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Targeting Solid Tumors with Bispecific T Cell Engager Immune Therapy

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Abstract

T cell engagers (TCEs) are targeted immunotherapies that have emerged as a promising treatment to redirect effector T cells for tumor cell killing. The strong therapeutic value of TCEs, established by the approval of blinatumomab for the treatment of B cell precursor acute lymphoblastic leukemia, has expanded to include other hematologic malignancies, as well as some solid tumors. Successful clinical development of TCEs in solid tumors has proven challenging, as it requires additional considerations such as the selectivity of target expression, tumor accessibility, and the impact of the immunosuppressive tumor microenvironment. In this review, we provide a brief history of blinatumomab, summarize learnings from TCEs in hematologic malignancies, and highlight results from recent TCE trials in solid tumors. Additionally, we examine approaches to improve the efficacy and safety of TCEs in solid tumors, including therapeutic combinations to increase the depth and durability of response.

INTRODUCTION

By leveraging our understanding of adaptive immunity, researchers have developed various T cell immunotherapies to restore and enhance T cells' antitumor activity. Immune checkpoint inhibitors are among the first immunotherapies to successfully restore the antitumor activity of exhausted T cells (Wei et al. 2018). However, as these benefit only a subset of patients, additional therapeutic approaches are needed. One such approach involves transducing T cells with receptors targeting tumor-associated antigens (TAAs). This approach, known as chimeric antigen receptor (CAR) T cell therapy, has been approved for the treatment of certain B cell malignancies and is being evaluated for the treatment of additional hematologic malignancies and solid tumors. Despite its promise, the manufacturing success of CAR T cells relies on the timely harvesting and in vitro culturing of T cells, making it a time-consuming process that may be prohibitive for patients with aggressive disease (June et al. 2018).

An off-the-shelf alternative modality to CAR T cells, T cell engagers (TCEs), redirects T cells to cancer cells by binding to CD3 on T cells and a TAA on tumor cells. This T cell engagement leads to CD3 clustering, T cell receptor (TCR)-dependent signaling, and release of perforin and granzyme, resulting in tumor cell killing (d'Argouges et al. 2009, Haas et al. 2009, Offner et al. 2006) (**Figure 1**). TCE-induced tumor cell killing is highly efficient, as serial killing occurs through the TCE bridging of a single T cell with multiple tumor cells over time (Hoffmann et al. 2005). TCE-induced T cell activation also causes cytokine release and T cell proliferation (Brischwein et al. 2007) (**Figure 1**), expanding the available T cell pool for additional

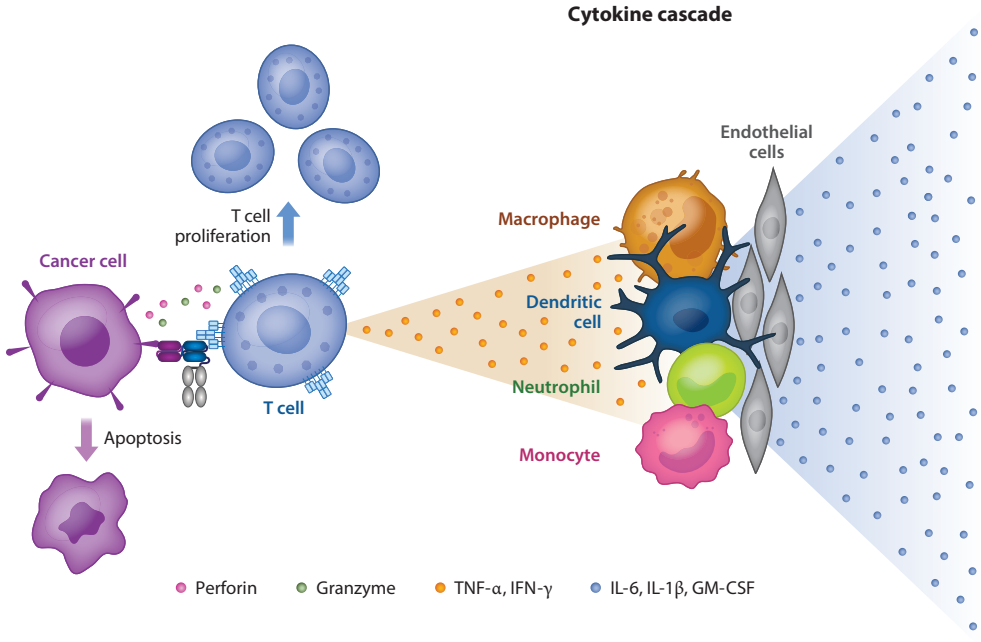


Figure 1

TCE engagement induces T cell–mediated cancer cell apoptosis, T cell proliferation, and cytokine release. Cytokines released from the T cell can stimulate the release of cytokines from other immune cells, as well as from endothelial cells, resulting in a cytokine cascade that potentially leads to CRS. Abbreviations: CRS, cytokine release syndrome; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon-gamma; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

