ORIGINAL RESEARCH ARTICLE: CLINICAL TRIALS AND OBSERVATIONS

Long-term Survival and T-Cell Kinetics in Adult Patients with Relapsed/Refractory B-Precursor Acute Lymphoblastic Leukemia Who Achieved Minimal Residual Disease Response Following Treatment with Anti-CD19 BiTE® Antibody Construct Blinatumomab

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Running head: Long-term survival after blinatumomab in ALL

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KEY POINTS

- 27% of patients (10 of 36) had survival of at least 30 months in a blinatumomab study in relapsed/refractory acute lymphoblastic leukemia
- Long-term survival might be associated with T-cell expansion, B cell depletion, and minimal residual disease response

ABSTRACT

This long-term follow-up analysis evaluated overall survival (OS) and relapse-free survival (RFS) in a phase 2 study of the bispecific T-cell engager (BiTE®) antibody construct blinatumomab in 36 adults with relapsed/refractory B-precursor acute lymphoblastic leukemia. In the primary analysis, 25 (69%) patients achieved complete remission with full (CR) or partial (CRh) recovery of peripheral blood counts within the first 2 cycles. Twenty-five patients (69%) had a minimal residual disease (MRD) response (< 10^{-4} blasts), including 22 CR/CRh responders, two patients with hypocellular marrow, and one patient with normocellular marrow but low peripheral counts. Ten of the 36 patients (28%) were long-term survivors (OS ≥ 30 months). Median OS was 13.0 months (median follow-up, 32.6 months). MRD response was associated with significantly longer OS (Mantel-Byar \( P = 0.009 \)). All 10 long-term survivors had an MRD response. Median RFS was 8.8 months (median follow-up, 28.9 months). A plateau for RFS was reached after approximately 18 months. Six of the 10 long-term survivors remained relapse-free, including four who received allogeneic stem cell transplantation (alloSCT) as consolidation for blinatumomab and two who received three additional cycles of blinatumomab instead of alloSCT. Three long-term survivors had neurologic events or cytokine release syndrome resulting in temporary blinatumomab discontinuation; all restarted blinatumomab successfully. Long-term survivors had more pronounced T-cell expansion than patients with OS < 30 months.
INTRODUCTION

The prognosis is poor for adult patients with relapsed/refractory (r/r) B-precursor acute lymphoblastic leukemia (ALL). Treatment with chemotherapy has been reported to result in median overall survival (OS) from 4.5 to 8.4 months.\textsuperscript{1-5} Five-year OS rates with chemotherapy are only 7\% to 10\%.\textsuperscript{1,2} Median OS is 5.8 months among patients who relapse after allogeneic stem cell transplantation (SCT), and 10 months among patients who relapse after chemotherapy only (without prior allogeneic SCT).\textsuperscript{5}

Blinatumomab, a CD19/CD3 bispecific T-cell engager (BiTE\textsuperscript{\textregistered}) antibody construct, leads to redirected lysis of CD19-positive (CD19\textsuperscript{+}) target B cells by inducing a transient cytolytic synapse between the target cells and T cells.\textsuperscript{6} In an exploratory dose-finding phase 2 study in adult patients with r/r B-precursor ALL (including patients in late first relapse > 12 months), 69\% of patients achieved a complete response (CR) or complete response with partial hematologic recovery (CRh), and 88\% of responders achieved a minimal residual disease (MRD) response within the first 2 treatment cycles.\textsuperscript{7} In addition, MRD response was seen in two patients with hypocellular marrow and in one patient with partial response (normocellular marrow but low peripheral counts). The study explored both constant dosing as well as single-step and double-step dosing to prevent severe cytokine release syndrome (CRS). In a confirmatory phase 2 study of 189 patients with r/r B-precursor ALL, including those with early relapse (less than 12 months) after first remission, 43\% achieved CR or CRh after two cycles of treatment with blinatumomab.\textsuperscript{8} Median relapse-free survival (RFS) was 5.9 months; median OS was 6.1 months.

