Cancer Biol Med 2025. doi: 10.20892/j.issn.2095-3941.2024.0470

REVIEW

Application and future prospects of bispecific antibodies in the treatment of non-small cell lung cancer

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ABSTRACT As the leading cause of cancer-related deaths, lung cancer remains a noteworthy threat to human health. Although immunotherapies, such as immune checkpoint inhibitors (ICIs), have significantly increased the efficacy of lung cancer treatment, a significant percentage of patients are not sensitive to immunotherapies and patients who initially respond to treatment can quickly develop acquired drug resistance. Bispecific antibodies (bsAbs) bind two different antigens or epitopes simultaneously and have been shown to enhance antitumor efficacy with suitable safety profiles, thus attracting increasing attention as novel antitumor therapies. At present, in addition to the approved bsAb, amivantamab, three novel bsAbs (KN046, AK112, and SHR-1701) are being evaluated in phase 3 clinical trials and many bsAbs are being evaluated in phase 1/2 clinical trials for patients with non-small cell lung cancer (NSCLC). Herein we present the structure, classification, and mechanism of action underlying bsAbs in NSCLC and introduce related clinical trials. Finally, we discuss challenges, potential solutions, and future prospects in the context of cancer treatment with bsAbs.

KEYWORDS Bispecific antibody; non-small cell lung cancer; novel antitumor therapy; structure; challenges

Introduction

Lung cancer, the leading cause of cancer-related deaths, is one of the most significant threats to human health. Non-small cell lung cancer (NSCLC) is the most prevalent subtype, accounting for nearly 85% of all lung cancer cases. Although targeted therapy and immunotherapy have improved the efficacy of NSCLC treatment, therapeutic resistance and poor patient response indicate the urgent need to find more effective therapies[1]. Bispecific antibodies (bsAbs) were first proposed by Nisonoff in the 1960s and are defined as antibodies that simultaneously bind two different antigens or epitopes[2]. BsAbs have shown encouraging efficacy as late-line treatment in numerous clinical trials, indicating that bsAbs may be effective treatments for overcoming resistance to existing targeted therapies and immunotherapies. Given the high overall response rate (ORR) and durable response demonstrated in a phase 1 trial (NCT02609776/CHRYSALIS), amivantamab was first approved in the US on 21 May 2021 for medical intervention in adult patients with locally advanced or metastatic NSCLC and epidermal growth factor receptor (EGFR) exon 20 insertion mutations that have progressed during or after platinum-based chemotherapy[3]. According to data from ClinicalTrials.gov, numerous bsAbs are being assessed in clinical trials for patients with NSCLC. However, the existing reviews concerning the use of bsAbs for the treatment of lung cancer have failed to describe the relevant bsAbs and clinical trials completely[4]. This article briefly introduces bsAbs, discusses the mechanisms of action and application in NSCLC treatment, and reviews related clinical trials in detail.

Production and classification of bsAbs

BsAbs refer to antibodies that bind two different antigens or epitopes simultaneously, which can enhance antibody targeting and cancer treatment efficacy. BsAbs were first proposed in the 1960s by Nisonoff[2] (**Figure 1**). BsAbs were mainly constructed using hybridoma or chemical recombination technology in the early stage. Hybridoma technology

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Received October 7, 2024; accepted March 5, 2025;
published online April 7, 2025.
Available at www.cancerbiomed.org

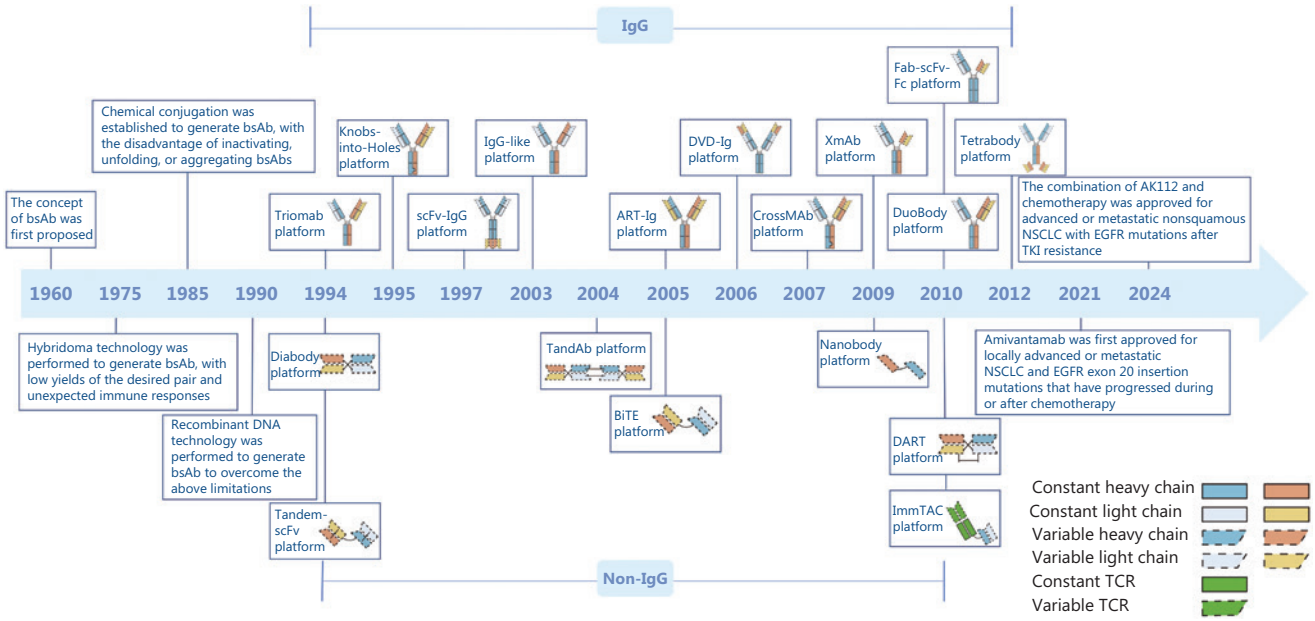

Figure 1 The evolutionary history map of the bsAbs. IgG subtype bispecific antibodies are placed above the timeline and the non-IgG subtype bispecific antibodies are placed below the timeline. Abs, antibodies; ART-Ig, asymmetric reengineering technology immunoglobulin; bsAb, bispecific antibody; BiTE, bispecific T-cell engager; CrossMab, cross-specific monoclonal antibody; DVD-Ig, dual variable domain-immunoglobulin; DART, dual-affinity re-targeting proteins; EGFR, epidermal growth factor receptor; Fab, fragment antigen-binding; Fc, fragment crystallizable; IgG, immunoglobulin G; ImmTAC, immune-mobilizing monoclonal T-cell receptors against cancer; NSCLC, non-small cell lung cancer; scFv, single-chain fragment variable; TandAb, tandem diabody; TKI, tyrosine kinase inhibitor; XmAb, xencor monoclonal antibody.

involves the formation of a quadroma by somatic fusing two different antibody-producing hybridoma cell lines to generate bsAbs[5]. Hybridoma cell A expressing mAb A and hybridoma cell B expressing mAb B were fused to generate a quadroma expressing bsAbs containing immunoglobulin heavy and light chains inherited from both parents (**Figure 2A**). However, this approach faced several challenges, including low yields of the desired bsAbs and immune side effects caused by misassembling heavy and light chains[2]. The stochastic pairing of two distinct heavy and light chains each can theoretically generate up to 10 possible molecular configurations, of which only 1 molecular configuration forms a functional bsAb[6].

Chemical recombination technology refers to the chemical reassociation of fragments derived from two different mAbs (**Figure 2B**)[7]. The initial chemical recombination strategies relied on manipulating the disulfide arrangements. With the advances in increasingly sophisticated antibody modification techniques, the number of available chemical crosslinkers increased, such as a bis-sulfone crosslinker, bis-maleimide chemistry, dibromomaleimide crosslinker, and pyridazinedione[8]. However, this method often led to the inactivation, unfolding, or aggregation of bsAbs[2]. With the development of bioconjugation and click chemistry-based technologies, chemical recombination technology has had a vital role in the construction of bispecific antibody–drug conjugates (bsADCs) with better yields in recent years, producing stable and homogeneous bsAbs[8].

The development of bsAbs has been significantly hindered due to limitations of these two strategies. The rapid advances in genetic engineering technologies have provided a promising alternative to overcome the limitations of hybridoma and chemical recombination technology, which could also make the antibody less immunogenic and better tolerated[9]. Genetic engineering technologies enhance specific activities, such as improving antigen affinity, modulating pharmacokinetics, and optimizing effector functions[10]. Gene recombination and protein engineering are the primary genetic engineering technologies used to construct bsAbs (**Figure 2C**)[11,12]. A second wave of bsAb production emerged due to advances in genetic engineering technologies[2]. Most of the bsAbs that are approved or in clinical trials are constructed by genetic engineering technologies.

BsAbs can be categorized into two distinct groups based on structure: IgG-like bsAbs containing Fc fragments; and non-IgG-like bsAbs without Fc fragments[13] (**Figure 2C**). IgG-like bsAbs are based on intact IgG structures and have better

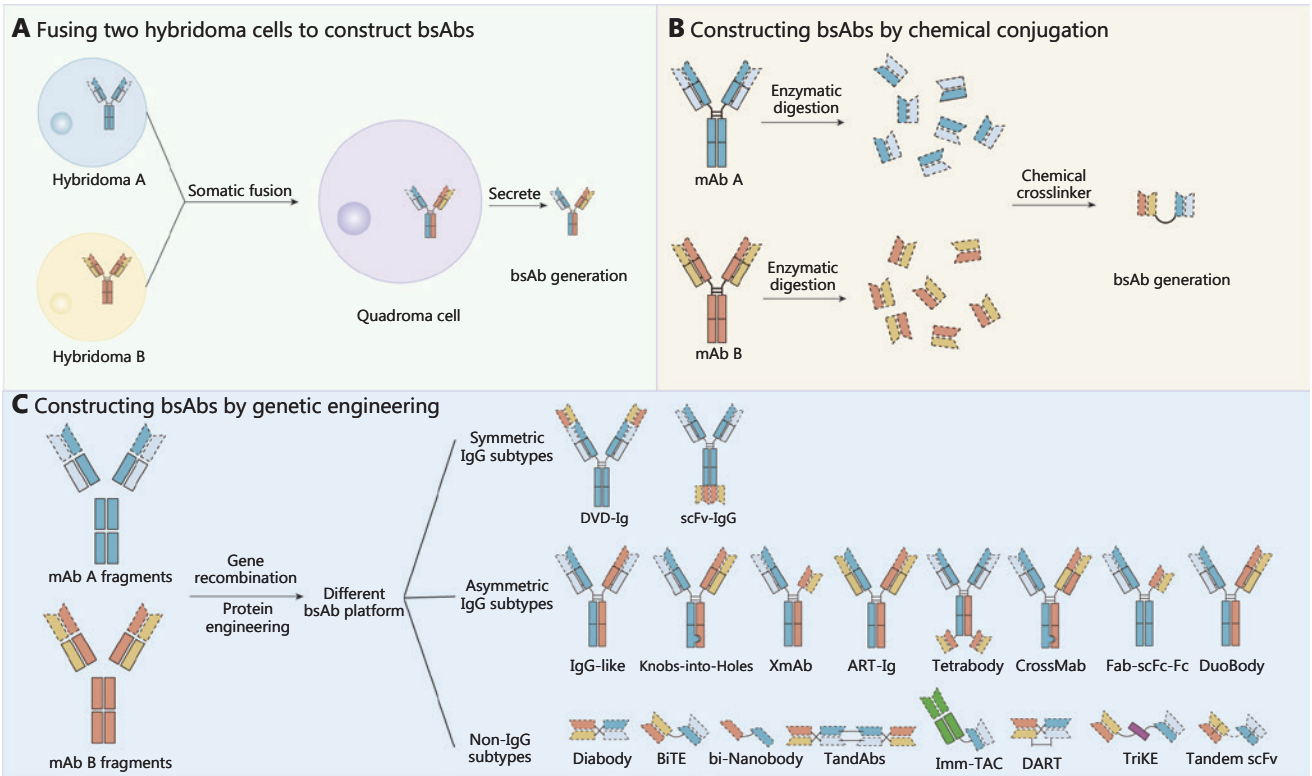

Figure 2 Three strategies for bsAb construction. (A) Fusing two hybridoma cells to construct bsAbs. Hybridoma cell A expressing mAb A and hybridoma cell B expressing mAb B are fused to generate a quadroma cell which expresses bsAbs. (B) Constructing bsAbs by chemical conjugation. Two mAbs were digested with enzymes to obtain antibody fragments. The fragments from mAb A and mAb B were then reassociated using chemical crosslinkers to construct bsAbs. (C) Constructing bsAbs by genetic engineering. Through genetic engineering technologies, fragments from two distinct mAbs are randomly recombined to construct bsAb in different platforms. The most common genetic engineering technologies used to construct bsAbs are gene recombination and protein engineering. BsAbs can be divided into three types: symmetric IgG subtype; asymmetric IgG subtype; and non-IgG subtype. ART-Ig, asymmetric reengineering technology immunoglobulin; bsAb, bispecific antibody; BiTE, bispecific T-cell engager; CrossMab, cross-specific monoclonal antibody; DVD-Ig, dual variable domain-immunoglobulin; DART, dual-affinity re-targeting proteins; Fab, fragment antigen-binding; Fc, fragment crystallizable; IgG, immunoglobulin G; ImmTAC, immune-mobilizing monoclonal T-cell receptors against cancer; scFv, single-chain fragment variable; TandAb, tandem diabody; TriKE, TriToxin-targeted killer engager; XmAb, xencor monoclonal antibody.

