blood advances

COUNTERPOINT BiTEs better than CAR T cells

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BiTEs and beyond

Bispecific proteins (recombinant proteins that simultaneously bind 2 different antigens) and chimeric antigen receptors (CARs) facilitate T-cell–mediated killing of malignant cells by redirecting autologous T lymphocytes to cell-surface antigens on cancer cells. Over recent years, bispecific antibodies have been engineered in >50 different formats, including dual-affinity retargeting proteins, tandem diabodies, and bi-nanobodies, but in oncology, the bispecific T-cell engagers (BiTEs) are the most developed and thus are the focus of this article.[1] Both BiTE and CAR approaches are independent of the specificity of the endogenous T-cell receptor and independent of major histocompatibility complex on tumor cells. Whereas both these platforms use single-chain variable fragments to recognize and target antigens expressed on tumor cells, the BiTE platform also uses one to recognize and bind T cells.[2]

CAR T-cell therapy

Tisagenlecleucel (tisa-cel) and axicabtagene ciloleucel (axi-cel) are the 2 CAR T-cell therapies currently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to treat adult patients with relapsed/refractory (r/r) B-cell malignancies. Although they share a common antigen target in the B-cell lineage surface protein CD19, they differ in their intracellular costimulatory domain (4-1BB vs CD28). Tisa-cel can also be used on a pediatric population and is indicated for patients <26 years with r/r B-cell precursor acute lymphoblastic leukemia (BCP-ALL).[3] Currently, the only BiTE with FDA and EMA approval is blinatumomab, which redirects $CD3^+$ T cells to $CD19^+$ leukemic blasts. It is approved for the treatment of r/r BCP-ALL, as well as BCP-ALL with minimal residual disease (MRD).[4,5]

BiTE vs CAR T-cell availability: "off the shelf" vs individualized good manufacturing practices production

Several aspects favor the application of bispecific T-cell–recruiting antibody constructs compared with the application of CAR T cells (Table 1). Bispecific antibody constructs are available off the shelf, whereas CAR T cells have to be engineered for each individual patient. This requires (1) a defined number of leukocytes and lymphocytes as a prerequisite for successful leukapheresis, depending on the CAR T-cell product and disease entity; (2) the isolation of T cells from the leukapheresis product; (3) transduction of these T cells with the vector that expresses the CAR; (4) expanding the transduced T cells to a sufficient number; (5) conditioning the patient; and (6) transfusing the patient with the CAR T cells. Although the production process is well established, it is only feasible in patients with sufficient peripheral counts, and each treatment involves several steps, each of which carries the possibility of error. Moreover, it is expensive and time consuming. In the JULIET trial, the median time from enrollment to infusion with tisa-cel was 54 days, and only 111 of 165 enrolled patients received cells.[6] Seven percent of patients did not receive the treatment because of manufacturing failure, and an unreported number of patients were ineligible for inclusion in the trial due to low circulating lymphocyte counts. In the ELIANA trial, 75 of 92 enrolled patients received tisa-cel, with a median of 45 days from enrollment to infusion. Seven cases had product-related issues.[7] However, in the pivotal ZUMA-1 trial, the manufacture of axi-cel failed for only 1 of 111 patients. The median time from leukapheresis to delivery was 17 days, and 101 of these patients received treatment.[8] The long turnaround times are clinically relevant, as patients carry a high intrinsic risk for disease progression during the production process. Optimized CAR T-cell logistics, including an increase in the number and sites of production, as well as changes in ex vivo culture time, will most likely shorten the time from harvesting to infusion.[9] In contrast, BiTEs are recombinant proteins that can be manufactured in large quantities without interpatient variability and can be rapidly used once the indication has been determined by the clinician, independent of peripheral lymphocyte counts. In the TOWER trial, 267 of 271 patients assigned to receive blinatumomab received the treatment.[4] However, allogeneic engineered cell products are in

