583	Phase 1/2	Advanced solid tumors
		NCT04477343	Phase 1	Metastatic pancreatic ductal adenocarcinoma
		NCT03161431	Phase 1	Melanoma
		NCT04245397	Phase 1	Myelodysplastic syndromes
AZD5069	CXCR2	NCT03177187	Phase 1/2	Metastatic castration resistant prostate cancer
		NCT02499328	Phase 2	Advanced solid tumors, metastatic head and neck squamous cell carcinoma
		NCT02583477	Phase 1/2	Metastatic pancreatic ductal adenocarcinoma
X4P-001	CXCR4	NCT02823405	Phase 1	Melanoma
		NCT02923531	Phase 1/2	Clear cell renal cell carcinoma
		NCT02667886	Phase 1/2	Clear cell renal cell carcinoma
Maraviroc	CCR5	NCT01785810	Phase 2	Metastatic colorectal cancer
		NCT03274804	Phase 1	Colorectal cancer
BMS-813160	Dual CCR2/5	NCT03184870	Phase 1/2	Colorectal cancer, pancreatic cancer
		NCT04123379	Phase 2	Non-small cell lung cancer, hepatocellular carcinoma
		NCT02996110	Phase 2	Advanced renal cell carcinoma
		NCT03767582	Phase 1/2	Locally advanced pancreatic ductal adenocarcinomas
		NCT03496662	Phase 1/2	Pancreatic ductal adenocarcinoma
FLX-475	CCR4	NCT03674567	Phase 1/2	Advanced cancer

Data were collected from ClinicalTrials.gov. Abbreviations: PD-L1, programmed death protein-ligand 1; VISTA, V-domain Ig suppressor of T cell activation; NCT, clinicaltrials.gov identification number; TLR, toll-like receptor; IDO1, indoleamine-2,3-dioxygenase-1; ARG, arginase; A2A, Adora2a; STING, stimulator of interferon genes; CSF1R, colony stimulating factor 1 receptor; RORγt, receptor-related orphan receptor gamma t; SHP2, Src homology-2-containing protein tyrosine phosphatase 2; PI3K, phosphoinositide-3 kinase; BTK, Brutons tyrosine kinase; CXCR, C-X-C chemokine receptor; CCR, C-C chemokine receptor.

Targeting immune checkpoints

Programmed death protein 1 (PD-1) or programmed death protein-ligand 1 (PD-L1) antibodies have a long-half life and only act on extracellular PD-1/PD-L1, that is, they cannot penetrate the tissue barrier. Therefore, the

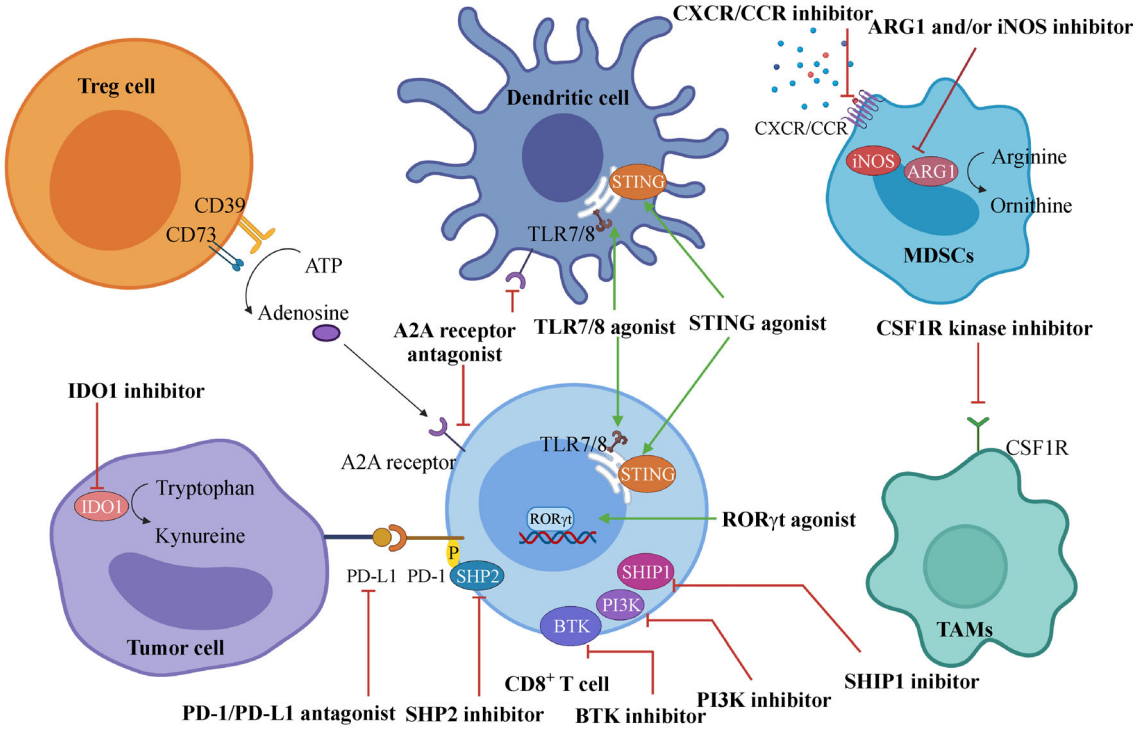

Fig. 1 Small molecule drugs and their targets in immunotherapy. This figure was created with BioRender.com. ATP, adenosine triphosphate; STING, stimulator of interferon genes; TLR, toll-like receptor; A2A, Adora2a; MDSCs, myeloid-derived suppressor cells; CXCR, C-X-C chemokine receptor; ARG1, arginase 1; IDO1, indoleamine-2,3-dioxygenase-1; PD-1, programmed death protein 1; PD-L1, programmed death protein-ligand 1; SHP2, Src homology-2-containing protein tyrosine phosphatase 2; SHIP1, SH2 domain-containing inositol-5′-phosphatase 1; PI3K, phosphoinositide-3 kinase; BTK, Brutons tyrosine kinase; RORγt, receptor-related orphan receptor gamma t; TAMs, tumor-associated macrophages; CSF1R, colony stimulating factor 1 receptor.

occurrence of irAEs must be anticipated and monitored. The advantages of small molecules are permeabilization, oral delivery, and dose modulation, which promote the development of small molecule inhibitors acting on the PD-1/PD-L1 pathway [5–7].

Companies Bristol-Myers Squibb (BMS) and Aurigene are leading the development of small molecule PD-L1 inhibitors, with molecules such as BMS-103, BMS-142, BMS-1166, CA-327, and CA170. Small PD-L1 inhibitors developed by BMS can induce the PD-L1 dimer by filling a deep hydrophobic channel-like pocket between two PD-L1 molecules and then blocking PD-1 binding [8,9]. Oral molecule, CA-327, shows anti-tumor activity in preclinical cancer models by inhibiting the PD-L1 and T cell immunoglobulin domain and mucin domain-3 (TIM-3) [10].

Developed by Aurigene, CA170 is an oral inhibitor that targets PD-L1 and the V-domain Ig suppressor of T cell activation (VISTA), and was reported as the pioneer of oral immunotherapy drugs among small molecule checkpoint inhibitors [11]. A CA-170 phase 2 clinical study is currently ongoing with data obtained from 15 non-small cell lung cancer cases and notable tumor reductions noted in six patients [12]. However, the affinity of small molecules to the target is worse than that of antibodies. Hence, off-targeting may occur and result in reduced efficacy and toxicity. Further mechanism explorations and clinical efficacy evaluations are needed. Although small molecule immune checkpoint inhibitors are mostly in preclinical and early clinical stages, these drugs will open a new avenue for tumor immunotherapy because of their pharmacokinetics and druggability advantages.