solubility and stability and a longer half-life than non-IgG-like bsAbs because IgG-like bsAbs contain Fc fragments. Moreover, IgG-like bsAbs also enhance tumor-killing effects by achieving antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cell phagocytosis (ADCP). The strategies used to construct IgG subtype bsAbs include CrossMab, Knobs-into-Holes (KiH), Triomab quadroma, Duo body, and dual variable domain-immunoglobulin (DVD-Ig). IgG-like bsAbs can be further classified into symmetric and asymmetric types, with the majority being asymmetric[6]. Symmetric bsAbs are designed by fusing two identical antibody fragments or conjugating single-chain variable fragments (scFvs) or single-variable domains to conventional antibodies *via* linker molecules. These constructs typically adopt a tetravalent structure with four binding sites, ensuring balanced antigen targeting while mitigating the risk of improper chain association. In contrast, asymmetric bsAbs incorporate distinct antigen-binding arms, forming heterodimers that offer enhanced flexibility in target selection[14]. Non-IgG-like bsAbs are based on scFvs, which are characterized by small size and strong permeability. The lack of an Fc fraction means that non-IgG-like bsAbs have many disadvantages, such as a short half-life, unstable structure, and low expression. The strategies used to construct bsAbs of this subtype include dual-affinity re-targeting proteins (DART), bispecific T-cell engager (BiTE), tandem diabodies (TandAbs), and bi-Nanobody[15,16]. The characteristics and relevant bsAbs of different platforms are listed in **Table 1**.

Table 1 Characteristics and relevant bsAbs of different platforms

Platform	Construction	Merit	Shortage	Technology	BsAbs in clinical trial	Refs.
Triomab	Triomab is composed of two half-antibodies, each containing one light chain and one heavy chain, derived from the mouse IgG2a isotype and the rat IgG2b isotype. The yields of the desired pair are low.	Addressing issues related to heavy chain and light chain mispairing	Low yields of the desired pair, immunogenic responses and immune side effects	Hybridoma technology	Catumaxomab	133
Diabody	Diabody is constructed in bacteria, slower dissociation kinetics, and an improved affinity for hapten.	Slower dissociation kinetics and an improved affinity for hapten	The formation of non-functional homodimers due to incorrect pairing of the variable domains.	Genetic engineering technology	MGD009	134
Knobs-in-Holes	"Knobs-in-Holes" technology facilitates the correct pairing of heavy chains, but does not prevent the incorrect pairing of light chains associated with the two distinct heavy chains.	Correct pairing of heavy chains	It does not prevent the incorrect pairing of light chains associated with the two distinct heavy chains.	Genetic engineering technology	Emicizumab Belantamab KN046	135,136
scFv-IgG	Cysteine residues are commonly introduced into the heavy- and light-chain regions of the scFv to form an intrachain disulfide bond.	Enhanced stability	Unforeseen molecular heterogeneity	Genetic engineering technology	CDX-527	137
TandAb	Two pairs of VL and VH domains are linked in a single polypeptide chain to form a TandAb.	Increased valency, stability, and therapeutic potential	Short half-life and low expression	Genetic engineering technology	Zenocutuzumab	138
ART-Ig	Mutations are introduced in the CH3 domains to create altered charge polarity across the Fc interface region.	Addressing the issue of heavy chain/heavy chain mispairing	Short half-life and unstable structure	Genetic engineering technology	AMG 420 REGN1979 AMG 701	139
BiTE	BiTE molecules can be rapidly cleared from circulation due to their short half-life. BiTEs bind a T cell-specific molecule and TAAs to redirect T cells.	BiTE can be rapidly cleared from circulation	Activation occurred in a strictly target-dependent manner	Genetic engineering technology	Blinatumomab AMG 596	71,140
DVD-Ig	DVD-Ig is created by adding additional antigen-binding units to the amino or carboxy termini of either light or heavy chains.	Targeting low-abundance proteins and longer dosing intervals	Enhanced immunogenicity and low yield	Genetic engineering technology	Lutikizumab ABT-981	141
CrossMAb	By utilizing different antibody formats and geometries through domain exchange or crossover.	Proper heavy chain pairing and correct association of light chains with the counterparts	Unrelated side products	Genetic engineering technology	Vanucizumab Faricimab AZD7789	142
XmAb	XmAb is a variant generated through structure- and sequence-based approaches to analyze the energetic landscape of paired variant combinations at the CH3 dimer interface.	Potent cytotoxic activity by NK cells and T cells	Enhanced immunogenicity and low yield	Genetic engineering technology	XmAb14045	143

Table 1 Continued

Platform	Construction	Merit	Shortage	Technology	BsAbs in clinical trial	Refs.
Nanobody	Nanobodies, derived from llama and camel heavy-chain-only antibodies, can be easily linked with short linkers, allowing for modulation of antigen-binding valency.	Efficient tissue penetration and enhanced epitope accessibility	Limited half-life	Genetic engineering technology	Ozoralizumab	144
Fab-scFv-Fc	Fab-scFv-Fc is composed of a light chain, a heavy chain, and a third chain incorporating both the Fc region and scFv.	Efficient manufacturing and purification	Short half-life and enhanced immunogenicity	Genetic engineering technology	MEDI-3902	12
DuoBody	DuoBody is engineered by leveraging the CH3/CH3 dimer interface of IgG4, enabling the generation of stable bispecific antibodies in both the IgG4 and IgG1 formats.	DuoBody mimics the natural Fab-arm Exchange process of human IgG4 *in vivo*	Poor permeability	Genetic engineering technology	Amivantamab Teclistamab Epcoritamab MGD013	145
DART	Two peptide chains link the opposite fragments, with VLA connected to VHB and VLB connected to VHA, and a disulfide bond at their C-termini that covalently fuses them together.	Improved stability, extended storage and serum stability	Reduced effective tumor penetration	Genetic engineering technology	MGD-009	146
ImmTAC	A stabilized and soluble T cell receptor is fused with an scFv recognizing CD3 to construct ImmTAC, enabling the targeting of processed antigenic peptides.	Improved affinity and exceptional stability	Activation occurred in a strictly target-dependent manner	Genetic engineering technology	IMCgp100	12,147
Tetrabody	Fusion of two monoclonal antibodies to form a tetravalent bispecific antibody.	Stable, monodisperse structure, potent anticancer activity, and extended *in vivo* half-life	Enhanced immunogenicity	Genetic engineering technology	AK104 AK112 AK130	148
TriKE	TriKE is engineered through the connection of two scFvs by polypeptide linkers, with the incorporation of human IL–15.	Promotion of the survival and proliferation of NKs	Immunogenic responses and immune side effects	Genetic engineering technology	GTB-3500	149
Biclonics	One residue in a CH3 domain is substituted with a positively charged lysine residue, while one or more residues in the second CH3 domain are substituted with negatively – charged glutamic acid or aspartic acid residues.	Preferential heavy chain heterodimerization	Unstable structure and enhanced immunogenicity	Genetic engineering technology	zenocutuzumab	144

ART-Ig, asymmetric reengineering technology immunoglobulin; bsAb, bispecific antibody; IgG, immunoglobulin G; BiTE, bispecific T-cell engager; CH3, heavy chain 3; DVD-Ig, dual variable domain-immunoglobulin; DART, dual-affinity re-targeting proteins; Fc, fragment crystallizable; Fab, fragment antigen-binding; ImmTAC, immune-mobilizing monoclonal T-cell receptors against cancer; IL-15, interleukin 15; NK, natural killer; scFv, single-chain fragment variable; TandAb, tandem diabody; TriKE, tri-specific killer engager; VL, variable region of light chain; VH, variable region of heavy chain.

Mechanisms of action underlying bsAbs

BsAbs can be split into the following two groups: bsAbs for immunotherapy; and bsAbs for targeted therapy. The mechanisms of action underlying bsAbs in the context of NSCLC treatment and relevant clinical trials are introduced herein. The framework of this review is shown in **Figure 3**.

BsAbs for immunotherapy

Moderating the immune response by binding multiple immunomodulatory molecules

The most well-known immunomodulators are immune checkpoints, which have a role in tumor immune evasion and can be divided into two categories: inhibitory checkpoints; and costimulatory checkpoints. Common inhibitory checkpoints

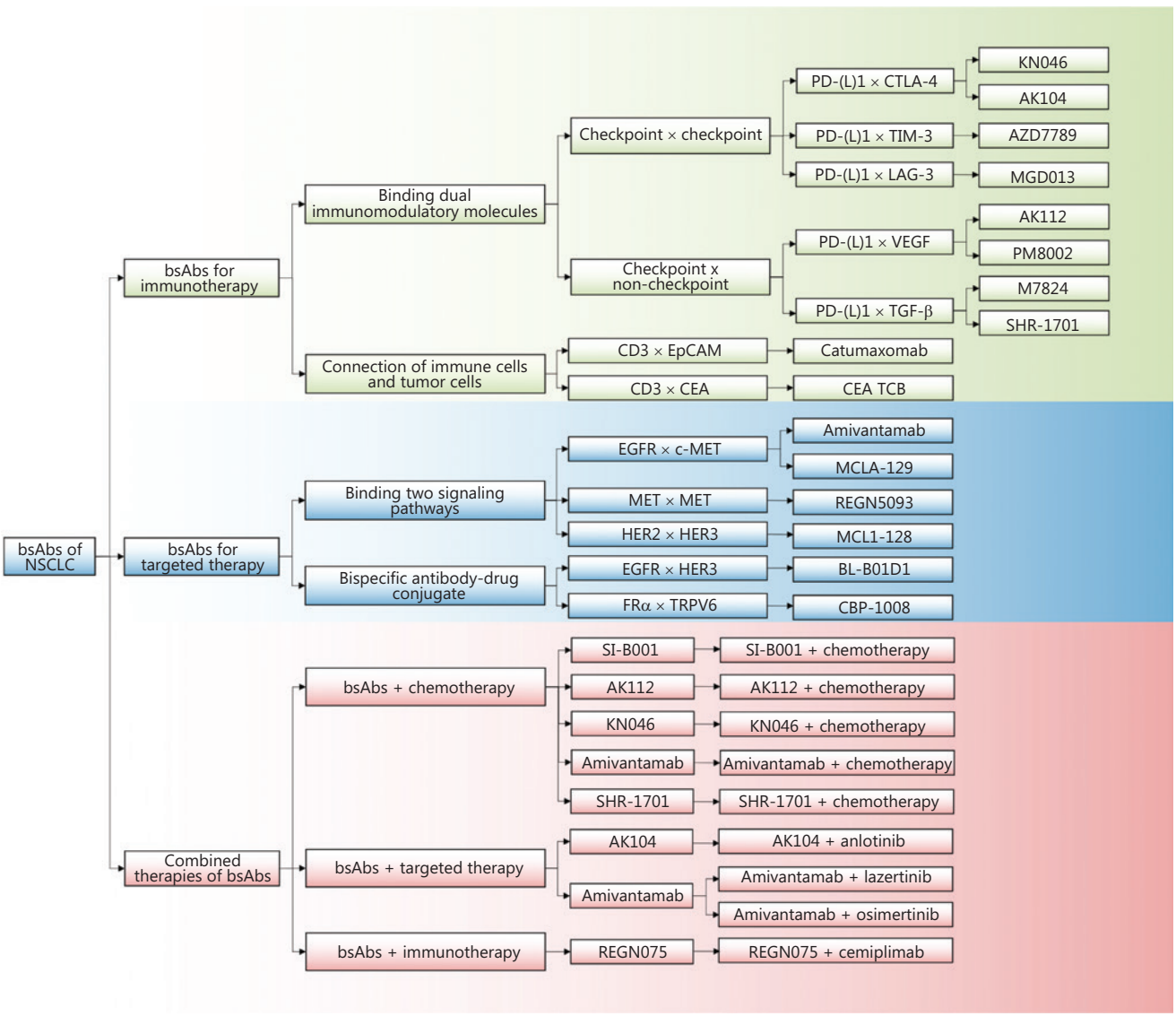

Figure 3 The framework of this review. CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; c-MET, cellular-mesenchymal epithelial transition; CEA, carcinoembryonic antigen; EpCAM, epithelial cell adhesion molecule; EGFR, epidermal growth factor receptor; FRα, folate receptor alpha; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; LAG-3, lymphocyte activation gene 3; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3; TGF-β, transforming growth factor β; TRPV6, transient receptor potential vanilloid 6; VEGF, vascular endothelial growth factor.

