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Review article

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The present and future of bispecific antibodies for cancer therapy

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Abstract

Bispecific antibodies (bsAbs) enable novel mechanisms of action and/or therapeutic applications that cannot be achieved using conventional IgG-based antibodies. Consequently, development of these molecules has garnered substantial interest in the past decade and, as of the end of 2023, 14 bsAbs have been approved: 11 for the treatment of cancer and 3 for non-oncology indications. bsAbs are available in different formats, address different targets and mediate anticancer function via different molecular mechanisms. Here, we provide an overview of recent developments in the field of bsAbs for cancer therapy. We focus on bsAbs that are approved or in clinical development, including bsAb-mediated dual modulators of signalling pathways, tumour-targeted receptor agonists, bsAb–drug conjugates, bispecific T cell, natural killer cell and innate immune cell engagers, and bispecific checkpoint inhibitors and co-stimulators. Finally, we provide an outlook into next-generation bsAbs in earlier stages of development, including trispecifics, bsAb prodrugs, bsAbs that induce degradation of tumour targets and bsAbs acting as cytokine mimetics.

Sections

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Introduction

Bispecific antibodies (bsAbs) have gained major interest in the past decade, given their unique and versatile modes of action (MoA). The approval of nine bsAbs for cancer therapy in the past 3 years (2021–2023) illustrates the evolution of this class of antibodies as novel therapeutic agents. The main reason for their success is the fact that bsAbs are capable of mediating therapeutic effects beyond those of natural monospecific antibodies[1,2], for instance, by recruiting immune effector cells to cancer cells or by targeting different signalling pathways with a single molecule. Importantly, bsAbs can exert multiple MoAs at the same time.

bsAbs act either in a combinatorial or an obligate manner, the latter meaning that the same MoA cannot be achieved by a sole combination of antibodies[1,2]. Combinatorial MoAs merge the activity of two different antibodies within one molecule, such as dual inhibition of target structures such as receptor tyrosine kinases (RTKs) in the case of amivantamab, or immune checkpoint inhibitors (CPIs) in the case of cadonilimab, in which each binding site can act independently from the other. In the obligate MoA, the activity of the bsAbs strictly depends on both specificities, either requiring simultaneous (spatial) binding of two targets[2], such as those used in T cell-engaging bsAbs such as blinatumomab (CD19 × CD3), or sequential (temporal) binding of two targets, such as those required in the delivery of a payload through biological barriers such as transferrin receptor-based bsAbs for delivery over the blood–brain barrier[3–6]. Notably, the combinatorial part of the MoA can benefit from bispecificity resulting in, for example, superior RTK inhibition or more selective checkpoint inhibition compared with the respective combination of antibodies.

An increasing number of different approaches are being studied for cancer therapy (Fig. 1). These approaches include dual targeted signalling inhibitors that act at different levels of tumour proliferation, vessel formation and metastases. They also include bsAb–drug conjugates, bispecific natural killer cell engagers (NKCEs) and innate immune cell engagers (ICE) as well as T cell-engaging bsAbs. Other approaches are bispecific CPIs (dual CPIs), co-stimulatory bsAbs and fusion proteins, bsAbs with combined checkpoint inhibitory and co-stimulatory features, as well as bsAbs specifically activated in the tumour microenvironment (TME) and novel ways of combining adoptive T cell therapy and therapeutic antibodies.

Notably, the discovery of new bsAbs is driven by the biology behind these approaches, which must be matched with an optimal bsAb design to choose the right format, affinity range and epitopes. Properties such as target choice, epitope locations, affinities, valencies, distance between binding sites, molecular size, flexibility and presence or absence of an Fc region and Fc-mediated effector functions can have a profound effect on functional properties and developability[7,8]. Antibodies can engage innate immune cells via their Fc portion binding to Fcγ receptors[8]. It may be desirable, however, for T cell engagers (TCEs) and other bsAbs to lack FcγR binding to avoid independent FcγR engagement of tumour targets — such as when a bsAb binds to the T cell receptor (TCR) — with the aim to avoid unspecific immune cell activation, recruitment and cytokine secretion, whereas for dual RTK-blocking bsAbs, FcγR function may be retained so that these bsAbs can mediate antibody-dependent cellular toxicity (ADCC) or antibody-dependent cellular phagocytosis. Accordingly, in bsAbs dependent on the intended MoA, FcγR binding is frequently abolished by introduction of mutations in the Fc portion[9] or retained/enhanced in cases where ADCC/antibody-dependent cellular phagocytosis is desired[8]. However, a functional Fc portion mediates IgG-like pharmacokinetic properties of bsAbs via neonatal Fc receptor (FcRn) recycling[10]. Mutations in the Fc portion cam modify binding to FcRn, and thus the antibody's half-life. In bsAbs that do not include an Fc portion, IgG-like pharmacokinetics can be achieved by other means, such as binding to human serum albumin (HSA) or other molecules that can extend the half-life.

A myriad of bsAb formats comprising more than several hundred types of molecules are available, with a diverse set of bsAb formats used in molecules approved and under clinical development[1,2,7,11–13]. Currently, most approved bsAbs and those in advanced stages of drug development are mainly used in cancer immunotherapy[14,15]. Various other entities that address novel drug delivery, such as bsAb-based antibody–drug conjugates (bsADCs), or dual targeting of cancer-associated signalling pathways, are also in development (Fig. 1).

Our recent search of data available from ClinicalTrials.gov, Cortellis and The Antibody Society revealed more than 300 clinical trials of more than 200 different bispecific molecules, with approximately 75% used to treat solid tumours and 25% to treat haematological malignancies (Fig. 1). Ten bsAb drugs are currently approved for cancer therapy, with nine approved in the United States and/or Europe and cadonilimab approved in China. Of these ten bsAbs, nine can be classified as TCEs. Catumaxomab, the first approved bsAb, was withdrawn from marketing in 2013 (Table 1 and Fig. 2). Notably, today a significant proportion of bsAbs in clinical development are already in the later stages (phases II and III). We found that bsAbs to treat solid tumours are dominated by immunomodulators, including dual CPIs (approximately 45%) and TCEs (approximately 33%), followed by bsAbs targeting dual pathways, ICEs and dual ADCs. By contrast, TCEs are the major class of bsAbs applied to treat haematological malignancies (approximately 75%), followed by ICEs, dual CPIs and NKCEs (Fig. 3).

The bsAbs in our data set address more than 60 different targets and more than 100 target combinations using >50 different bsAb formats. We acknowledge, however, that growth in the field is rapid and new bsAbs, potentially with novel targets and formats, have entered clinical trials since our analysis. Nevertheless, this Review aims to provide a comprehensive MoA-based overview of recent developments in the field of bsAbs developed as cancer therapies with a focus on bsAbs described in peer-reviewed publications as end of 2023.

bsAbs for dual receptor inhibition

Many cell surface proteins that are involved in cellular signalling, such as RTKs and related receptors, are validated targets for antibody-based therapies. Decades ago, research on these proteins, including the historic frontrunners epidermal growth factor receptor (EGFR; also known as HER1), HER2, vascular endothelial growth factor (VEGF) and antibodies that target them, provided the groundwork for virtually all subsequent antibody-based therapeutic developments. Although antibody-based therapies that bind one defined signalling entity are quite effective, disease-associated phenotypes are frequently triggered by more than one pathway. Such redundancies allow cells to overcome pharmacological growth inhibition or induction of cytotoxicity through a single target or pathway by using other compensatory signalling pathways. bsAbs that simultaneously modulate different disease-associated signalling receptors and/or pathways can reduce or overcome this limitation. Amivantamab (JNJ-61186372) targets EGFR and hepatocyte growth factor receptor (MET)[16]. Both receptors trigger proliferation of non-small cell lung cancer (NSCLC) and, consequently, blocking both can inhibit NSCLC growth more effectively than blocking just one pathway. Amivantamab is approved to treat a subtype of NSCLC that carries EGFR exon 20 insertion mutations[17].

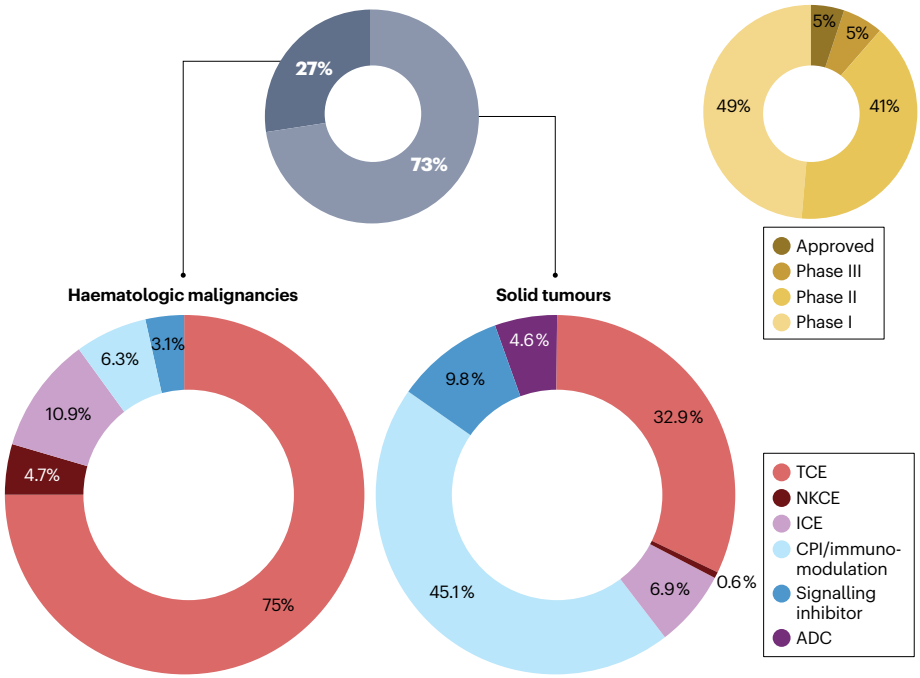

Fig. 1 | Bispecific antibodies in clinical development for cancer therapy. The more than 200 bispecific antibodies (bsAbs) currently in clinical development grouped according to cancer type, clinical stage and mode of action. Fifty per cent of bsAbs in clinical development are in phase II and phase III or already approved. Approximately three out of four bsAbs are developed to treat solid tumours and treatment of solid tumours is dominated by T cell engagers (TCEs; 32.9%) and bsAbs with checkpoint inhibition and/or immunomodulatory mechanisms of action (MoAs) (45.1%). By contrast, bsAbs for treatment of haematological malignancies are dominated by TCEs (75%) and other MoAs (21.9%) addressing immunological activities, such as engagement of natural killer cells and other immune cells through checkpoint inhibition and/or immunomodulation owing to the availability of highly tumour-selective or lineage-specific antigens. Of note, dual signal inhibition is used by approximately 10% of bsAbs developed for treatment of solid tumours. To date, bsAb antibody–drug conjugates (ADCs) are developed to treat solid tumours. CPI, checkpoint inhibitor; ICE, innate cell engager; NKCE, natural killer cell engager.