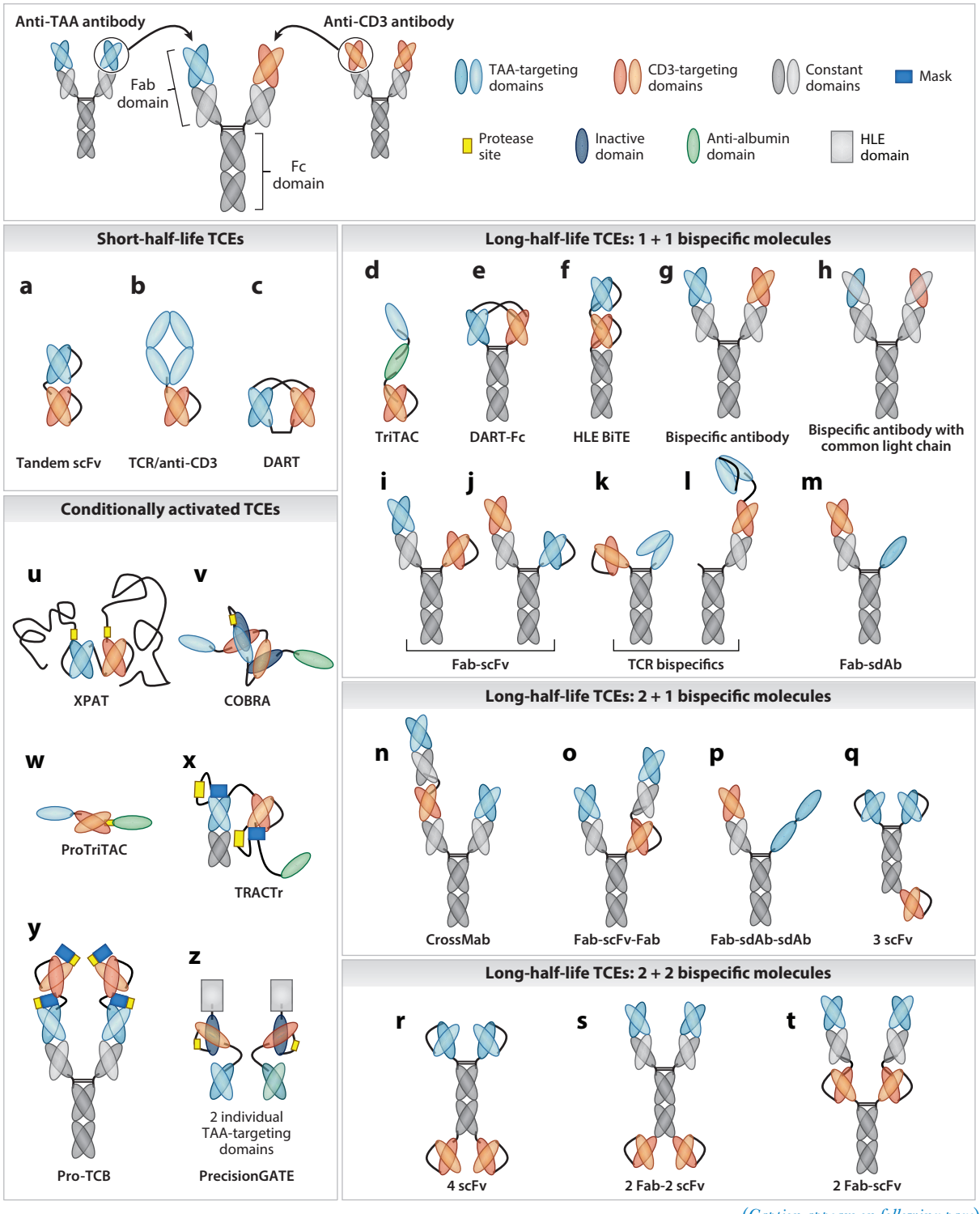
TCE-mediated tumor cell killing. Furthermore, because the TCE-mediated recognition of tumor cells depends on the TAA and not on the major histocompatibility complex (MHC), tumor cells cannot evade killing by downregulating expression of MHC class I molecules, a common approach to evading the adaptive immune response (Spranger & Gajewski 2018).

The first FDA (Food and Drug Administration)-approved TCE was the BiTE® (bispecific T cell engager) molecule blinatumomab (Bargou et al. 2008, Gökbuget et al. 2018, Topp et al. 2015) for the treatment of relapsed or refractory (r/r) B cell precursor acute lymphoblastic leukemia (B-ALL) and B-ALL with minimal residual disease (MRD) (Gökbuget et al. 2018, Kantarjian et al. 2017). Subsequently, TCEs have shown early evidence of antitumor activity in other hematologic malignancies, including multiple myeloma (MM), non-Hodgkin's lymphoma (NHL), acute myeloid leukemia (AML), and several solid tumor types. There are currently about 100 ongoing clinical trials to evaluate the efficacy and safety of TCEs for cancer treatment. However, challenges for successfully deploying TCEs remain. The adverse events of interest observed in the treatment of hematologic malignancies include neurotoxicity and cytokine release syndrome (CRS). Additional challenges for solid tumor treatment include the availability of tumor-specific antigens, limited intratumoral T cell availability, and immunosuppression in the tumor microenvironment (TME) (Goebeler & Bargou 2020). This review highlights how learnings from the use of TCEs in hematologic malignancies can guide the development of TCEs in solid tumors and preliminary efficacy and safety profiles for solid tumor TCEs. It also discusses approaches for mitigating CRS, improving the therapeutic index, and enhancing the antitumor activity.

KEY OBSERVATIONS AND LEARNINGS FROM T CELL ENGAGER TREATMENT OF HEMATOLOGIC MALIGNANCIES

TCEs have been in clinical testing for over two decades (Nagorsen et al. 2012), and to date, only blinatumomab, which targets the B-lineage antigen CD19 on normal and malignant B cells (Löffler et al. 2000), has been FDA approved. Blinatumomab comprises two single-chain variable fragments, one recognizing CD19 and the other recognizing CD3, connected by a flexible linker (**Figure 2**). The serum half-life of blinatumomab is ~2 h, and blinatumomab is administered by continuous intravenous (cIV) infusion to maintain active exposure. Based on the results from the phase III TOWER trial in which overall survival was significantly prolonged for patients with r/r B-ALL randomized to blinatumomab versus standard of care chemotherapy (Kantarjian et al. 2017), blinatumomab has been approved for the treatment of patients with r/r B-ALL. Subsequently, in the single-arm phase II BLAST trial, blinatumomab demonstrated a complete response rate of 78% (Gökbuget et al. 2018), resulting in approval for the treatment of MRD in patients with B-ALL.

