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CD3-Bispecific Monoclonal Antibodies: A Novel Therapeutic Approach for Complex and Multifactorial Diseases

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Abstract Monoclonal antibodies (mAbs) have witnessed significant advancements in recent years, offering promising therapeutic options for the management of complex and multifactorial diseases. Despite their success, conventional mAbs exhibit limitations such as restricted targeting capacity and suboptimal immune activation, which has driven the development of bispecific monoclonal antibodies (BsAbs) capable of engaging multiple antigens simultaneously. Among these, CD3-bispecific mAbs have emerged as a potent class of immunotherapeutics, capable of activating T cells and inducing T cell-mediated cytotoxicity against target cells, particularly in cancer immunotherapy. This review highlights several representative formats of BsAbs, elucidates their underlying mechanisms of action, and discusses current design strategies for CD3-bispecific mAbs. Emphasis is placed on optimizing their therapeutic efficacy while minimizing adverse effects, supported by recent drug development examples and clinical applications.

Keywords CD3-bispecific antibodies; monoclonal antibodies; T cell activation; cancer immunotherapy; bispecific antibody formats; immunotherapeutics

Introduction

Background of monoclonal antibodies (mAbs)

Since the discovery of the first monoclonal antibody (mAb) in 1975, this class of therapeutics has rapidly advanced and profoundly transformed modern medicine (1, 2). Produced by a single clone of B cells, monoclonal antibodies are highly uniform immunoglobulins capable of specifically recognizing and binding to particular antigenic epitopes (3, 4). The first mAb to receive approval from the United States Food and Drug Administration (FDA) was muromonab-CD3, commercially known as Orthoclone OKT3, in 1986; however, it was later withdrawn from clinical use due to significant toxicity concerns (3). Over time, to address the strong immunogenicity triggered by non-human protein sequences, monoclonal antibodies have evolved from murine to chimeric, humanized, and ultimately fully human forms (1). Today, they are

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extensively applied in disease treatment, diagnostic procedures, and biomedical research, offering new therapeutic possibilities for patients with complex conditions, including cancer, infectious diseases, cardiovascular disorders, and neurodegenerative illnesses (5). Structurally, antibodies are globular proteins, collectively termed immunoglobulins (Ig), and classified into five primary subtypes: IgA, IgD, IgE, IgG, and IgM (6). Among these, IgG stands out as the most widely utilized in clinical practice owing to its higher serum concentration, prolonged half-life, and potent effector functions (7). The IgG molecule adopts a characteristic Y-shaped configuration composed of two identical light chains and two identical heavy chains. Each chain consists of a variable domain and a constant domain, designated as variable light chain (VL), variable heavy chain (VH), constant light chain (CL), and constant heavy chain (CH), respectively. The variable regions, VL and VH, occupy the upper tips of the Y-shaped molecule, while the constant regions, CL and CH, are positioned beneath them. The CH region is further subdivided into CH1, CH2, and CH3 domains. Two heavy chains are connected via disulfide bonds, with light chains paired alongside, forming a symmetrical arrangement (Figure 1). The antibody's two upper arm-like structures, known as the antigen-binding fragments (Fab), are responsible for antigen recognition, while the lower segment, comprising the CH2 and CH3 domains, is termed the crystallizable fragment (Fc). This Fc region mediates interactions with immune effector cells, including macrophages and neutrophils, as well as with the complement system (6, 8).

In general, the functional mechanisms of monoclonal antibodies (mAbs) are categorized into two primary types: direct and indirect actions. Direct mechanisms are predominantly mediated by the Fab domain and involve processes such as inducing cell apoptosis, promoting receptor-mediated endocytosis, modulating interactions between receptors and their respective ligands, and either activating or inhibiting receptor functions along with their associated downstream signaling pathways. Conversely, indirect mechanisms are facilitated by the Fc domain and include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) (9). Additionally, to address challenges such as poor bioavailability and heightened systemic toxicity associated with non-targeted drug molecules, therapeutic agents can be conjugated to mAbs to enable selective targeting. This strategy, known as antibody-drug conjugation (ADC), allows for the precise delivery of cytotoxic drugs to disease-specific targets, thereby enhancing treatment efficacy and minimizing off-target effects (10).

Development of bispecific antibodies (BsAbs)

As previously discussed, monoclonal antibodies (mAbs) have been widely recognized as important therapeutic agents for the treatment of various diseases. However, in certain cases, their clinical application and therapeutic effectiveness are limited due to the specificity of their single-target approach (11). In cancer immunotherapy, for instance, most mAbs exert their effects by either blocking the interaction between specific ligands and their corresponding receptors or by engaging accessory immune cells via their Fc domains. In this scenario,

