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REVIEW

Stepping forward: T-cell redirecting bispecific antibodies in cancer therapy

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KEY WORDS

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Tumor microenvironment;
Cancer immunotherapy;
Combination therapy strategies

Abstract T cell-redirecting bispecific antibodies are specifically designed to bind to tumor-associated antigens, thereby engaging with CD3 on the T cell receptor. This linkage between tumor cells and T cells actively triggers T cell activation and initiates targeted killing of the identified tumor cells. These antibodies have emerged as one of the most promising avenues within tumor immunotherapy. However, despite success in treating hematological malignancies, significant advancements in solid tumors have yet to be explored. In this review, we aim to address the critical challenges associated with T cell-redirecting bispecific antibodies and explore novel strategies to overcome these obstacles, with the ultimate goal of expanding the application of this therapy to include solid tumors.

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1. Introduction

Cancer has an extremely high incidence and mortality rate worldwide, making it one of the significant public health issues currently, posing a severe threat to human health[1]. Immunotherapy, one of the most promising strategies for tumor treatment that harnesses the body's immune system to combat tumor cells selectively, has received significant attention internationally[2−5]. T-cell-based therapies hold a prominent position in the scope of immunotherapy, which mainly involves three primary categories: immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T-cell therapy, and bispecific antibodies, each addressing the disease through distinct mechanisms. ICIs could block T cell activation negative regulators, such as PD-1 and CTLA-4. By inhibiting the interaction between these checkpoint molecules and their ligands, these therapies rejuvenate the immune system's ability to combat cancer. However, ICIs induce long-term remission only in patients with specific cancer types and only in a minority of patients. CAR T therapy is to couple the potency of a T cell with the specificity of an antibody to kill diseased cells precisely. However, the production of CAR T cells necessitates *ex vivo* genetic modification and proliferation, typically of autologous T cells, which is a time-consuming, relatively inefficient, and costly process. Bispecific antibodies (BsAbs) are composed of two distinct antibodies, enabling them to bind to either two epitopes of the same antigen or two distinct antigens[6,7]. Due to the complexity of the tumor's immunosuppressive microenvironment and the immune escape mechanisms, single immune-modulating strategies still have significant limitations, such as their capacity only to solely target individual molecules, insufficient treatment, or side effects[8,9]. BsAbs, especially T cell-redirecting BsAbs, have emerged as a promising and progressive avenue in T-cell immunotherapy[10].

So far, over 100 BsAbs are undergoing clinical research. Among these, thirteen BsAbs have been granted market approval, with nine targeting the CD3 receptor (Figs. 1 and 2, and Table 1)[11−15]. In the architecture of a T-cell redirecting BsAb, one arm is designed to bind to the tumor cell surface antigen tumor-associated antigens (TAA), while the other arm is connected to the CD3ε subunit of the T cell receptor complex (TCR)[16-19]. These antibodies can remarkably bridge the tumor and T cells, facilitate interactions with T cells, and trigger cytotoxic responses[20,21]. In a concise overview, the T-cell redirecting BsAb establishes an immune synapse upon binding the target and T cells[21]. This synapse enables T cells to transport cytolytic proteins such as perforin and apoptosis-inducing proteins like granzymes) to the target cell membrane, effectively leading to tumor cell killing. The cytolytic activity of $CD3^+$ T cells is redirected towards tumor cells, promoting their elimination independent of major histocompatibility complex (MHC) molecules, thus bypassing the constraints of MHC limitation[6]. The synapse formation also triggers the cross-linking of TCR, subsequent activation of T cells, releasing pro-inflammatory cytokines, and induction of T cell proliferation. This review provides a broad overview of the current challenges and future perspectives for developing T-cell redirecting BsAbs for cancer treatment, which provides insights into the research and development of novel cancer therapeutics using BsAbs.

2. Challenges of T-cell redirecting BsAbs

T-cell redirecting BsAbs face numerous challenges in cancer immunotherapy, mainly toxicity, loss of TAA, the tumor microenvironment, infections, and tumor-lysis syndrome. Toxicity includes on-target off-tumor toxicities (target expression in healthy tissues), increased cytokine release leads to cytokine release syndrome (CRS), and immune effector cell-associated neurotoxicity syndrome (ICANS). The release of tumor cells after lysis also causes metabolic abnormalities. Furthermore, tumor cells can suppress T cell activation and evade immune surveillance through various mechanisms, such as antigen expression loss, antigen endocytosis, or shedding. Immunosuppression of the TME and infection also enhances the side effects of BsAbs (Fig. 3). The clinical presentation ranges from mild symptoms, such as fever, to severe symptoms, such as hypoxia and hypotension.

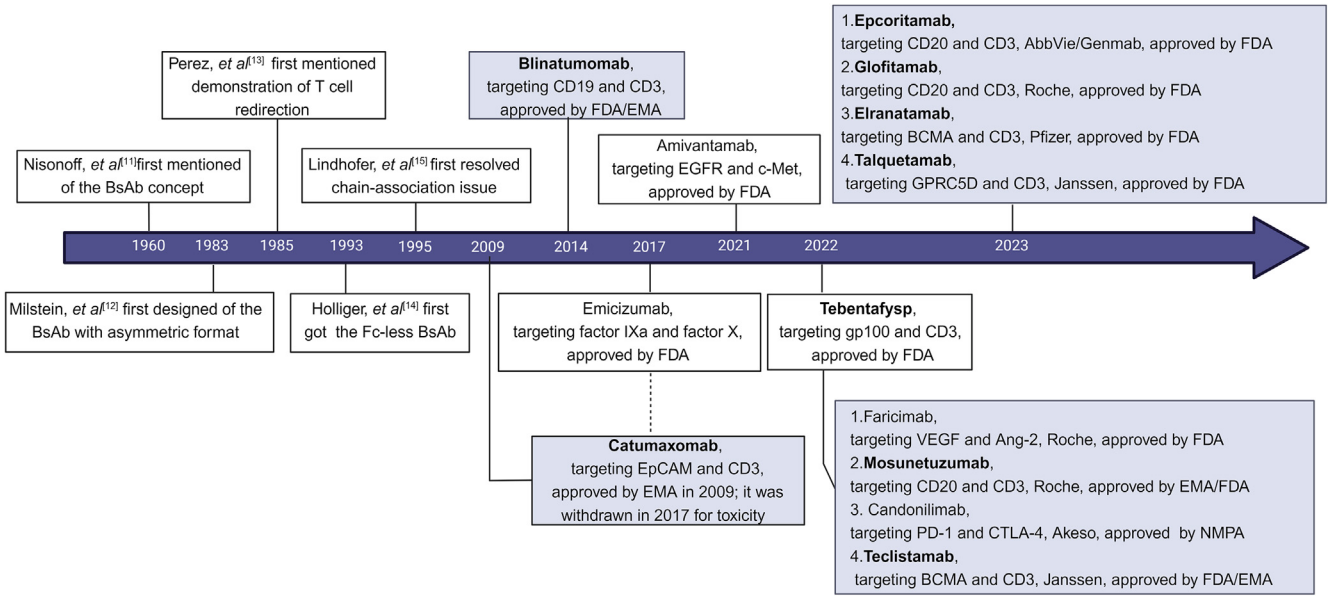

Figure 1 Timeline of some major advances in bispecific antibodies.

