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REVIEW

Bispecific antibodies in cancer therapy: Target selection and regulatory requirements

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Abstract In recent years, the development of bispecific antibodies (bsAbs) has been rapid, with many new structures and target combinations being created. The boom in bsAbs has led to the successive issuance of industry guidance for their development in the US and China. However, there is a high degree of similarity in target selection, which could affect the development of diversity in bsAbs. This review presents a classification of various bsAbs for cancer therapy based on structure and target selection and examines the advantages of bsAbs over monoclonal antibodies (mAbs). Through database research, we have identified the preferences of available bsAbs combinations, suggesting rational target selection options and warning of potential wastage of medical resources. We have also compared the US and Chinese guidelines for bsAbs in order to provide a reference for their development.

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1. Introduction

Over the last three decades, therapeutic antibodies have become a key component of cancer treatment due to their specificity and sensitivity[1]. The first monoclonal antibody, Muromonab-CD3 (OKT3), was approved for marketing in 1986[2], Since then, antibody-based drugs have developed rapidly and have become one of the most important types of drugs. In oncology therapy, monoclonal antibody drugs have demonstrated excellent therapeutic effects, such as Rituximab (anti-CD20) and Trastuzumab (anti-HER2), which have been approved for the treatment of B-cell malignancies and breast cancer with promising results[3,4].

Bispecific antibodies have been developed to address drug resistance and improve efficacy[5]. Combination therapies of monoclonal antibodies targeting different receptors or epitopes can enhance treatment efficacy and help to overcome drug resistance[6]. However, these therapies may also cause higher toxicity[7,8]. Bispecific antibodies can improve efficacy and safety by simultaneously recognizing and binding two different antigens or antigenic epitopes[9]. Additionally, they have the unique advantage of redirecting cytotoxic effector cells[10].

Bispecific antibodies have not been widely explored until the last decade, even though they have shown a specific benefit. Since the first bispecific antibody (Catumaxomab) was launched in 2009[11], nine bsAbs (seven for tumors) were approved for marketing, and five of them are coming to market in 2021 and 2022 (Table 1). Up to now, more than 200 drugs are being investigated in clinic, with 10 entering Phase III (Fig. 1) (https://www.cortellis.com/drugdiscovery/home)[12]. It can be expected that a large number of bispecific antibodies will come to market in the next 3−5 years, bringing the development of bispecific antibodies into a high-speed development period.

It is clear that the development of bispecific antibodies is in a rapid and early stage, and the market competition pattern is unclear. The similarity in target selection may lead to increased competition, but also limit therapeutic diversity and waste medical resources. To ensure a rational design and development strategy, it is important to summarize clinical data, analyze target selection, and clarify regulatory requirements. The FDA and NMPA have issued guidance on bsAbs in 2021 and 2022 respectively[13,14], which may help to provide policy regulation.

2. Structure

2.1. Formats

The selection of format and target determines the therapeutic effect, pharmacokinetic characteristics and stability of bispecific antibodies[15]. The abundance of structural forms provides more solutions to technical problems in bispecific antibody research. We will briefly introduce the format design to provide a better understanding.

According to the existence of the Fc (fragment crystallizable) region, bispecific antibodies can be divided into two categories: IgG-Based bsAbs and Fragment-Based bsAbs (Fig. 2).

2.1.1. IgG-based bsAbs

IgG-Based bispecific antibodies are similar in structure to native antibodies, and all have Fc regions. The Fc region is associated with multiple activities of bispecific antibodies, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cell phagocytosis (ADCP)[16]. Furthermore, the Fc region of bsAbs may contribute to an increase in half-life[17]. Additionally, the Fc region facilitates the purification of bsAbs and also promotes their stability and solubility[18,19].

However, the IgG-Based bsAbs are also associated with various disadvantages, such as the side effects due to the off-target binding of active Fc domain to FcRs (Fc receptors)[20], and the chain-associated issue[21].

New formats are being developed to address these problems. For instance, the recently launched mosunetuzumab (anti-CD3/CD20 bispecific antibody) adopted the classic knobs-into-holes format to ensure to correct heavy chain assembly[22]. This technology has a large amino acid on one chain to create a “knob” and a smaller amino acid on the other chain to create a corresponding “hole”[23], which is helpful for the correct assembly of two heterologous antibody heavy chains, thus solving the “chain-associated issue”. The mismatch between non-homologous heavy and light chains is another common problem. A new approach is the CrossMab format, which was created based on the Knobs-into-holes format and further solves the problem of light chain mispairing[24]. Faricimab (anti-ang-2/VEGF) is designed in this format and is currently approved for the treatment of diabetic macular edema and neovascular (wet) age-related macular degeneration (nAMD)[25].

2.1.2. Fragment-based bsAbs

Fragment-based bsAbs are composed of the variable light and heavy domains from two antibodies, or the Fab units, and lack the Fc region which distinguishes them from IgG-Based bsAbs[26,27]. These fragments are bound together by linkers (*e.g.*, disulfide bonds or non-covalent interactions) and different pharmacokinetic properties than the IgG-Based bsAbs[28]. Fragment-based bsAbs showed several advantages, including high yield, low cost, good tumor penetration, and the ability to overcome chain-related issues[15,29,30]. Due to their low molecular weight, BiTE (bispecific

Table 1 Approved bispecific antibodies for cancer therapy.

Name	Targets	Developer	Time to market	Indication
Catumaxomab	CD3 × EpCAM	Trion pharma	2009 (EMA)	Malignant ascites
Blinatumomab	CD3 × CD19	Amgen	2014(FDA), 2015(EMA)	ALL
Mosunetuzumab	CD3 × CD20	Roche	2022(EMA), 2022(FDA)	R/R FL
Tebentafusp	CD3 × gp100	Immunocore	2022(FDA), 2022(EU)	Uveal melanoma
Teclistamab	CD3 × BCMA	Janssen	2022(EU), 2022(FDA)	R/R MM
Amivantamab	EGFR × cMET	Janssen	2021(FDA)	NSCLC
Cadonilimab	PD-1 × CTLA-4	Akeso	2022(NMPA)	R/M CC

ALL, acute lymphoblastic leukemia; NSCLC, non-small-cell lung carcinoma; R/M CC, relapsed or metastatic cervical cancer; R/R FL, relapsed or refractory follicular lymphoma; R/R MM, relapsed or refractory multiple myeloma.