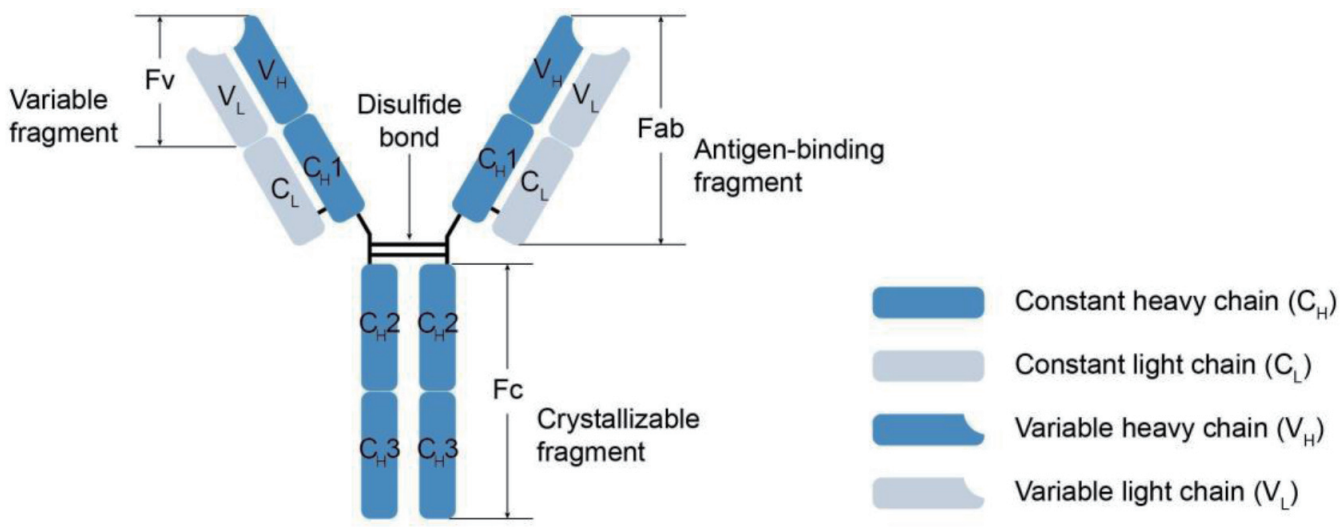

Figure 1. Structure of an IgG antibody

the adaptive immune system, particularly T cells, remains largely uninvolved in the antitumor response, thereby restricting the clinical efficacy of mAbs (6, 12). Consequently, the cytotoxic effects on target cells predominantly rely on Fc domain-mediated indirect mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). Furthermore, T cell activity can be suppressed by immune checkpoints — for example, through the binding of programmed death-1 (PD-1) receptors on T cells to programmed death-ligand 1 (PD-L1) on tumor cells as well as through other tumor immune evasion mechanisms, including reduced expression of the molecular markers targeted by mAbs and increased expression of compensatory receptors or antigens on tumor cell surfaces. These adaptive resistance pathways contribute to immune evasion and drug resistance (10, 13-16). Ultimately, the restricted single-target nature of mAbs often accounts for their limited clinical efficacy in complex disease scenarios.

To address these limitations, bispecific monoclonal antibodies (BsAbs), which can simultaneously bind to two distinct antigens or epitopes, have been developed. Many BsAbs are designed to target cluster of differentiation (CD) antigens on T cells predominantly CD3 while concurrently binding to tumor-associated antigens (TAAs) on tumor cells. This dual targeting enables BsAbs to physically bridge T cells and tumor cells or redirect T cells to tumor sites, thereby activating and amplifying T cell-mediated cytotoxic responses against diseased cells and enhancing therapeutic outcomes (14). Additionally, BsAbs can help alleviate immunosuppressive mechanisms by redirecting effector cells toward target cells and, in some cases, by directly targeting immune checkpoints such as PD-1 and PD-L1 to overcome T cell inhibition. Moreover, BsAbs can concurrently block multiple receptors or neutralize different ligands, thereby inhibiting both primary disease-driving pathways and compensatory mechanisms (10). Owing to these advantages, BsAbs have rapidly advanced in recent years, showing considerable potential for increased therapeutic efficacy and specificity. Several BsAb-based therapeutics have already gained regulatory approval and are now available for clinical use (6).

Overview of Immune Cell Targets

The immune system consists of a complex network of specialized cells that collectively defend the body against invading pathogens and microorganisms. Among these, immune cells such as T cells, macrophages, and natural killer (NK) cells play pivotal roles in both innate and adaptive immunity, making them key therapeutic targets in the ongoing development of bispecific monoclonal antibodies (BsAbs). By modulating the activity and functional responses of these immune cells, significant advancements have been made in the treatment of complex diseases, including cancer, autoimmune disorders, and infectious diseases. Mechanistically, CD3 — a membrane protein complex found on the surface of T cells — is one of the most commonly targeted molecules for T cell activation in BsAb design. CD3 is composed of four distinct polypeptide chains: CD3γ, CD3δ, CD3ε, and CD3ζ, with CD3ε serving as the primary mediator of signal transduction (17, 18). Upon engagement of CD3ε and initiation of intracellular signaling cascades, T cells become activated, proliferating and releasing cytotoxic cytokines such as tumor necrosis factor-alpha (TNFα) and interferon-gamma (IFNγ), which contribute to the induction of apoptosis in target cells. The first monoclonal antibody targeting CD3, muromonab-CD3 (commercially known as Orthoclone OKT3), was introduced for the prevention of organ transplant rejection by modulating T cell responses. However, its clinical use was later discontinued due to the occurrence of severe toxicities (14, 19, 20).

Beyond T cells, macrophages and NK cells, through their Fc gamma receptors (FcγRs) — also referred to as IgG receptors — are critical components of immune-mediated cytotoxicity and are additional targets in antibody-based therapies. FcγRs exist in several subtypes, including FcγRI, FcγRIIa, FcγRIIIa, and FcγRIIb. Based on their structural and functional characteristics, FcγRIIb is classified as an inhibitory receptor, while the remaining subtypes function as activating receptors, capable of engaging accessory immune cells and promoting the indirect cytotoxic mechanisms of mAbs (21). Moreover, the neonatal Fc receptor (FcRn) plays an essential role in maintaining mAb stability and longevity. Expressed on the surface of various cell types, particularly accessory immune cells, FcRn binds to the Fc region of antibodies following endocytosis, preventing their lysosomal degradation and facilitating their recycling back into circulation — thereby extending the half-life and therapeutic effects of these biologics (21, 22).

Common Formats of Bispecific Antibodies (BsAbs)

IgG-like BsAbs

Based on their structural characteristics, bispecific antibodies (BsAbs) can broadly be classified into two major categories: IgG-like BsAbs and non-IgG-like, or fragment-based, BsAbs (8, 23). IgG-like BsAbs resemble the conventional IgG antibody structure, containing both functional Fc and Fab regions, while fragment-based BsAbs lack the Fc domain. Among the IgG-like formats, trifunctional bispecific antibodies (TriomAbs) are notable for possessing two distinct antigen-binding arms alongside an Fc domain. This enables them to simultaneously engage with three different cell types (11). Specifically, the two Fab domains can bind both tumor cells and effector cells, while the Fc domain interacts with accessory immune cells such as macrophages and NK cells (Figure 2A). These immune cells, together with T cells, can exert antitumor activity by secreting cytotoxic cytokines like TNFα and IFNγ, or by inducing apoptosis through mechanisms such as antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) (11). A key example is *catumaxomab*, the first TriomAb approved for clinical use, which targets CD3 on T cells and epithelial cell adhesion molecule (EpCAM) on tumor cells. This dual-targeting mechanism bridges T cells and tumor cells, triggering cytotoxic responses while simultaneously recruiting accessory cells via its Fc region to enhance ADCC and ADCP.

Conjugation of Two mAbs

Apart from TriomAbs, BsAbs can also be generated through the chemical conjugation of two distinct, fully intact monoclonal antibodies, including those already approved for clinical use. In this format, known as *IgG-IgG*, the CH3 domains of two separate IgGs are chemically linked, preserving the original antibody structures while combining their specificities (10, 24). The typical production process involves introducing thiol groups into one mAb using Traut's reagent and maleimide groups into the other with sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC). These reactive groups subsequently undergo a thiol-maleimide coupling reaction to produce a chemically conjugated BsAb capable of targeting two distinct antigens (25-29). One such candidate, *CD20Bi*, was produced by conjugating *Orthoclone OKT-3* (anti-CD3) with *Rituxan* (anti-CD20). Though it showed promise in early-phase trials, it did not progress beyond phase III.

