Cancer Immunology, Immunotherapy (2022) 71:1549–1567
https://doi.org/10.1007/s00262-021-03097-x

REVIEW ARTICLE

Targeting the immune checkpoint B7-H3 for next-generation cancer immunotherapy

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Received: 18 February 2021 / Accepted: 21 October 2021 / Published online: 5 November 2021

Abstract

Immune checkpoint inhibitors (ICIs) for programmed death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1) have become preferred treatment strategies for several advanced cancers. However, response rates for these treatments are limited, which encourages the search for new ICI candidates. Recent reports have underscored significant roles of B7 homolog 3 protein (B7-H3) in tumor immunity and disease progression. While its multifaceted roles are being elucidated, B7-H3 has already entered clinical trials as a therapeutic target. In this review, we overview the recent results of clinical trials evaluating the antitumor activity and safety of B7-H3 targeting drugs. On this basis, we also discuss the challenges and opportunities arising from the application of these drugs. Finally, we point out current gaps to address in the understanding of B7-H3 function and regulation in order to fully unleash the future clinical utility of B7-H3-based therapies for the treatment of cancer.

Keywords Immune checkpoint · B7-H3 · Clinical trials · Immunotherapy

Introduction

With the discovery of immune checkpoint molecules, related immunotherapies have gradually been pillars of cancer treatments, which are currently shifting to the front-line for advanced non-small-cell lung cancer (NSCLC) and melanoma [1–3]. However, response rates to immune checkpoint inhibitors (ICIs) are still limited to a very small subset of patients [4], encouraging the need for identifying new ICI candidates. In fact, several other immune checkpoints are already under investigation, such as the B7 homolog 3 protein (B7-H3). B7-H3 is a single-pass type I transmembrane protein discovered in 2001 [5, 6], which belongs to the B7 family. While B7-H3 is low or undetectable in normal tissue, it is widely expressed in cancers [7]. Importantly, B7-H3 has been found in solid tumors with low or negative PD-L1 expression like pulmonary invasive mucinous adenocarcinoma [8], prostate cancer [9], and soft tissue sarcoma [10]. Furthermore, it has been found on keratinocytes, fibroblast synovial cells, endothelial cells [11, 12], and tumor-associated vasculature (TAV) [13].

Unlike its well-known homolog B7-H1 (PD-L1), the isoforms of B7-H3 are different between humans and mice [5, 6]. In mice, the extracellular domain contains an immunoglobulin variable (IgV)-like and an immunoglobulin constant (IgC)-like domain. While in humans, it contains one or two pairs of identical domains owning to exon duplication, both of which were encoded by a gene on chromosome 15 [5, 14] (Fig. 1A). Despite B7-H3 has been originally described as a co-stimulatory molecule, a growing number of studies have shed the light on an immune-inhibitory function of

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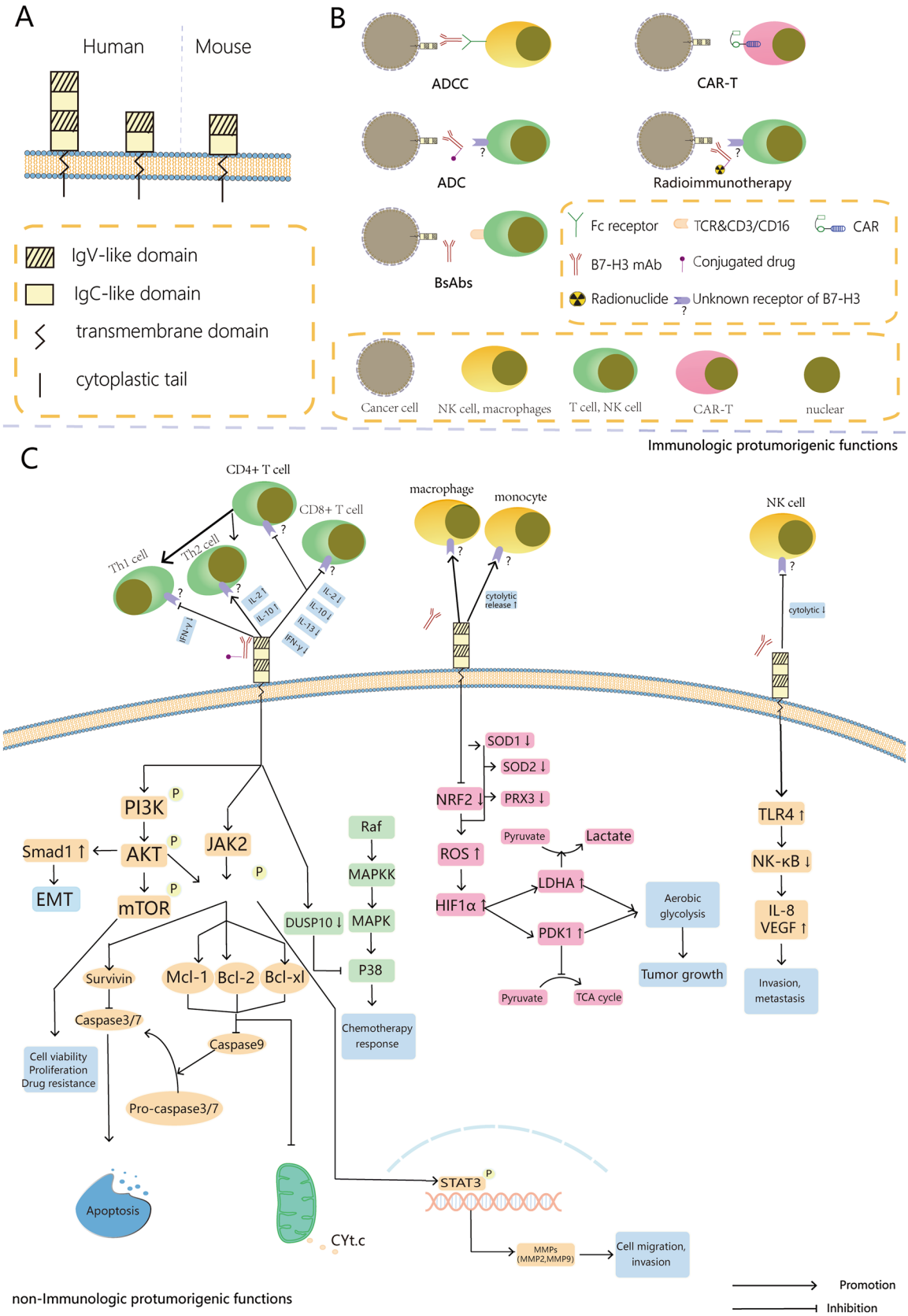
A
Human
Mouse
IgV-like domain
IgC-like domain
transmembrane domain
cytoplastic tail
B
ADCC
CAR-T
ADC
Radioimmunotherapy
BsAbs
Fc receptor
TCR&CD3/CD16
CAR
B7-H3 mAb
Conjugated drug
Radionuclide
Unknown receptor of B7-H3
Cancer cell
NK cell, macrophages
T cell, NK cell
CAR-T
nuclear
Immunologic protumorigenic functions
C
CD4+ T cell
CD8+ T cell
Th1 cell
Th2 cell
macrophage
monocyte
NK cell
IL-2↓
IL-10↓
IL-13↓
IFN-γ↓
cytolytic release↑
cytolytic↓
PI3K
AKT
JAK2
Smad1↑
EMT
mTOR
Raf
MAPKK
MAPK
P38
DUSP10↓
Survivin
Mcl-1
Bcl-2
Bcl-xl
Caspase3/7
Caspase9
Pro-caspase3/7
Cell viability
Proliferation
Drug resistance
Chemotherapy response
Apoptosis
CYt.c
SOD1↓
SOD2↓
NRF2↓
PRX3↓
ROS↑
HIF1α↑
Pyruvate
Lactate
LDHA↑
PDK1↑
TCA cycle
Aerobic glycolysis
Tumor growth
TLR4↑
NK-κB↓
IL-8
VEGF↑
Invasion, metastasis
STAT3
MMPs (MMP2,MMP9)
Cell migration, invasion
non-Immunologic protumorigenic functions
Promotion
Inhibition