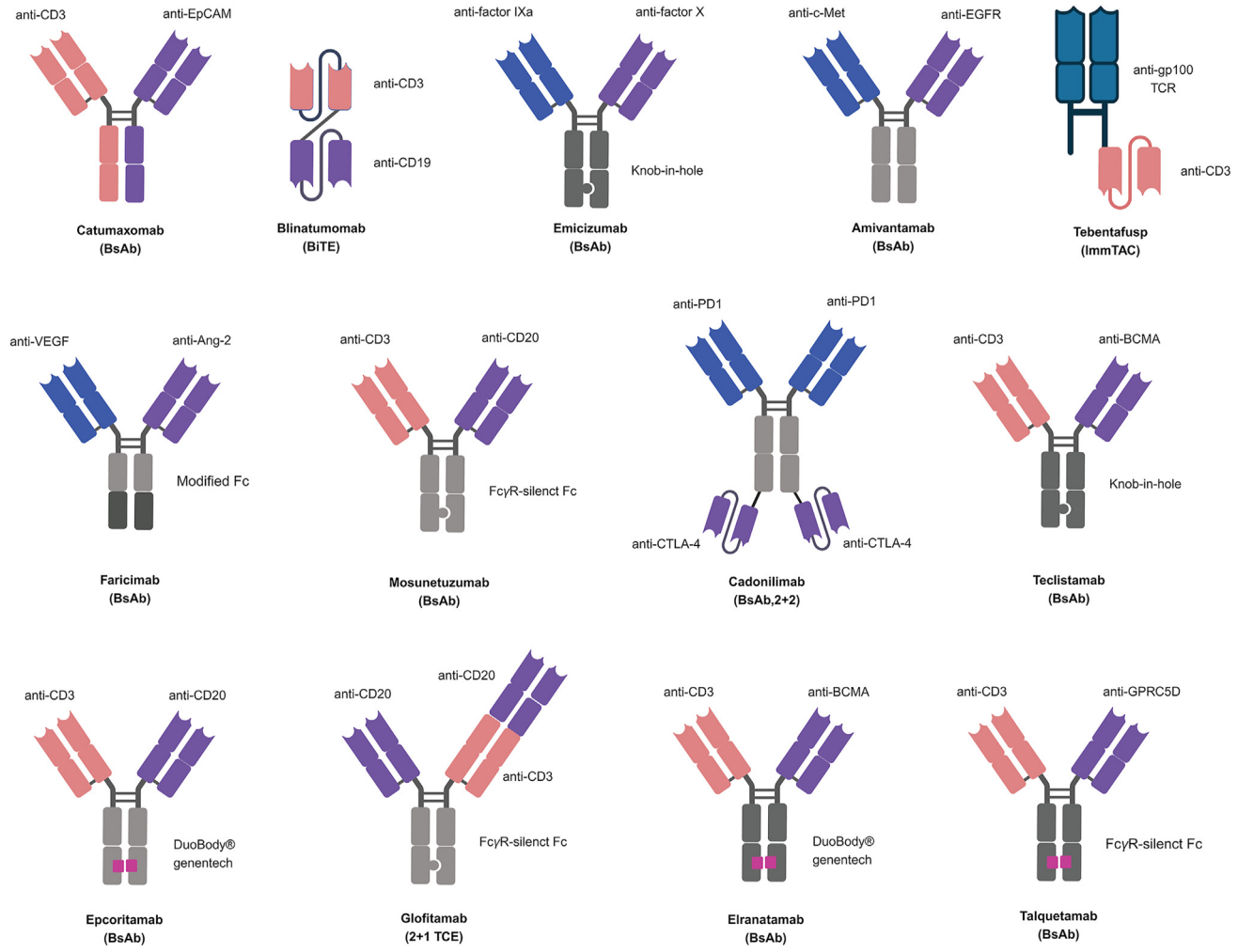

Figure 2 Schematic structures of thirteen bispecific antibodies that have been approved. Bispecific antibodies recognize antigens (blue and purple) by two different antigen-binding arms, and most bispecific antibodies are established on IgG1/4 scaffolds. IgG: immunoglobulin G; CD3: cluster of differentiation 3; EpCAM: epithelial cell adhesion molecule; CD19: cluster of differentiation 19; EGFR: estimated glomerular filtration rate; c-Met: cellular-mesenchymal epithelial transition factor; VEGF: vascular endothelial growth factor; Ang-2: angiopoietin-2; PD-1: programmed cell death protein 1; CTLA-4: cytotoxic T lymphocyte associate protein-4; BCMA: B cell maturation antigen; CD20: cluster of differentiation 20.

2.1. Toxicity

2.1.1. On-target, off-tumor toxicity

The first challenge in T-cell redirecting BsAbs immunotherapy is identifying and differentiating therapeutic targets in normal cells from those in tumor cells. Most membrane proteins are conserved between tumor and healthy cells[22]. Ideal TAAs are limited to a small fraction of tumors, and solid tumor TAAs are usually expressed on tissues of healthy organs[23,24], resulting in varying degrees of off-tumor toxicity[25]. Some proteins with significant expression differences in solid tumors are intracellular proteins, but traditional antibodies cannot penetrate the cells[26,27]. These intracellular proteins require recognition through HLA class I molecules, such as ImmTACs or TCR-like antibodies[28–30]. TCR-like CD3 BsAbs still exhibit on-target-off-tumor side effects in clinical trials. For example, dose-limiting toxicity was observed in clinical trials of tebentafusp. Tebentafusp is a bispecific protein composed of a soluble T-cell receptor incorporating single-chain variable fragments targeting the CD3 domain[31–33]. This high-affinity, high-specificity T-cell receptor targets a 9-amino acid peptide derived from intracellular gp100 protein through proteolytic degradation, presented by HLA molecules on the surface of target cells (including melanocytes and melanoma cells). By targeting tumor-associated antigens specifically shared by tumors, these T-cell receptor bispecific molecules can recruit T cells and direct them toward tumors. Patients treated with tebentafusp experienced skin-related adverse events, possibly due to the recognition of gp100-expressing melanocytes. In order to improve the selectivity and specificity of tumor antibodies and reduce off-tumor toxicity, many researchers have designed novel T-cell redirecting BsAbs. For example, they enhance the binding to tumor cells by adding a second tumor-associated antigen (TAA) binding domain. If the affinity to the TAA is too high, it may lead to binding to low-expressing TAAs on healthy cells. On the other hand, if the affinity to the TAA is too low, it may affect the efficiency of T-cell redirecting BsAbs. Besides, the design of antibodies can be improved to develop BsAbs with a modulatory activity that is only activated in the acidic tumor microenvironment. The acidic pH is due to the

Table 1 Overview of clinical advances in T-cell redirecting bispecific antibodies in the treatment of tumors.

Drug	Target	Indication	Phase	Clinical trial	Status	Last update posted	Research institution
Cibisatamab	CD3 × CEA	Metastatic colorectal cancer	II	NCT04826003	Recruiting	2023/8/1	Roche Holding AG
Nivatrotamab	CD3 × GD2	Neuroblastoma, small cell carcinoma, osteosarcoma	II	NCT03033303	Active, not recruiting	2023/7/5	Y-mAbs Therapeutics, Inc. Memorial Sloan Kettering Cancer Center
BNT-142	CD3 × CLDN6	Solid tumor	I/II	NCT05262530	Recruiting	2023/6/22	BioNTech SE
GEN-1047	CD3 × B7−H4	Breast cancer, endometria cancer, ovarian cancer	I/II	NCT05180474	Recruiting	2023/4/21	Genmab A/S
GEN1044	CD3 × 5T4	Locally advanced or metastatic solid tumor(s), prostate cancer, esophageal cancer	I/II	NCT04424641	Terminated	2023/2/1	Genmab A/S
CD30 biAb-AATC	CD3 × CD30	Pediatric cancer, Hodgkin disease, CD30-positive diffuse large B-cell lymphoma	I/II	NCT05544968	Not yet recruiting	2022/9/19	Medical College of Wisconsin Research Foundation
CM-350	CD3 × GPC3	Advanced solid tumor	I/II	NCT05263960	Recruiting	2022/6/9	Keymed Biosciences Inc.
RO-7444973	CD3 × MAGEA4	Solid tumor	I	NCT05129280	Terminated	2023/8/4	Roche Holding AG
A-337	CD3 × EpCAM	EpCAM expressing solid tumours	I	CTR20232278	Not yet recruiting	2023/8/2	Evive Biotech
Runimotamab	CD3 × HER2	Solid tumor	I	NCT03448042	Recruiting	2023/7/13	Genentech, Inc.
ERY-974	CD3 × GPC3	Hepatocellular carcinoma (HCC)	I	NCT05022927	Recruiting	2023/7/10	Chugai Pharmaceutical Co., Ltd.
AMG-199	CD3 × MUC17	MUC17-positive solid tumors	I	NCT04117958	Terminated	2023/6/22	BeiGene Pharmaceuticals (Guangzhou) Co., Ltd.
BA-1202	CD3 × CEA	Advanced solid tumor	I	NCT05909241	Not yet recruiting	2023/6/22	Luye Pharma AG
Vixtimotamab	CD3 × CD33	AML, childhood	I	NCT05077423	Terminated	2023/5/30	Amphivena Therapeutics, Inc.
BA-3182	CD3 × EpCAM	Advanced adenocarcinoma	I	NCT05808634	Not yet recruiting	2023/4/11	BioAtla, Inc.
EMB-07	CD3 × ROR1	Advanced/metastatic solid tumors	I	NCT05607498	Recruiting	2023/3/17	EpimAb Biotherapeutics, Inc.
CX-904	CD3 × EGFR	Solid tumor	I	NCT05387265	Recruiting	2023/2/28	Amgen, Inc.
JNJ-63898081	CD3 × PSMA	Neoplasms	I	NCT03926013	Completed	2022/11/7	Janssen Research & Development LLC
GBR-1342	CD3 × CD38	HER2 expressing solid tumors	I	NCT02829372	Terminated	2020/10/9	Ichnos Sciences, Inc.
PF-6671008	CD3 × CDH3	Neoplasms	I	NCT02659631	Terminated	2020/5/6	MacroGenics, Inc.

