REVIEW SERIES

Bispecific T-cell engagers in childhood B-acute lymphoblastic leukemia

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Abstract

Immunotherapy has revolutionized treatment for a wide variety of cancers yet its use has been relatively limited in childhood malignancies. With the introduction of bispecific T-cell engagers (BiTE®) and chimeric antigen T-cell receptor technologies, previously refractory patients have attained remission, including molecularly negative states of disease, thus providing the possibility of long-term cure. Blinatumomab is a widely available CD3-CD19 BiTE that has dramatically changed the landscape of therapy for some children with precursor-B acute lymphoblastic leukemias (B-ALL) and lymphoblastic lymphomas. Challenges remain with using BiTE in a broader population although the appeal of now-confirmed reduced toxicity and deeper molecular remissions suggests that this approach will be an essential part of future treatment of childhood B-ALL. Herein, we review some of the pertinent literature covering clinical trials with blinatumomab and address future approaches and combination trials including BiTE.

Introduction

Bispecific T-cell engager (BiTE®) immunotherapy has revolutionized the treatment of B-lineage malignancies in children. These agents offer tumor antigen-directed T-cell-mediated killing with favorable toxicity profiles in clinical practice.[1] Blinatumomab, a CD19/CD3 BiTE, has shown remarkable efficacy in reducing subsequent relapse and improving overall survival in patients with relapsed and refractory (R/R) B-cell acute lymphoblastic leukemia (B-ALL) compared with conventional multi-agent chemotherapeutic regimens and is the only BiTE with regulatory approval for use in children.[2-4] As such, blinatumomab received accelerated approval initially by the United States Food and Drug Administration (US FDA) for the treatment of R/R and minimal residual disease (MRD)-positive B-ALL in adults and children and has subsequently received regulatory approval in more than 50 countries worldwide. Despite the successes of blinatumomab, response is variable, and resistance has been reported. This highlights the need for additional BiTE therapies as well as strategies to overcome the likely class-specific limitations of these agents, such as improved efficacy in extramedullary disease and targeting resistance mechanisms including antigen loss, lineage switch, and microenvironmental inhibition.[1,2,5-7]

Mechanism of action

BiTE employ a targeted, immune-directed strategy through the simultaneous binding of tumor-associated antigens and a component of the patient's own T-cell receptor, most commonly a CD3ε within the T-cell receptor complex.[8] BiTE traffic T cells directly to the designated antigen on the tumor cell of interest, inducing T-cell activation and targeted cell lysis in the absence of co-stimulatory signaling. Vital to their function, BiTE are small molecules devoid of the antibody constant region typical of monoclonal antibodies. Instead, they consist of two single-chain variable fragments (scFv). This allows the formation of tight immune synapses, facilitating perforin and granzyme-mediated target cell lysis.[1,8] Blinatumomab transiently links CD19 on B cells and many B-cell malignancies to CD3 on a patient's endogenous cytotoxic T cells. CD19 represents an ideal target for B-cell malignancies as it is expressed in nearly all stages of B-cell development. Additionally, in most patients, CD19 is ex-

pressed on nearly 100% of B-lymphoid malignant cells.[9] In the presence of CD19, blinatumomab can independently activate resting T cells, leading to upregulation of activation markers, release of inflammatory cytokines, and induction of robust T-cell proliferation *in vitro*.[10] Like other BiTE, blinatumomab causes T-cell activation independently, and these activated T cells have the potential to eliminate multiple target cells without additional stimulation by the drug.[11] In preclinical models, repeated target lysis of CD19/CD3 double-positive T cells is presumed to represent transfer of CD19 from B cells to T cells when synapses form, triggering subsequent cytolysis.[11] This capacity of blinatumomab, in conjunction with its small size of 54.1 kDa and transient direct action, likely contributes to the improved efficacy and favorable toxicity profile observed with this BiTE compared with other antibody-targeted approaches.

