Recent advances of bispecific antibodies in solid tumors

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Abstract
Cancer immunotherapy is the most exciting advancement in cancer therapy. Similar to immune checkpoint blockade and chimeric antigen receptor T cell (CAR-T), bispecific antibody (BsAb) is attracting more and more attention as a novel strategy of antitumor immunotherapy. BsAb not only offers an effective linkage between therapeutics (e.g., immune effector cells, radionuclides) and targets (e.g., tumor cells) but also simultaneously blocks two different oncogenic mediators. In recent decades, a variety of BsAb formats have been generated. According to the structure of Fc domain, BsAb can be classified into two types: IgG-like format and Fc-free format. Among these formats, bispecific T cell engagers (BiTEs) and triomabs are commonly investigated. BsAb has achieved an exciting breakthrough in hematological malignancies and promising outcome in solid tumor as showed in various clinical trials. In this review, we focus on the preclinical experiments and clinical studies of epithelial cell adhesion molecule (EpCAM), human epidermal growth factor receptor (HER) family, carcinoembryonic antigen (CEA), and prostate-specific membrane antigen (PSMA) related BsAbs in solid tumors, as well as discuss the challenges and corresponding approaches in clinical application.

Keywords: BsAb, Solid tumor, EpCAM, CEA, PSMA, HER family, Radioimmunotherapy

Background
Although great progress has been achieved in the treatment for cancer, it is still difficult to be cured due to tumor recurrence, drug resistance, etc. [1]. Therefore, there is a critical need for the development of new treatment for those refractory or recurrent patients. Compared with other conventional therapeutic approaches, immunotherapy has a specific advantage [2]. Monoclonal antibody (mAb), tumor vaccine, immune checkpoint blockade [3–5], and most recently CAR-T and bispecific antibody (BsAb) are powerful tools for the immunologic treatment of cancer [6–8].

In the mid-1980s, the BsAb was proposed to the treatment of cancers. Until recently, BsAb is intensively investigated [9]. BsAb can enhance tumor killing in a non-MHC-restricted manner by redirecting effector cells (e.g., T cells, NK cells, macrophages, and monocytes) to the tumor cells [10, 11]. Moreover, BsAb not only offers an effective linkage between therapeutics (e.g., immune effector cells, radionuclides) and targets (e.g., tumor cells) but also simultaneously blocks two different oncogenic mediators such as anti-epidermal growth factor receptor (EGFR) × anti-HER2 and anti-EGFR × anti-c-MET [12, 13]. With the development of advanced technology, many different BsAb formats have been proposed. According to the Fc domain, BsAb can be classified into two types: IgG-format and non-IgG-format [14]. The IgG-like molecules containing Fc domain retain Fc-mediated effector functions such as antibody-dependent cell mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP) [15], mainly including quadrroma, knobs-into-holes, scFv-IgG, and (IgG)2. The Fc-free BsAbs include TandscFv, DART, TandAb, F(ab')2, Diabody, and ImmTAC [14] (Fig. 1). Triomabs and BiTEs are the most advanced BsAb formats among various BsAb molecules [16]. BiTEs are fusion proteins consisting of two single-chain variable
fragments (scFv) connected by a short peptide linker. One of the scFvs binds to CD3 on T cells and the other to a surface antigen on tumor cells [17]. Blinatumomab, as a BiTE antibody against CD19/CD3, has been approved for the treatment of relapsed/refractory B-precursor acute lymphoblastic leukemia (ALL) by the US Food and Drug Administration (FDA) in December 2014 [18]. Due to the absence of Fc domain, BiTEs showed short serum half-lives, which hamper its clinical application. The permeability is an important issue in the treatment of solid tumors, and the permeability of BiTEs is greater than triomabs due to its small molecular mass (55KDa) [19]. Triomabs, as an IgG-like molecule, could bind two different tumor antigens simultaneously and interact with the FcR expressed on NK cells, macrophages, and dendritic cells through the Fc domain [20]. Because of the existence of Fc domain, triomabs show slower clearance from the blood than BiTEs. Nevertheless, the strong immunogenicity and compromised permeability of triomabs are just caused by Fc domain [21]. In 2009, catumaxomab, a triomab co-targeting EpCAM/CD3, was approved for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive cancers [22].

The interaction of T cells and tumor cells, which is mediated by BsAb, initiates the killing process of T cells, including activation of CD3, formation of immunologic synapses, activation and proliferation of T cell, secretion of cytokines and cytotoxic granules, and lysis of tumor cells [23]. The activated CD8+ and CD4+ T cells lyse cancer cells predominantly through perforin and granzyme B. The activated T cells secrete various cytokines such as IFN-γ, TNF, IL-2, IL-6, and IL-10 [24]. In addition to the above mechanism, triomabs can recruit other immune cells such as NK cells, macrophages which could kill tumor cells and mediate the co-stimulation between T cells and accessory cells [20]. Simultaneously recruiting and activating different immune effector cells to the tumor site result in potent tumor-cell elimination by the different immunologic killing mechanisms mentioned above [25] (Fig. 2).

At present, although the clinical outcome of BsAB is less satisfied in solid tumors than in hematologic malignancies [26, 27], there are abundant ongoing studies,
and some products have entered clinical trials. In this review, we will mainly summarize the correlated studies of BsAb in solid tumors (Table 1) and discuss the challenges and corresponding approaches in clinical application. This review will focus on commonly expressed antigens on solid tumors, such as EpCAM, HER family, CEA, and PSMA, and BsAB targeting these antigens are also extensively investigated and have demonstrated a great potential in cancer immunotherapy.

**Targeting antigens**

**EpCAM**

EpCAM (CD326, 17-1A) is a 39–40 KDa transmembrane glycoprotein that functions as adhesion molecule [28, 29]. EpCAM is expressed by majority of normal epithelial tissues including lung, colon, pancreas, bile ducts, breast, as well as embryonic stem cells [30, 31]. Similar to CD44, CD133, and CD166, EpCAM is also considered as a cancer stem cell (CSC) marker [32–34]. The expression of EpCAM is correlated with epithelial cell proliferation, differentiation, and migration [35–37]. EpICD, as the intracellular domain of EpCAM, is associated with the Wnt pathway which regulates gene transcription when translocated into the nucleus resulting in cell proliferation and tumor formation [32, 38]. EpCAM is expressed on certain carcinomas including ovarian cancer, breast cancer, lung cancer, pancreas cancer, colorectal cancer, head and neck squamous-cell carcinoma (HNSCC), and gastric cancer [39, 40]. Overexpression of EpCAM was detected in 35.6% breast cancer samples by the immunohistochemical method and was related to poor prognosis [41]. A study demonstrated that high expression of EpCAM was a poor prognostic indicator of breast cancer with node-positive [42]. Similarly, a retrospective study found that EpCAM was overexpressed in 68.8% epithelial ovarian cancer (EOC) and was associated with reduced survival time, especially in stage III–IV and poorly differentiated subtype [43]. EpCAM was highly expressed in 86.5% of HNSCC patients [35]. Moreover, high level of EpCAM expression was detected in more than 90% of HNSCC patients [35]. Therefore, bspecific antibody targeting EpCAM provides an attractive choice for immunotherapy of those cancers. Triomab (e.g., catumaxomab) and BiTE (e.g., MT110) are two major types of anti-EpCAM x anti-CD3 BiAbs.