Targeting innate immunity

Pattern recognition receptors are key members in innate immunity that can distinguish pathogen-associated molecular patterns and promote T cell effector function [13]. Toll-like receptor (TLR) 7/8 is located in the endosome of cells. By improving the identification of foreign organisms, small molecule TLR agonists activate immune response. Imiquimod, a TLR7 agonist developed as topical cream by the Minnesota Mining & Manufacturing Company (the United States) has been used for superficial basal cell carcinoma [14]. This drug has also shown anti-tumor activity in a phase 2 clinical trial for patients with bladder

cancer [15]. Motolimod (VTX-2337), an agonist of TLR8, can mediate the release of IL-18 and activate natural killer (NK) cells [16]. Resiquimod (R848), a TLR7/8 agonist, helps macrophages acquire an anti-tumorigenic phenotype [17]. These TLR7/8 agonists are mostly in phase 1/2 clinical trials (Table 1).

Stimulator of interferon genes (STING) participate in the innate immune recognition of immunogenic tumors [18]. The activation of the STING pathway contributes to tumor regression in mouse models [19]. STING agonists might also improve the activation of dendritic cells (DCs) and T cells [20]. In June of 2019, Aduro announced the results of a phase 1b clinical trial for a small molecule STING antagonist (ADU-S100) combined with spartalizumab. However, only 6 out of the 83 patients with lymphoma or advanced solid tumors exhibited remarkable responses [21]. In hope of achieving relatively improved results, Aduro is currently preparing to combine ADU-S100 and Keytruda for head and neck cancers in a phase 2 clinical trial. The STING small molecule antagonist MK-1454 is also in a phase 2 clinical trial (NCT04220866).

In addition to antibodies for checkpoint modulation and cell therapy, pattern recognition receptor agonists and STING agonists provide a new approach for small molecules to prompt innate immune members to contribute to anti-tumor immune strategies. Although TLR agonists are promising targets that may exhibit synergistic effects with existing immunotherapy strategies, future research must consider that the TLR pathway is associated with gastric and pancreatic tumorigenesis [22,23]. Additional studies are required to further assess the safety of these small molecule agonists.

Targeting amino acid metabolism

The TME contains diverse immunocytes. Tumor-associated macrophages (TAMs) support tumor invasion and metastasis. Treg cells and myeloid-derived suppressor cells (MDSCs) are linked to immunosuppression in the TME. Small molecule drugs navigating metabolic pathways might strengthen the anti-tumor immunity by metabolical reprogramming of tumor and immune cells in the TME [24].

Indoleamine-2,3-dioxygenase-1 (IDO1) participates in the degradation of tryptophan to kynurenine, and selective inhibition of IDO1 enhances NK cell proliferation and reduces conversion to Treg cells [25]. BMS-986205 is one highly-efficient oral IDO1 inhibitor that can shrink bladder tumors when combined with ICIs from a phase 1/2a study [26]. IDO1 inhibitor navoximod has also shown acceptable safety and tolerance in a phase 1 clinical trial of advanced solid tumors, but its combination with atezolizumab was not beneficial [27]. A recent phase 3 trial, ECHO301, tested the efficacy of IDO1 inhibitor epacadostat combined with pembrolizumab in melanoma; however, the reaction was not better than that for pembrolizumab alone [28].

Small molecule arginase 1 (ARG1) or inducible nitric oxide synthase (iNOS) inhibitors targeting MDSCs or TAMs might overcome immunosuppression and aid the restoration of immune function [29]. ARG1 inhibitor CB-1158 promotes the production of inflammatory cytokines and increases $CD8^+$ T cell tumor infiltration [30]. CB-1158 is now under phase 1/2 clinical trials and is also being combined with a small molecule PD-1 blockade (Table 1). Transient treatment with CB-839, an inhibitor of glutaminase 1, also enhances cytotoxic lymphocyte-mediated anti-tumor responses [31].

Treatments targeting the amino acid metabolism of tumor and/or immune cells in the TME can produce a synergistic effect with existing immunotherapy approaches. However, the unexpected efficacy of IDO1 inhibitor epacadostat combined with pembrolizumab in clinical trial suggests that much efforts are need to further understand the metabolic mechanisms of immune cells to improve the effectiveness of combination therapies.

Targeting adenosine signaling

Ectonucleotidases CD73 and CD39 participate in the dephosphorylation of adenosine triphosphate to produce adenosine, which binds to the Adora2a **(**A2A) receptor, activates adenosine signaling, and amplifies the immunosuppressive effects of Treg cell [32]. In preclinical studies, the efficacy of ICIs have been enhanced using a combination of A2A receptor antagonists [33]. Preliminary evidence from a phase 1b clinical trial showed that A2A receptor inhibitor CPI-444 combined with atuzumab exhibits disease control in refractory renal cell carcinoma [34]. Other phase 1/2 studies have also assessed the safety of A2A receptor antagonists used alone or combined with ICIs in advanced tumors (Table 1). Given the immunosuppressive role of adenosine signaling in the TME, small molecule antagonists targeting A2A receptor show potential as therapeutics.

Targeting cytokine signaling

Small molecules can regulate the tumor immune response by influencing specific cytokine-mediated pathways. Retinoic acid receptor-related orphan receptor gamma t (RORγt) is a member of the nuclear receptor superfamily of transcription factors and plays an important role in the differentiation of cytokine interleukin-17 expressing immune cells [35]. RORγt agonists enhance anti-tumor immunity by activating Th17 cells and reducing Treg proliferation [36]. RORγt agonist, LYC-55716 in

combination with an ICI, is currently undergoing a phase 1 clinical trial (Table 1).

Galunisertib, a transforming growth factor-beta (TGF-β) receptor I inhibitor, suppresses Smad family member 2 phosphorylation and was granted orphan drug designation for the treatment of liver cancer by the European Medical Agency and the FDA in the United States in 2013 [37]. Galunisertib combined with a PD-L1 blockade can enhance the expression of immune-related genes and modulate T cell immunity in colorectal and breast cancer mouse models [38].

Although the relationship between these cytokines and immune regulation has been established, only a few of these drugs are currently undergoing clinical trials, possibly because they mediate complex signaling pathways. Their effects on tumor cells and immune cells in the TME and the risks of combination drugs must be paid attention.

Targeting oncogenic phosphatases and kinases

Phosphatases and kinases that regulate signal transduction are potential targets for small molecule drugs. Src homology-2-containing protein tyrosine phosphatase 2 (SHP2) is involved in the downstream signaling of PD-1, which suppresses T cell function [39]. Owing to its crucial role in T cell activation, SHP2 has emerged as a treatment strategy. In colon cancer xenograft models, SHP2 inhibitor SHP099 combined with an anti-PD-1 antibody showed better reducing ability for tumor load than monotherapy [40]. The SHP2 inhibitor RMC-4630s is currently under phase 1/2 clinical trials, and its pharmacokinetic profile and safety are also being evaluated (Table 1).

Colony stimulating factor 1 receptor (CSF1R) is activated by phosphorylation; pexidartinib, an oral CSF1R inhibitor, decreases TAMs and increases $CD8^+$ T cells when used in combination with a dendritic cell cancer vaccine in mesothelioma mouse models [41]. Two clinical trials of pexidartinib monotherapy and two clinical trials of pexidartinib combined with monoclonal antibodies in advanced tumors are currently ongoing.

3-α-Aminocholestane, a small molecule inhibitor of lipid phosphatases SH2 domain-containing inositol-5′-phosphatase 1 (SHIP1), can strengthen the antitumor response of NK and T cells in mouse models [42]. IPI-549, a phosphoinositide-3 kinase (PI3K)-γ inhibitor, can inhibit neutrophil migration and increase the antitumor efficacy of $CD8^+$ T cells [43,44]. IPI-549 used alone or in combination with ICIs is currently under investigation (Table 1). Ibrutinib, an inhibitor of Brutons tyrosine kinase (BTK), can also enhance T cell function in leukemia [45].

These small molecule drugs targeting phosphorylases and kinases usually affect tumor cell signal transduction. Additional research is needed to clarify their overall influence on tumor and immune cells prior to clinical trials.