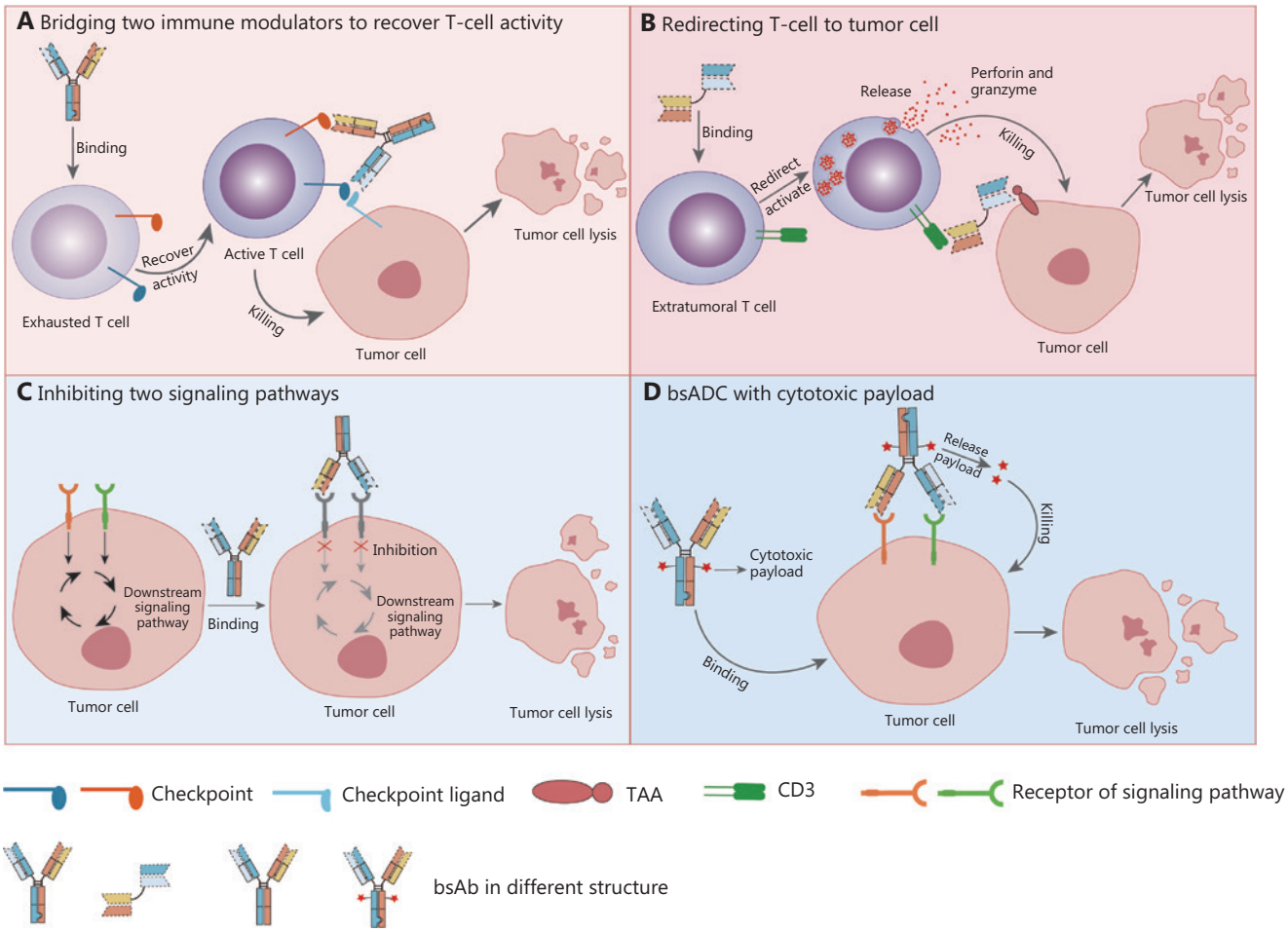

Figure 4 Mechanisms of action underlying bsAbs for the treatment of NSCLC. (A) Bridging two immune modulators to recover T-cell activity. After binding to two immune modulators on the T cell surface, bsAbs can restore the activity of exhausted T cells to initiate a tumor-killing immune response. (B) Redirecting T cell to tumor cell. BsAbs can bind the CD3 on the T cell surface and TAA on the tumor cell surface to redirect T cell. Then, T cells will release perforin and granzyme to kill tumor cells. (C) Inhibiting two signaling pathways. The survival of tumor cells relies on multiple signaling pathways. By targeting two receptors on the surface of tumor cells that are integral to these pathways, bsAbs can inhibit downstream signaling, thereby inducing tumor cell death. (D) bsADC with cytotoxic payload. After linking to tumor cells, bsADC release cytotoxic payload to kill tumor cells. bsADC, bispecific antibody-drug conjugate; CD3, cluster of differentiation 3; bsAb, bispecific antibody; TAA, tumor-associated antigen.

include programmed death-1/programmed death-ligand 1 (PD-1/PD-L1), cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), T-cell immunoglobulin and mucin domain-containing protein (TIM-3) and lymphocyte activation gene 3 (LAG-3). Immune checkpoint inhibitors (ICIs) have undoubtedly been the paramount accomplishment in tumor treatment over the past decade, revolutionizing the field of oncology[17]. Unfortunately, the ORR of patients with NSCLC receiving ICIs is only 20%[18]. Many NSCLC patients develop resistance over time and relapse, indicating that single mAb therapy has limited efficacy. Although combination therapy can increase the response rate, combination therapy may result in an increased number of treatment-related adverse events (TRAEs) and immune-related adverse events (irAEs)[19], thus forming the basis for the use of bsAbs to simultaneously target two checkpoint proteins. BsAbs modulate the immune response by binding multiple immunomodulatory molecules to enhance antitumor efficacy (**Figure 4A**).

BsAbs targeting two checkpoints

Inhibitory checkpoints, the "brakes" of the immune system, are vital for the maintenance of self-tolerance. Inhibitory checkpoints decrease the intensity of the immune response and terminate continued activation to alleviate or prevent host damage

caused by chronic inflammation and autoimmunity under normal physiologic conditions[20]. ICIs blocking inhibitory checkpoints induce an effective antitumor immune response[21] and markedly increase the survival rate and ORR of NSCLC patients[22]. Although > 100 mAbs are approved for clinical application by the Food and Drug Administration (FDA), patients have an enduring response to only a few mAbs, indicating the limited efficacy of mAbs[23]. Through the upregulation of alternative inhibitory checkpoints, mAbs may result in incomplete blockage, leading to ICI resistance[24]. This shortcoming can be overcome by developing bsAbs that bind two inhibitory checkpoints simultaneously to achieve an enhanced antitumor effect.

PD-1/PD-L1 × CTLA-4

PD-1, which is upregulated on effector T cells after activation, restrict the activity of T cells in the context of the inflammatory response[25]. The major PD-1 ligand is PD-L1, which is expressed in NSCLC cells and may inhibit the antitumor immune response[26]. Blocking the PD-1/PD-L1 axis can inhibit tumor growth and restore the functional activity and number of CD8+ T cells[27]. CTLA-4 was the first targeted immune checkpoint[25]. CD28 receptors located on T cells that bind to CD80 or CD86 costimulatory molecules on antigen-presenting cells (APCs) are crucial for effectively activating T cells[28]. CTLA-4 and CD28 have common ligands, and compared to CD28, CTLA-4 has greater overall binding affinities to CD80 and CD86[29]. By strongly binding to CD80 and CD86, CTLA-4 decreases the activation of T cells and actively delivers inhibitory signals. CTLA-4 and PD-1 are usually co-expressed in tumor-infiltrating lymphocytes (TILs). Although combining anti-PD-1 and anti-CTLA-4 antibodies is the most effective and powerful combination therapy for numerous malignancies to date, combining anti-PD-1 and anti-CTLA-4 antibodies also significantly increases immune-mediated toxicity, which may reduce the opportunity for combination with other therapies. Compared to the combination of two monoclonal antibodies, bsAbs that simultaneously target two inhibitory checkpoints can achieve maximum clinical therapeutic effects while minimizing toxicity[30].

KN046

KN046, a novel bsAb that is fused with the human wild-type IgG1 Fc fragment, can simultaneously bind to PD-L1 and CTLA-4 with increased affinity for PD-L1, which enables KN046 to target tumors expressing high levels of PD-L1. A phase 2 trial (NCT03838848) that evaluated KN046 in metastatic NSCLC patients as second-line treatment, revealed an ORR of 14.1%, a median progression-free survival (mPFS) of 3.7 months, and a median overall survival (mOS) of 18.4 months among 64 patients. The incidence of grade ≥ 3 TRAEs was 42.2%. The most frequent TRAEs were infusion reactions (10.9%), hepatic dysfunction (4.7%), and pneumonia (3.1%). KN046 was shown to be effective and relatively safe for advanced NSCLC as a second-line treatment[31].

Cadonilimab/AK104

Cadonilimab, also known as AK104, is a human symmetric IgG1 tetravalent bsAb that targets PD-1 and CTLA-4. Cadonilimab has distinctly lower toxicity in the clinical setting because cadonilimab does not bind Fc receptors, thereby minimizing ADCC, ADCP, and interleukin-6 (IL-6)/IL-8 release[32,33]. The effect of AK104 on patients with selected advanced solid tumors was evaluated in the NCT04172454 phase 1b/2 study. Among the 53 evaluable patients previously treated for advanced NSCLC, the ORR was 5.7% and the disease control rate (DCR) was 35.8%. TRAEs occurred in 39 patients (73.6%) and grade ≥ 3 TRAEs occurred in 6 patients (11.3%). The most frequent TRAEs were increased aspartate aminotransferase (AST) levels (22.6%), increased alanine transaminase (ALT) levels (18.9%), and weight loss (13.2%; **Table 2**)[34]. Cadonilimab was approved for use in China for patients with recurrent or metastatic cervical cancer who have received or are receiving platinum-based chemotherapy in June 2022[35]. We look forward to more supportive research results to promote the use of cadonilimab in the treatment of NSCLC. A range of studies investigating the therapeutic efficacy of cadonilimab in NSCLC, such as NCT05816499, NCT05812534, and NCT05377658, are ongoing.

