CARs versus BiTEs: A Comparison between T Cell–Redirection Strategies for Cancer Treatment

Clare Y. Slaney1,2, Pin Wang3, Phillip K. Darcy1,2, and Michael H. Kershaw1,2

ABSTRACT

The redirection of T cells against tumors holds much promise for the treatment of cancer. Two main approaches for T-cell redirection involve their genetic modification with chimeric antigen receptors (CAR), or the use of recombinant proteins designated bispecific T-cell engagers (BiTE). These approaches have demonstrated dramatic effects in patients with hematologic cancers, although limited effect against solid cancers. Here, we review and compare the successes and challenges of these two types of immunotherapies, with special focus on their mechanisms, and discuss strategies to improve their efficacy against cancer.

Significance: CAR and BiTE cancer therapies have generated much excitement, but although the therapies are potentially competitive, information directly comparing the two is difficult to obtain. Here, we present the fundamentals of each approach and compare the range and level of functions they can elicit from T cells, and their efficacy against cancers.

INTRODUCTION

Immunotherapy that utilizes the body’s immune system against tumors is a promising field of cancer research. In recent years, excising technologies and platforms have been developed for the intricate design and production of anticancer immune reagents using antibodies in the form of single-chain variable fragments (scFv; Fig. 1). The scFv technology has been adopted and used in some promising cancer immunotherapies, including bispecific T-cell engager (BiTE) and chimeric antigen receptor (CAR). Both strategies use scFv to direct cytotoxic T lymphocytes (CTL) to specific surface antigens on cancer cells to facilitate a polyclonal T-cell response to tumor antigens.

A BiTE is a recombinant bispecific protein that has two linked scFvs from two different antibodies, one targeting a cell-surface molecule on T cells (for example, CD3ε) and the other targeting antigens on the surface of malignant cells. The two scFvs are linked together by a short flexible linker (Fig. 1). By binding to tumor antigens and T cells simultaneously, BiTEs mediate T-cell responses and killing of tumor cells. Importantly, the T-cell/target cell adherence facilitated by BiTE is independent of MHC haplotype (1).

A variety of formats for bispecific antibodies have been developed in addition to BiTEs, including bispecific IgG, diabodies, and conjugates, which have been the subject of some comprehensive recent reviews (2, 3). BiTEs have the advantages of relatively simple recombinant production and purification, and a molecular weight enabling tissue penetration. BiTEs have also advanced through the regulatory framework, gaining FDA approval for certain indications, and will be the focus of this review.

The other area that has a high level of excitement for using scFv technologies is the CAR T-cell approach for adoptive cell transfer. A CAR is a synthetic receptor comprising an extracellular tumor antigen recognition domain, which is fused to the CD3ζ chain for intracellular signaling and contains one or more costimulatory domains (Fig. 2). The binding of scFv to tumor cells triggers the T-cell receptor (TCR) intracellular domain and leads to T-cell responses against antigen-expressing cells.

Various molecular formats of CAR have been developed, differing in their extracellular, transmembrane, and cytoplasmic domains, which have been the subject of some previous excellent reviews (4). In this review, we focus on CARs in which the predominant cytoplasmic signaling domains are derived from CD3ζ and the costimulatory molecules CD28 or CD137, which endow T cells with significant antitumor activity, and which have received FDA approval for the treatment of some hematologic malignancies.
Recent advances and positive clinical results from BiTE and CAR T-cell therapies have generated hope and excitement. However, both therapies are facing similar dilemmas, such as toxicity concerns in hematologic cancers and a lack of response in solid cancers. In this article, we review and compare the recent progress in the application of BiTE and CAR T-cell therapies, summarize the current understanding of their anticancer mechanisms, and discuss strategies to improve their efficacy against cancers (Table 1).

MECHANISM KEY POINTS

T-cell Types

T-cell populations are made up predominantly of two cell subsets, expressing either CD4 or CD8. The main function of CD8+ T cells is considered to be cytolysis of tumor cells, whereas CD4+ T cells secrete cytokines to regulate immune responses. In the CAR T-cell approach, CD4+ T cells and CD8+ T cells are usually mixed and genetically modified with CAR and infused back to the patients in clinical trials. Although there is no doubt that CD8+ CAR T cells are the major players in killing cancer cells, CD4+ T cells armed with a CAR can also be activated via CAR and showed cytolytic activities. Although CD4+ CAR T cells eradicated leukemia cells more slowly, these CD4+ CAR T cells persisted longer in vivo (5).