The first analysis of the phase 2 dose-finding study analyzed OS with a median follow-up of 12.1 months.\textsuperscript{7} The long-term follow-up analysis presented here evaluated OS at a median follow-up of 32.6 months. We evaluated clinical characteristics, including disease-related medical history before blinatumomab treatment, outcomes of blinatumomab treatment (including
hematologic and MRD responses to blinatumomab, adverse events, consolidation with allogeneic SCT, and relapses), and T-cell and B-cell kinetics during treatment.
PATIENTS AND METHODS

Study Design

This report describes a follow-up analysis of relapse and overall survival; the methods of the primary analysis were described elsewhere. This was an open-label, multicenter, exploratory, single-arm, phase 2 study in adult patients with r/r B-precursor ALL conducted in collaboration with the German Study Group for Adult Acute Lymphoblastic Leukemia. The target population was Philadelphia chromosome (Ph)-negative and Ph-positive patients with primary refractory disease or relapse. Key exclusion criteria were Ph-positive ALL eligible for dasatinib or imatinib treatment; autologous SCT within 6 weeks and allogeneic SCT within 3 months before the start of blinatumomab treatment; history or presence of clinically relevant central nervous system (CNS) pathology, active CNS leukemia, active graft-versus-host disease (GVHD) and/or immunosuppressive therapy for GVHD within 1 week of blinatumomab treatment start, or active infections. The study protocol was approved by the Paul-Ehrlich-Institute and each study site’s independent ethics committee, and written informed consent was obtained from each patient, in accordance with the Declaration of Helsinki. Toxicity and efficacy data were reviewed by an independent data monitoring committee. ClinicalTrials.gov Identifier: NCT01209286.

Study Procedures

The first 2 cycles of blinatumomab were administered to induce remissions. A bone marrow aspirate or biopsy was obtained before the first blinatumomab cycle and on day 29 of each cycle; cytomorphology and MRD were assessed in central reference laboratories. Hematologic complete remission with full recovery of peripheral blood counts (CR) was defined by ≤ 5% blasts in the bone marrow, no evidence of circulating blasts or extra medullary disease, platelets > 100,000/µL, hemoglobin ≥ 11 g/dL, and absolute neutrophil count (ANC) > 1,500/µL. Hematologic complete remission with partial recovery of peripheral blood counts (CRh) was defined by the same criteria but a lower minimum of peripheral blood counts (platelets
> 50,000/µL, hemoglobin ≥ 7 g/dL, and ANC > 500/µL). An MRD response was defined as MRD < 10^-4 by allele-specific real-time quantitative polymerase chain reaction for clonally rearranged immunoglobulin and/or T-cell receptor genes (sensitivity ≥ 10^-4).9

Each treatment cycle was 6 weeks, including 4 weeks of continuous intravenous infusion and a 2-week treatment-free interval. The dose-finding stage used the following dosing schedules: Cohort 1 (n = 7) received blinatumomab 15 µg/m^2/day; Cohort 2a (n = 5) received 5 µg/m^2/day in week 1 and then 15 µg/m^2/day; Cohort 2b (n = 6) received blinatumomab 5 µg/m^2/day in week 1, 15 µg/m^2/day in week 2, and then 30 µg/m^2/day. In the extension stage, Cohort 3 (n = 18) used the dosing schedule from Cohort 2a. In case of CR or CRh, consolidation treatment with up to 3 additional cycles of blinatumomab and/or allogeneic SCT was permitted. After one incidence of grade 4 CRS, prephase treatment with dexamethasone (≤ 24 mg for 1-5 days) and/or cyclophosphamide (200 mg/m^2 for 1-4 days) was allowed. Each patient had mandatory intrathecal CNS prophylaxis with methotrexate 15 mg, cytarabine 40 mg, and dexamethasone 4 mg administered by a spinal tap during screening and on day 29 of each cycle. Intravenous dexamethasone 16 mg or equivalent was given within 1 hour of treatment start. Adverse events were collected throughout the study and graded by the Common Terminology Criteria for Adverse Events (Version 4.0).10

