Pediatric posttransplant relapsed/refractory B-precursor acute lymphoblastic leukemia shows durable remission by therapy with the T-cell engaging bispecific antibody blinatumomab

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ABSTRACT

We report on posttransplant relapsed pediatric patients with B-precursor acute lymphoblastic leukemia with no further standard of care therapy who were treated with the T-cell engaging CD19/CD3-bispecific single-chain antibody construct blinatumomab on a compassionate use basis. Blast load was assessed prior to, during and after blinatumomab cycle using flow cytometry to detect minimal residual disease, quantitative polymerase chain reaction for rearrangements of the immunoglobulin or T-cell receptor genes, and bcr/abl mutation detection in one patient with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blinatumomab was administered as a 4-week continuous intravenous infusion at a dosage of 5 or 15 μg/m^2/day. Nine patients received a total of 18 cycles. Four patients achieved complete remission after the first cycle of treatment; 2 patients showed a complete remission from the second cycle after previous reduction of blast load by chemotherapy. Three patients did not respond, of whom one patient proceeded to a second cycle without additional chemotherapy and again did not respond. Four patients were successfully retransplanted in molecular remission from haploidentical donors. After a median follow up of 398 days, the probability of hematologic event-free survival is 30%. Major toxicities were grade 3 seizures in one patient and grade 3 cytokine release syndrome in 2 patients. Blinatumomab can induce molecular remission in pediatric patients with posttransplant relapsed B-precursor acute lymphoblastic leukemia and facilitate subsequent allogeneic hematopoietic stem cell transplantation from haploidentical donor with subsequent long-term leukemia-free survival.

Introduction

Patients with relapsed B-lineage acute lymphoblastic leukemia (ALL) after allogeneic stem cell transplantation remain a therapeutical challenge.[1] For these patients, subsequent hematopoietic stem cell transplantation (HSCT) offers the only curative treatment.[1,2] Despite several advances over the last decade which led to significantly reduced transplant-related mortality, relapse rates are still high and represent the major cause of death in these patient cohorts.[3-6]

Level of pre-transplant minimal residual disease (MRD) has been shown to be an important adverse prognostic parameter for estimating the risk of relapse; MRD, therefore, influences long-term event-free survival after allogeneic stem cell transplantation.[7-12] In particular, patients who relapsed posttransplant have an extremely poor prognosis and need to achieve another hematologic remission or an even more advantageous very low or negative MRD level before proceeding to a subsequent SCT.[6] Additional chemotherapy will often be of limited benefit and may be associated with high toxicity.[7,8] Moreover, with duration and progression of relapsed ALL, treatment faces multi-drug resistance which constitutes an unsolved problem.[13-15] Therefore, new therapeutics with known but also alternative mode of action and lower toxicity profile are necessary.[16-19] The novel class of bispecific single-chain antibody construct (BiTE) blinatumomab is currently under investigation. Blinatumomab recruits and activates T cells through CD3 of the T-cell receptor (TCR) complex for redirected lysis of malignant cells and non-malignant cells expressing CD19. Thus, it leads to close targeted cell-cell contact enabling the formation of cytolytic synapses which is the prerequisite for leukemic cell lysis.[20-22] Pediatric B-precursor ALL blasts show a stable and sufficient expression of CD19 to make it a good target for immunotherapeutic approaches.[23-28] Furthermore, an excellent standard of detection of MRD levels can be achieved with high specificity and sensitivity. Multicolor flow cytometry enables complex patient-specific aberrant immunophenotyping of the blasts with a regular detection level of $1x10^{-4}$. Established MRD monitoring by real-time quantitative polymerase chain reaction (PCR) or reverse transcriptase PCR reaches an even

 doi:10.3324/haematol.2013.100073
The online version of this article has a Supplementary Appendix.
Manuscript received on October 27, 2013. Manuscript accepted on April 4, 2014.
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lower detection threshold.[11,29] In a phase I study in patients with relapsed non-Hodgkin lymphoma, continuous infusion of blinatumomab showed dosage-dependent induction of remission,[30] and in a recent phase II trial in adult B-lineage ALL, blinatumomab was able to induce and consolidate hematologic remission in 80% of patients with chemorefractory MRD.[31] Given these results, we used this agent in pediatric patients with refractory B-lineage ALL at extremely high risk. All patients had had a first or subsequent posttransplant relapse and had not demonstrated a sufficient response to previous applied chemotherapy. Therefore, blinatumomab was administered on a compassionate use basis in order to induce another remission and facilitate a subsequent HSCT from a haploidentical family donor.

