Trends in Cancer

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Feature Review

Bispecific antibodies: advancing precision oncology

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Bispecific antibodies (bsAbs) are engineered molecules designed to target two different epitopes or antigens. The mechanism of action is determined by the bsAb molecular targets and structure (or format), which can be manipulated to create variable and novel functionalities, including linking immune cells with tumor cells, or dual signaling pathway blockade. Several bsAbs have already changed the treatment landscape of hematological malignancies and select solid cancers. However, the mechanisms of resistance to these agents are understudied and the management of toxicities remains challenging. Herein, we review the principles in bsAb engineering, current understanding of mechanisms of action and resistance, data for clinical application, and provide a perspective on ongoing challenges and future developments in this field.

Highlights

Bispecific antibodies (bsAbs) offer a novel approach to anticancer therapy by targeting different antigens via a range of mechanisms of action.

Manipulating bsAb structures allows generation of multiple formats to optimize molecular function for specific clinical contexts.

Thus far, a total of seven bsAbs have received regulatory approval for use in hematological malignancies and four in select solid tumors, with notable clinical efficacy.

The toxicities associated with the use of bsAbs need bespoke management strategies and expertise in clinical use.

Understanding mechanisms of resistance to bsAbs is crucial to rational combination regimens or sequencing with other therapeutics to improve patient outcomes.

Introduction

Distinct from other forms of immunotherapy and targeted therapies, bsAbs can target multiple antigens. The unique structure and the multiple design possibilities allow for a range of mechanisms of action, including redirecting immune cells to the **tumor microenvironment (TME)** (see Glossary) or blockade of driver signaling pathways. To date, 11 bsAbs have received regulatory approval for use in cancer, ten of them by the US Food and Drug Administration (FDA) (Table 1). Specifically, seven have received regulatory approval across hematological malignancies and four in selected solid cancer indications for use as standard-of-care treatments, with a plethora of ongoing clinical trials [1] (Table S1 in the supplemental information online). However, clinical application of this class of agents presents challenges and toxicities, such as cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), infusion-related reactions (IRRs), and opportunistic infections [2–5]. Some of these adverse events may vary in severity and presentation depending on the targeted cell. In this review, we provide an overview of bsAb design principles, mechanisms of action, current therapeutic applications, limitations, challenges in clinical use, and future developments.

Manipulating molecular structures to optimize function

The structure and biological function of endogenous bivalent antibodies have informed the design of bsAbs [6,7] (Figure 1). Methods in producing engineered antibodies have enabled the generation of bsAb formats with various structures, composition, and pharmacological properties (Boxes 1 and 2; Figure 2). With bsAbs, no single format can be considered universally optimal, given the need and opportunity to tailor individual bsAbs for various clinical contexts and specific therapeutic objectives [8].

Modulation of Fc-dependent immunogenicity

The Fc region of an antibody is responsible for mediating effector functions, including **antibody-dependent cellular cytotoxicity (ADCC)**, complement-dependent cytotoxicity (CDC), and

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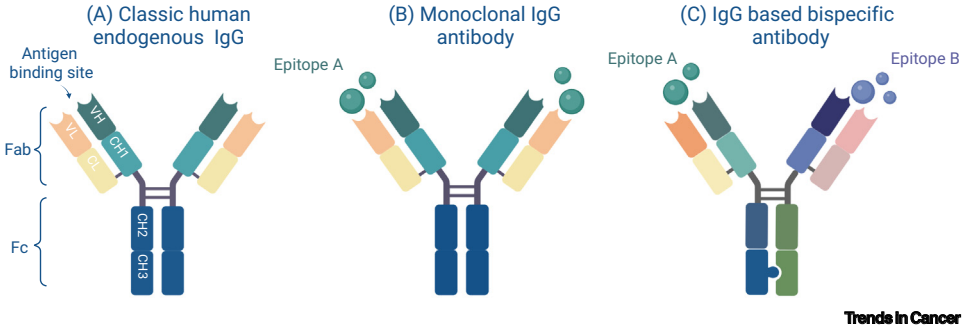

Figure 1. Structure of endogenous and engineered antibodies. (A) Human immunoglobulins are glycoprotein molecules comprising two pairs of identical light and heavy chains linked together in a Y-shaped structure by disulfide and noncovalent bonds. The light chain comprises a variable (VL) and constant (CL) domain, while the heavy chain has one variable (VH) and three constant (CH1–3) domains. The antigen-binding fragment (Fab) has two antigen-binding sites, one in each arm of the 'Y', which bind a single antigen with high affinity, rendering human immunoglobulins bivalent but monospecific. The crystallizable fragment (Fc), which classifies the immunoglobulin (Ig) into five isotypes based on the heavy chain isoforms (IgA, IgD, IgE, IgG, or IgM), is responsible for interacting with Fc receptors on the surface of effector cells, such as leukocytes. (B) Monoclonal antibodies (mAbs) are monospecific toward one epitope of a single antigen. (C) Bispecific antibodies (bsAbs) with distinct antigen-binding domains are designed to target two different epitopes, usually on two separate antigens.

antibody-dependent cellular phagocytosis (ADCP) [9], through its binding to FcγRIIIa on natural killer (NK) cells, complement components, and FcγRIIa in macrophages, respectively [10]. However, Fc-related effector functions may also produce undesirable effects, such as non-specific activation of immune cells leading to Fc-dependent CRS [11]. The Fc region of IgG-based bsAbs can be manipulated to mitigate these unfavorable effects and/or enhance antitumor activity to create a balance in Fc functionality [12].

Box 1. The making of bispecific antibodies 'building blocks'

BsAbs are broadly categorized into Fc based (IgG-like and IgG-appended) or fragment based (IgG-less or IgG-free) based on the presence or absence of the Fc region in their structure [206] (Figure 2 in the main text). Fc-based bsAbs commonly mirror the modular structure of endogenous IgG molecules, which have a favorable plasma distribution profile and serum stability compared with other Ig isotypes. Fragment-based bsAbs usually represent a simpler structure with a smaller size and at least two variable domains. Generally, bsAbs are synthesized by fusing two different heavy chains, and two different light chains. The two variable binding domains allow simultaneous targeting of two epitopes that are leveraged for therapeutic effects.

Fragment-based manufacturing is based on a structure with two antigen-binding arms, usually represented by the scFv or the single-domain Ab (sdAb). ScFv is a fusion protein comprising heavy chain and light chain variable domains [207], while sdAb (also known as nanobody) only has the heavy chain antigen-binding variable domain (VHH) [208]. Some of the most common formats in clinical use and development include bispecific TCEs (BiTEs®), dual-affinity retargeting molecules (DARTs®), and tandem diabodies (TandAbs®) [206]. BiTEs® comprise two scFvs, one of which is specific for tumor-associated antigen while the other is specific for CD3, expressed on T cells. A flexible linker connects the two domains and allows unrestricted rotation and versatile interaction between the two targets [27]. DARTs® have a diabody backbone carrying two polypeptide chains, with the VH connected by a disulfide bond [28,206], leading to a more stable molecule with potentially higher levels of T cell activation [209]. Improved stabilization can also be achieved with the TandAb® format, a tetravalent molecule comprising two diabodies linked together, carrying two binding sites for each antigen. In addition, due to their molecular weight, TandAbs® have a longer half-life compared with smaller molecules [26,206]. Similarly to BiTEs®, BiKEs are heterodimeric bispecific scFvs that target NK cells. TriKes additionally incorporate an IL-15 crosslinker that enhances NK stimulation [210].

The production of IgG-based bsAbs requires two distinct light chains and two distinct heavy chains for binding to two different epitopes. This can be achieved by heterodimerization of different light and heavy chains that result in asymmetric IgG molecules. Another method is to fuse additional binding sites with a different binding specificity to the N or C terminus of the heavy or light chains. The latter, also known as appended IgG [211], can be constructed with antigen binding domains (commonly scFv, scFab, or sVD) added via short peptide linkers that enable flexible valency.

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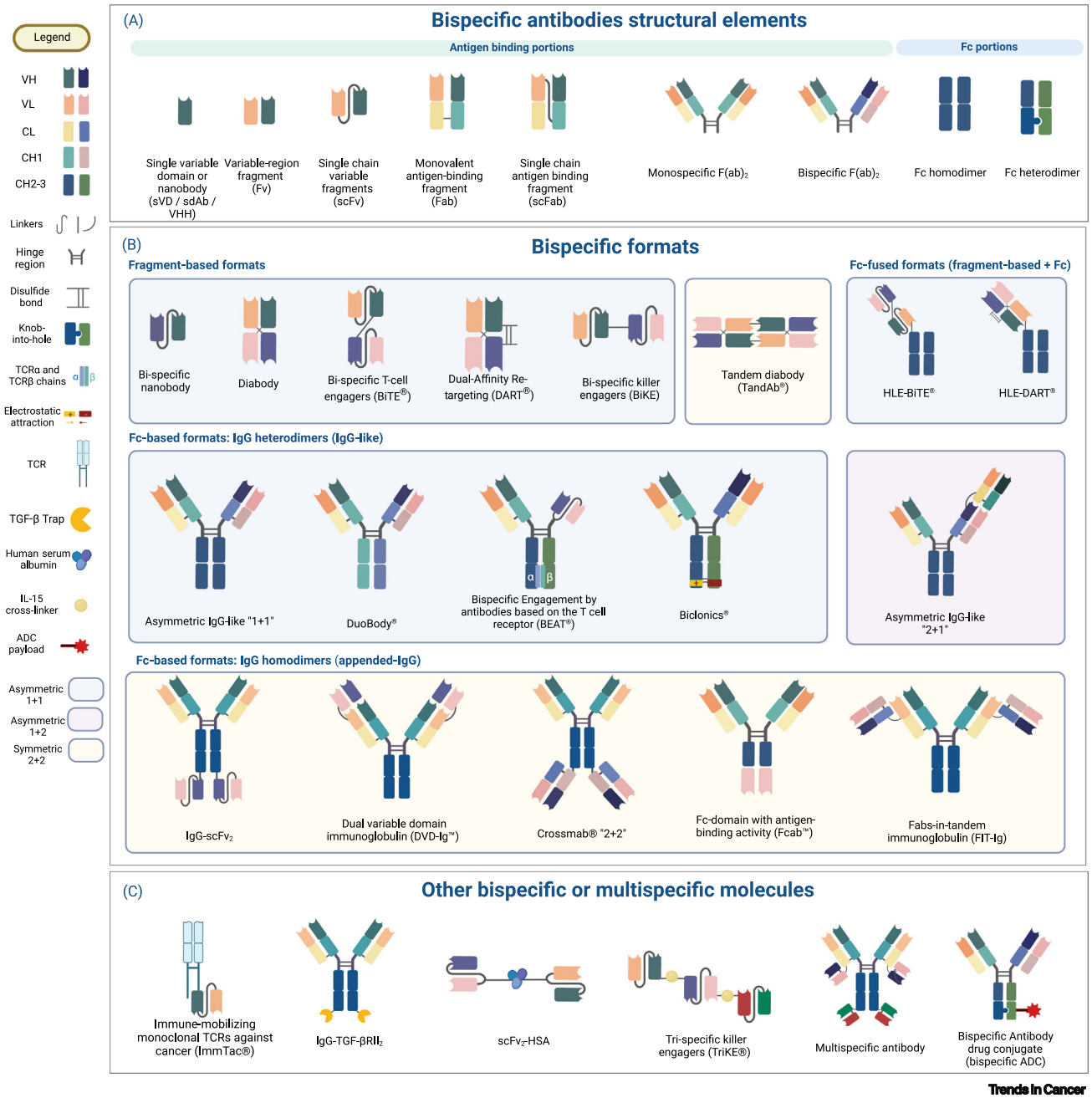

Figure 2. Bispecific antibody (bsAb) design and engineering: 'building blocks'. (A) Structural elements from the antigen-biding portion [sVD, Fv, scFv, Fab, scFab, and F(ab')2], and the crystallizable fragment (Fc), necessary to build different bispecific formats. (B) Examples of fragment-based (bispecific nanobodies, diabody, BiTe®, DART®, BiKe, and TandAb®®); Fc-fused formats, constructed by adding an Fc portion to a fragment-based bispecific antibody format (BiTE®-HLE and DART®-HLE); Fc-based heterodimers (classic IgG-like asymmetric antibodies '1+1 or '2+1', DuoBody®, BEAT®, and Biclonics®) and appended-immunoglobulin (Ig)-G bispecific antibodies, constructed by adding other structural elements to a IgG homodimer Fab portion (DVD-Ig™ and FIT-Ig) or Fc portion (IgG-scFv2, Fcab™, and Crossmab® 2+2). (C) Representation of other molecules of interest, including other bi- or trispecific molecules (ImmTac®, IgG-TGF-βRII2, scFv2-HSA, and TriKE®®), and examples of the structure of multispecific antibodies and bispecific antibody–drug conjugates. Abbreviations: TCR, T cell receptor; TGF, transforming growth factor.