Analysis of Lymphocyte Subpopulations

T-cell and B-cell kinetics were assessed in each patient. Using methods that were described previously,11 peripheral blood mononuclear cells were isolated at various time points before and throughout the first and second cycle of blinatumomab treatment and stained with fluorescent-labeled antibodies against the following cell surface markers: CD3+/CD13-/CD14- or CD3+/CD45+ (T cells); CD3+/CD45RA-/CD197- (effector memory T [TEM] cells); and CD19+/CD13-/CD14- or CD19+/CD45+ (B cells). Flow cytometry data was collected on a FACSCanto II (Becton Dickinson, Heidelberg, Germany), or Navios 10/3 instrument (Beckman...
Coulter, Krefeld, Germany). Statistics were analyzed by the software FCS Express (De Novo Software, Glendale, CA, USA), or Kaluza (Beckman Coulter). Percentages of lymphocyte subpopulations were multiplied by absolute lymphocyte numbers from a differential blood count to calculate absolute cell numbers for each lymphocyte subpopulation. The analysis set included all available and/or evaluable data points (T and TEM cells, before blinatumomab infusion [=baseline, on day 1] and on days 8, 15, 22, and 29 of each cycle; B cells, same schedule but also on day 3 of each cycle) regardless of blinatumomab dosing regimen.

**Statistical Analysis**

Relapse-free survival was measured from the time of first CR or CRh to hematologic or extramedullary relapse or death resulting from any cause. Patients still in remission at data lock were censored at the time of last remission status assessment. Overall survival was measured from the time of first blinatumomab dose to death resulting from any cause. Kaplan-Meier methods were used to estimate the probability of RFS and OS over time, providing median and 95% confidence interval (CI). A Mantel-Byar test was conducted to evaluate the OS benefit associated with achieving an MRD response versus not achieving an MRD response, and Simon-Makuch plots were used to visualize the survival estimates over time between those who did and did not achieve an MRD response at each event time. A log-rank test was conducted to compare OS between patients with prior allogeneic SCT versus those without prior allogeneic SCT.

Long-term survivors were defined as patients with OS ≥ 30 months. The definition of long-term overall survival by duration of at least 30 months is based on published data, which show most events occurring within the first 24 months. Patients were grouped by MRD response and by OS duration (< 30 months or ≥ 30 months). Summary statistics were provided for each subgroup, including clinical characteristics before blinatumomab treatment, use of allogeneic SCT before/after blinatumomab, treatment response and relapse, and adverse events.
RESULTS

Treatment Response

Thirty-six patients (Table 1) were treated at nine centers in Germany between October 6, 2010, and June 19, 2012, with follow-up ongoing. Patient disposition according to treatment response is shown in Figure 1. As described previously for the primary analysis,7 the rate of CR/CRh was 69% (25 of 36 patients). Seventeen patients (47%) achieved CR and 8 patients (22%) achieved CRh as a best response during the treatment period (Table 2). The other patients had partial remission (n = 2) or hypocellular bone marrow (n = 4); or were refractory to treatment (n = 4) or unevaluable (n = 1). Twenty-two of 25 patients with CR/CRh (88%) had an MRD response (three responders did not achieve an MRD response). An additional three patients with bone marrow that did not fulfill the criteria for partial hematologic recovery had an MRD response. Thus, 25 of 36 patients (69%) who were treated with blinatumomab had an MRD response.

Overall Survival

At a median follow-up time of 32.6 months (range, 0.8–41.9), median OS was 13.0 months (95% CI, 8.5–21.9 months) (Figure 2A). A plateau was reached for OS after approximately 33 months. The Mantel-Byar odds ratio was 0.33 (P = .009), indicating a 67% risk reduction associated with an MRD response (Figure 2B). A difference in OS between patients with and without prior allogeneic SCT was not detected (log-rank P = .640; Figure 2C).

Relapse-free Survival

At a median follow-up time of 28.9 months (range, 0.5–34.5), median RFS was 8.8 months (95% CI, 5.7–13.2 months) among all 25 patients with CR/CRh. At approximately 18 months, a plateau was reached for RFS with six patients not having a documented relapse after this time (Figure 3). Of the six patients with long-term RFS, four patients underwent allogeneic SCT as consolidation for blinatumomab and two patients received three additional cycles of

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blinatumomab instead of allogeneic SCT. The three patients with CR/CRh and no MRD response had relapses after 0.5, 2.0, and 9.0 months, respectively.