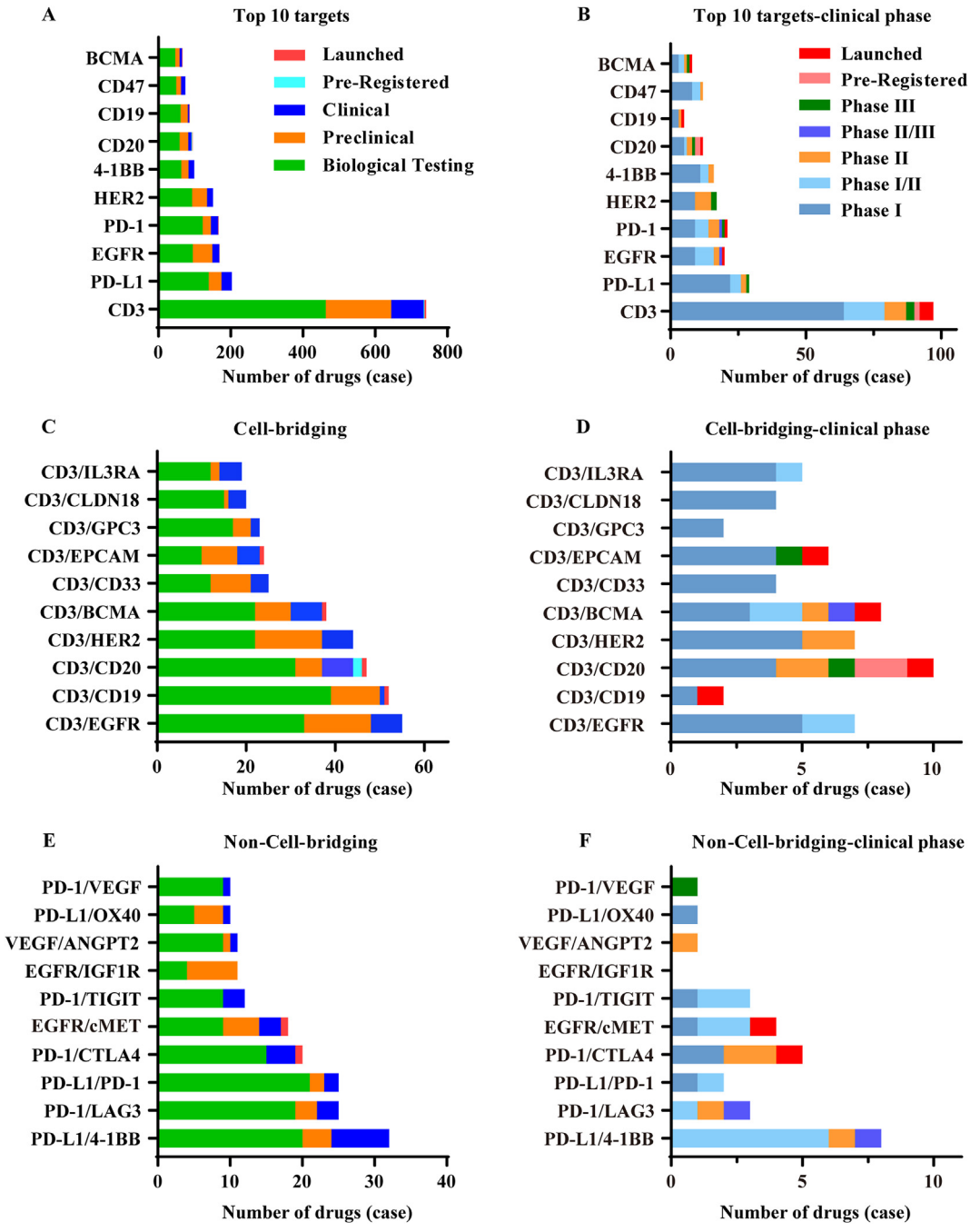

Figure 1 Preferred targets and combinations. (A) The top 10 most widely investigated targets. (B) The top 10 most widely investigated targets−clinical stage. (C) Top 10 selected target combinations of cell-bridging bsAbs and (D) their clinical phases. (E) Top 10 selected target combinations of non-cell-bridging bsAbs and (F) their clinical phases. Information was obtained from Cortellis Drug Discovery Intelligence (https://www.cortellis.com/drugdiscovery/home)[12].

T-cell engager) antibodies are more readily metabolized *in vivo*, with a typical half-life of only 2−4 h[31,32]. To increase the half-life of fragment-based bsAbs, antibodies have been designed to be fused to an Fc region or albumin-binding molecules[33]. Half-Life Extended (HLE) BiTE is a novel format that builds upon the classical BiTE format by fusing it to an Fc domain, significantly increasing its serum half-life[34]. Studies have shown that CD19 HLE BiTE® is an effective treatment for CD19-positive malignancies, with a half-life of 210 h after a single intravenous injection, which could be suitable for once-weekly dosing[35].

2.2. *Affinity and valency*

2.2.1. *Affinity*

The affinity of bispecific antibodies is a major factor influencing overall tolerability and cytokine release[36]. For CD3-targeting T-cell engagers, the affinity of the CD3 arm is a key factor in the success of T-cell bispecific antibodies (T-bsAbs). The CD3 arm with too high affinity would lead to excessive release of cytokines and affect the tissue distribution of bsAbs, limiting their reach to the target site[37,38]. In one study, PSMA/CD3 bispecific

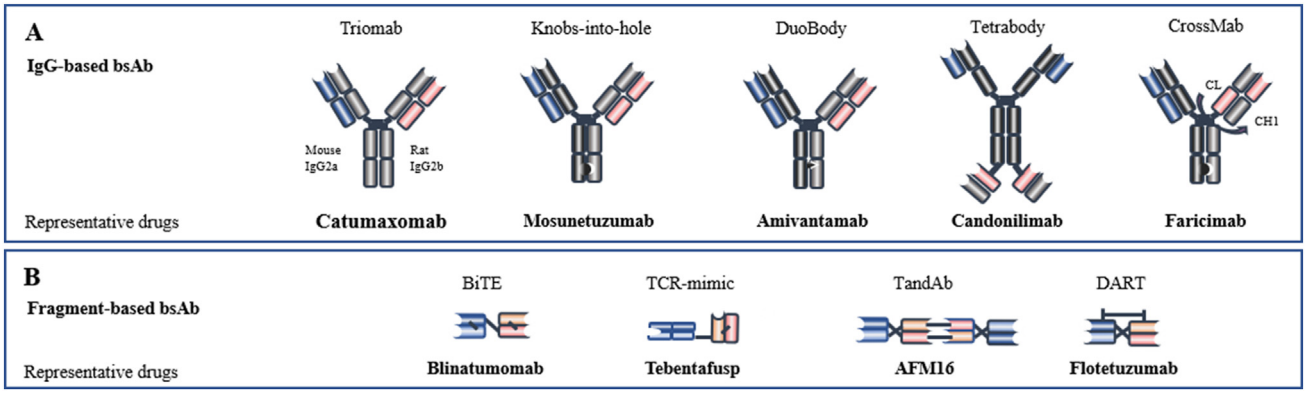

Figure 2 Representative bispecific antibodies and their format. According to the existence of the Fc region, bispecific antibodies can be divided into two categories: (A) IgG-based bsAbs and (B) Fragment-based bsAbs. BiTE, bispecific T-cell engager; TandAb, tandem diabody; DART, dual affinity retargeting.

antibodies with lower CD3 affinity were reported to be more effective in killing tumor cells and reducing the incidence and severity of cytokine release syndrome (CRS) in prostate cancer patients compared to bsAbs with high CD3 affinity[39]. Thus, a proper affinity is essential for drug distribution and efficacy.

