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Mechanism of Action and Pharmacokinetics of Approved Bispecific Antibodies

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Abstract

Bispecific antibodies represent a significant advancement in therapeutic antibody engineering, offering the ability to simultaneously target two distinct antigens. This dual-targeting capability enhances therapeutic efficacy, especially in complex diseases, such as cancer and autoimmune disorders, where drug resistance and incomplete target coverage are prevalent challenges. Bispecific antibodies facilitate immune cell engagement and disrupt multiple signaling pathways, providing a more comprehensive treatment approach than traditional monoclonal antibodies. However, the intricate structure of bispecific antibodies introduces unique pharmacokinetic challenges, including issues related to their absorption, distribution, metabolism, and excretion, which can significantly affect their efficacy and safety. This review provides an in-depth analysis of the structural design, mechanisms of action, and pharmacokinetics of the currently approved bispecific antibodies. It also highlights the engineering innovations that have been implemented to overcome these challenges, such as Fc modifications and advanced dimerization techniques, which enhance the stability and half-life of bispecific antibodies. Significant progress has been made in bispecific antibody technology; however, further research is necessary to broaden their clinical applications, enhance their safety profiles, and optimize their incorporation into combination therapies. Continuous advancements in this field are expected to enable bispecific antibodies to provide more precise and effective therapeutic strategies for a range of complex diseases, ultimately improving patient outcomes and advancing precision medicine.

Key Words: Bispecific antibody, Mechanism of action, Pharmacokinetics, Immunotherapy, Dual-targeting therapy, Antibody engineering

INTRODUCTION

The advent of antibody therapy has profoundly transformed modern medicine. The concept of using antibodies for therapeutic purposes dates to the late 19th and early 20th centuries (von Behring, 2024). The introduction of hybridoma technology in the 1970s enabled the production of monoclonal antibodies (mAbs) and facilitated extensive research on disease treatment (Kohler and Milstein, 1975). However, mAbs have not sufficiently addressed drug resistance in numerous diseases, particularly where tumor biology or underlying mechanisms are not fully understood (Aldeghaither *et al*., 2019; Torka *et al*., 2019). As a result, further research is needed, especially in the area of combination therapy, to address significant unmet medical needs. In the 1990s, advances in recombinant DNA technology and innovative engineering techniques led to the development of bispecific antibodies (BsAbs), designed to overcome the limitations of mAbs. BsAbs are engineered immunoglobulins that simultaneously bind to two distinct epitopes or antigens (Nisonoff and Mandy, 1962; Milstein and Cuello, 1983). Unlike mAbs which target a single antigen, BsAbs can exhibit a variety of mechanisms, including linking immune cells to cancer cells or simultaneously targeting different antigens or pathways (Haas *et al*., 2009; Nguyen *et al*., 2016; Lejeune *et al*., 2020). This diversity enables BsAbs to be used in various therapeutic strategies against cancer and

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other diseases. BsAbs are designed to simultaneously block multiple signaling pathways, thereby addressing the complex pathophysiological features characteristic of cancers and autoimmune diseases (Yarden and Shilo, 2007; Seshacharyulu *et al*., 2012).

The first clinically successful BsAb, catumaxomab (Removab®), was approved by the European Medicines Agency (EMA) in 2009 (withdrawn in 2017) for the treatment of malignant ascites in patients with epithelial cell adhesion molecule (EpCAM)-positive cancers (Tiller and Tessier, 2015). Since then, numerous BsAbs that target various diseases have demonstrated significant success in clinical development. BsAbs offer several advantages, particularly in oncology, where they enhance therapeutic efficacy by recruiting immune cells to cancer cells through simultaneous targeting of two distinct antigens. Additionally, because tumors often develop resistance to therapies targeting a single antigen, BsAbs have the potential to overcome this resistance by simultaneously targeting multiple antigens. They can also target multiple pathways to achieve effects similar to those of combination therapies, potentially leading to synergistic effects and improved clinical outcomes (Zhu *et al*., 2015). Furthermore, BsAbs can target diseases more precisely and selectively, thereby reducing off-target effects and enhancing their safety profile (Chen *et al*., 2021).

BsAbs have the potential to improve therapeutic efficacy, resistance and target specificity over conventional mAbs (Zhu *et al*., 2015). However, the intricate structure and dual-targeting nature of BsAbs impose distinct challenges on their pharmacokinetic (PK) profiles, necessitating thorough PK studies during their development. Moreover, the PK of BsAbs plays a crucial role in their development and clinical applications. A comprehensive understanding of the structure, mechanism of action (MOA), and PK of BsAbs is critical for optimizing their design and clinical applications. Such insights are essential to fully leverage their therapeutic potential. Building on this foundation, this review aimed to provide a comprehensive overview of the structural and PK profiles of currently approved BsAbs. This analysis serves as a basis for understanding the potentials and challenges associated with the development of BsAbs in this rapidly evolving field.

STRUCTURE DESIGN OF BISPECIFIC ANTIBODIES

Basic structural components

Variable regions of antibodies composed of variable heavy (VH) and variable light (VL) chains constitute the antigen-binding sites (Samuel and Naz, 2008). These regions are responsible for the specificity and affinity of antibodies toward their respective antigens (Sela-Culang *et al*., 2013). The fragment crystallizable (Fc) region is a constant part of the antibody that interacts with cell surface receptors and the complement system (Al-Taie and Sheta, 2024). Modifications of the Fc region can significantly enhance the functionality of BsAbs by extending their half-life, modulating immune effector functions, and improving their overall stability (Roopenian and Akilesh, 2007; Strohl, 2015; Carter and Lazar, 2018). These antibodies are typically engineered to possess two distinct fragment antigen-binding domains (Fab) arms, each specific to a different antigen (van Gils and Sanders, 2017). This configuration leverages the structural stability and effector functions inherent to conventional IgG antibodies while enabling bispecific binding (Schaefer *et al*., 2011; Lewis *et al*., 2014). BsAbs can be classified into two categories: IgG-like and non-IgG-like (Fig. 1, Table 1).

IgG-like bispecific antibodies

IgG-like BsAbs closely resemble conventional IgG antibodies but are engineered to possess dual specificity (Krishnamurthy and Jimeno, 2018). The Duobody platform utilizes a precise process in which two IgG1 molecules are individually engineered and recombined to generate bispecific antibodies. Each IgG1 molecule is independently engineered with specific mutations in the CH3 domain to facilitate correct chain pairing and association. After production and purification, the two IgG1 molecules undergo a controlled Fab-arm exchange, resulting in the formation of a bispecific antibody with high efficiency and yield (Labrijn *et al*., 2014). Another method of achieving precise pairing is through the use of the 'knobs-into-holes' approach, which introduces structural modifications in the CH3 domains to facilitate accurate chain association. The 'knobs' in one CH3 domain are designed to fit into complementary 'holes' in the opposite CH3 domain, ensuring that the heavy chains pair correctly and reducing the risk of mispairing (Ridgway *et al*., 1996). This method is particularly advantageous compared to other BsAb platforms such as Duobody, which rely on more flexible chain exchange methods that might result in less controlled pairing and potential off-target effects. CrossMAbs are notable IgG-like BsAb for their precise engineering, which ensures the correct pairing of heavy and light chains, thereby enhancing the stability and functionality of the antibody (Klein *et al*., 2019). CrossMAbs prioritize precise chain alignment, ensuring that the BsAb retains both its structural integrity and functional specificity. This precision minimizes unintended interactions while simultaneously enhancing the therapeutic efficacy of these antibodies, making them particularly effective in the clinical setting, especially in therapies requiring dual antigen targeting (Brinkmann and Kontermann, 2017). Each format embodies a unique strategy for the design and engineering of BsAbs, with the objective of optimizing their therapeutic potential by integrating specificity, stability, and functionality. In the following section, we describe the structures of approved BsAbs (Table 1).