Recently, data from a randomized phase III study showed superiority of amivantamab plus chemotherapy compared with chemotherapy alone in this subset of patients[18]. Several additional EGFR-targeting bsAbs that address different second targets on tumours are in clinical development. For example, petosemtamab (MCLA-158) binds EGFR and LGR5, a cancer stem cell-associated cell surface receptor. Early development of this bsAb was significantly supported by advanced tumour organoid technologies[19]. Petosemtamab is currently being evaluated in a phase I/II trial in patients with solid tumours, including advanced head and neck squamous cell carcinoma (NCT03526835).

Other bsAbs address target combinations of different members of the HER family, among which EGFR and HER2 are the most prominent. EGFR and HER2 form homodimers as well as heterodimers with each other and other HERs, including HER3, on the surface of tumour cells. Zenocutuzumab (MCLA-128) binds HER2 and HER3 (ref. 20), and its efficacy is currently being tested in patients with solid tumours that harbour neuroregulin-1 fusions, which trigger the formation of heterodimeric HER2–HER3 complexes. Another advanced HER-binding bsAb is izalontamab (SI-B001), which targets EGFR and HER3 (ref. 21) and has been tested in patients with locally advanced or metastatic epithelial tumours (NCT05020769). Finally, biparatopic bsAbs can target just one antigen, such as HER2, at different positions in different domains[22–24]. Biparatopic bsAbs build on the observation that combined targeting of HER2, such as with the monoclonal antibodies trastuzumab and pertuzumab, increases antitumour efficacy in preclinical models of NSCLC and breast cancer[25]. Examples of such biparatopic HER2-bsAbs in clinical development for the treatment of breast cancer are zanidatamab[26], MBS301 (ref. 27) and KN026 (ref. 28) (Table 2).

bsAbs for ligand-receptor inhibition

Receptor activation can be inhibited either by interfering with receptor dimerization or complexation, or by blocking the ligand-binding sites of the receptor. In cancer, often ligands and receptors from several complementary or compensatory pathways contribute to tumour progression and treatment resistance. bsAbs can simultaneously block two different ligands, either soluble or cell membrane-attached, or a combination of a ligand and a receptor[29]. This approach is not only addressed with bsAbs but also by bispecific or biparatopic scaffold-based binders, which we do not cover here[30–32]. Dual targeting of VEGF and angiopoietin 2 (ANG2) to block activation of VEGFR and angiopoietin-1 receptor (TIE2), respectively, two pathways involved in angiogenesis, has been the subject of several clinical trials in solid tumours including the angiogenic switch of micrometastases[33]. BI836880 is a trispecific nanobody (Nb) fusion protein consisting of three nanobodies targeting VEGFA, ANG2 and HSA, the latter to prolong its serum half-life[34]. This compound is currently undergoing evaluation in phase I studies in patients with head and neck squamous cell carcinoma, NSCLC and other solid tumours. Of note, vanucizumab, a bsAb targeting VEGFA and ANG2 that showed antitumour, anti-angiogenic and anti-metastatic effects in preclinical models in combination with chemotherapy[35], did not improve progression-free survival over VEGF inhibition in combination with chemotherapy in patients with colorectal cancer[36].

The concept of combining VEGF inhibition with another antagonistic activity was further employed in bsAbs targeting VEGF and delta-like canonical Notch ligand 4 (DLL4). DLL4 is a cell surface ligand that activates the Notch-1 receptor pathway, which has a central role in tumour angiogenesis[37]. Several bsAbs targeting VEGF and DLL4 have entered clinical trials, including navicixizumab, dilpacimab and CTX-009/ABL001. Navicixizumab, a tetravalent bsAb with four binding sites two of which are based on single-chain variable fragment (scFv) fusions, demonstrated promising clinical activity in combination with paclitaxel with manageable toxicity in patients with platinum-resistant ovarian cancer, indicating that this approach might provide clinical benefit in certain indications[38]. By contrast, a phase II study of dilpacimab, another tetravalent bsAb, in combination with folinic acid, fluorouracil and irinotecan (FOLFIRI) revealed safety concerns and did

Table 1 | Approved bispecific antibodies for cancer therapy

bsAb	International non-proprietary name	Targets	MoA	Format	Year of first approval/ region[a]	Indications	Company
Removab	Catumaxomab	EpCAM×CD3ε	TDCC	Quadroma mouse/rat 1+1	2009 Withdrawn EU 2013	Ovarian ascites, intraperitoneal	Trion Pharma/ Fresenius
Blincyto	Blinatumomab	CD19×CD3ε	TDCC	BiTE 1+1	2014 United States/EU, Japan	ALL	Amgen
Rybrevant	Amivantamab	EGFR×MET	Signalling inhibition, ADCC	Duobody 1+1	2021 United States/EU	NSCLC EGFR exon 20 insert mutation	J&J
KIMMTRAK	Tebentafusp	gp100-HLA-A*02× CD3ε	TDCC	scFv-TCR fusion 1+1	2022 United States/EU	Uveal melanoma	Immunocore
Lunsumio	Mosunetuzumab	CD20×CD3ε	TDCC	KiH 1+1 IgG	2022 United States/EU	Relapsed/refractory follicular NHL	Roche group
Kaitanni	Cadonilimab	PD1×CTLA4	Dual checkpoint inhibition	IgG-scFv tetrabody 2+2	2022 China	Hepatocellular carcinoma	Akeso Bio
Tecvayli	Teclistamab	BCMA×CD3ε	TDCC	Duobody 1+1	2022 United States/EU	Relapsed/refractory multiple myeloma	J&J
Columvi	Glofitamab	CD20×CD3ε	TDCC	CrossMAb 2+1	2023 United States/EU	Relapsed/refractory DLBCL	Roche group
(T)Epkinly	Epcoritamab	CD20×CD3ε	TDCC	Duobody 1+1	2023 United States/EU, Japan	Relapsed/refractory DLBCL	Genmab, Abbvie
Talvey	Talquetamab	GPRC5D×CD3ε	TDCC	Duobody 1+1	2023 United States/EU	Relapsed/refractory multiple myeloma	J&J
Elrexfio	Elranatamab	BCMA×CD3ε	TDCC	bsAb 1+1	2023 United States/EU	Relapsed/refractory multiple myeloma	Pfizer

ADCC, antibody-dependent cellular cytotoxicity; ALL, acute lymphocytic leukaemia; BCMA, B cell maturation antigen; BiTE, bispecific T cell engager; bsAb, bispecific antibody; DLBCL, diffuse large B cell lymphoma; EGFR, epidermal growth factor receptor; EpCAM, epithelial cellular adhesion molecule; GPRC5D, G-protein-coupled receptor class C group 5 member D; MoA, mechanism of action; NSCLC, non-small cell lung cancer; NHL, non-Hodgkin lymphoma; scFv, single-chain variable fragment; TCR, T cell receptor; TDCC, T cell-dependent cellular cytotoxicity. [a]Region of approval limited to the United States, the European Union (EU), Japan and China; products may also be approved in other countries. Status as of end of 2023.

not improve clinical outcome in patients with metastatic colorectal cancer compared with treatment with bevacizumab and FOLFIRI[39].

Based on positive results from combination therapy with VEGF antibodies and CPIs[40], the concept was further adapted to design bsAbs targeting VEGF and either PDL1 on tumour cells or PD1 on T cells, thus linking anti-angiogenesis with an immuno-oncology approach[41]. The most advanced molecule using this approach is ivonescimab (AK112), directed against PD1 and VEGF. A marketing application for ivonescimab is currently under regulatory review in China. Other bsAbs target PDL1 and VEGF, including PM8002, which is undergoing evaluation in a phase II/III study (NCT05756972) of patients with NSCLC.

bsAbs for receptor activation

Many antitumour responses are mediated through activation of cell surface receptors, including immune responses or cell death through induction of apoptosis. One approach for cancer therapy is the targeted delivery of receptor-activating ligands such as growth factors, cytokines and (co-)immuno-stimulatory ligands by fusing them to an antibody or antibody fragment to induce local or tissue-specific agonistic activity, and thereby cellular responses[42,43]. A growing number of these antibody–ligand fusion proteins are entering clinical trials[44].

Receptor activation can also be achieved using agonistic antibodies. Members of the tumour necrosis factor (TNF) superfamily comprising death ligands (such as TNF ligand superfamily member 10 (TNFSF10); also known as TRAIL), and their respective death receptors (such as TRAIL receptors 1 and 2; also known as DR4 and DR5), or ligands with co-stimulatory activity on immune cells (such as TNFSF9; also known as 4-1BBL) and their receptors (such as TNFRSF9; also known 4-1BB or CD137) have attracted particular attention in cancer therapy. However, monoclonal antibodies delivered rather disappointing results in clinical trials[45–48]. The reason is that many TNFRSFs require cross-linking of more than two receptors for activation, which can only be achieved by Fc–FcγR-mediated hyperclustering of antibodies on cell surfaces, and as such is dependent on the presence of sufficient numbers of FcγR-expressing innate immune cells in the TME[49]. As an alternative, bsAbs directed against a target antigen and a TNFRSF member have been shown to act as powerful and tumour-selective agonistic molecules mimicking the activity of surface-displayed ligands[46]. This is especially eminent for DR4 and DR5, with DR5 requiring a clustering of several receptors for activation. Notably, bsAbs against DR5 and a surface antigen, such as fibroblast activation protein-α (FAPα; also known prolyl endopeptidase FAP), cadherin 17 (CDH17) or folate receptor-α (FRα), not only efficiently induce receptor activation but also avoid sequestration by decoy receptors[50]. RG7386 (RO6874813), a tetravalent bsAb targeting FAPα on cancer-associated fibroblasts and DR5, triggered potent and selective tumour cell killing in vitro and in vivo in mice with FAPα-expressing stroma and entered clinical testing, but was

subsequently discontinued due to strategic portfolio prioritization[51]. Furthermore, BI905711, a tetravalent bispecific IgG-scFv fusion protein targeting DR5 and CDH17 currently in a phase I clinical study, showed a greater than 1,000-fold gain in efficacy on CDH17-positive target cells, translating into potent and selective antitumour activities in colorectal cancer models[50]. The same format is used in a bsAb targeting DR5 and FOLR1, an ovarian cancer-enriched receptor, shown to mediate cytotoxicity in *cis* (on the same cell) and in *trans* (on two different cells), with FOLR1 acting as a clustering point for efficient DR5 activation[52].