Clinical trials, mixing CAR-equipped CD4+ and CD8+ T cells at a fixed ratio in treating patients, have generated remarkable responses. A study using a defined ratio of CD4+:CD8+ CAR T cells treating refractory B-cell acute lymphoblastic leukemia (B-ALL) achieved bone marrow disease remission in 27 of 29 evaluable patients (6). In that study, 27 patients received a 1:1 ratio of CD4+:CD8+ CAR T cells, whereas 2 patients received alternate ratios. Of the 2 patients receiving alternate ratios, one relapsed and one achieved minimal residual disease. The low patient numbers and intratrial differences in treatment regimens did not allow a statistically meaningful comparison between patients receiving a 1:1 and other ratios. Interestingly, a similar approach using a defined mixture of CD4+ and CD8+ CAR T cells in patients with relapsed/refractory B-cell non-Hodgkin lymphoma (NHL) achieved 64% complete responses (7). This was comparable with other studies using CAR T cells of variable composition (8, 9), although differences in doses and treatment regimens did not allow a direct comparison of efficacy.
effector (T EFF) T cells. It is now evident that less differentiated that T SCM cells had greater resistance to cell death caused by first 6 weeks after infusion (10). Preclinical studies showed related with their (T SCM), central memory (T CM), effector memory (T EM), and antigen-experienced or activated subtypes: stem cell memory frequency of T SCM cells within the transferred lymphocytes cor-

Table 1. Comparison of CAR T cells and BITEs

<table>
<thead>
<tr>
<th></th>
<th>CAR T cell</th>
<th>BITE</th>
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<tbody>
<tr>
<td>Structure</td>
<td>A synthetic gene construct encoding an scFv against tumor antigen linked to activation and costimulatory motifs.</td>
<td>A recombinant protein composed of two linked scFvs; one binds to CD3 on T cells and the other to target a tumor antigen on tumor cells.</td>
</tr>
<tr>
<td>Effector cell types</td>
<td>Engineered CD8+ and CD4+ T cells (5). Less-differentiated subsets displaying better antitumor activity in vivo (T SCM and T CM; ref. 10).</td>
<td>Endogenous CD8+ and CD4+ T cells (13). Antigen-experienced T EM, but not T N, effective (14).</td>
</tr>
<tr>
<td>Immune synapse</td>
<td>Atypical (15).</td>
<td>Typical (17–19).</td>
</tr>
<tr>
<td>Serial killing</td>
<td>Yes (16).</td>
<td>Yes (22).</td>
</tr>
<tr>
<td>Killing mechanisms</td>
<td>Perforin and granzyme B (16), Fas/Fas-L, or TNF/TNF-R.</td>
<td>Perforin and granzyme B (17).</td>
</tr>
<tr>
<td>Trafficking</td>
<td>Active. Trafficking of CAR T cells involves comprehensive interactions between various molecules and cell–cell interactions (57).</td>
<td>Passive. Biodistribution depends on factors related to rates of diffusion through vascular endothelium, fluid flow rates, and interaction with target.</td>
</tr>
<tr>
<td>Toxicity</td>
<td>CRS, neurotoxicity, B-cell aplasia (31, 49).</td>
<td>CRS, neurotoxicity, B-cell aplasia (62, 64).</td>
</tr>
<tr>
<td>Clinical applications</td>
<td>Pretreatment lymphodepleting regime using cyclophosphamide and fludarabine. Premedicate with anti-CD20 and an H1-antihistamine. One infusion.</td>
<td>No lymphodepletion regime required. Premedicate with dexamethasone. Repeat administration necessary, including continuous i.v. infusion regimens.</td>
</tr>
<tr>
<td>FDA approval</td>
<td>Yesera was approved to treat adult patients with relapsed/refractory B-cell lymphoma in 2017. Kymriah was approved to treat patients up to 25 years of age with refractory/B-ALL in 2017.</td>
<td>Blinatumomab was approved to treat relapsed/refractory B-ALL in 2014 and 2017.</td>
</tr>
<tr>
<td>Other characteristics</td>
<td>Individually produced for each patient. “Off the shelf” reagents.</td>
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In addition to cell subsets, T cells can exist in several differentiation states that have different functional abilities. T cells can be classified into naive T cells (T N), and four main antigen-experienced or activated subtypes: stem cell memory (T SCM), central memory (T CM), effector memory (T EM), and effector (T EFF) T cells. It is now evident that less differentiated CAR T cells have better efficacy in vivo. In CD19-CAR–treated patients with relapsed/refractory B-cell malignancies, the frequency of T SCM cells within the transferred lymphocytes correlated with their in vivo expansion and persistence during the first 6 weeks after infusion (10). Preclinical studies showed that T SCM cells had greater resistance to cell death caused by repetitive encounter with antigen and had better migration ability to secondary lymphoid organs (10). It is proposed that T CM are capable of self-renewal and give rise to shorter-lived T EM and T EFF cells that are incapable of self-renewal (11). Therefore, although T CM and T EFF can be present at high frequency at the peak of the anticancer immune response, their persistence is poor. Accordingly, strategies favoring the generation and preservation of T SCM and T CM have been applied in the CAR T-cell therapy field (12).

