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Review

Bispecific T-cell engagers for the recruitment of T cells in solid tumors: a literature review

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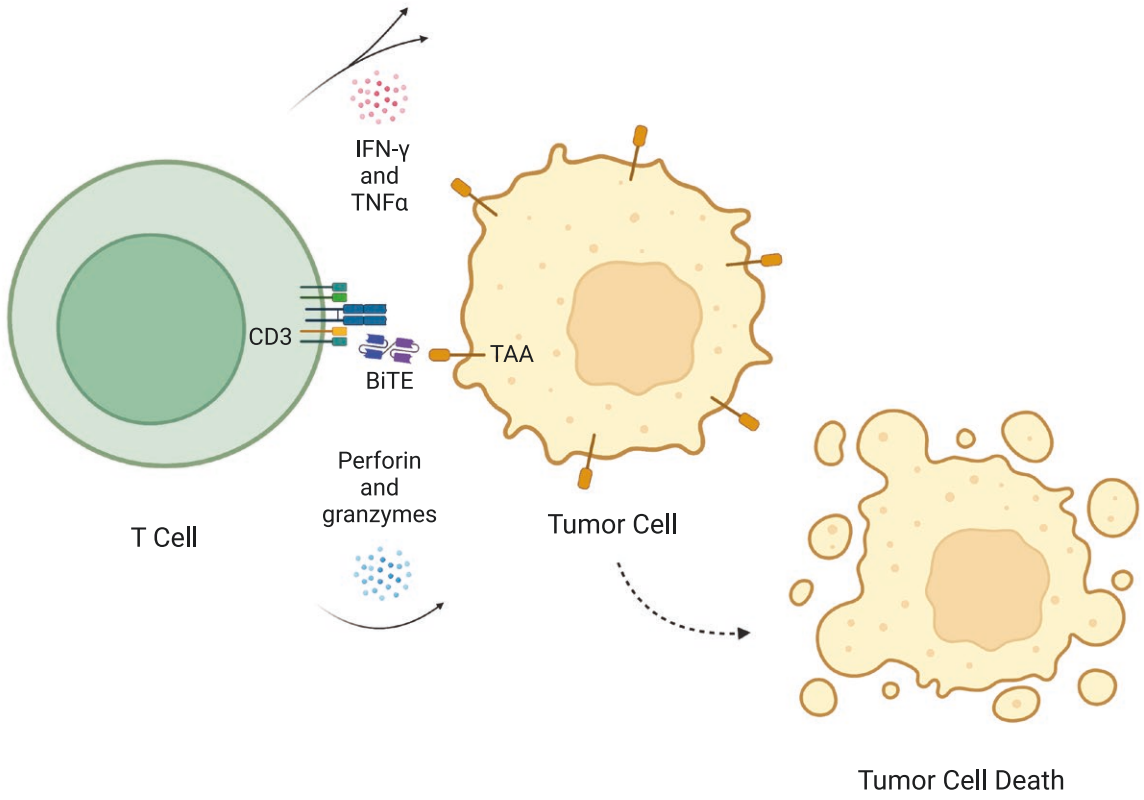
Abstract

In the past decade, T-cell-based immunotherapies have grown to become some of the most promising treatments for cancer. Following the success of immune checkpoint inhibitors, other T-cell-based therapies emerged including CAR-T cells and bispecific T-cell engagers (BiTEs). BiTEs have the unique ability to crosslink T cells and tumor cells independently of major histocompatibility complex (MHC) restriction. They do not rely on TCR specificity or the CD4+/CD8+ costimulatory molecules, overcoming tumor MHC downregulation and low-affinity TCR binding. However, like many other immunotherapies, BiTEs have shown limited success beyond the treatment of hematological malignancies. BiTEs for the treatment of solid tumors still face challenges. Studies in gastrointestinal tumors have revealed Fc toxicity, short half-lives, and immunotoxicity, leading to Fc-silenced half-life extended BiTEs with humanized single-chain variable fragments. Studies in prostate tumors, lung tumors, and malignant gliomas have identified promising targets in PSMA, DLL3, and EGFRvIII, respectively, but also highlighted the problems of on-target off-tumor and BiTE-specific toxicities and inaccessible or immunosuppressive tumor microenvironments. Ongoing research to overcome these limitations remains an interesting field to follow, as BiTEs have the potential to be a powerful tool, especially when used in combination with other immunotherapies.