Table 1. Comparison of blinatumomab vs CD19 CAR T cells

	Bispecific antibody constructs	CAR T cells
FDA approval	Blinatumomab: pediatric and adult patients with r/r ALL, MRD^+ (0.1%) ALL (first or second remission)	Axi-cel: adult with r/r (>2 prior Tx lines) DLBCL, PMBCL Tisa-cel: r/r ALL <26 y of age, adults with r/r (>2 prior Tx lines) DLBCL
EMA approval	Blinatumomab: pediatric (>1 y of age) r/r ALL and adults with r/r Ph^- ALL, adults with MRD^+ (0.1%) Ph^- ALL (first or second remission)	Axi-cel: adults with r/r (>2 prior Tx lines) DLBCL, PMBCL Tisa-cel: r/r ALL <26 y of age; adults with r/r (>2 prior Tx lines) DLBCL
Design	Recombinant soluble protein	Retro- or lentiviral-transduced CAR T cells
Availability	Off the shelf	17-54 d from patient presentation to CAR T transfusion (national vs international patient recruitment, different trial design)[6,8]
Manufacturing failures	Not applicable	≤10%[38]
Manufacturing and dosing variability	Not applicable	CAR T-cell product variability due to differences in T-cell subset composition, CAR transduction efficacy, number of viable CAR T cells; number of transfused CAR T cells differs from 0.2×10^6 to 6×10^8[8,26]
Effector cell	Endogenous CD4 and CD8 T cells	Engineered, commonly using autologous CD4 and CD8 T cells
Effector cell function	Relies on endogenous T-cell composition and function at time of infusion	Relies on T-cell composition and function at time of leukapheresis; further modulation of CAR T function after transfusion through patient- and disease-related parameters (eg TME)[26]
Lymphodepletion prior to start of therapy	No lymphodepletion required	Lymphodepletion with cyclophosphamide and fludarabine prior to CAR T-cell transfusion mandatory (tisa-cel: exceptions in case of WBCs $<1\times10^9$/L within 1 wk prior to transfusion)[39]
Safety: AE ≥III	CRS:4.9%, ICANS: 9%, hematotoxicity: neutropenia: 28%; lymphopenia: 1.5%; decrease in white-cell count: 5.2%; decrease in platelet count: 6.4% (lower rate of infections compared with SOC; short half-life (<2 h), interruption possible[4]	CRS: 46%; ICANS: 12%-32%; hematotoxicity: ≤23%-45%; JULIET trial: cytopenia not resolved by day 28: 32%, CAR T cells persist for months/years[6,7]
Neoplasia through genetic interference, genotoxic side effects	Not applicable	Rare[40]
B-cell aplasia	Recovery after completion of infusion: 6-18 mo[41]	Months to years depending on persistence of functional CAR T cells; hypogammaglobulinemia for months to years[15]
ORR	44% in r/r ALL, 78% in MRD^+ ALL[4,5]	81% in r/r ALL, 54%-82% in r/r DLBCL[6-8]
Financial toxicity	Product: US$72 000; average no. of cycles: 1-2; in-hospital days: r/r setting: 9 d within the first cycle (MRD setting: 3 d), 2 d second cycle; additional costs: pump equipment, possible IgG-replacement therapy for 6-12 mo	Products: > US$350 000; no. of treatment applications: 1; additional costs: logistics, leukapheresis, lymphodepleting chemotherapy, average 10-d in-hospital stay (outpatient to long-term stay, including ICU), possible IgG-replacement therapy for months to years[37]
Target antigen	CD19 target antigen loss: 3%-21%[42]	CD19 target antigen loss: 7%-35%[23,26]
Multiple target antigens	High flexibility to combine different BiTE constructs given in parallel or sequentially, dual-specific BiTE constructs in clinical trials, individualized combinatorial approach of targeting BiTE construct and immunomodulatory construct feasible	Various dual-targeting CAR T-cell constructs in clinical trials; possibility to apply simultaneously vs sequentially
PD-L1 upregulation in target cells	PD-L1 upregulation commonly observed[43]	PD-L1 upregulation commonly observed[26]
T-cell exhaustion	Potentially, reversal through treatment-free intervals (induction: 4 wk on, 2 wk off; maintenance: 4 wk on, 8 wk off)	Preclinical work: drug-induced cessation of CAR receptor signaling to prevent or reverse exhaustion; genetically engineered CAR T cells to counteract exhaustion[35,44]

AE, adverse event; ICU, intensive care unit; IgG, immunoglobulin G; Ph, Philadelphia chromosome; PMBCL, primary mediastinal B-cell lymphoma; SOC, standard of care; Tx, treatment; WBCs, white blood cells.

preclinical and early clinical development and, with further development, should enable off-the-shelf allogeneic CAR T cell[10] or CAR natural killer cell[11] therapy. In addition to easier access, third-party cell donors might help to overcome the issues of lymphopenia and disease- and patient-related T-cell dysfunction that compromise the success of adoptively transferred autologous cell products. On the other hand, graft-versus-host disease and rejection of CAR T cells might counteract the benefit of allogeneic cell products.[12]