First, emicizumab (Hemlibra®) is modeled on the structure of a human IgG antibody, maintaining the typical Y-shaped configuration. The Fab regions are engineered to bind to different antigens that target the factors IXa and X. The Fc region remains unmodified, providing an extended half-life through interaction with the neonatal Fc receptor for IgG (FcRn) while minimizing immune-mediated clearance (Kitazawa *et al*., 2012; Kitazawa and Shima, 2020). Amivantamab (Rybrevant®) is designed to simultaneously target the epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition factor (MET), both of which are crucial receptors in oncogenic signaling pathways. Utilizing the Duobody platform, amivantamab precisely controls the pairing of heavy and light chains, thereby enhancing structural stability and dual antigen binding capability. Additionally, the Fc region maintains the typical structure of IgG antibodies (Cho *et al*., 2023). Faricimab (Vabysmo®) is designed to target both vascular endothelial growth factor A (VEGF-A) and angiopoietin-2 (ANG-2). It utilizes CrossMAb technology to precisely align the heavy and light chains. This dual binding enhances its structural sta-

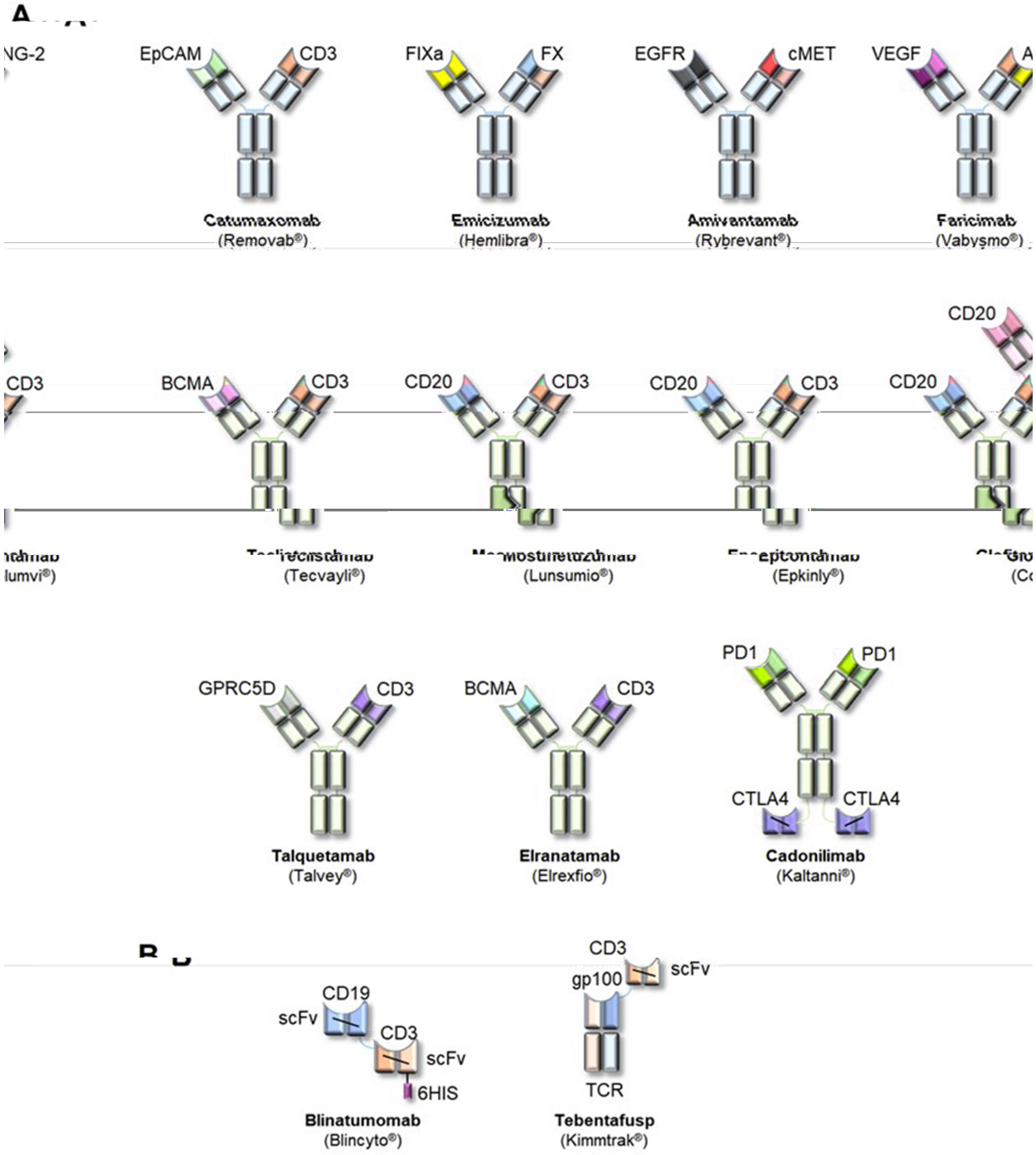

Fig. 1. Structure of approved bispecific antibodies. (A) Bispecific antibodies with IgG-like structures. The figure presents the approved BsAbs with IgG-like structures. These structures consist of two heavy chains (with constant regions depicted in light blue and VH domains highlighted in various colors) and two light chains (with constant domains also shown in light blue and VL domains in different colors). (B) Non-IgG-like bispecific antibodies. The figure is organized according to the approval date. ANG-2, angiopoietin-2; BCMA, B cell maturation antigen; cMET, c-mesenchymal-epithelial transition factor; CTLA4, cytotoxic T lymphocyte-associated antigen 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FX, coagulation factor X; FIXa, coagulation factor IXa; GPRC5D, G-protein-coupled receptor class C group 5 member D; gp100, glycoprotein 100; PD1, programmed cell death protein 1; scFv, a single-chain variable fragment; TCR, T-cell receptor; VEGF, vascular endothelial growth factor; 6HIS, 6X his-tag.