Additionally, bispecific antibodies can achieve high selectivity against tumor cells by decreasing the affinity of arms to tumor-specific antigens (TSA). HER2 T-cell-dependent bispecific antibody (TDB) is a bsAb with two low-affinity HER2 arms which has been reported to have high tumor specificity. It has a strong binding ability to cells with high HER2 expression, while the binding rate to low HER2-expressing cells is low. Clinical data has shown that this bsAb has better tolerability compared to CAR-T (chimeric antigen receptor T) cell therapies targeting HER2[40].

2.2.2. *Valency*

Valency refers to the number of binding sites in the antibody that can be used to bind antigens. It is another important factor in the design of bispecific antibodies, as it can affect the efficacy of the antibody[31]. Monovalent and multivalent designs can be used to achieve different levels of efficacy. Glofitamab is an example of a bsAb with a 2:1 valency against CD20 of B cells and CD3 of T cells. It has been shown to have 40-fold higher *in vitro* anti-tumor activity than 1:1 valency bsAbs[41]. This demonstrates the importance of considering all structural features when designing bispecific antibodies, as well as the need for a comprehensive screening process to obtain an optimal product[42–44].

3. Classification of antibodies based on target selection

According to the NMPA guidelines, bispecific antibodies can be classified into three categories based on their mechanism of action: bridging cells, bridging receptors, and bridging cytokines. Additionally, the classification of bridging receptors and cytokines has been added to accommodate special bispecific antibodies, such as SHR-1701 (targeting TGF-β and PD-L1). All four types are shown in Fig. 3 to make it easier to understand.

3.1. *Bridging cells*

Bispecific antibodies (BsAb) can redirect cytotoxic effector cells to tumor cells. From a mechanistic perspective, this type of bispecific antibody can recruit immune cells (such as T cells and NK cells) to the tumor area to exert cytotoxic effects. One antigen-binding site of the BsAb binds to specific antigens expressed on tumor cells, while the other one bridges and activates effector cells such as macrophages and cytotoxic T lymphocytes (CTL)[45,46]. CD3 is the most common targeted protein expressed on effector cells, which can activate the anti-tumor activity of T cells. Some emerging target proteins are also classified into this category, such as TCR and CD16A (Table 2).

3.1.1. *Targeting cytotoxic effector cells*

3.1.1.1. CD3 targeting T cell engagers. Binding of T-bsAbs to CD3 has been shown to be a promising cancer therapy due to its ability to activate T cells without the restriction of the major histocompatibility complex (MHC) and directly induce tumor-associated antigens (TAA) and immune cells to form immune synapses (IS)[47]. Furthermore, they can induce tumor cell necrosis or apoptosis through the production of perforin and granzyme A/B[47,48], as well as the stimulation of death ligands such as the Fas−FasL pathway[49]. However, they may also cause serious side effects. Catumaxomab, the first commercially available bispecific antibody for the treatment of malignant ascites, was withdrawn from the market in 2017 due to its potential to cause adverse events such as cytokine release syndrome (CRS) and T-cell-mediated hepatotoxicity[11,50–52]. Therefore, it is important to consider various factors when designing a bispecific antibody to ensure its safety and efficacy.

3.1.1.2. TCR targeting $\gamma\delta$ T cell engagers. CD3 is widely distributed on the surface of T lymphocytes, and anti-CD3 bsAbs can activate the majority of T cells, including some immunosuppressive cells such as regulatory T cells (Tregs)[53,54]. Targeting specific T-cell subsets with bispecific antibodies is a promising approach to improve the efficacy and selectivity of T-bsAbs[55]. By selectively activating immune cells, it is possible to avoid the activation of immunosuppressive Tregs and reduce the risk of adverse events. For example, targeting Vγ9Vδ2 T cells, a small cell subpopulation (1%−10%) of the peripheral blood T cells, has shown promising therapeutic activity due to their conserved T-cell receptor (TCR) that recognizes malignant cells without relying on MHC[56,57]. 7D12-5 GS-6H4 is a novel bispecific antibody against Vγ9Vδ2 T cells and EGFR (epidermal growth factor receptor) that has been shown to induce activation of Vγ9Vδ2 T cells and promote apoptosis of colorectal cancer cells in a mouse xenograft