The adverse events of interest for blinatumomab include neurotoxicity and CRS. In patients with B-ALL, neurologic events occurred in approximately 65% of patients (Blinatumomab 2018). Most commonly, neurologic events are grade 1 to 2 in severity, and consist of headache or tremor that onsets within the first 2 weeks and subsequently resolves. While the cause of neurotoxicity has not been fully defined, potential mechanisms include the adhesion of activated T cells to vasculature and transmigration across the brain endothelium followed by B cell–dependent cytokine release (Klinger et al. 2020) and direct engagement of CD19-expressing blood/brain barrier–associated mural cells by blinatumomab-activated T cells (Parker et al. 2020). Cytokine release occurs with TCE-mediated T cell activation (**Figure 1**) and may lead to the development of CRS, a systemic inflammatory condition that can result in a range of symptoms from mild fever or rash to more severe symptoms such as vascular leakage or multiorgan failure. CRS was observed in 15% of blinatumomab-treated patients with r/r B-ALL and in 7% of patients with MRD-positive



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Examples of various TCE formats. The anti-TAA and anti-CD3 domains of a TCE are derived from separate antibodies and combined into one bispecific antibody. (*Top*) A representative TCE comprising an Fc domain and two unique Fab domains. (*a–c*) Short-half-life, (*d–t*) long-half-life, and (*u–z*) conditionally activated TCE formats. Molecules with a long half-life include (*d–m*) 1 + 1 bispecific molecules (with one anti-CD3 binding domain and one anti-TAA binding domain), (*n–q*) 2 + 1 bispecific molecules (with one anti-CD3 binding domain and two anti-TAA binding domains), and (*r–t*) 2 + 2 bispecific molecules (with two sets of binding domains targeting CD3 and TAA). TCEs include (*a*) anti-CD19 blinatumomab or anti-PSCA GEM3PSCA, (*b*) anti-gp100 tebentafusp, (*c*) anti-CD123 flotetuzumab, (*d*) anti-PSMA HPN424, (*e*) anti-B7-H3 MGD009, (*f*) anti-PSMA AMG 160, (*g*) anti-GUCY2C PF-07062119, (*h*) anti-MUC16 REGN4018, (*i*) anti-SSTR2 TCE tidutamab, (*j*) anti-HER2 BEAT GBR 1302, (*k*) anti-MAGEA4/8 IMA401 TCER, (*l*) anti-survivin ABBV-184, (*m*) anti-PSMA AMG 340, (*n*) anti-CEA cibisatamab, (*o*) anti-STEAP1 AMG 509, (*p*) anti-BCMA TNB-383B, (*q*) anti-PSMA APVO442, (*r*) anti-CD123 APVO436, (*s*) anti-PSMA CC-1, and (*t*) anti-LRRC15 QL315.T5. Abbreviations: BCMA, B cell maturation antigen; BEAT, bispecific engagement by antibodies based on the T cell receptor; BiTE, bispecific TCE; CEA, carcinoembryonic antigen; COBRA, conditional bispecific redirected activation; DART, dual-affinity retargeting; Fab, antigen-binding fragment; HLE, half-life extended; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; scFv, single-chain variable fragment; sdAbs, single domain antibodies; TAA, tumor-associated antigen; TCB, T cell engager bispecific antibody; TCER, bispecific T cell–engaging receptors; TCR, T cell receptor; TRACTr, tumor activated T cell engager; TriTACs, trispecific T cell–activating constructs; XPAT, XTENylated protease-activated T cell engager.

B-ALL (Blinatumomab 2018). While the pathophysiology of CRS is an area of active investigation, it may be initiated by the release of tumor necrosis factor-alpha and interferon-gamma from activated T cells, which subsequently stimulate cytokine release, including interleukin 6 (IL-6) from other immune cells and endothelial cells (Shimabukuro-Vornhagen et al. 2018) (**Figure 1**). IL-6 is considered a central mediator of the clinical manifestations of CRS (Lee et al. 2014, 2019). In blinatumomab-treated patients, CRS was most common during the first cycle and was most severe after the first dose (Klinger et al. 2012, Nägele et al. 2017). Subsequent cycles typically induce less cytokine release. Approaches to managing CRS include corticosteroid prophylaxis and step dosing, in which a lower dose is administered before the target dose (Kantarjian et al. 2017). The step-dosing concept is based on the observation that the first physiologic dose of a TCE initiates the greatest rise in IL-6, and systemic cytokine peaks are attenuated following subsequent doses, potentially reflecting the attenuation of T cells (Hijazi et al. 2018, Hosseini et al. 2020). The step dosing for blinatumomab in r/r B-ALL is 9 μg per day for 1 week, followed by 28 μg per day for 3 weeks in cycle 1. With the combination of corticosteroids and step dosing, CRS was manageable in patients treated with blinatumomab and usually did not result in treatment discontinuation (Gökbuget et al. 2018, Kantarjian et al. 2017, Topp et al. 2015).

TCEs are also being evaluated in other hematologic malignancies, including NHL, MM, and AML. In NHL, blinatumomab demonstrated the ability of a TCE to deplete target cells in tissues beyond the blood or bone marrow, including lymph node and liver (Bargou et al. 2008), indicating that TCEs can penetrate and activate T cells in solid tissue. The active dose of blinatumomab in NHL (112 μg/day) is higher than the active dose in B-ALL (28 μg/day), potentially due to lower biodistribution of blinatumomab in tissues compared with blood or bone marrow (Bargou et al. 2008, Dufner et al. 2019). In NHL, TCEs targeting CD19 (TNB-486), CD20 (odronextamab, epcoritamab, glofitamab, and mosunetuzumab), and CD22 (JNJ-75348780) are being evaluated in both indolent and aggressive NHL. In MM, TCEs targeting BCMA, GPRC5D, or FCRH5 have shown promising early activity (Chari et al. 2020, Cohen et al. 2020, Costa et al. 2019, Garfall et al. 2020, Harrison et al. 2020, Lesokhin et al. 2020, Madduri et al. 2020, Rodriguez et al. 2020, Topp et al. 2020). Except for blinatumomab, all of these molecules are large (~112 to ~200 kDa) and antibody-like (**Figure 2**), with serum half-life values of several days, allowing them to be dosed weekly or less frequently. Most are administered by an intravenous (IV) bolus injection, resulting in a high maximum drug concentration (Cmax) that is associated with cytokine release, and CRS remains the primary adverse event across indications. In the treatment