Conceptually, this MoA can also be transferred to co-stimulatory members of the TNFRSF family that also depend on receptor clustering, including 4-1BB, OX40 and CD40 (Fig. 3) using bsAbs directed against tumour-associated antigens (TAAs), but also against other targets such as PDL1, thus combining co-stimulation with checkpoint inhibition (see below).

bsAbs for targeted payload delivery

Two conceptually different approaches exist for the use of bsAbs in targeted delivery of payloads such as cytotoxic agents or radioactivity. The first approach, pre-targeted therapy, uses one binding specificity of the bsAb to target tumour cells and the other to subsequently capture a payload on the tumour. bsAbs in this category bind to cancer targets such as carcinoembryonic antigen (CEACAM5) or HER2 on tumours, and subsequently capture radioactive-labelled payloads/complexes that are recognized by the second binding specificity of bsAbs[53–55]. Recently, a novel compelling self-assembling bispecific pre-targeting concept

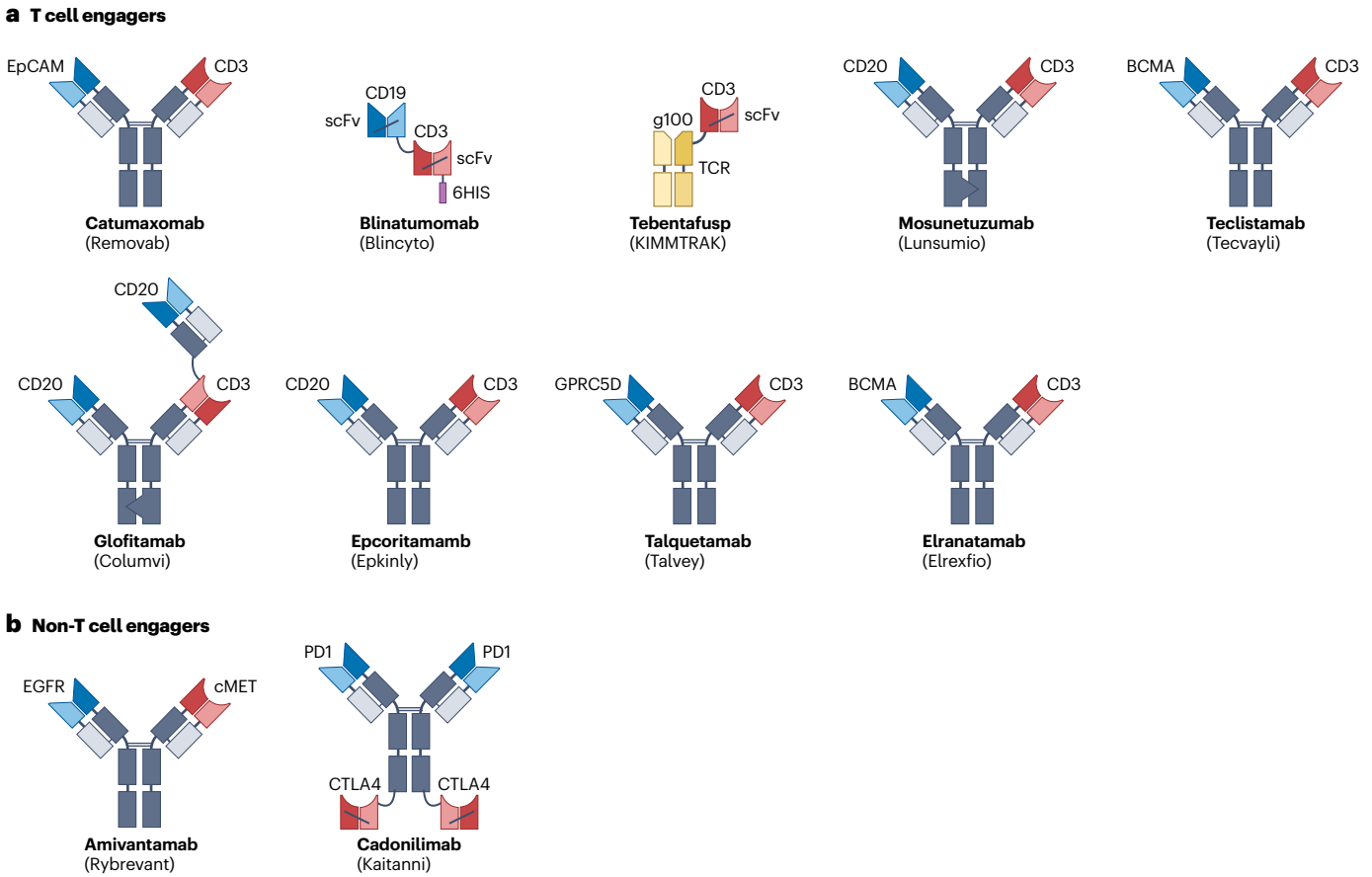

Fig. 2 | Overview of approved bispecific antibodies for cancer therapy. **a**, Bispecific T cell engagers (TCEs). **b**, Other bispecific antibodies (bsAbs) (non-T cell engagers (non-TCEs)). Six of the 11 approved bsAbs have an IgG-like structure composed of 2 heavy chains (constant regions shown in dark grey and VH domains shown in dark blue or dark red) and 2 light chains (constant domain shown in light grey and VL domains in light blue or light red). Most of them have a 1 + 1 stoichiometry — one binding site for each antigen — and one further TCE utilizes a 2 + 1 stoichiometry by fusing an additional Fab fragment to one of the heavy chains. The other bsAbs are either Fc-free fusion proteins of two single-chain variable fragment (scFv) fragments (blinatumomab) or a scFv fragment fused to a T cell receptor (TCR) fragment (tebentafusp), or are IgG-scFv fusion proteins with a 2 + 2 stoichiometry. Except for catumaxomab, which was produced by the hybrid-hybridoma technology combining a mouse and a rat hybridoma, all other bsAbs are produced by recombinant technologies. For the IgG-like heterodimeric molecules, correct assembly of the two different heavy chains is solved by using mutations in the CH3 domain to force heterodimerization — such as the knobs-into-holes technology — or controlled Fab-arm exchange (cFAE), which allows assembly of IgG-like molecules from two different monospecific IgGs. cFAE also solves the light chain problem, that is the cognate pairing of light and heavy chains. Alternatively, further modifications in the Fab arms, such as the CrossMab approach, allow to enforce correct light and heavy chain pairings. Of note, all recombinant IgG-like bsAbs comprise an Fc region with silent or reduced effector functions, which was achieved by aglycosylation or protein engineering by introducing mutations into the hinge/CH2 domain to avoid or reduce Fcγ receptor and complement binding. BCMA, B cell maturation antigen; EGFR, epidermal growth factor receptor; EpCAM, epithelial cellular adhesion molecule; GPRC5D, G-protein-coupled receptor class C group 5 member D.