CEA: carcinoembryonic antigen; GD2: disialoganglioside GD2; CLDN6: claudin-6; GPC3: glypican-3; MAGEA4: melanoma-associated antigen A4; HER2: human epidermal growth factor receptor 2; ROR1: inactive tyrosine-protein kinase transmembrane receptor; EGFR: epidermal growth factor receptor; PSMA: prostate-specific membrane antigen; CDH3: cadherin-3.

lactic acid produced by the glycolytic pathway used by tumor cells. The acidic environment creates an immunosuppressive microenvironment that helps tumor cells evade the immune system while also affecting the bioavailability of classical antibodies. Researchers have developed selective antibodies and CAR-T cell therapy technologies based on acidic pH, which can increase the TME targeting of antibodies and CAR-T cells and reduce on-target, off-tumor toxicity. BA3182, a conditionally active biologic, possesses two binding domains for EpCAM and CD3ε. *In vitro* studies reveal that BA3182 can specifically and reversibly bind to the target proteins EpCAM and CD3 under the acidic conditions characteristic of the tumor microenvironment. Conversely, at the physiological alkaline pH of 7.4 found in normal cells, its binding affinity is considerably diminished. *In vivo* research indicates that BA3182 offers a therapeutic index surpassing non-CAB variants by over a hundredfold, underscoring the immense potential of CAB BsAbs compared to their traditional counterparts (NCT05808634).

Furthermore, by combining bispecific antibodies with other forms of treatment, such as immunomodulators and small molecule drugs, comprehensive strategies can significantly enhance the selectivity and specificity of bispecific antibodies, maximize their therapeutic effects, and simultaneously reduce off-target toxicity to normal tissues.

2.1.2. CRS

Cytokine release syndrome (CRS) emerges due to excessive activation of immune cells, marking a severe immune-related adverse reaction[33]. CRS is characterized by a substantial surge in pro-inflammatory cytokines within the bloodstream, including IL-6, IL-10, TNF-α, and IFN-γ, leading to a cytokine storm.

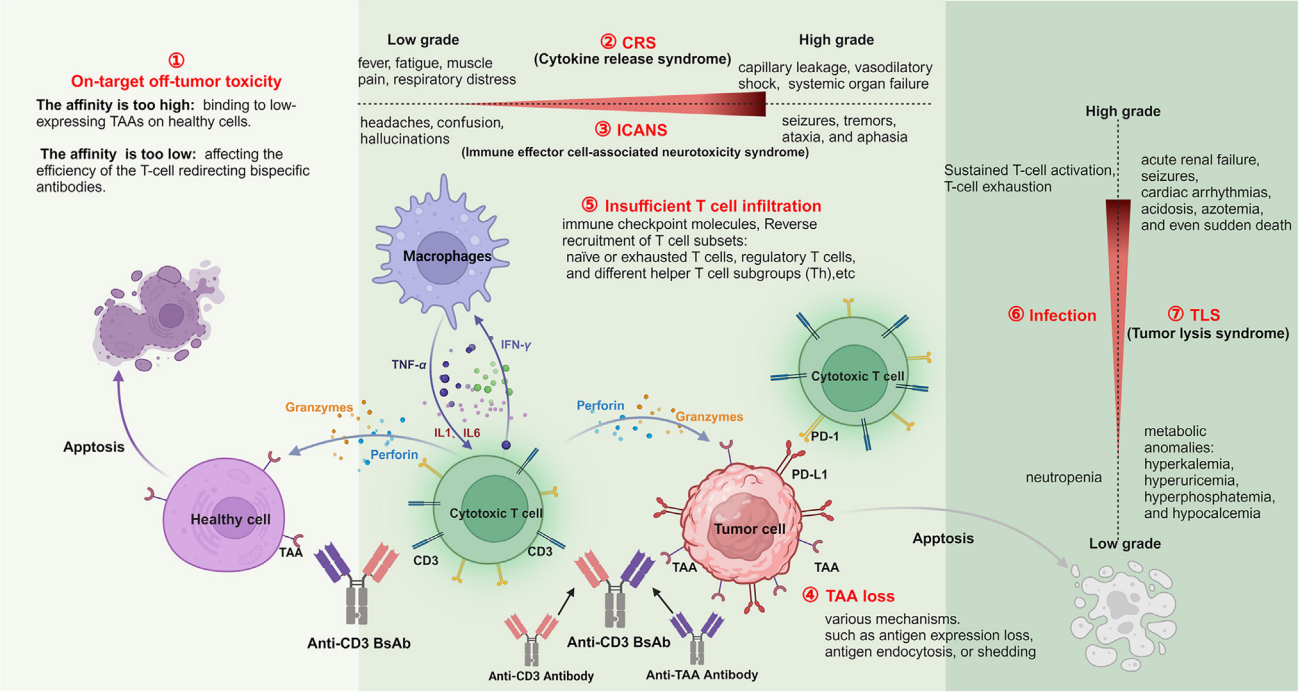

Figure 3 Challenges of T-cell redirecting bispecific antibodies in cancer immunotherapy. ① On-target, off-tumor toxicity. A subset of solid tumor antigens is expressed in tissues of healthy organs, resulting in varying degrees of off-tumor toxicity. ② Cytokine release syndrome (CRS). A significant surge of proinflammatory cytokines due to overactivation of immune cells leads to a cytokine storm. ③ Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). ④ TAA loss. Tumor cells can suppress T cell activation and evade immune surveillance through various mechanisms, such as antigen expression loss, antigen endocytosis, or shedding. ⑤ Tumors microenvironment (TME). It includes insufficient T cell infiltration and reverse recruitment of T cell subsets. A significant feature of solid tumors is the presence of an immunosuppressive TME. Secondly, in addition to recruiting effector T cells, T-cell redirecting bispecific antibodies also recruit other immune cells, which may lead to drug resistance. ⑥ Infection. Infections are common in patients receiving bispecific antibody therapy, and combination therapy increases the risk of infection. ⑦ Tumor-lysis syndrome (TLS) is attributed to the breakdown of tumor cells, which release their contents into the systemic circulation, resulting in metabolic abnormalities.

Clinical manifestations of CRS include fever, fatigue, muscle pain, respiratory distress, capillary leakage, vasodilatory shock, and systemic organ failure[34].

The pioneering BsAb drug, Catumaxomab, showed promising efficacy in treating malignant ascites. However, its strong immunogenicity gave rise to pronounced hepatotoxicity and triggered a high-intensity cytokine storm when it went off-target, leading to its withdrawal from the market in 2017[35]. CRS is the predominant toxicity concern in therapies involving BsAbs that bridge T cells[36]. Existing pharmacological interventions, such as the immunosuppressant agent dexamethasone and the IL-6 receptor inhibitor tocilizumab, offer promising avenues for mitigating CRS. In 2017, due to insufficient endogenous immune response to clear patients' cancer, Ishiguro and colleagues designed a BsAb targeting CD3/GPC3 for solid tumors. In a monkey model, the main adverse event was cytokine storm, mitigated by pre-treatment with dexamethasone without compromising anti-tumor efficacy. In the dose-escalation phase I trial, 29 subjects were enrolled, with a dose range of 0.003−0.81 μg/kg[37–39]. The results showed that more than 20% of the patients experienced CRS reactions and fever. The dose of 0.81 μg/kg was found to be intolerable; however, doses lower than 0.81 μg/kg were generally well-tolerated, and adverse events could be managed with corticosteroids[40] and anti-IL-6 receptor inhibitors (NCT05022927).

TNB-383B, co-developed by Tenebio and AbbVie, is a novel BsAb featuring a unique structural configuration: two αBCMA molecules on one arm, a singular αCD3 arm, and a silent IgG4 Fc segment[41]. This design endows it with a potent T-cell activating capacity and a low-affinity anti-CD3 activity, which balances robust cytotoxic effects with minimized cytokine release. In phase I clinical trials, TNB-383B is evaluated for safety, tolerability, and pharmacokinetics, including its maximum concentration and half-life in the circulatory system. Preliminary data suggest a dosage-dependent therapeutic response, with an impressive 80% overall response rate (ORR) at doses equal to or exceeding 40 mg[42]. Common side effects encountered in the trials include CRS, fatigue, headaches, reduced blood cell counts, infections, and nausea.