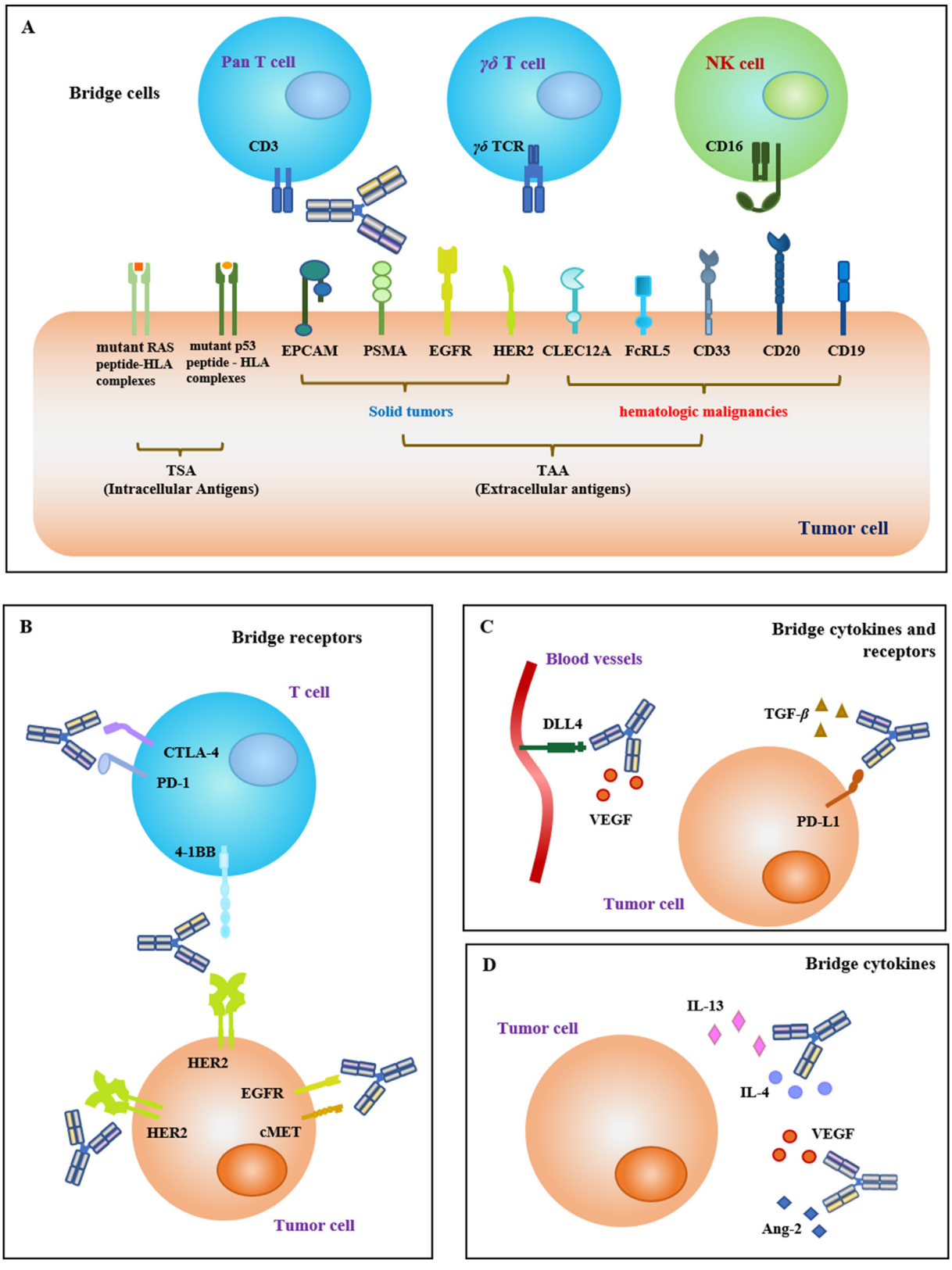

Figure 3 Bispecific antibodies in cancer therapy would be classified into four categories based on mechanism and target selection. (A) BsAbs that bridge immune effector cells to tumor cells, including pan T cells, γδ T cells and NK (natural killer) cells, etc. (B) BsAbs that bridge receptors from the same or different cells. (C) BsAbs that bridge cytokines and receptors. (D) BsAbs that bridge two cytokines.

Table 2 BsAbs bridge two cells in clinical stages.

Bridge immune cell	Bridge tumor cell	Name	Indication	Phase	Clinical trial
CD3	BCMA	BI836909	R/R MM	I	NCT03287908
	CD123	APVO436	AML	I	NCT03647800
	CD19	AMG562	DLBCL	I	NCT03571828
	CD20	GEN3013	DLBCL	I/II	NCT03625037
	CD33	GEM333	AML	I	NCT03516760
	CD38	GBR1342	R/R MM	I	NCT03309111
	CEA	RG7802	Solid tumors	I	NCT02650713
	CLEC12A	MCLA-117	AML	I	NCT03038230
	DLL3	AMG757	AML	I	NCT03541369
	EGFR	AFM24	Advanced solid tumor	I/II	NCT04259450
	EpCAM	MT110	Solid tumors	I	NCT00635596
	FcRH5	RO7187797	MM	I	NCT03275103
	FLT3	AMG427	AML	I	NCT03541369
	GD2	NCT03541369	SCLC	I/II	NCT04750239
	Glypican-3	ERY974	Solid tumors	I	NCT02748837
	gpA33	MGD007	Colorectal carcinoma	I	NCT02248805
	GPRC5D	ERY974	Solid tumors	I	NCT02748837
	HER2	BTRC4017A	Solid tumors	I	NCT03448042
	MAGE-A4 (HLA-A*02:01)	IMC-C103C	Select advanced solid tumors	I/II	NCT03973333
	MUC17	AMG199	MUC17-positive solid tumors	I	NCT04117958
	MUC16	REGN4018	Recurrent ovarian cancer	I/II	NCT03564340
	NY-ESO-1 (HLA-A*02:01)	GSK01	Select advanced solid tumors	I/II	NCT03515551
	P-cadherin	PF-06671008	Neoplasms	I	NCT02659631
	PRAME (HLA-A*02:01)	IMC-F106C	Select advanced solid tumors	I/II	NCT04262466
	PSCA	GEM3PSCA	NSCLC	I	NCT03927573
	PSMA	JNJ-63898081	Neoplasms	I	NCT03926013
	SSTR2	Xmab18087	Neuroendocrine tumor	I	NCT03411915
	STEAP1	AMG509	Prostate cancer	I	NCT04221542
	5T4	GEN1044	Malignant solid tumors	I/II	NCT04424641
γδTCR	CD1d	LAVA-051	CLL	I/II	NCT04887259
	PSMA	LAVA-1207	Metastatic castration resistant prostate cancer	I/II	NCT05369000
CD16A	BCMA	RO7297089	R/R MM	I	NCT04434469
	CD30	AFM13	NHL	I/II	NCT04074746
	EGFR	AFM24	Advanced solid tumor	I/II	NCT04259450

AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung carcinoma; R/R MM, relapsed or refractory multiple myeloma; SCLC, small-cell carcinoma.

model[58]. This novel therapy avoids the activation of immunosuppressive Tregs and is effective in killing tumors.

3.1.1.3. CD16A targeting NK cell engagers. AFM13 is a novel tetravalent bispecific antibody developed by Affimed that targets CD16A and CD30[59]. CD16A activates NK cells, increases the release of pro-inflammatory cytokines and chemokines, and enhances the anti-tumor capacity of NK cells[60]. The cytotoxicity of NK cells induced by AFM13 is strictly dependent on the presence of CD30. In a phase I clinical trial for the treatment of Hodgkin's lymphoma, AFM13 significantly induced activation of NK cells in peripheral blood, showing strong anti-tumor activity and good tolerability[61]. This demonstrates the potential of bispecific antibodies to selectively activate NK cells and provide more potent and durable anti-tumor activity.

3.1.2. Targeting tumor cells

3.1.2.1. Targeting tumor-associated antigens. Monoclonal antibodies targeting tumor-associated antigens (TAAs) such as CD19, CD20 or HER2 (human epidermal growth factor receptor 2) have shown good clinical efficacy in treating cancer[62–64]. However, due to the low expression of these targets on normal cells, the drugs can also cause the killing of normal cells during treatment. To reduce the risk of adverse effects, bispecific antibodies offer an advantage over monoclonal antibodies in terms of selectivity and specificity. By adjusting the affinity and valency of the antibody arms, bispecific antibodies can be designed to target TAAs on the surface of tumor cells while reducing damage to normal cells. This allows for more targeted and effective treatment of cancer with fewer side effects.

3.1.2.2. Targeting tumor-specific antigens. Distinct from tumor-associated antigens (TAAs), tumor-specific antigens (TSAs) are only expressed in tumor cells. Targeting TSAs theoretically avoids the toxicity to normal cells and has a higher safety profile. Mutant proteins expressed by mutated proto-oncogenes and tumor suppressor genes (*e.g.*, *RAS* and *p53*) can become potential TSAs[65,66]. These mutated proteins are often intracellular proteins that are difficult to target directly by antibodies. However, it has been found that the hydrolyzed mutant proteins can bind to human

leukocyte antigens (HLA) in the form of short peptides to form peptide-HLA (pHLA) complexes that present on the cell surface[67]. These peptides are also known as mutation-associated neoantigens (MANAs), which can be used as targets for bispecific antibody design.

Targeting these MANAs allows the design of bispecific antibodies with higher selectivity to redirect T cells to TSA-expressing tumor cells. TCR-mimic antibodies, also known as MANA-directed antibodies (MANAbodies), have been developed and have shown promising results in clinical trials. A MANA antibody targeting mutated RAS has been developed and has been shown to activate T cells and kill tumor cells in cancers with KRAS mutations, such as pancreatic, colorectal, and lung cancers[68,69].