Clinical Characteristics

Patients were divided into three groups. The first group included the 10 patients who were long-term survivors, defined as OS $\geq$ 30 months after start of blinatumomab treatment, all of whom achieved an MRD response. The second group included the 15 patients with MRD response who were not long-term survivors. The third group included the 11 MRD nonresponders, none of whom were long-term survivors. The main clinical characteristics for each subgroup are summarized in Table 1. A detailed listing of clinical characteristics for all 36 patients on study is provided in Supplemental Table 1. Based on individual patient-level data, the three patient groups had a similar number of prior salvage treatments, prior allogeneic SCT, prephase treatment with dexamethasone and/or cyclophosphamide, and administration of immune globulins. Median blast count was numerically lowest in the long-term survivors and highest in patients with no MRD response, but these differences were not statistically significant.

Treatment responses, follow-up treatment, and relapses are summarized by MRD response and duration of survival categories in Table 2. Seven of the long-term survivors achieved a CR (70%), but two patients with CRh (20%) and one patient with hypocellular bone marrow (10%) were also among the long-term survivors. None of the 11 patients without an MRD response was a long-term survivor. Best response to blinatumomab, follow-up treatment, and relapse for individual long-term survivors are shown in Table 3.

Long-term Survivors With Allogeneic SCT as Consolidation for Blinatumomab

Six of the 10 long-term survivors underwent allogeneic SCT as consolidation for blinatumomab (Table 2). Three of six patients previously received allogeneic SCT before blinatumomab treatment. Five of six patients were still alive at the last follow-up for this analysis, including the three patients who received allogeneic SCT before blinatumomab. One of the three patients
achieved hypocellular bone marrow as treatment response to blinatumomab; the other patients achieved a CR or CRh as the best response to blinatumomab treatment.

**Long-term Survivors Without Allogeneic SCT as Consolidation for Blinatumomab**

Four of the 10 long-term survivors did not undergo allogeneic SCT as consolidation for blinatumomab. All four achieved a CR or CRh as the best response to blinatumomab treatment. One of these four patients received two prior allogeneic SCT. All four patients were still alive at the last follow-up for this analysis. Two of these four patients are in ongoing remission without further treatment. One patient has been alive for 30.0 months since the start of blinatumomab treatment (the patient had a reversible grade 4 CRS). The other patient has been alive for 36.9 months since the start of blinatumomab treatment (the patient had a reversible grade 3 neurological event). The other two patients relapsed after blinatumomab treatment. One of these patients had two CD19-positive relapses more than 12 months after having received 5 cycles of blinatumomab but responded to blinatumomab after each relapse with an MRD-negative remission (3 cycles of retreatment each time). The other patient had a CD19-negative relapse during the third cycle of blinatumomab. This patient achieved another remission after FLAG-IDA chemotherapy and received allogeneic SCT as consolidation for the chemotherapy. The patient was still alive at a follow-up of 41.9 months. Relapses in the central nervous system were not reported.

**Adverse Events in Long-term Survivors**

Adverse events associated with blinatumomab treatment have been previously described in detail for all 36 patients enrolled in the study. Among the long-term survivors, three had treatment-related adverse events (neurologic events or CRS) that resulted in treatment interruption (Table 4). All three patients received no other treatment than blinatumomab. Two of the three patients are still in remission; one patient had a CD19-positive relapse that was successfully retreated with blinatumomab.
T-Cell and B-Cell Kinetics

Expansion of CD3+ T cells, which was analyzed by kinetics of median cell counts during treatment cycles 1 and 2, was predominantly observed in MRD responders with OS ≥ 30 months, in both cycle 1 and cycle 2 (Figure 4A). Increased T-cell numbers appeared to contract toward baseline during the 2-week treatment-free interval between cycles 1 and 2, mimicking a naturally occurring T-cell response also consisting of a T-cell activation, expansion, and contraction phase. MRD responders with OS < 30 months showed some T-cell expansion in cycle 1, but not in cycle 2, whereas T-cell expansion was absent in MRD nonresponders in both cycles. Of note, the most pronounced CD3+ T-cell expansion was observed in a long-term survivor with an MRD response who experienced a grade 3 neurologic adverse event but did not receive any other treatment than five cycles of blinatumomab (data not shown).