CAR T cells can persist and expand in patients and are typically given as a single transfusion (as in the ZUMA-1 trial). However, the dose of CAR T cells used in these trials varies and also differs among recipients within a single trial. The JULIET trial used a median dose of a total of 3.0×10^8 viable CAR T cells with a range from 0.1×10^8 to 6.0×10^8, the ELIANA trial used a median of 3.1×10^6 CAR T cells per kilogram, but with a range from 0.2×10^6 to 5.4×10^6 cells per kilogram. BiTEs, on the other hand, can be manufactured in a large quantity in a single batch, enabling precise

dosing and repeated use. In contrast to CAR T cells, blinatumomab has an in vivo half-life of 2 to 4 hours and requires continuous IV infusion. In the TOWER trial of blinatumomab, patients received 2 cycles of induction therapy followed by up to 3 cycles of consolidation therapy if necessary and then ≤12 months of maintenance therapy. The induction and consolidation therapies were 6-week cycles consisting of 4 weeks on and 2 weeks off, whereas the maintenance therapy was 4 weeks for every 12 weeks. The great advantage of this approach is an increase in the safety profile, as the infusion can be stopped at any time, thereby reversing immune activation and immune-related adverse events.

Safety: "on-off" vs "dividing drug"

Common adverse events of BiTE and CAR T-cell therapies are cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS). Grade ≥3 CRS and neurologic events were observed in the ZUMA-1 trial in 32% of treated patients.[8] In the JULIET trial, grade ≥3 CRS and neurologic events occurred in 22% and 12% of treated patients, respectively[6]; in the ELIANA trial, these cases were 46% and 13%, respectively.[7] The expansion and persistence of CAR T cells make it difficult to "stop" CAR T-cell treatments if toxicity is observed. Pharmacological immunosuppression, such as using tocilizumab and/or corticosteroids, is necessary to manage these toxicities.[13] In contrast, because of its short half-life, blinatumomab treatment can be interrupted or discontinued if necessary, without prolonged effect. Dexamethasone was used in the TOWER trial prophylactically to prevent CRS and neurologic events; thus, blinatumomab's safety in this regard cannot be compared with tisa-cel or axi-cel. However, adverse events of grade 3 or higher occurred in 87% of patients treated with blinatumomab in the TOWER trial, which is lower than observed in the ZUMA-1 trial (95%) and similar to those rates in the JULIET (89%) and ELIANA (88%) trials. However, looking at grade ≥3 CRS and ICANS in blinatumomab-treated patients, the event rate was much lower compared with the CAR T trials, with 4.9% for CRS and 9% for ICANS. The mitigation of CRS was achieved through implementing dose steps in addition to prophylactic anti-inflammatory drugs (initially dexamethasone, prospectively tocilizumab). This approach enables escalation of the titrated BiTE dose while maintaining a favorable safety profile. Clearly, intertrial comparisons are problematic per se and are further complicated by differences in toxicity grading systems,[14] trial design, inclusion and exclusion criteria (including disease entities [TOWER and JULIET (r/r ALL vs ZUMA-1 and ELIANA (r/r diffuse large B-cell lymphoma [DLBCL])]), and patient cohorts (eg, average age within the JULIET trial was 11 years of age, whereas the other trials were conducted on adults). A third very common toxicity of CAR T-cell therapy consists of prolonged and severe cytopenia that can predispose for severe infectious complications.[15] CAR T-cell–associated hematotoxicity is related to mandatory lymphodepleting chemotherapy prior to CAR T-cell infusion and immunomodulation through CAR T cells. This is in sharp contrast to blinatumomab treatment in which responding patients often recover their neutrophil counts while receiving blinatumomab infusion, resulting into a lower rate of short-term infectious complications.[4] After either blinatumomab or CD19 CAR T-cell infusion, long-term B-cell aplasia and hypogammaglobulinemia have been reported, although it is more profound after CAR T-cell therapy. Thus, the overall safety profile appears to be better for BiTE molecules than for CAR T cells.