As the most common and promising targets, PD-(L)1 and CTLA-4 have crucial roles in NSCLC immunotherapy. The efficacy and safety of anti-PD-(L)1 and anti-CTLA-4 combination therapy were evaluated. However, TRAEs occurred in 78.0% of patients and grade ≥ 3 TRAEs occurred in 34.0% patients of patients receiving nivolumab and ipilimumab[36]. BsAbs simultaneously binding PD-(L)1 and CTLA-4 were developed to enhance anti-tumor efficacy and reduce toxicity and TRAEs. TRAEs occurred in 73.6% of patients and grade ≥ 3 TRAEs occurred in 11.3% of patients receiving AK104. The incidence of grade ≥ 3 TRAEs was clearly reduced compared to combination nivolumab and ipilimumab. However, the incidence of grade ≥ 3 TRAEs was 42.2% in patients receiving KN046. In addition, investigation of biomarkers warrants further study. A recent study showed that CD74 can be used as a

Table 2 Clinical trials of bsAbs for NSCLC as monotherapy that have posted results

NCT number	Phase	Indications	Enrollment	BsAb	Target	Treatment	Outcomes				
							ORR	mPFS	mOS	TRAE	Grade ≥ 3 TRAE
NCT03838848	2	Metastatic NSCLC who failed first line treatment	64	KN046	PD-1 × CTLA-4	KN046	14.1%	3.70 months	18.40 months	NA	42.2%
NCT04172454	1b/2	Patients who had failed previous platinum-based doublet chemotherapy and were immunotherapy naïve	30	AK104	PD-1 × CTLA-4	AK104	10.0%	1.91 months	19.62 months	76.7%	10.0%
NCT02517398	1	Adults with advanced NSCLC that progressed following chemotherapy and was primary refractory or had acquired resistance to anti-PD-(L)1 treatment	62	M7824	PD-L1 × TGF-β	M7824	4.8%	1.40 months	7.30 months	73.5%	22.9%
NCT03774979	1	PD-L1+ advancer/metastatic NSCLC	57	SHR-1701	PD-1 × TGF-β	SHR-1701	44.2%	NA	NA	NA	21.1%
NCT02609776	1	EGFR Exon 20 Insertion–Mutated NSCLC	50	amivantamab	EGFR × c-MET	Amivantamab	40.0%	8.30 months	22.80 months	NA	6.0%
NCT05194982	1	NSCLC (EGFR mut)	40	BL-B01D1	EGFR × HER3	BL-B01D1	67.5%	5.60 months	NA	NA	NA
		NSCLC (EGFRwt)	62				40.3%	5.40 months	NA	NA	NA
NCT04995523	1/2	Advanced NSCLC who had prior CPI treatment and a PD-L1 tumor proportion score ≥ 1%	80	AZD2936	PD-1 × TIGIT	AZD2936	NA	NA	NA	46.3%	5.0%

BsAb, bispecific antibody; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; c-MET, cellular-mesenchymal epithelial transition; HER3, human epidermal growth factor receptor 3; mOS, median overall survival; mPFS, median progression free survival; NSCLC, non-small cell lung cancer; ORR, overall response rate; PD-L1, programmed death-ligand 1; PD-1, programmed death-1; TGF-β, transforming growth factor β; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TRAE, treatment related adverse event.

biomarker to predict the treatment response to AK104. Patients with high expression of CD74 protein had superior progression-free survival (PFS) and overall survival (OS)[37]. In addition, among receiving KN046, those with high CD8 expression had a longer median (m)OS and combination PD-L1 and CD8 had the potential to predict the KN046 response[38].

PD-1/PD-L1 × TIM-3

TIM-3, encoded by HAVCR2, is a marker of dysfunctional CD8+ T cells[39]. Antibodies targeting TIM-3 promote an antitumor immune response that relies on T cells and IFN-γ[40]. Alternative immune inhibitory molecules, especially TIM-3, might lead to adaptive resistance in patients receiving anti-PD-1 therapy by increasing expression; resistance is prevented when anti-PD-1 antibodies are combined with anti-TIM-3 antibodies[41]. Co-blocking PD-1 and TIM-3 can lead to regression of tumors in preclinical models and enhance the antitumor T-cell response in patients with advanced tumors. Nearly 60% of regulatory T cells (Tregs) in lung cancer patients express TIM-3[39].

AZD7789

AZD7789 is a novel IgG1 bsAb that simultaneously binds PD-1 and TIM-3. AZD7789 may overcome anti-PD-1 resistance. The safety and preliminary efficacy results of a phase 1/2 clinical trial (NCT04931654) were presented at 2023 European Society for Medical Oncology (ESMO). Among 39 patients with stage IIIB-IV NSCLC receiving previous anti-PD-(L)1 treatment, 82% experienced TEAEs, 23% of which were grade ≥ 3. TRAEs occurred in 41% of patients and no grade ≥ 3 TRAEs were observed. The most common TRAE was asthenia, which occurred in 8% of patients. Among all 18 patients evaluated, 7 had stable disease[42].

TIM-3 is a biomarker of exhausted T cells and the potential mechanism of adaptive resistance. However, few bsAbs targeting PD-(L)1 and TIM-3 have been developed for NSCLC treatment. Researchers have developed an experimental system to predict the response of bsAbs targeting PD-(L)1 and TIM-3 based on multi-omic analyses[43], which may help more accurately select the population most likely to benefit.

PD-1/PD-L1 × LAG-3

LAG-3/CD223 is a type I transmembrane protein expressed on CD4+ and CD8+ T cells, natural killer (NK) cells, and natural killer T (NKT) cells. LAG-3/CD223 is constitutively expressed on plasmacytoid dendritic cells (pDCs) and Tregs. LAG-3 can be cleaved to form soluble LAG-3 (sLAG-3), which is positively correlated with T cell antitumor activity and DC migration. LAG-3 is expressed on TILs in 41.5% of NSCLC patients[44]. LAG-3 expression in NSCLC cells was not reported until a study in 2020 revealed that LAG-3 is expressed in lung cancer cell lines and that LAG expression is associated with the NSCLC clinical stage[45]. LAG-3 expression correlates with recurrence-free survival (RFS), PD-1 expression on TILs, and PD-L1 expression on tumor cells in patients with NSCLC[46]. LAG-3 enhances the ability of Tregs to exert inhibitory effects and inhibits CD8+ effector T cell function[25]. Dual blockade of the LAG-3 and PD-1 pathways can achieve a more effective antitumor response compared to blocking LAG-3 or PD-1/PD-L1 alone[47].

MGD013/tebotelimab

MGD013, also known as tebotelimab, is a first-in-class PD-1 × LAG-3 bsAb containing an Fc fragment that enhances the antitumor T-cell response compared to anti-PD-1 and anti-LAG-3 antibodies alone or in combination and can restore the function of exhausted T cells[48,49]. NCT03219268, a phase 1 study, was conducted to evaluate the efficacy of MGD013 in patients with unresectable or metastatic neoplasms. Among 29 NSCLC patients, the ORR was 17.2% and the DCR was 58.6%. The incidence of grade ≥ 3 TRAEs was 18% and the most common TRAEs were fatigue (15.6%), rash (8.3%), and hypothyroidism (7.8%)[50].

LAG-3 is expressed on exhausted T cells and targeting LAG-3 may reinvigorate antitumor immunity[51]. A recent study revealed that LAG-3^{hi}PD-1^{hi} memory CD4+ T cells are potential biomarkers for predicting the response to PD-1 × LAG-3 bsAb[52]. MGD013 showed a satisfactory safety profile with 18% grade ≥ 3 TRAEs in NSCLC treatment. However, the paucity of enrolled patients and the dearth of relevant clinical trials both point to the importance of further research.

BsAbs targeting checkpoints and non-checkpoints

Several non-checkpoint molecules, such as growth factors (GFs) and cytokines, also affect the intensity of the antitumor immune response in addition to immune checkpoints. GFs and cytokines exert pro- or anti-tumor effects on the initiation and progression of malignancies. Numerous antibodies targeting GFs or cytokines are approved for monotherapy or combination therapy with ICIs for several cancer types[23].

PD-1/PD-L1 × vascular endothelial growth factor (VEGF)

VEGF (also referred to as VEGFA) has crucial roles in vascular homeostasis in various tissues, the progression and metastasis

of several cancers, and retinopathologic changes in eye diseases that cause blindness. VEGF is correlated with invasion, metastasis, recurrence, and prognosis in the majority of human cancers[53]. MAbs that target VEGF, such as bevacizumab, have shown potent antitumor effects in several human cancer types.

AK112/ivonescimab

AK112 (also known as ivonescimab) is a novel IgG1 tetravalent PD-1 × VEGF bsAb that is designed to overcome PD-1-mediated immunosuppression and concurrently suppress tumor angiogenesis in the TME. AK112 was developed *via* TETRABODY technology. AK112 exhibits enhanced binding affinity and antitumor effects[54]. According to the results presented at the 2024 WCLC from NCT05499390, a phase 3 trial testing AK112 in patients with PD-L1-positive advanced NSCLC, AK112 exhibited excellent antitumor ability and tolerable toxicity. Among all 398 enrolled patients, 198 received AK112 and 200 received pembrolizumab. Notably, among patients who received AK112, the ORR was 50% and the DCR was 89.9%. The mPFS was 11.14 months. Among patients who received pembrolizumab, the ORR was 38.5% and the DCR was 70.5%. The mPFS was 5.82 months. Among patients who received AK112, TRAEs occurred in 89.9% and grade ≥ 3 TRAEs occurred in 29.4%. The most common TRAEs were proteinuria (31.5%), increased aspartate aminotransferase activity (19.8%), and hypercholesterolemia (16.2%). AK112 significantly improved the mPFS compared to pembrolizumab (11.14 months *vs.* 5.82 months) and showed tolerable toxicity.

PM8002

PM8002 is an IgG1 bsAb that targets PD-L1 and VEGFA. The tolerance, safety, PK, and antitumor efficacy of PM8002 have been evaluated in patients with advanced solid tumors in a phase 1/2a clinical trial (ChiCTR2000040552). Among the 263 enrolled patients, the overall ORR was 15.2% and the DCR was 75.4%. Among 27 patients with NSCLC, the ORR was 18.5%. TRAEs occurred in 181 (68.8%) patients, 48 (18.3%) of whom were grade ≥ 3. The most frequent TRAEs were proteinuria (17.5%), hypertriglyceridemia (11.4%), and increased aspartate transaminase (AST) levels (9.9%)[55]. In addition, according to the results of the clinical trial, NCT05918445, which was presented at the 2024 American Society of Clinical Oncology (ASCO) meeting, among 36 evaluable EGFR-T790M-positive NSCLC patients the ORR with PM8002 was 19.4% and the DCR was 69.4%. Among all 61 enrolled patients, TRAEs occurred in 85.2% and grade ≥ 3 TRAEs occurred in 19.7%. The most common TRAEs were proteinuria (54.1%), hypertension (24.6%), hypothyroidism (21.3%), and hypoalbuminemia (19.7%).

The combination of AK112 and chemotherapy was first approved in May 2024 for the treatment of NSCLC patients who progressed after tyrosine kinase inhibitor (TKI) therapy[56]. We will discuss this combination in more detail in the combination therapy section. However, studies on biomarkers for bsAbs targeting PD-(L)1 and VEGF are inadequate.

PD-1/PD-L1 × transforming growth factor β (TGF-β)

TGF-β is a multifunctional polypeptide cytokine that has a complex and paradoxical role in tumors at different phases of tumor progression. TGF-β exerts tumor-inhibiting effects in the early stage and tumor-promoting effects in the late stage[57]. In addition to supporting tumor growth, metastasis, invasion, and therapy resistance, recent research has revealed that TGF-β regulates cancer metabolic reprogramming and drives tumor metabolism in the tumor microenvironment (TME)[58]. TGF-β facilitates lung cancer cell invasion and metastasis by reducing SH2B3 to increase anoikis resistance and epithelial–mesenchymal transition (EMT)[59]. Although TGF-β seems to be a promising therapeutic target and anti-TGF-β antibodies have been evaluated in several clinical experiments, anti-TGF-β therapy has not achieved satisfactory results because of adverse systemic effects. PD-1/PD-L1 and TGF-β activate immunosuppressive signaling pathways in the TME and inhibiting TGF-β is helpful for restoring sensitivity to anti-PD-L1 therapy. BsAbs that simultaneously target PD-1/PD-L1 and TGF-β exhibit superior antitumor activity[60].