Targeting chemokine receptors

The chemokine superfamily consists of a large number of ligands and receptors that participate in the homing, retention, circulation, and activation of immune cells [46]. C-C chemokine receptor (CCR) 2 inhibitor PF-04136309 depletes macrophages and inflammatory monocytes from the primary lesion and premetastatic liver, thereby enhancing antitumor immunity, depressing tumor growth, and reducing metastasis [47]. Inhibiting C-X-C chemokine receptor (CXCR) 4 may also reduce the accumulation of macrophages in the TME [48]. Plerixafor, a CXCR4 antagonist, has achieved good results as a chemosensitizer in phase 1/2 leukemia clinical trials [49]. Other ongoing clinical trials of small molecule drugs targeting chemokine receptors have focused on CCR2/5 antagonist BMS-813160, CCR4 inhibitor FLX475, and CXCR2 antagonist AZD5069 (Table 1).

The small molecule targeting of chemokine receptors is often used in combination with ICIs and chemotherapeutics in clinical trials. Given the important role of chemokines in the TME, the combinational strategies may provide meaningful clinical benefits. At present, numerous small molecule drugs have been developed to target the extracellular or intracellular pathways in adaptive or innate immunity; however, most of them are in the early stage of clinical trials. Additional basic experiments and clinical trials are urgently required to clarify their mechanism, clinical efficacy, and pharmacokinetics.

Bispecific antibodies (bsAbs)

An overview

First described in the 1960s [50], bsAbs are special molecules that can bind two antigens or one antigen with different epitopes. The technological innovation of bsAbs subsequently developed in antibody engineering and biology (Fig. 2) [51]. At present, only three bsAbs are approved for global marketing: catumaxomab (CD3/EpCAM) [52], blinatumomab (CD3/CD19) [53], and emicizumab (FIXa/FX or Hemlibra) [54].

BsAbs are utilized in various ways, including receptor-activation, receptor-blocking, receptor-internalization, receptor-clustering, or retargeting of cytotoxic effector cells [55]. Cancer is a complicated and polyfactorial disease. Compared with monospecific monoclonal antibodies, bsAbs can synchronously bind two individual epitopes or antigens for greater impact and better treatment effects. Multi-combined regions in one antibody could help regulate diverse functional pathways in cancer, thus

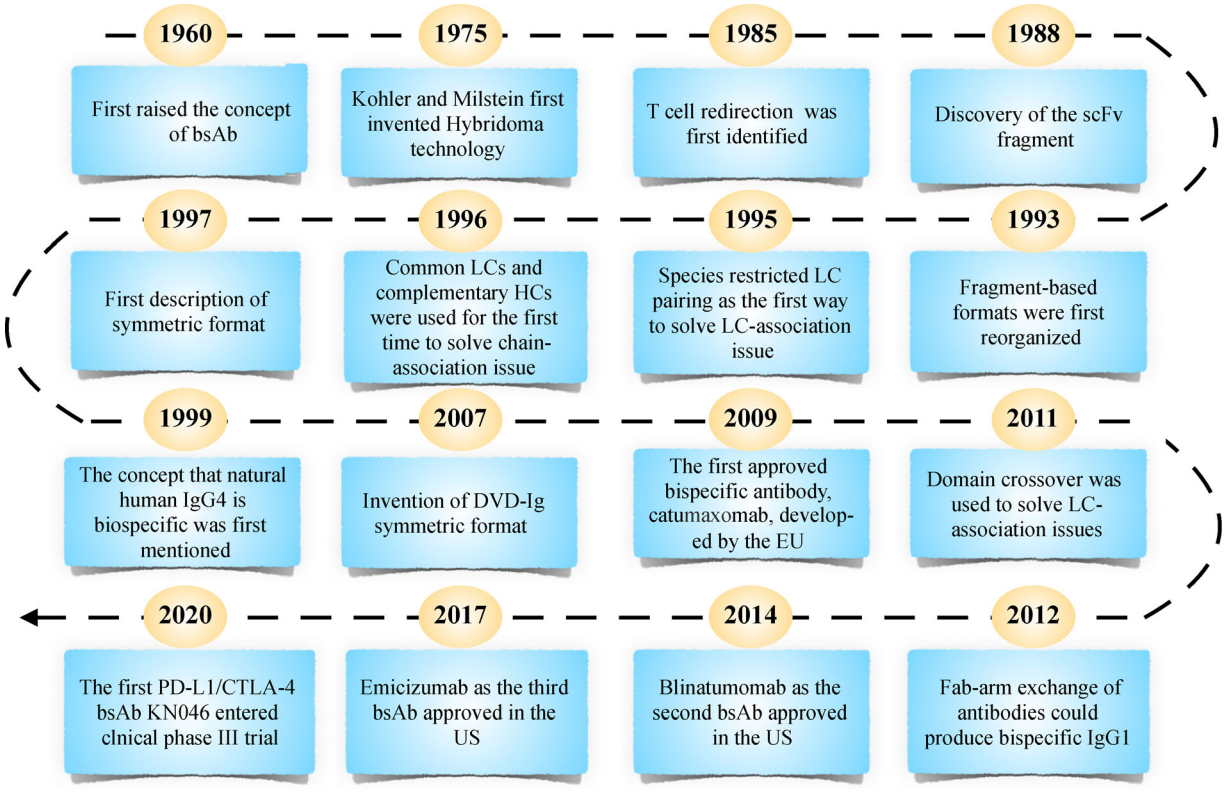

Fig. 2 Timeline of the conceptual and technical innovations contributing to the therapeutic bsAb landscape. bsAb, bispecific antibodies; DVD, dual variable domain; EU, European Union; Fab, antigen binding fragment; HC, heavy chain; Ig, immunoglobulin; LC, light chain; scFv, single-chain variable fragment.

avoiding drug resistance and decreasing the side effects on intravital tissues [56–59].

With the rapid development of gene engineering antibodies and immunology, the construction, technology platform, product research, and development of bsAbs are continuously being innovated at high speed. BsAbs are expected to be the next generation of biological therapeutics for tumors, autoimmune illness, contagious diseases, diabetes, Alzheimer's disease, and osteoporosis [51,60]. However, several challenges have been encountered during their development, namely, how to prevent poisoning and immunogenicity due to neo-antigenic determinants, how to meet the threshold for sensitizing diverse molecular mechanisms, and how to ensure the manufacturing quality [61].

Preparation method of bsAbs

BsAbs contain two different antigen binding domains that cannot be found in nature and can only be prepared artificially. Chemical coupling [62], two-hybrid method [63], and genetic engineering [64] are the most common preparation techniques for bsAbs. The most attractive application is the realization of new biological functions and therapeutic mechanism of action (MOA). However, new MOAs pose undiscovered risks that cannot be estimated in preclinical research. The indeterminacy over their safety is the major hurdle in the exploration of bsAbs. Molecular imaging studies could be used to create predictive models for the pharmacokinetic parts of bispecific constructs and to develop optimal dosing strategies [65].

Structure types

The basic structure of bsAbs consists of two pairs of heavy-light polypeptide chains connected by interchain disulfide and noncovalent bonds resembling a "Y" shape compound, including antigen binding fragments (Fabs) and a fragment crystallizable region (Fc). BsAbs could help immune cells target tumor cells by binding to one surface antigen expressed on cancer cells and to a second antigen expressed on immune cells, such as NK cells or effector T cells. The fusing of the antitumor binding domain with the Fc receptor (FcR) or the anti-CD3 binding domain may help produce bsAbs that can recruit immune cells. FcR is the terminal area of the antibody that interplays with the neonatal receptor, which results in

lethal immune-mediated effects [66,67].