Graphical Abstract



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Introduction

A breakthrough in the field of cancer treatment came in T-cell-based immunotherapies, proving that we can target T cells to promote the eradication of malignant cells [1, 2]. The most notable success is that of immune checkpoint inhibitors (ICIs), antibodies that block negative regulators of T-cell responses, such as CTLA4 and PD-1, to promote T cell eradication of malignant cells [3]. Such regulators are normally used to limit T-cell responses but are often co-opted by tumor cells in an attempt to evade the immune response [4]. Despite the success of ICIs in some advanced tumors such as melanoma, response rates remain limited [1]. One key reason for this is low tumor immunogenicity; many tumor-associated antigens (TAAs) are derived from self-proteins, but T cells with corresponding T-cell receptors (TCRs) are often deleted during central tolerance or have low affinity for the antigen [5]. Another reason is tumor cell downregulation of major histocompatibility complex (MHC) class I molecules, resulting in a lack of T-cell infiltration into the tumor site, i.e. a cold phenotype [2].

Several immunotherapies have been developed in an attempt to redirect T cells to cold tumors, most notably CAR-T cells and T-cell engaging bispecific antibodies (bsAbs). CAR-T cells are genetically engineered T cells that display a chimeric antigen receptor (CAR) that targets TAAs [1, 6]. While proving effective for the treatment of hematological cancers (especially lymphomas), autologous CAR-T cells require complex and costly manufacturing that delays the availability of treatment by 2–4 wk, and success has not translated over to solid tumors [6]. Alternatively, T-cell engaging bsAbs are antibodies that simultaneously engage TAAs and cell surface molecules on T cells. The most common and promising subclass is bispecific T-cell engagers (BiTEs). BiTEs have also experienced success in haematological malignancies, exemplified by the remarkable efficacy and approval of blinatumomab for the treatment of B-cell precursor acute lymphoblastic leukemia [7]. Use in solid tumors, however, is still in development. Table 1 compares the aforementioned immunotherapies.

BiTE structure and mechanism of action

In general, human antibodies bind much stronger to their cognate antigen than TCRs, but the two variable domains target the same antigen [5]. BsAbs take advantage of this strong binding but target two antigens in order to redirect immune cells or deliver toxic drugs to tumor cells [14]. The most common approach has been to redirect T cells via CD3 molecules expressed in TCRs to tumor cells via a TAA [1]. BsAbs have been created in more than 50 different formats and can be split up into IgG-like, which have Fc domains, and non IgG-like, which are Fv-based [8]. IgG-like bsAbs have a longer half-life and are able to recruit additional immune cells, while Fv-based bsAbs lacking Fc domains (such as BiTEs) are much smaller and have shorter half-lives [1, 5, 8, 15–17]. Spiess *et al.* [15] provide a good summary of the various bsAb formats.

BiTEs consist of two single-chain variable fragments (scFvs) linked by a peptide [1] (Fig. 1). Each scFv has one heavy and one light chain variable region connected via a flexible linker [18]. One scFv is taken from an anti-CD3 monoclonal antibody (anti-CD3ε) and a second from an anti-TAA antibody [2]. Normally, T cells are activated by the TCR binding to peptides presented via MHC and co-stimulatory signals such as CD28 [19]. In contrast, BiTEs engage T cells through the CD3 subunit. This means that BiTEs can crosslink T cells and tumor cells independently of MHC restriction, costimulatory molecules, or TCR specificity, thus overcoming tumor MHC downregulation and low-affinity TCR binding [12, 20, 21].

Binding of BiTEs causes activation of T cells and their release of granzymes and perforin onto the tumor cells, causing cell lysis (shown in Fig. 2). This happens through transient interactions or formation of an artificial immunological synapse resembling a synapse formed by a TCR-MHC peptide bond [21]. BiTE-activated T cells also secrete cytokines such as TNF-α, IFN-γ, IL-6, Il-4, and IL-10 and show a dramatic increase in number [2, 20] (Fig. 2). Calcium is required for perforin multimerization and pore formation, and calcium chelation inhibits target-cell lysis, suggesting that this is the main mechanism by which BiTEs induce killing of tumor cells [2]. However, there is also some evidence to suggest that BiTEs can induce the bystander effect, or killing of cells that are near the target cell but negative for the target TAA. This is suggested to be via TNF-α and IFN-γ induced expression of ICAM-1 and CD95, molecules that stabilize T-cell synapses, on bystander cells [22]. This is particularly important for solid tumors as they often have heterogeneous TAA expression. Lastly, BiTEs might contribute to epitope spreading by activating T cells that happen to be specific to tumor antigens other than the target [12].