Bintrafusp alfa (BA)/M7824

BA, also known as M7824, is a novel bsAb that fuses the extracellular domain of human TGF-β receptor II (TGF-βRII) to the C-terminus of heavy chains in the IgG1 anti-PD-L1 antibody to sequester TGF-β more effectively in the TME. BA enhances T-cell activity and increases the number of TILs to exert better antitumor effects through increased affinity-based binding to TGF-β and increased sequestration of TGF-β by targeting PD-L1 on the cell surface and co-localization[61]. BA shows promising antitumor efficacy and manageable tolerability according to the results of NCT02517398, a phase 1 trial evaluating BA in patients with metastatic or locally advanced solid tumors. The ORR was 17.5% in patients receiving a 500-mg dose and 25.0% in patients receiving a 1200-mg dose. The ORR was 21.3% in all 80 patients. TRAEs occurred in 55 patients (68.8%) and 23 (28.8%) were grade ≥ 3. The most common TRAEs were pruritus (21.3%), maculopapular rush (18.8%),

and decreased appetite (12.5%)[62]. In addition, the results for advanced NSCLC patients who progressed to primary refractory or acquired resistance to anti-PD-L1 therapy after chemotherapy have recently been published. Among 62 patients, the ORR was 4.8%, the mPFS was 1.4 months, and the mOS was 7.3 months. TRAEs occurred in 73.5% of patients, 22.9% of whom had grade ≥ 3 TRAEs. The most common TRAEs were asthenia (27.7%), pruritus (22.9%), and decreased appetite (16.9%; **Table 2**)[63]. However, according to the results of a phase 3 trial (NCT03631706) that compared the efficacy of M7824 and pembrolizumab in PD-L1+ NSCLC patients, those treated with M7824 had a shorter mOS (21.1 months *vs.* 22.1 months) and more TRAEs[64].

SHR-1701

SHR-1701 is a novel PD-L1 × TGF-β bsAb that fuses an anti-PD-L1 mAb with the TGF-βRII extracellular domain. NCT03774979 is an open-label, phase 1 study that was conducted in 2021 to investigate the safety, tolerability, and clinical activity of SHR-1701 in patients with metastatic or locally advanced solid tumors. According to the results presented at the 2021 ASCO meeting, in the cohort consisting of EGFR+ NSCLC patients TRAEs occurred in 50.3% of patients, grade 3 TRAEs occurred in 7.4% of patients, and no grade 4 or 5 TRAEs occurred among all 27 enrolled patients. The most common TRAEs were increased ALT levels (14.8%), fatigue (11.1%), anemia (11.1%), and anorexia (11.1%). Among the 24 evaluable patients, the ORR was 16.7% and the DCR was 50.0% (**Table 2**).

Given the high-level expression of PD-(L)1 and TGF-β in the TME[65], bsAbs that simultaneously target PD-(L)1 and TGF-β to precisely enrich the TME and reduce side effects were developed. The two bsAbs showed encouraging antitumor effects and manageable tolerability with < 30.0% developing grade ≥ 3 TRAEs. However, the latest results of the relevant trials have not been published and the exploration of biomarkers was inadequate. In addition, a phase 2 trial (NCT04580498) is underway to explore the efficacy and safety of SHR-1701 as neoadjuvant therapy, the latest results of which are promising[66].

Connection between immune cells and tumor cells

CD3 is a critical surface molecular marker of T cells. BsAbs can simultaneously bind CD3 on the surface of T cells and tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs) on the surface of tumor cells, thereby redirecting T cells to tumor cells and facilitating binding (**Figure 4B**). This binding can lead to T-cell activation and immune synapse formation[67], through which activated T cells secrete perforin and other granzymes, eventually leading to tumor cell lysis[68]. These bsAbs are also called T-cell engagers, which can overcome tumor immune evasion caused by loss of MHC class I expression[69]. In addition, bsAbs linking other molecules on the surface of T cells, such as PD-1, to TAAs/TSAs can also redirect T cells and facilitate binding with tumor cells[70]. In addition to targeting CD3 to recruit an extensive range of T cells, several effector cells or immune cell subtypes, such as γδ T cells, NK cells, and invariant natural killer T (iNKT) cells, can be promising targets for bsAbs engaging immune and tumor cells[71].

CD3 × epithelial cell adhesion molecule (EpCAM)

EpCAM is a 39–42 kDa L-type transmembrane glycoprotein consisting of 314 amino acids that are encoded by the TACSTD1 gene. EpCAM is often overexpressed in many tumors, such as rectal cancer, NSCLC, esophageal cancer, and prostate cancer[72,73]. EpCAM directly affects the cell cycle and cell proliferation and can upregulate the proto-oncogenes, c-myc and cyclin A/E[74]. Therefore, the inhibition of EpCAM can restrain the growth of tumor cells.

Catumaxomab

Catumaxomab is a quadroma bsAb targeting CD3 and EpCAM that was approved for the treatment of patients with EpCAM-positive malignant ascites by the European Union in 2009 and is also the first bsAb approved for marketing[75]. Although catumaxomab was withdrawn from the market for commercial reasons in 2017; relevant studies are still ongoing. Because 86.5% of NSCLC patients express EpCAM[76], we believe that CD3 × EpCAM bsAbs may be effective for treating NSCLC. Unfortunately, the results of a phase 1 study assessing the safety and tolerability of intravenous catumaxomab treatment in NSCLC patients were disappointing[77].

CD3 × carcinoembryonic antigen (CEA)

CEA is a common tumor biomarker that is overexpressed in several solid tumors, such as colorectal carcinoma (CRC), pancreatic carcinoma (PanCa), gastric carcinoma (GC), NSCLC, and other carcinomas[78]. CEA is a 180 kDa glycoprotein that is anchored to the cell surface *via* glycosylphosphatidylinositol (GPI) and belongs to the CEA-related cell adhesion molecules (CEACAMs) immunoglobulin family. CEA is involved in

endothelial cell functions, such as cell proliferation, adhesion, and migration.

CEA TCB/RO686889

CEA TCB is a novel IgG-based bsAb that binds the CD3 epsilon chain (CD3e) located on T cells and to the CEA located on tumor cells. CEA TCB is characterized by a longer circulatory half-life, T-cell activity within tumors, and killing of tumor cells independent of immune cell activation in the peripheral blood. The CEA TCB molecule is characterized by monovalent binding to CD3e, owing to a novel Fc fraction that abrogates binding to complement components and Fc gamma receptors (FcγRs). The level of CEA expression is the best indicator for predicting the activity of CEA TCB and 10,000 CEA binding sites/cells is the threshold for the activity of CEA receptors. Moreover, CEA-TCB efficiently affects poorly infiltrated and non-inflamed T cells and can alleviate the immunosuppressive TME by converting immune-cold, PD-L1-negative tumors to immune-hot, PD-L1-positive tumors[79]. A phase 1/2 study (NCT03337698) focusing on the effects of several combination immunotherapy-based therapies, including CEA TCB, on patients with metastatic NSCLC is ongoing.

In addition to the above TAAs/TSAs, several other tumor markers are targeted by bsAbs to bind immune and tumor cells. An increasing number of new drugs, such as GEN1044 (5T4 × CD3) and CC-1 (PSMA × CD3), are currently in preclinical or clinical trials. Although numerous encouraging results have been reported, several clinical trials evaluating bsAbs for NSCLC have not published any results. The relevant clinical trials are listed in **Table 3**.

In this section we introduce the use of bsAbs for immunotherapy in NSCLC patients. Most bsAbs exert the antitumor effects by binding dual immunomodulatory molecules and few bsAbs connect immune and tumor cells. Although most of these drugs exhibit encouraging efficacy and acceptable safety profiles, studies of biomarkers are inadequate. Therefore, we expect more studies involving biomarkers to help select the population most likely to benefit more precisely.

BsAbs for targeted therapy

BsAbs that block two signaling pathways

Targeted therapies, such as those that inhibit signaling pathways by targeting receptor tyrosine kinases (RTKs), also have crucial roles in the treatment of malignancies. RTKs constitute a cell-surface receptor family that have a critical role in modulating cell proliferation, differentiation, migration, survival, and metabolism. RTK mutations and abnormal activation of intracellular signaling pathways are important pathogenic mechanisms of numerous cancers under pathologic conditions. Blocking or attenuating RTK activity is a promising treatment strategy[62]. Several TKIs and monoclonal antibodies, such as trastuzumab, cetuximab, and cabozantinib, have exhibited potent antitumor activity. However, drug resistance resulting from the activation of alternative signaling pathways or other mutations severely limits the application of TKIs[80]. BsAbs that simultaneously target two signaling pathways may enhance the antitumor response and overcome drug resistance (**Figure 4C**).

EGFR × cellular-mesenchymal epithelial transition (c-MET)

EGFR, a member of the ERBB receptor tyrosine kinase superfamily, is one of the most essential RTKs and has crucial roles in the proliferation, angiogenesis, and metastasis of cancer cells. Moreover, EGFR mutations have crucial roles in lung cancers, especially NSCLC, among which 15% of patients present with EGFR mutations[80]. c-MET, a member of the RTK family, is a proto-oncogene that promotes several biological processes, such as the proliferation, development, migration, and invasion of cells under physiologic conditions, and can lead to oncogenesis and tumor progression, especially in NSCLC. Interestingly, in patients with acquired resistance, the MET gene copy number (GCN) is increased and the combination of MET and EGFR inhibition can restore drug sensitivity[81]. This phenomenon suggests that simultaneously blocking EGFR and MET may enhance antitumor effects.

Amivantamab

Amivantamab, a fully human monoclonal IgG1 bsAb developed *via* the Genmab DuoBody® technology platform, exerts antitumor effects by simultaneously binding EGFR and c-MET to inhibit downstream signaling cascades. Amivantamab has an enhanced ability to bind to FcγRIIIa and enhances antitumor activity through several mechanisms, such as Fc-mediated ADCC *via* interactions with Fcγ receptors on NK cells and cytokine production and phagocytosis *via* interactions with Fcγ receptors on monocytes and macrophages[3]. A phase 1 study, NCT02609776 (CHRYSALIS), evaluated the safety, PK and preliminary efficacy of amivantamab in advanced NSCLC patients. Among 39 response-evaluable patients with EGFR exon 20 insertion mutations, the ORR was 36%, and the clinical benefit rate was 67.0%. Among all 50

Table 3 Ongoing clinical trials evaluating bsAbs for immunotherapy in patients with NSCLC or solid tumors

NCT number	Study state	Indications	Treatment	Target	Phase
NCT05585034	Recruiting	Advanced solid tumors	XmAbÂ®808	B7-H3 × CD28	Phase 1
NCT05607498	Recruiting	Locally advanced/metastatic solid tumors or relapse/ refractory lymphoma	EMB07	CD3 × ROR1	Phase 1
NCT05461287	Recruiting	Advanced solid tumor	QLS31904	DLL3 × CD3	Phase 1
NCT05403554	Recruiting	Mesothelin expressing solid cancers	NI-1801	Mesothelin × CD47	Phase 1
NCT05263180	Recruiting	Advanced or metastatic Solid tumors	EMB-09	PD-1 × OX40	Phase 1
NCT05293496	Recruiting	Advanced solid tumors	Lorigerlimab	PD-1 × CTLA-4	Phase 1
NCT05645276	Recruiting	Advanced malignant tumors	AK129	PD-1 × LAG-3	Phase 1
NCT05577182	Recruiting	Advanced malignancies	INCA32459-101	PD-1 × LAG-3	Phase 1
NCT04777084	Recruiting	NSCLC	IBI318	PD-1 × PD-L1	Phase 1
NCT05028556	-	Metastatic or locally advanced solid tumors	Y101D	PD-1 × TGF-β	Phase 1
NCT03809624	Recruiting	Solid tumors	INBRX-105	PD-L1 × 4-1BB	Phase 1
NCT05200013	Recruiting	Advanced solid tumors	BAT7104	PD-L1 × CD47	Phase 1
NCT05101109	Recruiting	Progressive, locally advanced (unresectable) or metastatic solid tumors	BL501	PD-L1 × LAG-3	Phase 1
NCT05638334	Recruiting	Advanced solid tumors	S09501	PD-L1 × OX-40	Phase 1
NCT05537051	Not yet recruiting	Advanced solid tumors	PM8001	PD-L1 × TGF-β	Phase 1
NCT04954456	-	Advanced or metastatic malignancies	QLS31901	PD-L1 × TGF-β	Phase 1
NCT05607563	Recruiting	Advanced tumor	PM1009	TIGIT × PVRIG	Phase 1
NCT05180474	Recruiting	Malignant solid tumors	GEN1047	CD3 × 5T4	Phase 1/2
NCT05543330	Not yet recruiting	Malignant pleural effusions caused by NSCLC	M701	EpCAM × CD3	Phase 1/2
NCT05559541	Recruiting	Advanced solid tumors	AK104	PD-1 × CTLA-4	Phase 1/2
NCT04597541	Active, not recruiting	Advanced solid tumors	Ivonescimab	PD-1 × VEGF	Phase 1/2
NCT05689853	Recruiting	Advanced solid tumors	Ivonescimab	PD-1 × VEGF	Phase 1/2
NCT05229497	Recruiting	Advanced malignant tumors	Ivonescimab	PD-1 × VEGF	Phase 1/2
NCT05214482	Recruiting	Advanced malignant tumors	Ivonescimab	PD-1 × VEGF	Phase 1/2
NCT05159388	Recruiting	Solid tumors	PRS-344/S095012	PD-L1 × 4-1BB	Phase 1/2
NCT05425602	Not yet recruiting	Advanced/metastatic solid tumors	MAX-40279-01	PD-L1 × CTLA-4	Phase 1/2
NCT03440437	Active, not recruiting	Advanced malignancies	FS118	PD-L1 × LAG-3	Phase 1/2
NCT04262466	Recruiting	Select advanced solid tumors	IMC-F106C	PRAME × CD3	Phase 1/2