However, most MANAs are expressed at low levels on the cell surfaces, making the identification more difficult. When developing MANA antibodies, there are higher requirements for structures and valence optimization[70].

3.2. *Bridging receptors*

BsAbs targeting two tumor receptors have been extensively studied due to their high efficacy and low toxicity. As previously mentioned, tumor-associated antigens (TAAs) are also expressed in normal tissues, leading to undesired toxicity. Targeting two TAAs or different epitopes of the same antigen would increase selectivity and reduce toxicity. In addition, dysregulation of multiple proteins is often observed in malignant tumors. Designing bispecific antibodies to inhibit compensatory pathways is beneficial for improving efficacy and overcoming resistance.

3.2.1. *Receptors on tumor cells*

3.2.1.1. Bridging two separate receptors. Simultaneously inhibiting two tumor-associated proteins can produce a stronger therapeutic effect because it can target multiple pathways involved in tumor growth and progression, thus providing a more comprehensive approach to treating cancer. Additionally, it can reduce the risk of drug resistance, as it is more difficult for the tumor to develop resistance to two drugs at once.

It is clear that EGFR inhibitors have shown promising results in the treatment of various cancers, including NSCLC (non-small-cell lung carcinoma) and colon cancer[71–74]. However, mutations of EGFR and activation of compensatory pathways can lead to drug resistance[75]. To overcome this, the combination of two drugs to simultaneously block compensatory pathways has been developed, such as the combination of EGFR and cMET inhibitors, leading to the development of EGFR/cMET bsAbs[76]. Amivantamab (JNJ-61186372) is an example that has been approved by the FDA on May 21, 2021 for the treatment of adult patients with locally advanced or metastatic NSCLC (non-small-cell lung carcinoma)[77–79].

3.2.1.2. Bridging different epitopes of the same receptor. Trastuzumab and pertuzumab are monoclonal antibody drugs targeting the HER2 protein, but they have different binding sites[80,81]. The combination of trastuzumab and pertuzumab has been shown to be effective in treating HER2-positive breast cancer, as it can target two different antigen-binding sites on the same receptor. This combination has been approved by the FDA for the treatment of HER2-positive advanced breast cancer, in combination with chemotherapy[82]. The use of this combination has been shown to be more effective than Trastuzumab alone, as it can block compensatory pathways that can lead to drug resistance.

Zanidatamab (ZW25) is a bispecific antibody that targets two epitopes of HER2, combining the binding sites of trastuzumab (HER2 ECD4) and pertuzumab (HER2 ECD2)[83]. It has shown promising results in the treatment of HER2-positive breast cancer and gastroesophageal adenocarcinoma (GEA). In a phase I clinical trial, ZW25 in combination with docetaxel had an overall response rate (ORR) of 90.5%, which was higher than the ORR of 80.2% in the standard first-line treatment group (pertuzumab, ttrastuzumab, and chemotherapy)[84,85]. The FDA has granted ZW25 fast-track designation in combination with standard chemotherapy for patients with high-HER2-expressed GEA[86].

3.2.2. *Receptors on immune cells*

Immune cells have a variety of regulatory proteins on their surface, including a series of immune checkpoint proteins, which regulate the activation, proliferation and anti-tumor activity of immune cells[87]. Stimulating or inhibiting the relevant pathways in a rational manner can induce stronger immune clearance effects[88,89]. CTLA-4 and PD-1/PD-L1 are important immune checkpoint proteins, and the activation of these two pathways can significantly inhibit the activation of immune cells such as T cells, resulting in tumor cells "immune escape"[90,91]. CTLA-4 and PD-1/PD-L1 inhibitors promote the activation of immune cells in the tumor microenvironment (TME), which in turn leads to the apoptosis of tumor cells[92,93]. These immune checkpoint inhibitors (ICIs) have become important treatment options for tumors, however, drug resistance and side effects such as immune-related adverse events (IrAEs) are also present[94]. To address these issues, novel strategies such as combination therapies and novel drug delivery systems are being developed to improve the efficacy and reduce the toxicity of immune checkpoint inhibitors[95].

Cadonilimab is a bispecific antibody designed to target both PD-1 and CTLA-4, which is based on the Tetrabody format, providing enhanced efficacy and lower toxicity (Fig. 4A). The co-expression of CTLA-4 and PD-1 on tumor-infiltrating lymphocytes is widespread, while peripheral T cells are lacking. This reduces the tetravalent binding of cadonilimab to peripheral T cells and increases its enrichment in the TME. Additionally, the modified Fc region of cadonilimab helps to avoid Fc-mediated toxic effects, resulting in a higher specificity and lower toxicity[96,97]. On June 29, 2022, the NMPA approved its marketing for the treatment of patients with recurrent or metastatic cervical cancer (R/M CC) who have failed prior platinum-containing chemotherapy[98].

Another excellent bsAb design is FS120, which is a dual agonistic targeting 4-1BB and OX40 with the tetravalent format (Fig. 4B)[99]. Activating the 4-1BB pathway stimulates the activation and proliferation of T cells[100]. However, monotherapy with agonist antibodies to 4-1BB may induce serious toxicities, limiting the development of 4-1BB mAbs[101,102]. In the design of FS120, the binding arm targeting 4-1BB can be activated only after the simultaneous binding of OX40, which will lead to increased selectivity and reduced toxicities. While ensuring safety, the antitumor effect of FS120 is improved compared to the combination of mAbs[99].

3.2.3. *Receptors on tumor and immune cells*

Bispecific antibodies (bsAbs) can be used to target both immune cells and tumor cells (Table 3), activating the anti-tumor activity