Catumaxomab (Removab) is an intact trifunctional bspecific antibody consisting of a murine IgG2a targeting EpCAM, a rat IgG2b targeting CD3, and Fc fragment recruiting different immune effector cells [44]. It was
| BsAb                  | Targets       | Format       | Function                              | Diseases                                  | Phase      | Stage    | Identifier         |
|----------------------|---------------|--------------|---------------------------------------|-------------------------------------------|------------|---------|-------------------|
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Ovarian cancer                            | II         | Completed | NCT00189345       |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | EpCAM-positive tumor, malignant ascites   | II/III     | Completed | NCT00836654       |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Gastric cancer, adenocarcinoma           | II         | Completed | NCT00648933       |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Gastric adenocarcinomas                  | II         | Active, not recruiting | NCT01504256 |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Ovarian cancer                            | II         | Completed | NCT01246440       |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Gastric peritoneal carcinomatosis         | II         | Terminated | NCT01784900       |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Epithelial cancer                        | I          | Terminated | NCT01320020       |
| Catumaxomab          | EpCAM/CD3     | Triomab      | Redirect T cells and accessory cells  | Malignant ascites                        | III        | Completed | NCT00822809       |
| MT110                | EpCAM/CD3     | BiTE         | T cell recruitment                    | EpCAM-positive solid tumors              | I          | Completed | NCT00635596       |
| Ertumaxomab          | HER2/CD3      | Triomab      | Redirect T cells and accessory cells  | Breast cancer                            | II         | Terminated | NCT00452140       |
| Ertumaxomab          | HER2/CD3      | Triomab      | Redirect T cells and accessory cells  | Breast cancer                            | II         | Terminated | NCT00522457       |
| Ertumaxomab          | HER2/CD3      | Triomab      | Redirect T cells and accessory cells  | Her2 positive advanced solid tumors      | I/II       | Terminated | NCT01569412       |
| HER2Bi-aATC          | HER2/CD3      | Triomab      | Redirect T cells and accessory cells  | Her2 positive neoplasms                  | I          | Recruiting | NCT02662348       |
| EGFRBi-aATC          | EGFR/CD3      | T cells armed with BsAbs | Activated T cells | Advanced solid tumors                  | I          | Terminated | NCT01081808       |
| MDX-447              | EGFR/CD64     | 2(Fab)       | Active monocytes                      | Brain and central nervous system tumors | I          | Completed | NCT00005813       |
| MM-111               | HER2/HER3     | HSA body     | Blockade of 2 receptors               | HER2 amplified solid tumors              | I          | Completed | NCT00911898       |
| MM-111 + Herceptin   | HER2/HER3     | HSA body     | Blockade of 2 receptors               | Breast cancer                            | I          | Completed | NCT01097460       |
| MCLA-128             | HER2/HER3     | Full Length IgG | Blockade of 2 receptors              | Malignant solid tumors                   | II         | Recruiting | NCT02912949       |
| MM-141               | HER2/HER3     | scFv-IgG     | Blockade of 2 receptors               | Hepatocellular carcinoma                 | I          | Completed | NCT01733004       |
| MM-141               | HER2/HER3     | scFv-IgG     | Blockade of 2 receptors               | Pancreatic cancer                        | I          | Recruiting | NCT02399137       |
| LY3164530            | EGFR/MET      | OrthoFab-IgG | Blockade of 2 receptors               | Advanced or metastatic cancer            | I          | Active, not recruiting | NCT02221882 |
| TargomiRs            | EGFR/EDV      | Unclear      | Delivery of nanoparticles             | MPM and NSCLC                           | I          | Recruiting | NCT02369198       |
| EGFR(-)-EDV-Dox      | EGFR/EDV      | Unclear      | Delivery of nanoparticles             | Glioblastoma                            | I          | Recruiting | NCT02766699       |
| AMG211               | CEA/CD3       | BiTE         | T cell recruitment                    | Gastrointestinal cancer                  | I          | Recruiting | NCT02760199       |
| AMG211               | CEA/CD3       | BiTE         | T cell recruitment                    | Gastrointestinal cancer                  | I          | Recruiting | NCT0291614        |
| AMG211               | CEA/CD3       | BiTE         | T cell recruitment                    | Gastrointestinal adenocarcinomas         | I          | Recruiting | NCT01284231       |
| ROX995688            | CEA/CD3       | IgG-based    | T cell recruitment                    | CEA-positive solid tumors                | I          | Recruiting | NCT02324257       |
| ROX995688+Atezolizumab| CEA/CD3       | IgG-based    | T cell recruitment                    | CEA-positive solid tumors                | I          | Recruiting | NCT02550713       |
| ROX6895882           | CEA/IL2       | ScFv-IgG     | The delivery of cytokines             | CEA-positive solid tumors                | I          | Completed | NCT02004106       |
| TF2                  | CEA/HS5       | Dock and lock | Radioimmunotherapy                    | Colorectal cancer                        | I          | Completed | NCT00860860       |
| Study Code | Target | Modality | Tumor Type                          | Phase | Status    | Clinical Trial ID  |
|-----------|--------|----------|------------------------------------|-------|-----------|--------------------|
| TF2       | CEA/ HSG Dock and lock | Radioimmunotherapy | Small cell lung cancer | I/II | Completed | NCT01221675       |
| TF2       | CEA/ HSG Dock and lock | Immuno-PET | Medullary thyroid carcinoma | I/II | Completed | NCT01730638       |
| TF2       | CEA/ HSG Dock and lock | Immuno-PET | Breast carcinoma expressing CEA | I/II | Ongoing    | NCT01730612       |
| TF2       | CEA/ HSG Dock and lock | Radioimmunotherapy | Colorectal cancer | I    | Terminated | NCT01273402       |
| TF2       | CEA/ HSG Dock and lock | Immuno-PET | Colorectal cancer | II   | Completed | NCT02587247       |
| Anti-CEAxanti-DTPA | CEA/di-DTPA-131 scFv-IgG | Radioimmunotherapy | Medullary thyroid carcinoma | II   | Completed | NCT00467506       |
| BAY2010112 | PSMA/CD3 BiTE | T cell recruitment | CRPC | I    | ongoing    | NCT01723475       |
| MOR209/ES414 | PSMA/CD3 ScFv-Fc-scFv | T cell recruitment | mCRPC | I    | Recruiting | NCT02262910       |

The details of Table 1 derived from http://clinicaltrials.gov/
approved by the European Union for the treatment of malignant ascites in April 2009 [45]. The trifunction of catumaxomab was assessed by co-culture of tumor spheroids of FaDu cell line (HNSCC) with peripheral blood mononuclear cells (PBMCs). The results indicated that three functional parts of catumaxomab were essential for the entire antitumor activity [46]. Schmitt et al. investigated the opsonization mediated by catumaxomab with co-culture of tumor cells and PBMCs. In their studies, opsonization with catumaxomab caused the activation of PBMCs and destroyed EpCAM-positive tumor cells [44]. To evaluate the immunomodulatory effects of catumaxomab, Zitvogel et al. developed in vitro experimental model of malignant ascites system. They found that catumaxomab activated and transformed T cells to inflammatory CD4+ and CD8+ Th1 cells, and it stimulated the secretion of IFN-γ. In addition, catumaxomab promoted CD16+ cells to express TRAIL and costimulatory molecules CD40 and CD80 [22]. Another group investigated the immunological changes in six patients with malignant ascites after intraperitoneal administration of catumaxomab. They found the accumulation of NK cells, macrophages, and T cells in the peritoneal cavity. At the same time, CD69 and CD38, the activation molecular of T cells, were induced by the intraperitoneal (i.p.) catumaxomab infusion. Catumaxomab promoted the secretion of IFN-γ and IL-2, whereas its functions were inhibited in the immunosuppression microenvironment of ascites in vitro [47]. In an open-label, dose-escalation clinical trial, 16 patients with EPAM-positive solid tumors were enrolled and treated with catumaxomab. The antitumor efficacy was not optimal: two patients had stable disease, nine patients had disease progression, and the remaining patients were not evaluable. The study results determined that the maximum tolerated dose (MTD) of intravenous catumaxomab was 7 μg/kg. The cytokine release-related symptoms and hepatotoxicity were considered as the major adverse events (AEs) [48]. In a large phase II/III trial, 258 patients with malignant ascites due to epithelial cancer were enrolled. The total patients were comprised of 129 ovarian cancer patients and 129 non-ovarian cancer patients. In each group, 85 patients were treated with paracentesis plus catumaxomab, and the other 44 patients were treated with paracentesis alone. The difference of the time to next paracentesis in the catumaxomab group (77 days) and in the control group (13 days) was significant. The puncture-free survival and overall survival (OS) also exhibited beneficial trend in the catumaxomab group. In addition, compared with the control group, fewer signs and symptoms of ascites were observed in catumaxomab-treated patients. The side effects related to catumaxomab were reversible and manageable [49]. In another phase I clinical trial, a total of 21 patients with NSCLC were recruited, and 15 of them were treated with catumaxomab and were evaluated for the dose limiting toxicity (DLT) and MTD. The five dose-escalation levels ranged from 2 μg/kg to 7.5 μg/kg. In dose level IV and V, grade 3 and 4 elevations of ALT, AST, and γ-GT were observed, which were identified as the DLT. Nevertheless, the elevation of liver enzymes was reversible. The MTD was determined in dose level III (5 μg of catumaxomab). Moreover, it did not observe the HAMA/HARA (human anti-mouse/human anti-rat antibody) within 28 days in 15 evaluable patients [50]. Twenty-three patients with malignant ascites due to refractory ovarian cancer were treated with i.p. catumaxomab in phase I/II study. The i.p. of catumaxomab significantly decreased the ascites production. During the infusion, just 1 of 23 patients required a paracentesis. Promisingly, severe adverse events were not observed [51].

MT110 (solitomab) is a BiTE bispecific antibody consisting of two scFvs. One of the scFvs binds to EPAM expressed on tumor cells, and the other binds to CD3 on T cells [23]. BsAb which targets EPAM and CD3 prolonged the contact time between lymphocytes and cancer cells [52]. Via MT110, T cells could potently recognize and lyse target tumor cells. The mechanism of lysis predominantly depends on the pore forming and apoptosis. The lysis process including caspase activation, PARP cleavage, and DNA fragmentation was mainly mediated by granzyme B and perforin [23]. MT110 showed potent antitumor effect against chemotherapy-resistant ovarian cancer cell lines. When incubated with autologous tumor-associated T cells and EPAM+ ovarian cancer cells derived from ascites, MT110 upregulated the expression of T cell activation markers and enhanced its cytotoxicity to malignant cells [53]. CSCs might be responsible, at least partly, for the resistance to chemotherapy and recurrence of hepatocellular carcinoma (HCC) [54, 55]. EPAM was considered as a CSC marker in HCC [56]. On the basis above, Blaudszum et al. generated an EPAM/CD3 BiTE using the scFvs of anti-EPAM monoclonal antibody 1H8 and anti-CD3 monoclonal antibody. Their results indicated that 1H8/CD3 effectively eradicated CD133+ EPAM+ HCC CSCs and EPAM+ HCC cells in vitro and in vivo [57]. EPAM/CD3-BiTE potently killed the colon cancer cell line at a low effector-to-target ratio in vitro and significantly restricted the ovarian cancer growth in a xenograft model [58]. Another study showed that MT110 eliminated colorectal cancer cells and stem cells [59]. Some studies also demonstrated that MT110 could eradicate the primary cancer cells and the CSCs of pancreatic cancer in vivo and in vitro [60, 61].