One strategy to mitigate ADCC effects is through selection of different IgG subclasses, such as IgG2 or IgG4, which exhibit lower binding to FcγRs compared with IgG1 [13]. Additionally, amino acid substitutions (e.g., L234F, L235E, and N297G) in the Fc region create tailored Fc silent mutations that prevent nonspecific immune cell activation derived from the crosslinking of CD3 and Fcγ receptors. Consequently, this modification facilitates the successful redistribution of T cells to the TME and decreases Fcγ-mediated C1q complement activation [14]. This strategy may be beneficial for bsAbs aiming to restore immune function, such as **T cell engagers (TCEs)**, or those targeting **immune checkpoints (ICPs)**. In other scenarios, optimization of the interactions with different FcγRs to enhance immune cell activation may be desirable to further promote antitumor activity, especially in those bsAbs blocking activated signaling pathways, such as epidermal growth factor (EGFR) or human epidermal growth factor 2 (HER2) [15]. For example, ADCC activation via FcγRIIIa, the only Fc receptor expressed on NK cells, can contribute to NK-mediated cytotoxicity and enhance the antitumor response [16]. In particular, the strategy of removing or reducing the core fucose from Fc N-glycans has been shown to increase IgG1 Fc binding affinity to the FcγRIIIa, leading to enhanced ADCC activity [17]. This has been applied in monoclonal antibodies (mAbs), such as trastuzumab, and further expanded to the field of bsAbs, such as amivantamab (EGFR × cMET, DuoBody®) [18]. Thus, optimization of the interactions with different FcγRs to enhance immune cell activation may further promote antitumor activity, especially in those bsAbs blocking activating signaling pathways [15,18].

Pharmacokinetics and half-life extension optimization

BsAb formats can be manipulated to alter their biodistribution and **pharmacokinetics (PK)** profile. Similar to most circulating serum proteins, circulating IgG molecules bind neonatal fragment crystallizable receptor (FcRn) in acidified endosomes, and the antibody is then returned to the bloodstream when the IgG-FcRn complex dissociates at physiological (neutral) pH [19]. This FcRn-mediated recycling mechanism protects IgGs from intracellular degradation. This can help maintain the serum concentration and extend the serum half-life of the antibody. Modification of the pH-dependent binding to FcRn is being explored to tailor bsAb PK properties [20,21]. For example, Q311R and M428L mutations in the Fc region improved dissociation from FcRn at pH 7.4, with resultant enhanced bioavailability, prolonged serum persistence, and enhanced efficacy [22,23]. Different selections of IgG subclasses can also modify the bioavailability of Fc-based bsAbs to impact the drug half-life, in addition to impacting effector functions, as discussed in the preceding text [24].

In contrast to Fc-based bsAbs, fragment-based bsAbs are smaller in size and exhibit high tissue permeability [25]. Consequently, these molecules may achieve more efficient distribution within the TME, which is particularly relevant to solid tumors with dense and complex structures. However, fragment-based bsAbs also have a shorter half-life and rapid clearance in circulation owing to their low molecular weight [6,26], which, in turn, can compromise tissue penetration. As such, maintaining biological effects may require multiple dosing or continuous infusions [27,28], which translates to increased healthcare burden and costs. Subcutaneous administration of bsAbs, which decreases the distribution and drug release kinetics to prolong drug persistence in circulation, is a potential solution under investigation (NCT04521231). Preliminary data for subcutaneous blinatumomab (CD3 × CD19, BiTE®) show PK and pharmacodynamic profiles consistent with continuous intravenous infusion [29]. Another strategy that prolongs the half-life of non-Fc-carrying molecules is fusion of the fragment to other molecules, such as human serum albumin [30], which functions as a natural vehicle with a plasma half-life of 19 days [31]. Moreover, classic BiTEs®, a subcategory of TCEs, can be redesigned by adding an Fc domain [32], resulting in longer half-life second-generation molecules, termed half-life extended BiTEs (HLE-BiTE®), which are under preclinical [33] and clinical investigation (NCT05740566). Similarly, DART®

Glossary

Antibody specificity: ability of an antibody to specifically recognize and bind to a given antigen or molecule. The specificity is based on the 3D structure of the antigen-binding site of the antibody and is essential for the recognition and neutralization of pathogens, detection and elimination of cancer cells, and regulation of immune responses.

Anti-drug antibodies (ADAs): specific antibodies produced in response to therapeutic drugs. The generated antibodies recognize the drug and can interfere with its efficacy or lead to side effects. ADAs are a concern in the development of new drugs because they can impact treatment efficacy, safety, and PK.

Antibody-dependent cellular cytotoxicity (ADCC): immune response mediated by Fc receptors, where, effector cells, such as natural killer (NK) cells, recognize and destroy target cells coated with antibodies. This process has a key role in the elimination of infected and cancerous cells.

Antibody-dependent cellular phagocytosis (ADCP): process in which macrophages or neutrophils phagocytose and destroy cells that have been recognized by antibodies. This process is mediated by the binding of antibodies to specific antigens on the surface of target cells, stimulating the activation of phagocytes. ADCP has a crucial role in the immune system response to pathogens.

Immune checkpoints (ICPs): molecules on the surface of immune cells (e.g., PD-1 and CTLA-4) regulating pathways that help to modulate the intensity and time of immune responses. These pathways are essential to avoid autoreactive responses; however, when dysregulated, they can facilitate immune evasion by tumors and pathogens.

Pharmacokinetics (PK): study of how the body interacts with an administered drug for the duration of exposure. It encompasses the processes of drug absorption, distribution, metabolization, and elimination.

Tumor-associated antigens (TAA): molecules expressed by tumor cells but not necessarily specific to them; they can also be present on some healthy endogenous cells. TAAs can be used as immune targets when developing immunotherapies.

T cell engager (TCE): class of bsAb that binds to tumor cells and T cells

molecules can also be fused with an Fc region, resulting in a DART-Fc compound [34]. Definitions of BiTEs® and DART® are provided in Box 1 and represented in Figure 2.

Valency determination

BsAbs may be subclassified based on the number of antigen-binding sites present. Symmetric bsAbs are constructed by either fusion of two identical antibody fragments or by the addition of single chain variable fragments (scFv) or single variable domains (VHH) directly to canonical antibodies through linkers [35]. Usually, such molecules have a tetravalent (2+2) structure with four binding sites (two binding sites for each antigen), which contribute to a balanced approach to targeting both antigens and avoiding the issues of improper chain association. Cadonilimab (PD-1 × CTLA-4, IgG-scFv$_2$) [36] is an example of a tetravalent bispecific IgG1 backbone with two symmetrical scFvs (Figure 2).

Asymmetric bsAbs combine different antigen-binding arms, resulting in a heterodimer that provides flexible target selection [6] (Figure 2). BsAbs with a 1+1 format, such as mosunetuzumab (CD3 × CD20, IgG-like) [37], are characterized by the presence of two antigen-binding sites (one for each of the two antibody arms) targeting different antigens (bivalent antibody). Other molecules can show one binding site for one antigen, and two binding sites for the other antigen in a 1+2 format, resulting in a trivalent BsAb. As an example, glofitamab (CD3 × CD20, IgG-like) [37] comprises two arms with one antibody targeting one antigen, while the other arm targets a different antigen, resulting in a bsAb with three antigen-binding sites.

Improving on-target off-tumor toxicity profile

'On-target off-tumor' toxicity refers to unwanted effects of antibody binding to its cognate antigen expressed on normal tissue in addition to the tumor. Accordingly, the likelihood of on-target off-tumor toxicities may be increased with bsAbs targeting two different antigens compared with mAbs [38]. However, in bsAbs, the **specificity** and affinity of the different binding sites can be selectively optimized for a better toxicity profile.

As an example, if one arm has a high propensity for on-target off-tumor toxicity because the antigen is expressed on both tumor and normal tissue, the alternate arm can be designed intentionally to target an antigen that is highly expressed on tumor or tumor stromal cells (e.g., HER2, PSMA, EGFRvIII, or FAP). The aim is a net effect or overall affinity of the dual targets to favor tumor-specific interaction of the bsAb [39]. This is a potential approach to address hepatotoxicity of mAbs targeting 4-1BB, due to the Fc-gamma receptor interaction with 4-1BB signaling in Kupffer cells [38]. Bispecific molecules targeting both 4-1BB and a second antigen expressed on tumor or stromal cells require an established *in trans* cell bridging, a physical connection that results in increased cytotoxicity in the TME [40]. This strategy, along with bsAbs with an altered or absent Fc region, allows for specific targeting and prevention of systemic side effects and hepatotoxicity observed with previous 4-1BB agonists, and potentially enhances antitumor efficacy [41,42]. An example of this is ABL503 (PD-1 × 4-1BB, IgG-scFv2). Preclinical studies showed ABL503 to be well tolerated, with low risks of liver toxicity, and signs of superior activity compared with a combination of the corresponding mAbs [43].

Another example of engineering to address on-target off-tumor activity is TG-1801 (NI-1701) (CD47 × CD19, Kλ body). CD47 is not only upregulated on tumor cells to evade macrophage-mediated phagocytosis, but also constitutively expressed on most normal cells, whereas CD19 is B cell specific. On TG1801, the CD19- and CD47-targeting arms are engineered with high and low binding affinity, respectively. This net effect is increased selectivity for CD19-expressing malignant B cells that have upregulated CD47, with decreased on-target off-tumor effects on CD47-expressing normal cells (erythrocytes, platelets, etc.) to overcome the limitation of CD47

simultaneously. This brings both cells in contact, facilitating the engagement of T cells in the antitumor response.

Tumor microenvironment (TME): area that encompasses the cellular and noncellular components surrounding a tumor, including tumor, immune and stromal cells, and blood vessels. It has a key role in tumor growth, invasion, and response to therapies in solid tumors.