of CRS, early and aggressive supportive care, early use of vasopressors, and prompt initiation of anticytokine therapy are critical to achieve the best outcomes (Lee et al. 2019). To prevent CRS, researchers are evaluating corticosteroids, step dosing, and the investigational use of cytokine-neutralizing antibodies (e.g., tocilizumab) (Bannerji et al. 2020; Hutchings et al. 2020, 2021; Li et al. 2019; Olszewski et al. 2020). Additional approaches include the coadministration of TAA-blocking antibodies that limit the amount of engageable antigen (Dickinson et al. 2020), subcutaneous dosing that results in slow TCE absorption from the injection site and reduces Cmax (Matasar et al. 2020), and the use of TCEs containing modified CD3-binding domains (e.g., lower affinity and/or targeting a unique epitope) (Malik-Chaudhry et al. 2021, Trinklein et al. 2019). If efficacy and durability of response are comparable across TCEs, success may depend on a more favorable safety profile or patient convenience.

In AML, there are clinical trials evaluating CD33, CD123, CLEC12A (CLL-1), and FLT3. While some promising results have been observed, these are often limited to patients with a lower disease burden (Ravandi et al. 2020a,b). The main adverse event is CRS, but additional safety findings include transaminase elevation and some neurologic events (Aldoss et al. 2020; Ravandi et al. 2020a,b; Subklewe et al. 2019). Approaches to improve patient response and safety include optimizing dose and schedule and evaluating cytokine prophylaxis treatments.

In hematologic malignancies, several factors are associated with enhanced TCE efficacy, including increased TCE exposure, homogeneous TAA expression, and T cell number and fitness. Successful application of TCEs in solid tumors will require considering these factors, as well as addressing the additional challenges of solid tumor accessibility, lack of tumor-specific TAAs, T cell infiltration, and immunosuppression in the TME.

CLINICAL RESULTS IN SOLID TUMORS

Supplemental Material >

The development of TCEs for solid tumors has lagged behind that of TCEs for hematologic malignancies (**Supplemental Table 1**), and first-generation compounds have failed to demonstrate significant antitumor activity. One TCE, catumaxomab, was approved by the European Medicines Agency to treat malignant ascites of epithelial cancers based on an improved puncture-free survival versus paracentesis alone, but it was later withdrawn from the market (Heiss et al. 2010, Linke et al. 2010). Catumaxomab, a rat/mouse hybrid TCE that targets EpCAM and CD3, also has a functional Fc domain that binds Fcγ receptor–expressing accessory immune cells, which may induce antigen-independent T cell activation and natural killer cell recruitment. Intraperitoneal administration of catumaxomab was associated with adverse events in the gastrointestinal tract and liver (Burges et al. 2007, Linke et al. 2010), which precluded further dose escalation.

Other first-generation TCEs in solid tumors faced similar challenges. In a phase I study, IV treatment with the anti-EpCAM BiTE molecule solitomab/AMG 110 led to one unconfirmed partial response and stable disease in 17 of 54 patients (Kebenko et al. 2018). Dose-limiting toxicities included diarrhea and increased liver enzyme levels (Kebenko et al. 2018). Development of the carcinoembryonic antigen (CEA)-targeted BiTE molecule AMG 211 was discontinued after a phase I study because antidrug antibodies developed in all patients treated at doses >3.2 mg (Moek et al. 2018). Development of other early TCEs, including those targeting HER2, CDH3, GPC3, EGFRvIII, and B7-H3, has been slow or deprioritized.

Newer TCEs that were directed against tumor targets with less normal tissue expression and that used formats that did not include an Fc domain with effector function have shown improved safety and efficacy profiles. Several clinical studies are ongoing in solid tumors, including TCEs targeting prostate-specific membrane antigen (PSMA), the Notch pathway member delta-like ligand 3 (DLL3), CEA, somatostatin receptor 2 (SSTR2), and the melanoma-associated antigen

glycoprotein (gp) 100. Pasotuxizumab (BAY2020112/AMG 212), a PSMA-targeted BiTE molecule, demonstrated 50% decline in prostate serum antigen (PSA50) or better responses in three of nine prostate cancer patients treated at doses of ≥20 μg/day by cIV infusion. Two of these responses lasted over 1 year and one responder had complete regression of soft tissue metastases and marked regression of bone metastases as assessed by PSMA-positron emission tomography/computed tomography (Hummel et al. 2021). The study was stopped before the maximal tolerated dose was reached due to a change in sponsor. Subsequently, the half-life extended (HLE) anti-PSMA BiTE molecule AMG 160 produced PSA50 or better responses in 34.3% (12/35) of patients and RECIST (response evaluation criteria in solid tumors) partial responses in 13.3% (2/15) of patients across dose levels (Tran et al. 2020). Another PSMA-targeted TCE, HPN424, also showed PSA declines (8 of 44 patients with a PSA50 response) across dose levels (1.3 to 120 ng/kg) (Bendell et al. 2020). CRS was the main adverse event observed in studies with PSMA TCEs. In small-cell lung cancer (SCLC), AMG 757, an HLE BiTE molecule targeting DLL3, demonstrated responses at target doses ≥0.3 mg and had an acceptable safety profile (Borghaei et al. 2020). Furthermore, confirmed partial response was observed in 20% of patients across dose ranges, with a median duration of response of 8.7 months (Owonikoko et al. 2021). In neuroendocrine tumors, the anti-SSTR2 TCE tidutamab (XmAb® 18087) induced a disease control rate of 43% (6/14) across dose levels (0.1 to 1.0 μg/kg) and demonstrated dose-limiting toxicities of nausea and vomiting associated with SSTR2 target expression in the gastrointestinal tract (El-Rayes et al. 2020). In CEA-positive solid tumors including metastatic colorectal cancer, the anti-CEA TCE cibisatamab was evaluated both alone and in combination with the anti–programmed death-ligand 1 (PD-L1) monoclonal antibody atezolizumab, with interim response rates of 6% (2/31) and 18% (2/11), respectively, at doses ≥60 mg (Tabernero et al. 2017). Although cibisatamab treatment was associated with adverse events in the gastrointestinal tract attributed to CEA target expression, the safety profile was considered manageable. Promising results have also been seen with tebentafusp (IMCgp100), an affinity-enhanced TCR fused to an anti-CD3 binding domain. This TCE recognizes a peptide from gp100 presented by HLA-A2*01, and in a phase II evaluation of 127 patients with metastatic uveal melanoma it produced an objective response rate of 5%, which was accompanied by a reduction in target lesion in 44% of patients (Sacco et al. 2020). Overall, these preliminary reports of efficacy are highly encouraging and provide proof of concept for TCE activity in solid tumors.