Mus110 is a BiTE bispecific antibody to murine EPAM and murine CD3, and its structure is similar to MT110. In breast cancer and lung cancer mouse models, Mus110 showed potent antitumor activity as low as 5 μg/kg, but mice could tolerate a high dose of mus110.
up to 400 μg/kg [31]. Studies have shown that adverse
events of mus110 in mice were mainly due to an acute T
cell activation. The therapeutic window and target-related
side effects of mus110 in mice might be a prediction for
MT110 in human [62, 63]. As we all know, compared with
triomabs, BiTEs are not able to mediate ADCC, CDC, and
ADCP for lacking Fc region. However, the BiTE antibody
against EpCAM and CD16 recruited innate immune cells
and then induced effective ADCC, as well as enhanced the
killing of human carcinoma overexpressing EpCAM [64].

HER family

The receptor tyrosine kinase family known as the HER
family consists of four members: EGFR (also known as
ErbB1/HER1), HER2, HER3, and HER4. HER1–4 play a
pivotal role in controlling and regulating cell growth, dif-
ferentiation, migration, and death [69, 70]. EGFR as a
tumor-associated antigen overexpressed on the cell sur-
face of various malignant tumors, such as NSCLC, glio-
blastoma, pancreatic cancer, HNSCC, renal cancer, and
colonctal cancer (CRC) [71]. HER3 has been identified as
a critical molecule in the interaction with ligand as well as PI3K signaling pathway [72]. Compared with
other members, HER4 is less known to us. Study
demonstrated that HER4 is a favorable prognostic
marker for OS in patients with breast cancer [73].

Based on the above reasons, the HER family members are as
attractive targets for immunotherapy, especially in the
application of BsAb.

The application of anti-EGFR monoclonal antibody
(such as cetuximab and panitumumab) in EGFR over-
expressing tumors has been marketed for many years [74].
However, there are studies demonstrated that the therape-
utic outcome of anti-EGFR mAbs is not well satisfying
in patients with KRAS and BRAF genes mutated CRC
[75]. T cell engaged BiTE antibodies using the binding do-
 mains of cetuximab, and panitumumab remained potent
antitumor activity in KRAS and BRAF mutation of CRC
cell lines and in xenograft models [76]. Glioblastoma over-
expressed wild type EGFR, EGFRvIII, and HER2, so they
were all considered as attractive immunotherapy targets
[77]. BsAb that target EGFR and HER2 may be an
effective strategy for the treatment of glioblastoma.

A group examined the antitumor activity of the ATC armed
with chemically heteroconjugated anti-CD3 × anti-HER2
(HER2Bi) and/or anti-CD3 × anti-EGFR (EGFRBi). It was
demonstrated that the armed ATC significantly killed ma-
lignant glioma lines (U87MG, U118MG, and U251MG)
and primary glioblastoma lines. Moreover, the increased
secretion of three Th1 cytokines (IFN-γ, GM-CSF, and
TNF-α) and one Th2 cytokine (IL-13) had been detected
[78]. EGFRBi-armed CIK cells showed significant antitu-
mor effects in EGFR-positive glioblastoma in vitro and
in vivo [79]. A clinical study led by Solomon et al.
examined the safety of EGFR-targeted, paclitaxel-loaded
minicells (EGRminicellsPac). Among 22 patients that
completed cycle 1 treatment, ten patients achieved stable
disease, and 12 had progressive disease. The most com-
mon treatment-related AEs were chills and pyrexia. The
number of 1 × 1010 CIK cells was considered to be the
MTD. In general, the study reported that the EGRminicellsPac
could be safely administered to patients with ad-
vanced solid tumors [80].

Evidences showed that fully human HER2/CD3 BsAb
potently delayed the growth of breast cancer by stimulat-
ing the activation and proliferation of tumor-infiltrating
lymphocytes [81]. More recently, a phase I trial was con-
ducted by Lum and colleagues. Eight castrate resistant
prostate cancer (CRPC) patients were treated with
HER2Bi-armed ATC at infusion 2.5, 5, 10, and 20 billion
units. One patient achieved partial response, and three of
seven patients had a remarkable decline in their PSA
levels. The Th1 cytokines of two patients had increased.
In addition, no dose limiting toxicities were observed [82].
Another phase I clinical trial was conducted to test the
safety and efficacy of HER2Bi-armed ATC in combination
with interleukin 2 (IL-2) and granulocyte-macrophage
colony stimulating factor (GM-CSF) in 23 patients with
stage IV breast cancer. Thirteen of 22 evaluable patients
achieved a stable disease condition, and the remaining
patients had progressive disease. The median OS for all
patients was 36.2 months, 57.4 months for the HER2 3+
group, and 27.4 months for the HER2 0-2+ group. The
major side effects including chills, fever, headache, fatigue,
and hypotension were controllable and reversible. The
MTD was not reached. Encouragingly, this strategy in-
duced endogenous cytotoxicity and cytokine responses in
evaluable patients [83]. Ertumaxomab, as a trifunctional
antibody, could eliminate tumor cell lines regardless of
HER2 expression level. However, trastuzumab-mediated
cytotoxicity depends on the high expression of HER2
since the HER2 binding sites for trastuzumab and
ertumaxomab are located in different positions [84]. A phase I clinical trial was conducted by Kiewe et al. to determine the safety and efficacy of ertumaxomab in patients with metastatic breast cancer. Fifteen of 17 enrolled patients completed the study with three ascending doses of ertumaxomab (10–200 Ag). There were 5 out of 15 evaluable patients showed antitumor response including one with complete response, two with partial responses, and two with stable disease. The patients infused with 150 μg/kg and 200 μg/kg developed severe toxicities. Therefore, 100 μg/kg is suggested as the MTD.

Human anti-mouse antibody (HAMA) was induced in 4 out of 16 evaluable patients (25%) on day 41 [85]. Fourteen patients with HER2-positive advanced solid tumors were enrolled in another phase I trial. Patients were treated with the trifunctional antibody ertumaxomab in a weekly escalating dosing regimen. The clinical response to ertumaxomab treatment was seen in 3 out of 11 evaluable patients, including one partial remission and two disease stabilizations. The treatment-related toxicities were mild and completely reversible [86].

The tetramerized bispecific antibody targeted EGFR and CD16 simultaneously and then exhibited cytotoxicity against EGFR-expressing tumor cells [87]. MDX-447 is a bispecific antibody that combined humanized Fab anti-Fcry1 (CD64) and humanized Fab anti-EGFR [88]. Another bispecific antibody MDX-210 co-targeting HER2/neu and Fcry1 increased the efficacy in vitro when combined with granulocyte-colony stimulating factor (G-CSF) in breast cancer patients overexpressing HER2/neu [89]. Therefore, a phase I clinical study was conducted to determine the safety and efficacy of MDX-447 with and without recombinant human G-CSF in patients with advanced solid tumors. The study results indicated that MDX-447 alone was generally well tolerated, but the combination of MDX-447 and G-CSF was not [90].

Targeting individual members of HER family such as EGFR or HER2 led to limited antitumor activity. The BsAb not only redirected effector cells to the target tumor cells but also bound to two receptors and blocked the downstream signaling pathway. Anti-EGFR/HER2 bispecific antibody effectively suppressed the growth of breast tumor [8]. Another BsAb targeting HER2/HER3 overcomes the heregulin-induced resistance to PI3K inhibition in prostate cancer [91]. MM-111, a bispecific antibody consisting of human anti-HER2 and anti-HER3 scFv linked by modified human serum albumin (HSA) blocked HER3 and PI3K pathway in the HER2-overexpressing cells and inhibited tumor growth in xenograft models. MM-111 combining with trastuzumab or lapatinib showed potent antitumor ability in the HER2-overexpressing tumors [92]. The phosphorylation of EGFR and HER3 activated the downstream Ras/MAPK and (PI3K)/AKT signaling pathways which contributed to the cell growth and proliferation. The monospecific antibodies of EGFR or HER3 cannot completely inhibit the proliferation and survival signals [93, 94]. Sliwkowski et al. constructed a two-in-one antibody against HER3 and EGFR (namely MEHD7945A) and tested the function in vitro and in vivo. The study results showed that MEHD7945A not only potently inhibited receptor phosphorylation of EGFR and HER3 but also enhanced gemcitabine-mediated cytotoxicity in vitro and in vivo. Besides, the dermatologic toxicity of MEHD7945A was significantly less than monospecific antibody in xenograft models [95]. In addition, the binding of HER family members and other receptors also enhanced the therapeutic outcome. For instance, EGFR × c-MET bispecific antibody JNJ-61186372 enhanced the killing of EGFR mutant lung cancer cells [9]. BsAb co-targeting EGFR and VEGFR2 promoted the antitumor activity by inhibiting phosphorylation of the receptors and blockade PI3K/AKT and MAPK signaling pathways [96]. EGFR and the insulin-like growth factor-1 receptor (IGF-1R) play an essential role in cell proliferation and tumor progression. Therefore, the bispecific antibody XGER targeting EGFR and IGF-1R exhibited potent antitumor efficacy [97]. Negrin et al. investigated the ability of BsAb anti-HER2 × cancer antigen-125 (CA125) with CIK cells against primary ovarian carcinomas. The results suggested that the cytolytic activity of CIK cells with BsAb was significantly higher than CIK cells alone [98]. Study showed that in primary breast cancer, 65% were positive for CEA, 19% were positive for HER2, and 12% expressed both antigens. Therefore, the bispecific antibody simultaneously targeting HER2 and CEA on the same cell obviously enhanced tumor localization [99].