Box 2. 'Blend and bond': heterodimerization of light and heavy chains to form Fc-based bispecific antibodies

Generating IgG-based bsAbs requires successful heterodimerization of antibody chains. In this process, the potential mispairing (or homodimerization) of the antibody chains needs to be addressed. The first bsAbs were generated by chemical conjugation of two different, purified mAbs or by fusing two hybridomas resulting in a quadroma cell line [212]. This process leads to both heavy chain homo- and heterodimers with a heterogeneous product variable in the conjugation sites and ratios, which can compromise the overall functionality, stability, and proportion of the resulting bsAbs [213].

There are methods to avoid homodimerization and potential promiscuous pairing of heavy and light chains (Figure I). 'Knob-into-hole' technology involves the introduction of specific mutations into the constant regions (Fc domains) of antibody heavy chains. These mutations are strategically placed in the CH3 domain to create a 'knob' (usually a protruding amino acid residue) on one heavy chain and corresponding to a complementary 'hole' on the other heavy chain, creating steric interactions [214]. The DuoBody® platform uses the single mutations K409R and F405L in each CH3 domain of the two parental IgG1s mimicking the Fab-arm exchange (FAE) process naturally occurring in IgG4 antibodies. This process, known as 'controlled FAE', promotes heterodimer formation [215]. In addition, a common method to avoid heavy chain mispairing is the introduction of mutations to generate charge alterations in the CH3 domain that lead to electrostatic attraction between heterodimers and repulsion between homodimers, used by platforms such as DEKK (salt-bridge interactions) [216], ART-Ig® [217], or CRIB™. The latter adds this method in addition to the previously described knob-into-hole [218]. 'Strand-exchange engineered domain' (SEED) method is another example of CH3 manipulation to prevent heterodimers. This uses alternating segments of human IgA and IgG in the CH3 domain to promote the assembly of complementary sequences [219]. The BEAT® bispecific molecule achieves heavy chain heterodimerization by mimicking the natural association of the T -cell surface receptors α and β between the two CH3 [220].

The CrossMab® method involves the exchange of different domains within the Fab-fragment and can involve the crossover of complete or part of the Fab domain [221]. It reduces the heavy chain–light chain mispairing, allowing each to retain specificity for a distinct antigen. The Wuxibody® platform substitutes the CH1/CL region with the α and β chains from the TCR constant domain, which prevents mispairing between noncognate heavy and light chains [222]. To maintain the correct heterodimerization of the heavy chains, these platforms can also incorporate other methods, such as the knob-into-hole technology or charge interactions [222,223].

These are nonexhaustive examples of bsAb engineering aimed at overcoming challenges associated with heterogeneity, stability, and functionality. Other novel platforms are also in development.

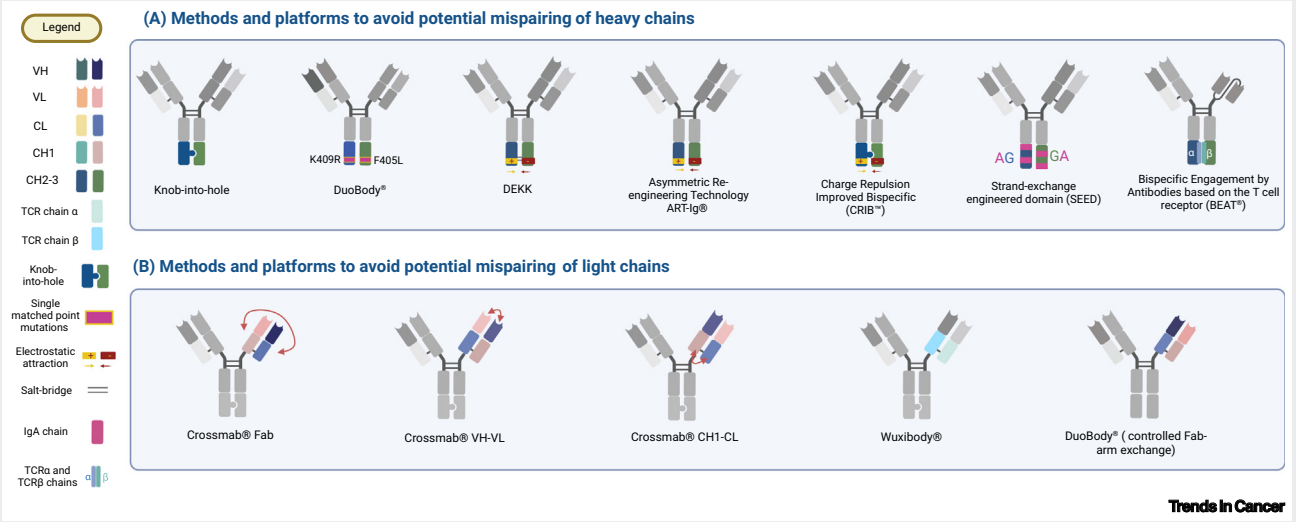

Figure I. Heterodimerization of light and heavy chains. Representation of various platforms designed for minimizing the mispairing of antibody chain. (A) Knob-into-hole (KiH), DEKK, ART-Ig®, CRIB™, SEED. and BEAT® technology aim to avoid the mispairing of heavy chains. (B) Different Crossmab® methods and Wuxibody® antibodies prevent the mispairing of light chain. In these platforms, prevention of heavy chain mispairing is additionally promoted by KiH technology. The DuoBody® platform addresses both potential heavy and light chain mispairing through single point mutations in the CH3 domain and post-production controlled Fab-arm exchange. Abbreviations: CL, light constant domain; CH, heavy constant domain; LH, light variable domain; TCR, T cell receptor; VH, heavy variable domain.

monospecific mAbs [44]. The drug demonstrated early signs of clinical activity and was generally well tolerated [45].

Delivering bsAbs as prodrugs with conditional activation in the TME may be another avenue to mitigate unwanted effects on healthy tissue [46]. One of these strategies is the use of protease-cleavable peptide masks in the variable fraction of the bsAb [47,48]. This method not only limits

bsAb activation in healthy tissues expressing the target antigen, but allows conversion to an active form by specific enzymes present in the tumor tissue. An example of selected activation in the TME is TAK-280 (CD3 × B7H3, COBRA T cell engager), a prodrug that is only activated in hypoxic conditions by tumor-associated proteases, which facilitates T cell action only against B7H3-expressing tumor cells. TAK-280 is being evaluated in patients with metastatic solid tumors within a Phase 1 trial [49].

Mechanisms of action of bispecific antibodies

The biological target of bsAbs dictate their mechanisms of action and therapeutic effect, and bsAbs can be classified accordingly, as discussed in the following section (Figure 3).

Immune cell engagers

Bispecific TCEs are designed to link endogenous $CD4^+$ and $CD8^+$ T cells with tumor cells by simultaneously binding to the CD3e subunit of T cell receptor (TCR) complexes and the selected **tumor-associated antigen (TAA)** [50,51]. This interaction activates T cells, some of which are cytotoxic or demonstrate proinflammatory activity facilitating tumor cell killing [52]. The impact that TCEs have on T cells with anti-inflammatory polarization, such as regulatory $CD4^+$ T cells, remains unclear, but it is expected that their activation upon TCE engagement is detrimental to the effector function of neighboring T cells and overall tumor cell death. TCEs can also trigger T cell margination and proliferation to reshape the TME, as reported following treatment with glofitamab in B cell non-Hodgkin lymphoma (B-NHL) [53]. The TCE-induced TME changes likely result from secretion of cyto/chemokines following the initial rounds of T cell activation, and recruitment of more T cells and other immune cells [53].

TCE-induced T cell killing of cancer cells is thought to be independent of antigen recognition via major histocompatibility complex (MHC) class I/II or costimulatory molecules [54]. However, recent data from longitudinal profiling of bone marrow T cell repertoires in multiple myeloma (MM) demonstrated that efficient T cell expansion following treatment with a TCE (CD3 × BCMA) relies heavily on peptide-MHC class I [55]. These data suggest that peptide-MHC class I are required as an additional costimulatory signal for T cell responses following the administration of at least some TCEs. It remains unclear whether TCEs can also lead to tumor-specific long-lived T cell memory formation and mount effective responses upon antigen recall.

New immune cell engagers that target a specific repertoire of T cells are in development. T cells are mainly divided into αβ and γδ T cells according to the expressed TCR. LAVA-051 (Vγ9Vδ2-T × CD1d, bispecific nanobody, Gammabody®) is an example of a γδ TCE designed to target the Vδ2-TCR chain and CD1d, expressed by leukemia and myeloma cells [56].

NK cells can exert spontaneous, antigen-independent cytotoxicity against cancer cells [57]. Bispecific killer cell engagers (BiKEs) redirect NK cells toward the tumor. Unlike T cells which express somatically rearranged antigen receptors, NK cells express stochastic combinations of activating receptors [58]. CD16 (low-affinity IgG Fc region receptor III) is the most potent activating receptor on NK cells, triggering cytokine release when activated and enabling ADCC [59]. Accordingly, BiKEs targeting CD16 (and other receptor molecules) and TAA have been developed. Importantly, NK cells preferentially kill MHC I-deficient tumor cells, making BiKE an attractive therapeutic strategy given that the loss of MHC I expression is a cancer cell immune evasion mechanism for both primary and adaptive resistance, particularly with ICP blockade [60]. There is increasing evidence for the clinical efficacy of BiKE to support their further development. For example, AFM13 (CD30xCD16A, TandAb®) shows preclinical activity that is strongly dependent on CD30 [61]. Subsequently, early Phase trials in patients with $CD30^+$ lymphoma showed significant