◂**Fig. 1** Graphical Abstract. (**A**) B7-H3 is a single-pass type I transmembrane protein with three domains: an extracellular domain, a transmembrane, and an intracellular domain. In mice, there is one (IgV)-like and (IgC)-like domain (2IgB7-H3). In humans, it contains one (2IgB7-H3) or two (4IgB7-H3) pairs of identical domains owning to exon duplication; (**B**) Overview of therapeutic tools targeting B7-H3 currently under investigation in clinical trial. (**C**) Roles of B7-H3 in the tumor microenvironment, including immunologic and non-immunologic pro-tumorigenic functions

B7-H3 in tumors [6, 15] (See Fig. 1C). A detailed review of the multifaceted roles of B7-H3 in tumor immunity has been recently published by Li et al. [16]. Besides, B7-H3 has also been reported to carry non-immunological functions by promoting cancer cell invasion and chemoresistance through a variety of mechanisms, including anti-apoptosis, pro-proliferation, metabolic reprogramming, and pro-angiogenesis [17].

In recent years, more than 20 clinical trials on B7-H3 have been carried out to evaluate the curative efficacy of B7-H3 targeting therapies. In this review, we provide an overview of these therapies and the latest results of preclinical and clinical studies (Table 1). In addition, we describe the current unknowns on B7-H3 biology and challenges and opportunities of current and future B7-H3 targeting therapies.

B7-H3 as a tumor-associated antigen targetable with antibody-based therapies

Several therapeutic antibodies targeting B7-H3-expressing cells have been developed in the last decade (Fig. 1B, Table 1). Preclinical studies have been successfully completed leading to multiple dose-escalation phase I/II trials with safety and tolerability as primary endpoints. Few clinical trials have also included antitumor activity as a secondary endpoint. Based on the anticipated completion date, final reports for the majority of clinical trials would be published within the next two or three years.

Antibody–drug conjugates

Mechanism of action

Antibody–drug conjugates (ADCs) are emerging as promising anticancer therapies with the recent FDA approval of several ADCs for the treatment of metastatic solid tumors [18–20]. ADCs are formed from three different components: a humanized antibody, a linker and a drug payload. Their primary function is to target a tumor-associated surface protein and deliver the cytotoxic payload upon internalization of the antibody-antigen complex. Based on these mechanisms, ADCs have the potential to efficiently eradicate tumors while minimizing off-target toxicities. Nevertheless, the efficacy and safety of ADCs rely on critical parameters including antibody affinity to its target, high expression of the target in tumors and low/no expression in normal tissue, target internalization and payload cytotoxic potency. Tubulin inhibitors monomethyl auristatins (MMAE, MMAF) have been commonly used for ADC development [21]. Duocarmycins represent a novel class of ADC payloads, leading to DNA alkylation and cell death [22]. They are attractive tools because they can act on dividing and non-dividing cancer cells which make hypoxic, chemoresistant and cancer stem-like tumor cells sensitive to these ADC payloads [23]. In addition, duocarmycins are cell-permeant and upon cell death, and they can diffuse through membranes of neighboring tumor cells resulting in bystander killing. Unlike MMAE, duocarmycins are not substrates of multidrug resistance proteins (MDR) which cause resistance to ADC through drug efflux outside the cell. To date, two ADCs targeting B7-H3, namely MGC018 and DS-7300a, are currently under investigation in clinical trials. MGC018 is a humanized IgG1 monoclonal antibody against B7-H3 developed by Macrogenics that contains a cleavable linker-duocarmycin payload [24]. DS7300a (Daiichi Sankyo Inc) is a humanized IgG1 anti-B7-H3 conjugated with a cleavable linker and an exatecan derivative (DXd) that inhibits topoisomerase I and DNA replication in dividing cells [25].