Similar to CAR T-cell therapy, BITE therapies involve polyclonal T-cell responses, which are independent of MHC and TCR recognition and costimulation. It has been demonstrated that when incubated with BITEs, both CD8+ and CD4+ T cells can be activated and induce target tumor cell death, with CD8+ cells killing faster than CD4+ T cells (13). In contrast to CAR T-cell therapy, where less differentiated T cells showed better efficacy, in BITE treatment, antigen-experienced T-cell subsets mediate BITE-induced tumor cell death, whereas naive T cells are not activated when engaged by BITE (1). In an early study, Bargou and colleagues reported that following injection of the anti-CD19 BITE blinatumomab in patients with relapsed and refractory NHL, T cells were redistributed and activated. T cells transiently declined in numbers within 24 to 48 hours, as the T cells adhered to blood vessels endothelium and/or extravasated. T cells then returned to baseline numbers with an activated phenotype, driven by an expansion of CD4+ and CD8+ T EM cells. T-cell levels eventually exceeded pretreatment levels, and this increase was due to the expansion of T CM cells, but not naive T cells (14). Despite some differences in the optimal cell phenotype for each approach, both therapies mediated tumor cell killing independent of MHC and provide an opportunity to redirect T-cell cytotoxicity to tumor cells.

T-cell Cytotoxicity

CAR T cells can induce target tumor cell death through the formation of synapses, cytokine secretion, perforin/granzyme B release and/or death receptor ligation. Interestingly, CAR-mediated immune synapse formation has been reported to differ from the traditional TCR-induced synapse, in that the CAR-induced synapse has a much smaller actin ring and diffuse distribution of LCK. In addition, the microtubule-organizing center is distant from the immune synapse in comparison with the classic TCR-induced synapse, which is
proximal to the LCK accumulation. The adhesion molecule LFA1 does not accumulate at the synapse, which could result in rapid detachment from the dying tumor cells, thereby enabling CAR T cells to act rapidly (15). In fact, CAR T-cell serial killing (one T cell kills multiple targets) requires a significantly shorter time than TCR-mediated serial killing of cancer cells (16).

Similar to CAR T cells, it has been reported that, when incubated with BiTEs, both CD8+ and CD4+ T cells could be activated to kill target tumor cells in vitro, via perforin and granzyme B (17), although roles for TNF and FAS do not appear to have been investigated. However, initial studies suggest that differences exist between the synapse formed between T cells and target cells when mediated by BiTEs compared with CARs. The linkage of tumor cells and T cells by BiTE leads to the formation of cytolytic synapses that are similar to the ones formed during regular cytotoxic T-cell recognition (17–19). Once activated, these T cells start to proliferate and also upregulate the expression of activation markers, such as CD69 and CD25 (20). Although the reason for the difference in synapses formed by CARs and BiTEs is not clear, it potentially arises because BiTEs engage the natural CD3 complex, whereas CARs do not.

Both the size and the position of the epitope to the cell surface determine the extent of BiTE-mediated cancer cell killing (21). Activated T cells kill the target cancer cells via triggering apoptosis, as evidenced by caspase 3/7 activation, PARP cleavage, DNA fragmentation, and membrane blebbing (17). Similar to CAR T cells, it has been shown that BiTEs can induce serial killing by T cells, with one T cell able to kill several target cells (22). However, unlike CAR redirected T-cell function, BiTE-directed function is sensitive to the dose of BiTE administered. Although this may have advantages in tuning the response to avoid an overreaction and potential toxic levels of cytokine, an excess of BiTE may completely coat CD3 and target antigen separately, thereby reducing the opportunity for coengagement of T cells and target cells (23–25).

Although direct cancer cell lysis is seen as the most potent means to reduce cancer burden by T cells, large amounts of cytokines can be secreted by both CAR- and BiTE redirected means (26). These effector cytokines have the potential to change the tumor microenvironment and induce endogenous antitumor immunity. This potential was evident in a study that demonstrated the ability of CAR T cell–secreted cytokines to change the recruitment and activation of endogenous macrophages and create a favorable milieu for antitumor immune response (27). Interestingly, studies using both BiTEs and CARs suggest that cytokines secreted upon target cell ligation can contribute to lysis of antigen-negative tumor cells in close proximity to the antigen-specific engagement, so-called bystander lysis (28, 29), although this is not always the case for all target antigens (30).