**CEA**

CEA is a 180–200 KDa glycoprotein that belongs to the CEA-related cell adhesion (CEACAM) superfamily [100, 101]. CEA is expressed at low levels in various normal tissues including colon, stomach, esophagus, tongue, cervix, and prostate [102]. Under physiological conditions, CEA is expressed on the apical surface and luminal portion of normal epithelial cells [101]. But in cancer tissues, CEA is overexpressed and lose the polarized distribution. Besides, CEA was cleaved from the surface of cancer cells by phospholipase, which resulted in the increase of serum CEA [103]. Blood levels of CEA are currently used as a diagnostic and prognostic marker, as well as a monitoring index in patients after treatment [104, 105]. It was demonstrated that serum levels of soluble CEA did not affect the tumor suppression by CEA/CD3 BsAbs [106, 107]. CEA-overexpressing malignant cancers included colorectal, gastric, lung, breast, pancreatic, and other cancers [108]. CEA, as a well characterized tumor-associated antigen (TAA), plays a vital role in cancer adhesion, migration, and invasion [109]. Thus,
it has become a pivotal target for the immunotherapy, including antibody-based treatments of CEA-positive solid tumors [110].

MEDI-565 (AMG211, MT111), as a BiTE antibody mediating T cell-directed cytotoxicity towards CEA positive tumor cells, was positively correlated with CEA antigen density regardless of the mutational status of the tumor cell lines, including BRAF, KRAS, PTEN, PI3KCA, and TP53 [111]. MEDI-565 recognized a nonlinear epitope in the full-length but not a short splice variant of CEA. The CEA splice variant neither affected the binding of MEDI-565 and full-length CEA nor inhibited MEDI-565-induced T-cell activation and cytotoxicity [112]. In vitro, normal donor- and cancer patient-derived T cells redirected by MEDI-565 induced cytotoxicity to CEA-positive tumor cells which were derived from patients with metastatic colorectal cancer and previously treated with chemotherapy. In mice xenografted model, the MEDI-565 also significantly inhibited tumor growth at a low concentration (1 ng/ml) without the assistance of costimulatory agents [106]. In a multicenter phase I, open-label study (NCT01284231), a total of 39 patients with advanced gastrointestinal adenocarcinomas were enrolled and were intravenously injected with MEDI-565 over 3 h on days 1 through 5 in 28-day cycles with dexamethasone premedication. The study results demonstrated that 11 patients had stable disease. The median overall survival for 39 patients was 5.5 months, and the MTD of MEDI-565 was 5 mg. Nausea, vomiting, abdominal, and fatigue were considered as the most common adverse effects. During the treatment, high-level antidrug antibodies were detected in 19 patients. Like other BiTes, MEDI-565 showed rapid clearance and a short half-life [113]. As we know, recent researches have focused on checkpoint blockade. MEDI-565 in combination with anti-PD1 and/or anti-PD-L1 antibodies could significantly enhanced T cell cytotoxicity activity [114]. Bacac et al. developed the CEA TCB (RO6958688) that is a novel IgG-based T-cell bispecific (TCB) antibody. CEA TCB is a head-to-tail 2:1 T cell bispecific antibody that harbors bivalent binding site for CEA and monovalent binding site for CD3 [115]. CEA TCB significantly eliminated the CEA-expressing xenograft tumor in mice models. Moreover, number of immune cells infiltrating in the tumor tissues were observed, which resulted in a highly inflamed tumor microenvironment. The group also demonstrated that the activity of CEA TCB positively related to CEA expression. The efficient target cell lysis required at least approximately 10,000 CEA-binding sites [116]. Another study results suggested that CEA TCB potently increased the number of tumor-associated T cells and induced death of tumor cell within 24 h in vitro and in vivo. In addition, the investigators also observed the prolonged interactions between multiple T cells and tumor cells in vivo by fluorescence imaging [117]. A phase I study (NCT02324257) led by Hoffmann-La Roche which focused on the safety and feasibility of CEA TCB to patients with advanced CEA-positive solid tumors is currently ongoing.

A novel bispecific antibody BiSS (Bispecific antibody with Single domain, Single domain antibodies) was constructed by tandemly linking two single domain antibodies, anti-CEA, and anti-CD16. BiSS exhibited potent recruitment of NK cells and cytotoxicity to CEA-positive tumor cells, HT29 and LS174T. In an in vivo study, BiSS also significantly limited cancer progression [118]. Another single domain antibody-based bispecific antibody (anti-CD16 × anti-CEA) ss-Fc fused with CH3 “knobs into holes” also showed the potent antitumor activity in vitro and in vivo [119]. Compared with single-chain tandem scFvs (e.g., BiTE), two-chain diabodies induced T cell-activated proliferation in a target cell-dependent manner, which could reduce the toxicity to normal tissues [120]. However, unbalanced expression of the two chains of diabody is a limitation for the function of it. The incorporation of a 2A self-processing peptide derived from foot-and-mouth disease virus and a two-chain diabody gene balanced the secretion of diabody chains and maximized the final amount of assembled diabody [121]. Due to the important role in the cell cycle, tumor necrosis factor alpha (TNF-α) is considered as a potential tumor treatment strategy. However, the concentration of TNF-α is not high enough to exhibit its antitumor activity. Therefore, Azria et al. developed a bispecific antibody anti-TNF-α × anti-CEA in combination with TNF-α and radiotherapy to enhance the tumor growth control of pancreatic tumor xenografts in nude mice [122].

Radioimmunotherapy (RIT) is a molecular targeted therapy that uses mAb targeting specific tumor antigens to deliver radionuclides to tumor sites and kill tumor cells [123]. The application of RIT in treatment of hematologic malignancies achieved a gratifying outcome, but not in solid tumor [124]. The main restriction of RIT in solid tumor is bone marrow exposure and dose-limiting hematological toxicity caused by the moderate tumor/non-tumor ratios [125]. With the development of recombinant and humanized mAbs, pretargeting radioimmunotherapy (pRIT) using BsAb is becoming a potential therapeutic approach [126]. The classical two-step protocol of pRIT is to administer the BsAb to blood with adequate time for tumor uptake and clearance of excess BsAb in circulation, and then infuse the radiolabeled hapten, finally excess radiolabeled hapten is cleared from the bloodstream [127] (Fig. 3). Due to the extensive expression profile of CEA in several cancers, pretargeting BsAbs anti-CEA × anti-hapten were studied by numerous investigators.
TF2 is a trivalent humanized bispecific antibody that composed of two anti-CEA Fab fragments and an anti-histamine-succinyl-glycine (HSG) Fab fragment linked by the dock and lock method [128, 129]. The pre-targeting strategy using bispecific anti-CEA × anti-diethylenetriaminepentaacetic acid (DTPA) antibody and radiolabeled peptide significantly improved the tumor/blood ratios due to the rapid clearance of the radiolabeled peptide from the circulation [130].