Response rates: r/r and MRD settings

Clearly, challenges in production, manufacturing, and safety should be balanced against response rates. In the ZUMA-1 trial, axi-cel treatment achieved an overall response rate (ORR) of 82%, including a 54% complete response (CR) with ≥1 year of follow-up, and 52% overall survival rate at 18 months in refractory large B-cell lymphoma. Tisa-cel achieved a 52% ORR, including a 40% CR rate, in adult patients with r/r DLBCL in the JULIET trial. In children and young adults with BCP-ALL with ≥3 months of follow-up, tisa-cel achieved a CR rate of 81%. In patients with r/r BCP-ALL, blinatumomab treatment achieved a 44% CR rate with full, partial, or incomplete hematologic recovery, as compared with the 25% achieved by chemotherapy. However, a direct comparison of the response rates is invalid due to the differences in patients treated in each trial. Age was a particularly variant factor between study cohorts. Blinatumomab was given to adults with a median age of 41 years, whereas the median age in the ELIANA trial was 11 years. Although the first phase 1 trial with blinatumomab was conducted in patients with B-cell neoplasia,[16] further developments in r/r DLBCL were compromised by the need for higher dosing, which led to an increase in ICANS. To mitigate adverse events, dose steps were implemented, which were again hampered by disease progression.[17,18] Novel half-life–extended constructs (CD19 HLE BiTE) and "full-size" antibodies have entered clinical trials with improved pharmacokinetics. The most advanced construct, the CD20 × CD3 T-cell–bispecific mosunetuzumab, has a rendered ORR of 37% in aggressive lymphoma with a CR rate of 19%.[19] Several other clinical trials are currently recruiting patients for single or combinatorial approaches. Interestingly, a common denominator of response was identified across trials: patients treated in the setting of MRD had a significantly better response and long-term survival compared with patients with a high tumor load.[5,20] A comparison of clinical trials revealed that the recurrence-free survival in patients (n = 255) treated with blinatumomab in the MRD setting (MRD cutoff: 10^{-3}) was 35.2 months vs 7.3 months in the r/r setting (n = 271).[4,21] For CAR T cells, the number of reported patients treated in the MRD setting is much lower, and no MRD-focused trials have yet been reported. Park et al[22] reported on long-term follow-up of CD19-CD28 CAR T cells in a pediatric BCP-ALL population (n = 53). After 29 months, the median event-free survival time was 6.1 months; however, in the subgroup of MRD-positive patients, that figure rose to 10.6 months. The data strongly support the use of blinatumomab in MRD-positive patients with BCP-ALL.

Antigen escape

Tisa-cel, axi-cel, and blinatumomab all target CD19, and loss of this surface marker plays a key role in the development of resistance to these treatments.[23] Notably, the incidence of CD19 loss was lower in patients receiving blinatumomab (12% to 21% in ALL) compared with tisa-cel and axi-cel (9% to 25% in ALL and 27% to 35% in DLBCL).[24-26] A potential explanation for this clinical observation might be the difference in dosing schedule, that is, intermittent vs continuous exposure to CD19-directed immunotherapy. Targeting different tumor antigens, either simultaneously or sequentially, might be a strategy for bypassing this path of resistance. Dual-specific antibody constructs and CAR T cells are being developed to counteract monotargeting escape. Different technological approaches are evolving, such as bicistronic CAR T cells,

tandem CAR T cells, and CAR T-cell products for 2 different targets administered together or sequentially. Pan et al[27] demonstrated in a small pediatric BCP-ALL population the feasibility of sequentially administering CD19 CAR T cells followed by CD22 CAR T cells. Although this might overcome immune escape due to loss of one antigen, it might be more feasible to generate a library of BiTE constructs for individualized sequential application. In that sense, the BiTE platform offers more flexibility in choosing and changing the targeting domain compared with the CAR T platform, thereby enabling individualized targeting strategies during the course of the disease.

Adaptive immune escape mechanism

However, most disease relapses do not feature loss of the target antigen but present with other immune-related escape mechanisms, including the upregulation of inhibitory checkpoint molecules, most commonly PD-L1.[28] To reverse this adaptive immune escape mechanism, several anti–PD-1 or anti–PD-L1 monoclonal antibodies are currently used in combination with blinatumomab and CAR T cells. Other novel formats, such as the multifunctional antibody construct that targets a tumor-associated antigen with high affinity and blocks an inhibitory checkpoint molecule with low affinity, will be tested.[29] Alternative constructs elicit a combination of simultaneous blockade of immune checkpoint molecules and costimulation[30] or provide targeting and stimulating within one construct.[31] Also, the CAR T-cell platform enables different strategies to be used to block the inhibitory PD-1 signal, including CRISPR-Cas9–mediated PD-1 disruption. However, the BiTE platform offers a higher flexibility for combinatorial and sequential approaches from a toolbox of targeting and immunomodulatory antibody constructs. As stated, the upregulation of immune checkpoint molecules is an escape mechanism common to both BiTE and CAR T-cell therapy, and these can be expressed on both activated and exhausted T cells. Furthermore, T-cell subset composition and function determine the response to BiTE treatment.[32,33] However, in the case of CAR T cells, T-cell composition and function at time of leukapheresis also influence CAR T function and are further modulated through patient- and disease-related parameters after transfusion. Therefore, both platforms rely on T-cell context, and it is unclear to what extent the ex vivo production of CAR T cells can overcome T-cell dysfunction at the start of the process.[12]