Table 1. Structural designs and clinical indications of bispecific antibodies

Structural design	Drug name (trade name)	Format	Targets	Mode of action	Indication	Approval status
IgG	Emicizumab (Hemlibra®)	Common light chain	Factor IXa, Factor X	Co-factor for blood clotting	Hemophilia A	2017 (FDA)
	Amivantamab (Rybrevant®)	Duobody	EGFR, MET	Dual inhibition of EGFR and MET	NSCLC with EGFR exon 20 insertion mutation	2021 (FDA)
	Faricimab (Vabysmo®)	CrossMab	VEGF-A, ANG-2	Dual inhibition of VEGF and ANG-2	Neovascular AMD	2022 (FDA)
	Teclistamab (Tecvayli®)	Duobody	BCMA, CD3	T cell engager	Multiple myeloma	2022 (FDA)
	Mosunetuzumab (Lunsumio®)	Knobs-into-holes	CD20, CD3	T cell engager	Relapsed/refractory non-Hodgkin lymphoma	2022 (FDA)
	Epcoritamab (Epkinly®)	Duobody	CD20, CD3	T cell engager	Relapsed/refractory diffuse large B-cell lymphoma	2023 (FDA)
	Glofitamab (Columvi®)	CrossMAb	CD20, CD3	T cell engager	Relapsed/refractory diffuse large B-cell	2023 (FDA)
	Talquetamab (Talvey®)	Duobody	GPRC5D, CD3	T cell engager	Multiple myeloma	2023 (FDA)
	Elranatamab (Elrexfio®)	Modified IgG	BCMA, CD3	T cell engager	Multiple myeloma	2023 (FDA)
	Cadonilimab (Kaltanni®)	Tetrabody	PD-1, CTLA-4	Dual immune checkpoint inhibition	Relapsed/refractory cervical cancer	2023 (NMPA)
	Catumaxomab (Removab®)	Triomab	EpCAM, CD3	T cell engager, Fc-mediated effects	Ovarian ascites	2009 (Withdrawn)
Non-IgG	Blinatumomab (Blincyto®)	BiTE	CD19, CD3	T cell engager	B cell precursor ALL	2014 (FDA)
	Tebentafusp (Kimmtrak®)	ImmTAC	gp100, CD3	T cell engager	Uveal melanoma	2022 (FDA)

bility and functional efficacy, while the unmodified Fc region supports extended half-life and immune system interaction. These structural optimizations are specifically designed to address the pathological angiogenesis and vascular instability characteristic of diseases such as neovascular age-related macular degeneration (nAMD) and diabetic macular edema (DME) (Regula *et al*., 2016; Patel *et al*., 2018; Panos *et al*., 2023). Teclistamab (Tecvayli®) is an antibody developed using the Duobody platform, which incorporates Fab-arm exchange technology to enable dual antigen binding. This format preserves the structural integrity and stability of the antibody while allowing dual antigen binding. The modified Fab regions of teclistamab direct T cells to tumor cells, thereby enhancing T cell-mediated cytotoxicity. Through the Duobody platform, teclistamab achieves both specificity and structural stability, making it a potent therapeutic agent. Mosunetuzumab (Lunsumio®) is based on an IgG1 scaffold incorporating knobs-into-holes technology. This design facilitates heterodimerization, ensuring correct heavy chain pairing, while reducing the likelihood of chain mispairing and incorrect chain formation. As mentioned above, this technology helps to stabilize the antibody structure and improves its functional efficacy. The structural design of mosunetuzumab has been optimized to provide effective and durable anti-tumor activity in patients with relapsed or refractory (R/R) B-cell lymphoma. Epcoritamab (Epkinly®), developed using the Duobody platform by Genmab and AbbVie, is designed to target both CD20 on B cells and CD3 on T cells, facilitating T-cell-mediated cytotoxicity against B cell malignancies (Thieblemont *et al*., 2023). This BsAb uses Duobody technology inspired by Fab-arm exchange, designed to preserve the regular structure of IgG antibody (Labrijn *et al*., 2014). Glofitamab (Columvi®) is a BsAb engineered to target CD20 on B cells and CD3 on T cells, thus promoting T-cell-mediated cytotoxicity against B-cell malignancies (Dickinson *et al*., 2022). This BsAb utilizes a distinctive 2:1 configuration (CrossMAb) featuring two binding sites for CD20 and one for CD3 (Dickinson *et al*., 2022). Glofitamab preserves the conventional Y-shaped structure of IgG antibodies, which comprises two heavy and light chains that support its bispecific functionality. The Fc region of glofitamab incorporates the knobs-into-holes strategy in the CH3 domains, ensuring correct chain association, which further stabilizes the antibody structure and contributes to its overall functional integrity (Salvaris *et al*., 2021). The Fab regions were designed to ensure high-affinity binding to both CD20 and CD3, thereby optimizing the redirection of T cells to recognize and eliminate B cells. Additionally, a 2:1 ratio enhances the specificity and effectiveness of the antibody by increasing the avidity of CD20, thereby enhancing its therapeutic potential for treating B cell lymphomas (Dickinson *et al*., 2022). Talquetamab (Talvey®) is a BsAb that targets GPRC5D and CD3 to direct T cells toward cancer cells (Einsele *et al*., 2024; Li *et al*., 2024). This BsAb maintains a conventional Y-shaped IgG structure comprising two heavy and two light chains (Duobody) (Chiu *et al*., 2019). Interaction with FcRn extends its half-life and enhances its stability (Sun *et al*., 2020). Fab regions were engineered to specifically bind to GPRC5D and CD3. Furthermore, an asymmetric format was applied to ensure the correct pairing of heavy and light chains (Cooke *et al*., 2018). Elranatamab (Elrexfio®) is also a BsAb engineered to target B-cell maturation antigen (BCMA) on multiple myeloma cells and CD3 on T cells, thereby facilitating T cell-mediated cytotoxicity against BCMA-expressing myeloma cells (Lesokhin *et al*., 2023; Tomasson *et al*., 2023). Structurally, it is engineered with an IgG2Δa format (modified IgG), which provides greater structural stability compared to the conventional IgG2 structure and is known to reduce immune-related adverse effects (van de Donk *et al*., 2023). This precise engineering enhances the antibody's ability to redirect T cells toward myeloma cells, significantly improving its therapeutic potential in the treatment of R/R multiple myeloma. Cadonilimab (Kaltanni®) is a uniquely designed tetravalent BsAb (tetrabody) that simultaneously targets both PD-1 and CTLA-4. It retains a symmetric IgG1 format, which enhances structural stability, and its Fab and Fc regions are engineered to achieve dual specificity. The tetravalent structure with four antigen binding sites offers a significant advantage over conventional BsAbs by increasing antigen binding affinity and improving therapeutic efficacy. This configuration helps to reduce off-target effects and elicit a more efficient immune response. The Fab region is designed to bind to PD-1, while the Fc region is modified to bind to CTLA-4 (Keam, 2022; Klein *et al*., 2024). Catumaxomab originally approved in 2009 but withdrawn in 2017 for commercial reasons, is a rat-mouse hybrid trifunctional IgG2 antibody (Triomab). It consists of two half antibodies, each containing a light chain, and a heavy chain derived from mouse IgG2a and rat IgG2b isotypes, respectively. It has two antigen-binding arms. Its Fc domain is engineered to interact with Fc receptors on immune cells, facilitating immune-mediated cytotoxicity through its unique trifunctional structure (Chelius *et al*., 2010).

In summary, IgG-like BsAbs represent a class of therapeutic agents that retain their structural stability and functional properties while simultaneously targeting multiple antigens. These antibodies, with their optimized design and engineering, hold significant promise in precision medicine, particularly for the treatment of complex diseases such as cancer. Further research should focus on expanding the clinical applications of BsAbs, exploring strategies to enhance their safety and efficacy, and ensuring their effective integration into therapeutic regimens.