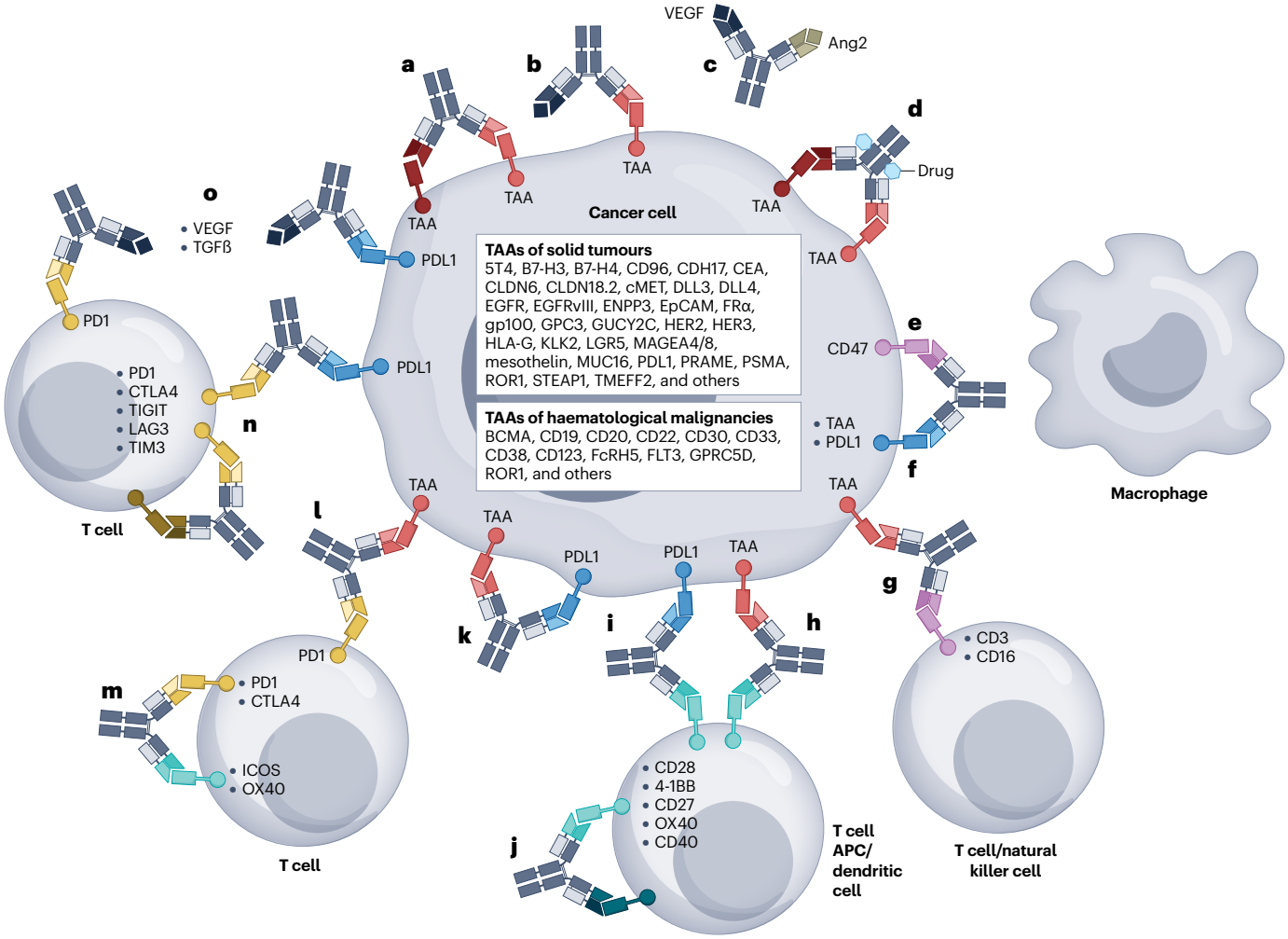

Fig. 3 | Modes of action of bispecific antibodies currently in clinical trials. **a**, Signal inhibition by receptor blockade of two different signalling receptors (indicated by different colours). **b**, Signal inhibition by inhibition of a soluble ligand (blue) and a receptor. **c**, Signal inhibition by dual blockade of two different ligands. **d**, Drug delivery by bispecific antibody (bsAb) drug conjugates (conjugate indicated in light blue). **e**, Targeted blockade of the 'don't eat me signal' (CD47–SIRPα interaction) resulting in phagocytosis by macrophages. **f**, Blockade of the 'don't eat me signal' in combination with checkpoint inhibition. **g**, Effector cell engagement through CD3 as part of the T cell receptor (TCR) on T cells or CD16 (FcγRIIIa) on natural killer cells. **h**, Tumour cell binding-mediated immune cell (co-)stimulation. **i**, PDL1 binding-mediated immune cell (co-)stimulation. **j**, Dual immune cell co-stimulation. **k**, Tumour-targeted PDL1 pathway inhibition. **l**, Tumour-targeted PD1 pathway inhibition. **m**, Checkpoint binding-mediated immune cell (co-)stimulation. **n**, Dual checkpoint inhibition. **o**, Dual checkpoint and soluble transforming growth factor-β (TGFβ) signalling inhibition by ligand blockage. Examples of target molecules and tumour-associated antigens (TAAs) for solid tumours and haematological malignancies are shown. Note: bsAbs can exert multiple modes of action (MoAs) at the same time. Ang2, angiopoietin 2; APC, antigen-presenting cell; BCMA, B cell maturation antigen; CDH17, cadherin 17; DLL4, delta-like canonical Notch ligand 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cellular adhesion molecule; FRα, folate receptor-α; GPRC5D, G-protein-coupled receptor class C group 5 member D; HLA, human leukocyte antigen; ICOS, inducible T cell co-stimulatory; LAG3, lymphocyte-associated gene 3; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; VEGF, vascular endothelial growth factor.

termed SADA was described using a modified p53 tetramerization domain to prolong tumour retention of GD2 × DOTA. GD2 x DOTA – a bsAb that recognizes GD2, a disialoganglioside with high expression in cancer, and the radioactive payload ^{177}Lu-DOTA – assembles in the TME and is retained there, but without tetramerization it is rapidly cleared from the periphery as it lacks half-life extension[56]. So far, few bsAbs in clinical trials use this MoA, likely owing to the complex logistics involved with pre-targeting of radioactivity, which limits the general applicability.

The second approach achieves specific targeting of tumour cells by delivery of a cytotoxic payload attached to bispecific antibody–drug conjugates (bsADCs) by simultaneously addressing two epitopes or two targets on tumour cell surfaces. Examples of bsADCs in clinical development include zanidatamab zovodotin (ZW49), the biparatopic HER2-binding antibody zanidatamab conjugated to an auristatin-derived cytotoxic payload[57], the biparatopic MET × MET bsADC REGN5093 (ref. 58) or izalontamab brengitecan (BL-B01D1), an EGFR × HER3 bsAb linked to a novel TOPO-I inhibitor (NCT05194982) (Table 2).

bsAbs for cancer immunotherapy

Most bsAbs used in cancer immunotherapy either function via T cell-driven natural or endogenous immunity, for example by boosting pre-existing antitumour responses and/or overcoming checkpoint inhibition, or by providing synthetic immunity through, for instance, bsAb-driven engagement, activation and recruitment of immune cells (Fig. 3). In this section we provide an overview of the most prominent MoAs of bsAb and multi-specific antibodies comprising CPIs, effector cell engagers – including TCEs and ICEs – and co-stimulatory bsAbs.

Dual CPIs

Numerous monospecific antibodies that interfere with immune checkpoints, such as CTLA4 or PD1/PDL1, are approved for cancer immunotherapy, including ipilimumab, tremelimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab or cemiplimab[59]. Expanding checkpoint inhibitory antibody therapies to include bsAbs may reduce undesired side effects and enhance the efficacy observed with the monospecific antibody therapeutics. The first aspect, safety of checkpoint inhibition, includes prevention of autoimmune-related events and/or undesired activation of immune cells. With this goal, bsAbs that selectively target the checkpoint inhibiting functionality and TAAs such as RTKs, and hence aim to focus its activity preferentially on tumours, are in development. The second aspect, efficacy of checkpoint immunotherapy, is addressed by bsAbs that simultaneously target two different checkpoint proteins to increase efficacy and reduce the probability of resistance, or provide complementary immune-stimulating signals. It should be noted that a common design principle of these bsAbs is to improve the selectivity for T cells that express two checkpoint proteins, such as CTLA4/PD1 or PD1/lymphocyte-associated gene 3 (LAG3) that are likely to be more tumour-reactive, with the goal to provide a superior therapeutic index – by, for example, lowering the incidence of autoimmune adverse events – than with combined checkpoint blockade[60]. Exploratory work for tumour-targeted checkpoint inhibition includes development of bsAbs that target HER-family RTKs on the surface of tumour cells, combined with blockade of PD1/PDL1 signalling as a second functionality, thereby directing a PDL1 binder to tumour cells. Specific tumour targeting of those molecules resulted in more specific and increased activity towards EGFR-expressing tumour cells[61]. In a similar manner, a bsAb that specifically bound both HER2 and PDL1 was shown to specifically block PDL1 activity in HER2-expressing tumour cells, resulting in greater therapeutic activity than each entity by itself or when applied in combination but not tethered as a bsAb[62,63].

Table 2 | Overview of selected bispecific antibodies in late-stage[a] clinical development

bsAb	INN	Targets	Format	Indications	Company
T cell engagement (T cell-mediated cytotoxicity)					
REGN1979	Odronextamab	CD20×CD3e	Veloci-bi (1+1)	NHL	Regeneron
REGN5458	Linvoseltamab	BCMA×CD3e	Veloci-bi (1+1)	Myeloma	Regeneron
AMG 757	Tarlatamab	DLL3×CD3e	Fc-BiTE	Small cell lung cancer	Amgen
LP-000	Catumaxomab	EpCAM×CD3e	Quadroma (1+1)	Peritoneal carcinomatosis	LintonPharm
Dual checkpoint inhibition					
KN046	Erfonrilimab	PDL1×CTLA4	Nb-Nb-Fc (2+2)	NSCLC, PDC	Alphamab
XmAb20717	Vudalimab	PDL1×CTLA4	IgG-scFv (1+1)	Solid tumours	Xencor
MEDI5752	Volrustomig	PD1×CTLA4	DuetMab (1+1)	NSCLC, renal cell carcinoma	Astra Zeneca
AZD2936	Rilvegostomig	PD1×TIGIT	DuetMab (1+1)	NSCLC	Astra Zeneca
MGD013	Tebotelimab	PD1×LAG3	DART-Fc (2+2)	Solid and haematological tumours	Macrogenics
SHR-1701	Retlirafusp alfa	PDL1×anti-PDL1×TGFβR2	IgG-fusion protein	Solid tumours	Suzhou Suncadia Biopharmaceuticals
Checkpoint and pathway inhibition					
PM8002	–	PDL1×VEGF	IgG-VHH (2+2)	NSCLC	Biotheus
AK112	Ivonescimab	PD1×VEGF	IgG1-scFv$_2$ (2+2)	NSCLC	Akeso
Dual pathway inhibition					
SI-B001	Izalontamab	EGFR×HER3	IgG1-scFv$_2$ (2+2)	NSCLC	Baili
CTX-009	—	VEGF×DLL4	IgG1-scFv$_2$ (2+2)	BTC	Compass
MCLA-128	Zenocutuzumab	HER2×HER3	Biclonics	Breast cancer	Merus
Biparatopic pathway inhibition					
ZW25	Zanidatamab	HER2(D2)×HER2(D4)	Fab/scFv-Fc(het) (1+1)	Gastric cancer, breast cancer	Zymeworks
KN026	Anbenitamab	HER2×HER2	IgG (cL,Fc$_{het}$) (1+1)	Stomach cancer	Alphamab

BiTE, bispecific T cell engager; BCMA, B cell maturation antigen; bsAb, bispecific antibody; BTC, biliary tract cancer; cL, common light chain; DART, dual-affinity re-targeting; DLL4, delta-like canonical Notch ligand 4; EGFR, epidermal growth factor receptor; EpCAM, epithelial cellular adhesion molecule; Fc$_{het}$, heterodimerizing Fc; LAG3, lymphocyte-associated gene 3; Nb, nanobody; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; PDC, pancreatic ductal adenocarcinoma; scFv, single-chain variable fragment; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor. [a]Late stage includes pivotal phase II, phase II/III and phase III clinical studies.

Another bsAb-mediated targeted immunotherapy approach included the combination of PDL1 blockade with a binder that recognized chondroitin sulfate proteoglycan 4 (CSPG4) on melanoma. Specific tumour targeting enhanced the specificity, activation status and efficacy of antitumour T cells[64]. A conceptually related approach includes a PDL1 binder chemically conjugated with melanocyte stimulating hormone. Thereby, PDL1 inhibiting activity became directed to the melanocortin-1 receptor on melanoma cells[65].

Examples of targeting two checkpoint proteins simultaneously without additionally including common tumour cell surface targets include bsAbs that simultaneously bind PD1 and PDL1 on tumour cells. Wan et al. analysed the combined activity elicited by such bsAbs in high-grade serous ovarian cancer organoids in co-culture with immune cells. The bsAbs mediated superior efficacy and induced phenotypic changes in both T cells and natural killer cells, including alteration of the activation state and cytotoxicity, when compared with monospecific binders[66]. The bsAb LY3434172 also uses this approach[67]. By fully inhibiting the PD1/PDL1 pathway, LY3434172 showed increased antitumour activity compared with each parental antibody or with combinations of both, again demonstrating that tethering in the form of bsAbs matters and can increase efficacy.

Expanding beyond the boundaries of the PD1/PDL1 approach, bsAbs that include additional immune receptors such as CTLA4 are in development or approved. Simultaneous targeting of PD1 and CTLA4 is the MoA of the bsAb cadonilimab. Cadonilimab mediated increased avidity-driven target binding to high-density PD1 and CTLA4 due to tetravalency, which, in conjunction with Fc mutations to abolish FcγR engagement, likely is the basis for its favourable therapeutic index[68–70]. Notably, cadonilimab also mediated *trans*-binding between $PD1^+$ and $CTLA4^+$ T cells[68]. In a phase I/Ib study in patients with advanced-stage solid tumours, cadonilimab showed antitumour activity and a favourable safety profile with a low incidence of immune-related adverse events[71]. Cadonilimab is approved in China to treat relapsed or metastatic cervical cancer that progressed on or after platinum-based chemotherapy[72]. Other bsAbs in advanced clinical trials that combine the same specificities are MGD019 and MEDI5752. MGD019 combines PD1 and CTLA4 binders, and acceptable safety and evidence of combinatorial blockade and objective responses in multiple solid tumours have been observed in an ongoing clinical study[73]. MEDI5752, which is currently also undergoing clinical evaluation, binds PD1 and CTLA4 (ref. 74) and showed PD1-mediated targeting, as it preferentially saturated CTLA4 on $PD1^+$ T cells, and caused rapid internalization and degradation of PD1 (ref. 75). In contrast to cadonilimab, MGD019 and MEDI5752 seem to show stronger avidity-driven selectivity gain for double-positive T cells.