SAFETY CHALLENGES OBSERVED WITH SOLID TUMOR T CELL ENGAGERS

Safety challenges associated with solid tumor TCEs include CRS and damage to normal, antigen-expressing tissues. Preliminary results from TCEs in solid tumors have reported CRS rates that are generally higher (19%–91%) than those associated with blinatumomab (7%–15%), although overall CRS remains manageable and reversible (Bendell et al. 2020, Borghaei et al. 2020, El-Rayes et al. 2020, Middleton et al. 2020, Tran et al. 2020). Differences in CRS grading systems complicate cross-trial comparisons of CRS severity (Lee et al. 2014, 2019). Prophylactic approaches like those used in hematologic malignancies are being evaluated. Although these approaches reduce CRS, they may also impact efficacy, as corticosteroids dampen the T cell response and step-dosing delays administration of the target dose. Typically, step doses are administered several days apart to allow cytokine levels to return to acceptable levels between doses; however, a more aggressive approach to step dosing could reduce the time needed to reach the target dose. In preclinical studies of anti–glypican 3 (ERY974), step doses (threefold increase) were given daily for 10 days with manageable cytokine release (Ishiguro et al. 2017), suggesting that more rapid step dosing may be tolerated.

Two factors associated with cytokine release include antigen accessibility and abundance (normal and tumor tissue) and high-affinity CD3 engagement (Leong et al. 2017, Zuch de Zafra et al. 2019). The role of antigen abundance was first observed in hematologic malignancies ALL and AML, where patients with a higher burden of disease frequently experienced increased incidence and severity of CRS (Ravandi et al. 2020a,b; Topp et al. 2014). The role of antigen accessibility was demonstrated by the increased incidence of CRS observed in blinatumomab-treated ALL versus NHL patients (Coyle et al. 2018, Topp et al. 2014) where ALL blasts are easily accessible in the blood and bone marrow, whereas lymphoma cells are less accessible in the lymph nodes. Antigen accessibility and abundance may also be a factor in solid tumors. Different rates of CRS have been observed among patients treated with anti-PSMA TCEs compared with the anti-DLL3 BiTE AMG 757, potentially reflecting the higher abundance or accessibility of PSMA relative to DLL3. Ongoing evaluation of TCEs in solid tumors will refine the understanding of antigen-associated factors influencing CRS in solid tumors.

Cytokine release can also be influenced by characteristics of the anti-CD3 binding domain, as described earlier. The majority of TCEs evaluated clinically bind a similar CD3 epitope with high affinity (dissociation constant K_D < 100 nM) (Wu & Cheung 2018). The high-affinity binding to CD3 delivers cytotoxicity at picomolar concentrations and also induces strong cytokine release (Leong et al. 2017, Zuch de Zafra et al. 2019). TCEs that induce less cytokine release are now being evaluated clinically in hematologic malignancies (Rodriguez et al. 2020, Trinklein et al. 2019); however, it is unknown if these will be successful in solid tumors, as cytokines may be important for sustained antitumor activity by increasing T cell serial killing or proliferation, factors that may be critical in solid tumors. Ultimately, the CD3 affinity of a TCE may need to be tailored for the specific use, based on balancing the risk of cytokine release and the need to provide T cell support.

Damage to normal, antigen-expressing tissues is a concern for TCEs targeting antigens that are expressed by both tumor and normal tissue. On-target, off-tumor toxicity was a limiting factor for the anti-EpCAM BiTE molecule AMG 110 and has also been observed with the anti-SSTR2 TCE tidutamab (XmAb 18087) and the anti-CEA TCE cibisatamab. Approaches to limit normal tissue activity include targeting tumor-selective antigens, if possible, and using TCE engineering approaches (described below) to limit activity to tumors if tumor-selective antigens are not available.

APPROACHES TO IDENTIFY NEW TARGETS AND INCREASE THE THERAPEUTIC INDEX

TAAs fall into three general categories: tumor-specific, tumor-selective, and non-tumor-selective. Tumor-specific antigens include those arising from epigenetic or genetic changes and represent the greatest opportunity for selectively targeting tumors. Unfortunately, tumor-specific antigens are rare and many of them are intracellular and not accessible to a standard TCE. Notable exceptions include EGFRvIII, often found in glioblastoma, or p95HER2, which is detected in ~40% of HER2-positive tumors (Rius Ruiz et al. 2018). Tumor-selective antigens include those that are overexpressed by the tumor or differentially located (e.g., apical versus basolateral localization) compared with normal tissue. An example is DLL3, which is expressed on the majority of SCLC and other neuroendocrine tumor cells, with low, mainly cytoplasmic expression in select normal tissues including brain (Giffin et al. 2021). Nontumor-selective antigens are the remainder of antigens that are commonly expressed on both tumors and normal tissue.