Clinical progress and efficacy

Despite decades of advances in outcomes of children with B-ALL, 10-15% of patients will relapse.[12] The 5-year overall survival of these patients in the era of intense, conventional multi-agent chemotherapy is between 35% and 50%.[13,14] Blinatumomab has demonstrated efficacy in improving the event-free survival and overall survival in these patients, with a significant reduction in severe adverse events.[1-4,8] Following the early successes of blinatumomab in adults with relapsed, refractory, and MRD-positive B-ALL, a phase I/II study examining blinatumomab in children with R/R B-ALL was conducted.[15-17] This study established the recommended dose of blinatumomab of 5 $\mu g/m^2$ with escalation to 15 $\mu g/m^2$ after 1 week of therapy to modulate cytokine release syndrome, observed primarily in patients with high disease burden in the bone marrow. With 39% of patients achieving complete remission with monotherapy, blinatumomab exhibited previously unobserved single-agent antileukemic activity, including MRD-negative responses in patients with multiply R/R B-ALL, with a tolerable toxicity profile.[3] These findings launched a series of pediatric trials and led to the accelerated approval of blinatumomab for R/R B-ALL in children.

In children and young adults with intermediate and high-risk B-ALL in first relapse, the Children's Oncology Group (COG) study AALL1331 demonstrated a 2-year event-free survival of 54% with blinatumomab treatment after reinduction compared to 39% in those treated with chemotherapy.[4] While the improvement in event-free survival was not statistically significant, this study was terminated early because of the substantial toxicity seen in the chemotherapy arm and MRD advantages for blinatumomab. The international, expanded-access RIALTO trial found that 59% of children with multiply relapsed or refractory disease achieved a complete remission within two cycles of blinatumomab and 65% of patients proceeded to allogeneic transplant with a trend toward improved overall survival in this cohort through the follow-up period of 18 months.[18] Substitution of one cycle of blinatumomab for a third consolidative chemotherapy cycle in pediatric patients with high-risk, first-relapse B-ALL significantly improved event-free survival (69% *vs*. 43%) in an important study run in parallel in Europe,[19] with nearly identical findings observed in AALL1331.[4,9] This effect was seen across subgroups and was independent of MRD following two cycles of consolidation therapy.[4,19]

AALL1331 also studied blinatumomab for patients with low-risk B-ALL in first relapse, randomizing patients to receive either blinatumomab or chemotherapy as block 3 of consolidation. Patients in the blinatumomab arm had the drug intercalated into two continuation blocks. This significantly improved disease-free survival and overall survival in the two-thirds of patients with bone marrow relapse with or without extramedullary relapse for patients with low-risk disease. Patients with isolated extramedullary relapse fared poorly in both arms, particularly those with isolated central nervous system (CNS) relapse, and it is known that blinatumomab, like other BiTE, does not cross the blood-brain barrier with any consistency. This study highlighted the inefficacy of blinatumomab in treating CNS leukemia, which can be mitigated by CNS-directed therapy delivered concurrently with blinatumomab cycles, typically on days 1 or 1 and 15 of each 28-day cycle of blinatumomab depending on individual patients' factors, and including single or multi-agent intrathecal agents. AALL1331 was the first trial to suggest that extramedullary disease may be more refractory to blinatumomab, a finding that was not appreciated in the intermediate and high-risk arm of AALL1331. It is important to note that the intensity of CNS-directed therapy was reduced compared to that of previous studies of relapsed B-ALL, an important consideration for future trials[2] and when delivering standard-of-care therapy. Currently, the International Frankfurt Berlin Münster (IBFM) group, the COG, and individual institutions are assessing the utility of blinatumomab in improving disease-free survival as an addition to up-front therapy in newly diagnosed standard-risk B-ALL in combination with conventional chemotherapy and, in some cases, other immunotherapeutic agents. In the Cancer Research United Kingdom (CRUK)-sponsored AllTogether-1 study conducted in the UK and European Union, approximately 8,000 children, adolescents, and young adults up to the age of 29 years will be enrolled in a series of risk-adapted arms. Importantly, AllTogether-1 will treat patients with Down syndrome with blinatumomab in the intermediate-risk group. This population of patients may benefit from receiving less cytotoxic chemotherapy and the concerted effort to identify both toxicity and response for patients with Down syndrome will contribute to a wider understanding in this context.

Blinatumomab recently demonstrated remarkable efficacy

Table 1. Summary of selected studies with blinatumomab in pediatric patients with B-lineage malignancies.