Non-IgG-like bispecific antibodies

Non-IgG-like BsAbs have a more compact and flexible structure than IgG-like BsAbs. One strategy involves dimerizing single-chain variable fragments (scFvs) by incorporating a peptide linker between the two domains, resulting in the formation of two antigen-binding sites oriented in opposite directions (Holliger *et al*., 1993). Bispecific T cell Engagers (BiTEs) are compact molecules composed of two scFvs connected by a flexible linker that is structurally designed to target CD3 on T cells and tumor-specific antigens (Huehls *et al*., 2015; Tian *et al*., 2021). For example, blinatumomab (Blincyto®), the first BiTE approved by the FDA, is composed of two scFvs engineered to specifically target and bind to CD19 expressed on the surface of B cell acute lymphoblastic leukemia (ALL) cells and CD3 on T cells (Wu *et al*., 2015). The small size and flexibility of BiTEs facilitate effective immune synapse formation and potent T cell-mediated cytotoxicity, enabling rapid tumor tissue penetration and clearance from non-target tissues, thereby improving the therapeutic index (Carrasco-Padilla *et al*., 2022). Dual-affinity re-targeting (DART) molecules were developed by MacroGenics as an advanced variation of diabodies distinguished by their enhanced stability and binding affinity (Johnson *et al*., 2010). DART molecules consist of two

Fv fragments, each comprising a VH domain from one antibody and a VL domain from another, resulting in two unique antigen binding sites. In contrast, diabodies are composed of two chains, each pairing a VH and VL domain from different antibodies, arranged in a head-to-tail configuration. DART molecules enhance stability and binding affinity of diabody through the incorporation of proprietary disulfide linkages and optimized amino acid sequences, ensuring efficient chain paring and overall structural stability. Tandem diabodies (TandAbs), tetravalent derivatives, are formed by the polymerization of two diabodies connected by polypeptide chains, resulting in molecules with two antigen binding sites for each target antigen. TandAb is designed to enhance the efficacy of cancer immunotherapy. It connects two scFvs to form BsAbs that create a strong link between tumor cells and immune cells. Targeting two distinct antigens allows TandAbs to increase specificity and reduce off-target effects, enabling more precise tumor recognition than conventional mAbs. The reduced size of TandAbs improves penetration into tumor tissues and increases fluidity in the bloodstream, allowing rapid clearance from non-target areas, thereby improving the therapeutic index (Kipriyanov *et al*., 1999). Additionally, their small size contributes to increased fluidity in the bloodstream, enabling swift clearance from non-target areas (Ahmad *et al*., 2012; Monnier *et al*., 2013). In addition to various scFv-based strategies that simultaneously target both tumor and immune cells, there is the direct use of T cell receptor (TCR) fusion. The structure of the immune mobilizing monoclonal TCR against cancer (ImmTAC) is engineered to combine the unique specificity of TCRs, which recognize tumor-associated peptides presented by major histocompatibility complex (MHC) Class I molecules, with an antibody fragment that binds to CD3 on T cells (Hua *et al*., 2022). This design allows for the precise redirection of T cells towards cancer cells. The small size and enhanced stability of ImmTAC molecules improve tumor penetration and enable sustained T cell-mediated cytotoxicity. For example, tebentafusp (Kimmtrak®) is a TCR-based ImmTAC therapy that has shown promising efficacy by targeting glycoprotein 100 (gp100), a tumor-associated antigen, and engaging CD3 on T cells to promote targeted cytotoxicity in melanoma cells (Nathan *et al*., 2021; Howlett *et al*., 2023).

BsAbs demonstrate enhanced efficacy by simultaneously targeting multiple antigens, thereby potentially overcoming the limitations of single-target therapies. However, these trials have revealed challenges in clinical applications, including the induction of immune responses and stability-related issues. Non-IgG-like BsAbs, which generally exhibit a smaller size and a more flexible structure than IgG-like antibodies, may exhibit lower immunogenicity owing to improved tissue penetration (Fan *et al*., 2015; Li *et al*., 2020). Nonetheless, the immunogenicity is not uniformly low and can vary based on specific structural configurations (Hermeling *et al*., 2004; Baker *et al*., 2010). Increased immunogenicity has the potential to trigger adverse immune reactions, complicating their clinical application (Chirmule *et al*., 2012; Ratanji *et al*., 2014; Pineda *et al*., 2016). Additionally, reduced stability may lead to a shorter half-life, necessitating more frequent dosing (Cumber *et al*., 1992; Sanz *et al*., 2005). Ongoing engineering modifications to improve stability and reduce immunogenicity are critical for the broader adoption of non-IgG-like BsAbs in therapeutic settings.

MECHANISMS OF ACTION OF APPROVED BISPECIFIC ANTIBODIES

The MOA of BsAbs is diverse and depend on their specific design and target (Sun *et al*., 2023). These mechanisms are based on the ability of BsAbs to bring together two targets, cancer cells and immune cells, thereby facilitating specific intercellular interactions and enhancing therapeutic effects (Huang *et al*., 2020). They are particularly promising for immunotherapy of cancer, hematological disorders, autoimmune diseases, and infectious diseases (Hosseini *et al*., 2021). Several key mechanisms are commonly employed for their therapeutic actions (Table 1, Fig. 2).

T cell redirection and activation

One of the most prominent applications of BsAbs is in cancer immunotherapy where they are used to redirect and activate T cells against tumor cells (Qi *et al*., 2023). T cell engagers (TCEs) are a specialized class of BsAbs designed to harness the body's immune system to combat cancer by bringing T cells close to tumor cells. These engineered antibodies possess two distinct binding sites: one that targets a specific antigen on the surface of cancer cells, and the other that binds to a component of the TCR complex. This dual-targeting mechanism facilitates the formation of an immunological synapse between T cells and cancer cells, leading to a potent immune response against the tumor (Qi *et al*., 2023; van de Donk and Zweegman, 2023; Surowka and Klein, 2024). The primary MOA of TCEs involves several critical steps that result in the activation and recruitment of T cells to the tumor site.

TCEs simultaneously bind to both tumor antigens and T cells. One arm of the TCE is designed to specifically bind to a tumor-associated antigen (TAA) expressed on the surface of cancer cells. Antigens such as CD19, CD20, B cell maturation antigen (BCMA), CD123, human epidermal growth factor receptor 2 (HER2), or EGFR, are often overexpressed in tumor cells and serve as markers for TCE targeting (Baeuerle and Wesche, 2022; Cattaruzza *et al*., 2023). The other arm of the TCE binds to the CD3 epsilon (ε) subunit of the TCR complex on T cells (Qi *et al*., 2023). CD3 is a key component of TCR and plays a crucial role in T cell activation and signaling (Gil *et al*., 2002; Tailor *et al*., 2008).

Upon simultaneous binding to the tumor antigen and the CD3 subunit on T cells, TCE facilitates the formation of stable immunological synapses (Baeuerle and Wesche, 2022). This synapse brings T and cancer cells into proximity, creating an environment conducive to T cell activation (Strohl and Naso, 2019). Engagement of CD3 triggers T cell activation, leading to a cascade of intracellular signaling events that results in T cell proliferation and cytokine secretion (Smith-Garvin *et al*., 2009). Activated T cells release cytotoxic molecules, such as perforin (pore-forming proteins) and granzymes (apoptosis-inducing proteases), which penetrate and destroy cancer cells. This targeted cytotoxic response ensures that tumor cells are efficiently eradicated while minimizing damage to healthy tissues (Dustin, 2014).