Approaches to identify tumor-specific or -selective TAAs include immunization with tumor-derived cells, or analysis of tumor and normal tissue transcriptomes, proteomes, and metabolomes. Immunization approaches have yielded targets such as EpCAM and gpA33 (Moore et al. 2018);

transcriptome data have been mined to identify targets such as DLL3 (Giffin et al. 2021, Saunders et al. 2015) and CLDN18.2 (Helftenbein et al. 2008). Proteomic-based approaches have been used to identify tumor-selective peptides presented by MHC class I molecules. TCE targeting of tumor-specific or -selective antigens can be achieved using a number of standard TCE formats (**Figure 2**). To leverage differences in antigen expression levels, researchers may use 2 + 1 formats, which have two lower-affinity TAA-binding domains (Nolan-Stevaux 2020) (**Figure 2**). Additional TCE engineering approaches, including conditional activation, are being evaluated to target nontumor selective antigens.

Conditional activation of TCEs is an emerging field where TCEs are administered in an inactive form and become activated at tumors by tumor-expressed proteases, differential pH, or the presence of metabolites. This approach was pioneered with peptide-masked antibodies (Desnoyers et al. 2013) and extended to peptide-masked TCE molecules (Boustany et al. 2018). Recently, several approaches for conditional TCE activation have been reported, including a ProTriTAC format (Lin et al. 2020), a conditional bispecific redirected activation format (COBRA) (Dettling et al. 2019), and XTENylated protease-activated TCEs (XPATs) (Cattaruzza et al. 2020) (**Figure 2**). Conditional activation of TCEs could also be achieved by requiring binding of two TAAs for activity (Deshaies 2020), as demonstrated for CAR T cells (Lajoie et al. 2020), or by a combination of protease masking and dual TAA binding, as shown by the PrecisionGATE format (**https://www.revitope.com/**).

APPROACHES TO BUILD ON THE EARLY PROMISE OF BISPECIFIC T CELL ENGAGERS

The encouraging antitumor activity of TCEs in solid tumors (Bendell et al. 2020, Borghaei et al. 2020, El-Rayes et al. 2020, Tran et al. 2020) highlights the promise of this approach. However, clinical development of TCEs in solid tumors is still in early stages and it remains to be seen whether deep and durable responses can be achieved as monotherapy. Indeed, many of the mechanisms that limit the activity of other immunotherapies in solid tumors, such as the immunosuppressive TME and inadequate T cell availability, may also impact TCE efficacy. In addition, the ability of TCEs to induce T cell activation independent of peptide-MHC engagement may result in T cell–tumor cell interactions that are unable to deliver optimal costimulatory or cytokine signals. Preclinical studies have begun to model these potential mechanisms of resistance and identify novel combinatorial approaches to enhance TCE activity.

Enhance T Cell Engager–Mediated T Cell Activation

T cell activation, expansion, and differentiation are regulated by three primary signals: TCR engagement (signal 1), and signals from costimulatory receptors (signal 2) and cytokines (signal 3) (Goebeler & Bargou 2020) (**Figure 3**). CD28, the prototypical costimulatory receptor, likely plays a critical role in priming and expansion of naïve tumor-specific T cell populations in tumor-draining lymph nodes (TDLNs) during initiation of the antitumor T cell response. As these tumor-specific T cells are activated and begin to proliferate, they express additional receptors, such as 4-1BB, ICOS, and OX40, that can further enhance T cell proliferation and survival, both in the TDLNs and at the tumor site. Notably, these latter receptors have unique expression patterns across and within $CD4^+$ and $CD8^+$ T cell compartments and can function to shape T cell responses by mediating expansion and survival of specific T cell populations. Similarly, cytokines shape the T cell response through induction of specific differentiation and expansion programs, with IL-2, IL-7, IL-12, and IL-15 being particularly relevant to the antitumor response. Importantly, many of these costimulatory and cytokine signals are normally delivered during interactions

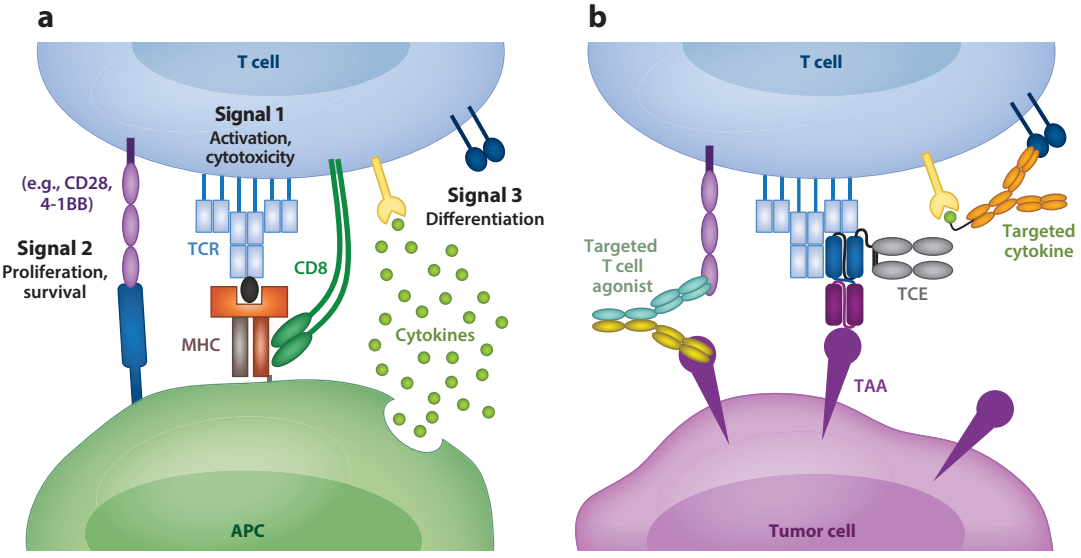


Figure 3

Key differences between peptide-MHC-mediated T cell activation during interactions with a professional APC and TCE-mediated T cell activation during interactions with a tumor cell. (*a*) Antigen-specific $CD8^+$ T cell interactions with a professional APC involve forming a complex between the TCR, a cognate peptide-MHC class I molecule, and the CD8 coreceptor, resulting in the generation of signal 1. Professional APCs can also express high levels of costimulatory receptors and cytokines that can provide signals 2 and 3, further promoting T cell expansion and effector differentiation. (*b*) TCE-mediated activation of a $CD8^+$ T cell through interactions with a TAA-expressing tumor cell. A TCE may induce qualitatively different TCR signals compared with a cognate peptide-MHC complex due to the absence of coreceptor engagement and other biophysical binding properties. Signals 2 and 3 may also be attenuated or absent, depending on the phenotype of the tumor cell. As such, current approaches to enhance TCE activity have focused on modulating TCE-TCR binding kinetics and delivering signals 2 or 3 through combination therapy. Examples of therapeutic combinations currently being explored include bifunctional antibodies capable of mediating TAA-dependent costimulatory receptor signaling or targeted cytokine delivery to a T cell. Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex; TAA, tumor-associated antigen; TCR, T cell receptor.