In regards to the type of effectors cells being recruited, a study of an anti-CD19/CD3 BiTE showed that the majority of cell lysis is mediated by cytotoxic T cells, with CD4+ T cells involved in a delayed fashion [23]. In vivo and in vitro studies of BiTEs in hematological malignancies have shown efficacy at very low doses (10–100 pg/ml) and low T cell to target cell ratios (<1:90) (due to multiple target cell lysis by each T cell), making them an encouraging therapy [12, 20].

Efficacy in solid tumors

Despite being best known for success in hematological malignancies, the first ever approved bsAb was for use in solid tumors; catumaxomab was approved by the European Medicines Agency (EMA) in 2009 for the treatment of malignant ascites in patients with epithelial cell adhesion molecule (EpCAM)-positive carcinomas [24, 25]. EpCAM is a glycoprotein commonly expressed in many healthy tissues, most notable in the gastrointestinal tract, but overexpressed in many solid tumors including carcinomas of the breast, ovaries, lungs, pancreas, colon, head and neck squamous cells, and stomach [26]. Catumaxomab, a T-cell redirecting bsAb against epCAM/CD3, showed positive results in phase II/III trials [27]. There was a significant decrease in the frequency of paracentesis and symptoms of ascites, increase in overall survival, and lack of non-manageable/reversible side effects [24]. However, a trial to test administration of catumaxomab via intravenous (iv) infusion was much less successful and had to be stopped before reaching the maximum tolerated dose (MTD) due to dose-limiting liver toxicity, with one patient experiencing fulminant fatal acute liver failure [2]. The drug was eventually withdrawn for commercial reasons. The side effects were attributed to the binding of the intact Fc region to Fc receptors on Kupffer cells [13]. As a result, modern bsAbs all have absent or silent Fc regions.

Table 1. Summary comparison of notable T-cell immunotherapies

	ICI	CAR-T cells	BiTEs
Structure	Monoclonal antibody targeting immune checkpoint inhibitor proteins [1]	T cells genetically engineered to present a TAA-targeting TCR [1, 6]	Recombinant antibody with two scFv regions; one targeting a TAA and one targeting CD3, a part of the TCR complex in T cells [1]
Effector cell	Endogenous T cells	Engineered T cells	Endogenous T cells
MAO	ICIs block receptor-ligand interactions to inhibit negative regulators of T cells, thereby promoting T cell-mediated destruction of tumor cells	CAR-T are T cells with engineered TCRs that have been designed to recognize TAAs, promoting T cell-mediated destruction of tumor cells	BiTEs simultaneous target the TCR complex on T cells and antigens on tumor cells to promote T cell-mediated destruction of tumor cells (independently of MHC restriction and TCR specificity)
Personalized	No	Yes[a] [6]	No
Production	Hybridoma technology to produce monoclonal antibodies [1]	Genetic engineering of (patient's[a]) T cells in vitro [1, 6]	Protein engineering of antibodies from mammalian cells lines [1]
Availability	Off the shelf [3]	Delayed ≈ weeks	Off the shelf [8]
Half-life	Medium ≈ days	Long ≈ weeks-months [6, 8]	Short ≈ hours
Lymphodepletion prior to therapy	No	Routine [6, 8]	No
Administration	Repeat dosing [3, 9]	Single infusion [6]	Repeat dosing [7]
FDA/EMA approvals	Pembrolizumab, Nivolumab, Atezolizumab, Durvalumab, Avelumab, Cemiplimab, Dostarlimab, Ipilimumab, Relatlimab [8–10]	Idecabtagene vicleucel, lisocabtagene maraleucel, ciltacabtagene autoleucel, tisagenlecleucel, brexucabtagene autoleucel, axicabtagene ciloleucel [11]	Blinatumomab [7]
Indications	Mostly solid tumors, some hematological malignancies [1, 9]	Hematological malignancies approvals only [1]	Hematological malignancies approvals only [1]
Advantages	Durable responses, can be curative even in metastatic disease, generally well tolerated compared with chemotherapy, broad-spectrum activity [1]	Works independently of MHC expression, and endogenous TCRs, therapy be tailored to patient's tumor [1, 6]	Works independently of MHC and TCR expression, easy production, promising results in solid tumors [1, 8, 12]
Disadvantages	Only effective in a small proportion of patients (checkpoint-, MHC-, and TCR-expression dependent), ineffective in cold tumors, autoimmune toxicities [1, 9]	Long, complex, and expensive production, remained effect and toxicity after tumor clearance (due to long half-life), proving ineffective for solid tumors, antigen dependent, requires lymphodepletion, can lead to CRS or neurotoxicity, may have on-target off-tumor effects (due to antigen expression in healthy tissue) [1, 6, 8, 13]	Short half-life requiring continuous infusion, antigen dependent, can lead to CRS or neurotoxicity, may have on-target off-tumor effects (due to antigen expression in healthy tissue) [1, 6]

[a]For autologous CAR-T cells.
MAO, mechanism of action.