In addition to cytotoxicity, activated T cells secrete cytokines, including interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which further enhance the immune response by recruiting additional immune cells and promoting inflammation at the tumor site (Burkholder *et al*., 2014; Jorgovanovic *et al*., 2020). T cell engagement also stimulates

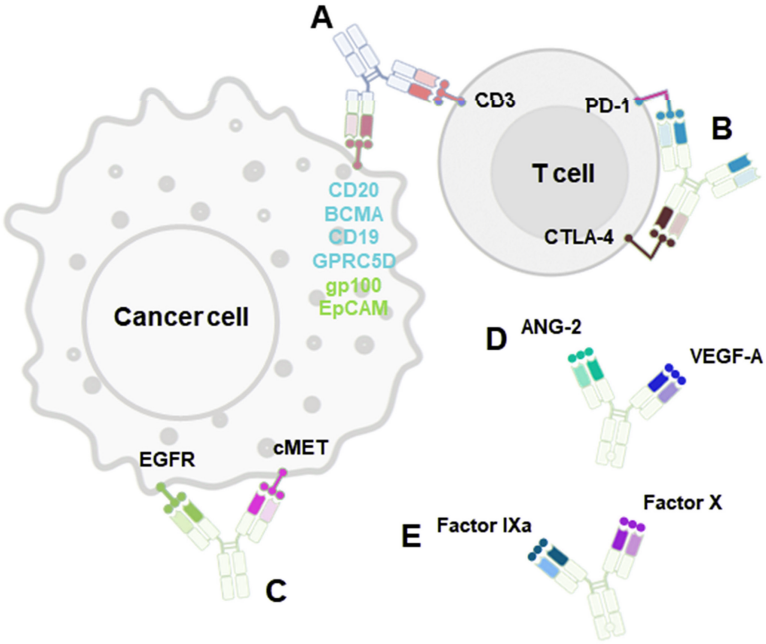

Fig. 2. Modes of action of approved bispecific antibodies. (A) Effector cell engagement through CD3 on T cells. CD20 (mosunetuzumab, epcoritamab, glofitamab), BCMA (teclistamab, elranatamab), CD19 (blinatumomab), and GPRC5D (talquetamab) on hematological tumors; gp100 (tebentafusp) and EpCAM (catumaxomab) on solid tumors. (B) Checkpoint binding-mediated immune cell co-stimulation; PD-1/CTLA-4 (cadonilimab). (C) Cell signal inhibition by the blockade of two signaling receptors; EGFR/MET (amivantamab). (D) Cell signal inhibition by the neutralization of soluble two ligands; VEGF-A/ANG-2 (faricimab). (E) Coagulation factor replacers. Factor X/Factor IXa (emicizumab). ANG-2, angiopoietin-2; BCMA, B cell maturation antigen; cMET, c-mesenchymal-epithelial transition factor; CTLA4, cytotoxic T lymphocyte-associated antigen 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FX, coagulation factor X; FIXa, coagulation factor IXa; GPRC5D, G-protein-coupled receptor class C group 5 member D; gp100, glycoprotein 100; PD-1, programmed cell death protein 1; VEGF-A, vascular endothelial growth factor-A.

T cell proliferation, leading to an expanded population of effector T cells capable of recognizing and attacking tumor cells throughout the body (Ahmed *et al*., 2023).

Several TCEs have been approved for clinical use, demonstrating their effectiveness in the treatment of various types of cancer: mosunetuzumab (Kang, 2022a), epcoritamab, glofitamab (Dickinson *et al*., 2022; Thieblemont *et al*., 2023), teclistamab and elranatamab (Kang, 2022b), blinatumomab (Goebeler and Bargou, 2016), talquetamab (Keam, 2023), tebentafusp (Hua *et al*., 2022), catumaxomab (Chelius *et al*., 2010).

Immune checkpoint inhibition

Immune checkpoints are regulatory mechanisms in the immune system that are crucial for maintaining self-tolerance and for adjusting the duration and amplitude of physiological immune responses in peripheral tissues, thereby reducing collateral tissue damage (Pardoll, 2012; Taefehshokr *et al*., 2020). However, tumors can exploit these pathways to evade immune surveillance. Representative immune checkpoint targets include programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T lymphocyte–associated antigen 4 (CTLA-4, alternatively known as CD152) (Pardoll, 2012). PD-1 is a protein on the surface of T cells that is engaged by its ligands PD-L1 or PD-L2 to inhibit T cell activation (Keir *et al*., 2008; Han *et al*., 2020). PD-L1 is expressed on cancer cells and other cells within the tumor microenvironment, helping the tumor evade immune detection (Keir *et al*., 2006). CTLA-4 is another inhibitory receptor on T cells that downregulates immune responses by outcompeting the stimulatory receptor CD28 for binding to B7 molecules (CD80/CD86) on antigen-presenting cells (APCs) (Taefehshokr *et al*., 2020). It mainly acts in the lymph nodes to dampen early-stage T cell activation (Buchbinder and Desai, 2016).

BsAbs can be designed to simultaneously target two distinct immune checkpoints: PD-1 and CTLA-4. This approach was designed to overcome the limitations of therapies that target a single checkpoint and provide more comprehensive activation of the immune system (Farhangnia *et al*., 2023). Tumors can develop resistance to single checkpoint inhibitors through various mechanisms such as upregulating alternative checkpoints or increasing immunosuppressive cell populations (Barrueto *et al*., 2020). Dual blockade can counteract these resistance mechanisms by targeting multiple pathways simultaneously, thereby reducing the ability of the tumor to adapt and escape immune attacks (Tojjari *et al*., 2023). Several BsAbs are being developed and tested in clinical trials, and an example of an approved BsAb with dual immune checkpoint blockade is cadonilimab for the treatment of relapsed or metastatic cervical cancer. This antibody simultaneously blocks PD-1 and CTLA-4, enhancing T cell activation and reducing Treg-mediated suppression (Keam, 2022; Klein *et al*., 2024).

Receptor tyrosine kinases (RTKs) targeting

BsAbs simultaneously inhibit two distinct molecular pathways involved in disease progression, thereby offering a

broader and more effective therapeutic approach (Huang *et al*., 2020). By targeting both angiogenic and inflammatory pathways, which are crucial for tumor growth and disease progression, BsAbs enhance treatment efficacy and reduce the likelihood of resistance development. An example of a BsAb approved to block multiple pathways is amivantamab (Syed, 2021). This antibody targets EGFR and MET, two critical receptors involved in oncogenic signaling (Cho *et al*., 2023). Amivantamab has been approved for the treatment of non-small cell lung cancer (NSCLC) with EGFR exon 20 insertion mutations, where it disrupts these pathways to inhibit tumor growth and promote cancer cell death (Syed, 2021; Cho *et al*., 2023). By blocking both EGFR and MET, amivantamab provides a comprehensive approach to interfere with tumor growth and survival mechanisms.