In November 2023, the BsAb targeting CD3/CD20, Glofitamab, was approved by the NMPA for marketing in China. Before that, in March 2023, Glofitamab was approved for marketing in Canada; in June, it was approved by the US Food and Drug Administration (FDA) for marketing. Glofitamab, a 2 + 1 CD3/CD20 BsAb, targets T cells to malignant B cells to exert its anti-tumor effects[43,44]. In a Phase II clinical trial for the treatment of aggressive lymphoma, CRS was the most common adverse reaction, occurring in the majority (approximately 63%) of patients, mainly at Grade 1 (47.4%) and Grade 2 (11.7%), and one patient discontinued the trial due to CRS.

BsAbs currently demonstrate potential effectiveness in treating certain malignant tumors; however, CRS, as a significant adverse effect issue, remains to be addressed. As a result, future research may focus more on the design and improvement of BsAbs. These

antibodies aim to maximize the targeted effects on tumor cells while minimizing the impact on healthy tissues and controlling the host's inflammatory response. Additionally, drugs such as dexamethasone used in pre-treatment have shown potential in reducing CRS induced by BsAbs treatment. Future therapies may adopt more of this kind of strategy, which includes using more advanced immunosuppressants or inflammation pathway-specific inhibitors to decrease the toxic side effects associated with treatment.

2.1.3. *Neurotoxicity*

Neurotoxicity of BsAbs in tumor immunotherapy is often an indirect consequence, transpiring concurrently with CRS or shortly after CRS[45−49]. Nevertheless, it can also occur independently of CRS[41,42]. This form of neurotoxicity is referred to as ICANS and is characterized by a range of symptoms, including headaches, confusion, hallucinations, seizures, tremors, ataxia, and aphasia[39]. The risk of ICANS can be mitigated through dose escalation and premedication[45]. Supportive care interventions for ICANS-affected patients involve the administration of tocilizumab (when CRS is concurrent), corticosteroids, or antiepileptic drugs.

Neurotoxicity has been observed in a subset of patients with B-cell hematological malignancies who underwent T-cell engager therapy[50−56]. Blinatumomab, a T-cell redirecting BsAb, has been developed to target CD19, an antigen expressed on B-cell acute lymphoblastic leukemia cells. In a multicenter, single-arm, phase 2 clinical trial, blinatumomab rarely induced adverse events in tumor immunotherapy; they were largely transient and resolved effectively. However, a majority of these adverse events exhibited a notable association with a high rate of neurotoxicity (all grades at 47%−53%; grade 3 or higher at 7%−13%)[57−60]. Compared to treatments targeting other antigens, CD19-directed BsAbs demonstrated a heightened frequency of neurotoxicity, a phenomenon potentially attributed to the presence of CD19 on cells in the blood−brain barrier[61].

Clinicians can formulate precision therapeutic strategies by deepening our understanding of the risk factors and mechanisms underlying neurotoxicity. These strategies involve adjusting dosages and selecting appropriate antibody treatments contingent on a patient's clinical symptoms, genetic predispositions, and immune system status. Enriched knowledge of ICANS paves the way for innovative support treatments. The future holds promise for BsAbs and related immunotherapies to reduce side effects and enhance patient safety while effectively combatting cancer.

2.2. *TAA loss*

The development of T-cell redirecting BsAbs is predicated on identifying and TAAs. Nevertheless, tumor cells can inhibit T cell activation and circumvent immune detection through several strategies, including losing antigen expression, antigen endocytosis, or shedding. The immune system's elimination of cells exhibiting tumor-specific antigens can give rise to variations of the tumor that lack these antigens. Additionally, analogous to viral "antigenic drift", tumor cells can evade T cell-mediated destruction by modifying their antigenic profile through epitope mutations. The CD19 antigen has emerged as an effective target for the immunotherapeutic treatment of acute lymphoblastic leukemia, yet resistance to the BsAb blinatumomab, targeting CD3/CD19, has been observed during treatment[62]. Flow cytometry analysis of tumor tissues from resistant patients revealed mutations in the CD19 antigen, indicative of TAA loss. Further analysis showed no structural anomalies in the CD19 gene, suggesting the presence of complete antigen loss escape variants, as opposed to single-epitope loss or splice variants.

BsAb research faces obstacles in treating mature solid tumors such as gastrointestinal cancer, melanoma, and glioma due to the scarcity of identifiable tumor-specific antigens and the underlying heterogeneity of solid tumor cells. Therefore, selecting optimal targets predominantly found on tumor cells and displaying minimal expression on healthy cells to reduce collateral damage to normal tissue while enhancing treatment specificity is crucial. Given tumors' diverse nature and variability, therapies concentrating on a single antigen may prove insufficient to eradicate cancer cells. Future research should pivot towards combination therapies that concurrently target multiple antigens, thereby increasing the likelihood of therapeutic success and mitigating the risk of resistance emergence.

2.3. *Tumors microenvironment*

2.3.1. *Insufficient T cell infiltration*

Compared to hematologic malignancies, one distinguishing feature of solid tumors is the presence of an immunosuppressive TME[63,64]. The TME encompasses a complex interplay of elements, including immune suppressor cells, stromal cells, immune suppressor cytokines, and soluble molecules, all inhibiting antitumor immunity[29]. Both tumor cells and immune suppressor cells express immune checkpoint molecules, which upregulation often leads to tumor cell immune evasion[64−66]. Moreover, tumor cells employ other tactics to evade immune system surveillance. Under normal circumstances, there exists a dynamic balance between molecules of immune activation and immune suppression[67], collectively maintaining the stability of the body's immune system[68]. However, excessive expression of immune suppressor factors on tumor or T cells disrupts T cell activity, leading to exhaustion and restraining the infiltration of $CD8^+$ T cells into the tumor microenvironment[69−71]. Inadequate tumor-infiltrating lymphocytes into tumors may also be attributed to prolonged antigen stimulation in the TME[71], causing a decline in the frequency of tumor-specific T cell clones and a reduction in effector T cells, culminating in T cell exhaustion[72,73].

2.3.2. *Reverse recruitment of T-cell subsets*

T-cell redirecting BsAbs are designed to interact with tumor target cells and effector T cells, as brief contact can promote the formation of an immune synapse between T cells and tumor cells[74]. Afterward, the presence of perforin and granzyme in the immune synapse is observed[75], subsequently facilitating the elimination of tumor cells by T cells. Most cytotoxic T cells (CTL), such as $CD8^+$ T cells, $CD4^+$ T cells, $\gamma\delta$T cells, and NKT cells, can participate in tumor cell lysis through this mechanism[76]. $CD8^+$ T cells within CTL are the primary effector cells[77]. Intravenous administration of T cell redirecting bispecific antibodies has shown the ability to recruit or activate CTL cells directly, alongside recruiting other immune cells[78]. However, this recruitment is not exclusive and may encompass various T cell subgroups, including those with suppressive functions such as naïve or exhausted T cells, regulatory T cells, and different helper T cell subgroups (Th). This indiscriminate recruitment could lead to drug resistance, weakening the therapeutic potential of tumor cell killing by $CD8^+$ T cells. Notably, a clinical study has indicated that resistance to Binatumomab arises from $CD3^+$ $CD4^+$ CD25 hi*FoxP3*$^+$ Treg cells[20], which hinder the dissolution of $CD8^+$ T cells and suppress their tumor-killing capabilities. Additionally, the recruited $CD4^+$ T cells,

predominantly Th1 and Th17, often release cytokines and spark a cytokine storm. Therefore, enhancing the specificity of BsAbs can lead to better cell killing and reduce cytotoxic effects. Furthermore, exploring alternative targets for T cell recruitment or activation, such as CD28, emerges as a strategic avenue to address the non-selective recruitment of T cells by BsAbs in immune therapy.

2.4. *Infection*

Infections are frequent in patients treated with BsAbs, although their frequency varies considerably across diverse clinical studies[79,80]. Apart from tumor type, this could be associated with the extent of prior immunosuppressive therapy. The frequency of neutropenia caused by BsAbs may also fluctuate among hematological malignancies[81–83]. Furthermore, sustained T cell activation mediated by BsAbs may lead to T cell exhaustion, thereby increasing the risk of infection. Additionally, the combinations of T-cell redirecting BsAbs with other treatment strategies may also increase the incidence and severity of infections.

2.5. *Tumor-lysis syndrome (TLS)*

TLS represents a metabolic emergency attributed to the breakdown of a substantial number of tumor cells, which release their contents into the systemic circulation. This phenomenon results in metabolic anomalies like hyperkalemia, hyperuricemia, hyperphosphatemia, and hypocalcemia[84]. These metabolic abnormalities can lead to acute renal failure, seizures, cardiac arrhythmias, acidosis, azotemia, and even sudden death. Although BsAbs can potentially reduce tumor burden, the development of TLS may also occasionally occur. Patients at risk of TLS, particularly those with elevated tumor burden or impaired renal function, should receive prophylaxis measures such as uric acid-lowering medications and adequate hydration[85-88].