In an effort to avoid these Fc-dependent dose-limiting toxicities (DLTs), an EpCAM/CD3 BiTE called Solitomab (MT 110 or AMG 110) was developed. A phase I dose-escalation trial of Solitomab was the first BiTE ever tested for the treatment of solid tumors (specifics in Table 2) [28]. Solitomab was administered via continuous iv infusion over at least 4 wk to 65 patients. According to response evaluation criteria in solid tumors (RECIST), 1 patient (2%) had a partial response (PR), 17 (31%) had stable disease (SD), and 28 (52%) had progressive disease (PD) [29]. However, dose escalation to therapeutic levels was unattainable due to DLTs in 15 (23%) patients, mostly commonly grade ≥3 diarrhea, elevations in liver function tests, and increases in lipase, with one fatal case of treatment-related diarrhea [29]. The adverse events (AEs) were likely related to targeting of EpCAM expressed on normal liver bile ducts and GI epithelium. This first highlighted the importance of choosing tumor-specific TAAs to avoid on-target off-tumor toxicity, an ongoing challenge in BiTE development.

As of March 2022, there are still no BiTEs (or bsAbs of any kind) approved for use in solid tumors. However, many BiTEs targeting recognized TAAs including carcinoembryonic

antigen (CEA), epidermal growth factor receptor (EGFR), prostate specific membrane antigen (PSMA), delta-like ligand 3 (DLL3), claudin-18.2 (CLDN18.2), and mucin 17 (MUC17) are currently in development [30, 32, 34, 36–38, 41, 43–45, 47–50, 52]. An overview can be found in Table 2.

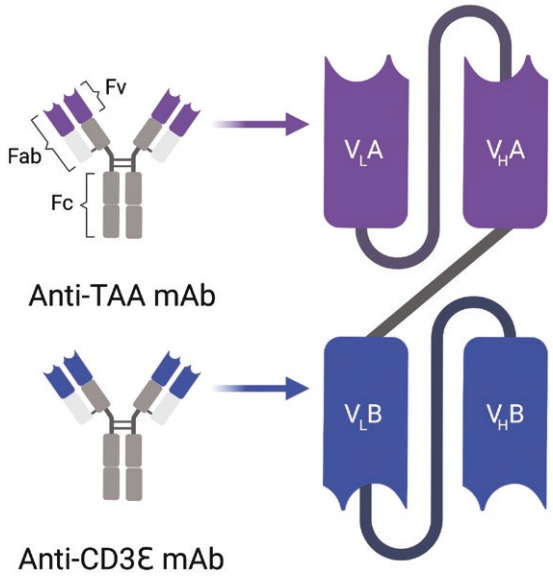

Figure 1. BiTE structure consists of two scFvs; a set of heavy and light chain variable regions targeting a TAA (V_HA and V_LA) linked via a peptide chain to a set of heavy and light chain variable regions targeting CD3ε (V_HB and V_LB) [14]. There are no Fc regions. Fab antigen binding fragment, Fv variable fragment, Fc fragment crystallizable, mAb monoclonal antibody. Created with BioRender.com

Gastrointestinal tumors

CEA is a glycoprotein expressed in healthy colon, stomach, esophagus, tongue, cervix, and prostate tissue [26]. In many cancers, CEA is overexpressed and no longer limited to the apical/luminal surface of epithelial cells [26]. MEDI-565 (AMG 211 or MT111) is a CEA-targeting BiTE that inhibited CEA-expressing tumor growth in models [33]. A phase I trial administered MED-565 via intermittent iv infusion to treat gastrointestinal adenocarcinomas [30], and 11 (28%) patients achieved SD, but no responses occurred. Serious AEs consisted of diarrhea, vomiting, pyrexia, cytokine release syndrome (CRS), and hypoxia. The MTD was found to be 5 mg, but 19 (49%) patients also had antidrug antibodies and the half-life of MEDI-565 was very short [31]. Results from the trial were disappointing, highlighting issues with short half-lives and immunogenicity. In an effort to make dosing easier, many modern BiTEs have silenced Fc domains to increase half-life (half-life extended [HLE] BiTEs).