**T-cell Numbers and Persistence**

The resolution of malignant disease requires large numbers of responding T cells and their continued action over prolonged time periods. In the case of CAR T cells, these requirements are best achieved by expansion in vivo and long-term engraftment following transfer. In some hematologic malignancies, CAR T cells have been demonstrated to expand up to, or even exceeding, 1,000-fold (31, 32) and to reach greater than 20% of circulating lymphocytes (33). In clinical studies targeting CD19 on B-cell malignancies, absolute counts of circulating CAR T cells can vary among patients, but still achieve levels from several thousand per mL of blood up to several hundred thousand per mL (8, 34, 35).

In the case of BiTEs, a large pool of antigen-experienced T cells can be exploited to provide substantial numbers of redirected T cells, with less reliance on expansion. Nevertheless, increased numbers of circulating T cells have been demonstrated following BiTE administration, suggesting expansion of the pool of T cells with the potential to respond against tumor cells. Increases of the order of 2- to 4-fold in circulating T cells have been described (14, 36, 37). However, because the number of antigen-experienced T cells (able to respond independently of costimulation) comprises only a proportion of total T cells, the actual level of expansion of responding T cells is likely several folds higher than that reflected in increases in total circulating T cells.

The provision of large numbers of tumor-reactive T cells in the solid-tumor setting presents different challenges for the BiTE and CAR T-cell approaches. Whereas extensive expansion and prolonged persistence has been demonstrated for CAR T cells in hematologic cancers, little if any expansion of CAR T cells is usually demonstrated when treating solid cancers (38–40), except for isolated reports of expansion exceeding 100-fold (41, 42). CAR T cells directed against blood cancers may receive further costimulatory signals from cancer cells, or other antigen-positive leukocytes, in a microenvironment that is less immunosuppressive than that found in solid tumors. This may explain the observed differences in T-cell expansion in liquid cancers compared with solid cancers. Approaches combining CAR T-cell transfer with vaccines, to provide T-cell support away from the tumor microenvironment, are being used to enhance CAR T-cell proliferation and persistence in the solid-tumor setting (43, 44).

Because the BiTE approach is potentially less reliant on T-cell expansion, it may be more successful against solid tumors. However, the success of this approach is dependent on the sustained loading of BiTEs on T cells, which may be compromised during the process of penetration of solid tumors.

Differences between the CAR and BiTE approaches also exist when considering tumor recurrence. CAR T cells are able to engraft long-term and provide an ongoing source of tumor-reactive T cells to respond against recurring tumors, even before they become evident. This has been demonstrated in mouse tumor models, in which mice that rejected a primary tumor also rejected a secondary challenge with tumor cells (43). In contrast, without continuous administration of BiTEs, recurrent tumors have the opportunity to grow large before detection, which may render subsequent treatment with BiTEs less effective.

**CLINICAL APPLICATIONS**

CAR T-cell therapies have generated fantastic responses in B-cell malignancies and revolutionized the field of cancer immunotherapy. CD19 CAR T-cell therapy has been tested in treating different B-cell malignancies, including NHL (7, 45,
Slaney et al. reported that CAR T-cell treatment targeting IL13Rα2 has been effective in glioblastoma (60). Interestingly, a recent inspiring study demonstrated CAR T-cell therapy targeting neuronal-specific enolase (NSE) in glioblastoma and other solid cancers (40, 59), and EGFRvIII in colorectal cancer (56, 57). A number of tumor antigens have been targeted in solid-cancer clinical trials, including BCMA in multiple myeloma (54), and Lewis Y in acute myeloid leukemia (AML; ref. 55) have been tested for CAR T-cell therapy. Without this, significant clinical benefit is rarely observed (51).

Given the remarkable initial response rate observed using CAR T cells in some blood cancers, but a significant relapse frequency at present, the question remains as to whether this form of therapy would be best applied as a stand-alone treatment or as a bridge to stem-cell transplantation. The use of CAR T cells as a bridge to transplant has some attractive features including dramatic reduction in disease burden often to undetectable levels, perhaps enabling a subsequent less-intensive conditioning regimen prior to transplant. In the CAR T-cell setting, transplantation following conditioning would have the added benefit of eliminating CAR T cells to allow recovery of normal B cells, because B-cell aplasia can persist long-term in a substantial proportion of patients with leukemia/lymphoma achieving complete responses. This is likely to continue to be an important option for clinicians in the treatment of hematologic cancers. However, the increased cost of an additional transplant procedure, together with transplant-related toxicities if using allogeneic donors, indicates that with improvements to cell production and efficacy, and controlled gene expression or inclusion of suicide genes for CAR T cells, a stand-alone approach would be an ultimate goal.