CD3+ T-cell expansion was associated with increasing numbers of CD3+ TEM cells, which play an important role in blinatumomab-induced apoptosis of target B cells due to their large cytotoxic potential (Figure 4B). Other memory T-cell subsets like CD8+ TEMRA cells or CD4+ TCM cells also added to overall CD3+ T-cell expansion (data not shown). Of note, MRD nonresponders not only showed no CD3+ T-cell and CD3+ TEM^cell expansion but also had the lowest absolute cell counts of these important T-cell populations.

Additionally, kinetics of CD19+ B-cell depletion differed between MRD responders and nonresponders (Figure 4C). Median B-cell depletion was ≤ 1 cell/µL at day 3, complete at day 8, and sustained throughout treatment cycles 1 and 2 in MRD responders regardless of OS duration. In contrast, in patients without an MRD response, median duration of B-cell depletion lasted 22 days, with detectable return of peripheral B cells before start of treatment cycle 2 at day 43.
DISCUSSION

This long-term follow-up analysis to the primary analysis of the first phase 2 study of blinatumomab in 36 adult patients with r/r B-precursor ALL demonstrated a median OS of 13.0 months with 32.6 months of follow-up and a median RFS of 8.8 months with 28.9 months of follow-up. Previous studies reported chemotherapy treatment of patients with r/r ALL results in median OS ranging from 4.5 to 8.4 months.\(^1\)\(^-\)\(^5\) Recently published results for inotuzumab ozogamicin, an anti-CD22 monoclonal antibody drug conjugate, in r/r ALL showed a median OS of 7.3 months at a weekly dosing schedule.\(^13\) Furthermore, one previous study reported that OS with salvage chemotherapy in relapsed ALL is shorter among patients who relapse after allogeneic SCT.\(^5\) Many of the patients in the present study relapsed after allogeneic SCT before they received blinatumomab, but there was no difference in OS between patients with and without prior allogeneic SCT.

In this follow-up analysis, 10 of the 36 patients initially treated with blinatumomab were long-term survivors, defined by an OS of \(\geq\) 30 months, and six of the 25 patients who achieved CR/CRh had long-term RFS. Although our results are preliminary and collection of larger data sets is warranted, the analysis points to some factors that may have influenced long-term outcomes after blinatumomab treatment. All long-term survivors previously achieved an MRD response. These results suggest that achievement of an MRD response with blinatumomab treatment in r/r B-precursor ALL may translate into clinical benefit in terms of long-term survival. Of the six long-term survivors who remained relapse-free after blinatumomab treatment, two received blinatumomab only and four received blinatumomab followed by allogeneic SCT. These data suggest that the use of allogeneic SCT as consolidation for blinatumomab is a feasible treatment concept that warrants testing in larger trials. Based on individual patient-level data, prephase treatment with dexamethasone or cyclophosphamide did not have a negative impact on long-term survival. This is in line with previous observations from the large
confirmatory phase 2 study of 189 patients, in which a multivariate analysis showed no effect of
dexamethasone prephase treatment on response.8

We also examined the outcome of patients who either received blinatumomab as their only
therapy or who received retreatment with blinatumomab after relapse. Three long-term survivors
received no other treatment after blinatumomab infusion. All three patients experienced
neurologic adverse events or CRS that resulted in temporary interruption of blinatumomab
treatment. Similar toxicities were reported in a phase 1 study of autologous T cells expressing
the 19-28z chimeric antigen receptor (CAR) specific for the CD19 antigen,14 suggesting that
toxicities associated with agents that target CD19-expressing cells may be similar and possibly
independent of the mechanism of T-cell activation. The long-term outcomes of these three
patients illustrate that long-term survival following blinatumomab treatment may be achieved
with no other subsequent treatment, even in relapsed ALL. One long-term survivor in this study
who relapsed twice responded to retreatment with blinatumomab both times, suggesting that
blinatumomab might be an alternative to chemotherapy for treatment of relapses in patients with
r/r ALL after blinatumomab-induced CR followed by blinatumomab maintenance treatment.15
Additional research is required to confirm the activity and tolerability of blinatumomab
retreatment in this setting. One patient without allogeneic SCT as consolidation for
blinatumomab had a CD19-negative relapse and achieved another remission following
chemotherapy. Blinatumomab might have prolonged the interval between chemotherapy
regimens, thus possibly enhancing sensitivity to the subsequent chemotherapy. Larger data
sets are needed to confirm this observation.