Dual-Variable Domain Immunoglobulin (DVD-Ig)

Another IgG-like BsAb format is the *dual-variable domain immunoglobulin* (*DVD-Ig*), which incorporates two antigen-binding domains per Fab arm. Built upon an intact IgG backbone, this format links a single-chain variable fragment (scFv) from a second mAb to the variable region of each heavy chain using a short peptide linker (30, 31). The resulting symmetrical BsAb structure possesses two antigen-binding sites per Fab arm, making it tetravalent and capable of binding up to four antigens simultaneously (Figure 2C). This enhanced valency improves therapeutic potential. For example, researchers have engineered a DVD-Ig BsAb to target both epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 2 (VEGFR2), two proteins implicated in triple-negative breast cancer (TNBC). This BsAb was constructed by combining the intact IgG structure of cetuximab (anti-EGFR) with the scFv of ramucirumab (anti-VEGFR2) via a glycine linker, achieving superior antitumor activity compared to the individual mAbs (30).

"Knob-in-Hole" (KIH) Technology-Generated BsAbs

A significant challenge in producing asymmetrical BsAbs is the unwanted mispairing of heavy chains during assembly, which compromises product quality. To overcome this, "*knob-in-hole*" (*KIH*) technology was developed. This approach involves introducing a bulkier amino acid substitution into the CH3 domain of one heavy chain, creating a "*knob*," and replacing a corresponding residue in the other chain with a smaller amino acid, forming a complementary "*hole*" (32). The amino acid substitution for the "knob" is T336Y, while Y407T creates the "hole" (33) (Figure 2D). This ensures the correct pairing of heterodimeric heavy chains. An example of a KIH-structured BsAb is a molecule designed to treat TNBC by targeting EGFR and PD-L1. In this case, one antigen-binding arm comprises the VL and VH regions of *cetuximab* along with a standard constant region, while the other arm consists of the scFv of *atezolizumab*, forming an asymmetrical antibody configuration. The KIH format in the CH3 domain facilitates correct heavy chain assembly and BsAb functionality (34). Numerous additional IgG-like BsAb formats exist but are not described here in detail (Figure 2E) (10, 35).

Fragment-Based Bispecific Antibodies (BsAbs)

One important category of bispecific antibodies is the fragment-based BsAbs, which differ from IgG-like BsAbs by lacking an Fc region. Among these, one of the most notable formats is the tandem single-chain variable fragment (tandem scFv). This

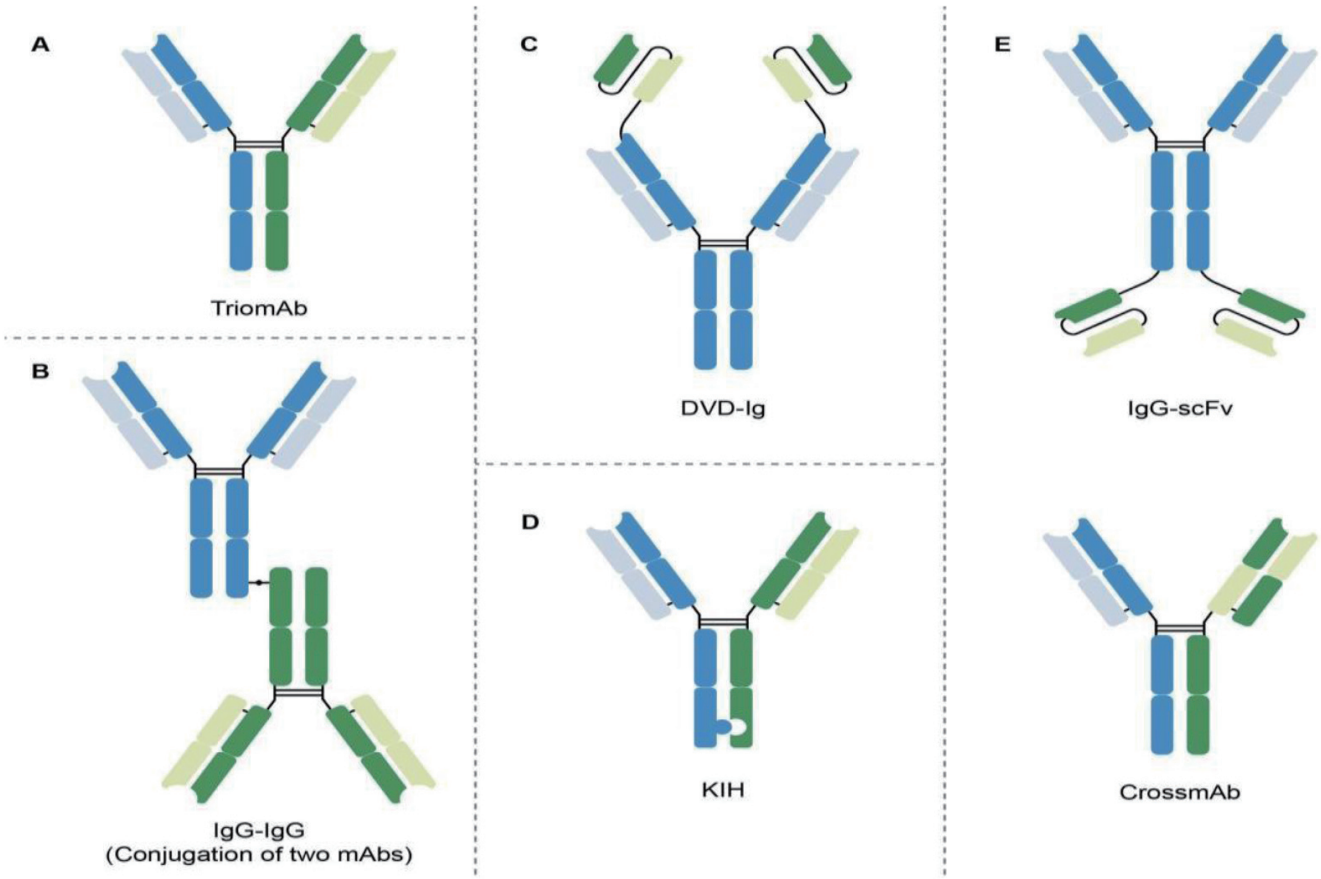

Figure 2. Examples of IgG-like BsAbs structures

structure consists of two distinct single-chain variable fragments (scFvs), each derived from different monoclonal antibodies, connected in sequence to create a compact, bispecific molecule. Bispecific T cell engagers (BiTEs) represent a well-established application of this format, particularly in oncology, as they can simultaneously bind T cells and tumor cells, effectively redirecting immune responses toward malignancies (6). In these constructs, the scFvs retain their antigen specificity and are covalently linked by a non-immunogenic peptide chain featuring glycine-serine motifs arranged as SGGGG repeats (6, 8). The fragment-based configuration avoids interactions between Fc domains and Fcγ receptors on accessory cells, significantly reducing the risk of immune-related adverse effects such as cytokine release syndrome (CRS), especially during T cell activation. Moreover, their smaller molecular size improves tumor tissue penetration and molecular flexibility. However, this advantage comes at the cost of a shorter systemic half-life, since the absence of an Fc region eliminates neonatal Fc receptor (FcRn)-mediated recycling, leading to faster clearance from circulation (22, 36). Noteworthy examples of clinically approved BiTEs include blinatumomab, which targets both T cells and malignant B cells (37), and tarlatamab, which engages T cells and tumor cells (38). Many other BiTE candidates are currently in various stages of clinical evaluation, further emphasizing the potential of this therapeutic strategy (39).