Neutralization of soluble ligands

BsAbs bind and neutralize soluble ligands, preventing them from interacting with their receptors, and thereby modulating disease processes (Kontermann, 2012). A prime example of this dual ligand targeting capability is faricimab, an FDA-approved BsAb. Faricimab is specifically designed to simultaneously bind and inhibit both ANG-2 and VEGF-A, two key players in pathological angiogenesis (Shirley, 2022). It neutralizes VEGF-A to prevent it from promoting abnormal blood vessel growth and leakage, while also inhibiting ANG-2, which competes with angiopoietin-1 (ANG-1) to bind to Tie-2 receptors (Shirley, 2022). This inhibition helps restore vascular stability and reduce inflammation by allowing ANG-1 to effectively activate the Tie-2 receptor (Joussen *et al*., 2021; Shirley, 2022). This dual-action approach not only controls nAMD and DME more comprehensively but also allows for less frequent dosing with intervals of up to 16 weeks, thus enhancing patient convenience and treatment adherence (Shirley, 2022; Ferro Desideri *et al*., 2023).

Coagulation factor replacers

BsAbs also act as enzyme or cofactor mimics, facilitating reactions by bringing enzymes and substrates together or by substituting missing or dysfunctional cofactors in enzymatic pathways (Kang *et al*., 2022). Among the approved BsAbs, emicizumab is a notable cofactor mimic (Scott and Kim, 2018; Blair, 2019). It specifically targets the activated factors IXa and X in the coagulation cascade, mimicking the function of missing factor VIII in patients with hemophilia A by bridging factors IXa and X to promote clotting and significantly reduce bleeding episodes (Blair, 2019).

PHARMACOKINETICS OF BISPECIFIC ANTIBODIES

During drug development, PK involves absorption, distribution, metabolism, and excretion (ADME) in the body. PK studies are essential for predicting a drug's blood concentration, duration of action, systemic distribution, cost reduction, and personalized treatment (Meibohm and Derendorf, 2002; Reichel and Lienau, 2016; Kantae *et al*., 2017). PK studies contribute to determining the optimal dosing time to maximize the efficacy and minimize the toxicity of BsAbs (Gibbs *et al*., 2020). Table 2 and 3 summarizes the PK profiles of the FDA-approved BsAbs and the binding affinity and protein binding properties of BsAbs, respectively.

Table 2. Pharmacokinetic profiles of bispecific antibodies

Drug name (trade name)	Absorption	Volume of distribution	Clearance	Half-life	Ref.
Blinatumomab (Blincyto®)	Intravenous	5.27 L	3.11 L/h	~2.1 h	Food and Drug Administration, 2024c
Emicizumab (Hemlibra®)	Subcutaneous	10.4 L	0.24 L/day	~30 days	Food and Drug Administration, 2024e
Amivantamab (Rybrevant®)	Intravenous	5.34 L	360 ± 144 mL/day	~13.7 days	Food and Drug Administration, 2024b
Tebentafusp (Kimmtrak®)	Intravenous	7.56 L	16.4 L/day	~7.5 h	Food and Drug Administration, 2024i
Faricimab (Vabysmo®)	Intravitreal	5.91 L	Not available	~12.0 days	Food and Drug Administration, 2024a
Teclistamab (Tecvayli®)	Subcutaneous	5.63 L	0.472 L/day	~15 days	Food and Drug Administration, 2024j
Mosunetuzumab (Lunsumio®)	Intravenous	5.49 L	1.08 L/day	~16.1 days	Food and Drug Administration, 2024g
Epcoritamab (Epkinly®)	Subcutaneous	25.6 L	0.53 L/day	~33.0 days	Food and Drug Administration, 2024f
Glofitamab (Columvi®)	Intravenous	5.61 L	0.674 L/day	~7.6 days	Food and Drug Administration, 2024d
Talquetamab (Talvey®)	Subcutaneous	10.1 L	0.90 L/day	~17.7 days	Food and Drug Administration, 2024h
Elranatamab (Elrexfio®)	Subcutaneous	7.76 L	0.324 L/day	~22 days	Food and Drug Administration, 2023
Cadonilimab (Kaltanni®)	Subcutaneous	6.1 L	Not available	~4.76 days	Keam, 2022; Li *et al*., 2024
Catumaxomab (Removab®)	Intraperitoneal	Not specified	Not available	~2.13 days	Ruf *et al*., 2010b

Table 3. Binding affinity and protein binding properties of bispecific antibodies

Drug name (trade name)	Target 1	Target 1 (K_d)	Target 2	Target 2 (K_d)	Plasma protein binding (%)	Manufacturer
Blinatumomab (Blincyto®)	CD19	1.5×10^{-9}	CD3	2.6×10^{-7}	5	Amgen Inc.
Emicizumab (Hemlibra®)	FIXa	1.5×10^{-6}	FX	9×10^{-7}	97	Roche
Amivantamab (Rybrevant®)	EGFR	1.4×10^{-9}	MET	4×10^{-11}	90	Johnson & Johnson
Tebentafusp (Kimmtrak®)	gp100	2.4×10^{-11}	CD3	3.8×10^{-8}	85	Immunocore
Faricimab (Vabysmo®)	VEGF	2×10^{-8}	Ang-2	1×10^{-9}	75	Roche
Teclistamab (Tecvayli®)	BCMA	1.8×10^{-10}	CD3	2.8×10^{-8}	80	Johnson & Johnson
Mosunetuzumab (Lunsumio®)	CD20	6.8×10^{-8}	CD3	4×10^{-8}	78	Roche
Epcoritamab (Epkinly®)	CD20	2.4×10^{-9}	CD3	4.7×10^{-9}	95	Genmab A/S
Glofitamab (Columvi®)	CD20	N/A	CD3	4×10^{-10}	77	Roche
Talquetamab (Talvey®)	GPRC5D	4.21×10^{-9}	CD3	2.5×10^{-8}	70	Johnson & Johnson
Elranatamab (Elrexfio®)	BCMA	3.8×10^{-11}	CD3	1.7×10^{-9}	70	Johnson & Johnson
Cadonilimab (Kaltanni®)	PD-1	1×10^{-10}	CTLA4	4.1×10	60	Akeso Inc.
Catumaxomab (Removab®)	EpCAM	5.6×10^{-10}	CD3	4.4×10^{-9}	70	Fresenius Biotech