BsAbs can be divided into two categories according to their structure: one contains the Fc region, and the other lacks the Fc region. These types can be further classified into asymmetric IgG-like bsAbs, symmetric IgG-like bsAbs, and non-IgG-like bsAbs [68]. IgG-like bsAbs can achieve effector functions, and non-IgG-like binding antibody (bAbs) are diminutive, which can improve penetration. IgG-like bAbs contain three arms/binding sites: two Fab arms and an Fc arm. The IgG-like bsAb structure promotes Fc domain-mediated effects and defends the physical properties endowed by the FcR [69,70]. A unique kind is asymmetric IgG-like bsAbs that possess an integrated Fc and a couple of distinguishing arms combining different antigens; some examples include M802, M701 [51], KN026 [71], MBS301 [72], IBI318 [73], IBI315 [74], and KN046 [75].

Symmetric IgG-like bsAbs are composed of an IgG-like Fc and a pair of symmetric arms formed by the association between different Fabs, single-chain variable fragment (scFV), and variable domain of heavy chain (VHH); these include EMB-01 [76], ES101 [77], K193 [78], AK104 [79], SI-B001 [80], and MGD013 [81]. Non-IgG-like bsAbs lack the Fc domain and exert the corresponding effect mechanism mainly through the characteristics of antigen binding; these include SHR-1701 [82], IMM0306 [83], and HX009 [84].

Mechanisms of bsAbs

BsAbs have manifold targets and special MOA.

T cell redirection

BsAbs characteristically target the antigen connected to T cells. By bonding to T cells and cancer cells, they can redirect the toxicity of effector T cells and obliterate cancer cells [85,86].

Double checkpoint inhibition

BsAbs can block PD-1 or lymphocyte-activation gene 3 (LAG3), PD-L1, TIM-3, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) interaction, thereby activating tumor immune response [87–90]. A number of current clinical trials are targeting the above two immune checkpoints [91–94].

Co-localized blockage

SHR-1701 can simultaneously block the PD-L1 immune checkpoint and TGF-β on cancer cells. The aforementioned combination therapy could also increase the antineoplastic effect compared with mono-treatment in cancer cell pathways [95,96].

Dual signaling inhibitions

EMB-01 (EGFR/MET) has shown promising effectiveness in numerous preclinical tests. EGFR and MET signaling paths are partly complementary and mediate the restriction of signal pathways [97–99]. SI-B001 (EGFR/HER3) activates the downstream pathways and inhibits tumorigenesis [100,101].

Tumor targeted immune-modulators

Tumor-targeted immune-modulators are intended to be combined with tumor-associated antigen (TAA) and immune-regulating receptors (PD-1/CD47) to improve immune-treatment by orientating cancer cells. Such modulators include IBI315(HER2/PD-1) [74] and IMM0306 (CD47/CD20) [102].

Biparatopic bsAbs (bpAbs)

BpAbs combine two non-overlapping sites of identical antigen to cement Ab-Ag reciprocity and enhance the cancer cellular targeting of monoclonal antibodies [103]; these include KN026 (HER2/HER2) [104] and MBS301 (HER2/HER2) [105].

Research status

Many multinational pharmaceutical companies and biotechnology companies have committed to developing bsAb-related drugs. Many Chinese companies are also involved in the research and development of bsAbs, some of which have entered the clinical or clinical application stage.

More than 100 bsAb constructions and 200 clinical trials and over 30 technology research platforms, including CrossMab (Roche), CRIBTM (China), ItabTMv (China), and FIT-IgTM (China), have been conducted over the past decade [61]. Despite starting later than other countries, Chinese bsAb development has rapidly progressed. By using the aforementioned bsAb technology research platforms, China has created 18 bsAb structures and initiated 25 clinical trials (Table 2). As of August 2020, PD-L1 and CTLA-4 are the most commonly studied targets in China [106]. In particular, 10 bsAb and 41 clinical trials were noted for both China and other countries (Table 3) [106]. 90 bsAb structures and 149 clinical trials are currently being studied outside of China (Table 4).

AK104 (PD-1/CTLA-4) is under a recent phase 2 multicenter study on advanced gastric adenocarcinoma. The common targeted cancer bsAb simultaneously blocks the

Table 2 BsAbs under clinical development in China as of August 2020

Antibody name	Targets	Clinical studies	Phase	Cancer type
MBS-301	HER2 × HER2	NCT03842085	Phase 1	Her2 positive recurrent or metastatic malignant solid tumor
IBI-318	PD-1 × PD-L1	NCT03875157	Phase 1	Advanced malignancy
IBI-322	PD-L1 × CD47	NCT04338659	Phase 1	Advanced malignancies
IBI-315	PD-1 × HER2	NCT04162327	Phase 1	Advanced solid tumor
A-319	CD3 × CD19	NCT04056975	Phase 1	Relapsed or refractory B cell lymphoma
		CTR20190205	Phase 1	Relapsed or refractory B cell lymphoma
M701	CD3 × EpCAM	NCT04501744	Phase 1	Malignant ascites
M802	HER2 × CD3	NCT04501770	Phase 1	Her2 positive advanced solid tumor
IMM0306	CD47 × CD20	CTR20192612	Phase 1	Refractory or recurrent CD20 positive B cell non-Hodgkin's lymphoma
KN-026	HER2 × HER2	CTR20190853	Phase 2	Her2 positive advanced solid tumor
EMB-01	EGFR ×c-MET	CTR20190241	Phase 2	Advanced or metastatic solid tumors
KN-046	PD-L1 × CTLA-4	NCT04469725	Phase 2	Thymic carcinoma
		NCT04474119	Phase 3	Non-small cell lung cancer
		NCT04521179	Phase 2	Her2 positive solid tumors
AK-104	PD-1 × CTLA-4	CTR20182027	Phase 1/2	Advanced solid tumor and advanced or metastatic gastric adenocarcinoma or gastresophageal junction adenocarcinoma
		CTR20200779	Phase 2	Hepatocellular carcinoma
		CTR20202184	Phase 2	Locally advanced unresectable or metastatic highly unstable satellite or mismatch repair defective solid tumor, gastric carcinoma and colorectal cancer
MGD-013	PD-1 × LAG-3	NCT04009460	Phase 1	Solid tumors
		CTR20200549	Phase 2	Advanced hepatocellular carcinoma
HX-009	PD-1 × CD47	CTR20192299	Phase 1	Advanced solid tumor
M7824	PD-L1 × TGF-β	NCT04396886	Phase 2	Recurrent or metastatic carcinoma
SHR-1701	PD-L1 × TGF-β	CTR20182404	Phase 1	Advanced solid tumor
		CTR20181823	Phase 1	Advanced solid tumor
SI-B001	HER3 × EGFR	CTR20200502	Phase 1	Locally advanced or metastatic epithelial tumors
K193	CD3 × CD19	CTR20191955	Phase 1	Refractory or recurrent B cell non-Hodgkin's lymphoma

Abbreviations: HER, human epidermal growth factor receptor; NCT, clinicaltrials.gov identification number; PD-1, programmed death protein 1; PD-L1, programmed death protein-ligand 1; CTR, Clinical Trial Registry; EpCAM, epithelial cell adhesion molecule; c-MET, cellular-mesenchymal epithelial transition; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; LAG-3, lymphocyte-activation gene 3; TGF, transforming growth factor; EGFR, epidermal growth factor receptor.

PD-1 and CTLA-4 immune regulatory checkpoints, resulting in the potential suppression of double checkpoints and antineoplastic activity [107]. Developed by Alphamab, KN046 (PD-L1/CTLA-4) is currently in a phase 3 trial. Some studies have recently reported treatment-related toxic side effects of anti-CTLA-4 antibody [108,109]. Compared with each parental mAbs, KN046 can improve the safety and efficacy [110].

Developed by Biokin, SI-B001 is an anti-HER3 × anti-EGFR bsAb that is currently in a phase 1 trial and could firsthand activate the downstream paths and inhabit tumorigenesis [100,101].

BsAbs have great clinical potential because of their unique characteristics that cannot be found in monoclonal antibodies. Most bsAbs are in clinical or preclinical research. Adverse reactions, such as cytokine storms, neurotoxicity, and production processing, are the main problems for this therapy. Designing a reasonable antibody structure according to different effect mechanisms is the focus of bsAb research and development. The continued development of clinical studies and advances in upstream and downstream technology will hopefully help to solve these bsAb-related problems.