Dual inactivation is also being explored by combining PD1/PDL1 blockade with LAG3 or TIM3 inhibition. Examples are IBI323 and FS118, both of which bind PDL1 and LAG3 and showed combinational enhanced dual efficacy in preclinical models[76–78]. LY3415244 combined blockade of PDL1 and TIM3, as a different immune modulator[79], but development of the molecule was terminated due to immunogenicity observed in a phase I study. Most recently, clinical phase I dose escalation and expansion data for the PD1 × LAG3 bsAb tebotelimab showed promising activity in patients with solid tumours or haematological malignancies as a monotherapy and in combination with the HER2 antibody margetuximab[80]. Dual ligand inhibition is another bsAb-based approach for checkpoint inhibition. PDL1 and transforming growth factor-β (TGFβ) are receptor-binding ligands that have complementary and non-redundant immunosuppressive activity[81]. TGFβ is not only immunosuppressive but can further induce upregulation of pro-angiogenic factors and activation of cancer-associated fibroblasts. Several bsAbs targeting PDL1 and TGFβ are currently in development, such as the bsAb-like antibody fusion protein bintrafusp alfa (M7824). In this case, whereas PDL1 binding is achieved by a 'standard' antibody binding module, TGFβ is trapped by an extracellular domain of human TGFβ receptor II[82]. Bintrafusp alfa showed activation of both the innate and the adaptive immune systems in preclinical studies, resulting in better inhibition of tumour growth and metastasis than treatment with the individual anti-PDL1 antibody or TGFβ trap modules alone[82,83], and is currently in clinical trials. Retlirafusp alfa (SHR-1701), another bifunctional fusion molecule that targets PDL1 and TGFβ in a similar fashion[84], is undergoing evaluation in phase III studies in patients with cervical cancer and gastric or gastroesophageal junction cancer (Table 2). YM101 is a TGFβ × PDL1 bsAb in preclinical development[85]. A surrogate of YM101, Y101D, is being evaluated in patients with advanced-stage solid tumours (NCT05028556)[86]. All approaches that counteract the inhibitory TGFβ and PD1/PDL1 pathways simultaneously showed evidence of higher antitumour activity in in vivo models compared with individual binders alone but have failed so far to deliver clinical proof of concept. Targeted TGFβ signalling blockade has also been implemented and assessed in preclinical studies with bsAbs that block TGFβ signalling in $CD4^+$ T cells. For this purpose, the non-immunosuppressive CD4-targeted antibody ibalizumab was combined with a TGFβ trap in a bsAb-like manner similar to that described above. This resulted in targeted TGFβ signalling blockade in helper T cells which can release an antitumour response[87].

Inhibition of PD1 via recruitment of phosphatase by bsAbs is a novel MoA that has been recently reported[88]. This is achieved by a single domain (VHH) fused to a scFv that links PD1 to the promiscuous cell surface phosphatase CD45, which in turn triggers intracellular dephosphorylation. Such an approach enhanced the inhibition of checkpoint blockade compared with PD1 antibody-mediated ligand blockade alone[88]. Following the same principle, bsAbs can link the cell surface phosphatase CD45 with the inhibitory natural killer cell surface receptors NKG2A or Ly49 for subsequent dephosphorylation[89]. Such bsAbs can block and simultaneously dephosphorylate their targets, and may be more potent antagonists than the respective monospecific antibodies and their combination of inhibitory signals to enhance natural killer cell and T cell antitumoural activities. Biparatopic targeting of different epitopes on CD73 with bsAbs is another bsAb-related approach to counteract immunosuppressive activities or enhance immune competence directed at tumours. CD73 generates immunosuppressive adenosine, and its bsAb-mediated inhibition and depletion by internalization is more effective than with mono-paratopic CD73 antibodies[90]. Finally, it must be mentioned that bifunctional antibody derivatives that are not bsAbs in a strict sense play a growing role in cancer immunotherapy. Such bifunctional molecules include T cell *cis*-targeted cytokines that elicit one functionality via an antibody-derived binding entity, preferentially PD1 targeting, and the other functionality by a fused cytokine derivative. Examples of such molecules that enhance antitumoural immune responses are PD1-targeted IL-15 (such as ASKG915, IAP0971), IL-21 (such as latikafusp) or IL-2 derivatives (such as PD1-IL2v/eciskafusp alfa, IBI363)[91–98].

Effector cell engagers

T cell engagers. During the past two decades, TCEs that specifically bind to a tumour surface antigen and the CD3ε chain of the TCR have dominated this class of bsAbs, with several hundred TCEs described

and more than 100 reaching clinical development[99,100]. In fact, the first bsAb approved for marketing was the mouse/rat chimeric epithelial cellular adhesion molecule (EpCAM) × CD3ε bsAb catumaxomab[101], which was approved in the European Union (EU) in 2009 for intraperitoneal treatment of malignant ovarian ascites, but subsequently withdrawn from marketing for commercial reasons. It is currently being assessed again in clinical trials in China for intraperitoneal administration. Applied systemically, catumaxomab showed severe infusion reactions and a high incidence of immunogenicity, likely due to its mouse/rat chimeric constant regions and retained FcγR binding, which resulted in unspecific FcγR and TCR activation without antigen binding. Subsequently, next-generation TCEs were designed to lack an Fc portion, as exemplified by the tandem scFv-based CD19 × CD3ε bispecific T cell engager (BiTE) blinatumomab[102], which was first approved in 2014 for the treatment of B cell precursor acute lymphocytic leukaemia (B-ALL). However, the therapy requires continuous infusion owing to a lack of recycling through binding to the FcRn, which rescues IgG from intracellular lysosomal degradation by recycling it from the sorting endosome to the cell surface. Based on the success of blinatumomab, BiTEs targeting various targets in haematological and solid tumours were developed, but ultimately did not proceed to late-stage clinical trials[103] owing to the suboptimal pharmacokinetic properties of BiTEs and related short half-life. Attempts were made to achieve an IgG-like half-life of bsAbs by either incorporating FcγR-binding inert Fc domains (silenced Fc) or HSA-binding moieties, and currently most TCEs in development are IgG-based and/or have IgG-like pharmacokinetics.

Of note, the majority of TCEs use one single monovalent binding site for CD3 as the trigger molecule on T cells to avoid systemic activation of T cells as a consequence of TCR cross-linking in the absence of simultaneous target binding. They then use either one (1 + 1) or two (2 + 1) binding sites for the tumour target, as bivalent binding can provide advantages in terms of tumour selectivity and potency[104,105]. The CD20 × CD3ε bsAb mosunetuzumab[106] and the B cell maturation antigen (BCMA) × CD3ε bsAb teclistamab[107] were the first heterodimeric 1 + 1 IgG-based TCEs approved in 2022 for the treatment of relapsed/refractory follicular non-Hodgkin lymphoma (NHL) or multiple myeloma, respectively. In 2023, the heterodimeric 2 + 1 CD20-TCE glofitamab[104,108] and the 1 + 1 CD20-TCE epcoritamab[109] were approved for treatment of relapsed/refractory diffuse large B cell lymphoma (DLBCL), as well as the 1 + 1 G-protein-coupled receptor class C group 5 member D (GPRC5D)-TCE talquetamab[110] and BCMA-TCE elranatamab[111] for relapsed/refractory multiple myeloma (Table 1). Marketing applications for the CD20-TCE odronextamab[112] and the BCMA-TCE linvoseltamab are awaiting regulatory decisions. Given their promising clinical efficacy and complete response rates in patients with relapsed/refractory B cell and plasma malignancies — including activity in patients who relapsed after CAR-T cell therapies[107,108,113,114] — as well as their straightforward availability as off-the-shelf therapies and the possibility for subcutaneous administration[115], it can be expected that TCEs will become a major alternative to CAR-T cell therapies, particularly as they start being developed for earlier lines of treatments and are readily available to patients[116].

Immune mobilizing monoclonal TCRs against cancer (ImmTACs) are TCEs that combine engineered TCRs that recognize human leukocyte antigen (HLA)-presented peptide antigens with anti-CD3 scFv, enabling targeting of intracellular TAAs. Notably, the short-lived ImmTAC tebentafusp, which comprises a soluble TCR targeting an epitope of melanocyte lineage-specific antigen glycoprotein (gp100) presented by HLA-A*02:01 fused to a CD3-targeting scFv[117], was approved in early 2022 for the treatment of uveal melanoma[118], opening a novel intracellular target space for TCEs in solid tumours. Notably, tebentafusp mediates an overall survival benefit although it induced only a low number of clinical responses so that direct tumour cell killing may not be its only MoA. In the meantime, various TCEs, either based on recombinant TCRs or using antibodies that are TCR-like (TCR mimetics), entered clinical development[119,120], including TCEs specific for WT1 (ref. 121) and mutated neoantigens such as KRAS[122] or p53 (ref. 123). Importantly, high-throughput kinetic screening[124] and generic TCR-like libraries can enable easier development of highly selective TCRs and TCR mimetics for use in TCEs[125]. Interestingly, using the covalent KRAS^{G12C} inhibitor sotorasib, a TCR-like TCE could be generated that specifically recognized the HLA-presented sotorasib–peptide conjugate and subsequently induced T cell killing of KRAS^{G12C}-mutant cells[126,127].

The occurrence of cytokine release syndrome, largely due to on-target T cell activation, has been a major challenge for the clinical development of TCEs[128,129]. Although in many cases this can be managed by pre-treatment with steroids and step-up dosing, more recent efforts have investigated the use of CD3ε antibodies with reduced CD3ε affinity with the goal to uncouple T cell killing from cytokine secretion[130–136].

As is the case for CAR-T cells, due to the very high killing potency of TCEs a highly tumour-specific and/or lineage-specific expression of the target antigen is required in order to avoid on-target off-tumour toxicity[137,138]. This is the case in haematological cancers for the B cell and plasma cell antigens, such as CD19, CD20, CD79b (ref. 139), BCMA[140,141] and FcRH5 (ref. 142), that are generally believed to be found only on B cell and plasma cell malignancies and the non-essential B cell and plasma cell lineage. However, even for antigens such as CD19, expression may not be absolutely absent from normal tissues and there are reports suggesting CD19 expression on mural brain cells/pericytes, which surround the endothelium and are critical for blood–brain barrier integrity, as an on-target, off-tumour mechanism relating to neurotoxicity[143]. By contrast, in the case of acute myeloid leukaemia, typical antigens such as CD33 (refs. 144,145) or CD123 (refs. 146–148) are also found on the myeloid haematopoietic lineage, making their clinical development more challenging.