MGC018 (Macrogenics)

Preclinical studies with MGC018 in several patient-derived xenograft models (breast cancer, prostate cancer, head and neck cancer) showed strong antitumor activity with > 95% tumor reduction [24]. No in vivo data with MGC018 have been conducted to demonstrate bystander killing, drug intracellular uptake or off-tumor on-target toxicity. MGC018 does not cross-react with mouse B7-H3 precluding assessment of potential toxicity in normal tissue. Bystander killing activity of MGC018 has only been observed in vitro with co-culture of B7-H3-positive and B7-H3-negative tumor cells. Interestingly, TAV has been shown to upregulate B7-H3 expression, while no expression was detected in normal vasculature [26]. In a follow-up study, Seaman et al. generated an ADC (named m276 conjugated to MMAE) targeting both mouse and human B7-H3 [27]. Using a mouse tumor xenograft model, they show that this ADC was effective against tumor cells as well as TAV supporting a dual therapeutic activity. MGC018 is currently tested in phase I/II clinical study (NCT03729596[1]) with patients presenting advanced solid cancers, including metastatic castrate resistant prostate cancer, NSCLC, triple negative breast cancer, squamous cell carcinoma of head and neck and melanoma [28]. Interim analysis with 29 patients enrolled in the study

Table 1 Clinical trials with B7-H3-targeting immunotherapies

Drug(s)	Type of mAb	ID Number	Phase	Recruitment Status	Country	Estimated enrollment	Masking	Dose	Age (years)	Cancer type	Outcomes
MGC018	ADC	NCT03729596[1]	I/II	Recruiting	MacroGenics	182	None	Not mentioned	> 18	Advanced Solid Tumor, Metastatic Castrate Resistant Prostate Cancer, Non-Small Cell Lung Cancer, Triple Negative Breast Cancer, Squamous Cell Carcinoma of Head and Neck, Melanoma, Prostate Cancer	5/9 metastatic prostate cancer patients with reduction in PSA levels of ≥ 50%, and 3/3 melanoma patients showed partial response with ≥ 24% tumor volume reduction. One patient experienced a grade 4 AE (neutropenia)
MGA271	ADCC	NCT01391143[3]	I	Terminated	United States	179	None	0.01-15 mg/kg	> 18	Melanoma, prostate carcinoma and other B7-H3^{+} tumors	43.5% of patients (20/46) experienced stable disease (> 12 weeks) and tumor shrinkage (2%-69%), common AEs were observed, including fatigue, nausea, chills, and vomiting
MGA271	ADCC	NCT02923180[4]	II	Not recruiting	United States	33	None	15 mg/kg (weekly) IV	> 18	Prostate Cancer	Primary endpoints are safety and tolerability. 3/32 patients showed marked tumor infiltration of CD8 + T cells in MGA271-treated samples

Table 1 (continued)

Drug(s)	Type of mAb	ID Number	Phase	Recruitment Status	Country	Estimated enrollment	Masking	Dose	Age (years)	Cancer type	Outcomes
^{124}I-8H9	Radioimmunotherapy	NCT01502917[17]	I	Recruiting	United States	64	None	0.25–12 mCi	2–21	Brain Cancer, Brain Stem Glioma	Some patients presented with grade 3 AEs (hemiplegia, skin infection, and anxiety) but no grade 4 or grade 5 AEs was reported suggesting an acceptable safety profile
^{131}I-8H9	Radioimmunotherapy	NCT00089245[18]	I	Not recruiting	United States	120	None	10–100 mCi	All	Brain and Central Nervous System Tumors Neuroblastoma Sarcoma	Nearly 50% of patients were alive at 36 months post-treatment in a setting where overall survival at 36 months is usually less than 10%
^{131}I-8H9	Radioimmunotherapy	NCT01099644[19]	I	Not recruiting	United States	54	None	30–50 mci/m2	> 1	Peritoneal Cancer	Three patients (n = 1 each) had transient, self-limiting, possibly therapy-related grade 3 toxicities: neutropenia, hepatic transaminase elevation and thrombocytopenia. 6/7 DSRCT patients treated without evaluable disease remain in remission at a median of 11.1 months post 131I-8H9

Table 1 (continued)

Drug(s)	Type of mAb	ID Number	Phase	Recruitment Status	Country	Estimated enrollment	Masking	Dose	Age (years)	Cancer type	Outcomes
DS-7300a	ADC	NCT04145622[2]	I-II	Recruiting	United States	160	None	Stating at 0.8 mg/kg	> 18	Advanced Solid Tumor, Malignant Solid Tumor	Ongoing
MGA271	ADCC	NCT02982941[5]	I	Completed	United States	25	None	Starting at 10 mg/kg (weekly) IV	1–35	Neuroblastoma, Rhabdomyosarcoma, Osteosarcoma, Ewing Sarcoma, Wilms Tumor, Desmoplastic Small Round Cell Tumor	Ongoing
MGA271	ADCC	NCT02381314[6]	I	Completed	United States	24	None	Not mentioned	> 18	Melanoma Non-Small Cell Lung Cancer	Ongoing
MGA271	ADCC	NCT02475213[7]	I	Not recruiting	MacroGenics	145	None	Not mentioned	> 18	Melanoma Head and Neck Cancer Non-Small Cell Lung Cancer Urethelial Carcinoma	Ongoing
MGA271	ADCC	NCT04634825[8]	II	Recruiting	United States	80	None	Not mentioned	> 18	Head and Neck Cancer	Ongoing
DS-5573a	ADCP	NCT02192567[9]	I	Terminated	Japan	19	None	0.1–30 mg/kg	> 20	Advanced solid malignant tumor	Ongoing

Table 1 (continued)