Beyond BiTEs, several alternative fragment-based BsAb formats have been developed. Tandem antibodies (TandAbs) are another example, featuring tetravalent structures with two binding sites for each of two different antigens. Structurally, TandAbs are composed of two identical polypeptide chains that associate non-covalently, forming homodimers with increased molecular weight and extended circulation half-life relative to smaller formats like BiTEs (40). Another promising strategy involves fusing antigen-binding fragments to albumin. In these designs, either two separate scFvs are individually linked to albumin, or a bispecific fragment containing two scFvs is attached to a single albumin molecule (10, 41). Albumin acts as a natural plasma carrier with a biological half-life of approximately 19 days, thereby significantly prolonging the in vivo persistence of the BsAb molecule (41, 42). Additionally, other fragment-based BsAb formats have been explored, though these variants fall outside the scope of the current discussion

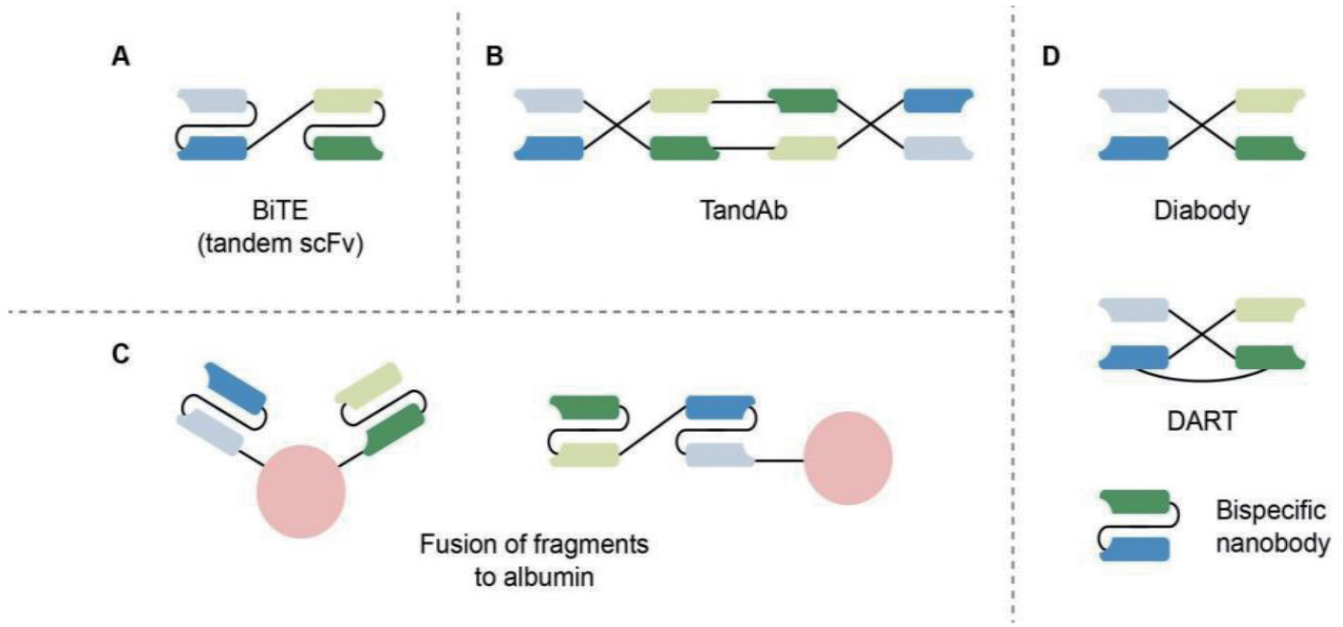

Figure 3. Examples of fragment-based BsAbs structures

(10, 43).

Mechanisms of Action (MoA) of Bispecific Antibodies (BsAbs)

The mechanisms by which bispecific antibodies exert their therapeutic effects can generally be categorized into three main strategies: modulation of disease-associated signaling pathways, recruitment and activation of immune cells, and the targeted delivery of therapeutic payloads (Figure 4). In the first mechanism, BsAbs regulate signaling pathways that drive disease progression by interacting with key molecular targets. This can involve several approaches, such as simultaneously inhibiting two different receptors, neutralizing two ligands, activating multiple receptors, or combining receptor activation and inhibition within the same pathway. Other mechanisms include blocking ligand-receptor interactions, preventing receptor dimerization, or activating one receptor while inhibiting a second (Figure 4A) (15, 43). The primary aim of this approach is to disrupt the transmission of pathological signals through pathways associated with tumor-associated antigens (TAAs) or immune checkpoints (8, 12). Notable examples include BsAbs designed to target both EGFR and VEGFR2, or EGFR and PD-L1, for the treatment of triple-negative breast cancer (TNBC). Importantly, in this strategy, BsAbs are not required to engage both targets simultaneously, and such antibodies are often referred to as *combinatorial BsAbs* in the literature, indicating their ability to bind independently to their respective targets (13-15).

Another major therapeutic mechanism involves the recruitment and activation of immune cells, particularly T cells, to enhance antitumor immunity. One limitation of conventional monospecific monoclonal antibodies is that T cells are often excluded from their mechanism of action and can even be suppressed through immune checkpoint pathways (16). BsAbs address this issue by acting as molecular bridges between immune effector cells and target cells, a strategy dependent on their ability to simultaneously bind both. These antibodies, referred to as *obligate BsAbs*, redirect immune cells, such as T cells, toward tumor cells, thereby promoting targeted immune responses (14, 16). In cancer therapy, BiTEs exemplify this approach. One of the BiTE's antigen-binding domains engages a TAA on the tumor cell, while the other binds to CD3 on the T cell surface, triggering T cell activation, proliferation, and the release of cytotoxic cytokines, ultimately leading to tumor cell lysis (Figure 4B). Additionally, the presence of Fc domains in some BsAb formats has raised concerns regarding safety and unintended immune activation through Fcγ receptor interactions. To mitigate these risks, newer generations of CD3-bispecific antibodies are being engineered to minimize or eliminate Fc domain functionality, either by reducing Fc receptor affinity or completely omitting the Fc region, as seen in tandem scFv-based BiTEs (14).