Similarly, many of the major (epithelial) solid tumour antigens such as EGFR, HER2, EpCAM, tumour-associated calcium signal transducer 2 (also known as cell surface glycoprotein Trop-2), MUC1, CEACAM5 and glypican 3 can also be found in (essential) normal tissues. As a consequence, and likely owing to the more immune-suppressive TME, the development of TCEs (and CAR-T cells) in solid tumours experienced less progress during the past years[149]. Importantly, it has to be recognized that for TCEs, as compared with ADCs[150], the requirements for tumour selectivity are stricter owing to the higher potency, and solid tumour ADC targets such as HER2 and TROP-2 may not be targetable with conventional TCEs. At the same time, ADCs as opposed to TCEs may benefit from stronger bystander killing of antigen-negative tumour cells due to the release of the toxic payload in the TME. As a consequence, few tumour-selective antigens seem to be feasible targets for TCEs, including neoantigens such as p95-HER2 (ref. 151) and EGFRvIII[152–154], or targets such as MUC16 (ref. 155), claudin 18.2 (ref. 156), DLL3 (ref. 157) and STEAP1 (ref. 158). For the DLL3-targeting TCE tarlatamab, currently under priority review by the US Food and Drug Administration (FDA), promising phase I clinical data have been reported in small cell lung cancer with an overall response rate of 23%[157]. A subsequent phase II study confirmed activity with durable objective responses and promising survival outcomes in patients treated with a 10 mg dose every 2 weeks[159]. Based on these data, a randomized pivotal

study that compares tarlatamab with the standard of care has been initiated (NCT05740566). Similarly, xaluritamig, a 2 + 1 STEAP1 TCE with avidity-driven activity enabling selectivity for tumour cells with high expression of STEAP1 compared with normal cells, demonstrated promising clinical activity in a phase I dose escalation study in patients with metastatic castration-resistant prostate cancer in a late-line treatment setting[160,161]. Taken together, these proof-of-concept data show that the common assumption that TCEs are only active in haematological cancers, and especially B cell malignancies, does not apply, and that TCEs also have potential for the treatment of certain solid tumours as long as on-target, off-tumour activity can be managed.

Diverse approaches are being used to overcome the solid tumour selectivity challenge. For example, tumour antigen binders with reduced affinity in bsAbs bivalent for the tumour antigen (2 + 1 formats) enable an avidity-mediated selectivity gain (also known as affinity tuning) with the goal to use expression differences between tumour and normal tissues as described for CEACAM5, HER2 and FOLR1 for improved tumour selectivity[105,162,163]. Alternatively, dual targeting and avidity-mediated selectivity gain are applied in trispecific TCEs that selectively kill tumour cells that co-express these targets, but do not affect normal cells expressing only one of these targets[164–167]. Furthermore, trispecific TCEs targeting two different tumour antigens such as CD19 and CD22 can prevent immune escape by antigen loss[168].

Although most conventional TCEs recruit both conventional T cells and unconventional innate-like γδ T cells (T cells that express TCRγ/δ rather than TCRα/β), a specific class of γδ TCEs has been designed to specifically recruit γδ T cells via TCRγδ with the goal to improve safety and efficacy by addressing only a T cell subset that can be further expanded in patients[169,170]. In preclinical studies, γδ TCEs showed potent antitumour activity as single agents[171–175]. Alternatively, bispecific tumour-targeted heterodimeric BTN2A1 and BTN3A1 antibody fusion proteins were made for this purpose[171]. As γδ T cell biology is not fully reflected in mouse models, the ultimate proof of concept for this approach will have to rely on clinical data from ongoing clinical trials, such as with the PSMA-targeted γδ TCE LAVA-1207 (NCT05369000).

Instead of CD3ε or TCRα/β-targeting bsAbs, peptide–MHC complexes can be applied to specifically recruit T cells specific for certain pMHC complexes, such as cytomegalovirus-derived peptides which are present due to naturally occurring and prevalent cytomegalovirus infection[176]. In an alternative approach, viral epitopes can be delivered to tumour cells in order to load the respective MHC molecules and recruit virus-specific T cells to attack these cells[177–179].

bsAbs have also been described for the more potent engagement of bsAb-mediated clustering and complement-dependent cytotoxicity[180]. Interestingly, by applying two separate antibody pairs, a kind of logic gate can be established to selectively eliminate cells characterized by co-expression of two antigens through complement-dependent cytotoxicity[181]. For example, using this approach B cells co-expressing CD20 and CD52 could be eliminated, whereas cells expressing either of these antigens alone were not killed[181].

With the substantial interest in adoptive CAR-T cell therapies, a specific class of TCEs, acting as bispecific adaptors between CAR-T cells and tumour cells, has been engineered to recruit specifically adoptively transferred T cells for the treatment of various cancers, while not engaging natural T cells[182–187]. One example of such bsAb-based adaptor CAR-T cell systems is the synthetic agonistic receptor (SAR)-T cell system, in which a bsAb recognizes a tumour antigen and a synthetic antigen receptor introduced into an adoptively transferred T cell[185]. Such approaches in the context of synthetic biology offer multiple ways to further engineer and control adoptive T cell therapy approaches beyond what conventional antibody therapies or classical CAR-T cell therapies can offer. Another alternative approach combining cell therapy and bsAbs is the ex vivo arming of T cells with multiple TCEs with the goal to overcome tumour heterogeneity. For this purpose, T cells were incubated ex vivo with TCEs targeting tumour antigens such as GD2, HER2, PSMA and STEAP1 and administered to tumour-bearing animals, in which they prevented tumour progression due to antigen loss[188].

Innate cell engagers. Although TCEs have dominated the field of effector cell engagers, substantial efforts are ongoing to develop next-generation ICEs comprising NKCEs, and bsAbs engaging myeloid-derived cells (MDCs) for phagocytosis, including neutrophils and macrophages/monocytes[189]. ADCC-mediating monoclonal antibodies – such as rituximab, cetuximab or trastuzumab – which require binding of Fc to innate immune cells, can be considered first-generation ICEs because ADCC/antibody-dependent cellular phagocytosis is critical to their MoA, in addition to other actions such as signalling inhibition. Based on this experience, several Fc-engineered IgG1 antibodies with enhanced ADCC function, as compared with conventional monoclonal antibodies, have been approved in the past decade, including obinutuzumab, mogamulizumab, tafasitamab, margetuximab and amivantamab[190]. Based on this experience and with the goal to further enhance the ADCC function beyond that of Fc-engineered IgG1 antibodies, various bispecific NKCEs[191] that target different surface molecules on natural killer cells, such as CD16, NKG2D, NKp46 or NKp30, have been developed as immunotherapies[192]. The most advanced of these NKCEs is a short half-life CD30 × CD16 bispecific bsAb for the treatment of Hodgkin lymphoma that is either used as a monotherapy, in combination with checkpoint inhibition or in conjunction with natural killer cell infusions[193–195]. More recently, half-life-extended tetravalent IgG-based bsAb formats targeting antigens such as EGFR or BCMA, as well as trispecifics making use of dual targeting for tumour selectivity, have been developed[196–199]. Symmetrical trispecific NKCEs that specifically target EGFR–PDL1 co-expressing cells via ADCC through their CD16-binding region were generated using two-in-one antibodies[200]. A different approach for engagement of natural killer cells relies on a bispecific fusion protein comprising NKG2D ligands, such as UL-16 binding protein 2 (ULBP2), fused to a scFv targeting HER2 for effective ADCC-mediated tumour cell killing[201]. Alternatively, bsAbs with retained FcγRIII binding and targeting NKp46 or NKp30 and various tumour antigens can provide potent antitumour efficacy[202–204]. As opposed to TCEs, where FcγR binding is undesired, these bsAbs rely on retained or enhanced FcγRIII binding for potent natural killer cell engagement. Notably, when an IL-2 variant was fused to a NKp46 bsAb, resulting in tetrafunctional molecules, the efficacy was strongly enhanced owing to the concomitant induction of natural killer cell expansion[205].

Another field of active investigation are bsAbs engaging phagocytic or MDCs as well as neutrophils for killing by binding, for example, to the FcαRI receptor (CD89) on macrophages and neutrophils[206,207] or the IgE receptor via bispecific IgE antibodies[208]. Alternatively, tumour-targeted bsAbs can block the CD47–SIRP1α interaction that is the basis of the 'don't eat me signal' on tumour cells specifically, so that these cells are attacked and phagocytosed by MDCs[209]. Bispecific CD19 x CD47 or mesothelin x CD47 antibodies combine tumour targeting and CD47 inhibition with effector cell recruitment via an effector competent Fc portion[210–212]. In this context, bsAbs with low affinity for CD47 are advantageous to overcome undesired binding to

CD47 on erythrocytes. Various groups applied this concept to engage PDL1 × CD47 co-targeting[213,214]. Alternatively, SIRPα fusion proteins can be applied to block the CD47–SIRPα 'don't eat me signal'[215].

Co-stimulatory bsAbs

An efficient, long-lasting and locally restricted antitumour immune response requires co-stimulatory and co-inhibitory signals to tightly regulate activation, differentiation and maintenance of cytotoxic T cells, natural killer cells and macrophages. Members of the TNFRSF family (such as 4-1BB, CD40, OX40, TNFRSF18 (also known as glucocorticoid-induced TNFR-related protein (GITR)), CD27 or CD30) as well as the CD28 immunoglobulin superfamily (such as CD28, CTLA4, PD1, inducible T cell co-stimulatory (ICOS), and B lymphocyte and T lymphocyte attenuator (BTLA)) and their corresponding ligands have an eminent role in mediating immunoregulatory signals, provided locally through cell–cell contacts, such as between antigen-presenting cells (APCs) and T cells[216]. These receptors provide the so-called signal 2 to T cells that is required to sustain the signal 1 provided by the TCR either through a peptide–MHC–TCR interaction or via a TCE cross-linking the TCR. Targeting these immunomodulatory pathways may enhance immunotherapy[217]. Studies using agonistic monoclonal antibodies directed against co-stimulatory receptors have shown that these often lead only to weak activation, but this activation can be strongly increased upon FcγR-mediated receptor super-clustering. Furthermore, factors such as stoichiometry between antibodies and receptors, binding affinity and epitope specificity can influence activity of co-stimulatory antibodies[216]. Thus, reducing the affinity of immunomodulatory antibodies can be used as a strategy to boost agonistic activities through increased receptor clustering[218], a principle frequently applied to bsAb agonists as well (see above). However, immune-related systemic adverse effects due to elevated production of chemokines and cytokines are a limiting factor in drug development of co-stimulatory antibodies[219]. Antibody–cytokine fusion proteins and bsAbs capable of providing co-stimulatory signals either in a target-dependent manner, by dual targeting of two different co-stimulatory receptors or by targeting of a co-stimulatory receptor and a CPI have been proposed to circumvent this limitation[220].