Drug(s)	Type of mAb	ID Number	Phase	Recruitment Status	Country	Estimated enrollment	Masking	Dose	Age (years)	Cancer type	Outcomes
MGD009	BiAb	NCT02628535[10]	I	Terminated	United States	67	None	0.3 ug/kg (every 2 weeks)	> 18	Mesothelioma, Bladder Cancer, Melanoma, Squamous Cell Carcinoma of the Head and Neck, Non-Small Cell Lung Cancer, Clear Cell Renal Cell Carcinoma, Ovarian Cancer, Thyroid Cancer, Breast Cancer, Pancreatic Cancer	Ongoing
4SCAR-276	CAR-T cell	NCT04432649[11]	I-II	Recruiting	China	100	None	Not mentioned	1–75	Solid Tumor	Ongoing
SCRICAR-B7H3(s)	CAR-T cell	NCT04185038[12]	I	Recruiting	United States	70	None	Not mentioned	1–26	Central Nervous System Tumor, Diffuse Intrinsic Pontine Glioma, Diffuse Midline Glioma, Ependymoma Medulloblastoma, Germ Cell Tumor, Atypical Teratoid/ Rhabdoid Tumor, Primitive Neuroectodermal Tumor, Choroid Plexus Carcinoma, Pineoblastoma, Childhood Glioma	Ongoing
4-1BBζ B7H3-EGFRt-DHFR	CAR-T cell	NCT04483778[13]	I	Recruiting	United States	68	None	Not mentioned	< 26	Advanced/metastatic cancer	Ongoing

Table 1 (continued)

Drug(s)	Type of mAb	ID Number	Phase	Recruitment Status	Country	Estimated enrollment	Masking	Dose	Age (years)	Cancer type	Outcomes
CAR.B7-H3	CAR-T cell	NCT04670068[14]	I	Recruiting	United States	21	None	Not mentioned	> 18	Epithelial Ovarian Cancer	Ongoing
B7-H3 CAR-T	CAR-T cell	NCT04077866[15]	I/II	Recruiting	China	40	None	Not mentioned	18–75	Recurrent Glioblastoma Refractory Glioblastoma	Ongoing
B7-H3 CAR-T	CAR-T cell	NCT04385173[16]	I	Recruiting	China	12	None	Not mentioned	18–75	Recurrent Glioblastoma Refractory Glioblastoma	Ongoing
^{131}I-8H9	Radioimmunotherapy	NCT03275402[20]	II-III	Recruiting	Spain	32	None	2 mCi in week 1, 50 mCi in week 2	< 18	Neuroblastoma, CNS Metastases, Leptomeningeal Metastases	Ongoing
^{131}I-8H9	Radioimmunotherapy	NCT04022213[21]	II	Recruiting	United States	55	None	80 mCi/m2	> 1	Desmoplastic Small Round Cell Tumor, Peritoneal Cancer, Peritoneal Carcinoma	Ongoing
177Lu-DPTA-omburtamab	Radioimmunotherapy	NCT04315246[22]	I-II	Not recruiting	United States	63	None	Dosimetry dose (5 weeks) ITV	> 18	Leptomeningeal Metastasis, Solid Tumor	Ongoing
177Lu-DTPA-omburtamab	Radioimmunotherapy	NCT04167618[23]	I-II	Recruiting	United States	40	None	Not mentioned	3–19	Medulloblastoma	Ongoing

was presented at the ASCO annual meeting 2021. Preliminary results showed antitumor activity in metastatic prostate cancer patients (5/9 patients with reduction in PSA levels of ≥ 50%) and melanoma patients (3/3 patients showed partial response with ≥ 24% tumor volume reduction). MGC018 showed an acceptable safety profile with manageable hematologic and dermal toxicity. One patient experienced a grade 4 adverse event (neutropenia). Primary outcome measures for this study are expected to be completed by March 2022.

DS-7300a (Daiichi Sankyo Inc)

Another B7-H3 ADC (DS-7300a) is under investigation in a dose-escalation phase I/II trial without reported results yet (NCT04145622[2]). Few data related to DS-7300a are available but a poster presentation at the ENA 2020 symposium reported its antitumor activity in preclinical tumor models [29].

Challenges and opportunities with B7-H3 ADCs

Since FDA approval of the first ADC in 2009 (gemtuzumab ozogamicin), 11 ADCs have been approved for clinical application and more than 80 other ones are under evaluation in clinical studies. To date, no duocarmycin-based ADCs have been approved for oncological practice yet. A HER2-targeting ADC conjugated with duocarmycin (Trastuzumab) has shown promising clinical efficacy in a phase I trial leading the FDA to grant Fast Track Designation status [30]. However, the study also reported that 71% of patients had ocular adverse events causing discontinuation for 10% of treated patients [30]. Despite considerable progress, these clinical data underscore several hurdles that remain to be overcome in ADC development including off-target toxicity, drug penetration, limited drug retention and stability of payload in circulation [31]. In addition, regulation and trafficking of the target need to be well understood as several mechanisms of resistance to ADC have been revealed in prior studies [32], including defective internalization of the target and reduced target expression in ADC-treated tumors [33, 34]. Such knowledge is critical to design rationalized ADC therapies and develop biomarkers that can identify patients who will benefit the most from this type of therapy. While targeting B7-H3 has already entered clinical trials, its regulation and trafficking in normal and tumor tissues has not been fully elucidated. B7-H3 expression has been detected in normal tissue and on the surface of immune cells but it is unclear if ADCs can cause life-threatening off-tumor on-target toxicity [35, 36]. In addition, B7-H3 can be shed from the cell surface as soluble protein or bound to extracellular vesicles [37–39]. It is currently unknown if soluble and EV-bound B7-H3 can act as decoys and impair tumor penetration and therapeutic efficacy of ADCs [40, 41].

Antibody-dependent cell-mediated cytotoxicity

Mechanism of action

Antibody-dependent cellular cytotoxicity (ADCC) represents another promising therapeutic strategy for many cancer types. While ADCs directly induce cell death through the delivery of cytotoxic payload, ADCC efficacy relies on the engagement of the host immune system to eradicate tumor cells [42]. It is one of the defense mechanisms the immune system used to contain an infection. Pathogen-infected target cells are coated by naturally produced antibodies, and the Fc region of the antibody is recognized by NK cells through affinity with Fc receptors. Upon binding, NK cells release cytotoxic molecules (perforin, granzyme B) causing infected target cell death. This natural immune mechanism has been recently exploited to develop synthetic therapeutic antibodies that can induce ADCC upon binding of tumor-associated surface antigens [43, 44]. Besides inherent barriers to antibody-based therapies (tumor penetration, soluble antigens, sensitive and specific recognition of tumor-associated surface antigens), the therapeutic efficacy of ADCC antibodies is also dependent on the binding affinity of the Fc receptor, NK cell tumor infiltration and degree of immune cell activation. Site-directed mutagenesis, glycoengineering of Fc portions of antibodies are currently used to enhance the potency of therapeutic ADCC antibodies [45]. Currently, there are 11 ADCC antibodies approved by the FDA or in clinical trials [45]. Two ADCC antibodies against B7-H3 are currently under investigation, namely MGA271 (Macrogenics) and DS-5573a (Daiichi Sankyo Inc).