between T cells and professional antigen-presenting cells (APCs) that have captured, processed, and presented tumor antigens in the context of MHC molecules within both TDLNs and tumor lesions. Antitumor T cell responses induced by a TCE are fundamentally different from those that develop against endogenous tumor antigens presented by MHC molecules. At the molecular level, signal 1 is normally delivered by low-affinity interactions between TCR and MHC complexes that include recruitment of the CD4 or CD8 coreceptor. In contrast, TCE-mediated TCR cross-linking is accomplished by relatively high-affinity binding to CD3 in the absence of coreceptor engagement. Therefore, TCE binding may lead to qualitative differences in TCR signaling that could impact T cell expansion and effector differentiation.

In addition to the molecular differences in signal 1, TCE-engaged T cells may not receive the same costimulatory or cytokine signals that are typically provided by professional APCs. The absence of these signals may negatively impact the T cell response by inducing nonresponsiveness or failing to support T cell differentiation (Subklewe 2021). In mouse models, the addition of a costimulatory 4-1BB agonist to TCE therapy can dramatically enhance tumor-associated $CD8^+$ T cell proliferation and expansion and tumor growth inhibition (Belmontes et al. 2021, Griessinger et al. 2020), and this combination may be superior to the combination of TCE

with programmed death-1 (PD-1) or cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) blockade (Sawant et al. 2019). TCE combination with a CD28 agonist (Correnti et al. 2018) may offer a similar benefit as a combination with 4-1BB; however, no head-to-head studies compare the TCE-potentiating activity of a 4-1BB agonist to a CD28 agonist, so it is unclear which approach is superior. While costimulatory receptor agonists enable TCE function in model systems, these combinations may impact the therapeutic window by potentiating cytokine release or normal tissue damage; thus, their applicability to the clinical setting remains to be determined.

Increase Intratumoral T Cell Density

Consistent with their critical role in antitumor immunity, $CD8^+$ T cell density in solid tumors has been shown to positively correlate with prognosis across a variety of solid tumor indications (Bruni et al. 2020) and to serve as a predictive biomarker of response to anti-PD-1/L1 therapy (Kim et al. 2019, Sun et al. 2018, Tumeh et al. 2014, Uryvaev et al. 2018) (**Figure 4*a***). Given that TCE activity requires both T cells and tumor cells, the baseline density of effector T cells in the tumor and the ability of TCE treatment to induce T cell expansion or recruitment from circulation may be critical factors determining TCE efficacy in solid tumors. To evaluate the importance of baseline T cell density for TCE activity, we have developed an immunocompetent mouse model in which TCEs

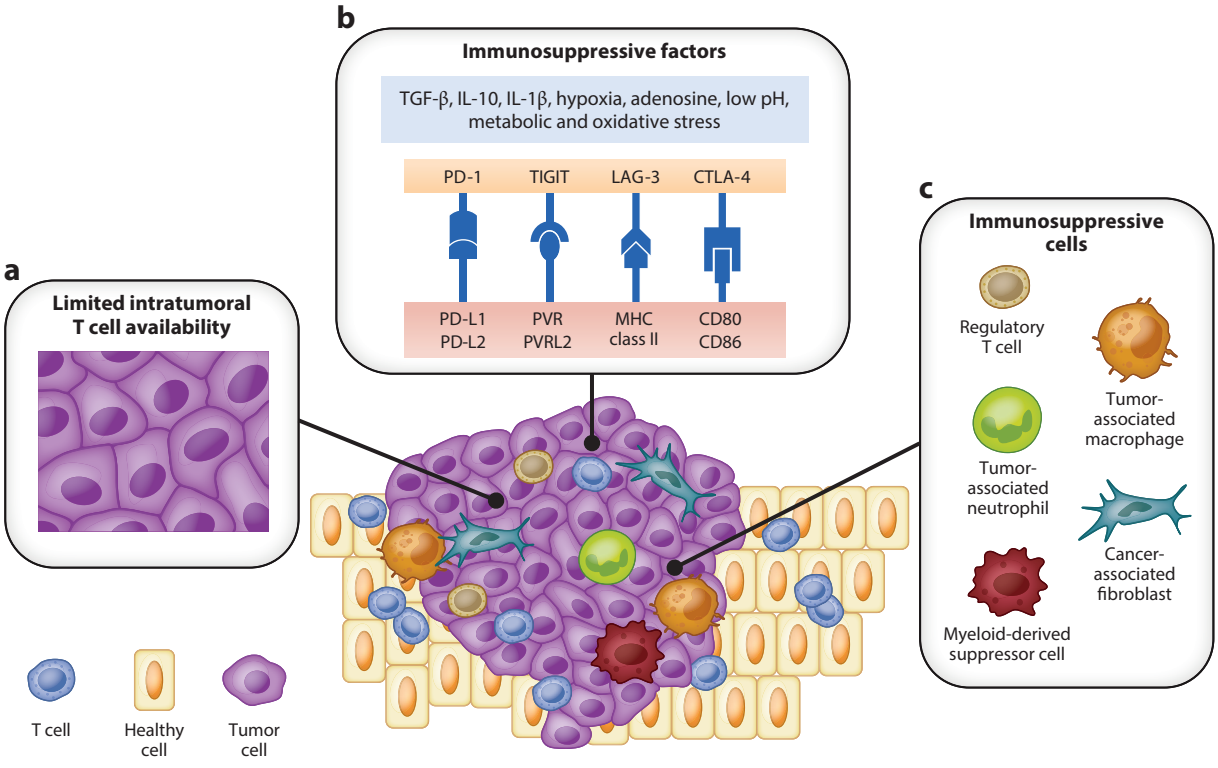


Figure 4

Barriers to TCE efficacy in solid tumors. (*a*) Limited T cell infiltration. (*b*) Immunosuppressive molecules and factors (*top*) and checkpoint proteins (*bottom*). (*c*) Cells contributing to immunosuppression in the tumor microenvironment. Abbreviations: MHC, major histocompatibility complex.