Although highly effective against CD19 B-cell malignancies, CAR T-cell therapy did not generate the same potent response against other lymphomas/leukemias. In addition, the loss of CD19 expression in patients can limit the CD19-CAR therapeutic benefit. Recently, a few additional CAR targets have been tested. CD22 in treating patients with ALL who are refractory to CD19 CAR T-cell therapy (52), CD20 in indolent B-cell and mantle cell lymphomas (53), BCMA maturation antigen (BCMA) in multiple myeloma (54), and Lewis Y in acute myeloid leukemia (AML; ref. 55) have been tested in the clinic and generated encouraging results.

In solid-cancer clinical trials, CAR T cells have not demonstrated the same dramatic effect as seen in hematologic cancers. The reasons are believed to be the solid-tumor immunosuppressive microenvironment, impaired T-cell trafficking, and the suboptimal phenotype of the infused CAR T cells (11, 56, 57). A number of tumor antigens have been targeted in solid cancers, such as GD2 in neuroblastoma (58), HER2 in glioblastoma and other solid cancers (40, 59), and EGFRvIII in glioblastoma (60). Interestingly, a recent inspiring study demonstrated that CAR T-cell treatment targeting IL13Rα2 has successfully eradicated a case of glioblastoma, and the complete response lasted several months (61). Although some of the trials have observed some response, the overall outcomes are less satisfactory than the ones used in blood cancers. There is great enthusiasm to improve CAR T-cell treatment efficacy against solid cancers in preclinical research, and this is discussed in the next section.

Similar to CAR T cells, BiTEs have been used in clinical trials targeting a variety of antigens. Blinatumomab was the first clinically tested and FDA-approved BiTE, receiving accelerated approval in 2014 for the treatment of CD19+ Philadelphia chromosome-negative (Ph−) relapsed and refractory B-ALL. In July 2017, the FDA further approved its use to include the treatment of Philadelphia chromosome-positive (Ph+) relapsed or refractory B-ALL.

A phase II trial (ALCANTARA, NCT02000427) enrolled 45 patients with Ph+ relapsed or refractory B-ALL for the blinatumomab treatment. In this trial, 16 patients (36%) achieved complete remission (CR) or CR with partial hematologic recovery, and the median duration of remission was 6.7 months (62). Thus, blinatumomab has led to promising results for patients with Ph+ relapsed/refractory ALL. A recent phase II trial (TOWER, NCT02013167) compared blinatumomab with standard-care chemotherapy in 405 patients with Ph+ relapsed and refractory B-ALL. Blinatumomab demonstrated a significant improvement in overall survival (OS) and higher rates of hematologic remission than chemotherapy. The median OS was 7.7 months with blinatumomab compared with 4 months with chemotherapy (63).

Apart from ALL, blinatumomab has also shown promise in treating NHL. The initial clinical studies used short infusions of blinatumomab and did not show efficacy but did show toxicities such as cytokine release syndrome (CRS) and neurotoxicity (14). Subsequently, lessons were learned from the ALL studies for continuous infusion of blinatumomab. A clinical trial using continuous infusion of blinatumomab 60 μg/m²/day in NHL (4-fold higher than ALL) has proven to be feasible. In this phase I trial, single-agent blinatumomab showed promising activity with an objective response rate of 69% (24/35 patients; ref. 64).

As with CAR T cells, treatment with BiTEs can lead to high initial response rates but a significant relapse rate, and the use of BiTEs in the hematologic setting offers an option as a bridge to transplantation. As with CAR T-cell therapy, dramatic reductions in disease and complete responses are observed in many patients but, in contrast to CAR T cells where T cells persist long-term, BiTEs require continuous administration to provide protection against disease recurrence. Therefore, hematopoietic stem-cell transplantation following BiTE therapy may be beneficial. Longer-term follow-up of complete responders receiving BiTEs (or CAR T cells) will be informative to determine the feasibility of using T-cell redirection as a stand-alone therapy.

Other tumor antigens have also been explored for BiTE therapy. For example, CD3–CD33 (AMG330) BiTE has been constructed to treat patients with relapsed/refractory AML (NCT02771501).