Study and publication date	Phase and aim	Results	Additional notes
2016 von Stackelberg A *et al.*[3]	Phase I/II. To determine the safety and pharmacokinetics of blinatumomab in children with R/R B-ALL.	Safety and dosing established: 5 μg/m^2 for 7 days, followed by escalation to 15 μg/m^2 in two 28-day cycles. CR rate of 39% (95% CI: 27-51%).	MRD-negative remissions noted across dose levels. All patients enrolled had highly refractory, multiply relapsed B-ALL.
2020 Locatelli F *et al.*[18]	Phase II: RIALTO. An open-label, single-arm, expanded access trial to determine the safety and efficacy of blinatumomab in pediatric patients with R/R BCP-ALL.	Tolerable safety profile. Of patients with ≥5% blasts, 59.2% had CR and 79.3% of those became MRD negative. Of patients with ≤5% blasts, 91.7% had an MRD-negative response.	-
2021 Brown PA *et al.*[4]	Phase III: COG AALL1331. To compare the survival of patients with high- and intermediate-risk first relapse of B-ALL treated with chemotherapy or chemotherapy plus blinatumomab.	2-year DFS of 54.4% for the blinatumomab arm *versus* 39% for the chemotherapy arm. One-sided *P*=0.03, non-significant. 2-year OS of 71.3% for the blinatumomab arm *versus* 58.4% for the chemotherapy arm (1-sided *P*=0.02), significant.	Study interpretation limited by early termination recommended by the safety monitoring committee due to toxicity and MRD advantages of blinatumomab, however possibly underpowering the primary endpoint.
2021 Locatelli F *et al.*[19]	Phase III. To evaluate EFS of children with high-risk B-ALL in first relapse following blinatumomab or chemotherapy as a third consolidative course prior to HSCT.	EFS in the blinatumomab arm was 69% *versus* 43% in the chemotherapy arm with a median follow-up of 22.4 months. OS was 85.2% in the blinatumomab arm *versus* 70.4% in the chemotherapy arm.	Incidence of adverse events in the blinatumomab *versus* chemotherapy arms was 31% *versus* 57%.
2023 Hogan LE *et al.*[2]	Phase III: COG AALL1331. To compare the survival of patients with low-risk first relapse of B-ALL treated with chemotherapy or chemotherapy plus blinatumomab.	4-year DFS of 61.2% ± 5% and OS of 90.4% ± 3% in blinatumomab arm *versus* 49.5 ± 5.2% DFS and 79.6 ± 4.3% OS in chemotherapy arm (*P*=0.89/*P*=0.11), non-significant.	For BM ± EM relapses, 4-year DFS/OS were 72.7 ± 5.8%/97.1% ± 2.1% for blinatumomab *versus* 53.7% ± 6.7%/84.8% ± 4.8% for chemotherapy (*P*=0.015/*P*=0.020). Poor outcomes for patients with CNS disease, although traditional CNS-directed therapy was reduced.
2023 Van der Sluis IM *et al.*[20]	Phase II. To assess the safety and efficacy of blinatumomab added to Interfant-06 in infants with *KMT2A*-rearranged ALL.	No toxic effects meeting the definition of the primary end point occurred. 2-year DFS of 81.6% and OS of 93.3% *versus* 49.4% and 65.8% in Interfant-06.	-

R/R: relapsed/refractory; ALL: acute lymphoblastic leukemia; CR: complete response; 95% CI: 95% confidence interval; MRD: minimal residual disease; BCP: B-cell precursor; DFS: disease-free survival; OS: overall survival; EFS: event-free survival; HSCT: hematopoietic stem cell transplantation; BM: bone marrow; EM: extramedullary; CNS: central nervous system.

when added to the Interfant-06 regimen for *KMT2A*-rearranged ALL in infants. Thirty infants were enrolled, each of whom received a single post-induction cycle of blinatumomab. The 2-year disease-free survival rate among these patients was 81.6% compared to 49.4% in the Interfant-06 trial, with corresponding overall survival rates of 93.3% and 65.8%, respectively. Additionally, 30% of patients experienced serious adverse events, and no fatal adverse events were reported.[20] The purposes and findings of selected studies of blinatumomab in children are summarized in Table 1. Intensification of chemotherapy has failed to significantly improve outcomes for these patients for nearly 40 years, and so the dramatic improvement with blinatumomab highlights a promising strategy for patients with infant ALL. The Interfant-21 study will prospectively seek to expand these very promising observations in the newest multinational study (NCT05327894) of infants under the age of 1 year with *KMT2A*-rearranged and multilineage/mixed phenotype acute leukemia.