The anti-CEA/anti-DTPA-indium complex BsAb combined with radiolabeled liposomes to carry high radio-nuclide activities and thus optimized the pre-targeting RIT of solid tumors [123]. The anti-CEA/anti-1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) bispecific antibody exhibited low tumor targeting and rapid blood clearance in a xenograft mouse model, but the specific tumor uptake and low normal tissue accumulation of the BsAb still improved the efficacy of RIT [131]. Karacay et al. made comparison between pretargeted peptide and directly radiolabeled IgG in a human colon cancer xenograft. The study demonstrated that the BsAb pre-targeting strategy sufficiently increased the dose of radioactivity in tumors and caused less hematologic toxicity than conventional RIT [132]. In order to increase tumor-to-blood ratios, the three-step protocol namely adding an avidin chase procedure on the basis of two-step was developed. The chase rapidly cleared the BsAb level from circulation, thereby reduced hapten concentration in blood and bone marrow exposure [133]. Thirteen patients with non-medullary thyroid carcinoma (non-MTC) and nine patients with medullary thyroid carcinoma (MTC) were enrolled in a phase I optimization clinical trial. The 75 mg/m² dose of BsAb was infused to 11 patients. 40 mg/m² dose of BsAb was infused to the remaining patients. Five days later, all patients received 1.9–5.5 GBq of 131I-di-DTPA. The results suggested that 40 mg/m² of BsAb and 5-day interval could be a better schedule for tolerable toxicity [134]. A phase II clinical trial (NCT00467506) was conducted to determine the efficacy and safety of anti-CEA × anti-DTPA BsAb and 131I-di-DTPA-indium bivalent hapten in patients with progressive metastatic MTC. Forty-five patients enrolled the study, but 42 completed the designed procedure and were evaluable for efficacy, adverse events, and response assessment. The study results indicated potent therapeutic responses including disease control in 76% of patients. Median progression-free survival (PFS) was 13.6 months, and median OS was 43.9 months. The main subacute adverse event was bone marrow exposure-related hematologic toxicity. Moreover, a significant increase of calcitonin doubling time (DT) in 56.7% of patients after pRIT was observed. The calcitonin DT was seen as an independent prognostic factor [135]. In another phase I/II trial (NCT01221675) by Centre René Gauducheau, nine patients with CEA-expressing advanced lung cancer were treated with TF2 and the IMP288 bivalent HSG peptide. The procedure included a pre-therapeutic imaging session.
and a therapy session. The pre-targeting delay was 24 or 48 h. In the end, one patient died (not considered treatment related) and eight patients were evaluated for pharmacokinetics, dosimetry, toxicity, and response. The study results suggested that increased the TF2 dose and shortened the pre-targeting delay were benefit for tumor uptake without increase of the toxicity to normal tissues. All patients were pretreated with an antihistamine and corticosteroid before each TF2 and peptide infusion thus only one patient was detected with human anti-human antibody (HAHA) against TF2 > 50 ng/ml [136].

The BsAb with radiolabeled hapten is a sensitive diagnostic tool. Bispecific antibody pre-targeting positron-emission tomography (PET) with a $^{68}$Ga- or $^{18}$F-hapten-peptide showed specific targeting in CEA-positive tumor and low ingestion in normal tissues and CEA-negative tumors [137]. BsAb anti-CEA × anti-hapten with an $^{124}$I-labeled hapten-peptide significantly increased tumor uptake and tumor-to-blood ratios in comparison to directly radiolabeled antibodies. Furthermore, the BsAb pre-targeting showed rapid clearance from normal tissues and clear visualization of tumor within 1–2 h [138]. TF2 pre-targeting CEA on the surface of tumor cells followed by the addition of Ga-labeled hapten could be obviously sensitive in the visualization of CEA [139]. Besides, it was proved that BsAb pre-targeting was highly selective for imaging micrometastatic tumor and showed better contrast ratio than $^{18}$F-FDG. Hence, single photon emission computed tomography (SPECT) and PET pre-targeted with TF2 could be a promising approach to improve imaging of metastatic CEA-positive malignancies [140]. Compared with conventional $^{99}$mTc-labeled CEA-specific F(ab’)$^\text{2}$, the BsAb pre-targeted $^{99}$mTc radiotracer increased 10-fold of the radioactivity signal and showed faster clearance and showed higher uptake ratio than the circulation and other normal tissues [141].

Fourteen patients with primary colorectal cancer were included and administered with anti-CEA × anti-Di-DTPA BsAb and a $^{111}$In-labeled di-DTPA peptide to assess the imaging effect. One of three patients that received $^{111}$In-peptide alone showed low tumor uptake. In 9 of 11 patients that received BsAb in combination with $^{111}$In-peptide, tumors were observed. The results suggested that pretargeting imaging was a promising diagnostic strategy using low dose BsAb and $^{111}$In-labeled peptide, with an optimal delay of 4 days between infusions of the two agents [142]. Another clinical trial on immune-PET using anti-CEA and 68Ga-labeled peptide in patients with metastatic medullary thyroid carcinoma showed that 30 h was the most favorable delay [143].

**PSMA**

PSMA is a membrane bound protein that is selectively expressed on the surface of prostate cancer cells as well as in the neovasculature of most solid tumors [144, 145]. PSMA is expressed across all stages of prostate cancer, and the expression level is inversely correlated with androgen levels [145]. PSMA plays an essential role in the progression of prostate cancer through MAPK-ERK1/2 and PI3K-AKT pathway [146]; besides, it can be used as the target of imaging agent to detect the metastatic tumor sites [147]. Therefore, PSMA is considered as an attractive target for the immunotherapy of prostate cancers.

BAY2010112 (AMG212, MT112), as a PSMA/CD3-bispecific BiTE antibody, bound to PSMA which was expressed in prostate cancer cell lines and PSMA cDNA transfected cell lines, and mediated T cells to eliminate target cells in vitro. The BiTE antibody potently suppressed tumor growth at a dose of 0.005 mg/kg daily intravenous (i.v.) administration [144]. BAY2010112 induced target-dependent activation and cytokine released by T cells. T cells exhibited potent cytotoxicity against PSMA-positive cell lines with the help of BAY2010112 in vitro. Compared to i.v. administration, subcutaneous (s.c.) injection of BAY2010112 significantly inhibited tumor formation and induced tumor regression in the subcutaneous xenograft immunodeficient NOD/SCID mice. In addition, the bioavailability of BAY2010112 was approximately 18% after s.c. administration in mice [148]. At present, a dose-escalation phase I clinical trial (NCT01723475) on BAY2010112 is ongoing. Patients with castration resistant prostate cancer will be recruited and treated with different dosages of BAY2010112. The primary objectives of this study are to determine the safety, tolerability, and MTD of BAY2010112. The secondary objectives are to assess the pharmacokinetics and the clinical efficacy of BAY2010112. MOR209/ES414 is a novel humanized BsAb which is designed to treat metastatic castration-resistant prostate cancer (mCRPC) by redirecting T cell cytotoxicity against prostate cancer cells expressing PSMA. MOR209/ES414 induced T cell activation and proliferation, and lysed tumor cells in vitro. In murine xenograft models, MOR209/ES414 also showed significant inhibitory effect on tumor and prolonged the survival time. The half-life period of MOR209/ES414 was 4 days in the peripheral blood of NOD/SCIDγ (NSG) mice [149]. A phase I study (NCT02262910) of MOR209/ES414 in patients with mCRPC is ongoing. The study is conducted by Aptevo Therapeutics to evaluate the tolerability, pharmacokinetics (PK), pharmacodynamics (PD), immunogenicity, cytokine response, and clinical activity of MOR209/ES414. Anti-PSMA × anti-CD3 BsAb could specifically bind to CD3-expressing Jurkat cells and PSMA-expressing C4-2 cells, as well as efficiently promoted the function of T cells to lyse target cells. PSMA × CD3 diabody showed efficient inhibition of tumor growth in C4-2 tumor xenografts [150, 151]. After activation, CD4+ and CD8+ T cells expanded and killed prostate cancer cells mainly through the perforin-
Conclusion
In this review, we concluded the current preclinical and clinical studies on BsAb against solid tumors, particularly anti-EpCAM, HER family, CEA, and PSMA. Taken together, the preclinical studies of BsAb showed potent antitumor efficacy, but the outcome of most clinical trials did not reach our expectation. In solid tumor, finding appropriate targets is the first step for the successful immunotherapy. The ideal target for BsAb would be the tumor specific antigens which are homogeneously expressed on the surface of malignant cell and play a critical role in tumorigenesis. In spite, there are many antigens expressed in various tumor cells; it also needs to make great efforts to seek out more appropriate antigens to improve the specificity. The drawback of Fc-free BsAb is short half-life caused by its small molecular mass. To overcome the limitation, investigators developed several approaches including chemical coupling of polyethylene glycol (PEG) to the small molecule protein, fusion to heavy chain fragments (Fc/CH3) or HSA. Among these approaches, fusion of recombinant BsAb molecules to HSA significantly increased the circulation time but did not reduce the binding ability of recombinant BsAbs [154]. In addition, several obstacles have remained to be overcome for a successful application of BsAb in solid tumor, such as toxicity to normal tissues and low tumor/blood ratios. For example, compared with two-step method of pRIT, the three-step method significantly increased the tumor/blood ratios [133]. Based on the catumaxomab, blinatumomab, solitomab and other BsAbs, Trivedi et al. summarized the challenges of clinical pharmacology, pharmacometrics, and bioanalysis of BsAb and the possible solutions [155]. Through continuous efforts, investigators could find better approaches to overcome these challenges.