Another study in humans (NCT02760199) showed that radioactively labeled MEDI-565 localized to CEA-expressing tumor tissue, suggesting on-target activity [35]. A second phase I trial was run where MEDI-565 was administered via continuous infusion to try and improve the therapeutic index [32]. There were no DLTs and initial changes in inflammatory and tumor markers were observed, but the study was terminated due to all patients with dosing above 3.2 mg

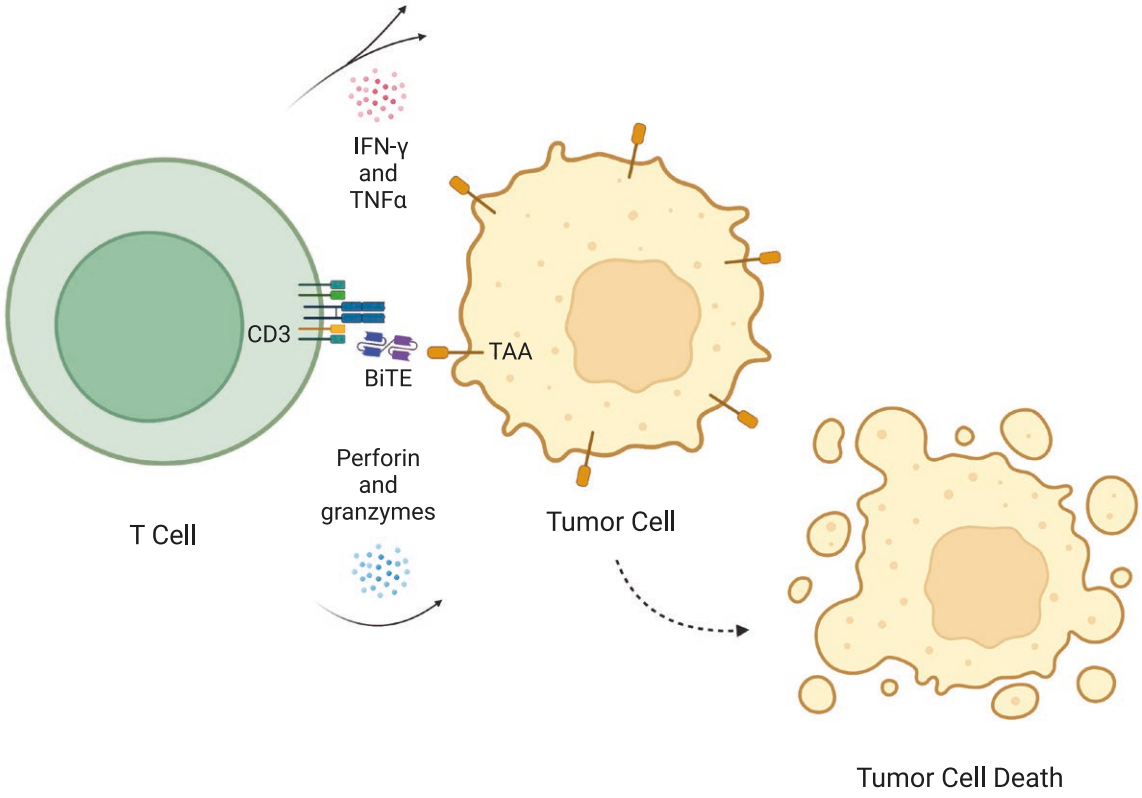

Figure 2. Mechanism of action of BiTEs. Binding to CD3 and the relevant TAA results in T-cell activation and perforin and granzyme release and subsequent cell lysis of the tumor cell, and cytokines release, possibly mediating bystander killing. In addition but not shown, the T cell undergoes proliferation and may contribute to epitope spreading. Created with BioRender.com