Indeed, little is known about the efficacy of blinatumomab in patients with mixed-phenotype acute leukemia, although as of the date of writing this review, three adult patients have been reported to respond at least transiently to blinatumomab; however, no prospective or randomized studies of mixed-phenotype acute leukemia treated with blinatumomab have been conducted to determine the true potential efficacy of the BiTE in this setting. The Interfant-21 study noted above, sponsored by the Princes Maxima Center in the Netherlands, will contribute important information regarding this very rare population of patients.

Toxicity

Randomized trials of blinatumomab in pediatric B-ALL thus far have demonstrated a decrease in grade ≥3 adverse events among patients receiving blinatumomab. AALL1331 found a significant difference in febrile neutropenia (3% *vs*. 48%), infections (5% *vs*. 51%), sepsis (0% *vs*. 11%), and anemia (13% *vs*. 58%) among low-risk patients receiving blinatumomab for block 3 compared to those receiving conventional chemotherapy,[2] Similar advantages favoring blinatumomab were observed in the intermediate- and high-risk groups.[4] These data are of particular importance for patients with Down syndrome, who are far more vulnerable to serious infectious complications and non-relapse morbidity and mortality.[21] As such, the frontline trial for standard-risk patients in COG was designed to substitute blinatumomab for some intensive elements of therapy in high-risk Down syndrome patients as well.

While blinatumomab exhibits a favorable overall toxicity profile, it still presents distinct and potentially significant adverse effects. Cytokine release syndrome (CRS), an on-target phenomenon, is seen in 4-22% of pediatric patients, with grade ≥3 syndrome occurring in 0-3% of patients.[2,4,18,19] The median time to onset of CRS is 24-48 hours following blinatumomab initiation, and the syndrome is correlated with a variety of cytokines, most notably interferon-γ and interleukin-6, and less so with tumor necrosis factors.[22] Tumor burden is correlated with the risk and severity of CRS, and reduction of disease burden prior to blinatumomab has demonstrated efficacy in reducing this risk.[22] Established as the standard of care, dose-escalation can mitigate the effects of CRS.[3] Additionally, patients with a high disease burden can be premedicated with dexamethasone in conjunction with slower dose-escalation.[22] In a 2022 meta-analysis examining blinatumomab safety in pediatric patients, no difference in the frequency of CRS was found between patients in the blinatumomab and chemotherapy arms. The AALL1331 trial did not provide details of CRS events in the chemotherapy arm.[23] Tocilizumab, an interleukin-6 receptor antagonist, is approved for use in CAR T-cell mediated CRS, as interleukin-6 is the predominant driver in this process. Treatment with tocilizumab has *not* been shown to reduce T-cell efficacy when used in this context[24] and has been given to adults and children with CRS caused by blinatumomab, although published evidence for its use in this setting is somewhat limited.[25]

Immune effector cell-associated neurotoxicity syndrome (ICANS) is an adverse effect associated with targeted immunotherapies. Manifestations include tremor, dizziness, confusion, encephalopathy, and, less commonly, seizure. Blinatumomab carries a higher risk of encephalopathy than does more conventional chemotherapy, with comparable risks of seizure.[23] The incidence of neurotoxicity is 3.7-24% in pediatric patients, with grade ≥3 adverse events in 2-3.6%.[2,4,18,19] The incidence is as high as 52% in adults, with 17% suffering grade ≥3 adverse events. Neurological abnormalities are typically present within the first 2 weeks of treatment with a median onset at 9 days in adults.[26] Given the short half-life of blinatumomab, temporary cessation of the drug with or without steroids is often sufficient to manage neurological toxicity, and most patients can be successfully retreated with the same or a reduced dose of blinatumomab to complete the recommended treatment course. Blinatumomab should be stopped at the onset of grade ≥3 adverse events, and dexamethasone can be added for patients with severe adverse events. Premedication with dexamethasone may be considered for subsequent doses, and secondary seizure prophylaxis at re-initiation of therapy should be started for patients with severe neurological adverse events. The choice of anti-seizure medication is left to the discretion of the treating physician, and is made easier by the lack of enzyme-inducing agent contraindications with BiTE therapies overall. It is recommended that blinatumomab be permanently discontinued following any seizure, grade 4 adverse event, or a delay in neurological recovery beyond 7 days.[27]