Absorption

The absorption route significantly influences bioavailability. BsAbs have poor stability in the gastrointestinal tract and low permeability across the gut wall, and consequently negligible oral bioavailability, similar to conventional therapeutic proteins. BsAbs can be administered via intravenous (IV), intraperitoneal (IP), or subcutaneous (SC) injection (Rathi and Meibohm, 2015). Although BiTE molecules are small, which may suggest rapid absorption kinetics, they are typically administered intravenously to achieve more rapid systemic exposure. IgG-like BsAbs, such as CrossMAbs, are commonly administered by SC injection due to their relatively large molecular size, which leads to slower but sustained absorption. This difference in profile between the two platforms can significantly influence the onset of therapeutic action and the overall duration of treatment. For example, the T cell engager blinatumomab is administered intravenously, providing rapid systemic availability, whereas elranatamab is administered subcutaneously, allowing for sustained absorption. IV administration delivers the drug directly into the bloodstream, thereby ensuring its immediate and complete bioavailability. This characteristic is particularly beneficial for BsAbs that require rapid therapeutic action, such as amivantamab, which is used to treat non-small cell lung cancer (NSCLC). Through this route, amivantamab quickly reaches the systemic circulation, enabling prompt engagement with its target antigens, EGFR and mesenchymal-epithelial transition factor (MET) (Cho *et al*., 2023). Similarly, tebentafusp, used to treat uveal melanoma, also benefits from IV administration, enabling rapid exposure and action (Food and Drug Administration, 2024i). Mosunetuzumab and glofitamab, both indicated for B cell non-Hodgkin's lymphoma, are also administered intravenously (Food and Drug Administration, 2024d, 2024g). This method allows these BsAbs to achieve rapid systemic concentrations, which are essential for their T cell-engaging mechanism to effectively target and eliminate malignant B cells. In contrast, SC administration offers the convenience of less frequent dosing and self-administration but generally leads to slower and more prolonged absorption (Stoner *et al*., 2014; Johnson *et al*., 2019). This route is exemplified by several BsAbs, including emicizumab, that are used to prevent bleeding in patients with hemophilia A. SC administration of emicizumab allows for sustained drug release, maintains stable therapeutic levels over time, and reduces injection frequency (Mahlangu *et al*., 2018). Teclistamab, epcoritamab, and talquetamab are additional examples of BsAbs administered subcutaneously, primarily for the treatment of multiple myeloma. These therapies benefit from the prolonged absorption profile of the SC route, allowing consistent therapeutic levels while minimizing the burden of frequent dosing (Haraya *et al*., 2017). Elranatamab, another BsAb used to treat multiple myeloma, also follows the same route of administration. The sustained release provided by SC injections ensures that patients can manage their condition with fewer injections, thus enhancing overall treatment adherence and comfort (Stoner *et al*., 2014; Jonaitis *et al*., 2021). Finally, cadonilimab designed for the treatment of advanced cervical cancer, is administered subcutaneously to benefit from sustained drug release and improved patient compliance. Understanding these differences in absorption profiles based on the route of administration is crucial for optimizing the dosing strategy of BsAbs and ensuring an appropriate balance between efficacy, safety, and patient compliance.

Distribution

In PK, the volume of distribution (V_d) is a critical parameter that provides insights into the distribution characteristics of a drug (Gillette, 1973). Higher V_d values indicate that the drug extensively permeates tissues and extracellular fluids, which is particularly important for drugs targeting widespread or deep-seated tissues such as tumors.

The differences in V_d among the BsAbs can be attributed to several complex factors. First, the molecular structure and size of each BsAb play crucial roles in determining the distribution of the drug beyond the central compartment. For BsAbs with a relatively large molecular size, their movement from the blood vessels to the interstitial tissue space occurs mainly convection rather than diffusion (Baxter *et al*., 1994). In the case of full-length IgG and F(ab')$_2$ (two linked antigen-binding fragments), convection is the dominant mechanism for extravasation. Larger IgG-like BsAbs, such as CrossMAbs, generally have a lower V_d due to their size and structural complexity, which limits their tissue penetration. In contrast, for BsAbs with relatively smaller molecular sizes, such as BiTE, DART, and nanobody, diffusion may also contribute to tissue distribution. In the case of smaller Fab fragments, diffusion plays a more significant role (Baxter *et al*., 1994). Smaller non-IgG-like BsAbs, such as BiTEs, have a higher V_d, allowing for more extensive tissue distribution. This difference in tissue penetration has significant implications for the therapeutic efficacy of these molecules, particularly in solid tumors where deeper tissue penetration is critical (Chen and Xu, 2017). Conversely, lower V_d values suggest that the drug remains within the plasma, potentially resulting in higher plasma concentrations and more rapid clearance (Øie, 1986; Toutain and Bousquet-Melou, 2004; Roberts *et al*., 2013). Larger or more complex molecules often exhibit restricted distribution and are primarily confined to the blood and lymphatic systems, whereas smaller molecules may exhibit more extensive tissue penetration (Liu, 2018). This insight is crucial for optimizing drug dosing and achieving therapeutic efficacy, while minimizing adverse effects.

Antigen specificity and target tissue distribution are also critical determinants of V_d. BsAbs targeting antigens that are primarily confined to the bloodstream or lymphatic system generally exhibit lower V_d values, whereas those targeting antigens with a broader tissue distribution tend to show higher V_d values, which can be attributed to increased tissue penetration. BsAbs that target cell surface antigens on B cell lymphoma and T cell, such as blinatumomab (BiTE, 55 kDa), typically have lower V_d values (5.27 L), indicating limited tissue distribution. Similarly, mosunetuzumab and glofitamab, bispecific antibodies with comparable pharmacokinetic characteristics, have V_d values of 5.49 L and 5.61 L, respectively, further supporting this distribution pattern (Food and Drug Administration, 2024b). In contrast, epcoritamab, which targets tissue-bound antigens, has a higher V_d (25.6 L) and a broader tissue distribution (Food and Drug Administration, 2024f). To optimize the uptake and penetration of BsAbs into solid tumors, several factors need to be carefully evaluated, including the appropriate binding affinities for tumor antigens and effector cells, as well as the optimal molecular size to achieve sustained systemic exposure (Chen and Xu, 2017).

The physicochemical properties of a drug, including lipophilicity, charge, and stability, play crucial roles in its distribution. For instance, lipophilic drugs are more likely to traverse cell membranes and accumulate in tissues, resulting in higher V_d, whereas hydrophilic drugs are more likely to remain confined to the vascular space (Liu *et al*., 2011). Administration routes can also influence V_d of BsAbs. For example, intravenously administered blinatumomab has a V_d of 5.27 L, indicating limited distribution, primarily within the blood and lymphatic systems. In contrast, subcutaneously administered epcoritamab (Duobody) has a V_d of 25.6 L, demonstrates extensive distribution into various tissues. The variability in distribution volumes among BsAbs highlights the differences in pharmacokinetic profiles (Gillette, 1973; Vugmeyster *et al*., 2012).

These factors are crucial for optimizing the pharmacokinetic profiles of BsAbs, enabling precise adjustment of dosing regimens to maximize therapeutic efficacy while minimizing adverse effects. The observed variability in V_d among BsAbs highlights the necessity for individualized PK evaluation during drug development and clinical applications. This approach ensures that each therapeutic agent is optimally deployed to achieve maximum efficacy across patient populations.

Metabolism

BsAbs undergo catabolism primarily via proteolytic enzymes in the reticuloendothelial system, particularly in the liver and kidneys (Vugmeyster *et al*., 2012). The Fc region allows antibodies to engage with FcRn receptors, which protects them from lysosomal degradation by diverting them away from the lysosome and facilitates their recycling back into circulation. This recycling process significantly prolongs its systemic presence, reduces the need for frequent dosing, and enhances the therapeutic window. Fc-containing BsAbs such as CrossMAbs and Duobody, benefit from FcRn-mediated recycling, which significantly prolongs their half-lives by protecting them from rapid proteolytic degradation. In contrast, smaller non-IgG-like BsAbs like BiTEs and TandAbs, which lack an Fc region, are more prone to rapid metabolism and therefore have shorter half-lives. These metabolic differences necessitate more frequent dosing of non-IgG-like BsAbs compared to IgG-like BsAbs, which maintain therapeutic levels for a longer duration due to FcRn recycling (Chen and Xu, 2017). Some BsAbs have been engineered with Fc modifications or albumin-binding domains to extend their half-life (Ebrahimi and Samanta, 2023). For example, blinatumomab and tebentafusp are characterized by rapid systemic clearance. This rapid clearance is attributed to their small size and the absence of Fc-mediated recycling, necessitating more frequent dosing in clinical applications. Conversely, BsAbs engineered with the Fc region, such as emicizumab, amivantamab, faricimab, mosunetuzumab, epcoritamab, and glofitamab, generally have longer half-lives.