Chimeric antigen receptor (CAR) T cell therapy

CAR T cells are T cells designed to express an artificial receptor that redirects the T cell toward tumor cell antigen. CAR T cell therapy is one of the most encouraging therapeutic strategies and has remarkable clinical potential. CARs are composed of four domains including the extracellular domain, the transmembrane (TM) domain, the intracellular domain, and an activation domain. The first-generation of CARs comprise an extracellular domain

Table 3 BsAbs under clinical development in both China and other countries as of August 2020

Antibody name	Targets	Clinical studies	Phase	Cancer type
KN-026	HER2 × HER2	NCT04165993	Phase 2	Metastatic breast cancer
		NCT03847168	Phase 1	Breast cancer
		NCT04040699	Phase 1	Her2 positive solid tumors
		NCT03619681	Phase 1	Breast cancer, gastric cancer
		NCT03925974	Phase 2	Gastric, gastresophageal junction cancer
EMB-01	EGFR × c-MET	NCT03797391	Phase 1/2	Neoplasm metastasis, non-small cell lung cancer
JNJ-61186372, JNJ-6372	EGFR × c-MET	NCT02609776	Phase 1	Non-small cell lung cancer
		NCT04077463	Phase 1	Carcinoma, non-small-cell lung
KN-046	PD-L1 × CTLA-4	NCT04040699	Phase 1	Her2 positive solid tumors
		NCT03838848	Phase 2	Advanced non-small cell lung cancer
		NCT03927495	Phase 2	Esophageal squamous cell carcinoma
		NCT03925870	Phase 2	Esophageal squamous cell carcinoma
		NCT03733951	Phase 1	Advanced solid tumors
		NCT04054531	Phase 2	Non-small cell lung cancer
		NCT03872791	Phase 1/2	Triple-negative breast cancer
		NCT03529526	Phase 1	Advanced solid tumors
AK-104	PD-1 × CTLA-4	NCT04380805	Phase 2	Recurrent or metastatic cervical cancer
		NCT04172454	Phase 1/2	Advanced solid tumors
		NCT04220307	Phase 2	Nasopharyngeal carcinoma
		NCT03261011	Phase 1	Advanced cancer
		NCT03852251	Phase 1/ 2	Advanced solid tumors
MGD-013	PD-1 × LAG-3	NCT04212221	Phase 1/2	Advanced hepatocellular carcinoma
		NCT03219268	Phase 1	Advanced solid tumors
		NCT04178460	Phase 1	Gastric cancer
		NCT04082364	Phase 2/3	Her2 positive gastric cancer, breast cancer
INBRX-105-1, INBRX-105, ES-101	PD-L1 × 4-1BB	NCT03809624	Phase 1	Metastatic solid tumors
		NCT04009460	Phase 1	Solid tumors
HX-009	PD-1 × CD47	NCT04097769	Phase 1	Advanced solid tumors
M7824	PD-L1 × TGF-β	NCT04246489	Phase 2	Uterine cervical neoplasms
		NCT04066491	Phase 2/3	Biliary tract cancer
		NCT04396535	Phase 2	Advanced lung non-small cell carcinoma
		NCT04220775	Phase 1/2	Recurrent head and neck squamous cell carcinoma
		NCT03631706	Phase 3	Non-small cell lung cancer
		NCT02517398	Phase 1	Solid tumors
		NCT03840915	Phase 1/2	Non-small cell lung cancer
		NCT03840902	Phase 2	Non-small cell lung cancer
		NCT03833661	Phase 2	Biliary tract cancer
SHR-1701	PD-L1 × TGF-β	NCT03710265	Phase 1	Solid tumors
		NCT03774979	Phase 1	Solid tumors
		NCT04282070	Phase 1	Nasopharyngeal carcinoma
		NCT04324814	Phase 1	Advanced solid tumors

Abbreviations: HER, human epidermal growth factor receptor; NCT, clinicaltrials.gov identification number; EGFR, epidermal growth factor receptor; c-MET, cellular-mesenchymal epithelial transition; PD-L1, programmed death protein-ligand 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; PD-1, programmed death protein 1; LAG-3, lymphocyte-activation gene 3; TGF, transforming growth factor.

linked to an intracellular domain without any co-stimulatory domain. However, no promising antitumor response was observed largely due to the lack of adequate activation [111]. As a solution, second- and third-generation CARs are being developed by adding one or two co-stimulatory domains, respectively, to enhance their activity [112,113].

Second-generation autologous (patient-derived) CAR T cell therapy has changed the treatment of hematologic malignancies; four CD19-targeting CARs have achieved

Table 4 BsAbs under clinical development excluding China as of August 2020

Antibody name	Targets	Clinical studies	Phases	Cancer type
Dilpacimab, ABT-165	VEGF × DLL4	NCT01946074	Phase 1	Advanced solid tumors
		NCT03368859	Phase 2	Neoplasms
MP0250	VEGF × HGF	NCT03136653	Phase 1/2	Relapsed multiple myeloma
		NCT03418532	Phase 1/2	EGFR positive lung cancer
		NCT02194426	Phase 1/2	Neoplasms
ABL-001, NOV-1501, TR-009	VEGF × DLL4	NCT02857868	Phase 1	Neoplasms
		NCT03595917	Phase 1	Chronic myeloid leukemia, acute lymphoblastic leukemia
		NCT03106779	Phase 3	Chronic myelogenous leukemia
		NCT04216563	Phase 2	Philadelphia chromosome negative, BCR-ABL1 positive chronic myelogenous leukemia
		NCT03292783	Phase 1	Advanced solid tumors
		NCT02081378	Phase 1	Chronic myelogenous leukemia, Philadelphia chromosome-positive acute lymphoblastic leukemia
		NCT03605277	Phase 1	Neoplasms
		NCT03906292	Phase 2	Chronic myeloid leukemia
		NCT03578367	Phase 2	Chronic myelogenous leukemia
Vanucizumab, RG-7221	ANGPT2 × VEGF	NCT01688206	Phase 1	Neoplasms
		NCT02141295	Phase 2	Colorectal cancer
		NCT02665416	Phase 1	Advanced or metastatic solid tumors
BI-836880	ANGPT2 × VEGF	NCT02689505	Phase 1	Neoplasms
		NCT02674152	Phase 1	Neoplasms
		NCT03972150	Phase 1	Neoplasms
		NCT03861234	Phase 1	Neoplasms
		NCT03468426	Phase 1	Non-squamous, non-small-cell lung cancer, neoplasms
Navicixizumab, OMP-305B83	VEGF × DLL4	NCT03035253	Phase 1	Metastatic colorectal cancer
		NCT03030287	Phase 1	Ovaries cancer, fallopian tube cancer
		NCT02298387	Phase 1	Advanced solid tumor malignancies
ZW-25	HER2 × HER2	NCT04224272	Phase 2	Her2 or HR positive breast cancer
		NCT02892123	Phase 1	Her2 positive cancers
		NCT03929666	Phase 2	Her2 positive gastresophageal adenocarcinoma
		NCT04276493	Phase 1/2	Breast cancer, gastric cancer, gastresophageal junction cancer
MCLA-128	HER2 × HER3	NCT03321981	Phase 2	Metastatic breast cancer
		NCT02912949	Phase 1/2	Harboring NRG1 fusion solid tumors
BCD-147	HER2 × HER2	NCT03912441	Phase 1	Neoplasms
BI-905677	LRP5 × LRP6	NCT03604445	Phase 1	Neoplasms
MP0274	HER2 × HER2	NCT03084926	Phase 1	Neoplasms
DuoBody-PD-L1x4-1BB, GEN-1046	PD-L1 × 4-1BB	NCT03917381	Phase 1/2	Solid tumors
REGN-5678	CD28 × PSMA	NCT03972657	Phase 1/2	Metastatic castration-resistant prostate cancer
FS118 mAb2, FS-118, LAG-3/PD-L1 mAb2	PD-L1 × LAG-3	NCT03440437	Phase 1	Advanced cancer
LY-3434172	PD-1 × PD-L1	NCT03936959	Phase 1	Advanced cancer
XmAb-23104	PD-1 × ICOS	NCT03752398	Phase 1	Advanced solid tumors
ABBV-428	MSLN × CD40	NCT02955251	Phase 1	Advanced solid tumors
ADC-1015, ATOR-1015	OX40 × CTLA-4	NCT03782467	Phase 1	Solid tumor
MCLA-145	PD-L1 × 4-1BB	NCT03922204	Phase 1	Advanced solid tumor, B cell lymphoma
MEDI-5752	PD-1 × CTLA-4	NCT03530397	Phase 1	Selected advanced solid tumors
MGD-019	PD-1 × CTLA-4	NCT03761017	Phase 1	Advanced solid tumor
PRS-343	HER2 × 4-1BB	NCT03330561	Phase 1	Her2 positive solid tumor
		NCT03650348	Phase 1	Her2 positive solid tumor