Predicting and improving the efficacy of blinatumomab and bispecific T-cell engagers

The efficacy of blinatumomab is variable, and while certain factors may aid in predicting response, understanding of the complex interplay between leukemia-intrinsic and environmental influences in the context of this therapy remains limited. Conventional indicators used to predict response to chemotherapy, such as age, duration of prior complete remission, lack of sensitivity to prior chemotherapy, and post-transplant relapse, do not appear to affect response to blinatumomab. Duration of therapy with blinatumomab is also individualized for patients, although common regimens include two or four 28-day cycles intercalated into a multi-agent chemotherapy regimen. The observation that conventional predictors of response do not match blinatumomab response aligns with the fact that blinatumomab bypasses many of the mechanisms associated with chemotherapy resistance.[28] Lower tumor burden has been associated with higher rates of complete remission across multiple studies,[3,18,19,28] influencing relapse-free and overall survival.[29] To identify additional factors affecting resistance or sensitivity to blinatumomab, pre-treatment samples from 44 adult B-ALL patients were analyzed using bulk tumor and single-cell sequencing: 55% of these patients achieved a complete remission, with a more favorable response observed in *CRLF2*-rearranged Philadelphia chromosome-like ALL (75%).[30] Other leukemogenic genetic aberrations did not predict response, though sample size

in most subgroups was small. Tumor cell immune response genes were enriched among those who achieved complete remission suggesting that blinatumomab-induced T-cell activation may be influenced by leukemia-intrinsic factors. There is growing evidence to suggest that endogenous T-cell function and T-cell subsets influence responses to blinatumomab and other immunotherapies. Enrichment of regulatory T cells in the peripheral blood predicted response to blinatumomab *in vitro*, influenced by interleukin-10-mediated T-cell suppression.[31] T-cell exhaustion markers, PD-1 and TIM3, were increased in samples from pediatric patients with poor response to blinatumomab, highlighting the role of the tumor microenvironment and immune escape/T-cell exhaustion in influencing BiTE efficacy.[5] Preclinical studies exploiting immune checkpoint inhibition in conjunction with blinatumomab have shown improved T-cell-mediated tumor killing and T-cell proliferation.[32] and in some cases, have reversed the T-cell exhaustion phenotype. These findings have prompted clinical trials combining these two targets. Phase 1 data on blinatumomab plus nivolumab, a PD-1 inhibitor, in adults demonstrated tolerable safety.[33] This same approach is being studied in COG study AALL1821 in pediatric patients in first relapse of B-ALL.

Low-risk patients with isolated extramedullary relapse had poor outcomes on AALL1331, particularly those with isolated CNS disease, which again is predictable based on blinatumomab's known poor penetrance of the CNS when delivered into the central circulation. Patients with isolated testicular disease did well with or without blinatumomab, suggesting that one limitation of blinatumomab in patients with B-ALL may be restricted to efficacy within the CNS.[2] It is unlikely, however, that trafficking to extramedullary sites is the sole limitation in extramedullary disease. Among adult responders to blinatumomab who experience subsequent relapse, 40% will do so at extramedullary sites, including sites outside of the CNS. While trafficking of BiTE may be a challenge in these cases, tumor microenvironmental factors may also restrict T-cell recruitment or efficacy in extramedullary sites. In support of this principle, B-ALL appears to be more sensitive than non-Hodgkin lymphoma (NHL) to blinatumomab at low doses, with higher-dose blinatumomab (60 μg/m^2/day) for NHL bridging this gap in response.[34] Studies are needed to examine the role of higher-dose blinatumomab in patients with extramedullary ALL.

CD19 loss or reduction in antigen density are described consequences CD19-targeted immunotherapies. In the largest retrospective real-world analysis of adult patients receiving blinatumomab, 9.8% of patients relapsed with CD19-negative disease, representing 34.2% of relapsed cases,[35] which is a higher percentage than what has been observed in clinical trials. In a meta-analysis reviewing 27 blinatumomab studies, 4.5% (median) of patients relapsed with CD19-negative disease, and 22.5% (median) of relapse cases overall. Findings in adults and children were comparable.[6] Regarding patients undergoing sequential therapy with blinatumomab and CAR-T cells, prior blinatumomab exposure has been associated with higher remission failure, subsequent remission loss, and antigen loss.[36] In the largest report to date, an analysis of 420 B-ALL patients, nonresponse to blinatumomab was independently associated with inferior event-free survival following CAR-T-cell therapy. Additionally, patients who received blinatumomab were more likely to exhibit CD19-dim or partial expression prior to CAR-T-cell treatment.[37] Notably, outcomes were comparable in blinatumomab-naïve patients and in blinatumomab responders. Therefore, while CD19 antigen loss is a mechanism by which leukemia can evade CD19-targeted immunotherapy, it is not the sole driver of treatment failure among these patients.