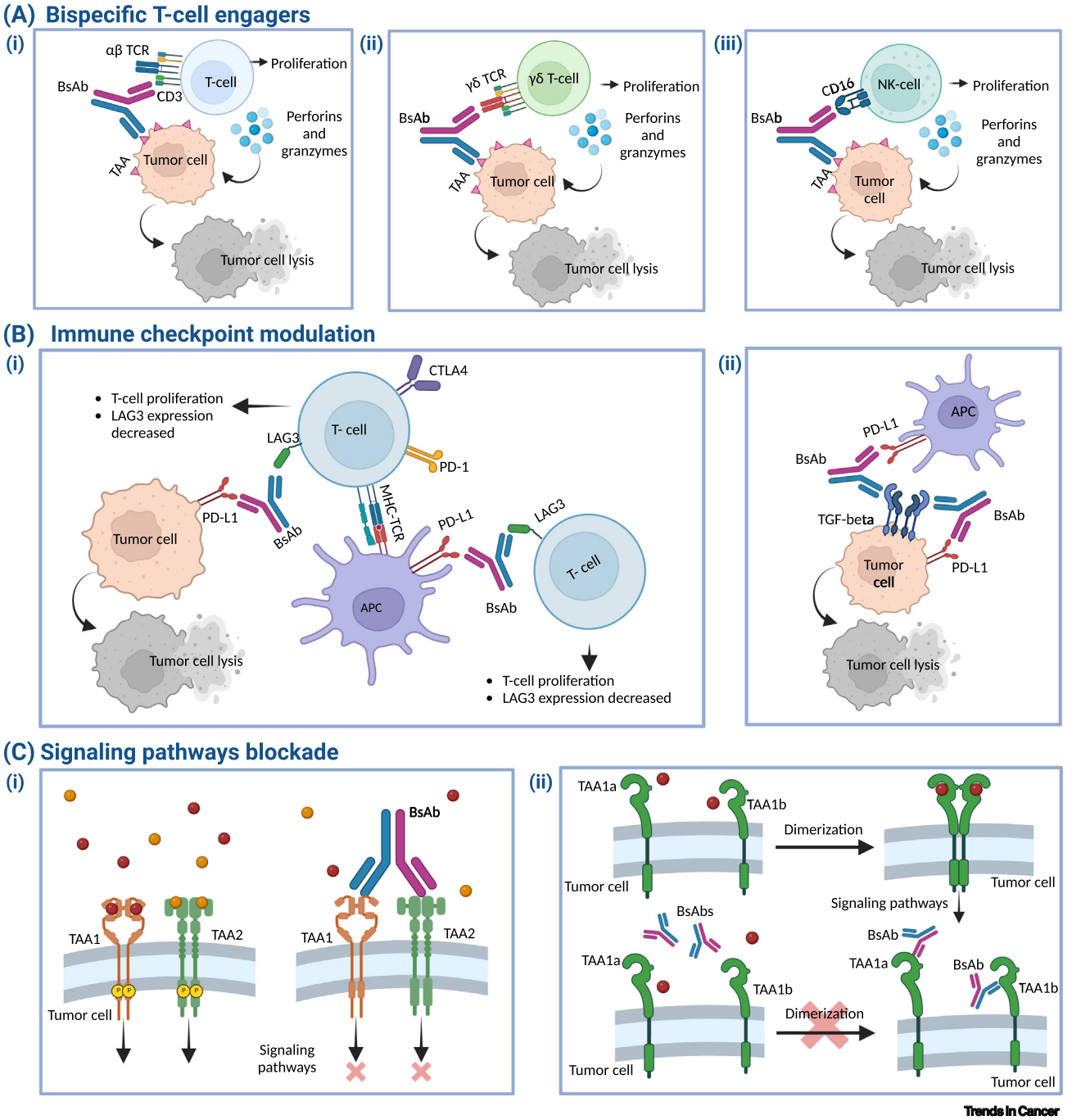

Figure 3. Mechanisms of action of bispecific antibodies (bsAbs). (A) Bispecific T cell engagers. (i) BsAb binds the CD3 subunit of the T cell receptor (TCR) and the selected tumor-associated antigen (TAA) to facilitate the formation of a synapse between the two cell types; the synapse activates T cells, which can release perforins and granzymes to lyse tumor cells. (ii) Bispecific γδ T cell engagers simultaneously bind Vγ9Vδ2 receptors and a specific TAA to activate T cells, release perforins and granzymes, and lyse tumor cells. (iii) Bispecific natural killer (NK) cell engagers simultaneously bind CD16 receptors and a specific TAA to activate NK cells, release perforins and granzymes, and lyse tumor cells. (B) Immune checkpoint (ICP) modulation. (i) Dual ICP-blocking bsAbs simultaneously bind lymphocyte-activation gene 3 (LAG3) on the surface of T cells and programmed death ligand 1 (PD-L1) on tumor or antigen-presenting cells, amplifying the activation of T cells, which release perforins and granzymes, and lyse tumor cell, decreasing LAG3 expression. (ii) BsAb targets ICP concurrently with a molecule involved in other signaling pathways. (C) Signaling pathway blockade. (i) BsAbs simultaneously bind epidermal growth factor receptor (EGFR) and cMET, blocking the ligand-induced phosphorylation,

(Figure legend continued at the bottom of the next page.)

antitumor activity as monotherapy or combined with pembrolizumab [62–64]. Another BiKE in clinical development is RO7297089 (BCMA × CD16A, IgG-scFv$_2$), with activity in MM [65].

Immune checkpoint modulation

ICP modulation therapies have revolutionized cancer care. However, limited efficacy in certain tumors, therapy resistance, and off-target toxicity are ongoing challenges [66].

Dual ICP-blocking bsAbs bind an inhibitory ICP receptor (e.g., PD-1, CTLA-4, LAG-3, or TIGIT) on the surface of the T cell with one arm, while the other arm binds another ICP receptor on the T cell, tumor cell, or antigen-presenting cell. While the co-expression of CTLA-4 and PD-1 on tumor-infiltrating lymphocytes (TILs) is frequently observed [67], this phenomenon is not commonly present in peripheral T cells. For this reason, an advantage of dual ICP-targeting bsAbs compared with mAb combinations is their ability to preferentially target TILs through avidity-mediated selection of ICP targets. For example, both volrustomig (PD-1 × CTLA-4, IgG1-like '1+1') [68] and cadonilimab [69] show preferential targeting of TILs co-expressing PD-1 and CTLA-4. Thus, targeting two ICPs by bridging two cell types or two molecules on the surface of the same cell favors the building of the immunological synapse in the intercellular space [70]. This strategy has been hypothesized to contribute to the limitation of the on-target off-tumor toxicity, and to potentially enhance the antitumor immune response and reduce immunosuppressive effects [71,72]. Nevertheless, clinical data comparing the efficacy of bsAb targeting the same epitopes as combination mAbs are lacking.

Furthermore, bsAbs may help to mitigate adaptive therapy resistance because single ICP blockade may result in compensatory upregulation of different checkpoint pathways to allow immune escape [73]. For example, FS118 (LAG-3 × PD-L1, Fcab^{TM}) was shown to decrease LAG-3 expression on T cells in mouse models, whereas LAG-3 expression increased upon treatment with mAbs individually targeting LAG-3 or PD-L1, even when the mAbs were used in combination [74]. This suggests that the bispecific nature of FS118 allows it to more effectively disrupt the inhibitory signals from both LAG-3 and PD-L1 simultaneously. By promoting the internalization and degradation of both targets, bsAbs may prevent the compensatory upregulation of other ICP molecules, which are often seen with single-target mAbs. Furthermore, the enhanced blockade of these pathways could alter signaling dynamics within the T cells, leading to a net reduction in LAG-3 expression as part of a feedback mechanism to regulate immune activation.

BsAbs can also engage costimulatory receptors signaling through the immunoglobulin B7–CD28 family, such as ICOS or CD28, or members of the tumor necrosis factor (TNF) superfamily, such as CD27, CD40, OX40, and 4-1BB. However, amplifying the T cell response so that it is in an appropriate and safe range is challenging and can lead to harm [75]. Addressing this risk is crucial for the successful development of these agents.

BsAbs can also redirect and activate immune cells through interactions with antigens involved in signaling pathways related to tumor development, angiogenesis, metastasis, and proliferation. This dual activity via vascular endothelial growth factor (VEGF) blockade in the TME and immunomodulation via PD-1 is the mechanism of action of AK112 (PD-1 × VEGF, IgG-scFv$_2$), which demonstrated activity in nonsmall cell lung cancer (NSCLC) [76]. Also of interest

promoting inhibition of the downstream signaling cascades, and stimulating receptor degradation. (ii) Biparatopic bsAbs bind two separate epitopes on the same target.
Abbreviations: APC, antigen-presenting cell; CD, cluster of differentiation; CTLA4, cytotoxic T lymphocyte antigen 4; MHC, major histocompatibility complex; PD-1, programmed death 1.

is the concept of dual ICP blockade with molecules modulating immunosuppressive pathways, such as transforming growth factor-beta (TGF-β) and PD-1/PD-L1, which are under investigation [77,78]. Other molecules, such as bintrafusp alfa (PD-L1 × TGF-β, IgG-TGF-βRII_2), have failed in clinical development [79].

Signaling pathway blockade

BsAbs can block two distinct antigens or various regions of a single antigen in signaling pathways. The dual targeting of driver signaling pathways can enhance bsAb treatment efficacy [80] and reduce resistance emergence [28]. There are multiple ways in which bsAbs can target two pathways. The main mechanism of action involves blocking receptor–ligand interactions, thereby inhibiting downstream signaling cascades. Second, bsAbs can induce internalization of the receptors, thus preventing receptor crosslinking (homodimerization and/or heterodimerization), resulting in downstream effects, such as suppression of angiogenesis and inhibition of cell proliferation [81]. Third, IgG-based bsAbs carrying the Fc structure can promote ADCP and ADCC, which can further induce apoptosis [10].

BsAbs can address emerging resistance to tyrosine kinase inhibitors (TKIs). This can occur through TKI-induced upregulation of alternative pathways, such as mesenchymal–epithelial transition (MET) pathway activation seen in *EGFR*-mutated NSCLC [82]. Amivantamab blocks ligand-induced phosphorylation, promotes inhibition of downstream signaling cascades, stimulates receptor degradation, and prevents drug resistance [18,83]. Furthermore, it overcomes the limitation of bivalent MET antibodies, which can cause receptor crosslinking and possibly result in tumor cell activation [6].

BsAbs targeting angiogenesis factors are in development. Under normal conditions, Delta-like ligand 4 (DLL4) signaling within blood vessels, together with VEGF activity, coordinates endothelial cell proliferation and angiogenesis [84]. Simultaneous inhibition with ABT-165 (VEGF × DDL4, DVD-Ig™) resulted in decreased tumor vessel perfusion and tumor growth inhibition in preclinical models, yielding antitumor effects that were greater than with either anti-DLL4 or anti-VEGF mAbs alone [85].

Biparatopic bsAbs refer to the binding of two separate epitopes on the same target, rather than targeting two different proteins. This may be relevant for targets such as HER2, where the extracellular component has four functional domains. Zanidatamab (HER2-ECD2 × *HER-ECD4, BEAT*®) can target HER2, ECD2, and ECD4 simultaneously, similar to the combination of trastuzumab and pertuzumab [86,87]. Furthermore, it leads to internalization of HER2 from the cell membrane [88], resulting in potent inhibition of HER2 signaling.

Clinical evidence

Several bsAbs have received regulatory approval for the management of various hematological and certain solid tumors (Table 1). The impact of bsAbs in cancer therapeutics will continue to broaden, given the ever-expanding number of clinical trials evaluating this class of agents (Table S1 in the supplemental information online).

Hematological malignancies

Acute lymphoblastic leukemia. Acute lymphoblastic leukemia (ALL) is characterized by the rapid proliferation of immature lymphoid cells, exhibiting a bimodal distribution in its incidence. The more substantial peak occurs during childhood, while a second peak occurs typically in individuals in their 50s, and is associated with a poorer prognosis. CD19 is the most widely expressed B lineage-specific antigen, present in nearly all cases of B cell ALL [89].