Abbreviations
ADCC: Antibody-dependent cell mediated cytotoxicity; ADCP: Antibody-dependent cellular phagocytosis; AE: Adverse event; ATC: Activated T cell; BiTEs: Bispecific T cell engagers; BsAb: Bispecific antibody; CA125: Cancer antigen-125; CART: Chimeric antigen receptor T cell; CDC: Complement-dependent cytotoxicity; CEA: Carcinoembryonic antigen; CRC: Colorectal cancer; CRPC: Castrate resistant prostate cancer; CS: Cancer stem cells; DLT: Dose limiting toxicity; DOTA: 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid; DT: Doubling time; DTPA: Diethylenetriaminepentaacetic acid; EpCAM: Epithelial cell adhesion molecule; FDA: Food and Drug Administration; G-CSF: Granulocyte-colony stimulating factor; GM-CSF: Granulocyte-macrophage colony stimulating factor; HSA: Human serum albumin; HSCC: Head and neck squamous-cell carcinoma; HSA: Human serum albumin; HSG: Histamine-succinyl-glycine; IGF-1R: Insulin-like growth factor-1 receptor; IL-2: Interleukin-2; mAb: Monoclonal antibody; MTD: Maximum tolerated dose; NSCLC: Non-small-cell lung cancer; OS: Overall survival; PBMCs: Peripheral blood mononuclear cells; PD: Pharmacodynamics; PEG: Polyethylene glycol; PET: Positron-emission tomography; PFS: Progression-free survival; PK: Pharmacokinetics; pRIT: Pretargeting radioimmunotherapy; PSMA: Prostate-specific membrane antigen; RIT: Radioimmunotherapy; scFv: Single-chain variable fragments; SPECT: Single photon emission computed tomography; TAA: Tumor-associated antigen; TNFα: Tumor necrosis factor alpha

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Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Authors’ contributions
SY and AL performed the selection of literature, drafted the manuscript, and prepared the figures. QL, HX, XY, and DJ collected the related references and participated in the discussion. RP participated in the discussion and revised the language. KW and XH carried out the design of this review and revised the manuscript. All authors contributed to this manuscript. All authors read and approved the final manuscript.

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References
1. Ja Y, Yun CH, Park E, Ercan D, Manuia M, Juarez J, et al. Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. Nature. 2016;534(7605):129–32.
2. Goel G, Sun W. Novel approaches in the management of pancreatic ductal adenocarcinoma: potential promises for the future. J Hematol Oncol. 2015;8:44.
3. Ma W, Gilligan BM, Yuan J, Li T. Current status and perspectives in translational biomarker research for PD-1/PD-L1 immune checkpoint blockade therapy. J Hematol Oncol. 2016;9(1):47.
4. Goel G, Sun W. Advances in the management of gastrointestinal cancers—an upcoming role of immune checkpoint blockade. J Hematol Oncol. 2015;8:86.
5. Falchi L, Sawai A, Deng C, Amengual JE, Colbourn DS, Lichtenstein EA, et al. High rate of complete responses to immune checkpoint inhibitors in patients with relapsed or refractory Hodgkin lymphoma previously exposed to epigenetic therapy. J Hematol Oncol. 2016;9(1):132.
6. Hirsch FR, Suda K, Wensi J, Bunn PA Jr. New and emerging targeted treatments in advanced non-small-cell lung cancer. Lancet. 2016;388(10048):1012–24.

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29. Balzar M, Briaire-de Bruijn IH, Rees-Bakker HA, Prins FA, Helfrich W, de Leij L, et al. Chimeric antigen receptor T cells: a novel therapy for solid tumors. J Hematol Oncol. 2017;10(1):78.

8. Pardoll DM. Immunology beats cancer: a blueprint for successful translation. Nat Immunol. 2012;13(12):1129–32.

7. Yu S, Li A, Liu Q, Li T, Yuan X, Han X, et al. Human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol. 1994;125(2):437–46.

26. Wu J, Fu J, Zhang M, Liu D. AFM13: a first-in-class tetravalent bispecific anti-CD19–anti-CD3 antibody construct. Int J Cancer. 2017;140(2):1255–67.

21. Morecki S, Lindhofer H, Yacovlev E, Gelfand Y, Slavin S, et al. Ep-CAM overexpression in node-positive breast cancer as a predictor of survival. J Clin Oncol. 2004;22(1):165–70.

16. Frankel SR, Baeuerle PA. Targeting T cells to tumor cells using bispecific antibodies to CD19-/CD3-bispecific single-chain antibody construct. Int J Cancer. 2009;125(2):437–46.

24. Hoffmann P, Hofmeister R, Brischwein K, Brandl C, Crommer S, Bargou R, et al. The coming of age of engineered multivalent antibodies. Drug Discov Today. 2015;20(5):588–94.

25. Mau-Sorensen M, Dittrich C, Dienstmann R, Lassen U, Buchler W, Martinius H, et al. Effective relief of malignant ascites in patients with advanced malignant ascites due to epithelial cancer: results of a prospective randomized phase II/III trial. Int J Cancer. 2010;127(9):2289–2309.

27. Zugaïra G, Klinger M, Benjamin J, Kischel R, Stienen S, Zugmaier G. Harnessing T cells to fight cancer with BiTE(R) antibody constructs—past developments and future directions. Immuno Rev. 2016;267(1):193–208.

28. Litvinov SV, Velders MP, Bakker HA, Fleuren GJ, Warnaar SO. Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol. 1994;125(2):2437–46.

29. Balzar M, Briaire-de Bruijn IH, Rees-Bakker HA, Prins FA, Helfrich W, de Leij L, et al. Epidermal growth factor-like repeats mediate lateral and reciprocal interactions of Ep-CAM molecules in homophilic adhesions. Mol Cell Biol. 2001;21(7):2570–80.

30. Gonzalez B, Denzel S, Mack B, Conrad M, Gires O. EpCAM is involved in maintenance of the murine embryonic stem cell phenotype. Stem Cells. 2009;27(8):1782–91.

9. Amann M, Brischwein K, Lutterbuse P, Parr L, Petersen L, Lorenzczewski G, et al. Therapeutic window of MuS10, a single-chain antibody construct bispecific for murine EpCAM and murine CD3. Cancer Res. 2006;66(1):143–51.

31. Munz M, Baueeie PA, Gires O. The emerging role of EpCAM in cancer and stem cell signaling. Cancer Res. 2009;69(14):5627–9.

32. Fossati M, Buzzonetti A, Monego G, Catzola V, Scambia G, Fattorossi A, et al. Nuclear signalling by tumour-associated antigen EpCAM. Nat Cell Biol. 2009;11(2):162–71.

9. Munz M, Kieu C, Mack B, Schmitt B, Zeidler R, Gires O. The carcinoma-associated antigen EpCAM upregulates c-myc and induces cell proliferation. Oncogene. 2006;25(34):5758–67.

47. Fossati M, Buzzonetti A, Monego G, Catzola V, Scambia G, Fattorossi A, et al. Effective relief of malignant ascites in patients with advanced ovarian cancer. Gynecol Oncol. 2006;103(2):483–8.

48. Mau-Sorensen M, Dittrich C, Dienstmann R, Lassen U, Buchler W, Martinius H, et al. The phase I trial of intravenous catumaxomab: a bispecific monoclonal antibody targeting EpCAM and the T cell coreceptor CD3. Cancer Chemother Pharmacol. 2009;63(3):341–9.

73. Fossati M, Buzzonetti A, Monego G, Catzola V, Scambia G, Fattorossi A, et al. The phase I trial of intravenous catumaxomab: a bispecific monoclonal antibody targeting EpCAM and the T cell coreceptor CD3. Cancer Chemother Pharmacol. 2009;63(3):341–9.

74. Fossati M, Buzzonetti A, Monego G, Catzola V, Scambia G, Fattorossi A, et al. Ep-CAM overexpression in node-positive breast cancer is associated with poor prognosis in node-negative breast cancer. Breast Cancer Res Treat. 2004;86(3):207–13.

75. Fossati M, Buzzonetti A, Monego G, Catzola V, Scambia G, Fattorossi A, et al. Effective relief of malignant ascites in patients with advanced ovarian cancer. Gynecol Oncol. 2006;103(2):483–8.

76. Schmitt M, Schmitt A, Reinhardt P, Thess B, Manfras B, Lindhofer H, et al. Oncoporation with a trifunctional bispecific (alphaCD3 x alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. Int J Oncol. 2004;24(5):841–8.

77. Schmitt M, Schmitt A, Reinhardt P, Thess B, Manfras B, Lindhofer H, et al. Oncoporation with a trifunctional bispecific (alphaCD3 x alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. Int J Oncol. 2004;24(5):841–8.

78. Schmitt M, Schmitt A, Reinhardt P, Thess B, Manfras B, Lindhofer H, et al. Oncoporation with a trifunctional bispecific (alphaCD3 x alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. Int J Oncol. 2004;24(5):841–8.

79. Schmitt M, Schmitt A, Reinhardt P, Thess B, Manfras B, Lindhofer H, et al. Oncoporation with a trifunctional bispecific (alphaCD3 x alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. Int J Oncol. 2004;24(5):841–8.