For treating solid tumors, CD3-coupled BiTEs specific for EPCAM (solitomab), CEA, and prostate-specific membrane antigen (PSMA) have all been tested. Limited responses were observed in treating patients with solid tumors, and toxicity was observed. In a trial using solitomab treating different...
solid tumors, transient elevation of liver enzymes and diarrhea as dose-limiting toxicities were observed. This may be due to the targeting of EPCAM to normal tissue in bowel epithelial and bile ducts. The toxicity precluded further dose escalation (65). Although some tumor response was observed, higher doses were limited by toxicity. New targets, such as gpc3 to treat hepatocellular carcinoma, have also been tested (66).

TOXICITIES
CAR T-cell therapy can be associated with some toxicities. The most commonly observed toxicity is CRS, a systemic response due to elevated levels of cytokines. Studies have demonstrated that high levels of 24 serum cytokines, such as IL6 and IFNγ in the first months after CAR T-cell infusion, were associated with severe CRS (67). CRS occurs several hours to 14 days after CAR T-cell infusion, and symptoms include fever, fatigue, anorexia, hypoventilation, tachycardia, and capillary leak, which can be life-threatening. It was demonstrated that the patients with highly elevated levels of IL6 are the ones with maximum CAR T-cell activation and proliferation (49). Anti-IL6 receptor antibody (tocilizumab) that blocks IL6 from binding to its receptor can reverse the life-threatening CRS without inhibiting CAR T-cell treatment efficacy (31).

Neurotoxicity, also termed CAR T cell–related encephalopathy syndrome (CRES), has also been reported (31, 48, 49). CRES is typically manifested by symptoms of confusion and delirium and occasionally by seizures and cerebral edema (6, 68). The pathogenesis of neurologic toxicity remains unclear, although it has been proposed that CRES could be due to cytokine-mediated endothelial activation and passive diffusion of cytokines into the brain (69) and/or trafficking of T cells into the central nervous system (CNS; ref. 68). Although CD19 CAR T cells were identified in the cerebrospinal fluid (CSF), levels of the transgene in the brain did not correlate with rates of high-grade CRES events (32). CRES is generally reversible using anti-IL6 receptor and/or corticosteroids (68, 69).

B-cell aplasia is also associated with CD19 CAR T-cell therapy, because CD19 is expressed on normal B cells, but injections of immunoglobulin can be used to maintain levels of circulating antibodies (49). Infections with microorganisms can also occur following CAR T-cell transfer, which was largely due to depletion of endogenous leukocytes by preconditioning regimens (70). Bacterial infections predominated, which included bacteremia and more localized infections to sites including gastrointestinal tissue. Viral and fungal infections were also experienced. These infections are largely manageable using antibiotics, although some fatalities have resulted.

Although leukodepleting preconditioning is not required for BiTE therapy, toxicities after blinatumomab treatment are common, but mostly manageable. The most frequent adverse effects were CRS and neurologic adverse effects including headache, tremor, aphasia, and seizure (62, 64). These effects might be associated with T-cell migration to the CNS or a transient cytokine storm in serum. Currently, the blinatumomab treatment dosage and administration schedule is based on body weight, and patients are required to be premedicated with dexamethasone, to reduce cytokine production while retaining cytolytic activity (71, 72). In addition, the treatment of blinatumomab also depleted normal B cells and plasma precursor cells, resulting in substantial hypogammaglobulinemia. This presents risk of infections, which can be reduced by administration of intravenous immunoglobulin.

RESEARCH IN ENHANCING TREATMENT EFFICACY AGAINST SOLID CANCERS
Both BiTE and CAR T-cell treatment strategies have produced remarkable effects in hematologic malignancies; however, the efficacy for both was generally poor against solid cancers. Considerable effort is being invested in enhancing the treatment efficacy against solid cancers.

For CAR T-cell therapy, the hurdles for treating solid cancers lie in the combination of the immunosuppressive effect of the tumor microenvironment and inefficient penetration of CAR T cells into tumors. The tumor microenvironment is a complex assortment of cell types including malignant cells, fibroblasts, endothelium, and immunoregulatory cells. Regulatory cell types include myeloid-derived suppressor cells (MDSC), M2 macrophages, and regulatory T cells (Treg; ref. 73). Using a preclinical model, Moon and colleagues demonstrated that CAR T cells were able to slow down tumor growth, but did not cause regression. The reason was the rapid loss of functions of CAR T cells when exposed to the tumor microenvironment in vivo. Diminished functions included reduction in the ability to secrete cytokines, killing ability, and phospho-ERK expression. When CAR T cells were isolated and rested away from the tumor, their hypofunction was reversed (74).