Table 3 Continued

NCT number	Study state	Indications	Treatment	Target	Phase
NCT05102214	Recruiting	Locally advanced or metastatic solid tumors	HLX301	TIGIT × PD-L1	Phase 1/2
NCT05390528	Recruiting	Locally advanced/metastatic solid tumors or lymphoma	HLX301	TIGIT × PD-L1	Phase 1/2
NCT05377658	Not yet recruiting	Resectable NSCLC	AK104	PD-1 × CTLA-4	Phase 2
NCT05215067	Recruiting	Advanced NSCLC	AK104	PD-1 × CTLA-4	Phase 2
NCT05247684	Not yet recruiting	Resectable NSCLC	AK112	PD-1 × VEGF	Phase 2

CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; DLL3, delta-like ligand 3; EpCAM, epithelial cell adhesion molecule; LAG-3, lymphocyte activation gene 3; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PVRIG, poliovirus receptor-related immunoglobulin domain-containing; PRAME, preferentially expressed antigen in melanoma; PSMA, prostate-specific membrane antigen; ROR1, receptor tyrosine kinase-like orphan receptor 1; TGF-β, transforming growth factor β; TIGIT, T cell immunoreceptor with Ig and ITIM domains; VEGF, vascular endothelial growth factor.

patients, 36.0% had grade ≥ 3 TRAEs. The most common AEs were rash (72.0%), infusion-related reactions (60.0%), and paronychia (34.0%)[82]. Based on the results of the CHRYSALIS trial, the FDA approved amivantamab on 21 May 2021 for the treatment of adult NSCLC patients with EGFR exon 20 insertion mutations that progressed during or after platinum-based chemotherapy. In addition, researchers updated the results concerning the therapeutic effects of amivantamab on NSCLC patients with MET exon 14 skipping mutations in the MET-2 cohort of CHRYSALIS at the 2022 ASCO meeting. Among 36 patients with ≥ 1 baseline disease assessment, the overall ORR was 33.3% and the clinical benefit rate was 58.3%[83]. Amivantamab shows encouraging antitumor activity in patients with MET exon 14 skipping mutations, including patients who have received prior MET inhibitor treatment. In addition to intravenous formulations, a subcutaneous (SC) formulation of amivantamab was developed *via* the Halozyme Therapeutics ENHANZE™ technology. The safety and PK of amivantamab SC administration were evaluated in a phase 1b study (NCT04606381/PALOMA).

MCLA-129

MCLA-129 is a novel ADCC-enhanced human EGFR × c-MET biclonic bsAb that was developed to overcome c-MET signaling-independent EGFR TKI resistance. MCLA-129 exerts antitumor effects *via* several mechanisms, such as ADCC and ADCP, and inhibits downstream signaling of EGFR and c-MET. MCLA-129 was shown to inhibit EGFR and c-MET activity in NSCLC cell lines to induce tumor regression and overcome c-MET signaling-independent EGFR TKI resistance[84]. According to the results of an open-label, dose-escalation, and expansion phase 1/2 study (NCT04868877), which evaluated MCLA-129 in patients with advanced NSCLC and other solid tumors, MCLA-129 showed preliminary antitumor activity and a favorable safety profile. Among the 13 evaluable patients, the DCR was 38.5% and no dose-limiting toxicity was observed[85]. In addition, a phase 1/2 study (NCT04930432) is underway to evaluate the safety, pharmacokinetics (PK), and antitumor effects of MCLA-129 in patients with advanced NSCLC and other solid tumors.

The above two bsAbs, which target EGFR and c-MET, both showed satisfactory antitumor efficacy. Amivantamab is undoubtedly one of the most widely used bsAbs in NSCLC treatment. Although amivantamab improves PFS compared to standard osimertinib, amivantamab also causes increased toxicity[86]. Importantly, structural complexity may increase immunogenicity and increase side effects. Amivantamab caused an infusion-related reaction in 69.0% of patients when administered for the first time. Researchers have tried to maintain antitumor efficacy and decrease side effects by optimizing the dosage forms. For example, subcutaneous amivantamab maintained efficacy and reduced administration time to improve tolerability in the PALOMA-3 study[87]. In addition, the exploration of biomarkers is worthy of attention. A recent study revealed that a high level of amphiregulin (AREG) expression is related to better amivantamab activity. AREG is a potential biomarker for predicting the response to amivantamab[88].

MET × MET

REGN5093

REGN5093 inhibits the growth of MET-driven tumor cells by concurrently binding two distinct epitopes of MET. NCT04077099 is a phase 1/2 study that evaluated the efficacy of REGN5093 in patients with MET-altered advanced NSCLC. Among the 36 patients who received the 2000-mg dose, a partial response occurred in 6 patients (16.7%). Among all 44 enrolled patients, 11 (25.0%) experienced grade ≥ 3 TEAEs[89].

Although MET plays a significant role in NSCLC progression, therapies targeting MET are not satisfactory because of the lack of predictive biomarkers[90]. Although the results revealed therapeutic effects and a manageable safety profile of REGN5093 in patients with MET-altered advanced NSCLC, additional clinical trials are needed.

HER2 × HER3

ERBB is a transmembrane RTK family consisting of ERBB1 (EGFR), ERBB2 (HER2), ERBB3 (HER3), and ERBB4 (HER4). Although HER3 has relatively weak tyrosine kinase activity owing to its ability to activate the signaling pathways of oncogenic EGFR and HER2, HER3 has still been linked to cancer[91]. Furthermore, HER3 expression is significantly associated with neuregulin-1 gene (NRG1) fusion in patients with lung cancer. NRG1 proteins, the ligands of HER3, induce HER3 to heterodimerize with HER2 to activate downstream ERBB-mediated signaling pathways. NRG1 fusions are uncommon in different kinds of cancers with an incidence of ≤ 1%, but in patients with invasive mucinous adenocarcinomas (IMAs) the incidence of NRG1 fusion is 10%–30%. Treatments targeting REBB are potential therapies for patients harboring NRG1 fusions[92].

Zenocutuzumab/MCLA-128

Zenocutuzumab, also known as MCLA-128, is a full-length human IgG1 biclonic bsAb that concurrently targets HER2 and HER3 and inhibits HER3 signaling at supramaximal NRG1 concentrations *via* a special "dock and block" mechanism. The arm targeting HER2 "docks" HER2 at the surface of tumor cells to increase the HER3 Fab concentration and the arm targeting HER3 "blocks" HER3 binding to NRG1 to prevent the formation of signaling dimers and downstream oncogenic signaling[93]. According to the results of NCT02912949, a phase 1/2 study evaluating the efficacy of zenocutuzumab in patients with solid tumors harboring an NRG1 fusion (eNRGy), zenocutuzumab showed encouraging antitumor efficacy and a well-tolerated safety profile. Among all 71 patients with measurable NRG fusions, the overall ORR was 34.0% and the ORR was 35.0% among 40 patients with NRG+ NSCLC. Among 208 patients who received zenocutuzumab monotherapy in phase 2, grade ≥ 3 adverse events occurred in < 5.0% of patients.

The prognosis of NSCLC patients with NRG1 fusion is not favorable because of the poor response to standard therapies[94]. Although NRG1 is a potential therapeutic target for NSCLC patients, the development of new drugs is not satisfactory. BsAbs targeting HER2 and HER3 exhibited encouraging efficacy in patients with NRG+ NSCLC, with a 35.0% ORR. Therefore, we look forward to more promising results.

BsADCs

BsADCs are novel antitumor drugs composed of an mAb conjugated to a cytotoxic agent *via* a chemical linker. ADCs combine the specific targeting ability of antibodies with the potent tumor killing effect of cytotoxic drugs to achieve superior antitumor efficacy[95]. BsADCs show more robust selectivity, enhanced internalization, and accelerated downstream cascades to exert greater antitumor activity and minimize toxicity compared to monospecific ADCs[96] (**Figure 4D**).

EGFR × HER3

BL-B01D1

BL-B01D1 is a novel EGFR × HER3 bsADC generated by attaching an EGFR × HER3 bsAb (SI-B001) to a novel inhibitor of topoisomerase I (Ed-01) *via* a stable cathepsin B cleavable linker. BL-B01D1 has superior tumor inhibitory effects in xenograft models compared to parental monospecific ADCs[97]. Based on results of NCT05194982, a phase 1 clinical study evaluating safety of BL-B01D1, PK and preliminary antitumor efficacy of BL-B01D1 in patients with locally advanced or metastatic solid tumors presented at the 2023 ESMO, BL-B01D1 showed encouraging antitumor efficacy and an adequate safety profile. Among 38 patients with EGFR mutant (EGFRmut) NSCLC, the ORR was 63.2% and the DCR was 89.5%. In addition, among 49 patients with EGFR wild-type (EGFRwt) NSCLC, the ORR was 44.0% and the DCR was 94.0%. The mPFS of patients with EGFRmut NSCLC and patients with EGFRwt NSCLC was 6.9 and 5.2 months, respectively. Among all 114 enrolled patients, the most common TRAEs were anemia (59.0%), leukopenia (59.0%), neutropenia (51.0%), and thrombocytopenia

(48.0%)[98]. These promising results in patients with NSCLC indicate that BL-B01D1 may be an effective drug for treating NSCLC.

Folate receptor alpha (FRα) × transient receptor potential vanilloid 6 (TRPV6)

CBP-1008

CBP-1008 is a novel bsADC that simultaneously targets FRα and TRPV6 and exhibits greater binding affinity to FRα than to TRPV6[99]. The safety, tolerance, and PK of CBP-1008 were assessed in patients with advanced solid tumors in a phase 1a/1b study (NCT04740398). Among all 178 enrolled patients, most AEs were mild-to-moderate. The most common grade ≥ 3 TRAEs were neutropenia (n = 85), decreased leukocyte count (n = 49) and anemia (n = 10). Among 82 evaluable platinum-resistant ovarian cancer (PROC) patients, the ORR was 25.6% and the DCR was 62.2%. According to the most recent results, CBP-1008 exhibited a manageable safety profile and antitumor efficacy in PROC patients[100]. We look forward to more clinical trials evaluating CBP-1008 in NSCLC patients because FRα and TRPV6 are highly expressed in lung cancer.

ADC is a novel and promising antitumor therapy. However, tumor heterogeneity, drug resistance, and TRAEs limit the use of ADCs. BsADCs could address the heterogeneity and resistance and maintain superior antitumor efficacy[101]. The exploration of the use of bsADCs in the treatment of NSCLC has been inadequate, and few results have been reported. Insufficient research on biomarkers has also limited the use of bsADCs.