Table 1. BsAbs with regulatory approval as of June 2024[a]

BsAb	Company	Target	Format	First approval	Indication	Pivotal clinical trial
Blinatumomab (MT103), Blincyto®	Amgen	CD3 × CD19	BiTE®	2014 FDA	Adults ALL Ph± Pediatric ALL Ph± MRD positive ALL; Consolidation of Ph– ALL	Phase 3 (TOWER, NCT02013167) Randomized
Tebentafusp (IMCgp100), Kimmtrak®	Medison/ Immunocore	CD3 × gp100-HLAA02:01	ImmTAC®	2022 FDA	Uveal melanoma	Phase 3 (NCT03070392) Randomized
Talquetamab (JNJ-64407564), Talvey®	Johnson & Johnson	CD3 × GPRC5D	IgG4 DuoBody®	2023 FDA	R/R MM after four previous lines	Phase 1/2 (MonumenTAL-1, NCT03399799/NCT04634552) Open-label, single-arm
Glofitamab (RO7082859), Columvi®	Roche (Genetech)	CD3 × CD20	IgG1-like '2+1'	2023 FDA	R/R DLBCL after two or more previous lines	Phase 2 (NP30179 Study, NCT03075696) Open-label single-arm
Mosunetuzumab (BTCT-4465A), Lunsumio®	Roche (Genetech)	CD3 × CD20	IgG1-like '1+1'	2022 FDA	R/R follicular lymphoma after two or more previous lines	Phase 2 (GO29781, NCT02500407) Open-label single-arm
Teclistamab (JNJ-64007957), Tecvayli®	Janssen	CD3 × BCMA	IgG4 DuoBody®	2022 FDA	R/R MM after four previous lines	Phase 1/2 (MajesTEC-1, NCT04557098) Open-label single-arm
Epcoritamab (GEN3013), Epkinly®	Abbvie	CD3 × CD20	IgG1 DuoBody®	2023 FDA	R/R DLBCL, high-grade B cell lymphoma and follicular lymphoma after two or more previous lines	Phase ½ (EPCORE NHL-1, NCT03625037) Open-label single-arm
Elranatamab (PF-06863135), Elrexfio®	Pfizer	CD3 × BCMA	IgG2a-like '1+1'	2023 FDA	R/R MM after four previous lines	Phase 2 (MagnetisMM-3, MCT04649659) Open-label single-arm
Amivantamab (JNJ-61186372) Rybrevant®	Janssen Biotech	EGFR × cMET	IgG1 DuoBody®	2021 FDA	EGFR ex20ins NSCLC	Phase 1 (CHRYSALIS, NCT02609776) Open-label single-arm
Cadonilimab (AK104)	Akeso	PD-L1 × CTLA4	IgG-scFv2 '2+2'	2022 China	Cervical cancer after platinum-based chemotherapy	Phase 2 (NCT04380805) Open-label single-arm
Tarlatamab (AMG757)	Amgen	CD3 × DDL3	BiTE®	2024 FDA	ES-SCLC after platinum-based chemotherapy	Phase 2 (DeLLphi-301, NCT05060016) Open-label, two-arms

BsAb	Population	Administration	Response	RFS/PFS	OS	CRS/ICANS/IRR (TRAE, all grades)	CRS/ICANS/IRR (Grade 3/4)	Refs
Blinatumomab (MT103), Blincyto®	R/R B cell precursor ALL	CIV	CR 34% versus 16%; Rem 76% versus 48%	7.3 versus 4.6m	7.7 versus 4.4m	CRS 14.2%	CRS 4.9%,	[90–96]
Tebentafusp (IMCgp100), Kimmtrak®	Uveal melanoma	IV	ORR 9% versus 5%	3.3 versus 2.9m	21.7 versus 16m	CRS 47%	CRS 1%	[122,123]
Talquetamab (JNJ-64407564), Talvey®	R/R MM	SC	ORR 74% (QW), 73% (Q2W)	QW 7.5 m; Q2W 11.9 m	Not available	QW: CRS 79%, ICANS 11% Q2W: CRS 75%, ICANS 11%	QW: CRS 3% Q2W: CRS 0%	[115,152]
Glofitamab (RO7082859), Columvi®	R/R DLBCL	IV	CR 39% Rem 52%	4.9 m	11.5 m	CRS 66%, ICANS 8%	CRS (G≥2) 18% Neurological event (G≥2) 15%	[107]
Mosunetuzumab (BTCT-4465A), Lunsumio®	R/R follicular lymphoma	IV	CR 60% Rem 80%	17.9 m	18 m OS 89.6%	CRS 42%	CRS 2%	[108]
Teclistamab (JNJ-64007957), Tecvayli®	R/R MM	SC	CR 39.4% Rem 63%	11.3 m	18.3 m	CRS 72.1% Neurotoxic event 14.5%	CRS 0.6% Neurotoxic event 0.6%	[113]
Epcoritamab (GEN3013), Epkinly®	R/R B cell lymphoma	SC	CR 38.9% ORR 63.1%	4.4 m	NR	CRS 49.7%, ICANS 6.4%	CRS 2.5%, ICANS 0.6%	[106,109, 110]
Elranatamab (PF-06863135), Elrexfio®	R/R MM	SC	CR 35.0% ORR 61%	NR	NR	CRS 57.7%	CRS 0%	[114]
Amivantamab (JNJ-61186372) Rybrevant®	EGFR Exon20ins NSCLC	IV	ORR 40% CB rate 74%	8.3m	22.8 m	IRR 66%	IRR 3%	[5,141]
Cadonilimab (AK104)	Pretreated cervical cancer	IV	ORR 33% DCR 52%	PFS 3.75m	17.51 m	No ICANS/CRS/IRR	No ICANS/CRS/IRR	[127]
Tarlatamab (AMG757)	Pretreated ES-SCLC	IV	ORR 50% (10 mg), 32% (100 mg)	PFS 4.9 m (10 mg), 3.9 m (100 mg)	OS 9 m 68% (10 mg) versus 66% (100 mg)	CRS 51%, ICANS 8% (10 mg) CRS 61%, ICANS 28% (100 mg)	CRS 1%, ICANS 0% (10 mg) CRS 6%, ICANS 5% (100 mg)	[144]

[a] Abbreviations: ALL, acute lymphocytic leukemia; CB, clinical benefit; CIV, continuous intravenous infusion; CR, complete response; CRS, cytokine release syndrome; DCR, disease control rate; DLBCL, diffuse large B cell lymphoma; EGFR, epidermal growth factor receptor; ES-SCLC, extensive-stage small cell lung -cancer; FDA, US Food and Drug Administration; ICANS, immune effector cell-associated neurotoxicity syndrome; IRR, infusion-related reaction; IV, intravenous; m, months; MM, multiple myeloma; MRD, minimal residual disease; NR, nonreached; NSCLC, nonsmall cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; Ph, Philadelphia chromosome; R/R., relapsed/refractory; Rem, remission; RFS, relapse-free survival; SC, subcutaneous; TRAE, treatment-related adverse event.

Blinatumomab has significant clinical activity in relapsed/refractory (R/R) B cell ALL with positive (Ph+) or negative (Ph–) Philadelphia chromosome. Notably, the TOWER and ALCANTARA Phase 3 trials in the adult population reported a complete remission rate (CRR) of ~35% [90,91], while the MT103-205 study in pediatric patients showed a CRR of 39% [92]. Blinatumomab received regulatory approval for use in R/R ALL in 2014. Since then, real-world data have shown that blinatumomab provides an overall survival (OS) of 12.2 and 16.3 months in the Ph– and Ph+ subgroups, respectively [93].

Blinatumomab has also received FDA approval for minimal residual disease-positive B cell ALL, based on the results of the Phase 2 BLAST study [94,95]. Subsequent studies continued to explore the efficacy of blinatumomab as part of the consolidation regimen in patients with newly diagnosed ALL. The results of the study E1910 [96] led to the FDA approval of blinatumomab in June 2024 for use in the consolidation phase of ALL in combination with chemotherapy, bringing blinatumomab into the frontline setting also for patients with Ph– ALL and MRD-negative disease.

Importantly, the clinical success of blinatumomab in ALL has also catalyzed further clinical investigations with other TCEs. One example is CN201 (CD3 × CD19, IgG4-like '1+1'), which showed a 75% complete response (CR) or CR with incomplete count recovery (CRi) at higher target doses; although, in this Phase 1 trial, only a minority of patients had received previous blinatumomab [97].

Optimal sequencing of blinatumomab and subsequent treatment with anti-CD19 therapies remains an open question [98].

Acute myeloid leukemia. Acute myeloid leukemia (AML) results from malignant clonal expansion of myeloid precursor cells and is a heterogeneous disease owing to the diversity of cytogenetic or molecular subtypes [99]. BsAbs targeting CD3 and CD33 or CD123, expressed on >99% [100] and 60–80% [101] of cases, respectively, have shown activity in the treatment of chemotherapy-refractory AML.

AMG330 (CD3 × CD33 BiTE®), showed a response rate of 19% in a Phase 1 trial in chemotherapy-refractory AML [102]. A better PK profile was achieved with AMG673 (CD3 × CD33, HLE-BiTE®), which is fused to the N terminus of a single-chain IgG Fc region, allowing an increased terminal half-life of 21 days. Preliminary data from a Phase 1 study (NCT03224819) showed that treatment with AMG673 led to reduced blasts in 12 of 27 evaluable patients with refractory AML [103]. Other CD33-targeting TCEs in development include AMV564 (CD3 × CD33, TandAb) (NCT03144245) and JNJ-67561244 (CD3 × CD33, DuoBody®) (NCT03915379).

Vibecotamab (CD3 × CD123, IgG-like '1+1') demonstrated a 14.8% objective response rate (ORR) in a Phase 1 study [104], with a higher (25.9%) ORR in patients with lower disease burden (marrow blasts ≤25%) Subsequently, a Phase 2 study of vibecotamab specifically in patients with AML in morphological remission with detectable minimal residual disease is underway (NCT05285813).

B cell non-Hodgkin lymphomas. B-NHL encompasses both aggressive and indolent diseases. Current management involves rituximab plus high-dose chemotherapy, autologous stem cell transplantation, and chimeric antigen receptor (CAR)-T therapy. Despite these treatments, relapse rates remain high. Recently, bsAbs have become an essential intervention for R/R B-NHL. Glofitamab and epcoritamab (CD3 × CD20, IgG1 DuoBody®) are TCEs targeting CD20 that received FDA accelerated approval for the treatment of R/R diffuse large B cell lymphoma (DLBCL) in patients previously treated with two or more previous lines of systemic therapy. Both

drugs have shown significant and comparable activity in patients with R/R DLBCL: a CR rate of 39% was seen with glofitamab in the NP30179 study [105], while epcoritamab showed a 38.9% CR rate in the EPCORE NHL-1 study [106]. Another promising CD20-targeting TCE is odronextamab (CD3 × CD20, IgG4-like, '1+1') with a 53% CR rate in patients with R/R DLBCL who had not previously received CAR-T cells, and a 27% CR rate in those who had previously received CAR-T cells [107].

In the setting of R/R follicular lymphomas, the TCE mosunetuzumab has shown a CR rate of 60% [108] in patients progressed on two or more lines of prior treatment, leading to FDA accelerated approval for its use as a third-line of treatment or beyond. In addition, epcoritamab has also received FDA accelerated approval in the same patient population. The EPCORE NHL-1 study reported a CR rate of 62.5% and 68% with epcoritamab in the pivotal [109] and dose optimization cohort [110], respectively for patients with R/R follicular lymphoma. There was also an improved safety profile in the latter, with fewer CRS and ICANS events (42% and 0%, respectively) compared with the data from the pivotal cohort (CRS 66% and ICANS 6%) [110]. Odronextamab has also demonstrated remarkable activity for patients with follicular lymphoma, with a CR rate of 72% [107].

Importantly, beyond the R/R setting, clinical trials are beginning to evaluate bsAbs as first-line B-NHL treatments in combination with current standard treatment options (NCT06047080, NCT05800366, NCT04914741, NCT03467373, NCT04663347, NCT05578976, and NCT05201248).