Building on our understanding of TLS, future research and clinical practices should prioritize the identification of reliable biomarkers for predicting TLS risk, thereby enabling prophylactic interventions before its onset. Given that acute renal failure is one of the principal complications associated with TLS, developing strategies and pharmaceuticals to preserve renal function represents a critical area of investigation.

3. Implications for treatment strategies

3.1. *Development of T-cell redirecting BsAbs in different formats*

3.1.1. *Antibody potency*

The design of BsAbs configurations can impact the target binding site[89]. A bivalent BsAb featuring one binding site for each target is called 1 + 1; introducing additional binding sites can result in tri-specific antibodies (2 + 1) or quadrivalent BsAb (2 + 2 or 1 + 3). Although traditional BsAbs maintain a 1:1 ratio of CD3 and TAA[90], enhancing the binding capacity through a 1 + 2 design may bolster the recognition of tumor cells while avoiding activation of CD3 on T cells in the absence of TAA. IGM2323 is a 10+1 type targeting CD20/CD3[91]. IGM-2323 is structured by the fusion of anti-CD3 scFv to the J chain, forming a 10:1 antibody form of anti-CD20: anti-CD3. IGM-2323 superimposed the TDCC effect on the action of CDC and played a significantly enhanced B-cell clearance effect, demonstrating the synergistic effect of BsAb (NCT04082936). Compared to rituximab, IGM-2323 has a 1000-fold increase in killing activity.

Among the BsAbs that have been marketed, Roche has two BsAbs targeting CD20/CD3-Mosunetuzumab and Glofitamab[92–96]. Although the two new drugs are dual antibodies with the same target, their structure and indications differ. Mosunetuzumab has a 1 + 1 structure, while Glofitamab has a 2 + 1 structure. The former is approved for follicular lymphoma and the latter for treating DLBCL. This 2 + 1 structure design makes it more binding to CD20 on the surface of B cells, induces rapid T cell activation and cytokine release, and leads to target cell lysis. The activity is 10−1000 times higher than traditional 1 + 1 BsAbs. In the Phase Ib/II GO40516 study, Mosunetuzumab and Polivy achieved an ORR of 65.0% in severely treated R/R NHL patients, with a CR of 48.3%. CRS occurred in 18%, and all adverse events occurred in the first treatment cycle and were grade 1 to 2. In the Phase I/Ib dose-escalation NP30179 study, Glofitamab alone achieved an ORR of 81.0% for patients with R/R NHL who received severe treatment and 100% ORR in the glofitamab plus Gazyva group. In the Phase Ib/II NP39488 study, Glofitamab and Polivy had an ORR of 73% over a 3.2-month follow-up period, including a CR of 51.5%, and no CRS above grade 3 was observed.

3.1.2. *Tri-specific antibody*

Among the successful cancer immunotherapies, approved BsAbs such as Blinatumomab have stimulated researchers' interest in other multispecific antibody therapies[97]. T-cell redirected tri-specific antibodies represent a promising multi-targeting immune intervention; some are currently in the clinical research stage (see Table 2). Tri-specific antibodies extend from BsAbs by introducing an extra antigen binding site, totaling three sites. This enables simultaneous interaction with target and effector cells (typically T cells or NK cells), enhancing the killing of target cells[97,98]. Compared to monoclonal and BsAbs, some tri-specific antibodies have two additional specific antigen binding sites, which are advantageous for increasing binding specificity, improving the accuracy of drug targeting to tumor cells, and reducing off-target toxicity[99,100]. Furthermore, some tri-specific antibodies bind to tumor antigens through one binding site; the second binding site on the other arm can specifically bind to the TCR on T cells, thereby activating the first signal pathway and guiding T cells to the vicinity of target cells to achieve the aggregation/localization of immune cells[101]. The third binding site on the arm triggers the second signal pathway, further activating immune cells. Those tri-specific antibodies have a strong cytotoxic effect on target cells through bridging and activating immune cells simultaneously or bridging immune cells and blocking dual signal pathways.

For example, Wu et al.[100] and Sun et al.[102] developed a T-cell redirected tri-specific antibody, SAR-442257, that interacts with CD38, CD3 and CD28. The engagement of CD3 and low-affinity CD28 affords efficient T cell activation ability, whereas the anti-CD38 antibody directs T cells to certain lymphomas, leukemias, and myeloma cells. *In vitro*, SAR-442257 showed 1000−10,000-fold higher cytotoxic activity against myeloma cells than CD38 monoclonal antibody. This promising approach is being explored for relapsed/refractory multiple myeloma (MM) and non-classical Hodgkin's lymphoma patients within phase I clinical trials (NCT04401020). Sun et al.[102] and Seung et al.[103] developed a HER2/CD3/CD28 tri-specific antibody based on the HER2/CD3 BsAb. Coupling an anti-CD28 variable domain to the anti-CD3 variable region in a humanized mouse model, regression of breast cancer cells mediated by $CD4^+$ and $CD8^+$ T cells was

Table 2 Representative clinical trials of the combination strategy.

Combination strategy	Antibody	Target	Phase	Clinical trial	Adaptive symptom	Source of information
Antibody potency	IGM2323, 1 + 10	CD3/CD20	I/II	NCT04082936	Non-Hodgkin lymphoma, follicular lymphoma, DLBCL	https://www.clinicaltrials.gov/
	Glofitamab, 2 + 1	CD3/CD20	Marketing	–	DLBCL	2023, FDA
Trispecific antibody	HPN-424	CD3/PSMA/albumin	I/II	NCT03577028	Prostatic cancer	https://www.clinicaltrials.gov/
	CMG1A46	CD3/CD19/CD20	I/II	NCT05348889	Acute lymphoblastic leukemia, non-Hodgkin lymphoma, hematologic neoplasms	
	PIT-565	CD2/CD3/CD19	I	NCT05397496	B-cell lymphoma, adult lymphoblastic lymphoma, non-hodgkin lymphoma	
	SAR-442257	CD3/CD28+/CD38	I	NCT04401020	Neoplasms, non-Hodgkin lymphoma, multiple myeloma	
	JNJ-80948543	CD3/CD20/CD79B	I	NCT05424822	B-cell chronic lymphocytic leukemia, non-Hodgkin lymphoma	
Pro-drug	AMX-818	CD3+HER2/EGFR	I	NCT05356741	Locally advanced or metastatic HER2-expressing cancers	Ref [180]
	ADG138	CD3+HER2	IND-enabling	–	Solid tumors	AACR poster, Adagene's official website
	CX-904	CD3+EGFR	I	NCT05387265	Solid tumours	AACR poster, Adagene's official website
Mobilization of other immune cells	AFM13	CD30/CD16	I/II	NCT04074746	Anaplastic large cell lymphoma, B-cell non-Hodgkin lymphoma, classic Hodgkin lymphoma	https://www.clinicaltrials.gov/
Reducing affinity of CD3 arm	Elranatamab	CD3/BCMA	IV	NCT06057402	Multiple myeloma	Ref [138–142]
	TNB-585	CD3/PSMA	I	NCT04104607	Castration resistant metastatic prostate cancer	Ref [126]
	MCLA-117	CD3/CLL	I	NCT03038230	Acute myeloid leukemia	Ref [130]
	NILK-2301	CD3/CEACAM5	Drug discovery	–	CEACAM5-expressing cancers	Ref [181]
Combining with chemotherapy drugs	Chemotherapy+ Mosunetuzumab	CD3+CD20	I	NCT05464329	Diffuse large B cell lymphoma, high-grade B-cell lymphoma, transformed B-cell lymphoma	https://www.clinicaltrials.gov/
Combining with radiation therapy	p53-SADA-BsAb	GD2	I	–	Non-small cell lung cancer, melanoma, Sarcoma	Ref [137,155–158]
Combining with oncolytic viruses	EphA2 TEA VV	EphA2	Drug discovery	–	Solid tumors	Ref [140]
	OAd-MUC16-BiTE	MUC16	Drug discovery	–	Ovarian cancer	Ref [165]
Combining with targeted therapy	BHKis + CD3/ CD19 BsAb	CD3+CD19	Drug discovery	–	Chronic lymphocytic leukemia	Ref [170]
Combining immune checkpoint inhibitors	M7824	PD-L1+TGFβ	II	NCT03840902	Non-small cell lung cancer	https://www.clinicaltrials.gov/
	SHR1701	PD-L1+TGFβ	I/II	NCT04407741	Solid tumors, lymphomas	

observed. The single-valent anti-CD28 utilized in this tri-specific antibody did not induce the severe cytokine release observed previously with the bivalent superagonist anti-CD28 agents. This antibody is currently undergoing phase I clinical trials (NCT05013554). T-cell redirecting BsAbs emerge as an ideal anti-tumor therapy, representing a multitargeted immunotherapeutic intervention with promising prospects for immunotherapy against human cancers.