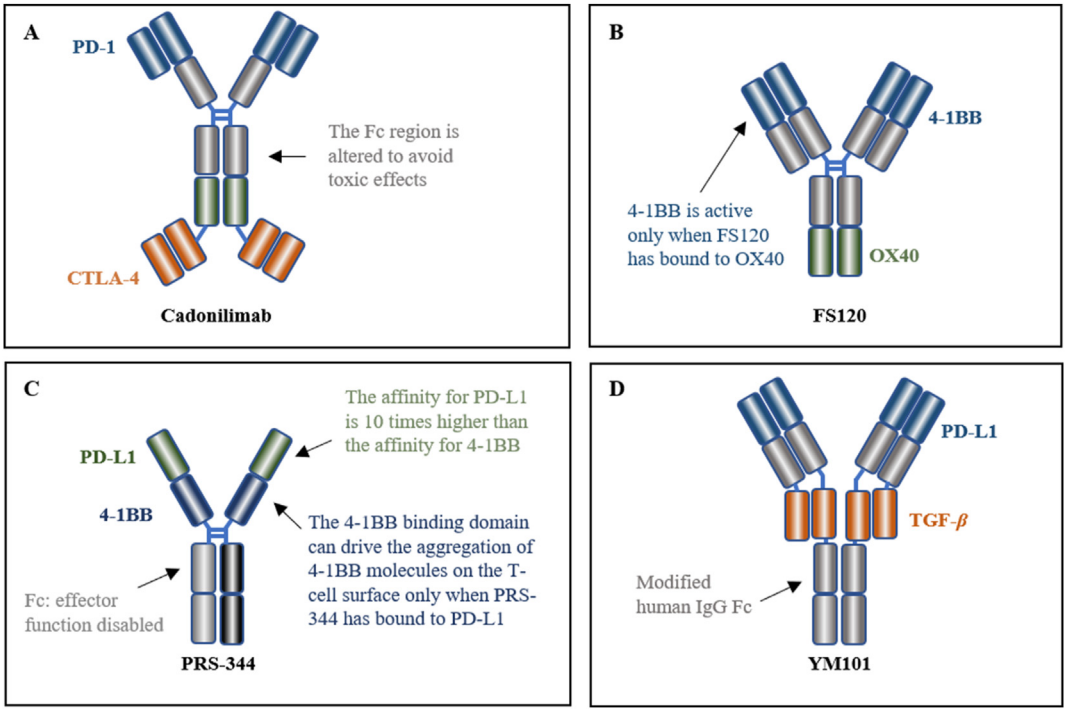

Figure 4 Representative bispecific antibodies with increased efficacy and reduced toxicity based on their unique structures.

Table 3 BsAbs bridge two receptors in clinical stages.

Classification	Target	Name	Indication	Phase	Clinical trial
Bridging two receptors on tumor cells	CD19 × CD47	TG-1801	B-cell lymphoma	I	NCT03804996
	CD20 × CD47	IMM0306	B-NHL	I	CTR20192612
	EGFR × cMET	EMB-01	Neoplasms	I/II	NCT05176665
	EGFR × HER3	Duligotuzumab	Head and neck cancer	I	NCT01911598
	EGFR × MET	LY3164530	Neoplasms	I	NCT02221882
	HER2 × HER2	Zanidatamab	$HER2^{+}/HR^{+}$ breast cancer	II	NCT04224272
	HER2 × HER3	Zenocutuzumab	Solid tumours harboring NRG1 fusion	II	NCT02912949
	HER3 × IGF-1R	MM-141	Pancreatic cancer	II	NCT02538627
	LRP5 × LRP6	BI905677	Neoplasms	I	NCT03604445
	PD-L1 × CD47	IBI322	Advanced malignant tumors lymphomas	I	NCT04338659
Bridging two receptors on immune cells	CD40 × 4-1BB	GEN1042	Malignant solid tumor	I/II	NCT04083599
	CTLA-4 × LAG-3	Xmab22841	Melanoma	I	NCT03849469
	CTLA-4 × OX40	ATOR-1015	Solid tumor	I	NCT03782467
	OX40 × 4-1BB	FS120	Advanced cancer	I	NCT04648202
	PD-1 × CTLA-4	AK104	Cervical cancer	II	NCT05227651
	PD-1 × ICOS	Xmab23104	Selected advanced solid tumors	I	NCT03752398
	PD-1 × LAG-3	Tebotelimab	Gastric cancer	II/III	NCT04082364
	PD-1 × TIM-3	RG7769	Solid tumors	I	NCT03708328
Bridging receptors on tumor and immune cells	CD40 × MSLN	ABBV-428	Advanced solid tumors cancer	I	NCT02955251
	HER2 × 4-1BB	PRS-343	HER2-positive solid tumors	I	NCT03330561
	PD-1 × PD-L1	IBI318	Advanced cutaneous squamous cell carcinoma	I/II	NCT04611321
	PD-L1 × 4-1BB	MCLA-145	Advanced cancer	I	NCT03922204
	PD-L1 × CTLA-4	KN046	Thymic carcinoma	II	NCT04925947
	PD-L1 × LAG-3	FS118	Advanced cancer	I/II	NCT03440437
	PD-L1 × TIM-3	LY3415244	Solid tumor	I	NCT03752177
	PSMA × CD28	REGN5678	Metastatic castration-resistant prostate cancer	I/II	NCT03972657

of the immune cells and directly acting on the tumor cells to induce apoptosis. The activated immune cells are usually tumor-infiltrating T cells that are already present in the TME[9], and this bsAbs-induced slow and sustained immune response increases the specificity and safety of the drug.

PRS-344 is a tetravalent antibody that targets PD-L1 and 4-1BB, which are located on the surface of tumor cells and immune cells, respectively (Fig. 4C). PRS-344 is designed to bind to PD-L1 first, which then enables the 4-1BB binding domain to drive the aggregation of 4-1BB molecules on the surface of T cells[103]. Compared to a 4-1BB monoclonal antibody, it has shown a significant reduction in hepatotoxicity in the clinic[104], and according to preclinical data, it has a better anti-tumor effect than the combination of two monoclonal antibodies[103].

3.3. *Bridging cytokines and receptors*

Abnormal regulation of cytokines is highly correlated with the development and progression of tumors[105]. Therapeutic approaches targeting cytokines have been reported, however, their development is limited by factors such as short half-life and high immunogenicity[106]. To overcome these limitations, many cytokine-based therapies have been adopted in combination therapies to improve efficacy and reduce toxicity[107], which is also the theoretical basis for designing bispecific antibodies (Table 4).

DLL4 is a receptor expressed in the vasculature and belongs to the Notch ligand family, which affects the formation of new vessels. Its expression is upregulated in various malignant tumors such as breast and bladder cancer[108,109]. However, DLL4 monoclonal antibodies have shown severe side effects in clinical trials[110]. To avoid its toxicity, navicixizumab was designed as a bispecific antibody targeting DLL4 and vascular endothelial growth factor (VEGF)[111]. This enables the antibody to better target the TME and has shown promising clinical activity and manageable toxicity in clinical trials for a range of solid tumors[112]. It has been granted a fast-track designation by the FDA for the treatment of heavily pretreated ovarian cancer[113].

TGF-β is an important cytokine that can promote immune escape of tumor cells in advanced stages and inhibit immune cell function in a non-redundant manner in combination with PD-L1[114,115]. When TGF-β inhibitors are used in combination with ICI, anti-tumor activity is increased[116]. YM101 is the first bispecific antibody targeting PD-L1/TGF-β developed on the Checkbody platform. It can enhance T cell infiltration, alter the immune microenvironment, induce effective clearance of tumors by immune cells, and is superior to single anti-TGF-β or PD-L1 antibodies[117].

3.4. *Bridging two cytokines*

Bispecific antibodies targeting two cytokines have been less studied in tumor therapy and remain to be further explored (Table 4). Vanucizumab is a promising new treatment for advanced solid tumors, as it has been shown to be effective in targeting both VEGF and Ang-2, two proteins that are involved in tumor growth and angiogenesis[118]. The safety and tolerability of the drug is comparable to other anti-VEGF or anti-Ang-2 inhibitors, making it a viable option for treating tumors such as breast cancer and gastric carcinoma[119–121].