In this section we introduce the use of bsAbs for targeted therapy in NSCLC patients. However, numerous clinical trials evaluating the use of bsAbs for targeted therapy in patients with NSCLC or other solid tumors have not published the results. The relevant clinical trials are listed in **Table 4**. EGFR is one of the most important targets for the targeted therapy of NSCLC. Although there are three generations of TKIs, drug resistance cannot be ignored. BsAbs targeting EGFR are potential ways to overcome drug resistance. In addition to monotherapy, combined therapy with bsAbs also exhibit promising efficacy and an acceptable safety profile. In May 2024 the combination of ivonescimab/AK112, pemetrexed, and carboplatin was first approved for patients with locally advanced or metastatic non-squamous NSCLC with EGFR mutations who progressed after TKI treatment[56]. In the next section we introduce combination bsAb therapies for the treatment of NSCLC.

Combination bsAb therapies

BsAbs + chemotherapy

SI-B001 + chemotherapy

SI-B001 is a novel EGFR × HER3 IgG-(scFv)2 bsAb that shows encouraging antitumor efficacy and a favorable safety profile when used alone or in combination with chemotherapy[102]. Docetaxel, a semisynthetic taxane, is one of the most important antitumor agents used in chemotherapy and can bind to β tubulin to arrest the cell cycle or induce apoptosis[103]. NCT05020457 is a phase 2 study that was conducted to evaluate the safety and efficacy of SI-B001 plus chemotherapy in patients with locally advanced or metastatic EGFR wild-type ALK wild-type NSCLC. According to the results presented at the 2023 ASCO meeting, among 22 evaluable patients in Schedule 2 of Cohort B who received SI-B001 in combination with docetaxel as second-line treatment after prior first-line anti-PD-(L)1 therapy plus platinum-based chemotherapy, the ORR was 45.5%, and the DCR was 68.2%. Among the 18 of 22 patients without actionable genomic alterations (AGA), the ORR was 50.0% and the DCR was 72.2%. Among all 55 enrolled patients, the most common grade ≥ 3 TRAEs were bone marrow suppression (17.0%), neutropenia (15.0%), and leukopenia (12.0%)[104].

AK112 + chemotherapy

AK112 is a novel bsAb that targets PD-1 and VEGF. Researchers presented work at the 2022 ASCO meeting that supported the promising antitumor efficacy and superior safety profile of AK112 in combination with chemotherapy. NCT04736823 is a phase 2 clinical trial that evaluated the efficacy of AK112 plus chemotherapy in NSCLC patients. The patients were divided into the following 3 cohorts: untreated NSCLC patients with wild-type EGFR/ALK (cohort 1); patients with EGFR mutations who failed prior anti-EGFR treatment or osimertinib treatment (cohort 2); and patients whose disease progressed after anti-PD-(L)1 therapy and platinum-based chemotherapy (cohort 3). The ORRs (DCRs) of cohorts 1, 2, and 3 were 53.5% (100%), 68.4% (94.7%), and 40.0% (80.0%), respectively. Among all 133 enrolled patients, TRAEs occurred in 115 patients (86.5%) and grade ≥ 3 TRAEs occurred in 38 patients (28.6%; **Table 5**)[105]. The latest results of a phase 3 trial (HARMONi-A) that evaluated AK112 plus chemotherapy in patients with EGFR-mutant non-squamous NSCLC

Table 4 Ongoing clinical trials evaluating bsAbs for targeted therapy in patients with NSCLC or solid tumors

NCT number	Study state	Indications	Treatment	Target	Phase
NCT04501770	Not yet recruiting	HER2-positive advanced solid tumors	M802	CD3 × HER2	Phase 1
NCT05442996	Not yet recruiting	Advanced or metastatic solid tumors	HLX35	EGFR × 4-1BB	Phase 1
NCT05360381	Active, not recruiting	Advanced or metastatic solid tumors	HLX35	EGFR × 4-1BB	Phase 1
NCT05150457	Recruiting	Advanced solid tumors	BNA035	EGFR × 4-1BB	Phase 1
NCT05387265	Recruiting	Advanced solid tumors	CX-904	EGFR × CD3	Phase 1
NCT04603287	Recruiting	Locally advanced or metastatic epithelial tumors	SI-B001	EGFR × HER3	Phase 1
NCT03526835	Recruiting	Advanced solid tumors	MCLA-158	EGFR × LGR5	Phase 1
NCT03842085	Recruiting	HER2 positive recurrent or metastatic malignant solid tumor	MBS301	HER2 × HER2	Phase 1
NCT05320874	Not yet recruiting	Advanced HER2-positive or expressing solid tumors	KM257	HER2 × HER2	Phase 1
NCT05380882	Recruiting	Advanced cancers	TQB2930	HER2 × HER2	Phase 1
NCT03650348	-	HER2-positive solid tumors	PRS-343	HER2 × 4-1BB	Phase 1
NCT05076591	Recruiting	HER2-expressing advanced solid tumors	IMM2902	HER2 × SIRPα	Phase 1
NCT04844073	Recruiting	Advanced or metastatic cancer	MVC-101 (TAK-186)	EGFR × CD3	Phase 1/2
NCT04930432	Recruiting	Advanced NSCLC and other solid tumors	MCLA-129	EGFR × c-MET	Phase 1/2
NCT05498389	Not yet recruiting	EGFR mutant lung cancer	EMB-01	EGFR × c-MET	Phase 1/2
NCT05523947	Recruiting	HER2 positive locally advanced or metastatic solid tumor	YH32367	HER2 × 4-1BB	Phase 1/2
NCT05299125	Recruiting	Recurrent/metastatic NSCLC with EGFR mutations	Amivantamab	EGFR × c-MET	Phase 2
NCT05588609	Recruiting	With or without molecularly defined cancers	Zenocutuzumab	HER2 × HER3	Phase 2
NCT05388669	Recruiting	EGFR-mutated advanced or metastatic NSCLC	Amivantamab	EGFR × c-MET	Phase 3
NCT04100694	Available	Advanced NRG1-fusion positive solid tumor	MCLA-128	HER2 × HER3	Not applicable

c-MET, cellular-mesenchymal epithelial transition; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; LRG5, leucine rich repeat containing G protein-coupled receptor 5; NRG1, neuregulin-1 gene; SIRPα, signal regulatory protein α.

were presented at the 2024 ASCO meeting. The ORR was 35.4% and the DCR was 83.2% in 161 patients who received placebo plus chemotherapy. The ORR was 50.6% and the DCR was 93.1% in 161 patients who received AK112 plus chemotherapy. TRAEs occurred in 98.1% of patients and grade ≥ 3 TRAEs occurred in 54.0% of patients. The most common TRAEs were leukopenia (65.2%), anemia (60.2%), neutropenia (60.2%), and thrombocytopenia (47.8%).

Table 5 Clinical trials of bsAbs for NSCLC as combined therapy that have posted results

NCT number	Phase	Indications	Enrollment	BsAb	Target	Treatment	Outcomes				
							ORR	mPFS	mOS	TRAE	Grade ≥ 3 TRAE
NCT04736823	2	Untreated advanced NSCLC, had no EGFR or ALK gene modifications	43	AK112	PD1 × VEGF	AK112 plus chemotherapy	53.5%	Not reach	NA	92.5%	22.5%
		Advanced NSCLC with EGFR-sensitive mutations, failed previous EGFR-TKI therapy	19			AK112 plus chemotherapy	68.4%	8.5 months	NA	90.7%	30.2%
		Advanced NSCLC who failed systemic platinum-based chemotherapy and anti-PD-1/PD-L1 treatments	20			AK112 plus chemotherapy	40.0%	7.5 months	NA	91.6%	26.5%
NCT04054531	2	Advanced NSCLC	87	KN046	PD-L1 × CTLA-4	KN046 plus chemotherapy	50.6%	5.8 months	26.6 months	92.0%	25.3%
NCT03838848	2	Patients with metastatic NSCLC who failed prior EGFR-TKI(s)	26	KN046	PD-L1 × CTLA-4	KN046 plus chemotherapy	26.9%	5.5 months	20.2 months	92.3%	57.7%
NCT04538664	1	Patients with advanced NSCLC with EGFR exon 20 insertions who had not received previous systemic therapy	153	Amivantamab	EGFR × c-MET	Amivantamab-chemotherapy	73.0%	17.2 months	24.4 months	100%	75.0%
NCT04988295	1	Locally advanced or metastatic EGFR-mutated NSCLC with disease progression on or after osimertinib monotherapy	263	Amivantamab	EGFR × c-MET	Amivantamab-lazertinib-chemotherapy	63.0%	6.3 months	NA	100%	92.0%
			131			Amivantamab-chemotherapy	64.0%	8.3 months	NA	100%	72.0%
NCT04487080	3	Patients with treatment-naïve, EGFR-mutated locally advanced or metastatic NSCLC	429	Amivantamab	EGFR × c-MET	Amivantamab plus lazertinib	86.0%	23.7 months	NA	100%	75.0%
NCT03774979	1	Recurrent or metastatic non-squamous NSCLC who had failed ≤ 2 lines of systemic treatment, driver gene-negative	10	SHR-1701	PD-1 × TGF-β	SHR-1701 plus bevacizumab	10.0%	6.2 months	NA	90.4%	29.4%

BsAb, bispecific antibody; c-MET, cellular-mesenchymal epithelial transition; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EGFR, epidermal growth factor receptor; mOS, median overall survival; mPFS, median progression free survival; NSCLC, non-small cell lung cancer; ORR, overall response rate; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TGF-β, transforming growth factor; TRAE, treatment related adverse event; VEGF, vascular endothelial growth factor.

Several clinical trials, such as Impower150, are underway to evaluate the efficacy and safety of atezolizumab plus bevacizumab and chemotherapy in NSCLC patients. According to the results posted for Impower150, TRAEs were observed in 94.1% patients and grade ≥ 3 TRAEs were observed in 60.4% patients[106]. Although grade ≥ 3 TRAEs occurred in 54.0% of patients receiving AK112 plus chemotherapy, which was lower than that patients receiving atezolizumab plus bevacizumab and chemotherapy, reducing toxicity must still be considered. The combination of AK112, pemetrexed, and carboplatin was first approved in May 2024 for patients with locally advanced or metastatic non-squamous NSCLC with EGFR mutations who had progressed after TKI treatment. However, the TRAEs cannot be ignored, with 98.1% of patients experiencing TRAEs and 54.0% with grade ≥ 3 TRAEs.

KN046 + chemotherapy

KN046 is a novel PD-L1 × CTLA-4 bsAb. NCT04054531 is a phase 2 clinical trial that was conducted to evaluate KN046 plus chemotherapy as a first-line treatment for NSCLC. Combined therapy with KN046 and platinum-based doublet chemotherapy exhibited promising antitumor activity and was tolerated according to the posted results. TRAEs occurred in 92.0% and grade ≥ 3 TRAEs occurred in 25.3% of the 87 enrolled patients. The most common grade ≥ 3 TRAEs were diarrhea (5.7%), increased ALT levels (4.6%), infusion-related reactions (3.4%), and rash (3.4%). Among 82 evaluable patients, the overall ORR was 50.6%, and the DCR was 87.7%[107]. In addition, according to the 2-year NCT04054531 follow-up results the mOS in both cohorts was > 2 years, showing that combining KN046 and chemotherapy as first-line treatment for NSCLC patients has promising clinical benefits and is well-tolerated[108]. NCT03838848 is a phase 2 study that was conducted to evaluate KN046 in patients with advanced NSCLC. According to the results presented at the 2023 ESMO, the ORR was 26.9%, the DCR was 80.8%, the mPFS was 5.52 months, and the mOS was 12.68 months among all 29 enrolled patients. The most common grade ≥ 3 TRAEs were infusion reaction (23.1%), decreased platelet cell count (15.4%), and anemia (11.5%; **Table 5**)[109].