Table 2. Summary of all BiTE clinical trials organized by tumor, chronologically

NCT	Phase and status	Indication	Intervention*	Target and type of BiTE	# P	Results (as per RECIST)	Ref.
General solid tumors							
NCT00635596	1 Completed	Advanced solid tumors	Solitomab/MT110/ AMG 110	CD3/EpCAM Canonical	65	1 (2%) PR 17 (31%) SD 28 (52%) PD 15 (23%) DLTs with 1 fatal outcome preventing dose escalation to therapeutic levels	[28, 29]
Gastrointestinal tumors							
NCT01284231	1 Completed	GI adeno-carcinoma	AMG 211/MEDI 565/ MT111	CD3/CEA Canonical	39	MTD found = 5 mg 11 (28%) SD 4 (10%) DLTs AEs included diarrhea, vomiting, pyrexia, CRS, hypoxia, raised ALT, and hypertension 19 (49%) developed anti-drug antibodies	[30, 31]
NCT02291614	1 Terminated	GI adeno-carcinoma	AMG 211/MEDI 565/ MT111	CD3/CEA Canonical	44	0 DLTs Initial changes in markers present All dosing > 3.2mg lead to anti-drug antibodies AEs included fatigue, nausea, abdominal pain, pyrexia, and diarrhea	[32, 33]
NCT02760199	1 Completed	Advanced gastro-intestinal cancer	AMG 211/MEDI 565/ MT111 labelled with 89Zr	CD3/CEA Canonical	9	Dose-dependent localization of MEDI-565 to CEA-specific viable tumor tissue observed	[34, 35]
NCT04117958	1 Recruiting	MUC17-positive solid tumors	AMG 199	CD3/MUC 17 HLE	165	Not available	[36]
NCT04260191	1 Active, not recruiting	Gastric and gastro-esophageal junction adeno-carcinoma	AMG 910	CD3/ CLDN18.2 HLE	16	Not available	[37]
Prostate tumors							
NCT01723475	1 Completed	Prostatic neoplasms	Pasotuxizumab/BAY 2010112/AMG 212/ MT 112	CD3/PSMA Canonical	47 (16 iv)	For IV: All ≥ AE, most commonly fever 13 ≥1 AE of grade 3/4 most commonly decreased lymphocytes (44%) and infections (44%) 1 serious AE of fatigue 3 (19%) ≥ 50% PSA reductions, 2 long-term (1+ year)	[38–40]
NCT03792841	1 Recruiting	Metastatic castration-resistant prostate cancer, prostate cancer	Acapatamab/AMG 160 (+ pembrolizumab, etanercept prophylaxis and cytochrome P450)	CD3/PSMA HLE	288	Out of 35 so far: 1 (3%) PR 5 (16%) SD 5 (16%) PD MTD not reached CRS (84%) most common AE 2 (6%) reversible DLTs	[41, 42]
NCT04631601	1/2 Recruiting	Metastatic castration-resistant prostate cancer	Acapatamab/AMG 160 (+enzalutamide, abiraterone, AMG 404)	CD3/PSMA HLE	159	Not available	[43]

Table 2. Continued

NCT	Phase and status	Indication	Intervention*	Target and type of BiTE	# P	Results (as per RECIST)	Ref.
NCT04702737	1 Recruiting	Neuro-endocrine prostate cancer	Tarlatamab/AMG 757	CD3/DLL3 HLE	60	Not available	[44]
Lung tumors							
NCT03319940	1 Recruiting	Small cell lung carcinoma	Tarlatamab/AMG 757 (+Pembrolizumab, CRS Mitigation Strategies)	CD3/DLL3 HLE	382	Out of 38 so far: 6 (16%) PR 11 (29%) SD 1 (3%) unconfirmed PR 17 (43%) CRS stage 1/2	[45, 46]
NCT04885998	1 Recruiting	SCLC	Tarlatamab/AMG 757 (+ AMG 404)	CD3/DLL3 HLE	50	Not available	[47]
NCT04822298	1 Recruiting	NSCLC	Acapatamab/AMG 160	CD3/PSMA HLE	50	Not available	[48]
NCT05060016	2 Recruiting	Relapsed/refractory SCLC	Tarlatamab/AMG 757	CD3/DLL3 HLE	160	Not available	[49]
Malignant gliomas							
NCT03296696	1 Completed	Glio-blastoma or malignant glioma	Etevritamab/AMG 596 (+ AMG 404)	CD3/EGFRvIII Canonical	30	Out of 7: 1 (12.5%) PR 2 (25%) SD 4 (50%) had PD at initial scan 1 (12.5%) discontinued treatment for PD All had AEs, 50% serious Most common grade ≥3 AEs headache and depressed consciousness (both 14%)	[50, 51]
NCT04903795	1 Not yet recruiting	Glio-blastoma or malignant glioma	hEGFRvIII-CD3 (+ Activated Cell Therapy)	CD3/EGFRvIII BRiTE	18	Not available	[52]

*(/) marks alternate names, NCT ClinicalTrials.gov identifier, #P number of participants (estimated or actual), Ref. references, DLT dose-limiting toxicity.

developing anti-drug antibodies [33]. This study showed the potential of BiTEs for treatment of solid tumors but once again highlighted the issue of immunogenicity. This prompted the ongoing development of humanized scFvs [53].

Other BiTEs targeting MUC17, a mucin overexpressed in gastric cancer, and CLDN18.2, an epithelial surface marker overexpressed in various cancers, are currently being tested in trials for patients with gastrointestinal tumors (see Table 2) [36, 37].