(*Continued*)

Antibody name	Targets	Clinical studies	Phases	Cancer type
RG-7769, RO-7121661	PD-1 × TIM-3	NCT03708328	Phase 1	Solid tumors
XmAb-20717	PD-1 × CTLA-4	NCT03517488	Phase 1	Solid tumors
XmAb-22841	CTLA-4 × LAG-3	NCT03849469	Phase 1	Solid tumors
MP0310	FAP × CD40	NCT04049903	Phase 1	Advanced solid tumor
AK-112	VEGF × PD-1	NCT04047290	Phase 1	Neoplasms malignant
GEN-1042	CD40 × 4-1BB	NCT04083599	Phase 1/2	Solid tumor, non-small cell lung cancer, colorectal cancer, melanoma
AGEN-1423, GS-1423	CD73 × TGF-β	NCT03954704	Phase 1	Advanced solid tumors
Tebentafusp (IMCgp100)	gp100/HLA-A*0201 × CD3	NCT03070392	Phase 2	Uveal melanoma
		NCT02889861	Phase 2	Malignant melanoma
		NCT02535078	Phase 1/2	Malignant melanoma
		NCT01209676	Early phase 1	Melanoma, advanced tumors
		NCT02570308	Phase 1/2	Uveal melanoma
		NCT01211262	Phase 1	Malignant melanoma
OXS-1550, DT-2219	CD19 × CD22	NCT02370160	Phase 1/2	Refractory or relapsed B-lineage leukemia
		NCT00889408	Phase 1	Leukemia, lymphoma
AFM-13	CD16 × CD30	NCT02321592	Phase 2	Hodgkin lymphoma
		NCT01221571	Phase 1	Hodgkin lymphoma
		NCT03192202	Phase 1/2	T cell lymphoma
		NCT04074746	Phase 1	Recurrent anaplastic large cell lymphoma, recurrent B cell non-Hodgkin lymphoma, recurrent classic Hodgkin lymphoma
		NCT04101331	Phase 2	Peripheral T cell lymphoma
		NCT02665650	Phase 1	Hodgkin lymphoma
Odronextamab, REGN-1979	CD3 × CD20	NCT02651662	Phase 1	Lymphoma
		NCT03888105	Phase 2	B cell non-Hodgkin lymphoma
		NCT02290951	Phase 1	Non-Hodgkin lymphoma, chronic lymphocytic leukemia
IMC-C103C	MAGE-A4/HLA *A0201 × CD3	NCT03973333	Phase 1/2	Advanced solid tumors
IMCnyeso	NY-ESO-1/HLA *A0201 × CD3	NCT03515551	Phase 1/2	Advanced solid tumors
Mosunetuzumab, RG-7828	CD3 × CD20	NCT03671018	Phase 1/2	B cell non-Hodgkin lymphoma
		NCT04313608	Phase 1	B cell lymphoma
		NCT03677141	Phase 1/2	B cell non-Hodgkin lymphoma
		NCT04246086	Phase 1	Follicular lymphoma
		NCT03677154	Phase 1/2	Diffuse large B cell lymphoma
		NCT02500407	Phase 1	Lymphocytic leukemia
OXS-3550, CD161533 TriKE	CD16 × CD33	NCT03214666	Phase 1/2	Acute myelogenous leukemia, mast cell leukemia
GEN-3013	CD3 × CD20	NCT03625037	Phase 1/2	Lymphoma
MCLA-117	CD3 × CLEC12	NCT03038230	Phase 1	Acute myelogenous leukemia, acute myeloid leukemia
Flotetuzumab, MGD-006	CD3 × CD123	NCT03739606	Phase 2	Acute and chronic myelogenous leukemia
		NCT04158739	Phase 1	Recurrent or refractory acute myeloid leukemia
MGD-007	CD3 × GPA33	NCT03531632	Phase 1/2	Colorectal cancer metastatic
		NCT02248805	Phase 1	Colorectal carcinoma
REGN-4018	CD3 × MUC16	NCT03564340	Phase 1/2	Recurrent ovarian cancer, recurrent fallopian tube cancer, recurrent primary peritoneal cancer
Cibisatamab, RO-6958688, RG-7802	CD3 × CEA	NCT02650713	Phase 1	Solid tumors
		NCT02324257	Phase 1	Solid tumors
		NCT03337698	Phase 1/2	Carcinoma, non-small-cell lung
		NCT03866239	Phase 1	Colorectal cancer

(*Continued*)

Antibody name	Targets	Clinical studies	Phases	Cancer type
AMG-701	CD3 × BCMA	NCT03287908	Phase 1	Relapsed or refractory multiple myeloma
AMG-160	CD3 × PSMA	NCT03792841	Phase 1	Metastatic castration-resistant prostate cancer, prostate cancer
AMG-330, MT-114	CD3 × CD33	NCT02520427	Phase 1	Relapsed or refractory acute myeloid leukemia
AMG-424	CD3 × CD38	NCT03445663	Phase 1	Relapsed or refractory multiple myeloma
AMG-427	CD3 × FLT3	NCT03541369	Phase 1	Relapsed or refractory acute myeloid leukemia
AMG-562	CD3 × CD19	NCT03571828	Phase 1	Diffuse large B cell lymphoma, mantle cell lymphoma, follicular lymphoma
AMG-596	CD3 × EGFRvIII	NCT03296696	Phase 1	Glioblastoma or malignant glioma
AMG-673	CD3 × CD33	NCT03224819	Early Phase 1	Acute myeloid leukemia
AMG-757	CD3 × DLL3	NCT03319940	Phase 1	Small cell lung carcinoma
AMV-564, TandAb T564	CD3 × CD33	NCT03144245	Phase 1	Acute myeloid leukemia
		NCT04128423	Phase 1	Locally advanced or metastatic solid tumors
		NCT03516591	Phase 1	Myelodysplastic syndrome
APVO-436	CD3 × CD123	NCT03647800	Phase 1	Acute myeloid leukemia, myelodysplastic syndrome
BI-836909, AMG-420	CD3 × BCMA	NCT02514239	Phase 1	Multiple myeloma
		NCT03836053	Phase 1	Relapsed or refractory multiple myeloma
RG-6026, RO-7082859	CD3 × CD20	NCT03533283	Phase 1	Non-Hodgkin's lymphoma
		NCT03467373	Phase 1	B cell lymphoma, non-Hodgkin lymphoma
		NCT04313608	Phase 1	B cell lymphoma
		NCT04246086	Phase 1	Follicular lymphoma
		NCT03075696	Phase 1	Non-Hodgkin's lymphoma
		NCT04077723	Phase 1	Lymphoma, non-Hodgkin
EM-901, CC-93269	CD3 × BCMA	NCT03486067	Phase 1	Multiple myeloma
ERY-974	CD3 × GPC3	NCT02748837	Phase 1	Solid tumors
GBR-1302	CD3 × HER2	NCT02829372	Phase 1	Her2 positive solid tumors
		NCT03983395	Phase 1/2	Breast cancer
GBR-1342	CD3 × CD38	NCT03309111	Phase 1/2	Multiple myeloma
GEM-333	CD3 × CD33	NCT03516760	Phase 1	Acute myeloid leukemia
GEM-3PSCA, GEM3PSCA	CD3 × PSCA	NCT03927573	Phase 1	Non-small cell lung cancer, breast cancer, pancreatic cancer, urogenital cancer
IGM-2323	CD3 × CD20	NCT04082936	Phase 1	Non-Hodgkin lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma
JNJ-67571244, JNJ-1244	CD3 × CD33	NCT03915379	Phase 1	Leukemia
JNJ-63709178, JNJ-9178	CD3 × CD123	NCT02715011	Phase 1	Leukemia
JNJ-64007957, JNJ-7957	CD3 × BCMA	NCT03145181	Phase 1	Hematological malignancies
JNJ-63898081, JNJ-8081	CD3 × PSMA	NCT03926013	Phase 1	Neoplasms
Orlotamab, MGD-009	CD3 × B7-H3	NCT03406949	Phase 1	Advanced solid tumors
		NCT02628535	Phase 1	Advanced solid tumors
Pasotuxizumab, AMG-212, (BAY2010112/MT112)	CD3 × PSMA	NCT01723475	Phase 1	Prostatic neoplasms
PF-06671008	CD3 × CDH3	NCT02659631	Phase 1	Neoplasms
PF-06863135, PF-3135	CD3 × BCMA	NCT03269136	Phase 1	Multiple myeloma
REGN-5458	CD3 × BCMA	NCT03761108	Phase 1/2	Multiple myeloma
RG-6194, BTRC-4017A	CD3 × HER2	NCT03448042	Phase 1	Solid tumors
TNB-383B	CD3 × BCMA	NCT03933735	Phase 1	Multiple myeloma
XmAb-13676, THG-338	CD3 × CD20	NCT02924402	Phase 1	B cell non-Hodgkins lymphoma, chronic lymphocytic leukemia
XmAb-14045, SQZ-622	CD3 × CD123	NCT02730312	Phase 1	Acute myelogenous leukemia, B cell acute lymphoblastic leukemia, blastic plasmacytoid dendritic cell neoplasm, chronic myeloid leukemia