Several mechanisms for CD19 antigen loss have been described. In an analysis of patients' samples with antigen loss following blinatumomab treatment, CD19 mutations universally involved the extracellular domain of the protein and included frameshift and nonsense mutations, splice-site variants, and in-frame deletions. These mutations were not present prior to exposure to blinatumomab, which was confirmed using targeted deep sequencing. One sample harbored a mutation in CD81, a chaperone protein that partners with CD19 to stabilize its surface expression. Thus, abnormalities in CD81 may contribute to a decrease or loss of CD19 expression in the absence of CD19 aberrations. Patients with hypodiploid ALL and chromosome 16 loss are likely more vulnerable to single allelic abnormalities in CD19, because its locus is on chromosome 16. This pattern was described in two of the evaluated samples. However, unlike observations in the setting of CAR-T-cell-mediated antigen loss, no samples had copy-neutral loss of heterozygosity at this locus.[30] In a retrospective analysis of blinatumomab-treated patients, patients with CD19-negative relapse maintained an identical cytogenetic profile to that at diagnosis.[28]

Lineage switch is a rare complication of CD19-directed therapies, including both blinatumomab and CAR T cells. Lineage switch to acute myeloid leukemia is most common following blinatumomab, although mixed-phenotype acute leukemia and unclassifiable leukemias have also been reported.[6] In a retrospective review of 161 relapsed B-ALL cases, nine patients experienced lineage switch.[37] *KMT2A* rearrangements are the most commonly described cytogenetic abnormalities in cases of lineage switch, although others including *TCF3-ZNF384* fusion, *FLT3*-ITD and *PAX5* polymorphisms have been described.[6] Lineage switch is a rare phenomenon, and its mechanisms are not well understood. Unsurprisingly, standardized treatment is lacking in these cases, and outcomes are generally very poor and also inconsistently reported. The prospective studies including patients with mixed-phenotype acute leukemia will be highly informative in this regard.

Pharmacokinetics and drug administration

The first human pharmacokinetic data for blinatumomab emerged from three phase I studies examining its safety in the context of R/R NHL. Patients in these trials received blinatumomab as intravenous (IV) infusions over 2-4 hours at doses ranging from 0.75-13 μg/m^2, two to three times weekly.[1,8] Grade 3 cytokine release syndrome, neurological toxicity and infections were experienced in the absence of objective clinical responses, leading to the early termination of these studies. Subsequent pharmacokinetic data determined the short serum half-life of blinatumomab in humans of 1.25-3 hours. Consequently, continuous IV infusion of blinatumomab was examined in a subsequent phase I study, with doses in the range of 0.5-90 μg/m^2/day over a 4- or 8-week period in NHL patients. Sustained persistence of blinatumomab was confirmed, allowing for predictable serum levels of the drug and dose linearity. Additionally, prolonged T-cell activation and reduced toxicity were demonstrated with the continuous infusion approach, with CD19-positive B-cell depletion at doses as low as 5-15 μg/m$^{2/}$day.[38]

Continuous IV infusion of blinatumomab has been widely accepted as the standard of care due to the drug's short half-life and demonstrated efficacy. However, this can be a major challenge for patients receiving this agent, particularly in pediatrics, in which access to medical centers or pharmacy services able to dispense the product may be limited, and disproportionally so for those patients/families with more limited resources and transportation access. Alternative means of more convenient and patient-friendly delivery, such as subcutaneous or extended half-life IV infusions, could be considered an unmet need for patients receiving blinatumomab. AMG562 is a half-life extended BiTE administered IV, engaging CD19-expressing cells and CD3 on T cells. In a phase I study in patients with R/R NHL, seven of nine patients treated with AMG562 had disease progression; serum pharmacokinetic data were unobtainable because of immeasurably low serum concentrations at most post-dose timepoints.[39] The study was, therefore, terminated early, and there are no other half-life extended BiTE in clinical trials to date.