MGA271 (Macrogenics)

Enoblituzumab (MGA271) is a Fc-optimized humanized IgG1 antibody against B7-H3, in which the Fc domain contains 5 amino acid changes to increase the affinity for activating FcγR (CD16A) and decrease the affinity for inhibitory FcγR (CD32B) [46]. A preclinical study using renal cell carcinoma and bladder cancer xenograft tumor models showed strong antitumor activity of MGA271 [46]. Following successful preclinical studies, MGA271 safety profile and efficacy have been evaluated in three clinical studies on patients with pediatric and adult solid tumors (NCT01391143[3], NCT02923180[4], NCT02982941[5]) (Table 1). The first two studies have been completed in 2019, but final results have not been reported yet. Interim results of NCT01391143[3] showed that 43.5% of patients (20/46) experienced stable disease (> 12 weeks) and tumor shrinkage (2–69%) [47]. Although no dose-limiting toxicity and no drug-related treatment discontinuation were observed in the interim study, common adverse events (AEs) were observed, including

fatigue, nausea, chills, and vomiting [47]. In another trial (NCT02923180[4]), primary endpoints are safety and tolerability and secondary endpoints include analysis of immune infiltration and tumor cell death in prostatectomy specimens. Preliminary results on 13/32 patients showed marked tumor infiltration of CD8 + T cells in MGA271-treated patients samples compared to age- and stage-matched untreated prostatectomy controls suggesting an antitumor immune response induced by treatment with MGA271 [48].

Meanwhile, the combination of MGA271 with other ICIs is currently investigated (NCT02381314[6], NCT02475213[7], NCT04634825[8]) (Table 1). In the trial of NCT02475213[7] [49], the patients with squamous cell carcinoma of the head and neck, NSCLC, and urethelial carcinoma showed clinical remission with the combination therapy.

DS-5573a (Daiichi Sankyo Inc)

DS-5573a is an afucosylated humanized anti-B7H3 IgG1 antibody binding to IgC1 and IgC2 immunoglobulin-like domains of human B7-H3 (Daiichi Sankyo Inc) [50], which has the potency of B7-H3-dependent ADCC and antibody-dependent cellular phagocytosis (ADCP) activity. In tumor xenograft mouse models with poorly differentiated breast adenocarcinoma, DS-5573a showed significant antitumor efficacy at doses of 0.003–3 mg/kg in vivo [50]. The only clinical trial on DS-5573a is an open-label phase I study (NCT02192567[9]) initiated in 2014 to evaluate the safety and pharmacokinetics of DS-5573a in patients with advanced solid tumors, but the study was terminated on the business decision in 2017. A derivative of DS-5573a conjugated with a radioligand (Zirconium-89) has been developed to serve as a molecular imaging probe in clinical studies to measure B7-H3 expression in different tumors [51].

Challenges and opportunities with B7-H3 ADCC

The ability of monoclonal antibodies to kill tumor cells largely depends on ADCC and ADCP. In addition to MGA271, some drugs depending on this mechanism are approved for use in several tumors, such as avelumab (anti-PD-L1) for the treatment of metastatic urothelial carcinoma [52], trastuzumab (anti-HER2) for the treatment of metastatic breast cancer [53] and cetuximab (anti-EGFR) for metastatic colorectal cancer [54]. Just like EGFR and HER2, B7-H3 is widely expressed in different tumors [55], but monoclonal antibodies for B7-H3 can be safer owing to the limited-expression level of B7-H3 protein in normal tissues. Additionally, according to the present results of NCT02923180[4] and NCT02982941[5], MGA271 not only promotes NK-mediated tumor cell killing directly but also increases tumor infiltration of CD8 + T cells. Combined with previous studies [56, 57], it supports the therapeutic potential of ADCC-related drugs and serves as a basis for future applications.

Bispecific antibodies

Mechanism of action

Bispecific antibodies are a class of therapeutic antibodies characterized by the ability to recognize two different antigens simultaneously. There are several types of bispecific antibodies based on their mechanism of action. ADC-derived bispecific antibodies can bind two tumor-associated surface antigens increasing specificity for tumor cells. Bispecific antibodies can target two immune checkpoint molecules (i.e., cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and PD-1) to increase antitumor immune response. Bispecific T-cell (BiTE) and NK-cell engagers (BiKE) act as a bridge between cancer cells and T-cells or NK cells, respectively. For instance, blinatumomab, used for the treatment of chronic lymphocytic leukemia, binds CD19 on tumor cells and CD3 on T-cells [58, 59]. The binding of BiTE to CD3 induces TCR-mediated signaling and antitumor immune response similar to antigen presentation. To date, only one bispecific antibody (Blinatumomab) has been approved for clinical use, but recent progress in antibody engineering has paved the way for more than 200 clinical trials testing bispecific antibodies for cancer treatment [60].

MGD009 (B7-H3 × CD3)

MGD009 is a bispecific anti-CD3 × anti-B7-H3 (B7-H3 Bi-Ab) antibody with one arm of the bispecific antibody binding to the CD3 component of the TCR complex on T cells, and the other arm recognizing B7-H3 on the surface of tumor cells. Following successful preclinical studies, a multicenter open-label trial was launched to study safety, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of MGD009 in advanced cancers (NCT02628535[10]) [61]. However, the study has been terminated on business decision and no further studies are anticipated with MGD009.