A third mechanism involves the delivery of therapeutic payloads directly to disease sites. Beyond modulating signaling pathways and immune activity, BsAbs can serve as carriers for cytotoxic drugs,

forming a class of therapeutics known as antibody-drug conjugates (ADCs) (Figure 4C) (24, 44). This approach addresses the limitations of conventional chemotherapeutic agents, which often lack selectivity and carry a high risk of systemic toxicity. BsAbs in ADC formats can be conjugated to cytotoxic payloads either through direct incubation methods or using *pretargeting strategies*, where BsAbs are first administered to bind their target antigens, followed by the delivery of the payload at a later stage (44, 45). Given their longer circulation time compared to most small-molecule drugs, BsAbs can effectively localize the payload to specific disease sites, reducing off-target effects while enhancing therapeutic outcomes.

Examples of CD3-Bispecific Monoclonal Antibodies (mAbs)

Several CD3-bispecific monoclonal antibodies have been developed and evaluated for clinical use, with some already receiving regulatory approval. These antibodies primarily function by bridging T cells with tumor cells to stimulate targeted immune responses.

Previously Approved CD3-Bispecific Monoclonal Antibodies

One of the earliest approvals in this category was catumaxomab (marketed as Removab), which received authorization from the European Medicines Agency (EMA) in 2009 for the treatment of malignant ascites. Catumaxomab belongs to the TriomAb class, possessing two antigen-binding sites and an Fc region. It can bind CD3 on T cells, EpCAM on tumor cells, and Fcγ receptors on accessory immune cells. This arrangement enables the redirection of immune cells towards tumor cells, promoting cytotoxic T cell activity, cytokine-mediated effects, phagocytosis, and antibody-dependent cellular cytotoxicity (ADCC) (12). Given the frequent overexpression of EpCAM in tumors, catumaxomab showed promising antitumor efficacy (46, 47). However, being a rat-mouse hybrid monoclonal antibody, it carried immunogenicity risks (8, 24, 48). Withdrawn in 2017, catumaxomab made a return to the market in 2025 under the trade name Korjuny, with new clinical investigations underway.

Another significant agent is blinatumomab (Blincyto), which received accelerated approval from the U.S. Food and Drug Administration (FDA) in 2014 for acute lymphoblastic leukemia (ALL), followed by full approval in 2017. Structurally a BiTE (bispecific T cell engager), blinatumomab consists of two single-chain variable fragments (scFvs) connected by a glycine-serine linker, targeting CD3 on T cells and CD19 on malignant B cells (49, 50-52). This bispecific format bridges T cells with malignant cells, inducing T cell activation and subsequent tumor cell apoptosis. Its design excludes an Fc region, minimizing Fcγ receptor-mediated adverse events such as cytokine release

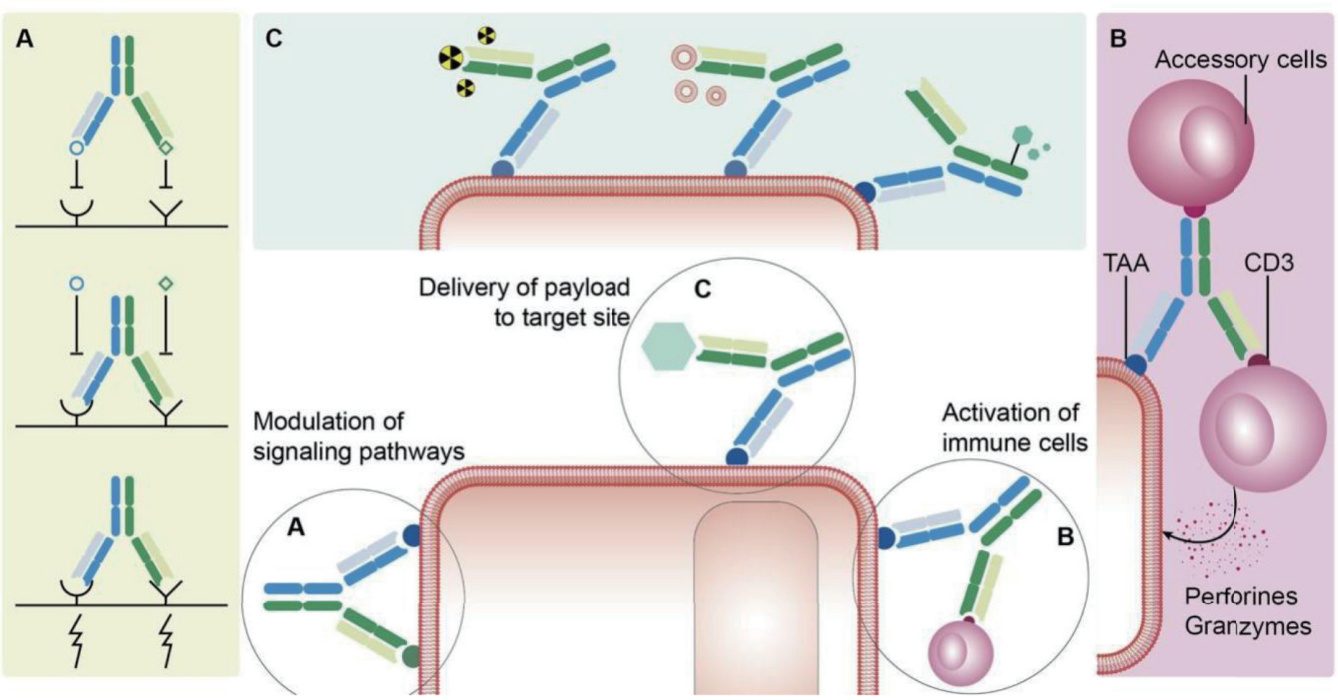

Figure 4. Schematic demonstration of the MoA of BsAbs

syndrome (CRS) (22, 43). However, the absence of the Fc region shortens its half-life to approximately 2 hours, necessitating continuous infusion for effective dosing — a factor contributing to its CRS risk (15, 53).

In recent years, several other CD3-bispecific antibodies have gained regulatory approvals, including teclistamab, mosunetuzumab, epcoritamab, glofitamab, talquetamab, elranatamab, tebentafusp, and tarlatamab. Most of these therapeutics have been approved for cancer and hematologic malignancies like ALL and large B cell lymphoma (54).