Early clinical trials of a subcutaneous form of blinatumomab for adults with R/R B-ALL are starting to return results. A phase Ib dose-escalation study assessed responses following daily to thrice weekly administration of subcutaneous blinatumomab in this population. Among the high-dose cohort, average concentrations at steady state were consistent with those of the approved IV blinatumomab regimen after a 26-day period. Overall, 64.3% of patients achieved an MRD-negative (MRD <10^{-4}) response, with 80% achieving these results in the high-dose cohort. Additionally, no dose-limiting toxicities were reported, although 85% of patients overall experienced a grade ≥3 adverse event.[40] While promising, additional data and longer duration of follow-up are necessary to determine efficacy and a more extensive comparison to the continuous IV infusion formulation in pediatric patients will be important. Only if non-inferior outcomes and similarly acceptable or better side effect profiles are observed in pediatric patients would this alternative be widely considered for use.

Future directions

In addition to demonstrating remarkable efficacy in pediatric B-ALL, blinatumomab has a favorable toxicity profile compared with chemotherapy. While this agent is associated with unique acute toxicities, they are rarely severe and usually manageable and reversible. Given the relatively recent approval of blinatumomab in pediatrics, late/long-term effects of this drug are not known. Conversely, late effects of conventional chemotherapy commonly used in ALL treatment have been extensively evaluated and are well-described. For example, osteopenia and osteonecrosis are well-described complications of glucocorticoid use. The incidence of osteonecrosis varies from 10% to upwards of 44.6% in adolescents treated for ALL,[41] and not infrequently necessitates surgical intervention or joint replacement, often at a very young age relative to the expected lifecycle of prosthetic joints.

Additionally, cardiac abnormalities are common and often progressive following anthracycline therapy among survivors of pediatric ALL, leading to increased cardiomyopathy and mortality.[42] Delayed intensification phases of therapy not only employ both steroids and anthracyclines, but remain a high-risk period for febrile neutropenia, infection, sepsis, the possibility of prolonged hospitalization, and occasionally fatal treatment-related toxicity. Each of these complications was reduced significantly when blinatumomab substituted chemotherapy in global clinical trials.[4,19] Therefore, the potential to substitute daunorubicin and glucocorticoids with blinatumomab or employ dose reductions in conjunction with blinatumomab to improve toxicities without compromising efficacy can be considered a next landmark to evaluate in pediatric ALL therapy overall and is already being done in adult treatment protocols for ALL.

The high costs of blinatumomab and the associated administration and preparation costs are important considerations, although a reduction in the above-described acute and late effects of chemotherapy would likely improve the overall financial burden of ALL therapy significantly. Additionally, the potential for generic formulations to become available when the drug patent expires would further reduce potential costs. At the time of this publication, there were 19 active studies listed in ClinicalTrials.gov for pediatric patients using blinatumomab (*classic.clinicaltrials.gov/ct2/results?cond=-leukemia&term=blinatumomab&cntry=&state=&city=&dist=&-Search=Search&recrs=a&recrs=d&recrs=f&age=0*; Accessed

16 December 2023), indicating a strong desire among the pediatric oncology community, and beyond, to explore a variety of combinations and strategies incorporating BiTE therapy. As more BiTE with targets of interest are developed, the portfolio of trials should expand appropriately.

As noted above, further development of subcutaneous blinatumomab is proceeding in adult patients and there is strong enthusiasm in the pediatric community to advance this agent to children as soon as appropriate adult data are confirmed. A subcutaneous formulation, if proven equally efficacious, would improve convenience and quality of life for patients and families during treatment, and would bring the potential for greater health equity to patients who are currently limited by travel and frequency of clinic visits or home care support necessitated by the continuous IV formulation. Currently, patients with fewer financial, transportation, and health care access resources to travel to treatment centers are limited in their ability to receive the continuous infusion formulations and have less access to what is now considered a new standard of care for many pediatric and adult patients with B-ALL and B-lymphoblastic lymphoma. Removing the current barriers inherent in the continuous infusion formulation could enhance patients' therapy experience substantially as long as efficacy is not compromised.