Long-term survivors (ie, MRD responders with OS ≥ 30 months) showed a higher degree of T-
cell and TEM-cell expansion during treatment cycles 1 and 2. The data suggest that T-cell
expansion might be a key factor for survival in the setting of r/r ALL. This is the first, albeit
limited, data set to support this hypothesis, and additional studies are needed to verify the
observation. Prior blinatumomab data in patients with relapsed non-Hodgkin lymphoma have indicated dose-dependent effects mainly on peripheral blood, bone marrow, and lymph node B cells while T-cell kinetics seemed less affected by blinatumomab dose. The onset of antileukemia responses following blinatumomab infusion occurs early during treatment, in most cases at the first assessment at the end of cycle 1. For nonresponders, additional cycles of treatment or an increased blinatumomab dose in the second cycle had little effect on improvement of antileukemia activity. In the current study, a dose increase to 30 µg/m²/day at day 15 of the first cycle did not result in a higher CR rate (data not shown). These results suggest that while kinetics of peripheral B-cell depletion seem to be associated with achieving a complete molecular response (ie, MRD negativity), the degree of T-cell expansion might be important not only for remission but also for long-term OS. Long-term survival (OS ≥ 30 months) was associated with a larger and repeated T-cell expansion, compared with only minor or even absent T-cell expansion at OS of < 30 months.

In conclusion, in this long-term follow-up analysis of an exploratory, dose-escalation phase 2 study of blinatumomab in adult patients with r/r B-precursor ALL, an MRD response to blinatumomab treatment was associated with significantly longer OS compared with patients who did not achieve an MRD response. All of the long-term survivors had an MRD response. Long-term survivors also had greater T-cell expansion. The data suggest that long-term survival after blinatumomab treatment may be associated with an MRD response and potentially also with a higher degree of T-cell expansion.
AUTHORSHIP
MT, NG, RCB, GZ, and MK designed the research. NG, MK, AV, MS, SN, HH, RM, CF, HD, AR, MB, MS, and MST performed the research. GZ, HH, MB, CH, HE, and RCB analyzed the data. GZ and MK wrote the first draft of the paper. All authors reviewed and contributed to the final paper.

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DISCLOSURES
NG, MB and HH have received research funding from Amgen Inc. NG, MB and RCB have consulted for Amgen Inc. NG, HH, RCB and MST have received honoraria from Amgen Inc. AV and MST have served on advisory boards from Amgen Inc. GZ, MS and MK are employees of Amgen Research (Munich) GmbH. CH is an employee of Amgen Inc. GZ, MS, MK and CH are shareholders in Amgen Inc. AV has served in advisory boards for Roche, Janssen, Gilead. AV has received honoraria from Pfizer and Roche and travel support from Roche, Pfizer, and Amgen Inc. MS, SN, AR, RM, CF, HE do not have conflicts to declare.
REFERENCES


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<th></th>
<th>MRD Responders</th>
<th>MRD Nonresponders</th>
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<tr>
<td></td>
<td>OS ≥ 30 Months</td>
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<td>≥ 2nd salvage</td>
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<td>Cyclophosphamide</td>
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2 MRD, minimal residual disease; OS, overall survival; SCT, stem-cell transplantation.

3 *All of the MRD nonresponders had OS < 30 months.
Table 2. Summary of response, follow-up treatment, and relapse

<table>
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<th>Outcome, n (%)</th>
<th>MRD Responders</th>
<th>MRD Nonresponders</th>
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<tbody>
<tr>
<td></td>
<td>OS ≥ 30 Months</td>
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<td>Best response†</td>
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<tr>
<td>CR</td>
<td>7 (70)</td>
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<td>CRh</td>
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<tr>
<td>Hypocellular bone marrow</td>
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<td>Partial remission</td>
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<td>Retreatment with blinatumomab</td>
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</tr>
<tr>
<td>Blinatumomab and allogeneic SCT</td>
<td>4 (40)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

AlloSCT, allogeneic stem cell transplantation; CR, complete remission; CRh, CR with partial recovery of peripheral blood counts; MRD, minimal residual disease; OS, overall survival.