Investigational CD3-Bispecific Antibodies

Several investigational CD3-bispecific antibodies remain under clinical evaluation. Ertumaxomab, a murine TriomAb, targets CD3 and HER2, facilitating the recruitment of both T cells and accessory immune cells against HER2-positive tumors. Early-phase trials demonstrated anticancer activity and associated adverse effects, including immunogenicity and inflammatory reactions (55-58). However, the sponsor halted development before phase II initiation (56). Similarly, FBTA05 (Bi20) targets CD3 and CD20, intended for conditions like non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Despite demonstrating potent antitumor activity in preclinical models (58, 59), clinical development was discontinued due to logistical limitations, including delayed patient recruitment.

CD20Bi and EGFRBi are chemically conjugated bispecific constructs produced via thiol-maleimide coupling. CD20Bi combines Orthoclone OKT-3 and Rituxan, while EGFRBi fuses OKT-3 with Erbitux. Both have shown potential in early-phase trials, notably through the generation of BsAb-armed T cells (BATs) for treating lymphomas, multiple myeloma, and glioblastoma (27, 29, 60-63). Yet, due to funding challenges and operational hurdles, later-stage trials were suspended.

Another notable BiTE is solitomab (MT110), which targets CD3 and EpCAM. Despite promising preclinical activity, its phase I trial encountered dose-limiting toxicities, likely due to EpCAM expression on healthy tissues, halting further clinical development (64, 65).

Conclusions

Monoclonal antibodies (mAbs) have established themselves as indispensable therapeutic agents in modern disease management. However, limitations associated with their singular antigen specificity and insufficient engagement of T cell-mediated cytotoxicity have restricted their therapeutic efficacy. To address these challenges, bispecific monoclonal antibodies (BsAbs) have been developed, designed with one antigen-binding domain targeting CD3 on T cells and the other recognizing tumor-associated antigens (TAAs). This dual-targeting capability allows BsAbs to redirect T cells toward diseased cells, enhancing targeted cytotoxicity. BsAbs have demonstrated considerable therapeutic potential, particularly in antitumor applications. Structurally, BsAbs are typically categorized into IgG-like and fragment-based formats. In the context of design strategies, it has been observed that interactions between the Fc domain of mAbs and FcγRs on accessory immune cells, especially during T cell activation, can trigger severe adverse drug reactions (ADRs) such as cytokine release syndrome (CRS). Consequently, approaches including Fc domain engineering, silencing, or deletion have been explored to mitigate these risks, giving rise to Fc-free BsAbs such as BiTEs. Although BiTEs offer enhanced tumor penetration due to their smaller molecular size, their lack of FcRn-mediated recycling leads to rapid systemic clearance and shorter half-life. To overcome this limitation, half-life extended BiTEs

Table 1. CD3-Bispecific mAbs Approved by FDA and EMA (as of April 2025)

Generic Name	Trade Name	Targets	Approval Year(s)	Agency
Catumaxomab	Removab/Korjuny	CD3 and EpCAM	2009 (withdrawn 2017), 2025	EMA
Blinatumomab	Blincyto	CD3 and CD19	2014	FDA, EMA
Teclistamab	Tecvayli	CD3 and BCMA	2022	FDA, EMA
Mosunetuzumab	Lunsumio	CD3 and CD20	2022	FDA, EMA
Tebentafusp	Kimmtrak	CD3 and GP100	2022	FDA, EMA
Epcoritamab	Epkinly	CD3 and CD20	2023	FDA, EMA
Glofitamab	Columvi	CD3 and CD20	2023	FDA, EMA
Talquetamab	Talvey	CD3 and GPRC5D	2023	FDA, EMA
Elranatamab	Elrexfio	CD3 and BCMA	2023	FDA, EMA
Tarlatamab	Imdelltra	CD3 and DLL3	2024	FDA

(HLE-BiTEs) have been developed by fusing BiTE fragments with engineered Fc domains or albumin, thereby prolonging their circulation time and reducing dosing frequency. Additionally, the binding affinity of the Fab regions significantly influences the safety and efficacy of BsAbs. Studies have shown that excessively high affinity to CD3 may result in rapid cytokine release and severe immune-related adverse events, while high affinity for tumor antigens risks off-target toxicity due to shared antigen expression on healthy tissues. Moderating these affinities has been found to improve safety without compromising therapeutic efficacy. In conclusion, the effective design of CD3-bispecific mAbs hinges on the careful selection of targeting sites and antibody formats, optimization of Fc domain properties, regulation of antigen-binding affinity, and enhancement of BiTE half-life collectively ensuring improved therapeutic performance and patient safety.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Nissim A, Chernajovsky Y. Historical Development of Monoclonal Antibody Therapeutics. In: Chernajovsky Y, Nissim A, editors. Therapeutic Antibodies. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008. p. 3-18.
2. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256(5517):495-7.
3. Liu JK. The history of monoclonal antibody development - Progress, remaining challenges and future innovations. Ann Med Surg (Lond). 2014;3(4):113-6.
4. Little M, Kipriyanov SM, Le Gall F, Moldenhauer G. Of mice and men: hybridoma and recombinant antibodies. Immunol Today. 2000;21(8):364-70.
5. Li J, Zhu Z. Research and development of next generation of antibody-based therapeutics. Acta Pharmacol Sin. 2010;31(9):1198-207.
6. Paredes-Moscosso SR, Nathwani AC. 10 years of BiTE immunotherapy: an overview with a focus on pancreatic cancer. Front Oncol. 2024;14:1429330.
7. Nelson PN, Reynolds GM, Waldron EE, Ward E, Giannopoulos K, Murray PG. Demystified…: monoclonal antibodies. Mol Pathol. 2000;53(3):111-7.
8. Krishnamurthy A, Jimeno A. Bispecific antibodies for cancer therapy: A review. Pharmacology & Therapeutics. 2018;185:122-34.
9. Schlothauer T, Herter S, Koller CF, Grau-Richards S, Steinhart V, Spick C, et al. Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. Protein Engineering, Design and Selection. 2016;29(10):457-66.
10. Kontermann RE. Dual targeting strategies with bispecific antibodies. MAbs. 2012;4(2):182-97.
11. Seimetz D, Lindhofer H, Bokemeyer C. Development and approval of the trifunctional antibody catumaxomab (anti-EpCAM×anti-CD3) as a targeted cancer immunotherapy. Cancer Treatment Reviews. 2010;36(6):458-67.
12. Sedykh SE, Prinz VV, Buneva VN, Nevinsky GA. Bispecific antibodies: design, therapy, perspectives. Drug Des Devel Ther. 2018;12:195-208.
13. Klein C, Brinkmann U, Reichert JM, Kontermann RE. The present and future of bispecific
antibodies for cancer therapy. Nature Reviews Drug Discovery. 2024;23(4):301-19.
14. Labrijn AF, Janmaat ML, Reichert JM, Parren PWHI. Bispecific antibodies: a mechanistic review of the pipeline. Nature Reviews Drug Discovery. 2019;18(8):585-608.
15. Guidi L, Etessami J, Valenza C, Valdivia A, Meric-Bernstam F, Felip E, et al. Bispecific Antibodies in Hematologic and Solid Tumors: Current Landscape and Therapeutic Advances. American Society of Clinical Oncology Educational Book. 2025;45(3):e473148.
16. Redman JM, Hill EM, AlDeghaither D, Weiner LM. Mechanisms of action of therapeutic antibodies for cancer. Mol Immunol. 2015;67(2 Pt A):28-45.
17. Mariuzza RA, Agnihotri P, Orban J. The structural basis of T-cell receptor (TCR) activation: An enduring enigma. J Biol Chem. 2020;295(4):914-25.
18. Brandão RO, Jiang X, Selvaraju S, Mohapatra P. Bispecific Antibodies: An Emerging Concept in Antibody-Based Cancer Therapies. In: Das Mohapatra A, Sahu PS, editors. Advances in Immunology and Immuno-techniques: Updated Concepts and Recent Applications. Singapore: Springer Nature Singapore; 2024. p. 57-87.
19. Benekli M, Hahn T, Williams BT, Cooper M, Roy HN, Wallace P, et al. Muromonab-CD3 (Orthoclone OKT3 ®), methylprednisolone and cyclosporine for acute graft-versus-host disease prophylaxis in allogeneic bone marrow transplantation. Bone Marrow Transplantation. 2006;38(5):365-70.
20. Chatenoud L, Ferran C, Legendre C, Thouard I, Merite S, Reuter A, et al. In vivo cell activation following OKT3 administration. Systemic cytokine release and modulation by corticosteroids. Transplantation. 1990;49(4):697-702.
21. Sepúlveda-Delgado J, Llorente L, Hernández-Doño S. A Comprehensive Review of Fc Gamma Receptors and Their Role in Systemic Lupus Erythematosus. International Journal of Molecular Sciences. 2025;26(5):1851.
22. Shah D, Soper B, Shopland L. Cytokine release syndrome and cancer immunotherapies – historical challenges and promising futures. Frontiers in Immunology. 2023;Volume 14 - 2023.
23. Karbyshev MS, Kalashnikova IV, Dubrovskaya VV, Baskakova KO, Kuzmichev PK, Sandig V. Trends and challenges in bispecific antibody production. Journal of Chromatography A. 2025;1744:465722.