4. Innovative bispecific antibody drugs

Bispecific antibodies offer unique advantages over monoclonal antibodies, including increased selectivity and efficacy. This increased selectivity makes them ideal for therapies that require high specificity, such as antibody–drug conjugates (ADC) drugs and chimeric antigen receptor (CAR) T-cell therapy. Furthermore, multispecific antibodies, such as trispecific and tetraspecific antibodies, are being developed to further increase selectivity and efficacy.

4.1. *Bispecific ADCs*

ADC drugs are a promising new drug design strategy that combines the specificity of antibodies with the high toxicity of small molecules[124]. Bispecific antibodies are particularly well-suited for this type of drug, as they offer increased specificity and endocytosis ability compared to monoclonal antibodies. This increased specificity helps to reduce the toxic side effects of the small molecule payload, while the endocytosis ability allows for more efficient transmembrane delivery of the ADC drug[125].

Bispecific ADCs targeting HER2 have been developed to improve the efficacy of HER2-targeted therapies[126]. ZW49, a bispecific antibody based on ZW25 (Fig. 5A), has demonstrated good antitumor activity and safety in clinical trials (ClinicalTrials.gov identifier: NCT03821233). Additionally, bsHER2xCD63$_{his}$-ADC, a bispecific antibody targeting HER2 and CD63, has been designed to improve the internalization and antitumor ability of HER2-based ADCs[127]. CD63 has the ability to regulate the transportation of proteins *via* endocytosis[128], which increases the endocytosis of ADC drugs and thus enhances their therapeutic efficacy[129].

4.2. *Trispecific/tetraspecific antibodies*

Combination therapy targeting synergistic pathways is an essential strategy for enhancing the efficacy of cancer therapy. T cells also

Table 4 BsAbs bridge cytokines or cytokines/receptors in clinical stages.

Classification	Target	Name	Indication	Phase	Clinical trial
Cytokines × receptors	TGF-β × CD73	GS-1423	Advanced solid tumors	I	NCT03954704
	TGF-β × PD-L1	SHR-1701	Squamous cell carcinoma of head and neck	II	NCT04650633
	TGF-β × EGFR	BCA101	Head and neck squamous cell carcinoma	I	NCT04429542
	VEGF × DLL4	OMP-305B83	Metastatic colorectal cancer	I	NCT03035253
	VEGF × PD-1	AK112	NSCLC	I/II	NCT04900363
Cytokines × cytokines	VEGF × Ang-2	Vanucizumab	Advanced solid tumors	I	NCT02665416

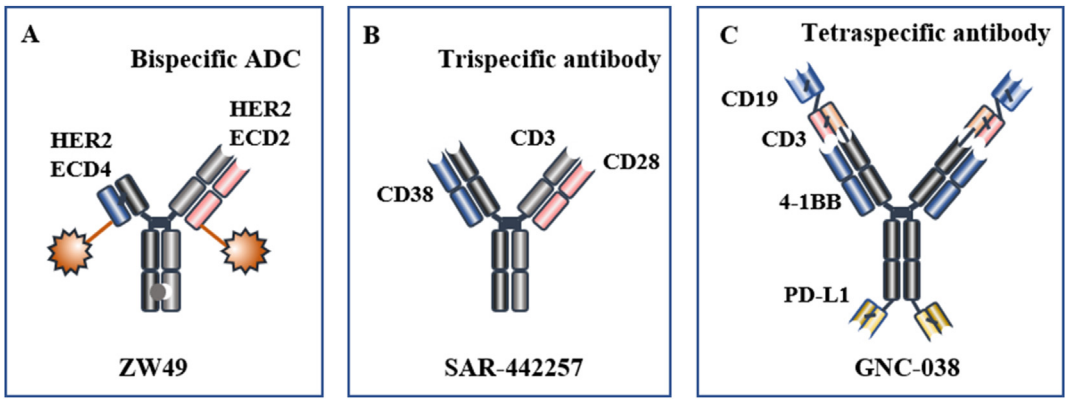

Figure 5 Representative innovative bispecific antibody drugs. (A) ZW49 is a bispecific ADC[122]. (B) SAR-442257 is a trispecific antibody[123]. (C) GNC-038 is a tetraspecific antibody (ClinicalTrials.gov identifier: NCT05192486).

require multiple signals for activation. Trispecific/tetraspecific antibodies, derived from bispecific antibodies, are thought to have a greater therapeutic potential (Fig. 5B and C).

Trispecific antibodies possess three distinct antigen-binding sites that can effectively bridge cells and stimulate immune cells more efficiently. SAR-442257 is a trispecific antibody targeting CD3/CD28/CD38. CD3 can recruit and activate T cells, while CD28 can further activate T cells and extend the duration of the immune response. CD38 domains have the ability to guide T cells to myeloma cells[130]. SAR-442257 is currently undergoing Phase I clinical trials to evaluate its therapeutic effects in relapsed/refractory multiple myeloma (R/R MM) and non-classical Hodgkin's lymphoma (R/R NHL) (ClinicalTrials.gov identifier: NCT04401020).

Tetraspecific antibodies possess four distinct antigen-binding sites, offering more options for target selection. GNC-038 is the first tetraspecific antibody to enter clinical trials. GNC-038 contains four antigen-binding sites: CD19, CD3, PD-L1, and 4-1BB. The CD3 and 4-1BB arms respectively activate the first and second signals of T cells, and the anti-CD19 and anti-PD-L1 domains target tumor cells[131]. GNC-038 stimulates peripheral T cells and facilitates T cell infiltration into tumor sites. It can overcome the immunosuppression in the TME and display antitumor activity *in vivo*. GNC-038 is currently in Phase I/II clinical trials to assess its effectiveness in non-Hodgkin's lymphoma, diffuse large B-cell lymphoma, and other lymphomas (ClinicalTrials.gov identifier: NCT05192486).

5. Guidance from FDA and NMPA

5.1. *Development of guidance*

Research on bispecific antibodies is unique and an increasing number of research institutions are engaging in it, necessitating industry guidance principles to regulate research and development, pointing out potential challenges to ensure successful research outcomes.

The FDA first issued draft guidance for *Bispecific Antibody Development Programs* on April 19, 2019, followed by a final version on May 24, 2021[13]. On April 11, 2022, the National Medicinal Products Administration (NMPA) published *the Technical Guidelines for Clinical Development of Bispecific Antibody Class Antitumor Drugs (Draft for Comments)*, with the final version released on November 9, 2022[14]. This marks the transition of bispecific antibodies from a "wild growth phase" to a more "scientific development phase" (Table 5). The European Union has yet to issue drug guidelines for bispecific antibody drugs, and the development of bispecific antibodies follows the guidelines for therapeutic protein drugs.

5.2. *Comments on the guidance*

The guidance issued by the US and China suggest various aspects that should be taken into consideration during the development of bispecific antibodies, such as design strategy, preclinical studies, quality control, drug metabolism and toxicity. It is essential to compare the guidelines between the US and China.