*All of the MRD nonresponders had OS < 30 months.
†Best response during the treatment period.
‡Excludes patients who relapsed and received chemotherapy before allogeneic SCT.
Table 3. Best response to blinatumomab, follow-up treatment, and relapse for individual long-term survivors

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Cohort</th>
<th>Best Response*</th>
<th>MRD Response</th>
<th>Allogeneic SCT After Blinatumomab</th>
<th>RFS Duration (Months)</th>
<th>Retreatment After Relapse</th>
<th>OS Duration (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2b</td>
<td>CR</td>
<td>Yes</td>
<td>No</td>
<td>17.5</td>
<td>Yes</td>
<td>38.6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>CR</td>
<td>Yes</td>
<td>Yes</td>
<td>34.5†</td>
<td>No</td>
<td>35.0</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>CRh</td>
<td>Yes</td>
<td>No</td>
<td>22.4†</td>
<td>No</td>
<td>30.0†</td>
</tr>
<tr>
<td>20</td>
<td>2b</td>
<td>CR</td>
<td>Yes</td>
<td>No</td>
<td>34.1†</td>
<td>No</td>
<td>36.9</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td>Hypocellular</td>
<td>Yes</td>
<td>Yes</td>
<td>N/A</td>
<td>No</td>
<td>29.7†</td>
</tr>
<tr>
<td>26</td>
<td>3</td>
<td>CR</td>
<td>Yes</td>
<td>Yes</td>
<td>11.5</td>
<td>No</td>
<td>31.9§</td>
</tr>
<tr>
<td>27</td>
<td>3</td>
<td>CR</td>
<td>Yes</td>
<td>Yes</td>
<td>23.5†</td>
<td>No</td>
<td>30.3</td>
</tr>
<tr>
<td>29</td>
<td>2a</td>
<td>CR</td>
<td>Yes</td>
<td>No†</td>
<td>2.8</td>
<td>No</td>
<td>41.9</td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>CRh</td>
<td>Yes</td>
<td>Yes</td>
<td>27.9†</td>
<td>No</td>
<td>30.2</td>
</tr>
<tr>
<td>32</td>
<td>3</td>
<td>CR</td>
<td>Yes</td>
<td>Yes</td>
<td>29.9†</td>
<td>No</td>
<td>30.3</td>
</tr>
</tbody>
</table>

CR, complete remission; CRh, CR with partial recovery of peripheral blood counts; MRD, minimal residual disease; OS, overall survival; RFS, relapse-free survival; SCT, stem cell transplantation.

*Best response during the treatment period.
†Patient was still relapse-free at the time of the analysis.
‡Patient had OS of ≥ 30 months after rounding to the nearest whole value.
§Death.
†Chemotherapy was administered after blinatumomab and before allogeneic SCT.
<table>
<thead>
<tr>
<th>Patient No.*</th>
<th>Blinatumomab Dose (µg/m²/day)</th>
<th>Cycle</th>
<th>Adverse Event, CTCAE grade</th>
<th>Blinatumomab Dose at Restart of Treatment (µg/m²/day)</th>
<th>Prophylaxis</th>
<th>Response, CRh, RFS, OS, Remission†</th>
<th>Alive/In Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>15</td>
<td>1</td>
<td>Convulsion grade 2</td>
<td>5</td>
<td>Clobazam</td>
<td>CRh RFS: 17.5 mo OS: 38.6 mo</td>
<td>Yes/No (Relapse)</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>2</td>
<td>Encephalopathy grade 3</td>
<td>5</td>
<td>No</td>
<td>CR RFS: 34.1 mo OS: 36.9 mo</td>
<td>Yes/Yes</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>1</td>
<td>CRS grade 4</td>
<td>5</td>
<td>Prephase dexamethasone‡</td>
<td>CRh RFS: 22.4 mo OS: 30.0 mo§</td>
<td>Yes/Yes</td>
</tr>
</tbody>
</table>

CR, complete remission; CRh, CR with partial recovery of peripheral blood counts; CRS, cytokine release syndrome; CTCAE, Common Terminology Criteria for Adverse Events (version 4.0); mo, months; OS, overall survival; RFS, relapse-free survival.

Long-term survivors were defined as patients with survival ≥ 30 months.