Multiple myeloma. MM is characterized by an uncontrolled monoclonal proliferation of plasma cells producing excess immunoglobulins. In MM, immunomodulatory drugs (lenalidomide), proteasome inhibitors (bortezomib), and mAbs (daratumumab) have improved patient outcomes, but therapy resistance remains problematic [111]. Recently, T cell redirection therapies with bsAbs targeting CD3 and plasma cell antigen CD38, B cell maturation antigen (BCMA), G-protein-coupled receptor family C group 5 member D (GPRC5D), and FcRH5, have shown promising activity in R/R MM [112].

The first bsAb that received FDA accelerated approval for the treatment of R/R MM was teclistamab (CD3 × BCMA, IgG4 DuoBody®), for patients previously treated with four or more lines of therapy, based on the MajesTEC-1 Phase 1/2 trial [113]. The CR rate was 39.4%, with progression-free survival (PFS) of 11.3 months and an OS of 18.3 months. Given the side effect profile (all-grade CRS 72.1%; G≥3 0.6%; all-grade neutropenia 70.9%, G≥3 64.2%), the availability of this drug is restricted, according to a Risk Evaluation and Mitigation Strategy [32]. Elranatamab (CD3 × BCMA, IgG2a-like, '1+1') is the most recent bsAb approved by the FDA for the treatment of R/R MM, following the results of the MagnetisMM-3 Phase 2 trial [114].

Talquetamab (CD3 × GPRC5D, IgG4 DuoBody®), is a first-in-class GPRC5D TCE, which resulted from the Phase 1/2 trial MonumenTAL-1 in R/R MM leading to FDA accelerated approval. This trial reported a similar ORR between its weekly or biweekly schedule (74% and 73%, respectively) and longer PFS in the biweekly schedule (PFS 7.5 versus 11.9 months). Interestingly, there was also a high ORR (63%) in patients with previous T cell redirection therapy [115]. Similar to other TCEs in R/R MM, the most frequent side effects included CRS (75–79%) and infections (58–65%).

Beyond the abovementioned bsAbs targeting BCMA and GPRC5D, there are no other bsAbs approved in R/R MM. Cevostamab (CD3 × FcRH5, IgG1-like, '1+1') has significant clinical activity (ORR 36.4–50%) in patients previously treated with targeted agents, including CAR-T cells and bsAbs [116], and is being evaluated in the Phase 1/2 CAMMA 2 trial in patients pretreated with anti-BCMA agent [117]. Other bsAbs under investigation include those targeting CD38 as ISB1342

(CD3 × CD38, BEAT®) [118]. The use of these agents may be useful to overcome loss of target-related resistance, particularly in those patients previously exposed to anti-BMCA T cell therapy.

Solid tumors

The development of bsAbs in solid tumors has been challenging, particularly compared with the noted success in hematological malignancies. Contributing factors include heterogeneity of antigen expression, an elevated risk of on-target off-tumor toxicity due to shared target expression in healthy tissues, and the presence of a stroma barrier with an immune-suppressive TME. Several bsAbs targeting different tumor antigens (Table S1 in the supplemental information online) have been investigated in solid tumors, although to date only four bsAbs (amivantamab, tebentafusp-tebn, cadonilimab, and tarlatamab) have received regulatory approval for specific solid tumor types. Catumaxomab (EpCAM × CD3, IgG2a-like '1+1') [119] was the first bsAb approved for solid tumors (in 2009) for the treatment of malignant ascites. However, this treatment was voluntarily withdrawn by the company in 2017 due to commercial reasons.

Immune-mobilizing monoclonal TCRs against cancer (ImmTAC) molecules (Figure 2) comprise affinity-enhanced TCRs with high specificity toward target peptide-human leukocyte antigen (pHLA), showing TCE-like activity [120]. ImmTACs, such as tebentafusp, have been commonly classified as a subtype of TCE bsAbs due to their similar mechanism of action. Tebentafusp-tebn is a first-in-class bispecific molecule comprising an engineered TCR targeting a gp100 epitope presented by HLA-A*02:01, and is fused to an anti-CD3 single-chain variable fragment [121]. In January 2022, tebentafusp received FDA approval for the treatment of patients with unresectable or metastatic uveal melanoma who are HLA-A*02:01 positive. Efficacy was demonstrated in the IMCgp100-202 Phase 3 study (NCT03070392) [122]. The 3-year analysis showed an OS of 21.6 months in the tebentafusp group compared with 16.9 months in the control group (pembrolizumab, Ipilimumab or dacarbazine, investigator's choice) [123]. As a tumor known for its resistance to conventional immunotherapy strategies [124], the success of the ImmTAC approach in uveal melanoma represents a significant proof of concept in overcoming such challenges, with ongoing work evaluating this approach with other types of antigen [125] and HLA. Despite the success of bsAbs in the setting of uveal melanoma, this disease represents only <5% of all melanomas and, so far, there are no bsAbs approved in the setting of cutaneous melanoma. FS222 (PD-L1 × CD137, IgG1-like, Fcab^{TM}), a bsAb in early-phase development and that has shown antitumor activity in patients with cutaneous melanoma previously exposed to immunotherapy [126], may hold promise in this setting.

Furthermore, other bsAbs targeting ICP have been shown to be active in solid tumors. Cadonilimab is in development as a monotherapy and with different combinations for the treatment of various solid tumors, including gynecological cancers [127–129], lung cancer [130,131], mesothelioma [132], gastric/gastroesophageal junction and esophageal cancer [133–135], hepatobiliary tumors [136,137], urothelial cancer [138], and head and neck cancer [139,140]. Cadonilimab was approved by the China National Medical Products Administration in June 2022 for use in patients with relapsed or metastatic cervical cancer who have progressed on or after platinum-based chemotherapy, based on the results of a Phase II study (NCT04380805) that showed an ORR of 33% and OS of 17.5 months in this population [127]. Cadonilimab is being further investigated in the front-line setting in combination with chemotherapy and antiangiogenics (NCT04982237), and with radiotherapy in locally advanced cervical cancer (NCT05687851, NCT05235516).

In lung cancer, amivantamab received FDA accelerated approval and conditional marketing authorization from the European Medicines Agency (EMA) in 2021 for the treatment of *EGFRex20ins* NSCLC after progression on platinum-based chemotherapy, following results of the CHRYSALIS

study (NCT02609776) [141]. In this study, amivantamab showed an ORR of 40% and an OS of 22.8 months. Following on from this success, the PAPILLON Phase 3 trial (NCT04538664) is evaluating the efficacy of amivantamab in combination with chemotherapy compared with chemotherapy alone as a first-line therapy for NSCLC with *EGFRex20ins* mutation. The results of the randomized Phase 3 PALOMA-3 trial [142] support further exploration of subcutaneous amivantamab as a viable route of administration. The trial demonstrated non-inferiority in PK parameters and ORR compared with the intravenous route. Additionally, improved duration of response, PFS, and OS with a better toxicity profile were observed. Beyond amivantamab, other bsAbs targeting EGFR and c-MET, such as bafisontamab (MET × EGFR, FIT-Ig®) (NCT03797391, NCT05176665) or MCLA-129 (MET × EGFR, Biclonic®) (NCT04868877), are under investigation.

Another target of interest in lung cancer is DLL3, which is highly expressed on the surface of small cell lung cancer (SCLC) with a low to null expression in normal lung tissue [143]. In May 2024, tarlatamab (CD3xDLL3, HLE-BiTE®) received FDA accelerated approval for R/R extensive-stage SCLC with disease progression following platinum-based chemotherapy, based on the results of the Phase 2 DeLLphi-301 study (NCT05060016). In this study, tarlatamab showed an ORR of 40% and OS of 14.3 months in patients with heavily pretreated SCLC [144]. A Phase 3 trial (NCT05740566) of tarlatamab is underway and an expanded access protocol is also active (NCT06064500).

New bsAbs are on the horizon for solid tumors. For example, anti-HER2 bsAbs include zanidatamab, which targets nonoverlapping domains of HER2, the TCE runimotamab (CD3 × HER2, IgG1-κ-like, '1+1'), and bsAbs simultaneously targeting HER2 and HER3 proteins, such as MM-111 (HER2 × HER3, $scFv_2$-HSA). Dual blockade of HER2/HER3 is also being evaluated in tumors harboring *NRG1* fusions [145].

Solid tumors are heterogeneous diseases commonly driven by multiple and complex molecular mechanisms. Identifying new key signaling pathways and targets continues to be important to advance bsAb applications. LGR5 has been recognized as a marker for adult stem cell populations regulated by Wnt signaling [146]. Petosemtamab (EGFR × LRG5, IgG1-like, Biclonic®), is in clinical development for patients with head and neck squamous cell carcinoma (HNSCC) [147], and has received breakthrough therapy designation for patients with pretreated HNSCC.

In the setting of gastrointestinal tumors, CLDN18.2 [148] appears to be a highly favorable target to explore. For example, IBI389 (CLDN18.2 × CD3, IgG-like) has shown preliminary activity in gastric and gastroesophageal junction tumors [149] and pancreatic adenocarcinoma [150].

Limitations and toxicities of bispecific antibodies

Cytokine release syndrome

CRS results from excessive and rapid immune cell activation leading to hypersecretion of circulating proinflammatory cytokines, such as interleukin (IL)-6, interferon-gamma (IFN-γ), and TNF-α [3,151]. The severity of CRS can range from mild and transient, to life-threatening with refractory hypotension, capillary leak syndrome, and multiorgan dysfunction. While any-grade CRS is common and expected with bsAb treatment (particularly those targeting CD3 given its mechanism of action), severe (G≥3) CRS is uncommon (Table 1). For example, the rate of any-grade CRS is 75–79% with talquetamab, but G≥3 events occur only in 0–3% of patients [152]. Timing of onset and severity of CRS depend on the agent and magnitude of immune cell activation [153]: CRS after treatment with the mAb rituximab typically occurs within minutes to hours, while CRS with adoptive T cell therapy occurs days to occasionally weeks after the infusion, coinciding with maximal *in vivo* T cell

expansion. The onset of bsAb-associated CRS generally occurs within 48 h after the first dose [154], and becomes more attenuated with subsequent treatment. Management of established CRS requires close monitoring, prompt and intensive supportive care, and frequently the use of anti-inflammatory agents [3]. Tocilizumab is an IL-6 receptor antagonist approved for the treatment of CRS [155,156], while siltuximab or clazakizumab [157] target IL-6 molecules directly and, therefore, prevent IL-6 from binding with soluble and membrane-bound IL-6 receptors. Preclinical and clinical data suggest that IL-6 receptor blockade does not interfere with antitumor activity [151,158]. The fact that cytokine induction and IL-6 release have been commonly associated with the first administration suggests that strategies such as step-up dosing or first-dose fractionation would help to achieve an optimal immune-system 'priming' with gradual activation rather than the early, uncontrolled inflammatory response expected with a fixed-dose regimen [159]. Prophylactic administration of tocilizumab is not currently recommended but is under investigation [160]. Other agents for CRS management, such as Janus kinase TKIs, are under investigation [161]. Although concerns regarding the use of corticosteroids compromising treatment efficacy were initially raised, there is not, to date, strong enough clinical data that preclude the use of steroids for the management of the toxicity from bsAbs. Prophylactic corticosteroids, particularly dexamethasone, are recommended as part of the CRS mitigation strategies in the prescribing label of different approved bsAbs [162]. In general terms, the recommendations advocate for the use in the first two cycles and further discontinuation if no CRS is experienced.