The documents issued by the FDA and China are programmatic, providing strategic guidance for the majority of requirements, while specific research protocols should be developed on a case-by-case basis. Considering the complexity and technical challenges of bsAbs, the guidance principles of the FDA and NMPA are open to communication regarding trial design and trial process. It is encouraged for research and development organizations to communicate with regulatory authorities in order to ensure the successful development of bsAbs.

Both guidance documents from the two countries include stringent requirements for efficacy and safety testing, such as immunogenicity testing and safety assessment. The FDA has established fundamental standards for pharmacology, toxicology, and safety evaluation, while the NMPA lacks such evaluation standards. Additionally, the NMPA provides the foundation for the design, selection and use of biomarkers, which are not mentioned in the guidance provided by the FDA.

Furthermore, the guidance of the two countries differs in terms of the selection of control groups. The FDA recommends comparing with the standard of care or placebo, while if monospecific products with the same antigens are approved for the same indication, then a comparison should be made with the monospecific products. On the other hand, the NMPA recommends selecting the optimal treatment regimen as a control. BsAbs are required to achieve a function that cannot be achieved by related monoclonal antibodies or monoclonal antibody combination therapy, which can bring valuable clinical benefits to patients.

At present, the development of bsAbs is still in its early stages, and there are few published guidelines that can be consulted. The guidance issued by the FDA and NMPA are both very instructive for the development of the bispecific antibody market. For bsAbs

Table 5 Guidance comparisons between FDA and NMPA[13,14].

Content	FDA	NMPA
Application scope	Bispecific antibodies, other types of bispecific protein products and multispecific products Not include antibody cocktails, polyclonal antibody products, or combination of monoclonal antibodies	Bispecific and multispecific antibodies in cancer therapy
Classification	BsAbs that bridge two target cells BsAbs that do not bridge cells	Classification by structure: Non-IgG based bsAbs; IgG based bsAbs Classification by target selection: Bridging cells; Bridging receptors; Bridging cytokines
Design of bsAbs	Not mentioned	Designing antibodies based on clinical needs, target selection and structure optimization
Scientific considerations		
CMC quality considerations	The development of the manufacturing procedures should be carried out according to standard monoclonal antibody development practices. Studies should be conducted on quality characteristics such as antigen specificity; affinity and on- and off-rates; avidity; potency; product-related impurities, fragments, homodimers, other mispaired species; stability; and half-life	Not mentioned
In vitro tests	Required to carry out, in combination with pharmacological experiments to support the scientific principle of bispecific antibodies	
Non-clinical trials	Pharmacology and toxicology experiments: the range of studies is similar to that of monoclonal antibodies; target expression profile and specificity should be considered when selecting models	Referring to the relevant guidelines that have been published in China, non-clinical studies were conducted to further support the rationality of the topic of bsAbs
Risk control for first-in-human (FIH) trials of innovative drugs	Not mentioned	Develop and strictly implement a risk management plan during clinical trials; scientifically and appropriately set the starting dose of FIH, the magnitude and speed of dose escalation study; and rationally define the dose limit toxicity (DLT)
Clinical pharmacology	Similar to research on monoclonal antibodies and other therapeutic protein products	
Pharmacodynamics	Necessary to consider the binding and impact of each target	
Optimal drug delivery strategy	Extended dose exploration studies can be conducted with no less than two candidate dosing regimens within the determined safe dose range	Factors such as pharmacology, toxicology, and pharmacokinetics should be evaluated comprehensively; Early dose escalation studies should be performed
Control selection	Comparison with the standard of care or placebo in many situations. If monospecific products with the same antigens approved for the same indication exist, then a comparison is conducted with the monospecific products	Comparison with the best standard treatment
Clinical trial establishment	Clinical studies should inform the benefit-risk assessment and support approval based on the specific targets and other clinical considerations. Sponsors are encouraged to discuss product development plans with the FDA's appropriate clinical review division	BsAbs should perform functions that are not achieved by the mAbs or combinations of mAbs, and have the potential to provide clinical value
PK assessment	Choose the bispecific antibody conformation associated with the bispecific antibody PK assessment (biologically active or inactive forms)	Not mentioned
Immunogenicity	Detection of immunogenic reactions of different structural domains of bsAbs using multiple methods	Immunogenicity risk assessment should be conducted and a risk management plan should be developed before clinical studies; Integrates clinical PK, PD and safety data during development to fully assess immunogenicity; Develop an immunogenicity study strategy based on immunogenicity risk; Detection of immunogenic reactions of different structural domains of bsAbs using multiple methods
Development of biomarker	Not mentioned	Design and use of biomarkers based on factors such as the mechanism of action, biological relationships between targets, clinical applications and data

that require approval from multiple countries, comprehensive considerations must be taken into account during the development stage to meet different regulatory requirements, such as pharmacodynamics, toxicology, and other *in vitro* tests.

6. Conclusions

Many successful bsAbs have been developed, providing a variety of successful templates and development experiences. Proper structural design and target selection are critical for ensuring the success of drug research, while maintaining the efficacy of combination therapy and reducing the corresponding toxicity, which is one of the core advantages of bispecific antibodies. Systematically understanding excellent examples of bispecific antibody design can help to develop novel therapeutic antibodies.

The development of bispecific antibodies has opened up new possibilities for the development of innovative drugs that can target multiple pathways simultaneously. The high selectivity of these antibodies makes them ideal for use as ADC drugs, which can improve selectivity against cancer cells and increase internalization for better clearance of tumor cells. Additionally, the development of trispecific and tetraspecific antibodies is expected to further enhance the anti-tumor effects of these drugs. Clinical trials are currently underway to evaluate the efficacy of these drugs, and the results of these trials will be eagerly awaited.

The collection of cases has revealed a high degree of similarity in target selection across multiple research institutions. CD3 is the most commonly chosen target for cell-bridging bsAbs, with 56 bispecific antibodies targeting both CD3 and CD19, including one marketed drug. For non-cell-bridging bsAbs, the most widely studied combination is PD-L1/4-1BB, with 32 bispecific antibodies. Bispecific antibodies offer the advantage of increased efficacy and reduced toxicity due to the rational combination of targets. However, the high similarity in target selection is unfavorable for study enrichment and may lead to wasted medical resources. Therefore, it is important to conduct a thorough review of the literature and market research before selecting a target for bispecific antibody development.

The rapid development of bispecific antibodies has led to an increased need for regulatory guidance. In 2021 and 2022, the US and China issued guidance to standardize the design strategy and drug evaluation of bsAbs, in order to maximize the unique benefits of bispecific antibodies. This guidance is intended to ensure the safety and efficacy of bsAbs, and to ensure that they are used in the most effective way. The global market for bispecific antibodies is growing rapidly, and sales of cancer-related bispecific antibodies are expected to reach $3.7 billion by 2027[132]. Despite the challenges that remain, bispecific antibodies offer unique advantages that make them a powerful therapeutic weapon. These advantages include increased efficacy, lower toxicity, and improved specificity, which can help to improve the effectiveness of cancer treatments.

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Author contributions

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Conflicts of interest

The authors declare no conflict of interest.

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