Amivantamab + chemotherapy

Amivantamab was shown to have outstanding performance as a monotherapy and encouraging performance as a combination therapy. NCT04538664 is a phase 3 study that assesses amivantamab plus chemotherapy in patients with locally advanced or metastatic NSCLC and EGFR exon 20 insertion mutations. According to the results presented at 2023 ESMO, the ORR was 73.0%, the DoR was 13.5 months, and the mPFS was 12.9 months among 153 patients receiving amivantamab plus chemotherapy. In contrast, the ORR was 43.0%, the DoR was 6.8 months, and the mPFS was 6.9 months in patients who received chemotherapy. TRAEs occurred in all patients and grade ≥ 3 TRAEs occurred in 75.0% of the patients. The most common TRAEs were neutropenia, paronychia, rash, anemia, and infusion-related reactions[110]. NCT04988295 is a phase 3 clinical trial evaluating amivantamab as a combined therapy in patients with locally advanced or metastatic NSCLC with the EGFR exon 19del or exon 21 L858R substitution after failing osimertinib therapy. According to the results posted by the 2023 ESMO, the ORR was 64.0% and the mPFS was 6.3 months among 131 patients receiving amivantamab plus chemotherapy. In contrast, the ORR was 36.0% and the mPFS was 4.2 months in patients receiving chemotherapy. TRAEs occurred in all patients and grade ≥ 3 TRAEs occurred in 72.0% of patients[111]. Notably, amivantamab plus chemotherapy achieved robust antitumor efficacy with a relatively high incidence of TRAEs.

SHR-1701 + chemotherapy

According to the results of NCT04580498 presented at the 2024 ASCO meeting, SHR-1701 plus chemotherapy has shown promising potential as a neoadjuvant therapy for NSCLC patients. The ORR was 58.0% among 97 patients who received SHR-1701 plus chemotherapy as neoadjuvant therapy. TRAEs occurred in 97.0% of patients and grade ≥ 3 TRAEs occurred in 73.0% of patients. The most common TRAEs were leukopenia (77.0%), anemia (70.0%), neutropenia (70.0%), and thrombocytopenia (53.0%).

BsAbs + targeted therapy

AK104 + anlotinib

Anlotinib is a novel TKI that targets multiple RTKs and inhibits the proliferation and angiogenesis of tumors[112]. AK104 (a PD-1 × CTLA-4 bsAb) and anlotinib combination therapy was evaluated in patients with NSCLC in a phase 1b/2 study (NCT04646330). This combination therapy showed promising antitumor efficacy and an acceptable safety profile. Among all 18 enrolled NSCLC patients with PD-L1 TPSs ≥ 1.0%, grade 3 TRAEs occurred in 1 patient (6.0%) and no grade 4 or 5 TRAEs were observed. The ORR was 62.5% and the DCR was 100% among the 8 evaluable patients.

Notably, the ORR was 80.0% among 5 evaluable patients with non-squamous NSCLC[113].

Amivantamab + lazertinib

Lazertinib is a novel third-generation TKI that was approved for the treatment of NSCLC in January 2021[114]. NCT02609776 is a phase 1 study that evaluated the safety, PK, and preliminary antitumor efficacy of amivantamab as monotherapy and in combination with lazertinib in patients with advanced NSCLC. According to the results presented at the 2021 ASCO meeting, ORR was 36.0% among 45 chemotherapy-naïve NSCLC patients who relapsed with osimertinib[115]. NCT04487080 is a phase 3 study that evaluated amivantamab plus lazetinib in patients with locally advanced or metastatic NSCLC. The latest results were presented at the 2023 ESMO. The ORR was 86.0% and the mPFS was 23.7 months among the 429 patients who received amivantamab plus lazetinib. TRAEs occurred in all patients and grade ≥ 3 TRAEs occurred in 75.0% of the patients. The most recent results from CHRYSALIS-2 were presented at the 2024 ASCO meeting. The ORR was 52.0% and the mPFS was 11.1 months among 105 atypical EGFR-mutated advanced NSCLC patients (**Table 5**).

Amivantamab + osimertinib

Osimertinib is an approved third-generation TKI that targets EGFR in NSCLC patients with acquired EGFR T790M resistance mutations[116]. NCT05801029 is a phase 2 study that was conducted to assess the efficacy and safety of amivantamab plus osimertinib as a first-line treatment in patients with EGFR mutations or locally advanced or metastatic NSCLC. This clinical trial was initiated on 18 July 2023 and we look forward to seeing the results.

BsAbs + immunotherapy

REGN7075 + cemiplimab

REGN7075 is a costimulatory bsAb that simultaneously targets EGFR and CD28. Cemiplimab is a monoclonal antibody against PD-1. Combination therapy with REGN7075 and cemiplimab is being evaluated in a phase 1/2 clinical trial (NCT04626635) in patients with advanced solid tumors. According to preliminary results for a dose-escalation cohort (up to 30 mg of REGN7075), TRAEs occurred in 14 (78.0%) patients and no grade ≥ 3 TRAEs were observed among all 18 enrolled patients. The most common TRAEs were fatigue (17.0%), increased AST levels (11.0%), diarrhea (11.0%), hypothyroidism (11.0%), pyrexia (11.0%), and maculopapular rash (11.0%)[117]. The antitumor efficacy has not been assessed due to the inadequate number of patients.

In this section we introduce combination therapies involving bsAbs and other therapies (chemotherapy, targeted therapy, and immunotherapy). The combination of bsAbs and chemotherapy is the most frequently used combination therapy. However, the results of clinical trials exploring the combination of bsAbs and radiotherapy (RT) are inadequate. Combination therapy has shown improved antitumor efficacy compared to monotherapy with bsAbs. However, the TRAEs also significantly increased.

Challenges and future prospects

Although bsAbs have exhibited superior antitumor efficacy and safety profiles compared to two mAb combination therapies, several challenges remain. We propose several methods to overcome these challenges.

Increased side effects caused by "on-target off-tumor" toxicity

Some solid tumor TAAs, which are also expressed in normal cells, may cause "on-target off-tumor" toxicity and increase side effects[118,119]. With advances in our understanding of tumor and immune cells, an increasing number of new targeting molecules, such as receptor tyrosine kinase-like orphan receptor (ROR1) and Notch signaling pathway members, have been discovered or used to generate novel bsAb forms for NSCLC treatment[120,121]. We expect more effective targets for the development of bsAbs to be discovered in the future. In addition to finding more effective targets that are abundantly expressed on tumor cells but scarcely expressed on normal cells, a novel "masking technology" is also a promising approach to alleviate toxicity through use of antibodies to reduce side effects in normal cells[122-124]. For example, bsAbs with XTEN masks that can be released by proteases within the TME can achieve more precise activation of bsAbs in tumors and reduce the effects on normal cells[125]. In addition, several drugs based on masking technology, such as CX-904, JANX008, TAK-186, and TAK-280, are being evaluated in phase 1 clinical trials (NCT05387265, NCT05783622, NCT04844073, and NCT05220098, respectively).

Insufficient infiltration of TILs and the immunosuppressive microenvironment limit bsAb efficacy

Insufficient infiltration of TILs in the TME and local immunosuppressive microenvironment may limit the efficacy of bsAbs[118,119]. RT has the potential to transform immune cell insufficient-infiltrating (immune-cold) tumors into immune cell sufficient-infiltrating (immune-hot) tumors. A recent study of pancreatic ductal adenocarcinoma showed that the use of a bsAb in combination with RT had greater antitumor effects compared to monotherapy[126]. In murine models with Lewis lung carcinoma lacking sufficient TILs, which respond poorly to anti-PD-(L)1 treatment, BA plus RT induced more significant tumor inhibition than BA or RT monotherapy did and prolonged survival[127]. Unfortunately, combination therapies involving bsAbs and RT for NSCLC treatment have not been thoroughly investigated. We encourage an increasing number of researchers to pay more attention to these types of combination strategies. Therefore, the combination with RT may be a promising therapy for patients with insufficient immune cell infiltration in the future. In addition, combining bsAbs with anti-PD-(L)1 ICIs can reverse the locally immunosuppressive environment to achieve better outcomes[119].

Increased TRAEs caused by increased immunogenicity

Although bsAbs can enhance antitumor effects by simultaneously binding two targets, both monotherapy and combination therapy cause increased toxicity. One of the main reasons is that the bsAb structure may increase immunogenicity. Neoantigens or the exposure of cryptic epitopes, a dysregulated immune system caused by anti-drug antibodies (ADAs) and the complexity of the bsAb structure could increase immunogenicity, which may trigger more TRAEs[128]. To address this question, optimizing the dosage forms and exploring the best route for administration are necessary. For example, infusion-related reactions (IRRs) were observed in patients who received amivantamab. Subcutaneous amivantamab can maintain efficacy and improve tolerability, with only 13.0% IRRs[87]. In addition, developing a reliable immunogenicity risk assessment system to guide the selection of bsAbs and formats in the clinic is necessary.

Explorations of biomarkers are insufficient

Inadequate research on biomarkers has hindered the selection of beneficial populations. In this review the exploration of the mentioned bsAb biomarkers was introduced, but the relevant studies were in the initial stage. The complexity of intratumoral heterogeneity was one of the determining factors for the patient response to bsAbs[129]. The development of spatial multiomics could help us to describe the TME more thoroughly and find predictive biomarkers of bsAbs. In addition, the levels of EGFR ligand expression correlated with the response to amivantamab, suggesting that monitoring the ligand expression levels of targets in peripheral blood could also help us identify biomarkers of bsAbs. We call for more research on prognostic biomarkers of bsAbs, which can promote the development of precision oncology.

Resistance to bsAbs failed to receive due attention

Although bsAbs have shown superior antitumor efficacy, drug resistance is still an unavoidable problem[130]. The stimulation of bsAbs may aggravate the exhaustion of T cells, which may lead to drug resistance to bsAbs[131]. In addition, the local immunosuppressive microenvironment is also an important reason for bsAb resistance[132]. There are several studies involving the mechanisms of drug resistance to bsAbs in hematologic malignancies. Owing to the lack of long-term follow-up data, relevant studies on NSCLC treatment are rare. More evidence from evidence-based medicine and relevant clinical trials is needed for further studies about bsAb resistance in NSCLC patients. Recently, the development of trispecific antibodies (tsAbs) has experienced rapid growth. tsAbs may be able to alleviate drug resistance to bsAbs.

More evidence-based medical evidence is needed to verify the superiority of bsAbs

Although combination therapies of two mAbs can enhance antitumor efficacy, the increased toxicity limits the use of combination toxicity. BsAbs are among the most promising novel antitumor therapies for the treatment of NSCLC and are expected to improve antitumor efficacy and reduce toxicity. For example, compared with the combination of nivolumab (anti-PD-1) and ipilimumab (anti-CTLA), the incidence of

grade ≥ 3 TRAEs was clearly lower in patients receiving AK104 (11.3% *vs.* 34.0%). In patients receiving AK112 plus chemotherapy, grade ≥ 3 TRAEs occurred in 54.0% of patients, which was lower than patients receiving atezolizumab (anti-PD-L) plus bevacizumab (anti-VEGF) and chemotherapy (60.4%)[106]. However, there are no clinical trials comparing the use of bsAbs with the combination of two matching drugs. More clinical trials and adequate mature data are needed to verify the superiority of the use of bsAbs over the combination of two drugs. The follow-up data and in-depth analysis of novel drugs are insufficient. The precise clinical and biological observations of the therapeutic benefits and adverse effects of bsAbs are expected to be popular research topics in the coming years.

Conclusions

With the development of immunotherapies for the treatment of NSCLC, the survival of patients with NSCLC has increased significantly. Unfortunately, only a few patients respond to treatment and many of these patients rapidly develop acquired resistance. BsAbs are promising therapies that may overcome acquired resistance and improve the therapeutic outcomes of NSCLC patients. In this review the application of bsAbs in the treatment of NSCLC was introduced and the related challenges, corresponding solutions, and future prospects were discussed. Although the use of bsAbs in solid tumors is limited compared to hematologic malignancies and only one bsAb has been approved for the treatment of NSCLC, the results from numerous clinical trials have shown that bsAbs may be a promising first- or later-line treatment for NSCLC patients. For many reasons, the research and development of bsAbs for solid tumors remain limited. BsAb, RT, and tsAb combined therapies are not thoroughly studied and merit more attention. We are confident that the use of bsAbs will change the treatment of NSCLC going forward.

Grant support

This work was supported by the National Natural Science Foundation of China (Grant No. 82272845) and the Natural Science Foundation of Shandong (Grant No. ZR2023LZL001).

Conflict of interest statement

No potential conflicts of interest are disclosed.

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Cite this article as: Wen J, Cui W, Yin X, Chen Y, Liu A, Wang Q, et al. Application and future prospects of bispecific antibodies in the treatment of non-small cell lung cancer. Cancer Biol Med. 2025; 22: 348-375. doi: 10.20892/j.issn.2095-3941.2024.0470