*Patient No. refers to patients listed in Supplemental Table 1.
†Alive at last follow-up/completed study in remission
‡Up to 24 mg dexamethasone per day for up to 5 days and/or 200 mg/m² cyclophosphamide per day for up to 4 days before blinatumomab infusion
§Patient first achieved remission on study day 99 and had to restart cycle 1 after interruptions.
FIGURE LEGENDS

Figure 1. Patient disposition

Of the 36 patients in the study, 25 achieved complete remission (CR) or CR with partial recovery of peripheral blood counts (CRh), and 11 patients did not achieve CR/CRh. Furthermore, 25 patients achieved minimal residual disease (MRD) response and 11 patients did not achieve MRD response. Although numerically identical, the MRD response subgroups overlapped, but were not the same as, the CR/CRh response subgroups.

Figure 2. Overall survival

(A) Overall survival in the entire patient population (N = 36) at a median follow-up of 32.6 months. Two patients who were alive at the last follow-up at 29.7 and > 29.9 months, respectively, were censored at these visits and were considered to have overall survival of ≥ 30 months after rounding to the nearest whole number. (B) Simon-Makuch analysis of overall survival in patients with and without minimal residual disease (MRD) response. (C) Overall survival with and without prior allogeneic stem cell transplantation in the entire patient population (N = 36) at a median follow-up of 32.6 months.

Figure 3. Relapse-free survival

Relapse-free survival (RFS) among responders (n = 25) at a median follow-up of 28.9 months.

Figure 4. T-cell and B-cell kinetics

T-cell and B-cell kinetics during cycle 1 (days 1 to 29) and cycle 2 (days 43 to 71) of blinatumomab treatment. (A) CD3⁺ T-cell expansion. (B) CD3⁺ TEM-cell expansion. (C) CD19⁺ B-cell depletion. Patients were grouped according to MRD response and duration of OS (< 30 months vs ≥ 30 months). Data shown are median cell values (interquartile range) with numbers of evaluable data points per patient subgroup at each time point given below. For clarity, initial T-cell redistribution during the first treatment week of cycles 1 and 2 is not shown.
Blinatumomab treatment
Received blinatumomab (n = 36)

Best hematologic response to blinatumomab
Achieved CR/CRh (n = 25)
Did not achieve CR/CRh (n = 11)
  Partial remission (n = 2)
  Hypocellular bone marrow (n = 4)
  Refractory (n = 4)
  Unevaluable (n = 1)

Minimal residual disease (MRD) response to blinatumomab
Achieved MRD response (n = 25)
  Achieved both CR/CRh and MRD response (n = 22)
  Achieved MRD response without CR/CRh (n = 3)
Did not achieve MRD response (n = 11)
  Achieved CR/CRh without MRD response (n = 3)
  Achieved neither CR/CRh nor MRD response (n = 8)

Long-term survival
Overall survival < 30 months (n = 26)
  MRD responders (n = 15)
  MRD non-responders (n = 11)
Long-term survival ≥ 30 months (n = 10)
  MRD responders (n = 10)
  MRD non-responders (n = 0)

Relapse and SCT among long-term survivors
Did not relapse after CR/CRh (n = 6)
  Blinatumomab + SCT (n = 4)
  Blinatumomab only (n = 2)
Relapsed after CR/CRh (n = 3)
  Blinatumomab + SCT (n = 1)
  Blinatumomab only (n = 2)
  Relapse not evaluable (hypocellular) (n = 1)
  Blinatumomab + SCT (n = 1)
Figure 2
Figure 3

Patients at risk

Time (Months)

Probability of Relapse-free Survival
Long-term survival and T-Cell kinetics in adult patients with relapsed/refractory B-precursor acute lymphoblastic leukemia who achieved minimal residual disease response following treatment with Anti-CD19 BiTE® antibody construct blinatumomab

Gerhard Zugmaier, Nicola Gökbuget, Matthias Klinger, Andreas Viardot, Matthias Stelljes, Svenja Neumann, Heinz-A. Horst, Reinhard Marks, Christoph Faul, Helmut Diedrich, Albrecht Reichle, Monika Brüggemann, Chris Holland, Margit Schmidt, Hermann Einsele, Ralf C. Bargou and Max S. Topp

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