(*Continued*)

Antibody name	Targets	Clinical studies	Phases	Cancer type
XmAb-18087, XENP-18087	CD3 × SSTR2	NCT03411915	Phase 1	Neuroendocrine tumor, gastrointestinal neoplasm
HPN-424	CD3 × PSMA	NCT03577028	Phase 1	Advanced prostate cancer
JNJ-64407564	CD3 × GPRC5D	NCT03399799	Phase 1	Hematological malignancies
		NCT04108195	Phase 1	Multiple myeloma
RG-6160 (BFCR4350A)	CD3 × FcRH5	NCT03275103	Phase 1	Multiple myeloma
NI-1701, TG-1801	CD19 × CD47	NCT03804996	Phase 1	B cell lymphoma
MCLA-158	EGFR × LGR5	NCT03526835	Phase 1	Advanced or metastatic solid tumors, colorectal cancer
ZW-49	HER2 × HER2	NCT03821233	Phase 1	Her2 positive cancers
SAR-440234	CD3 × CD123	NCT03594955	Phase 1/2	Leukemia
AFM-11	CD3 × CD19	NCT02848911	Phase 1	Leukemia
		NCT02106091	Phase 1	Relapsed or refractory B cell non-Hodgkin lymphoma
AFM-24	EGFR × CD16	NCT04259450	Phase 1/2	Advanced solid tumor
CCW-702	CD3 × PSMA	NCT04077021	Phase 1	Castration-resistant prostatic cancer
HPN-217	CD3 × BCMA	NCT04184050	Phase 1/2	Multiple myeloma in relapse
BI-905711	Cadherin-17 × TRAIL-R2	NCT04137289	Phase 1	Gastrointestinal neoplasms, cholangiocarcinoma, pancreatic neoplasms
MT110	CD3 × EpCAM	NCT00635596	Phase 1	Solid tumors

Abbreviations: VEGF, vascular endothelial growth factor; DLL4, delta like canonical notch ligand 4; NCT, clinicaltrials.gov identification number; HGF, hepatocyte growth factor; ANGPT2, angiopoietin 2; LRP, lipoprotein receptor related-protein; PSMA, prostate-specific membrane antigen; LAG-3, lymphocyte-activation gene 3; MSLN, mesothelin; TIM-3, T cell immunoglobulin domain and mucin domain-3; FAP, fibroblast activation protein; HLA, human leukocyte antigen; GPA33, glycoprotein A33; MUC16, mucin 16; BCMA, B cell maturation antigen; FLT3, FMS-liketyrosine kinase 3; EGFR, epidermal growth factor receptor; GPC3, glypican 3; PSCA, prostate stem cell antigen; CDH3, cadherin 3; SSTR2, somatostatin receptor 2; GPRC5D, G-protein coupled receptor family C group 5 member D; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; TRAIL-R2, TNF-related apoptosis-inducing ligand receptor 2; EpCAM, epithelial cell adhesion molecule.

FDA approval [114–117]. Clinical trials are also ongoing, and CAR T cells specific for CD30 (CD30-targeting CARs) have shown potential to treat Hodgkin's lymphoma (HL) in two phase 1/2 clinical trials (NCT02690545, NCT02917083) [118]. A clinical trial of anti-CD7 universal CAR-T (U-CAR-T) cells indicated that patients with T cell lymphoma displayed robust CAR-T cell expansion (NCT04264078) [119]. In a phase 1/2 clinical study (NCT01869166), anti-EGFR CAR-T cells were found to be a feasible therapeutic strategy for EGFR-positive patients with NSCLC [120].

However, the success of CAR T cell therapy is yet to be applied clinically. Several impediments have been encountered, namely, poor availability of tumor specific antigens, immunosuppressive characteristics of the TME, and variability in manufacturing quality and high processing costs [121–123]. The use of "off-the-shelf" allogeneic CAR T cells from healthy donors could potentially overcome these issues. Allogeneic T cells are primarily derived from peripheral blood mononuclear cells, embryonic stem cells, and induced pluripotent stem cells. Allogeneic CAR T products can markedly decrease the costs owing to industrialized and scaled-up production, thereby rendering CAR T treatment immediately accessible to a large number of patients due to the batch manufacturing of cryopreserved T cells. The use of allogeneic cells would also provide a high-quality product based on donor selection and allow for standardized dosing and re-dosing and a combination of CAR targets [122,123]. Other major issues must be addressed, including toxicities such as graft versus host disease (GVHD) and limited anti-tumor efficacy against solid tumors. Various safeguarding strategies, such as applying non-αβ T cells including γδ T cells [124], gene editing with αβ T cell receptor (TCR) deletion [125], and using virus-specific T cells [126] or donor-derived allogeneic T cells [127], are needed to improve the clinical safety of CAR T cell therapy. All these techniques have been designed to specifically reduce GVHD toxicity.

Although CAR T cell therapies have shown unsatisfactory efficacy in solid tumors, many promising methods can be applied for optimization. Improving CAR T structures [128] and combining with different treatment strategies such as chemotherapy [129], local therapy [130], checkpoint blockades [131], bsAbs [132], epigenetic modulators [133], vaccines [134], and oncolytic viruses [135] have all been explored to enhance the persistence and antitumor activity of CAR T cell therapy.

Despite the bumpy road ahead, the future of CAR T cell therapy looks promising because of the continuous evolution of advanced gene editing techniques and novel solutions. These innovations will help "off-the-shelf"

allogeneic CAR T cell therapy to be effective, safe, and perhaps even revolutionize cancer treatment.