Challenges and opportunities with B7-H3 Bi-Ab

Despite no clinical studies are ongoing with B7-H3 Bi-Ab, several groups have demonstrated the therapeutic potential of B7-H3 Bi-Ab in different malignancies including bladder cancer, melanoma, hematological cancer, and head and neck cancer [62–65]. Through a series of mouse model experiments, it found that B7-H3 Bi-Ab effectively inhibited tumor growth, thereby improving the overall survival of tumor-bearing mice [63, 64]. In 2021,

You et al. reported the efficacy of a bispecific antibody (B7-H3 × 4-1BB) to reduce tumor growth in an immunocompetent mouse model of colorectal cancer [66]. 4-1BB (CD137) is a co-stimulatory receptor, which is widely expressed in immune cells, including activated T cells, NK cells, and DCs. In this study, B7-H3 × 4-1BB elicited CD8 T cell mediated antitumor immune response. In addition, they found that B7-H3 × 4-1BB showed synergistic activity with PD-1 blockade. Altogether, these preclinical data support the future development of clinical trials investigating B7-H3 Bi-Ab for the treatment of advanced cancers.

Chimeric antigen receptor (CAR) therapy

Mechanism of action

Chimeric antigen receptor T cell and NK cell (CAR-T/NK) therapy is a form of immunotherapy that holds a lot of potential to treat cancers known as poorly immunogenic. CAR-T therapy consists of injecting patients' T cells that are genetically modified to specifically recognize a tumor-associated antigen. The chimeric antigen receptor is a fusion protein composed of the epitope binding site of a monoclonal antibody targeting the tumor antigen linked to costimulatory and signaling domains of T-cell receptor. It has been a stepping stone toward the treatment of hematological malignancies [67]. Recent progress made in genetic modification of CAR-T models and molecular profiling of tumors has opened new hopes for introducing CAR-T therapy in the armamentarium of oncologists for the treatment of advanced solid tumors [68]. In the last two years, several groups have designed and tested CAR-T cells targeting B7-H3 in preclinical tumor xenograft models. A B7-H3 CAR was generated with a single-chain variable fragment (scFv) derived from the anti-B7H3 antibodies, and the most common ones include MGA271 (Macrogenics) mAb 376.96 and 8H9. It also includes a costimulatory motif (from 4-1BB, CD28, and CD8 molecules) and a signaling domain (CD3ζ) [69–71]. The antitumor immune activity of B7-H3 CAR-T therapy has been confirmed in a series of hematologic and solid tumors [35, 72].

Clinical trials

Following the positive outcome of preclinical studies, seven ongoing clinical trials have been initiated to evaluate the safety and efficacy of B7-H3 CAR-T therapy in advanced pediatric and adult solid cancers (NC04432649[11], NCT04185038[12], NCT04483778[13], NCT04670068[14], NCT04077866[15], NCT04385173[16], NCT03198052). A first-in-human study was conducted on a 49-year-old woman with multiple recurrent anaplastic meningiomas [73]. MRI imaging showed reduced tumor growth in the treated tumor compared to untreated tumor lesions. An increase in inflammatory cytokines was observed in the cerebrospinal fluid after the last infusion, suggesting a therapeutic response to CAR-T. During the whole treatment, there were no AEs of grade 3 or higher.

Challenges and opportunities with B7-H3 CART

Altogether, preclinical results underscore the promising future for using B7-H3 as a tumor target for CAR-T therapy. Importantly, B7-H3 overexpression has been found in several pediatric cancers which are refractory to current immunotherapies. B7-H3 CAR offers new hopes for treating solid tumors with low immunogenicity. Despite B7-H3 represents one of the most promising cancer targets, inherent limitations of CAR-T therapy including treatment-related toxicities, tumor infiltration and persistence remain to be overcome. Recently, Lei et al. showed that B7-H3 CAR antitumor activity was enhanced in presence of SAHA (vorinostat), a deacetylase inhibitor used for the treatment of advanced cutaneous T-cell lymphoma trials [74]. SAHA mediated upregulation of B7-H3 on tumor cells and B7-H3 CAR on transduced T-cells. An increase in T-cell surface expression of B7-H3 CAR was associated with downregulation of immunosuppressive genes CTLA-4 and TET2 resulting in improved cytotoxic activity. While the molecular mechanisms of SAHA-mediated CAR-T function remain unclear, it represents a promising strategy to improve the therapeutic efficacy of CAR-T toward B7-H3-expressing tumors.

Radioimmunotherapy

Mechanism of action

Radioimmunotherapy (RIT) uses an antibody labeled with a radionuclide to deliver cytotoxic radiation to a target cell. It is a form of unsealed source radiotherapy. In cancer therapy, an antibody with specificity for a tumor-associated antigen is used to deliver a lethal dose of radiation to the tumor cells. The ability for the antibody to specifically bind to a tumor-associated antigen increases the dose delivered to the tumor cells while decreasing the dose to normal tissues. By its nature, RIT requires a tumor cell to express an antigen that is unique to the neoplasm or is not accessible in normal cells. Omburtamab (8H9) is a murine IgG1 monoclonal antibody produced by splenic lymphocytes of BALB/c mice immunized with neuroblastoma cells [75]. In 2001, Modak et al. first showed a high affinity of 8H9 for human brain tumors, childhood sarcoma, and neuroblastoma, while no

signal was observed for normal human tissues. A few years later, B7-H3 was reported to be the target for 8H9 antibody [76] and a humanized version of 8H9 has demonstrated anti-tumor activity through ADCC [77]. Based on in vitro and preclinical studies, the 8H9 antibody has been evaluated as a radioimmunotherapy agent. To date, two different antibodies are tested in clinical trials, iodine-131 labeled Omburtamab and lutetium-177 labeled Omburtamab.