For targeting the suppressive tumor microenvironment, a number of strategies have been tested. For example, using all-trans retinoic acid (75) or GM-CSF neutralization to disrupt MDSC function (76), targeting CD123 on macrophages (77), or fibroblast activation protein on cancer-associated fibroblasts (78) inhibiting adenosine 2A receptors (79) and altering metabolic components such as inhibiting protein kinase A activation (80) all demonstrated enhancement of CAR T-cell treatment efficacy in preclinical models of solid tumors. In addition, there has been great interest in using checkpoint inhibitors as adjuvant therapy for CAR T-cell treatment. Blocking a number of checkpoints, such as PD-1 (81) and LAG3 (82), has been shown to enhance CAR T-cell treatment in solid tumors.

Other strategies that have shown promising results include supplying CAR T cells with cytokine support to enhance their activity within the tumor microenvironment (83). Such approaches include inducing the local release of stimulatory cytokines, including IL12 (84), and transducing the IL7 receptor into CAR T cells to provide signal 3 for T-cell support (85). Inhibitory cytokines present in the tumor microenvironment can also be harnessed to provide support for T cells. In this approach, T cells are genetically modified with chimeric cytokine receptors. For example, chimeric cytokine receptors composed of the extracellular domain of the IL4 receptor fused to the intracellular domain of the IL7 receptor led to activation and proliferation of T cells in the presence of the normally inhibitory cytokine IL4 (86, 87).

To enhance CAR T-cell infiltration to the tumor bed, effort has been made to modify chemokine receptors on CAR T cells to enhance their migration toward tumors. For example, CAR T cells have been modified to express CCR2b, the chemokine

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receptor for CCL2 that is highly expressed by the target tumors (88). To facilitate the penetration into tumors, construction of CARs targeting VEGFR2 or αvβ3 integrin over-expressed on tumor vasculature has been reported (89, 90).

Similar to CAR T-cell therapy, the efficacy of BiTEs in treating solid tumors in preclinical models is modest. One of the major hurdles for BiTEs is their short half-life in serum. After injection, BiTEs are rapidly catabolized and cleared from the circulation, leading to a short serum half-life of around 2 hours (91). To overcome the limitation, current approaches include attaching BiTEs to heavy-chain fragments or other novel designs to increase the serum life. A number of these methods significantly increased the BiTE circulation time without hampering its binding to the target cells (92).

The other major hurdle for treating solid tumors using BiTE is the density and types of T cells already in the tumor bed. Small-animal PET and fluorescent imaging studies have demonstrated the penetration of BiTE into solid tumors (93). However, the profiles of the T cells already existing in the tumor are difficult to predict, as the intensity, location, phenotype, and activation phenotypes of these T cells vary dramatically among tumors (94). In preexisting T cells in the tumor may already have an exhausted phenotype or may be Tregs. Approaches in increasing T-cell infiltration into tumor tissue and combinational approaches to reverse T-cell exhaustion and targeting the tumor immunosuppressive microenvironment warrant further investigation to boost the efficacy of BiTE against solid cancers.

Recent enthusiasm surrounding BiTEs extends toward their production in vivo. One strategy is to produce genetically modified T cells for making BiTE to overcome the limitations of BiTEs’ short half-life and passive biodistribution. Technologies were developed to generate T cells that secrete BiTEs (ENG-T cells) targeting tumor antigens such as EPHA2 (95), CD123 (96), and CD19 (97). These ENG-T cells can recognize tumor cells in an antigen-dependent manner in vitro and in vivo, redirecting T cells to the target cancer cells, and have potent antitumor activity in cancer models (95, 96). Importantly, these ENG-T cells expanded in vivo and increased the production of BiTEs after activation, obviating the need for continuous infusion of engager molecules (95). Interestingly, a recent report compared the efficacy of RNA-electroporated ENG-T cells to the RNA-electroporated CAR T cells and concluded that the ENG-T cells showed more efficacy in the NALM6 leukemia model (98).

Another recent promising strategy is to use an oncolytic virus engineered to express a BiTE. Oncolytic viruses have been tested in the clinic and have demonstrated safety and some anticancer effects. Two recent studies engineered an oncolytic adenovirus to secrete an EGFR-targeting BiTE. The BiTE-expressing adenovirus induced a strong T-cell response against cancer cells both in vitro and in vivo (99, 100). The use of oncolytic virus in combination with BiTE or the oncolytic virus secreting BiTE is another strategy for changing the tumor microenvironment. Several oncolytic viruses are currently under development, and whether different strains of these viruses are better than others to be engineered to express and secrete BiTE is yet to be investigated. Post–oncolytic virus administration, large numbers of immune cells infiltrated to the tumors, and the addition of BiTE redirects infiltrating T cells against tumors (101).