24. Kontermann RE, Brinkmann U. Bispecific antibodies. Drug Discovery Today. 2015;20(7):838-47.
25. Ma J, Han H, Liu D, Li W, Feng H, Xue X, et al. HER2 as a promising target for cytotoxicity T cells in human melanoma therapy. PLoS One. 2013;8(8):e73261.
26. Han H, Ma J, Zhang K, Li W, Liu C, Zhang Y, et al. Bispecific anti-CD3 x anti-HER2 antibody mediates T cell cytolytic activity to HER2-positive colorectal cancer in vitro and in vivo. Int J Oncol. 2014;45(6):2446-54.
27. Ma P, He Q, Li W, Li X, Han H, Jin M, et al. Anti-CD3 x EGFR bispecific antibody redirects cytokine-induced killer cells to glioblastoma in vitro and in vivo. Oncol Rep. 2015;34(5):2567-75.
28. Lum LG, Thakur A, Pray C, Kouttab N, Abedi M, Deol A, et al. Multiple infusions of CD20-targeted T cells and low-dose IL-2 after SCT for high-risk non-Hodgkin's lymphoma: a pilot study. Bone Marrow Transplant. 2014;49(1):73-9.
29. Lum LG, Thakur A, Liu Q, Deol A, Al-Kadhimi Z, Ayash L, et al. CD20-Targeted T Cells after Stem Cell Transplantation for High Risk and Refractory Non-Hodgkin's Lymphoma. Biology of Blood and Marrow Transplantation. 2013;19(6):925-33.
30. Mohan N, Luo X, Shen Y, Olson Z, Agrawal A, Endo Y, et al. A Novel Bispecific Antibody Targeting EGFR and VEGFR2 Is Effective against Triple Negative Breast Cancer via Multiple Mechanisms of Action. Cancers (Basel). 2021;13(5).
31. Gu J, Ghayur T. Generation of dual-variable-domain immunoglobulin molecules for dual-specific targeting. Methods Enzymol. 2012;502:25-41.
32. Chen S, Li J, Li Q, Wang Z. Bispecific antibodies in cancer immunotherapy. Hum Vaccin Immunother. 2016;12(10):2491-500.
33. Ridgway JB, Presta LG, Carter P. 'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization. Protein Eng. 1996;9(7):617-21.
34. Mohan N, Agrawal A, Shen Y, Winarski KL, Endo Y, Dokmanovic M, et al. Comparative Characterization of Different Molecular Formats of Bispecific Antibodies Targeting EGFR and PD-L1. Pharmaceutics. 2022;14(7).
35. Velasquez MP, Bonifant CL, Gottschalk S. Redirecting T cells to hematological malignancies with bispecific antibodies. Blood. 2018;131(1):30-8.
36. Liu L, Lam CK, Long V, Widjaja L, Yang Y, Li H, et al. MGD011, A CD19 x CD3 Dual-Affinity Retargeting Bi-specific Molecule Incorporating Extended Circulating Half-life for the Treatment of B-Cell Malignancies. Clin Cancer Res. 2017;23(6):1506-18.
37. Kantarjian H, Stein A, Gökbuget N, Fielding AK, Schuh AC, Ribera JM, et al. Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. N Engl J Med. 2017;376(9):836-47.
38. Ahn MJ, Cho BC, Felip E, Korantzis I, Ohashi K, Majem M, et al. Tarlatamab for Patients with Previously Treated Small-Cell Lung Cancer. N Engl J Med. 2023;389(22):2063-75.
39. Ma J, Mo Y, Tang M, Shen J, Qi Y, Zhao W, et al. Bispecific Antibodies: From Research to Clinical Application. Frontiers in Immunology. 2021;Volume 12 - 2021.
40. Cochlovius Br, Kipriyanov SM, Stassar MJJG, Schuhmacher J, Benner A, Moldenhauer G, et al. Cure of Burkitt's Lymphoma in Severe Combined Immunodeficiency Mice by T Cells, Tetravalent CD3 × CD19 Tandem Diabody, and CD28 Costimulation1. Cancer Research. 2000;60(16):4336-41.
41. Dennis MS, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D, et al. Albumin Binding as a General Strategy for Improving the Pharmacokinetics of Proteins *. Journal of Biological Chemistry. 2002;277(38):35035-43.
42. Davé E, Ralph A, Oliver Z, Bruce C, E. CJ, Sarah D, et al. Fab-dsFv: A bispecific antibody format with extended serum half-life through albumin binding. mAbs. 2016;8(7):1319-35.
43. Herrera M, Pretelli G, Desai J, Garralda E, Siu LL, Steiner TM, et al. Bispecific antibodies: advancing precision oncology. Trends in Cancer. 2024;10(10):893-919.
44. Suurs FV, Lub-de Hooge MN, de Vries EGE, de Groot DJA. A review of bispecific antibodies and antibody constructs in oncology and clinical challenges. Pharmacology & Therapeutics. 2019;201:103-19.
45. Goldenberg DM, Sharkey RM. Novel radiolabeled antibody conjugates. Oncogene. 2007;26(25):3734-44.
46. Chames P, and Baty D. Bispecific antibodies for cancer therapy. mAbs. 2009;1(6):539-47.
47. Ruf P, Gires O, Jäger M, Fellinger K, Atz J, Lindhofer H. Characterisation of the new EpCAM-specific antibody HO-3: implications for trifunctional antibody immunotherapy of cancer. Br J Cancer. 2007;97(3):315-21.
48. Chelius D, Peter R, Patrick G, Matthias P, Reinhard L, Eva G, et al. Structural and functional characterization of the trifunctional antibody catumaxomab. mAbs. 2010;2(3):309-19.
49. Tian Z, Liu M, Zhang Y, Wang X. Bispecific T cell engagers: an emerging therapy for management of hematologic malignancies. Journal of Hematology & Oncology. 2021;14(1):75.
50. Löffler A, Kufer P, Lutterbuse R, Zettl F, Daniel PT, Schwenkenbecher JM, et al. A recombinant bispecific single-chain antibody, CD19 × CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. Blood. 2000;95(6):2098-103.
51. Nagorsen D, Baeuerle PA. Immunomodulatory therapy of cancer with T cell-engaging BiTE antibody blinatumomab. Experimental Cell Research. 2011;317(9):1255-60.
52. Bruzzese A, Martino EA, Labanca C, Caridà G, Mendicino F, Lucia E, et al. Therapeutic Strategies for Relapsed or Refractory B-Cell Acute Lymphoblastic Leukemia in Adult Patients: Optimizing the Use of Monoclonal Antibodies. European Journal of Haematology. 2025;n/a(n/a).
53. Einsele H, Borghaei H, Orlowski RZ, Subklewe M, Roboz GJ, Zugmaier G, et al. The BiTE (bispecific T-cell engager) platform: Development and future potential of a targeted immuno-oncology therapy across tumor types. Cancer. 2020;126(14):3192-201.
54. Lloyd K, Middelburg J, Ovcinnikovs V, Pencheva N,