Iodine-131 labeled omburtamab

The earliest clinical study was designed by Kramer et al. for the treatment of neuroblastoma [78]. They found that while ^{131}I-8H9 prolong overall survival (median OS 33 months) compared to previously published studies (median OS < 13 months), it is also associated with AEs in some patients, including self-limited fever, nausea, headache, transient grade 1 creatinine elevation, and grade 1 and 3 transient elevated serum transaminase. Self-limited myelosuppression was observed when the dose of ^{131}I-8H9 injections ≥ 40 mCi. In a phase I trial (NCT01502917[17]), some patients presented with grade 3 AEs (hemiplegia, skin infection, and anxiety), but no grade 4 or grade 5 AEs were reported suggesting an acceptable safety profile [79, 80]. In NCT00089245[18] [78, 81], nearly 50% of patients were alive at 36 months post-treatment in a setting where overall survival at 36 months is usually less than 10%. The same conclusion was observed in the trial of NCT01099644[19] for peritoneal cancer [82]. Currently, two clinical trials are focusing on the effect of ^{131}I-8H9 in other cancer types, including leptomeningeal metastasis of adult solid tumors, medulloblastoma and peritoneal cancer (NCT03275402[20], NCT04022213[21]) (Table 1), with no results released.

Lutetium-177 labeled omburtamab

Lutetium-177 is an ideal isotope for radiopharmaceutical therapy. Its half-life makes it easier to combine with biologically active compounds and extends the treatment time [83]. Studies have shown that Lutetium-177 can emit low-energy gamma rays and can be used to image tumors in real-time during treatment. In order to promote rapid radioactive clearance, the monoclonal antibody 8H9 was combined with the chelating agent diethylenetriamine pentaacetate and radiolabeled with Lutetium-177 [84]. In two clinical studies (NCT04315246[22] and NCT04167618[23]), possible adverse reactions and efficacy of lutetium-177 labeled Omburtamab in solid tumors were studied (Table 1), while the results are not published yet.

Challenges and opportunities with omburtamab

Modak et al. confirmed that 8H9 labeled with ^{131}I can provide a therapeutic dose of radiation to solid tumors and inhibit tumor cell growth through established xenografts [85]. In 2010, in patients with recurrence of neuroblastoma (NB), they further confirmed that 8H9 can improve survival, indicating that both radioimmunotherapies can inhibit the growth of CNS NS through the blood–brain barrier. But the similarities and differences between the two need to be further studied and compared. As 8H9 is an anti-B7-H3 antibody, whether it affects the central immune system after labeling with ^{131}I requires more research and discussion. In addition, because ^{131}I -8H9 is radioactive, its value in tumor imaging diagnosis should also be further explored. Since the current research on 8H9 is limited to brain tumors, its efficacy and mechanism would be studied in other solid tumors.

Targeting B7-H3 function for next-generation cancer immunotherapy

Immune checkpoint blockade

Considering the recent success of immune checkpoint blockade in advanced cancers, targeting the inhibitory function of B7-H3 is regarded as one of the most promising strategies for next-generation cancer immunotherapy. However, no ICI has been developed yet. Unlike PD-L1, the receptor of B7-H3 and the downstream events of B7-H3 binding to recipient cells remain poorly elucidated. The protein TREML2 (TLT-2) expressed on the surface of T-cells has been reported as the first identified murine B7-H3 receptor but one following study contradicted the initial finding [86, 87]. Given that mouse B7-H3 sequence and structure differs from its human counterpart, it is reasonable to think they may act via two different receptors [14, 86]. A second study used a high-throughput interaction screening platform and identified IL20RA as a B7-H3 receptor, but no functional outcome has been identified yet. Given that IL20RA [88, 89] has been mostly found on the surface of tumor cells, the nature (cis or trans) of IL20RA: B7-H3 interaction remains unclear. The limited success in discovering B7-H3 receptor(s) may also reflect a more complex mechanism involving additional interacting partners on the same cell surface (cis-interaction). To mediate its inhibitory activity, PD-L1 directly interacts with PD-1 trans [90]. A recent study revealed that PD-L1 can cis-interact with PD-1 on tumor cells and antigen-presenting cells [91]. Cis-interaction of PD-L1 and PD-1 inhibits the ability of PD-L1 to bind with T-cell PD-1 in trans resulting in improved T cell response. CD80 (B7-1) [92–94] has been identified as another PD-L1 cis-ligand and the heterodimer CD80: PD-L1 on the surface

of antigen-presenting cells was a critical mediator of the antitumor immune response. Therefore, further studies are needed to uncover the B7-H3 interactome on tumor cells and immune cells and identify protein interactions that mediate tumor immunity. Ultimately, this work will pave the way for developing new therapeutic strategies including immune checkpoint blockade.

Antibody-mediated degradation

While immune checkpoint blockade seems the most prevalent strategy to impair with B7-H3 function, intracellular degradation of the protein may also be a potent and durable therapeutic approach [95]. Other immune checkpoint molecules such as PD-L1 and CTLA-4 are subjected to antibody-mediated degradation [96, 97]. For instance, Ipilimumab (FDA-approved anti-CTLA-4) can induce lysosomal degradation of CTLA-4 by preventing interaction of intracellular CTLA-4 with LRBA, a regulator of CTLA-4 recycling from endosomes to cell surface. In the case of PD-L1, Tu et al. developed a novel antibody that prevents the binding of PD-L1 with CMTM6 resulting in PD-L1 destabilization at the plasma membrane and lysosomal degradation [96, 98, 99]. In a recent study, Durlanik et al. investigated the role of B7-H3 in the crosstalk between macrophages and cancer cells. They observed B7-H3 upregulation on the surface of macrophages when co-cultured with tumor spheroids. Strikingly, selective downregulation of B7-H3 expression on the surface of macrophages (~ 35%) was observed in the presence of anti-B7-H3 (clone EPNCIR122), while no effect was observed on tumor cells. Based on these findings, antibody-induced loss of B7-H3 in macrophages with EPNCIR122 clone may follow a similar mechanism to PD-L1 and CTLA-4 where anti-B7-H3 inhibits interaction with adaptor proteins responsible for its stability or trafficking at the cell surface. Since B7-H3 on tumor cells was unaffected by anti-B7-H3, it also points toward different regulatory mechanisms of cell-surface expression of B7-H3 in tumor and immune cells. Further studies are needed to elucidate the underpinnings of B7-H3 trafficking and leverage this knowledge to develop therapeutic antibodies that can inhibit B7-H3 function through intracellular degradation.