Advanced engineering strategies such as CrossMab and knobs-into-holes aim to enhance the structural stability and circulation time of BsAbs. CrossMab technology involves the exchange of domains between the heavy and light chains to maintain stability and functionality (Klein *et al*., 2019). For example, elranatamab and talquetamab have been developed using these methods to enhance their resistance to proteolytic degradation and to prolong their half-lives, thereby reducing the frequency of administration. Knobs-into-holes is another innovative approach that enhances heterodimer formation, thereby improving PK properties and reducing the clearance rate of BsAbs (Xu *et al*., 2015). Catumaxomab, a hybrid of murine and rat antibodies, poses various metabolic challenges.

Its non-human origin makes it more immunogenic and causes it to be rapidly cleared from the body. Additionally, it does not undergo FcRn-mediated recycling as efficiently as human or humanized antibodies, resulting in a shorter half-life and faster clearance.

Like conventional mAbs, BsAbs may undergo target-mediated drug disposition (TMDD) as clearance pathways. At lower doses, BsAbs that bind to cell membrane antigens may exhibit dose-dependent clearance, leading to faster elimination and shorter half-life. At higher doses, when the target-mediated clearance is saturated, PK become linear. In a xenograft mouse model, systemic bioavailability of catumaxomab decreased with increasing EpCAM-positive tumor burden and CD3-positive cells, indicating target-mediated disposition (Ruf *et al*., 2010a). This was further supported by tumor accumulation and decreased systemic availability. Conventional mAbs targeting soluble antigens at low levels typically show linear PK, while high endogenous levels or multiple binding epitopes can cause non-linear PK. BsAbs may exhibit similar behavior. As a biodistribution process, liver and spleen uptake BsAbs, suggesting that the decreased blood concentration may be attributed to the activity of the mononuclear phagocytic system (Datta-Mannan *et al*., 2016). BsAbs revealed predominant binding to liver sinusoidal endothelial cells, suggesting that the macrophage system also contributed slightly to the rapid clearance of BsAbs (Datta-Mannan *et al*., 2016).

Understanding the metabolic pathways of BsAbs is critical to optimize their therapeutic potential. Advanced engineering strategies are being developed to enhance these properties and improve the clinical efficacy of BsAbs. This variability in metabolic pathways highlights the necessity for individualized PK assessments during drug development to ensure that each therapeutic agent is effectively utilized across diverse patient populations.

Excretion

The contribution of renal clearance to BsAb elimination is considered to be influenced by the molecular size. For BsAbs with molecular weights below 70 kDa such as BiTE and DART, which fall under the renal filtration threshold, renal clearance may contribute significantly to the overall clearance and leading to short systemic persistence. BiTE molecules (approximately 55 kDa) are generally excreted more rapidly via the kidneys due to their small size and rapid clearance, whereas IgG-like BsAbs, including CrossMAbs, are excreted more slowly due to their longer half-life and FcRn recycling mechanisms (Chen and Xu, 2017). This results in less frequent dosing for IgG-like BsAbs, compared to BiTEs, which require more frequent dosing to maintain therapeutic efficacy. PK parameters, such as half-life, provide insights into the duration of action and dosing frequency required to maintain therapeutic levels. Blinatumomab (a small BsAb in BiTE) exhibits a short half-life of approximately 2.1 h in human, probably due to its small molecular size and lack of an Fc domain (Food and Drug Administration, 2024c). This rapid clearance necessitates continuous IV infusion to maintain therapeutic efficacy (Liu, 2018). Tebentafusp (a small BsAb in ImmTAC) also has a short half-life of approximately 7.5 h in human, probably due to its small molecular size of 77 kDa and lack of an Fc domain (Food and Drug Administration, 2024i). In contrast, cadonilimab is an anti-PD-1/CTLA-4 BsAb in tetrabody format with a molecular weight of approximately 200 kDa (Keam, 2022). The half-life of cadonilimab is approximately 4.76 days (Keam, 2022). Emicizumab (approximately 145 kDa) has a notably prolonged half-life of approximately 30 days, which facilitates less frequent dosing regimens and enhances patient compliance and convenience (Food and Drug Administration, 2024e). This extended half-life was attributed to the FcRn-mediated recycling process, which mirrors that of endogenous IgG, allowing sustained therapeutic levels with less frequent administration (Liu, 2018; Schmitt *et al*., 2021). In addition, adjusting the FcRn affinity presents the possibility of programming the half-life of therapeutic agents, thereby enhancing their PK and therapeutic efficacy (Mandrup *et al*., 2021).

These examples highlight the impact of molecular design on the PK and excretion patterns of BsAbs. For instance, molecules such as faricimab and glofitamab, which are engineered with Fc regions, benefit from FcRn-mediated recycling, leading to reduced clearance rates (Nicolo *et al*., 2021). This characteristic enables extended dosing intervals and enhances patient compliance, particularly in chronic treatment settings. Furthermore, current research efforts have focused on modifying BsAb structures, such as by incorporating albumin-binding domains or other half-life extension technologies, which offer promising opportunities for optimizing their PK (Kim *et al*., 2007; Nilvebrant *et al*., 2011). When tailored to specific clinical contexts and patient populations, these modifications could result in more personalized therapeutic strategies, ensuring that each BsAb achieves its full therapeutic potential (Vugmeyster *et al*., 2012; Xu and Vugmeyster, 2012; Ma *et al*., 2021). Future directions for BsAb development will likely focus on enhancing metabolic stability and excretion profiles using innovative engineering approaches. These advances are crucial for expanding the therapeutic applications of BsAbs in various diseases.

CONCLUSIONS

Advancements in BsAbs have marked pivotal developments in therapeutic antibody engineering, providing enhanced efficacy and specificity relative to conventional mAbs. Through simultaneous targeting of two distinct antigens, BsAbs can overcome the challenges frequently encountered with mAbs, including drug resistance and incomplete target coverage. The MOA of BsAbs, which enables the direct engagement of immune cells with cancer cells and the inhibition of multiple signaling pathways, offers a more targeted therapeutic approach for treating complex diseases. Nevertheless, the sophisticated structure and dual-targeting capability of BsAbs introduce distinct PK challenges that require comprehensive investigation to optimize their design and clinical utility. As engineering refinements continue to address these challenges, future research should focus on broadening the clinical applicability of BsAbs, enhancing their safety profiles, and developing strategies for their seamless integration into combination therapies. Continued innovation in BsAb technology has the potential to deliver novel therapeutic strategies for conditions that are currently challenging to treat with existing modalities, thereby significantly advancing the field of precision medicine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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