T-cell exhaustion

For both platforms, evolving T-cell exhaustion is highly relevant due to the fact that repeated antigen exposure takes place.[34] BiTE proteins are given as a continuous infusion with intermittent treatment-free intervals. The relevance and the necessary length of interruption to reverse T-cell exhaustion is unknown. The increasing interval of BiTE application during maintenance therapy (induction, 2 weeks; maintenance, 8-week treatment-free interval) is most likely sufficient to reverse an exhausted T-cell state. In the context of CAR T cells, in vitro studies have demonstrated the reversal of T-cell exhaustion through drug-induced regulation. In a preclinical model, dasatinib, an FDA-approved tyrosine kinase inhibitor, suppressed CAR T-cell activation via rapid and reversible antagonism of the CAR CD3 ζ chain, thereby diminishing exhaustion marker expression and restoring functionally.[35] This work demonstrated the potential to reinvigorate CAR T-cell function through drug-induced T-cell reprogramming. The use of adapter CAR T cells is aimed at combining the benefits of BiTE molecules with the power of ex vivo–activated CAR T cells. The adapter molecule recognizes the CAR expressed by the T cell with one arm and with the other a tumor-associated antigen. The approach allows targeting of several antigens simultaneously to decrease toxicity through an on-off adapter molecule with a short half-life and counteract T-cell exhaustion with treatment-free intervals. However, adapter kinetics, target antigen affinities, and antigen sinks are challenges that need to be overcome.[36]

r/r and MRD settings in BCP-ALL

Unfortunately, no trial has directly compared blinatumomab vs CAR T cells in patients with r/r BCP-ALL. Currently, blinatumomab is the only approved drug for treatment of MRD-positive BCP-ALL. Accordingly, blinatumomab is the preferred treatment of choice in this situation with high response rates (88/113 patients with MRD conversion) and a favorable safety profile. In the r/r setting, antigen loss and other adaptive immune escape strategies counteract the initial higher response rate of CD19 CAR T cells. The relevance of blinatumomab prior to treatment with CD19 CAR T cells is still under investigation with conflicting reports emerging. Considering the high rate of antigen loss, multitargeting adapter CAR T and dual-targeting CAR T cells appear a promising tool for combinatorial and/or sequential approaches. The combination of BiTEs as an adapter strategy for CAR T cells is currently being tested in early clinical trials.

Costs

Finally, both treatment platforms are associated with high financial toxicity. In this regard, BiTEs compare favorably to CAR T cells once the costs of production, logistics, treatment, days of hospitalization, and short- and long-term adverse events have been considered (Table 1).[37] Importantly, the long-term response rate to BiTEs and CAR T-cell therapy is critical to estimate the cost-effectiveness of these novel treatment platforms.

Summary and outlook

In the MRD setting, blinatumomab is the only drug approved for the treatment of BCP-ALL, demonstrating the importance of BiTEs in oncology. Furthermore, the BiTE platform provides an off-the-shelf product with a high safety profile and the possibility of dose titration and escalation, which are significant advantages over CAR T therapies. BiTE-based approaches are particularly promising against early-stage disease with low tumor burden (eg, in the MRD setting of BCP-ALL) and a still-functional T-cell compartment. BiTEs provide the advantage of flexibility of targeting multiple antigens simultaneously and sequentially and can be used in combination with chemotherapy, small molecules, and immunomodulatory drugs, such as checkpoint inhibitors. Looking ahead, we need predictive biomarkers to stratify patients to the treatment option with the highest likelihood of cure and mitigate clinical and financial toxicity. One can speculate that individualized biomarkers encompassing disease-, immune-, and patient-related parameters will guide personalized BiTE-based combinatorial approaches toward optimized safety profiles and response rates.

Are BiTEs better than CAR T approaches? This article sets out that case, but personally, I see room in the clinic for both. As well as personalized individual treatments using BiTEs or CAR T cells, one innovative way this could manifest itself is in the combination of BiTEs as an adapter strategy with universal CAR T cells that might

overcome the clinical stings of T-cell dysfunction while maintaining the benefits of BiTE constructs. BiTEs might therefore "assimilate" CAR T cells into a hybrid strategy that is very much led by BiTE technology.

For data sharing requests, e-mail the corresponding author, Marion Subklewe (marion.subklewe@med.uni-muenchen.de).

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