Regulation of B7-H3 expression and its impact on therapy development

Targeting B7-H3 glycosylation as anticancer therapy

In the past years, blocking the PD-1/PD-L1 axis has revolutionized cancer therapy. However, only a minority of patients (~ 30%) truly benefit from PD-1/PD-L1 inhibition [100]. More importantly, tumor PD-L1 expression is not a robust predictor of treatment response which represents a challenging scenario for patients and clinicians [101]. Recent progress in the understanding of PD-L1 expression and post-transcriptional regulation has revealed important regulatory mechanisms that can help design better therapeutic approaches and predictive tools [102–104]. For instance, glycosylation of PD-L1 has been shown to mediate its stability at the plasma membrane and its inhibitory activity through PD-1 binding [103, 104]. Targeting PD-L1 with specific antibodies against the glycosylated form promoted PD-L1 internalization and degradation resulting in enhanced T-cell cytotoxic activity [103]. Important lessons must be learned from heavily studied immune checkpoint molecules such as PD-L1 in order to establish rationalized studies investigating post-transcriptional regulation of B7-H3 and its impact on B7-H3 subcellular trafficking and function. While PD-L1 contains four glycosylation sites in its extracellular domain, B7-H3 has a total of eight glycosylation sites [86, 105, 106] because of the genomic duplication of its two immunoglobulin-like domains. Comprehensive analysis of B7-H3 glycosylation using mass spectrometry shows tumor-specific glycoforms with high levels of fucosylation in oral cancer [105]. A follow-up study identified the fucosyltransferase FUT8 as a critical mediator of B7-H3 glycosylation [106]. FUT8 enzymatic activity was necessary for B7-H3 stability at the plasma membrane and its inhibitory function on cytotoxic T-cells. Further studies are warranted to fully elucidate post-translational mechanisms of B7-H3 glycosylation and their role in its functional activity. Targeting B7-H3 glycosylation may be a promising therapeutic avenue to promote B7-H3 degradation and prevent its immunosuppressive activity.

Alternative splicing of B7-H3

Originally, two variants of B7-H3 have been identified. A full-length B7-H3 variant with 4Ig-like domains expressed in humans and a short variant with 2Ig-like domains expressed in mice [86]. A splicing variant of B7-H3 with 2Ig-like domains has also been reported in human cancer cell lines [86, 107, 108] and synovial monocytes, but it appears to remain a minor transcript compared to the full-length B7-H3. A recent study has revealed the first insights of B7-H3 splicing mediated by the regulator SRSF3, but it remains unknown functional roles of B7-H3 splicing in normal and malignant tissue [109]. Additionally, the functional differences between the short and full-length human B7-H3 isoforms remain controversial. In rheumatoid arthritis, 2Ig-B7-H3 was not found on the cell surface but in cytoplasmic compartments [108]. Using human T-cells, Sun et al. showed that 2Ig-B7-H3 recombinant protein induced T-cell

proliferation and release of IFNγ and IL-2 [110]. In contrast, 4Ig-B7-H3 reduced T-cell proliferation suggesting two distinct immune functions for B7-H3. Crystallography analysis showed 34% identity between the two isoforms, but they were both able to bind on the surface of activated peripheral blood mononuclear cells (PBMCs). The 2Ig-B7-H3 isoform can induce the release of TNFα and IL-6 from LPS-activated monocytes, while no effect was observed with the long isoform. While further studies are warranted to fully elucidate the specific roles and regulation of B7-H3 isoforms, it remains possible that human B7-H3 isoforms act differently on immune cell subsets and via two putative receptors.

"Soluble" B7-H3

To add to the complexity of B7-H3 regulation, additional forms of B7-H3 have been found in the extracellular compartment [38, 111, 112]. In 2008, Zhang et al. showed the presence of a soluble and functionally active form of B7-H3 in culture media of immune cells and tumor cells [111]. The release of soluble B7-H3 was inhibited by a pan-metalloprotease inhibitor suggesting proteolytic cleavage of B7-H3. This discovery was followed by several studies reporting high levels of soluble B7-H3 in the serum of cancer patients [37, 38, 113–116]. Besides proteolytic cleavage, soluble B7-H3 can be produced by alternative splicing resulting in an isoform lacking its transmembrane domain [38]. Most studies have used ELISA to measure levels of soluble B7-H3 in cell culture medium and patient serum. However, this method cannot discriminate between membrane-free B7-H3 and membrane-bound B7-H3. In line with this, B7-H3 has also been found on the surface of extracellular vesicles released by cancer cells suggesting that multiple forms of soluble B7-H3 can be found in culture medium and patient's biofluids [39, 117, 118]. To separate B7-H3-positive extracellular vesicles from unbound B7-H3, EV isolation/enrichment methods with size-exclusion chromatography, ultrafiltration or ultracentrifugation can be employed. In addition, advanced technologies such as nanoscale flow cytometry and single-particle interferometry are suitable to only detect B7-H3-positive extracellular vesicles. The recent groundbreaking findings on the immunoregulatory function of PD-L1-positive extracellular vesicles in local and systemic tumor immunity support the need to elucidate the regulatory mechanisms and functions of soluble B7-H3 [119, 120].

Conclusion

Targeting B7-H3 represents a promising therapeutic strategy at the dawn of the second revolution of cancer immunotherapy revolution. B7-H3 is highly expressed by several pediatric and adult cancers including cancers refractory to PD-1/PD-L1 therapies. Therefore, B7-H3 appears as a suitable tumor-associated antigen for the development of antibody-based therapies. While the first clinical trials to evaluate the safety and antitumor activity of B7-H3-targeting therapies are ongoing, B7-H3 function and regulation remain to be characterized, starting with the identification of the B7-H3 receptor. Further mechanistic studies are warranted to support the development of novel immunotherapeutic strategies, thereby opening new horizons for more efficacious treatments in human cancers.

Authors' contributions TW and FL conceptualized the study, CL and KX performed the literature search, CL, GZ, YK, RR, FL and TW drafted the work. CL, YK, KX and RR, generated the figure and the table. All authors read and approved the final manuscript.

Funding This study was funded by the National Natural Science Foundation of China (No. 31770963).

Availability of data and material Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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