Immune effector cell-associated neurotoxicity syndrome

ICANS is a serious neurological toxicity of immunotherapies including bsAbs. While it often co-occurs with CRS, it is considered a separate syndrome with distinct timing of onset and pathophysiology [163].

ICANS arises from the hyperactivation of immune effector cells, including from bsAb-redirected T cells. This leads to cyto/chemokine release that results in endothelial cell activation, disruption of the blood–brain barrier (BBB), and neuronal cell injury by neurotoxins [4]. The resultant vascular leak syndrome may have shared features with disseminated intravascular coagulation [163]. The reported incidence and severity of ICANS associated with bsAbs are variable but generally low, reported at <5% [154,164]. Host-related risk factors include pre-existing neurologic comorbidities or previous neurotoxicity with other immunotherapies [165,166]. Drug-related risk factors include smaller molecule size, bsAbs with a TCE mechanism of action, or targeting of tumor antigens also present in neural tissue (i.e., DLL3) [167]. Initial neurological features of ICANS can be subtle, including tremors, mild aphasia, apraxia, dysgraphia, and lethargy. Dysphasia may be a specific, early marker of severe neurotoxicity [168]. Symptoms may progress to delirium, seizures, or coma over hours to days [166]. The long-term effects of ICANS are unknown. Management of ICANS includes close monitoring for early signs of severe neurotoxicity and supportive care. Severe cases may require intensive care admission and airway protection. Whereas tocilizumab is an effective management of CRS, it does not efficiently penetrate the BBB and is ineffective in ICANS [166]. Corticosteroids are routinely used for the management of ICANS, and tocilizumab should be given only if there is concurrent CRS.

Infusion-related reactions

IRRs typically occur between 10 min and 4 h after the start of the administration, but can appear up to 24 h later [169]. The severity of symptoms can vary from mild–moderate to fatal [170,171]. IRRs are defined as 'Type B, bizarre' adverse drug reactions. These reactions are unpredictable, dose independent, and not related to the pharmacology of the molecule. IRRs can be divided into allergic reactions and nonallergic reactions, although the exact mechanism remains incompletely defined.

IRRs are common with mAb treatments but reports of bsAb-associated IRRs are sparse and incidence is variable, although the features are consistent with those reported with mAbs [141,170,172]. BsAbs that target dual signaling pathways or ICP are thought to be associated with higher risk, but most are G1/2 (Table S1 in the supplemental information online). Agents targeting EGFR and MET in particular have shown high IRR incidence, such as MCLA-129, with a reported IRR of 90% (all grades) [173], or amivantamab, which triggered IRRs in 67% of patients [5]. With the latter, most (90%) IRRs were G1/2 and occurred within the first 60 min of infusion and commonly in the first infusion, allowing the patients to continue the treatment in subsequent cycles [5]. Common symptoms include chills, dyspnea, flushing, nausea, chest discomfort, and vomiting. Management of IRRs includes antihistamines, steroids, analgesics, oxygen, and H2-receptor antagonists. Splitting the first dose to be administered over different days, slowing down the infusion rate, and premedication with steroids are proposed to limit this type of toxicity [141].

Opportunistic infections

The use of bsAbs can be associated with opportunistic infections [2], particularly in the setting of hematological malignancies. Host-specific conditions (e.g., immunosuppression related to underlying malignancy, or pre-existing cytopenias or hypogammaglobubinemia), direct effects of bsAbs treatment (e.g., on-target off-tumor effects of lymphocytes), and factors related to common adjunct therapies in this patient population (e.g., previous bone marrow transplant or CAR-T cell treatment) warrant due consideration with bsAb-associated infections. Infections associated with bsAbs can present variably, for example, as respiratory or line-related infections. Gram-negative bacterial infections are common, but fungal (e.g., *Aspergillus* spp.) and viral (such as cytomegalovirus) infections also occur [174].

Opportunistic infections in patients with MM treated with bsAbs targeting BCMA, GPRC5D, and FcRH5 are well documented. These bsAbs can cause on-target off-tumor toxicity to induce plasma cell aplasia (because the targets are expressed on both MM cells and normal plasma cells). This leads to reduced antibody production and hypogammaglobulinemia. A meta-analysis of bsAbs for MM showed that infection occurred in 40% of patients [175]. Another example reported that the risk of G≥3 neutropenia (39.2% versus 25.3%) and infection (30% versus 11.9%) was higher following BCMA-targeting bsAbs compared with non-BCMA targeting bsAbs, respectively [176]. This suggests infection relates to the target antigen expression on endogenous cells. One explanation is that BCMA has an essential role in the survival of long-lived plasma cells; thus, BCMA-targeted therapies, such as teclistamab or elranatamab, are associated with prolonged plasma cell aplasia [177].

Another hypothesized mechanism resulting in an increased infection risk is that bsAbs may trigger the activation of regulatory T cells [2], contributing to an immunosuppressive microenvironment. The use of IL-6 inhibitors or steroids for the management of severe bsAb-associated CRS has been linked with increased infection risks [178], although this remains controversial.

The adequate management of opportunistic infections during the treatment with bsAbs in patients with hematological malignancies, including monitoring, screening, and treatment decisions for bsAbs with an opportunistic infection, has been previously described [162].

Mechanisms of resistance

Current understanding of MoRs for bsAbs extends mostly from TCEs given that this is the biggest class of bsAbs. Broader immunotherapy MoRs have been previously reviewed [179].

Immune checkpoint proteins and costimulatory molecules

Cell culture and murine models have shown that upregulation of ICP is a potential MoR of TCEs predominantly in hematological malignancies. It has been reported that, in human AML cell lines, the expression of the inhibitory ligands PD-L1 and PD-L2 reduced the cytolytic activity of AMG 330 (CD3 × CD33, BiTE®) [180]. Another study showed that PD-L1 is not constitutively expressed at diagnosis of AML but expression was upregulated on AML cells upon treatment with AMG330 *ex vivo*. In the same experiment, blockade of the PD-1/PD-L1 interaction enhanced AMG 330-mediated AML cell lysis, T cell proliferation, and IFN-γ secretion [181]. This suggests that TCE-mediated immune escape via induced PD-L1 expression is partially reversible. Clinical data correlating PD-1/L1 status and therapy response are limited. In R/R B-NHL, biomarker analysis in a Phase 1 study of glofitamab showed that complete responses were associated with low PD-1 expression in pretreatment biopsy samples [53]. In patients with B-NHL, addition of an anti-PD-1 antibody augmented antitumor activity of odronextamab [182]. These data have prompted early-phase clinical trials of TCEs combined with anti-PD-1 antibodies, actively underway in hematological malignancies and solid tumors (NCT02879695, NCT03340766, NCT03512405, NCT03160079, NCT03605589, and NCT02650713).

Loss of target antigen expression

Cancer immune escape may be mediated by downregulation or loss of target antigen expression. Loss of CD19 occurs in 6–30% of patients who have progressed following blinatumomab [183–186]. Next-generation sequencing of samples from four patients with blinatumomab-resistant ALL showed disruption of CD19 membrane trafficking in the post-endoplasmic reticulum as a possible treatment-induced MoR. The same study showed retained expression of CD20 and CD22 in CD19-negative clones [187], suggesting concurrent targeting of other lymphoid antigens as a viable strategy to address blinatumomab resistance [188]. Testing of blinatumomab combined with inotuzumab (an anti-CD22 antibody–drug conjugate) is underway (NCT03739814). Following glofitamab treatment, patients with R/R aggressive B-NHL showed a high incidence of CD20 loss at the time of relapse [189]. These data support the need for further research to dissect the mechanisms of target loss and the need to explore other targets. Data on target antigen loss with TCE treatment in other cancer types are sparse. Analysis of longitudinal melanoma samples did not demonstrate loss of gp100 expression following treatment with tebentafusp [190]. In MM, baseline BCMA expression levels did not differ between responders and nonresponders to AMG420 (CD3 × BCMA, BiTE®) [191], but biallelic loss of BCMA conferred resistance to CAR-T cell therapy [192]. For on-treatment changes, one study reported downregulation of BCMA expression after TCE therapy in patients with R/R MM [193].

MHC-I loss and impaired IFN-γ signaling

MHC class I downregulation or loss may be a potential MoR associated with bsAbs in certain contexts. Impaired IFN-γ signaling is a possible MoR for bsAbs targeting HER2, where overexpression is a driver for gastric and breast cancers [194,195]. The kinase JAK2 transduces signals initiated by INF-γ, where expression can be downmodulated in T cells resistant to HER2-targeted bsAb [195]. The consequence of this IFN-γ pathway downmodulation is not entirely clear; however, studies have shown that IFN-γ increases the sensitivity of redirected T cell cancer cell killing with bsAb treatment [194,195]. Interestingly, the role of IFN-γ was reported to be independent of antigen presentation following HER-targeting with bsAbs [194,195]. The potential association between the IFN-γ pathway and antigen presentation as an MoR in other malignancies treated with bsAb remains unknown. It is of interest to understand whether some tumors downregulate MHC class I from an impaired IFN-γ pathway following bsAb treatment.

Anti-drug antibodies

As a protein therapeutic with large, complex structures, bsAbs can induce a sustained humoral response via non-self-recognition of amino acid sequences and structural motifs. Drug resistance from **anti-drug antibodies (ADAs)** results from formation of neutralizing antibodies (NAbs) against the variable regions of the bsAb, preventing target antigen engagement. Distinct from NAbs, ADAs that bind alternate regions of the antibody may have variable consequences that manifest across a spectrum, including clinically irrelevant effects, alteration of drug PK, or even life-threatening side effects, such as complement-mediated reactions, infusion reactions, or Type III hypersensitivity reactions [196–198]. The rates of ADAs in bsAbs have not been systematically documented but will relate to drug (e.g., inherent immunogenicity of the construct, dosing regimen) and/or host factors (e.g., functional impairment of immune response, including inherent, iatrogenic, or cancer-related factors). The incidence of NAb may be higher with antibody-based treatments against T cells or antigen-presenting cell targets compared with B cell targets [199]. The presence of pre-existing, as opposed to treatment-induced, ADAs has been reported with various other biotherapeutics [200], but little is known specifically in bsAbs.

Pasotuxizumab (CD3 × PSMA, BiTE®) has been investigated in patients with advanced prostate cancer (NCT01723475) [201]. While pre-existing ADAs were not observed, 96.7% of patients in the subcutaneous-administration cohort developed treatment-induced ADAs, with a median onset at cycle 2. Many cases progressed to higher titers, and none were transient. The emergence of ADA was associated with drug exposure loss and a corresponding rise in PSA levels, reflecting disease progression. By contrast, none of the patients in the intravenous infusion cohort developed ADAs. This suggests that the subcutaneous route is highly immunogenic, likely due to effective antigen presentation by skin-resident dendritic cells and epitope trafficking to secondary lymphoid tissue. Other reports of bsAb-induced ADA within clinical trials include LY3415244 (TIM-3 × PD-L1, IgG-like) in patients with solid tumors, which showed that 12/12 (100%) of patients developed ADAs, resulting in early termination due to ADA emergence [202]. For this reason, it is crucial to evaluate the emergence of ADAs during Phase 1 trials of bsAbs, ideally in a near real-time fashion, alongside assessment for NAbs and their potential impact on drug PK if detected.