At present, there is no specific guidance from regulatory authorities on what data are or would be needed to demonstrate that subcutaneous blinatumomab has both bioequivalence and therapeutic equivalence/non-inferiority and in general, the benchmarking for how best to transition between formulations can vary widely among different drug products. The pharmacokinetics of the continuous infusion formulation of blinatumomab are virtually identical in children and adults.[2,3,15,17,22] Once safety and toxicity have been established in adult patients, it may be possible to consider study designs that would allow pediatric patients to enroll on subcutaneous blinatumomab trials, including staggering pediatric patients to enroll one dose level behind adults as long as non-inferiority pharmacoequivalence can be proven. Similarly, enrolling patients who are post-pubertal but under the age of 18 onto adult clinical trials studying subcutaneous blinatumomab before enrolling younger patients would allow for careful consideration of the potential for unique considerations in pediatrics while shortening the duration of clinical development with the goal of making subcutaneous blinatumomab available to children more quickly. Study endpoints on these trials could include pharmacokinetic data, short-term efficacy including marrow and MRD responses at defined time periods with a longer follow-up, as has been done already with the continuous infusion formulation.

While blinatumomab is moving into frontline therapy for pediatric B-ALL, evidence for its efficacy in pediatric lymphomas is more limited. Blinatumomab has produced responses in diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma and more recently, relapsed/refractory Burkitt lymphoma among adults.[8,43] Phase I and II studies have shown promise in pediatric NHL,[34] though advanced phase trials of blinatumomab in children are warranted to potentially limit toxicity and improve outcomes in the upfront and relapsed settings.

Novel BiTE are under increasing investigation in liquid malignancies such as acute myeloid leukemia, multiple myeloma, and B-cell lymphomas. Odronextamab is a novel human CD20-CD3 bispecific antibody that has produced high response rates and durable remissions in R/R B-cell lymphomas in adults.[44] The phase I/II study NCT05991388 will evaluate the drug in children and adolescents with R/R B-cell lymphomas, although access to this drug is currently limited to compassionate use. A first-in-human trial of the CD33-CD3 BiTE AMG 330 demonstrated tolerability and anti-leukemic activity in patients with R/R acute myeloid leukemia, although further dose escalation evaluations are needed.[45] If efficacious, this compound could be considered for pediatric patients with ALL, multi-lineage acute leukemias, acute myeloid leukemia, or ALL cases with CD33 expression.

As discussed, coupling these agents with immune-checkpoint inhibition may overcome resistance to BiTE therapy. Further exploiting this mechanism are newly developed bifunctional checkpoint-inhibitory T-cell engagers (CiTE). These agents consist of a BiTE core with the addition of an anti-PD-1 or anti-PD-L1 to enhance efficacy and potentially limit off-target toxicity. This strategy has been demonstrated in a preclinical model targeting acute myeloid leukemia,[46] and additional strategies targeting exhaustion markers CTLA4 and TIM3 are being explored.[47]

Consideration of the role of blinatumomab in antibody-drug conjugate combinations and/or with CAR-T-cell therapy is beyond the scope of this review and is addressed elsewhere. There is also little known about the optimal duration of treatment with blinatumomab, including how many cycles are necessary or ideal, and whether or not blinatumomab should be used in phases of therapy other than those tested already. For example, blinatumomab in induction therapy such as in pre-phase[48] or induction cycles has not been studied sufficiently to date.

Consolidation therapy after stem cell transplant for patients with high MRD early after transplant or with early loss of B-cell aplasia after CAR-T-cell therapy has been suggested as an opportunity for blinatumomab use that has yet to be formally studied in children with B-ALL or B-lymphoblastic lymphoma. In summary, blinatumomab has transformed the care of children, adolescents, and adults with B-ALL and B-NHL, and is known to be both effective and no more toxic in patients with Down syndrome, bringing previously unobserved single-agent activity with negative MRD in refractory cases, advantages for toxicity over conventional multi-agent chemotherapy, and the ability for patients with refractory disease to proceed to more definitive therapies

including stem cell transplant at a higher rate, thus optimizing their chance for cure. In time, it is expected that these and many other important questions about how best to incorporate BiTE therapy for childhood ALL will be answered. New combinations of therapies, investigation in the front line, use in special populations including infants, and more patient-friendly formulations all lie ahead in the continuously rising trajectory of blinatumomab use, always with the goal of improving outcomes and quality of life for all.

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Contributions

KUL and LG conceived, researched, wrote, revised and edited the manuscript, and approved the initial and final submissions.

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