Prostate tumors

PSMA is a transmembrane protein with expression highly conserved to prostate tissue [1]. It is overexpressed in most prostate cancers and plays an essential role in progression of the tumor, making it an ideal target [26]. Pasotuxizumab (AMG 212, BAY 2010112, or MT 112) was the first PSMA-targeting bsAb tested in humans for the treatment of prostate cancer. A phase I trial administered Pasotuxizumab to 47 patients with castration resistant prostatic cancer [39], and 31 received subcutaneous (sc) injections but all developed antibodies [40], and 16 received continuous iv infusion and all subsequently developed ≥1 AE, most commonly fever (94%); 13 had ≥1 AE of grade ≥3, but there was no grade 5 AE and only 1 serious AE (fatigue). Overall, the safety profile was manageable [39, 40]. Iv-Pasotuxizumab receivers showed dose-dependent antitumor responses [measured as decreased serum prostate-specific antigen (PSA) levels], with 2 (12.5%) having long-term responses (14 and 19 mo). One of them showed complete regression of soft-tissue metastases and marked regression of bone metastases. Unfortunately, MTD was not reached due to changes in sponsorship during the trial, but the results were the first to demonstrate that BiTE can effectively treat solid tumors with reasonable safety [39, 40].

Other PSMA-targeting BiTEs are being tested for prostate cancer (Table 2) [41, 43, 44]. Acapatamab (AMG 160) is an HLE BiTE being tested alone and in combination with ICIs (pembrolizumab or AMG 404) in a phase I trial [41, 43]. As of May 2020, 32 patients have received ≥1 dose, but MTD has not been reached. CRS is the most common AE (84%) and 2 reversible DLTs have occurred. PSA reductions occurred in 15 patients (63%), with 6 having reductions >50%, showing promising results [42].

Lung tumors

DLL3 is a notch ligand that is highly expressed in small cell lung cancer (SCLC) but minimally in normal lung tissue, making it an excellent target. Tarlatamab (AMG 757), an HLE BiTEs targeting DLL3, is currently being tested alone and in combination with ICIs (pembrolizumab or AMG 404) for the treatment

of SCLC [44, 47, 49]. One on-going phase I trial has shown promising results in the 38 patients who have been evaluated, with 6 (16%) (+1 unconfirmed) PRs and 11 (29%) SDs. The most common AE was CRS (43%), but all were grade 1 or 2, reversible, manageable, and typically did not recur past the first cycle [46]. These positive results lead to recruitment for a phase 2 trial starting in September 2021 [35]. Additionally, Acapatamab is being tested in non-small cell lung cancer (NSCLC) where PSMA is expressed in 49%–85% of endothelial cells in the new-grown blood supply to tumors [48, 54].

Malignant gliomas

EGFR variant III (EGFRvIII) is mutant of EGFR that promotes tumor cell growth and is found in 1/3 glioblastomas [55]. Etevritamab (AMG 596) is an EGFRvIII targeting canonical BiTE that underwent a phase I trial with promising early results [50]. Out of 14 evaluable patients, all experienced AEs with half being serious (most grade ≥3 were headache and depressed consciousness), but none resulted in discontinuation. In patients with sufficient follow-up, 1 (12.5%) achieved a sustained PR and 2 (25%) had SD [51]. AMG 596 was discontinued due to portfolio prioritization but suggested that EGFRvIII BiTEs may be well-tolerated and potentially efficacious. Full results are not out yet but another BiTE targeting EGFRvIII is currently in trial [52].

Noticeably, BiTEs being used in the treatment of malignant gliomas are not HLE BiTEs, as these are unable to cross the blood brain barrier (BBB). A solution may be to attach the Fv of a humanized albumin-binding antibody, as this may actually facilitate BBB-crossing [12].

Main limitations and possible solutions

Toxicities

While clinical trials are showing promise for BiTE therapy in solid tumors, there are still many limitations to overcome. One is on-target off-tumor toxicity, as demonstrated first by Solitomab and many subsequent drugs [29]. In hematological cancers, toxicities such as B-cell or myeloid-cell depletion are often reversible as long as hematopoietic stem cells are not targeted, but toxicities in solid tumors are less forgiving and can lead organ failure and death [13]. EGFRvIII is a mutation exclusive to glioblastomas and PSMA is highly specific to the prostate, but many other TAAs are commonly expressed in various normal tissues. This highlights the need for good target TAAs; ones that are exclusively expressed on tumor cells and critical for tumor growth.

There is ongoing research into new TAAs to overcome these problems [56, 57]. Some new bsAb-targets that are currently being tested include CLDN18.2 (NCT04260191), MUC17 (NCT04117958), SSTR2 (NCT03411915), STEAP-1 (NCT04221542), GD2 (NCT03860207), B7-H4 (NCT05067972), and NVG-111 (NCT04763083) [6, 36, 37, 58–62]. Recent results from trials with GPC3, expressed highly in various carcinomas but limited in normal cells, show promise in CAR-T cell and bsAb therapies and present a good new target if CRS can be controlled [63].