Concluding remarks and future directions

BsAbs have shown high clinical efficacy in R/R hematological malignancies, with rapid developments across this field. Multiple clinical investigations are underway focused on application in the first-line setting, which will have particular relevance for patients unsuitable for traditional chemotherapeutics. However, questions remain as to the optimal timing and treatment sequencing of bsAbs, especially in diseases where CAR-T therapies are also available (see Outstanding questions). It is yet unknown whether bsAbs used in the first-line setting may contribute to terminal T cell exhaustion, impacting efficacy of subsequent therapies, neither is it definitively known whether CAR-T should be used before bsAbs.

Similar success of bsAbs has not been consistently observed in solid tumors, owing to inherent complexities, including heterogeneous antigen expression, stromal barriers to drug penetration, and an immunosuppressive TME. Over a decade of drug and clinical development has only led to four bsAbs with regulatory approval and impacted clinical care in solid cancers, which is a testament to these ongoing challenges. However, improvements in bsAb design and new understanding of therapy targets will no doubt accelerate development in solid cancers, and DLL3 and HER2-targeting bsAbs are such examples.

In broader efforts to optimize bsAb clinical application, the optimal combination bsAb regimen (i.e., with chemotherapy) is under investigation. It is of interest to assess whether the use of

Outstanding questions

Design and engineering principles have a crucial role in optimizing the function of bsAbs. How can the manufacturing process of bsAbs be optimized to increase efficacy, reduce costs, and ensure consistent quality?

The regulatory approval of bsAbs for various hematological malignancies marks a significant advancement in anti-cancer therapy. What are the economic implications of incorporating bsAbs into anti-cancer treatment regimens, and how can cost-effectiveness be optimized?

CRS and ICANS are significant toxicities impacting patient safety and treatment tolerability of bsAbs. How can we risk stratify patients at high risk of developing these toxicities, and what proactive measures can be implemented to mitigate their occurrence and severity?

Resistance to bsAbs presents challenges in maintaining treatment efficacy. What innovative therapeutic approaches and combinations can be explored to overcome or circumvent resistance mechanisms and enhance the durability of response to bsAbs?

BsAbs, such as TCEs, can modulate the TME. How can these bsAbs be further optimized to selectively maximize antitumor immune responses and induce antigen-specific, long-lasting T cell memory, particularly in the context of adaptive resistance?

BsAbs can induce antitumor immune responses that may affect the host immune system. What are the long-term effects of bsAbs on a patient's immune system and susceptibility to infections or secondary malignancies?

Given the rapid developments in the field of bsAbs, particularly in hematological malignancies, how can we optimize the timing and treatment sequencing of bsAbs, especially in relation to CAR-T therapies?

Considering the existing challenges, the development of bsAbs in solid tumors needs to be optimized. Are there specific bsAbs formats and target combinations that are most suitable for solid tumor therapeutics?

bsAb, as mono- or combination therapy, can induce lasting immunity (i.e., formation of memory T cells), which can continue immunosurveillance of tumor cells after therapy cessation and lend durable clinical responses. There are data to suggest that peptide–MHC presentation is required for efficient T cell responses following bsAb administration [55]; however, whether any of these expanded T cell clones specific to tumor antigens persist and mount an effective response upon antigen recall is not yet clear. It is also of interest to combine bsAbs with antibody–drug conjugates, because the lack of lymphopenia may render them ideal partners. One possibility is to debulk tumors with an effective antibody–drug conjugate, followed by the administration of bsAbs to trigger a sustained antitumor immune response (Figure 4).

Computational- and artificial intelligence-based methods hold great potential to accelerate the development and clinical application of novel bsAbs. How can we integrate these methods into the drug development pipeline to ensure rapid translation to the clinic?

Advancing bsAbs lies in innovative engineering, particularly to enhance clinical efficacy and address on-tumor off-target toxicities. Emerging design technologies, such as multispecific

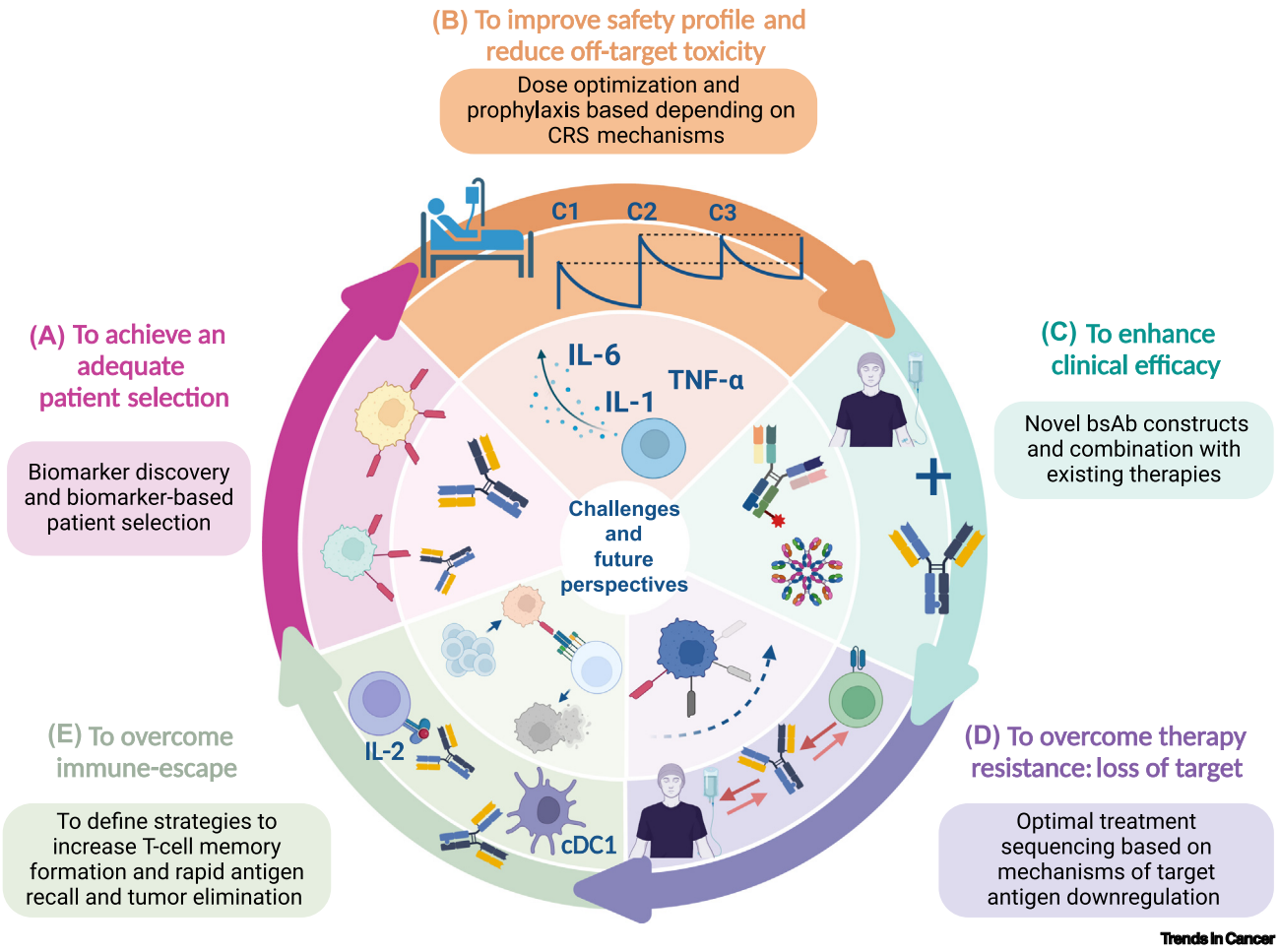

Figure 4. Challenges and future perspectives in bispecific antibodies (bsAbs). Potential preclinical strategies to address these challenges are represented in the inner part of the circular framework, while the strategies applicable to the clinical setting are represented in the external part of the circle. The circle is segmented into five sections (A–E), addressing distinct challenges. (A) The development of new biomarkers can be used for leveraging tailored bsAb therapy and improved clinical trial design based on biomarker patient selection. (B) A wider knowledge of cytokine release syndrome (CRS) physiopathology is necessary to define prophylactic strategies and treatment regimens and mitigate the risk of severe adverse reactions. (C) The optimization of specificity and pharmacokinetics, implementation of multispecific antibodies and conjugated molecules, and the combination with other modalities can maximize therapeutic efficacy. (D) A better understanding of the mechanisms of target downregulation is crucial for devising optimal treatment sequencing strategies. (E) Approaches such as facilitating crosstalk with other immune cells, including dendritic cells, or enhancing T cell activation with interleukin (IL)-2-targeting strategies may enhance the immune response effect and duration.

antibodies or the incorporation of new antigen targets, present such opportunities. Bispecific antibody–drug conjugates and strategies such as targeting multiple tumor antigens with multispecific antibodies (Figure 2) may offer further advances because they can induce synergistic effects, particularly in the setting of tumor heterogeneity, and potentially overcome resistance mechanisms that arise from the loss or downregulation of target. Interestingly, a possible strategy to enhance bsAb target specificity is to deplete endogenous cells expressing the targeted tumor antigen before bsAb administration. This has been implemented in patients with B cell lymphomas through pretreatment with the mAb obinutuzumab before treatment with a bsAb targeting CD20 to mitigate CRS episodes [161]. Furthermore, given the recognized role of dendritic cells on T cells during the active stage of anti-PD1 mAb treatment, a recent study reported that a bsAb targeting PD-1$^+$ T cells and conventional type 1 dendritic cells led to potent antitumor immunity in a preclinical model [203], thus offering a potentially new class of bsAb to our armamentarium (Figure 4). More broadly, in the context of constant innovation in molecule formats, a consistent, precise nomenclature when discussing antibody structure is critical to avoid confusion in the field. The Verified Taxonomy for Antibodies (VERITAS) classification and nomenclature scheme provides a uniform framework for naming antibodies with clear annotations of the assembled components and reference to the protein structure [204]. Finally, given the vast complexities in *de novo* antibody development, computational or artificial intelligence-based methods in construct design and target epitope-matching, combined with *in silico* predictions of epitope-binding affinities and PK, will be critical for fast-tracking clinical application of novel bsAbs at scale [205]. Looking forward, bsAbs represent a dynamic and promising frontier in cancer therapy, and ongoing research will unlock new therapeutic applications.

Acknowledgments

We thank Alan Russell and Michael Dickinson for their helpful suggestions and review of the manuscript. M.H is supported by a Sociedad Española de Oncología Médica (SEOM) fellowship and CRIS Cancer Out-Back program; G.P is supported by the CaixaResearch Advanced Oncology Research Program supported by 'la Caixa' Foundation (LCF/PR/CE07/50610001); E.G is supported by the CaixaResearch Advanced Oncology Research Program supported by 'la Caixa' Foundation (LCF/PR/CE07/50610001); L.L.S holds the BMO Chair in Precision Cancer Genomics; T.M.S. is funded through a collaboration with Roche; L.A. is supported by the Peter Mac Foundation Discovery Partner Fellowship.

Declaration of interests

None declared by authors.

Supplemental information

Supplemental information to this article can be found online at https://doi.org/10.1016/j.trecan.2024.07.002.

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