3.1.3. *Pro-drug*

T-cell redirected bispecific antibodies, exemplified by Blinatumomab (CD19/CD3), have succeeded in hematologic malignancies[104−107]. However, in solid tumors, the development of such BsAbs faces challenges, primarily due to T-cell overstimulation or on-target off-tumor effects causing damage to normal tissues[20,108]. Researchers are addressing these issues from multiple angles, one approach being tumor-specific activation of bispecific antibodies. The pro-drug design is used to reduce the specific killing of normal tissue cells by BsAb drugs[109], significantly improving the effectiveness and safety of BsAb drugs[110]. In normal tissues, BsAb drugs exist in the form of pro-body without activity[111]. The TME harbors abundant matrix metalloproteinases[112]. Upon reaching specific sites in the tumor area, they undergo enzymatic cleavage by certain enzymes, removing a small peptide segment that masks the binding site, thereby enabling the BsAb to exert its function[111,112].

Tianyan Pharmaceuticals' SAFEbody BsAb platform technology effectively employs this concept in design. ADG138 is a BsAb targeting HER2 × CD3, where the epitopes of HER2 and CD3 are masked[113]. Upon reaching the target site, the masking peptide is removed, exposing the Fab region of the antibody and revealing the binding sites for anti-HER2 and anti-CD3. This allows for the normal binding of the antibody to the antigens on tumor cells, recruiting and activating T cells to kill tumor cells selectively. CI107, a BsAb, consists of the cetuximab antibody targeting EGFR and the humanized SP34 antibody targeting CD3, fused in scFv form at the N-terminus of the cetuximab antibody heavy chain[114]. CI107 utilizes Tianyan Pharmaceuticals' SAFEbody platform to mask the epitopes of EGFR and CD3. Short masking peptides that can be specifically cleaved are fused at the N-termini of both the cetuximab antibody light chain and the scFv. *In vivo*, CI107 exhibited a potent, dose-dependent induction of tumor regression in established colon cancer xenografts mice. CI107 significantly reduced cytokine release and showed no elevation in AST levels. In contrast, administering the activated T cell bispecific antibody (TCB) increased IL-6, IFN-γ, and AST levels. These discernible differences underscore the compelling effect of dual masking on the EGFR and CD3 binding domains within CI107. This innovative approach substantially enhances the tolerability and safety profile of the probody TCB. Besides, the maximum tolerated dose of CI107 was determined in cynomolgus monkeys and was over 60 times higher than that of the unmasked TCB. Animals administered with CI107 exhibited significantly lower toxicity levels, further reinforcing the notion of an expanded safety margin for this novel therapeutic. This design prevents damage to normal tissues and side effects. CI107 has demonstrated good safety and efficacy in preclinical stages as well.

3.2. *Mobilization of other immune cells to precisely eradicate tumors*

Cell engagers could bridge tumor and immune cells to form an immune synapse-mediated cytotoxic effect[21,115]. In addition to bispecific T cell engager (BiTE), it can also mobilize other immune cells, such as NK cells[116]. The bispecific NK cell engager antibody redirects CD16A$^+$ NK cells to tumor cells and activates NK cells. Compared to BiTE, a noteworthy attribute of the bispecific NK cell engager lies in its ability to reduce CRS and neurotoxicity[117,118]. AFM13 is the pioneering tetravalent BsAb to target CD30 and CD16[119]. This dual targeting empowersAFM13 to bind CD16A on NK cells and CD30 on lymphoma cells, thereby mediating NK cell killing and clearance of cancer cells[120]. Interventions utilizing modified immune cells AFM13-NK and BsAbs AFM13 for the treatment of relapsed or refractory CD30$^+$ Hodgkin's or non-Hodgkin's lymphoma patients are currently in Phase I/II (NCT04074746)[121−123]. The application of AFM13 immunotherapy may benefit patients by enabling their immune system to attack tumor cells and potentially inhibit tumor growth and metastasis. Combining AFM13 with NK cells may potentiate tumor cell demise and suppression of tumor growth beyond the scope of AFM13 monotherapy.

3.3. *Reducing affinity of CD3 arm*

Studies have highlighted a significant trend in T-cell redirecting BsAbs associating affinity for CD3 with pharmacokinetic properties[124,125]. Intriguingly, the antibodies with a low-affinity CD3 arm exhibited slower clearance and higher drug exposure than a higher-affinity CD3 arm[126,127]. However, this correlation presents considerable challenges[21], such as the affinity for CD3 exerts a multifaceted influence on various properties crucial for therapeutic efficacy, including biodistribution, potency, and toxicity[128,129]. For instance, high CD3 affinity correlates with CRS and might result in a lack of potency and proper biodistribution. Ideally, optimizing the performance of a T-cell redirecting BsAb, such as the appropriate affinity of the CD3 and TAA arms, could result in the desired balance between potency, pharmacokinetics, and safety for meeting therapeutic objectives.

CD3/CLL1 TDB, a BsAb binding CD3 and CLL-1, is engineered to treat acute myeloid leukemia[130]. Notably, results in monkeys revealed that only the low-affinity CD3/CLL1 TDB was well tolerated and able to deplete target cells, compared to the high-affinity TDBs[130]. They provide evidence that using a low-affinity CD3 arm could circumvent toxicities related to CRS and provide acceptable safety while maintaining robust activity. Consequently, one of the most promising strategies to generate a promising BsAb involves pairing low-affinity CD3 antibodies with high-affinity antibodies targeting the TAA[130−133]. Besides, the target selection of antibodies depends on the different expression levels of TAAs, while varying structural formats yield different effects. For instance, compared to tandem scFv-based BsAbs, those based on Fab exhibit superior biophysical properties. Furthermore, Fab-based BsAbs retain the activity of their tandem scFv counterparts and possess unique biological activity[134]. Sebag et al.[135] developed a novel BsAb, Elranatamab, which optimizes affinity for binding to CD3 and the B cell maturation antigen BCMA for treating multiple myeloma[135−139]. Decreasing antibody affinity promotes T cell activation and enhances their anti-tumor activity against multiple myeloma, reducing off-target cell toxicity to some extent.

3.4. *Combining T-cell redirecting BsAb with other antitumor therapies*

T-cell redirecting BsAbs has demonstrated remarkable efficacy in tumor treatment[140]. However, the effectiveness achieved through a

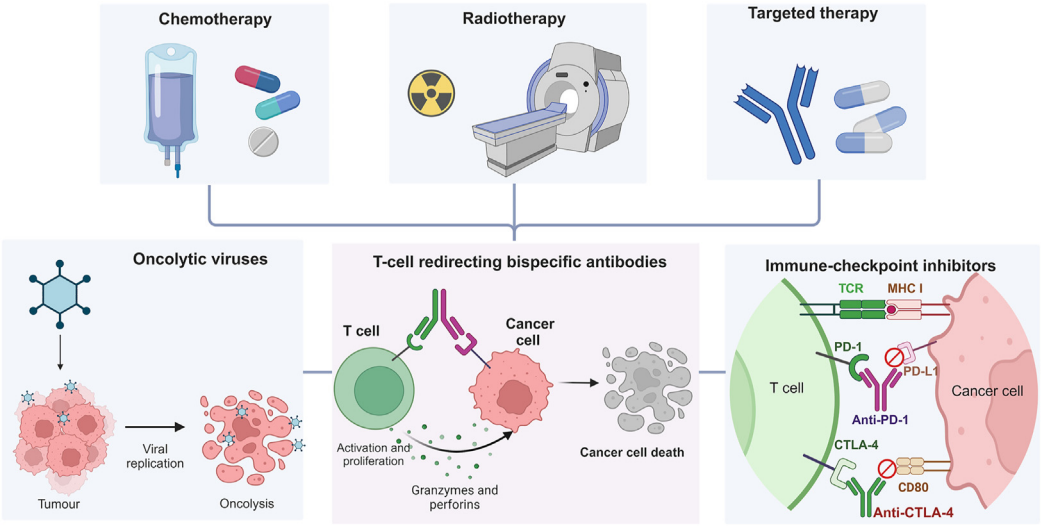

Figure 4 T cell-redirecting BsAbs combination therapy strategies. These include systemic chemotherapies, radiotherapy, targeted therapies, oncolytic viruses and immune-checkpoint inhibitors, which can create a synergistic effect, further enhancing the efficacy of tumor therapy.

single treatment approach is limited, and it is challenging to eliminate tumors solely relying on this treatment approach. Building upon the antitumor mechanisms of T-cell redirecting BsAbs, combining them with other treatment modalities, including systemic chemotherapies, radiotherapy, targeted therapies, and immunotherapies, creates a synergistic effect, further enhancing tumor therapy's effectiveness (Fig. 4).