Tumour-targeting co-stimulatory bsAbs. bsAbs that bind with one arm to a TAA and with the other arm to a co-stimulatory receptor on immune cells can locally mediate receptor clustering by acting in *trans* between the cancer cell and the immune cell to provide the signal 2. Co-stimulatory bsAbs currently in clinical trials include molecules directed against 4-1BB, CD40 and CD28 (ref. 48).

The induced expression of 4-1BB upon T cell activation makes it an attractive target for T cell co-stimulation to enhance CD8 T cell responses. Safety issues and poor efficacy in clinical trials using anti-4-1BB agonistic antibodies — such as urelumab and utomilumab — as monotherapy redirected efforts to alternative approaches focusing on bsAbs that can overcome these toxicities by introducing tumour-specific co-stimulation[47]. Approximately half of the cancer-targeted co-stimulatory bsAbs in clinical trials act through 4-1BB. Advanced bsAbs are PRS-343 (cinrebafusp), a tetravalent bsAb–anticalin fusion protein directed against HER2 and 4-1BB, which was found to be well tolerated with no overt and no relevant drug-related toxicities in IND-enabling studies[221]; FAP-4-1BBL (RG7872), which showed pharmacological activity as a monotherapy and in combination with PDL1 inhibition[222,223]; or englumafusp alfa, which is being studied as a signal 2 provider in combination with glofitamab for DLBCL[222]. Other targets in bsAbs for tumour-selective co-stimulation through 4-1BB currently in clinical trials include EGFR, PSMA, CLDN18.2, B7H4, CEACAM5, HER2, PDL1 (see below) and FAP[48].

Furthermore, bsAbs using CD28 as a co-stimulatory receptor constitutively expressed on naïve T cells are currently in clinical trials. Through ligation with either CD80 (also known as B7.1) or CD86 (also known as B7.2) on APCs, CD28 provides a necessary secondary signal for full T cell activation and survival[224]. This signal can also be provided by agonistic CD28 antibodies, even in the absence of a primary TCR engagement, as shown for the superagonist TGN1412, which dramatically failed in a phase I study due to massive cytokine release syndrome. It was later found that TGN1412 acts especially on regulatory T cells (T_{reg} cells) at low doses, whereas at high doses it activates both conventional T cells and T_{reg} cells, providing a window for selective targeting of T cell subsets, but also highlighting the delicate balance between T cell co-stimulation and hyperactivation[225]. Some bsAbs can provide this secondary co-stimulatory signal in a target-dependent manner to enhance antitumour T cell responses in a tumour-restrictive manner[226]. Notably, bsAbs can be designed to have limited activity and no toxicity when used alone, but potentiate T cell activation and antitumour activity of TCEs[227]. REGN5837 is a bsAb targeting CD22 and CD28 which has entered a phase I trial in combination with odronextamab, a TCE directed against CD20, for the treatment of NHL[228]. Tumour-targeting CD28 bsAbs can also synergize with PD1 to provide long-term antitumour immune response[229] and, currently, multiple bsAbs targeting TAAs such as EGFR, MUC16, PSMA, B7-H3, CD19, CD22 or PDL1 are in clinical trials in combination with checkpoint inhibition or as a single agent, respectively.

MDCs and dendritic cell-activating bsAbs. CD40 is expressed in a diverse set of cell types, including APCs, other MDCs and B cells, but is also expressed on a wide range of malignant cells[230]. Monoclonal agonistic CD40 antibodies can trigger antitumour effects by inducing maturation of APCs and activation of antigen-specific T cells and other immune cells, thus promoting a tumour-specific immune response, especially by priming cytotoxic T cells[231].

Besides agonistic CD40 antibodies, many of which have dose-limiting toxicities, bsAbs targeting CD40 and various TAAs such as CEACAM5, EpCAM or mesothelin have been developed[230]. As for other members of the TNF superfamily, receptor clustering plays a critical part in receptor activation. Combining a CD40 agonistic antibody with one against a tumour-targeting antigen in a bsAb allows the co-stimulatory activity to be directed to the TME, thereby increasing safety and efficacy[230]. Proof of concept was, for example, shown for tetravalent, bispecific bsAbs targeting CD40 and either CEACAM5 or EpCAM, which showed enhanced T cell cross-priming compared with monospecific CD40 antibodies[232]. A bsAb (ABBV-428) targeting mesothelin and CD40 demonstrated enhanced APC and T cell activation upon binding to mesothelin in vitro and in murine models, and showed an acceptable safety profile in a phase I trial[233,234]. To induce selective CD40 activation within the tumour but not in the periphery, FAP × CD40, a bsAb comprising two binding sites for CD40 and one binding site for FAP, was generated[235]. The antibody provides a strong FAP-dependent CD40-stimulating signal, demonstrated a good safety profile as well as tumour accumulation and intratumour immune activation, and is currently in a phase I trial. MP-0317 — a multi-specific designed ankyrin repeat protein (DARPin) that applies the same concept targeting FAP, CD40 and HSA for half-life extension — is in a phase I

clinical study[236]. Alternatively, bsAbs can be specifically directly targeted to dendritic cells via the marker Clec12a (ref. 237).

Dual co-stimulation with bsAbs. Dual targeting of two different co-stimulatory receptors on immune cells has been used to induce potent antitumour responses. FS120, a tetravalent bsAb directed against 4-1BB and OX40 expressed on activated T cells, activated CD4 and CD8 T cells in an FcγR-independent manner. This activity required co-engagement of both receptors for efficient immune cell activation and antitumour activity, supporting the concept of dual targeting of co-stimulatory receptors with tetravalent bsAbs to induce efficient receptor clustering and activation[238]. In animal studies, a surrogate for FS120 did not induce liver inflammation and hepatotoxicity, a limitation that had been observed for agonistic 4-1BB antibodies[238]. FS120 is currently in a phase I clinical trial. In a similar approach, a bivalent bsAb was developed for dual targeting of 4-1BB and CD40 (BNT-312, tecaginlimab), therefore targeting T cells and APCs[239]. Activation of APCs by CD40 leads to upregulation of co-stimulatory molecules and secretion of immuno-stimulatory cytokines, and thus to a robust CD8 T cell-mediated antitumour immune response independent of help by CD4 T cells.

bsAbs for dual checkpoint inhibition and co-stimulation. Antibodies against co-stimulatory receptors have furthermore been combined with CPIs to release T cells from inhibitory signals while simultaneously boosting T cell responses, especially in tumours that are resistant or refractory to checkpoint inhibition[240]. Most of the bsAbs currently in clinical trials use a combination of these signals in *trans* by targeting PDL1 on tumour cells and a co-stimulatory receptor on immune cells, with 4-1BB being the most prominent target. More than ten different bsAbs targeting PDL1 and 4-1BB are in phase I or phase II trials. The candidates in phase II include an IgG-anticalin fusion (PRS-344) where a ligand-binding anticalin scaffold represents the second specificity, a bivalent bispecific IgG antibody (acasunlimab, also known as GEN1046) and a trivalent, trispecific single-chain diabody-scFv fusion protein further comprising an HSA-binding site for half-life extension (NM21-1480)[240–242]. These types of bsAbs can potentiate the immune response beyond those of individual antibodies and their combination, further providing targeted co-stimulation against the PDL1-expressing tumour cells, and MDCs of the TME and tumour-draining lymph nodes[240]. In line with this, subsets of 4-1BB-positive tumour-infiltrating CD8 T cells within the TME express high levels of PD1, and are thus susceptible to treatment with PDL1 × 4-1BB bsAbs[241]. Notably, as 4-1BB signalling requires super-clustering, binding to PDL1 can lead to conditionally clustered and activated 4-1BB in the TME, reducing liver toxicities, as shown for a tetravalent, bispecific IgG-scFv (ABL503)[243]. Further bsAbs following this concept include a tetravalent, bispecific monoclonal antibody (FS222)[244] and bivalent, bispecific IgGs such as MCLA-145 (ref. 245). Likewise, CD28 co-stimulation and checkpoint inhibition can further be combined within one bsAb, as shown for a bsAb targeting PDL1 and a CD28 homologue, providing tumour selectivity through PDL1 expressed on tumour cells[246].

Checkpoint inhibition in combination with co-stimulation can be further achieved by dual targeting of T cells acting in *cis* within the tumour. Feasibility was demonstrated for ATOR-1015, an anti-OX40 IgG fused to an optimized version of the V-type domain of CD86 targeting CTLA4. Similar to 4-1BB, CTLA4 and OX40 are upregulated on activated T cells and expressed by T_{reg} cells, especially in the TME[247]. In contrast to GITR and ICOS, CD27 is expressed by naive T cells, and thus helps promote new T cell responses. A PDL1 × CD27 bsAb was more effective than the combination of PD1 blockade and CD27 co-stimulation while exhibiting a good safety profile in preclinical studies[248].

In conceptually similar approaches, binding sites for PD1 were combined with other co-stimulatory members of the TNF superfamily, including 4-1BB (such as with IBI319 (ref. 249)), GITR and ICOS (XmAb23104). GITR is also upregulated on activated T cells and constitutively expressed by T_{reg} cells, in which it plays a critical part in enhancing a nascent immune response. Proof of concept was demonstrated for PD1 × GITR-L bispecific fusion protein[250]. For some of these agents, the relative contribution of checkpoint inhibition as compared with the combined or respectively synergistic effect is not necessarily established.

Emerging concepts

The concepts supporting bsAb development have evolved quite rapidly from early exploratory approaches to clinical development, and bsAbs are now well established as a therapeutic principle. However, the field of bsAbs is still progressing to further explore and expand technologies and applications. Various early exploratory bsAb approaches pursued by academia and the biotech and pharmaceutical industries are promising, and a subset of these may provide therapeutic concepts that are not yet addressed by bsAbs currently in development. The scope of this Review does not allow us to cover all or many of such early approaches and comprehensive coverage may, in fact, be impossible in this fast-growing field. We therefore conclude our review by mentioning some emerging topics that we consider relevant and having the potential to provide promising candidates to further push the boundaries of bsAb-based cancer therapies.