Kemper K, van Hall T. Improving CD3 bispecific antibody therapy in solid tumors using combination strategies. Front Oncol. 2025;15:1548446.
55. Mitri Z, Constantine T, O'Regan R. The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. Chemotherapy Research and Practice. 2012;2012(1):743193.
56. Lim SM, Pyo K-H, Soo RA, Cho BC. The promise of bispecific antibodies: Clinical applications and challenges. Cancer Treatment Reviews. 2021;99:102240.
57. Kiewe P, and Thiel E. Ertumaxomab: a trifunctional antibody for breast cancer treatment. Expert Opinion on Investigational Drugs. 2008;17(10):1553-8.
58. Lameris R, de Bruin RCG, Schneiders FL, van Bergen en Henegouwen PMP, Verheul HMW, de Gruijl TD, et al. Bispecific antibody platforms for cancer immunotherapy. Critical Reviews in Oncology/Hematology. 2014;92(3):153-65.
59. Stanglmaier M, Faltin M, Ruf P, Bodenhausen A, Schröder P, Lindhofer H. Bi20 (fBTA05), a novel trifunctional bispecific antibody (anti-CD20 × anti-CD3), mediates efficient killing of B-cell lymphoma cells even with very low CD20 expression levels. International Journal of Cancer. 2008;123(5):1181-9.
60. Gall JM, Davol PA, Grabert RC, Deaver M, Lum LG. T cells armed with anti-CD3 × anti-CD20 bispecific antibody enhance killing of $CD20^{+}$ malignant B cells and bypass complement-mediated rituximab resistance in vitro. Experimental Hematology. 2005;33(4):452-9.
61. Thakur A, Huang M, Lum LG. Bispecific antibody based therapeutics: Strengths and challenges. Blood Reviews. 2018;32(4):339-47.
62. Lum LG, Thakur A, Kondadasula SV, Al-Kadhimi Z, Deol A, Tomaszewski EN, et al. Targeting CD138-/CD20+ Clonogenic Myeloma Precursor Cells Decreases These Cells and Induces Transferable Antimyeloma Immunity. Biol Blood Marrow Transplant. 2016;22(5):869-78.
63. Reusch U, Sundaram M, Davol PA, Olson SD, Davis JB, Demel K, et al. Anti-CD3 × Anti-Epidermal Growth Factor Receptor (EGFR) Bispecific Antibody Redirects T-Cell Cytolytic Activity to EGFR-Positive Cancers In vitro and in an Animal Model. Clinical Cancer Research. 2006;12(1):183-90.
64. Kebenko M, Marie-Elisabeth G, Martin W, Annette H, Ruth S-B, Barbara R, et al. A multicenter phase 1 study of solitomab (MT110, AMG 110), a bispecific EpCAM/CD3 T-cell engager (BiTE®) antibody construct, in patients with refractory solid tumors. OncoImmunology. 2018;7(8):e1450710.
65. Frey G, G. CAP, Haizhen L, Charles X, Christina W, Wen CH, et al. A novel conditional active biologic anti-EpCAM x anti-CD3 bispecific antibody with synergistic tumor selectivity for cancer immunotherapy. mAbs. 2024;16(1):2322562.
66. Staflin K, Zuch de Zafra CL, Schutt LK, Clark V, Zhong F, Hristopoulos M, et al. Target arm affinities determine preclinical efficacy and safety of anti-HER2/CD3 bispecific antibody. JCI Insight. 2020;5(7).
67. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res. 1986;46(12 Pt 1):6387-92.
68. Ahn M-J, Cho Byoung C, Felip E, Korantzis I, Ohashi K, Majem M, et al. Tarlatamab for Patients with Previously Treated Small-Cell Lung Cancer. New England Journal of Medicine. 2023;389(22):2063-75.