can engage mouse T cells directly (Belmontes et al. 2021). By profiling multiple tumor models, we found a correlation between TCE activity and the baseline T cell density. Tumors displaying a high density of baseline T cell infiltrate showed a stronger TCE response than those with low-density baseline T cell infiltrate (Belmontes et al. 2021). Posttreatment analysis of the tumor-associated T cell compartment revealed TCE-induced T cell activation but only limited T cell proliferation or recruitment, suggesting that TCE monotherapy may fail to expand the T cell compartment sufficiently to control a rapidly growing tumor. The addition of a 4-1BB agonist greatly increased proliferation and expansion of tumor-associated T cells, leading to significant tumor regression even in tumor models with extremely low baseline T cell infiltrate that were nonresponsive to immune checkpoint blockade. Notably, the ability of TCE treatment to recruit T cells into tumors may vary across different models and TCE targets (Benonisson et al. 2019, Li et al. 2018).

Overcome the Immunosuppressive Tumor Microenvironment

The TME of solid tumors contains a complex mixture of malignant, immune, and stromal cell populations that can suppress antitumor T cell responses through multiple pathways, including the upregulation of immune checkpoint receptor ligands, recruitment and expansion of immunosuppressive cell populations, and increased metabolic stress (Binnewies et al. 2018, Ho et al. 2020, Lim et al. 2020) (**Figure 4*b*,*c***). As TCEs function by directly activating tumor-associated T cells, many of the suppressive mechanisms present in the TME have the potential to negatively impact TCE efficacy. However, to date, only a few of these inhibitory pathways have been directly evaluated in the context of TCE activity.

The inhibitory receptor PD-1 plays a dominant role in the regulation of antitumor T cell responses and may directly inhibit TCE-mediated T cell activation and cytolytic activity. Multiple in vitro studies have shown that the potency of TCE-mediated tumor cell killing can be enhanced by the addition of PD-1 blockade (Feucht et al. 2016, Krupka et al. 2016, Laszlo et al. 2015), and an early clinical study investigating the combination of a CEA-targeted TCE with PD-L1 blockade in colorectal cancer showed a higher response rate in patients treated with combination therapy compared with those receiving TCE as monotherapy (Argilés et al. 2017). The overall impact on safety and antitumor activity of combination therapy with PD-1 checkpoint blockade and TCEs remains an area of active clinical investigation.

As discussed earlier, multiple cell types are found within the TME of solid tumors that function to suppress T cell recruitment, activation, and cytolytic activity (**Figure 4*c***). In the context of TCEs, regulatory T cells (Tregs) represent perhaps the most relevant immunosuppressive population, given their ability to be directly stimulated following TCE treatment. In a mouse model containing a high frequency of Tregs, we found that depletion of all $CD4^+$ T cells, which ablated both $CD4^+$ effector T cells and Tregs, was able to dramatically enhance TCE efficacy (Belmontes et al. 2021), presumably by removing Treg-mediated inhibition of $CD8^+$ T cell cytolytic activity; however, preclinical studies evaluating the effect of $CD4^+$ T cell depletion on TCE activity using different tumor models have not shown this potentiating effect (Benonisson et al. 2019, Li et al. 2018). Notably, higher circulating Treg frequency was associated with lower activity of blinatumomab in patients with ALL (Duell et al. 2017). Contrary to these findings, in vitro studies have demonstrated that under certain circumstances, TCE can induce Tregs to directly kill tumor cells through a granzyme-dependent mechanism (Choi et al. 2013). Additional preclinical and clinical work is needed to understand the role of Tregs in suppressing TCE-mediated effector T cell activation, the consequences of TCE-mediated stimulation of Tregs for their immunosuppressive activity, and the effects of combining a Treg-targeting therapy (e.g., anti-CTLA-4) with a TCE on both efficacy and safety.

CONCLUSIONS

The BiTE molecule blinatumomab provided clinical proof of concept for TCE antitumor activity in B cell malignancies and launched intense interest in TCE development across tumor indications. While efficacy of several TCE molecules has been demonstrated in multiple hematologic malignancies, proof of concept for TCE activity in solid tumor settings has only emerged recently. To successfully apply TCEs to solid tumors, researchers need to overcome several obstacles, starting with identification of tumor-specific antigens or TCE engineering options to enable tumor-specific engagement of TAAs that are expressed on normal tissue. Subsequently, the format, binding, and potency properties of the TCE need to be optimized to balance safety with activity. To deliver optimal activity in solid tumors, TCEs may need to be combined with agents that support T cell proliferation and survival or neutralize the immunosuppressive TME. Further characterization of tumor samples, pre- and post-TCE treatment, correlating tumor and immune cell components with efficacy will provide information on mechanisms of resistance and determine which combinations will be most beneficial. Emerging preclinical and clinical data reinforce the potential of the TCE modality to be a foundational component of cancer treatment.

DISCLOSURE STATEMENT

T.A., J.M.B., C.D.B., M.K., D.N., A.C., J.G.E., and F.M. are or were employees of Amgen Inc. and hold stock in Amgen Inc. T.A. and J.M.B. hold patents on products developed through Amgen Inc.

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