80. Schmitt M, Schmitt A, Reinhardt P, Thess B, Manfras B, Lindhofer H, et al. Oncoporation with a trifunctional bispecific (alphaCD3 x alphaEpCAM) antibody results in efficient lysis in vitro and in vivo of EpCAM positive tumor cells by cytotoxic T lymphocytes. Int J Oncol. 2004;24(5):841–8.
53. Ferrari F, Bellone S, Black J, Schwab CL, Lopez S, Cocco E, et al. Solitomab, an EpCAM/CD3 bispecific antibody construct (BiTE(R)), is highly active against primary uterine and ovarian carcinomas cell lines in vitro. J Exp Clin Cancer Res. 2015;34:123.

54. Martin-Padura I, Marighetti P, Agliano A, Colombo F, Lazzarol B, Rednardo M, et al. Residual dormant cancer stem-cell foci are responsible for tumor relapse after antiangiogenic metronomic therapy in hepatocellular carcinoma xenografts. Lab Invest. 2012;92(7):792–66.

55. Xu XL, Xing BC, Han HB, Zhao W, Hu MH, Xu ZL, et al. The properties of tumor-initiating cells from a hepatocellular carcinoma patient’s primary and recurrent tumor. Cancers. 2010;31(2):165–74.

56. Yamashita T, Budhu A, Fortunes M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. Cancer Res. 2007;67(22):10831–9.

57. Zhang P, Shi B, Gao H, Jiang H, Kong J, Yan J, et al. An EpCAM/CD3 bispecific antibody efficiently eliminates hepatocellular carcinoma cells with limited galectin-1 expression. Cancer Immunol Immunother. 2014;63(2):121–32.

58. Schröter B, Fichtner I, Lorenzewschi G, Kleindienst P, Brischwein K, da Silva A, et al. Eradication of tumors from a human colon cancer cell line and from ovarian cancer metastases in immunodeficient mice by a single-chain EpCAM-CD3-bispecific antibody construct. Cancer Res. 2005;65(7):2882–9.

59. Herrmann I, Baurepa PA, Friedrich M, Murr A, Filus F, Suttinger D, et al. Highly efficient elimination of colorectal tumor-initiating cells by an EpCAM/CD3-bispecific antibody engaging human T cells. PLoS One. 2015;10(10):e13474.

60. Cioffi M, Dorado J, Baurepa P, Aeberspaecher M, EpCAM/CD3-Bispecific T-cell engaging antibody MTI10 eliminates primary human pancreatic cancer stem cells. Clin Cancer Res. 2012;18(2):465–72.

61. Cioffi M, Aeberspaecher M.Immuno-targeting of pancreatic cancer stem cells: a new therapeutic strategy against a devastating disease? Oncoimmunology. 2012;1(4):560–2.

62. Amann M, Friedrich M, Lutterbuese P, Viesser E, Lorenzewschi G, Petersen L, et al. Therapeutic window of an EpCAM/CD3-specific BITE antibody in mice is determined by a subpopulation of EpCAM-expressing lymphocytes that is absent in humans. Cancer Immunol Immunother. 2009;58(1):95–109.

63. Amann M, D’Argouges S, Lorenzewschi G, Brischwein K, Kisic R, Lutterbuese R, et al. Antitumor activity of an EpCAM/CD3 bispecific antibody during long-term treatment of mice in the absence of T-cell energy and sustained cytokine release. J Immunother. 2009;32(5):452–64.

64. Valleria DA, Zhang B, Gleason MK, Oh S, Weiner LM, Kaufman DS, et al. Heterodimeric bispecific single-chain variable-fragment antibodies against EpCam and CD16 induce effective antibody-dependent cellular cytotoxicity against human carcinoma cells. Cancer Biother Radiopharm. 2012;3(8):274–82.

65. Morecki S, Lindhofer S, Yacovlev E, Han HB, Baskin SA, et al. Induction of long-lasting antitumor immunity by concomitant cell therapy with allogeneic lymphocytes and trifunctional bispecific antibody. Exp Hematol. 2008;36(8):997–1004.

66. Groth A, Salinikov AV, Ottinger S, Gladkich J, Liu L, Kalifatidis G, et al. New gene-immunotherapy combining TRAIL-lymphocytes and EpCAMxCD3 bispecific antibody for tumor targeting. Clin Cancer Res. 2012;18(4):1028–38.

67. Ren-Heidenreich L, Davol PA, Kouttab NM, Ellenbein GJ, Lum LG, Redirected T-cell cytotoxicity to epithelial cell adhesion molecule-overexpressing adenocarcinomas by a novel recombinant antibody, E8B2, in vitro and in an animal model. Cancer. 2004;100(3):1095–103.

68. Hoffmann SC, Wablitz GH, Sasmain Y, Moldenhauer G, Ludwig T. Functional analysis of bispecific antibody (EpCAMxCD3)-mediated T-lymphocyte and cancer cell interaction by single-cell forced spectroscopy. Int J Cancer. 2011;128(9):2096–104.

69. Yarden Y, Silvokov MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2002;3(2):127–37.

70. Baselga J, Svinar SM. Novel anticancer targets: revisiting ERBB2 and ERBB3. Curr Opin Pharmacol. 2001;1(1):29–36.

71. Ren-Heidenreich L, Davol PA, Kouttab NM, Ellenbein GJ, Lum LG. Redirected T-cell cytotoxicity to epithelial cell adhesion molecule-overexpressing adenocarcinomas by a novel recombinant antibody, E8B2, in vitro and in an animal model. Cancer. 2004;100(3):1095–103.

72. Schoberth A, Repp R, Heijnen IA, Buhring HJ, Deo YM, et al. Preclinical studies with FcγRII bispecific antibodies and granulocyte colony-stimulating factor-primed neutrophils as effector cells against HER-2/ HER-3-expressing breast cancer. Cancer Res. 1997;57(16):696–701.

73. Frye MG, Lipton A, Smith KM, Winston CB, Pfluger DG. A phase I trial of the epidermal growth factor receptor directed bispecific antibody MDX-447 without and with recombinant human granulocyte-colony stimulating factor in patients with advanced solid tumors. Cancer Immunol Immunother. 2008;57(12):1553–63.

74. Pozavasevic J, Kang JC, Kim D, Ober RJ, Ward ES. Antibody targeting of HER2/HER3 signaling overcomes heregulin-induced resistance to PI3K inhibition in prostate cancer. Int J Cancer. 2015;137(2):267–77.

75. McLaren MG, Huhlová A, Fernández M, Adams S, Paragas V, Oyama S, et al. Antitumor activity of a novel bispecific antibody that targets the ErbB2/Erbb3 oncogenic unit and inhibits heregulin-induced activation of ErbB3. Mol Cancer Ther. 2012;11(3):582–93.

76. Engelman JA, Janne PA, Mermel C, Pearlberg J, Mukhopadhyaya T, Fleet C, et al. ErbB3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. Proc Natl Acad Sci U S A. 2005;102(10):3788–93.

77. Garey P, Roche B, Santos C, Roche C, Santos E, et al. A human IgG like bispecific antibody co-targeting epidermal growth factor receptor and the vascular endothelial growth factor receptor 2 for enhanced antitumor activity. Cancer Biol Ther. 2016;17(12):139–50.
97. Schanzer JM, Wantha K, Moessner E, Hosse RI, Moser S, Crosdale R, et al. XGFRF, a novel affinity-matured bispecific antibody targeting IGFR-1R and EGFR with combined signaling inhibition and enhanced immune activation for the treatment of pancreatic cancer. MBs. 2016;8(4):81–77.

98. Chan JK, Hamilton CA, Cheung MK, Karimi M, Baker J, Gall JM, et al. Enhanced killing of primary ovarian cancer by retargeting autologous cytotoxic-induced killer cells with bispecific antibodies: a preclinical study. Clin Cancer Res. 2006;12(6):1859–67.

99. Horvitz M, Garambois V, Pourquier D, Goumou M, Rouanet P, Mani JC, et al. Targeting of human breast cancer by a bispecific antibody directed against two tumour-associated antigens: ErbB-2 and carcinoembryonic antigen. Tumor Biol. 2002;23(6):337–47.

100. Okawa S, Nakazato H, Konishi G. Primary structure of human carcinoembryonic antigen (CEA) deduced from cDNA sequence. Biochem Biophys Res Commun. 1987;142(2):511–8.

101. Hammarskog S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol. 1999;9(2):67–81.

102. Behr TM, Sharkey RM, Juweid ME, Dunn RM, Ying Z, Zhang CH, et al. Factors influencing the pharmacokinetics, dosimetry, and diagnostic accuracy of radioimmunodetection and radioimmunotherapy of carcinoembryonic antigen-expressing tumors. Cancer Res. 1995;56(8):1805–16.

103. Yamamoto M, Maehara Y, Sakaguchi Y, Mine H, Yamamata T, Korenaga D, et al. Distinct tumor localization in CEA and colorectal carcinoma. Hepato-Gastroenterology. 2004;51(55):147–51.

104. Rother M. Carcinoembryonic antigen monitoring for early detection of asymptomatic incurable metastatic colorectal cancer. J Clin Oncol. 2007;25(10):1293–4. author reply 1294.

105. Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, et al. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol. 2006;24(33):5313–27.