Other solutions to tackle on-target off-tumor toxicities include developing BiTEs as prodrugs that are activated in the tumor microenvironment (TME) by conditions such as low pH or increased proteolysis [13]. One group has developed a masked CD3-EGFR bsAb where binding of both ends is blocked until protease cleavage occurs in the TME [64]. In cynomolgus monkeys, the masked version has an MTD 60-fold higher than the non-masked bsAb, a longer half-life, and results in reduced serum cytokines and aspartate transaminase/alanine transaminase [64]. Prot-FOLR1-TCB is a similar pro-bsAb targeting folate receptor-1 [65]. COBRA T-cell engagers are a very new format which only have functional CD3-targeting domains in the TME after cleavage [66]. Prodrugs represent a creative solution and it will be exciting to see if they make it to clinic.

Another possible solution is to increase the avidity for the TAA by developing 2:1 BiTEs that have a second TAA binding domain. Two such bsAbs have been developed against HER2 and CEA and show anti-tumor activity without excessive toxicity in cynomolgus monkeys and humans, respectively [67, 68]. Alternatively, BiTEs can be delivered specifically to the tumor via oncolytic viruses (OVs) [69]. OVs encoding BiTEs would selectively infect tumor cells where the BiTEs would be expressed. There is evidence for this in mouse models but the idea is still in early development [70, 71]. An alternative yet to viruses may be to deliver immunotherapies via ultrasound-stimulated nanobubbles, which has had some early success in targeting solid tumors in mouse models [72, 73].

Beyond off-tumor toxicities, CRS and neurotoxicity are two of the most concerning AEs across all BiTE therapies, suggesting the need for good prophylactic treatments [1]. Mouse models suggest that CRS is mainly mediated by monocytes which are activated by TNF-α from T cells. Proposed solutions include TNF-α, IL-1β, or IL-6 inhibition, step-up dosing, subcutaneous administration, and reductions in CD3 affinity (though this requires balance against efficacy) [13].

Tumor microenvironment

The TME can limit BiTE action in two ways: inaccessibility and immunosuppression. Solid tumors may have deposition of extracellular matrix (ECM) in the stroma that acts as a physical barrier to immune cells. Consequently, few T cells are present in the tumor for BiTEs to activate. Solutions including targeting cancer-associated fibroblasts that produce ECM components. One elegant way to do this is to create OVs targeted to the tumor that encode fibroblast-targeting BiTEs. A study testing this in mice showed an effective response with decreased fibroblasts and increased antitumor activity [21].

Even when T cells make it to tumor cells, immunosuppressive cells including cancer-associated fibroblasts, myeloid-derived suppressor cells, and regulatory T cells await them [13]. In addition, chronic antigen stimulation is known to lead to "exhausted" T cells that upregulate CTLA-4 and PD1. Logical ways to counter this are to combine BiTEs with immunosuppressive cytokines and checkpoint inhibitors. ICI-BiTE combinations have shown positive results in vitro and are now being tested in clinical trials (Table 2) [69, 74]. One study testing the combination of a CEA-CD3 bsAb with atezolizumab (anti-PDL-1) compared with monotherapy showed better responses without increased toxicities, demonstrating the power of combination therapies [67].

Conclusion

Overall, this review highlights that BiTEs have great potential for the treatment of solid tumors. Early evidence from

EpCAM and CEA-targeting BiTEs revealed that important limitations (Fc toxicity and immunogenicity) that modern BiTEs have improved on. Evidence from past and ongoing trials with BiTEs targeting PSMA, DLL3, and EGFRvIII shows promising evidence. However, there are still key limitations that prevent them from being as successful as they have been in hematological malignancies, most notably toxicities (on-target off-tumor as well as BiTE specific), complex TMEs (inaccessible and immunosuppressive), and short half-lives (with HLEs now being tested to address this). The unique advantages and disadvantages of BiTEs suggest that they can complement CAR-T cells and ICIs for the best outcomes, and such combinations are seen in ongoing trials. Various solutions are being worked on to improve BiTEs, making for a dynamic field that will be interesting to follow.

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Author contributions

Laura Dewaele (Conceptualization, Data curation, Formal analysis, Investigation, Writing—original draft, Writing—review & editing) and Ricardo A. Fernandes (Conceptualization, Supervision, Writing—review & editing)

Ethical approval

Not applicable

Conflict of interest

The authors declare that they have no conflicts of interest.

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Data availability

No new data were created or analysed in this study. Data sharing is not applicable to this article.

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