3.4.1. Combining with chemotherapy drugs

One of the challenges in utilizing T-cell redirecting BsAbs for treating solid tumors lies in the limited infiltration of T-cells within the TME[20,141]. Traditional cytotoxic agents, like DNA intercalators[142,143], nucleotide analogs[144], or alkylating agents[145], are also being considered for combination with T-cell redirecting BsAbs. Chemotherapies are designed to target rapidly dividing cells directly[146]; thus, combining chemotherapy and T-cell redirecting BsAbs might improve therapeutic responses. Preliminary data substantiating this hypothesis have been reported. For example, ERY974, a T cell-redirecting antibody that targets glypican-3 and CD3, demonstrates modest antitumor effectiveness when used as a monotherapy in non-inflamed NCI−H446 xenograft tumors. This is due to the limited infiltration of ERY974-redirected T cells, primarily at the tumor-stromal boundary. Meanwhile, the cytotoxic agent paclitaxel monotherapy initially showed strong efficacy but could not prevent eventual tumor regrowth. However, ERY974 with chemotherapy synergistically and reciprocally increases antitumor efficacy in non-inflamed tumors by promoting T-cell infiltration into the tumor center and increasing ERY974 distribution in the tumor. This combination therapy may be effective for patients with non-inflamed tumors and those resistant to existing therapies. Chemotherapeutic cyclophosphamide is a potent antitumor drug as an alkylating agent that can alkylate the DNA of tumor cells within their cells[147−149]. At the same time, cyclophosphamide alone can inhibit tumor growth during the initial treatment phase; tumors often resurge within 2−3 weeks[150]. BCMA/CD3 BsAb monotherapy causes an increase in the number of $CD8^+$ T-cells. However, this excessive activation of T-cells can result in T-cell depletion, leading to temporary tumor regression followed by tumor recurrence after 3−4 weeks of treatment. Combining of BsAbs and cyclophosphamide can remarkably eradicate the tumor[150]. Experimental data proved that cyclophosphamide can inhibit the excessive activation of T cells and prevent T cell depletion. It can also inhibit the up-regulation of PD-L1 on the tumor surface, promote the renewal of memory T cells, and ensure that the T cells exhibit prolonged and highly effective antitumor activity. Therefore, the therapeutic strategy of combining T-cell redirecting BsAbs with chemotherapeutic drugs holds significant promise for further development[151-153].

3.4.2. Combining with radiation therapy

T-cell redirecting BsAbs and radiotherapy are two distinct domains in cancer treatment, each possessing non-overlapping cytotoxic effects[154,155]. A synergistic approach materializes through the combination of targeted immunotherapy, exemplified by tumor-targeting agents (BsAbs) and potent payloads (radioisotopes). This multifaceted strategy employs multiple stages, with BsAbs administered over 24 h, followed by a free radical scavenger, and a radioactive metal injection 4 h later. This orchestrated sequence seeks to overcome the limitations of conventional radiation therapy, including inadequate radioisotope uptake and elevated kidney retention. An article has reported a self-assembling and disassembling (SADA) BsAbs that fuses with p53 protein to form a p53−SADA−BsAbs tetramer. After administration, it forms dimers or monomers in the bloodstream, which can be cleared and reduce immunogenicity in rats after kidney filtration, with no short-term or long-term toxicity to the bone marrow, kidneys, or liver[137]. Notably, using anti-GD2 BsAbs in pretargeted radioimmunotherapy for neuroblastoma has showcased improved therapeutic effects in terms of tumor shrinkage, elimination of micrometastases, and prevention of relapse[156−158].

Radiation therapy operates by stimulating the immune system, thereby bolstering immune-stimulating cell infiltration within tumors, amplifying the immunogenicity of tumor cells, and augmenting antitumor immune responses. Nonetheless, it also engenders local or systemic immune suppression, occasionally even at low doses, culminating in temporary bone marrow inhibition and diminished lymphocyte counts. Radiation therapy can inadvertently impair $CD8^+$ T cells[159], pivotal for effective immune therapy response. Hence, in some instances, radiation

therapy might not accentuate the efficacy of combined immunotherapy and may attenuate its potency.

3.4.3. *Combining with oncolytic viruses*

Oncolytic viruses can infect, replicate, and lyse tumor cells while enhancing immune therapy and inducing increased permeability of the immune-tumor microenvironment, allowing for sufficient infiltration of $CD8^+$ T cells in the tumor microenvironment[160]. However, due to their non-targeted impact on normal cells, oncolytic viruses are often used in combination therapy strategies in clinical trials, resulting in promising advancements[161–164]. Yu et al.[140] engineered an oncolytic vaccinia virus (VV) that carries a BiTE antibody targeting the tumor cell surface antigen EphA2 and named it EphA2 TEA VV. In a mouse model of lung cancer xenografts, EphA2 TEA VV triggered CTL activation and redirection, outperforming conventional oncolytic vaccinia virus in achieving more potent antitumor outcomes. In another study, Wang et al.[165] designed an oncolytic adenovirus (OAd-MUC16-BiTE) carrying a BiTE antibody targeting MUC16 (mucin 16). Stable BiTE expression within tumor tissues was achieved post-local administration, mitigating systemic toxicity from systemic application. OAd-MUC16-BiTE mediated antigen-specific T cell activation and target cell lysis. Furthermore, it demonstrated the ability to "reverse" the tumor microenvironment, fostering CTL infiltration, amplifying T cell-mediated tumor cell elimination, and significantly enhancing anti-tumor efficacy.

The functional mechanism of oncolytic measles viruses harboring bispecific T-cell engagers (MV-BiTEs) hinges on the production and release of BiTE antibodies from tumor cells infected with BiTEs[166]. This triggers the assembly of multinucleated syncytia, culminating in the destruction of the tumor cells. Concurrently, BiTEs mobilize T cells to target and eliminate non-infected tumor cells, manifesting additional bystander effects. The therapeutic efficacy of MV-BiTEs was assessed in immunocompetent mouse models. Specifically, in the MC38-CEA model, MV-BiTE treatment extended the survival rates and effectively curbed tumor growth compared to the control group. Within the B16-CD20-CD46 model, MV-BiTE therapy augmented tumor-infiltrating T cells' number and activation levels, thereby bolstering anti-tumor immunity. These findings affirm that MV-BiTEs offer a potent, relapse-free treatment for solid tumors while inducing protective immunity.

Merging BsAbs with oncolytic viruses presents a promising strategy that supersedes the current limitations of individual BiTE treatments, offering a compelling avenue for solid tumor therapy. These studies underscore that combining oncolytic viruses and BiTE achieves a synergistic effect that overcomes the limitations of single-agent therapy.

3.4.4. *Combining with targeted therapy*

Targeted therapy is a precision treatment that designs corresponding targeted therapeutic drugs at the cellular and molecular levels to target specific cancer sites that have been identified. These drugs typically exert their effects predominantly on tumor cells, minimizing collateral damage to normal cells.

In small molecule targeted inhibitors, the primary targets are often vital kinases involved in cell signaling pathways, such as the mammalian target of rapamycin (mTOR), mitogen-activated protein kinase, and anaplastic lymphoma kinase. An exhaustive examination of 52 FDA-approved small molecule tyrosine kinase inhibitors revealed that select drugs aimed at mTOR, JAK, and Src kinases effectively suppress T cell proliferation[167]. When co-administered with T-cell redirecting BsAbs *in vitro*, mTOR and JAK kinase inhibitors diminished cytokine release related to CRS without undermining the tumor-killing potential of T-cell bispecific antibodies. In contrast, Src kinase inhibitor dasatinib successfully suppressed CRS-related cytokines but at the cost of impaired T cell cytotoxicity. Therefore, mTOR and JAK kinase inhibitors are preferable for mitigating CRS reactions in clinical settings without sacrificing anti-tumor effectiveness.