Strategies for changing the tumor microenvironment may greatly enhance BiTE treatment efficacy in solid cancers. It was reported that tumors upregulated PD-L1 post–BiTE administration (102) and adding anti–PD-L1 to AMG 330 treatment enhanced killing in vitro (103). Other inhibitor molecules to target include galectin 1, which has been documented to be linked with BiTE resistance (104). Enhancing tumor antigen presentation has also shown to be a good strategy for enhancing BiTE treatment efficacy. Adding the epigenetic modifier drugs panobinostat and azacitidine increased CD33 expression in some cell lines and enhanced AMG 330–induced cytotoxicity (105).

A few studies directly compared the functional efficacy of the two strategies in their antitumor effect (106). In an early study, Stone and colleagues compared the CAR and BiTE treatment antitumor response in vitro using the same anticancer scFv against a murine fibrosarcoma epite. 237. Although both strategies enhanced T cell–mediated antitumor effect, CAR T cells were more potent than the BiTE approach against cancers expressing lower levels of antigens (107). In a recent study, Hoseini and colleagues compared a bispecific antibody (specific for both CD3 and GD2), with a CAR that contained the same anti-GD2 scFv (106). Incubating GD2+ target cancer cells with anti-GD2 CAR T cells for 24 hours led to the depletion of the CARhi T cells, and, in contrast, in the presence of BC119, the exposure of untransduced T cells to GD2+ target cancer cells did not deplete the T cells. This GD2-specific CAR T-cell depletion severely compromised CAR T-cell cytotoxicity in vitro and impaired their in vivo antitumor efficacy. For treating the GD2+ tumors in immunocompromised mice, BC119 with untransduced T cells conferred rapid shrinkage of the established tumors, whereas CAR T cell–treated tumors showed a delayed regression for 2 weeks. The percentage of tumor-infiltrating lymphocytes (TIL) in the BC119 treatment was higher than that in the CAR T-cell treatment. Interestingly, the ratio of CD4+ and CD8+ TILs in BC119-treated mice was nearly equal, whereas the TILs in the CAR T-cell–treated mice were nearly all CD8+.

In the clinic, it is possible that patients refractory to one therapy respond to the other. For example, in a trial run by Maude and colleagues in 2014, 2 patients refractory to blinatumomab achieved CR after CD19-CAR treatment (49). This suggests that a lack of response to one immunotherapy against the same target may not preclude a successful therapy outcome with another, even with the same target. In addition, there could conceivably be some benefit from using CAR T cells and BiTEs simultaneously against tumors. A preclinical study targeting the alpha folate receptor using a CAR, while also targeting EGFR using an oncolytic virus encoding a BiTE, led to increased inhibition of tumors (111). This study suggested that combining these approaches, and oncolytic viruses, could address the issues of antigen heterogeneity and
immunosuppressive microenvironment of solid tumors to enhance antitumor responses.

CONCLUSIONS

We have summarized the current clinical status of the two immunotherapies BiTE and CAR T-cell treatment, their modes of action, toxicities, and preclinical development. Both platforms have demonstrated excellent efficacy in clinical trials against hematologic malignancies and were recently approved by the FDA for treating B-ALL. With their applications in the clinic, we will understand more about the anticancer mechanisms, investigate these treatments as single or combined methods in different situations, and eventually expand their success to other cancers.

Despite the excitement, a few issues still remain to be overcome. Interestingly, both platforms are facing the same dilemma: CRS and neurologic adverse effects and moderate anticancer effects in solid-tumor settings. Substantial effort has been invested in finding strategies for reducing toxicities and enhancing treatment efficacy against solid tumors, such as targeting the immunosuppressive microenvironment and enhancing T-cell infiltration to the tumors. Both treatments warrant further exploration in a wide variety of tumor settings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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REFERENCES


CAR T-cell and BiTE Therapies for Cancers


77. Ruella M, Klichinsky M, Kenderian SS, Sheshova O, Zieber A, Kraft DO, et al. Overcoming the immunosuppressive tumor microenvi-
107. Stone JD, Aggen DH, Schietinger A, Schreiber H, Kranz DM. A sensitivity scale for targeting T cells with chimeric antigen receptors (CARs) and bispecific T-cell Engagers (BiTEs). Oncoimmunology 2012;1:863–73.
CARs versus BiTEs: A Comparison between T Cell–Redirection Strategies for Cancer Treatment

Clare Y. Slaney, Pin Wang, Phillip K. Darcy, et al.

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