Trispecific TCEs with integrated co-stimulation

Co-stimulatory signals can be combined with bispecific TCEs to foster efficient T cell activation. This has been achieved using a combination of bispecific TCEs and co-stimulatory bsAbs as summarized above[222,227,228,251], but also by including a third binding site against a co-stimulatory receptor into the bispecific TCE, thus generating a trispecific antibody[252,253]. Two such trispecific TCEs are currently in clinical trials targeting a TAA and CD3, and further comprising an anti-CD28 binding site[254,255]. SAR443216 is a trivalent, trispecific immunoglobulin-based antibody directed against HER2 × CD3 × CD28 comprising a mutated IgG4 lacking Fc-mediated effector functions. This trispecific antibody, which is currently in phase I, is capable of activating primary CD4 and CD8 T cells, inducing T cell proliferation, release of cytokines and granzyme B as well as T cell-mediated killing of HER2-expressing tumour cells, including cells with low HER2 expression. In a similar approach, a binding site for CD38, which is overexpressed in multiple myeloma, was combined with CD3 and CD28 binding sites in the trispecific antibody SAR442257, which is also currently in phase I[254].

Prodrug approaches

One emerging approach aims at generating bsAbs with highly tumour-specific functionalities, by providing 'safety-critical' specificities as prodrugs that become activated selectively at the tumour. Some concepts that are applied for inactivation of antibodies to generate prodrugs that become reactivated at tumours are shown in Fig. 4. Conversion of prodrugs to functional bsAbs can be achieved by environmental triggers such as tumour-associated proteases[256]. For example, bsAbs with a steric mask can be activated proteolytically[257]. Protease-activated TCEs, so-called probodies[258], require the expression

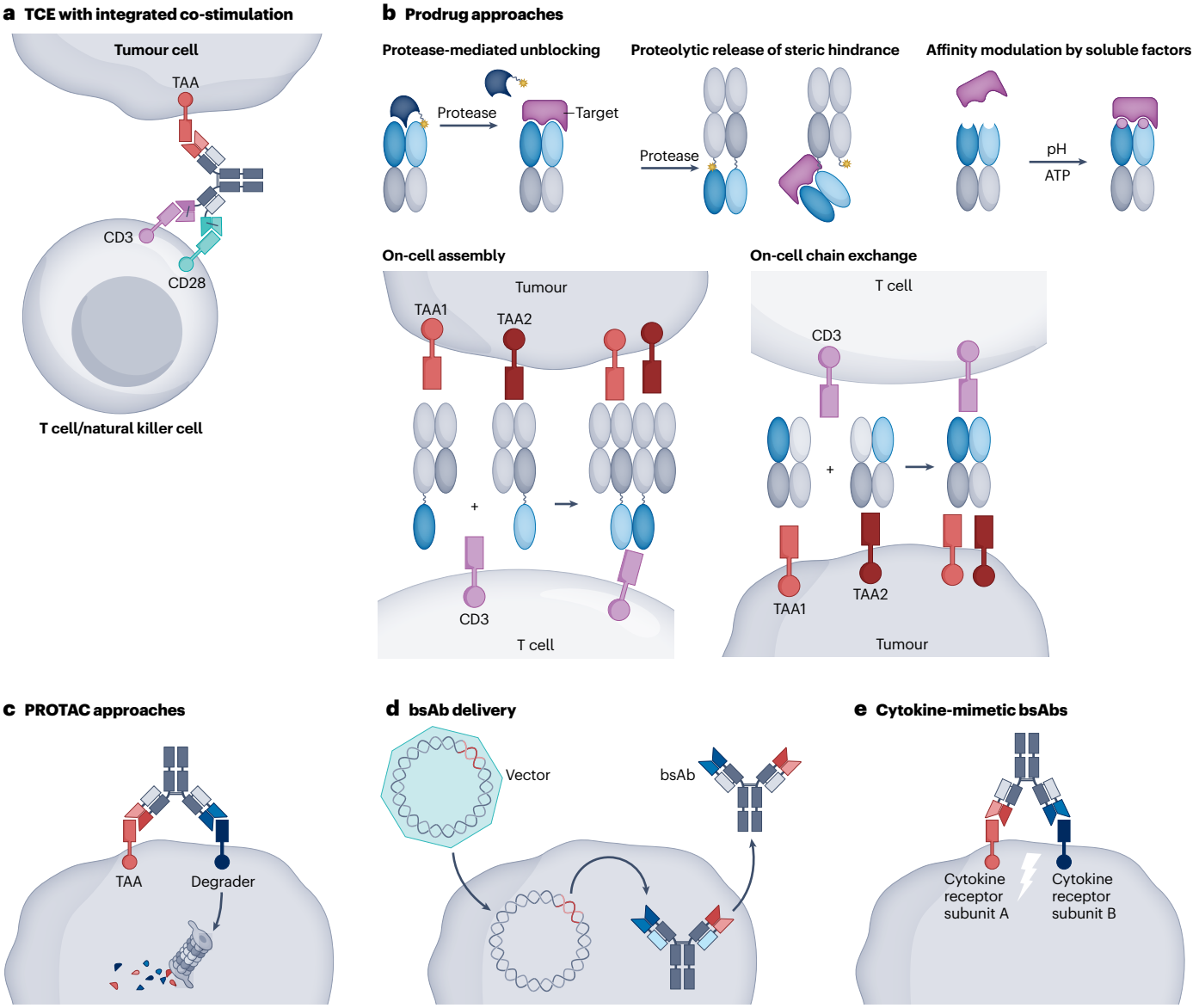

Fig. 4 | Emerging concepts in the field of bispecific antibodies. a, Trispecific T cell engagers (TCEs) with integrated co-stimulation for simultaneous T cell receptor (TCR) activation (signal 1) through CD3 binding and co-stimulation (signal 2) by binding to CD28. **b**, Mode of action of bispecific antibody (bsAb) prodrugs. General concepts for inactivating antibodies to generate prodrugs and reactivate those at the desired site of action include blockade of binding sites to inactivate and protease-induced activation of blocked antigen binding sites, affinity modulation in response to target environments such as acidic pH or ATP and assembly or exchange reactions due to local enrichment of complementary prodrugs via high local concentration. **c**, Proteolysis targeting chimera (PROTAC) approaches by binding to a surface antigen (tumour-associated antigen (TAA)) and a degrader moiety, such as a membrane E3 ligase, which results in internalization of a cell surface target and proteasomal degradation. **d**, bsAb delivery, for example via gene therapy, into CAR-T cells. **e**, Cytokine mimetic bsAbs triggering cytokine receptor pathways such as a IL-2Rβ–IL-2Rγ heterodimer by bringing the receptor domains into the steric vicinity, mimicking cytokine action.

of tumour-specific proteases, which are found in the TME, for their tumour-specific activation[259–263]. An alternative early-stage approach for dual tumour targeting relies on the tumour-specific assembly of a functional CD3ε antibody fragment from two bsAbs with split CD3ε binding moieties. Those entities need to be designed inactive as separate entities and in circulation so that they are only activated upon tumour antigen binding and subsequent in situ assembly, as has been described for hemibodies[264] or prodrug-activating chain exchange (PACE) molecules[265]. A major challenge for this class of molecules remains their production at scale as monomeric molecules and the prevention of premature assembly in solution in the absence of target binding, respectively. The targeted chain exchange-mediated reconstitution of a split type I cytokine is an example of how to achieve tumour-specific assembly[266]. Of note, recently an ATP-dependent switch introduced into the binding site of an anti-4-1BB antibody has been developed to increase tumour selectivity and to avoid on-target, off-tumour

toxicities. This switch uses the elevated ATP levels in tumours as a trigger, which might also be applicable to other binding entities[267].

PROTAC bsAbs

Another emerging concept and novel MoA only recently described uses bsAbs to inactivate proliferation-associated processes by targeted degradation. Similar to the emerging field of bispecific or multi-specific small-molecule proteolysis targeting chimeras (PROTACs), bsAbs can be designed to trigger the internalization and subsequent degradation of surface proteins involved in cancer development and/or progression[268]. Such bsAbs simultaneously bind to proteins targeted to be degraded, and to factors that trigger internalization and subsequent degradation of the bound target proteins, such as membrane-accessible E3 ubiquitin ligases or transferrin receptor[269–274]. Antibody–PROTAC concepts are still early in development and to date none has entered clinical studies to our knowledge, but the development of such entities is fully compatible with that of 'standard' bsAbs and offers unique possibilities in targeting novel pathways and bringing this concept to the clinic in the very near future.

bsAb delivery

Another important emerging concept in bsAb development is the local production and subsequent local delivery of bsAbs, such as a CLDN6 TCE through lipid nanoparticle-mediated mRNA delivery[275,276], oncolytic viruses[277–280] or gene therapy-like approaches including CAR-T cells[281–283].

Cytokine mimetic antibodies

Finally, bsAbs can also serve as cytokine mimetics or so-called synthetic cytokine agonists. In this context, scFvs or single-domain VHH-based approaches have been described to efficiently trigger cytokine signalling by mimicking IL-2 or IL-15, IL-18, type I interferon and IL-10 (refs. 284–286). Alternatively, tumour-specific cytokine receptor agonistic activity can be achieved with a split approach using the de novo IL-2 mimetic protein neoleukin[287,288]. Such antibody-based cytokine mimetics may have various applications in cancer immunotherapy.

Outlook

The field of bsAbs has made substantial progress recently, and by end-2023 more than a dozen bsAbs have been approved and many different approaches are being tested in the clinic. Nevertheless, we believe that, owing to the heterogeneous and adaptive nature of cancer, it is unlikely that any one of these approaches will be a universal cancer immunotherapy. Rather, antibodies will need to be tailor-made for certain applications and will rely on combination with other approaches to maximize their efficacy and safety window. Growth in the development of antibody therapeutics with multiple specificities is likely to occur in the following four areas in the near future: bsAbs targeting tumour-associated RTKs for tumours with defined dependencies; bsADCs for targeting tumours with increased selectivity; bispecific PROTACs for the targeted degradation of cell surface proteins applicable to various pathways; and multi-specific antibodies for cancer immunotherapy. In this latter field, particularly for very potent T cell-based therapies such as TCEs or CAR-T cells, the identification of highly tumour-selective antigens will be essential, as will be the means to specifically enable tumour-specific activity while sparing normal tissues, such as through activation by tumour-specific proteases and better regulation of effector functions. Another important aspect will be to provide dual checkpoint inhibition and/or complementary co-stimulatory signals to T cells to maintain and sustain the T cell response for prolonged periods of times, as has been demonstrated to be essential for the clinical success of CAR-T cell therapies. Importantly, such approaches will not only be applicable to the combination with TCEs but also for combination with endogenous immunity approaches. Another important aspect will be to foster the generation of a secondary immune response, such as by activation of the innate immune system, including natural killer cells, MDCs and dendritic cells. As with other drugs for cancer, applying bsAbs and multi-specific antibody-based therapies to patients early in their disease, enabled by improved diagnostics approaches and novel development paradigms will be essential to reach the goal of curing patients with cancer.

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Author contributions

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