Bruton tyrosine kinase inhibitors are the preferred therapeutic agents for patients with chronic lymphocytic leukemia (CLL)[168,169]. The efficacy of BHK in CLL can be enhanced by combining it with CD19/CD3 BsAbs[170]. Notably, BTKis actively disrupts CLL cells' immunosuppressive mechanisms, irrespective of ITK inhibition, thereby bolstering the cytotoxic activity of autologous T cells[171–174]. Elevated CTLA-4 expression in peripheral blood CLL cells has been documented, and its subsequent downregulation upon BTKi treatment suggests a mechanistic link that may enhance T-cell cytotoxic responses. Thus, combining BTKis with BsAbs is a robust strategy for achieving sustained CLL remission and counteracting drug resistance, which may have broader applications in treating other mature B-cell malignancies.

Targeted therapy has made substantial strides in cancer care, offering personalized treatments by engaging tumor-associated molecules. This not only heightens therapeutic efficacy but also minimizes adverse side effects. The combination of BsAbs can markedly elevate treatment success rates, underscoring the vast potential of this approach.

3.4.5. *Combining immune checkpoint inhibitors*

Even after BsAbs and T cells navigate multiple barriers to penetrate tumor sites, a host of elements can dampen their therapeutic efficacy. Exhaustion of T cells owing to upregulation of immune-checkpoint proteins during therapy has been described with various BiTEs, *in vitro* and patients. It is a potential immune escape mechanism underlying both intrinsic and acquired resistance. Combinatorial immunotherapy approaches have been designed to counteract T-cell dysfunction/exhaustion mechanisms using inhibitory checkpoint-blocking antibodies to improve the clinical outcomes of individuals receiving $CD3^+$ bispecific T-cell redirection therapies. Interestingly, resistance to blinatumomab treatment has been attributed to increased PD-L1 expression in $CD19^+$ leukemic cells[175]. In order to antagonize PD-L1 expressed by the leukemic cells, blinatumomab in combination with pembrolizumab (anti-PD-1 therapy) resulted in enhanced T cell-mediated cytotoxicity relative to that observed with the parental BiTE alone[176]. A large number of clinical trials are currently evaluating the safety and efficacy of combinatorial approaches for the treatment of relapsed/refractory acute leukemia or lymphoma patients (NCT03160079, NCT03605589, NCT03340766, NCT03512405).

Belmontes et al.[177] have found that combining PD-1 antibodies, CTLA-4 antibodies, and 4-1BB agonist antibodies with CD3 BsAbs can enhance the efficacy of CD3 BsAbs. Among them, 4-1BB has demonstrated the most substantial impact, particularly in amplifying $CD8^+$ T cells within tumor tissues and overcoming treatment resistance in solid tumors. One of the most rapidly advancing BsAbs, SMET12 (targeting EGFR/CD3), combined with PD-1 antibodies, is now in Phase II clinical trials.

Cibisatamab, TCB targeting CEA/CD3, is a novel T-cell redirecting BsAbs targeting CEA on tumor cells and CD3 on T cells[178]. Cibisatamab displays potent anti-tumor activity in preclinical models, leading to increased intra-tumoral T-cell infiltration and activation and upregulating PD-1/PD-L1. In the ongoing dose-escalation phase I studies (NCT02324257 and NCT02650713), activity appeared to be enhanced with doses in combination with atezolizumab, with a manageable safety profile.

An EGFR/CD3 molecule, SMET12, is currently in preclinical development for treating EGFR-positive advanced/metastatic solid tumors. Pharmacological studies demonstrate that SMET12 combined with Treprinumab (anti-PD-1 therapy) can effectively eliminate the immune suppressive environment caused by the expression of inhibitory receptors such as PD-1 after T cell activation[179], further exerting the anti-tumor function of SMET12 to achieve synergistic enhancement. It has significant advantages and potential good prospects for treating solid tumors.

4. Conclusions and prospects

T-cell immunotherapy constitutes a dynamic area in cancer treatment, with three main approaches currently in the spotlight: ICIs, T-cell redirecting BsAbs, and CAR-T cell therapy. Notably, T-cell redirecting BsAbs have achieved a revolutionary breakthrough in cancer therapy. Unlike ICIs, which release the general suppression of the cellular immune system and may trigger adverse reactions to non-tumor cells, T-cell redirecting BsAbs precisely engage T cells with tumor cells, enhancing T cell activation and proliferation. Their mechanism of action enables potentially quicker responses than ICIs by inducing direct T cell-mediated cytotoxicity. In contrast to personalized therapies like CAR-T, which are time-consuming and expensive due to the need for individualized collection, modification, and expansion of a patient's T cells, bispecific antibodies offer a more straightforward and cost-effective "off-the-shelf" solution[182]. They can be manufactured and stockpiled on a large scale, reducing treatment costs. Nevertheless, the progression of T-cell redirecting BsAbs in solid tumors is relatively slower, and there are still many challenges and areas for optimization, including CRS, targeted and non-targeted cytotoxicity, TAA loss, limited T-cell infiltration and suppression of T-cell activity in the tumor microenvironment.

CRS can be observed in patients treated with T-cell redirecting BsAbs such as Binatumomab, and the severity depends on the treatment type and target[89,150]. Preclinical studies using humanized mouse models have found that TNF-α produced by activated T cells is the primary mediator of CRS induced by T-cell redirecting BsAbs. This leads to massive secretion of inflammatory cytokines by monocytes[183]. To overcome this challenge, T-cell redirecting BsAbs could be designed using a low-affinity anti-CD3 to reduce the potential for dose-limiting toxicities associated with CRS. Besides, blocking upstream TNF-α and downstream IL-1β or IL-6 can induce tumor killing with reduced cytokine release[184,185].

The challenges posed by targeted and non-targeted cytotoxicity, the limited number of effector cells, and the suppressed T-cell activity within tumors are deeply intertwined[186,187]. These three obstacles are indispensable in tackling the limitations associated with T-cell redirecting BsAbs[188]. Most antibodies currently undergoing development are centered around differentiation markers[189]. Many investigational solid tumor targets, like CEA, EGFR, EpCAM, and HER2, fall within this differentiation marker category[190]. Noteworthy among these can be expressed in normal cells, albeit at limited levels, thereby narrowing the therapeutic window and circumscribing the potential for T-cell redirecting BsAbs design. To enhance the targeting of antibodies to tumor cells and reduce drug resistance, simultaneously targeting two different tumor antigens and tri-specific antibody development can be employed to address these challenges.

Many strategies have been devised to tackle the challenge of on-target off-tumor effects; one of the innovative approaches is utilizing a "mask" to shield the antibody epitope until it reaches the tumor site for conditional activation. The stimuli involved in this strategy include light, temperature, enzymes, pH, ions, small effect molecules, or rare antigens. In addition to the pro-drugs and cleavage enzymes mentioned earlier, structural modification of the antibody Fc domain has achieved significant breakthroughs. The TME caused by changes in tumor cell metabolism is characterized by acidic pH, a widely used parameter in targeted TME research. Low pH significantly impacts the TME, leading to immune suppression, immune escape, and disease progression. This acidic extracellular environment significantly affects the bioavailability of therapeutic antibodies. Harnessing this characteristic as a trigger for therapeutic antibody activation not only affords precise control but also mitigates drug toxicity linked to off-target effects. For instance, Sulea et al.[191] developed a pH-dependent HER2 antibody (bH1-P5P8) that exhibits heightened antigen binding in acidic pH compared to neutral pH, bolstering tissue-specific distribution and diminishing distribution-related side effects in normal tissues.

The objective of achieving a durable cure with limited toxicity is also a driving force in designing and developing new tumor immunotherapies. Efficacy in tumor treatment relies mainly on immune activation strategies, especially the cytotoxic activity of immune T cells within the tumor. However, the TME can affect the infiltrating T cells, curbing their proliferation and function, curtailing effector T-cell quantity and activity. Which may result in reduced or lost activity such as impaired proliferation and immune function. The emergence of T cell exhaustion, characterized by the expression of inhibitory receptors, represents a pivotal mechanism in tumor immune evasion. By countering these signals, interventions like ICIs have demonstrated considerable antitumor potency by revitalizing T-cell functionality within the tumor microenvironment. With the ongoing advancement of science and technology, researchers have proposed innovative approaches combining T-cell redirection bispecific antibodies with other strategies, including ICIs, immune activators, and oncolytic viruses. Current and upcoming clinical trials must prove the value of these combinatory approaches. As the number of BsAbs undergoing clinical trials increases, our understanding of the optimal structures and constructs for targeting particular tumor types is expanding. Such knowledge will improve the antitumor efficacy of T cell-engaging therapies, prevent disease relapse, and drive future advancements in this field.

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Author contributions

Xiaojing Qin, Wenjing Ning reviewed the literature and drafted the manuscript. Wenxin Luo and Xue Liu conceived and supervised the project. Other authors participated in the search and collation of literature. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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