Therapeutic cancer vaccines

Cancer vaccines trigger immune responses against tumor cells by amplifying and broadening antigen-specific T cells [136]. Tumor antigens, immune adjuvants, delivery vehicles, and formulations are the four key components of therapeutic vaccines and are vital for efficacy. Tumor antigens can be delivered in the form of genetic vaccines (DNA/RNA/viral), protein/peptide vaccines, and cell vaccines. Delivery method is also a major factor influencing vaccine efficacy [136].

Antigens for tumor vaccines include TAAs and tumor-specific antigens (TSAs). Early cancer vaccines focused on TAAs, self-antigens that have elevated levels on tumor cells but may also be expressed on normal cells. However, TAAs lacking tumor specificity increase the risk of autoimmune toxicities and have been unsuccessful in generating effective antitumor immune responses due to immune tolerance [136,137].

TSAs comprise antigens expressed by neoantigens or oncoviruses and are found exclusively in cancer cells. Neoantigen-based cancer vaccines are tumor-specific, can enhance a tumor-specific T cell response, and prevent toxicities caused by "off-target" damage. Recent development on bioinformatics technologies has enabled the systematic identification of tumor neoantigens; several promising studies have explored neoantigen cancer vaccines [138]. In a phase 1 clinical trial, Ott *et al.* reported a neoantigen vaccine that was formulated with up to 20 personized HLA-A/B-restricted peptides and has expanded neoantigen-specific T cells in patients with melanoma (NCT01970358) [139]. After a 4-year median follow-up of neoantigen vaccine therapy, a persistent T cell response was observed in patients with melanoma [140]. Neoantigen-specific T cells from peripheral blood also show the potential to migrate into intracranial tumors in glioblastoma after surgical resection cases in a phase 1b clinical trial (NCT02287428) [141]. These initial studies suggest that neoantigen-specific cancer vaccines are safe in patients with melanoma and glioblastoma. For further understanding on their therapeutic efficacy, in-depth studies must be conducted on the function of vaccine-induced T cells and the persistence of neoantigen-specific memory T cells.

Most therapeutic cancer vaccines are in ongoing trials, and their development can possibly enhance the efficacy of immunotherapy. In a phase 1b study of a neoantigen-based peptide vaccine NEO-PV-01 in combination with a PD-1 inhibitor, epitope spreading was detected post-vaccination and correlated with improved progression free survival in patients (NCT02897765) [142]. Compared with sunitinib monotherapy, sunitinib in combination with ilixadencel, a cell-based allogeneic off-the-shelf product, exhibited a higher overall response rate in patients with synchronous metastatic renal cell carcinoma [143]. In a clinical trial of personalized tumor lysate-pulsed DCs for patients with recurrent ovarian cancer, a vaccine plus therapy seemed to improve the overall survival compared with a low-dose cyclophosphamide and bevacizumab combination therapy [144].

Although the above preliminary findings are encouraging, numerous challenges remain to be addressed. First, further discovery of personalized neoantigen targets is required to maximize their effects. Second, delivery strategies are an important factor affecting vaccine efficacy; the effectiveness of different delivery methods varies among tumor types. Finally, when a vaccine is being combined with existing treatment approaches, the timing, sequence, and dose of combination therapy must be further explored.

Challenges and future direction

New immunotherapeutic approaches provide opportunities for further drug development and bring benefits to patients. However, challenges persist during their development. Therefore, further basic and clinical research is needed.

Assessment of combination therapy

Given that anti-tumor immunity involves various steps, rational combinations to modulate different biological steps might strengthen anti-tumor responses. Effective transformation from basic discovery to clinical application could be achieved by exploring the molecular mechanisms and optimizing the strategies and timing of combination therapy to maximize its effects. The combination of four components (anti-PD1 therapy, tumor antigen-targeting antibody, interleukin-2, and a T cell vaccine) that engage in innate and adaptive immune responses was reported to eliminate large tumors in mouse model [145]. However, most drugs are in the early stages of clinical trials with complicated combinations and pose various challenges, specifically how to maximize their synergistic effects and how to avoid combinational toxicities. For a partial solution, MORPHEUS and FRACTION platforms were designed to evaluate the safety and effectiveness of combination immunotherapies in multiple phase 1b/2 trials [146,147]. A novel Quick efficacy seeking trial (QuEST1) was also designed to assess different immunotherapy combinations in patients with prostate cancer [148]. The rational selection of the combination and dosage based on known molecular mechanisms to maximize their synergistic effects is yet to be elucidated.

Validated biomarkers

Over 3000 interventional clinical trials of immunological drugs either alone or in combination are being conducted globally [149]. Nevertheless, the clinical benefits of many novel immunotherapies cannot be determined at this stage. Strategies for the identification of valid biomarkers are essential in identifying patients who will benefit the most. Many current clinical trials include the detection of serial sampling of peripheral blood or tumor specimens for the analyses of corresponding biomarkers (such as NCT01928576 and NCT03220477). In a phase 2 study of immunotherapy combinations of motolimod and doxorubicin for ovarian cancer, statistically significant differences in the overall survival of motolimod-treated patients were observed in a subgroup of patients who experienced injection site reactions; this investigation may provide biomarkers to evaluate the efficacy of combinational immunotherapies [150]. Owing to the complex interactions required for effective treatment, the development of actionable information and identifying feasible markers that can accurately classify patients is imperative.

Autoimmune toxicities

The mechanisms of immune-related toxicities must be understood to produce the best personalized treatment approach. Small changes in the molecular structures of small molecules may lead to tremendous variations in efficacy and toxicity. Diverse challenges have emerged during the exploration of bsAbs, such as reducing toxicity and immunogenicity induced by neo-epitopes, satisfying thresholds for sensitizing various molecular pathways, and assuring the quantity and quality of bsAbs.

The application of CAR T is also not without concerns. This treatment can lead to adverse effects, such as cytokine release syndrome and on-target off-tumor toxicity. Early recognition of cytokine release syndrome and aggressive steroid administration in CAR T treatment are important [151]. Moreover, drug–drug interactions must be considered for the toxicities of combination treatments. In a phase 2 study, the combination of pembrolizumab plus oral azacitidine CC-486 was associated with an increase in treatment-related adverse events compared with the pembrolizumab plus placebo group. This phenomenon can be attributed to the intestinal and hematological toxicities noted for the oral formulation of azacitidine [152].

Improving manufacturing practices

The production of bsAbs, CAR T cells, and neoantigen-based vaccines is expensive and time consuming. In the development of biological products, optimizing the structures and workflows according to the biological mechanisms requires special attention. Designing a reasonable antibody structure according to different effect mechanisms is the focus of current bsAb research and development.

Complete CAR T cell therapy is complex compared with autologous products; however, allogeneic CAR T products offer the advantages of industrialized production and low costs [122]. Manufacturing some biological products is also time consuming, and the production of personalized vaccines is more expensive than off-the-shelf therapeutic agents. Vaccine preparation usually takes 3–5 months at best [139]. Technological developments, such as automated flow peptide production, might help promote peptide manufacturing and decrease the production time of personalized vaccines [153]. For these emerging immunotherapy drugs, their research, development, and production time and costs must be considered.

Conclusions

Immune checkpoint therapies, such as PD-1, PD-L1, and CTLA-4 antibodies, have made considerable headway in tumor treatments for the past decade. However, only a small number of cases respond to immunotherapy and are often accompanied by adverse reactions. Therefore, new treatment options are essential to enhance immunotherapy efficacy, overcome immunosuppression, and reduce toxicity. Understanding novel immuno-oncology therapeutic strategies allow us to provide additional opportunities for patients with advanced cancer. Small molecule drugs, bsAbs, CAR-T treatment, and cancer vaccines provide appealing avenues for immunotherapy. Related preliminary preclinical and clinical studies are already underway. Cost of treatment, lack of biomarker responses, and combination therapies targeting different immune mechanisms remain as challenges to be overcome. Nevertheless, these emerging strategies can bring about new opportunities for patients with cancer.

Compliance with ethics guidelines

Hongyun Zhao, Fan Luo, Jinhui Xue, Su Li, and Rui-Hua Xu declare that they have no conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval from relevant institutional review board or ethics committee.

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