106. Osada T, Hsu D, Hammond S, Hobeika A, Devi G, Clay TM, et al. Metastatic colorectal cancer cells from patients previously treated with chemotherapy are sensitive to T-cell killing mediated by CEA/CD3-bispecific T-cell-engaging BiTE antibody. Br J Cancer. 2010;102(1):124–33.

107. Lutterbuse R, Raum T, Kischel R, Lutterbuse P, Schlereth B, Schaller E, et al. Potent control of tumor growth by CEA/CD3-bispecific single-chain antibody constructs that are not competitively inhibited by soluble CEA. J Immunother. 2009;32(4):341–52.

108. Behr TM, Sharkey RM, Juweid ME, Dunn RM, Ying Z, Zhang CH, et al. Variables influencing tumor dosimetry in radioimmunotherapy of CEA-expressing cancers with anti-CEA and antimucin monoclonal antibodies. J Nucl Med. 1997;38(1):409–18.

109. Dallas MR, Liu G, Chen WC, Thomas SN, Wirtz D, Huso DL, et al. Divergent roles of CD44 and carcinoembryonic antigen in colon cancer metastasis. FASEB J. 2012;26(6):2648–56.

110. Khae PD, Shao-Xi Li, Kunoki M, Hirose Y, Arakawa F, Nakamura K, et al. Specifically targeted killing of carcinoembryonic antigen (CEA)-expressing cells by a retroviral vector displaying single-chain variable fragment antibody to CEA and carrying the gene for inducible nitric oxide synthase. Cancer Res. 2003;63(13):4703–12.

111. Oberst MD, Fuhrmann S, Muellgau K, Amann M, Cheng L, Lutterbuse P, et al. CEA/CD3 bispecific antibody MEDI-565/AMG 211 activation of T cells and subsequent killing of human tumors is independent of mutations commonly found in colorectal adenocarcinomas. MBs. 2014;6(6):1571–84.

112. Peng L, Oberst MD, Huong J, Brohaan P, Morehouse C, Leisiek K, et al. The CEA/CD3 bispecific antibody MEDI-565 (MT111) binds a nonlinear epitope in the folding loop but not a short splice variant of CEA. PLoS One. 2012;7(5):e36412.

113. Pishvaian M, Morse MA, McDevitt J, Norton JD, Ren S, Robbie GJ, et al. Phase 1 dose escalation study of MEDI-565, a bispecific T-cell engager that targets human carcinoembryonic antigen, in patients with advanced gastrointestinal adenocarcinomas. Clin Colorectal Cancer. 2016;15(4):345–51.

114. Osada T, Patell SP, Hammond SA, Osada K, Morse MA, Lyerly HK. CEA/CD3- bispecific T cell-engaging (BiTE) antibody-mediated T lymphocyte cytotoxicity maximized by inhibition of both PD1 and PD-L1. Cancer Immunol Immunother. 2015;64(6):677–88.

115. Bacac M, Klein C, Umana P. CEA TCB: a novel head-to-tail 2:1 T cell bispecific antibody for treatment of CEA-positive solid tumors. Oncoimmunology. 2016;5(8):e103498.
135. Slaun PY, Campion L, Boumaud C, Faivre-Chauvet A, Vuilze JP, Taieb D, et al. Phase II trial of anticarcinoembryonic antigen pretargeted radiolabeled therapeutic monoclonal antibody in advanced colorectal cancer: biomarker response and survival improvement. J Nucl Med. 2012;53(8):1185–92.

136. Bodet-Millin C, Ferrer L, Rauscher A, Masson D, Rbah-Vidal L, Faivre-Chauvet A, et al. Pharmacokinetics and dosimetry studies for optimization of pretargeted radioimmunotherapy in CEA-expressing advanced lung cancer patients. Front Med (Lausanne). 2015;2:94.

137. Schoffelen R, Sharkey RM, Goldenberg DM, Franssen G, McBride WJ, Ross EA, et al. Pretargeted immuno-positron emission tomography imaging of carcinoembryonic antigen-expressing tumors with a bispecific antibody and a 68Ga- and 18F-labeled hapten peptide in mice with human tumor xenografts. Mol Cancer Ther. 2010;9(4):1019–27.

138. McBride WJ, Zanzonico P, Sharkey RM, Noren C, Karacay H, Ross EA, et al. Bispecific antibody pretargeting PET (ImmunoPET) with an 124I-labeled hapten-peptide. J Nucl Med. 2006;47(10):1678–88.

139. Hall H, Velikyan I, Blom E, Lill J, Monazzam A, Pahlman L, et al. In vitro autoradiography of carcinoembryonic antigen in tissue from patients with colorectal cancer using multifunctional antibody TF2 and (67/68Ga)-labeled hapten by pretargeting. Am J Nucl Med Mol Imaging. 2012;2(2):141–50.

140. Sharkey RM, Karacay H, Vallabhajosula S, McBride WJ, Ross EA, Chang CH, et al. Metastatic human colon cancer: molecular imaging with pretargeted SPECT and PET in a mouse model. Radiology. 2008;246(3):497–507.

141. Sharkey RM, Cardillo TM, Ross EA, Chang CH, Karacay H, McBride WJ, et al. Signal amplification in molecular imaging by pretargeting a multivalent, bispecific antibody. Nat Med. 2005;11(11):1250–5.

142. Aarts F, Boerman OC, Sharkey RM, Hendriks T, Chang CH, McBride WJ, et al. Pretargeted radioimmunoconjugates in patients with primary colorectal cancer using a bispecific anticarcinoembryonic antigen CEA X anti-di(diehydrentetaminepentaacetic acid F(ab')2 antibody. Cancer. 2010;116(4 Suppl):1111–7.

143. Bodet-Millin C, Faivre-Chauvet A, Carlier T, Rauscher A, Bourgeois M, Cereto E, et al. Immuno-PET using an anticarcinoembryonic antigen bispecific antibody and 68Ga-labeled peptide in metastatic medullary thyroid carcinoma: clinical optimization of the pretargeting parameters in a first-in-human trial. J Nucl Med. 2016;57(10):1505–17.

144. Rajasekaran AK, Anilkumar G, Christiansen JJ. Is prostate-specific membrane antigen a multifunctional protein? Am J Physiol Cell Physiol. 2005;288(3):C975–81.

145. Cardillo MR, Gentile V, Di Silvio R. Correspondence re: Ghosh A and Heston WDW. Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer. J Cell Biochem. 2004;93(4):641–43.

146. Gaumilie LA, Dorchte K, Rahman MM, Grant CL, Stoddard C, Ferrer FA, et al. PSMA redirects cell survival signaling from the MAPK to the PI3K-AKT pathways to promote the progression of prostate cancer. Sci Signal. 2017;10(470).

147. Kiess AP, Banerjee SR, Mease RC, Rowe SP, Rao A, Foss CA, et al. Prostate-specific membrane antigen is a target for cancer imaging and therapy. Q J Nucl Med Mol Imaging. 2015;59(3):241–68.

148. Friedrich M, Baum T, Lutterbuese R, Voelkel M, Deegen P, Rau D, et al. Regression of prostate cancer xenografts in mice by AMG 212/BAY2010112, a novel PSMA/CD3-specific BiTE antibody cross-reactive with non-human primate antigens. Mol Cancer Ther. 2012;11(12):2664–73.

149. Hernandez-Hoyos G, Sebastian T, Brauer J, Chela A, da Silva FM, da Silveira M, et al. MOL89389/ES414, a novel bispecific antibody targeting PSMA for the treatment of metastatic carcinoma-resistant prostate cancer. Mol Cancer Ther. 2016;15(9):2155–65.

150. Baum V, Bühler P, Gierschner D, Hertenbach D, Fiala GJ, Schamel WW, et al. Antitumor activities of PSMAxCD3 diabodies by redirected T-cell lysis of prostate cancer cells. Immunotherapy. 2013;5(1):27–38.

151. Bühler P, Wolf P, Gierschner D, Schaber I, Katzenwadel A, Schulze-Seemann W, et al. A bispecific diabody directed against prostate-specific membrane antigen and CD3 induces T-cell mediated lysis of prostate cancer cells. Cancer Immunol Immunother. 2008;57(1):43–52.

152. Bühler P, Nolten E, Dopfer EP, Wolf P, Gierschner D, Wettersauer U, et al. Target-dependent T-cell activation by coligation with a PSMA X CD3 diabody induces lysis of prostate cancer cells. J Immunother. 2009;32(6):565–73.

153. Fortmuller K, Alt K, Gierschner D, Wolf P, Baum V, Freudenberg N, et al. Effective targeting of prostate cancer by lymphocytes redirected by a PSMA x CD3 bispecific single-chain diabody. Prostate. 2011;71(6):588–96.

154. Muller D, Karle A, Meissbinder B, Hofg I, Stork R, Kontermann RE. Improved pharmacokinetics of recombinant bispecific antibody molecules by fusion to human serum albumin. J Biol Chem. 2007;282(17):12650–60.

155. Trivedi A, Stienen S, Zhu M, Li H, Yuraszek T, Gibbs J, et al. Clinical pharmacology and translational aspects of bispecific antibodies. Clin Transl Sci. 2017;10(3):147–62.