Methods

Pediatric patients with posttransplant relapsed B-precursor acute lymphoblastic leukemia who consistently expressed CD19 were considered for blinatumomab treatment. Relapse was defined as more than 5% blasts in bone marrow. Multiparametric flow cytometry and quantitative PCR were used to detect and assess MRD.

The treatment approach with blinatumomab in the reported patient cohort was not a clinical study but a treatment for compassionate use. Data concerning Patients 2 and 3 have been previously published by Handgretinger *et al.*[32] All reported patients were treated at our institution between August 2008 and December 2011, and were registered with the local authorities of the University Children's Hospital, Tuebingen, Germany. The treatment for compassionate use in all 9 reported patients was conducted according to German ethical codes and regulations with the involvement of the Independent Ethics Committee of the University Children's Hospital, Tuebingen, Germany, and in accordance with the provisions of the Declaration of Helsinki. All patients and their representative in law gave informed consent before blinatumomab was started.

None of the patients in the study received antileukemic therapeutics during blinatumomab administration.

Patients received blinatumomab as continuous intravenous infusion (central venous catheter) at an initial dose of 5 or 15 μg/m^2/day for 28 days followed by a treatment-free interval of 7-14 days, adapted from an adult schedule.[30,31] This was defined as 1 cycle of treatment. In case of non-response, a dose escalation with increase of the initial dose to 15 μg/m^2/day was carried out or another chemotherapy cycle was added with or without subsequent stem cell boost before blinatumomab treatment was continued. Responders were to receive at least one additional cycle to consolidate remission status. Patients who achieved molecular remission proceeded to a subsequent transplantation from a haploidentical stem cell donor. Side-effects were assessed according to common terminology criteria for adverse events (CTCAE) v.4.

Monitoring of bone marrow and MRD detection

Microscopic examination of bone marrow aspirates and peripheral blood was assessed by Zeiss Axiolab microscope. Aberrant immunophenotype detected by multiparameter flow cytometry BD FACS LSR II,[33] as well as individual clonal immunoglobulin and T-cell receptor gene rearrangements[12,34] and bcr/abl translocation fusion genes measured by real-time quantitative PCR were used as MRD markers. MRD levels of all patients were assessed prior to, during and after blinatumomab administration. Flow cytometry-based MRD detection was performed as previously published.[11] MRD levels below 1x10^{-4} were defined as negative.

Flow cytometry

Flow cytometry monitoring was performed with regard to blast count and T-cell count ($CD3^+CD4^+$ and $CD3^+CD8^+$), as well as NK-cell count ($CD56^+CD16^+CD3^-$).

Statistical methods

GraphPad PRISM software v.5.04 was used for statistical analysis. Kaplan-Meier estimates for event-free survival probability and overall survival probability were calculated from the date of first blinatumomab administration to the date of relapse or the date of last follow up in continuous complete remission. In cases of non-response, the number of days until relapse is "0".

Details about clinical monitoring, acquisition of laboratory parameters and additional statistical analyses are available in the *Online Supplementary Appendix*.

Results

Outcome

Patients' characteristics are shown in Table 1. Six patients had received one previous allogeneic HSCT and 3 patients had had 2 or more previous allogeneic HSCTs; donors were matched unrelated, matched related and mismatched haploidentical related. Four patients had had no prior chemotherapy before blinatumomab was initiated. Five patients had received chemotherapy and one patient additional central nervous system (CNS) irradiation prior to the first blinatumomab cycle of treatment.

Between August 2008 and December 2011, 9 patients (median age 10 years) with posttransplant relapsed CD19 positive precursor ALL of the B-lineage, were treated with blinatumomab with a total of 18 cycles on a compassionate use basis (Table 2). The patient cohort includes 2 patients (Patients 2 and 3) who have already been reported by us in 2010.[32] Four of 9 patients (Patients 1, 2, 3 and 4) showed a remission within the first cycle of blinatumomab. Three of these responders (Patients 1, 2 and 4) had at least an M2 marrow prior to treatment. One patient (Patient 3) had a level of MRD 0.4x10^{-2}. In 2 patients (Patients 5 and 6), who were refractory to the first cycle of blinatumomab at a dose of 5 μg/m^2/day, chemotherapy was administered after blinatumomab treatment to stop leukemia progression. Remission was finally achieved by the second cycle of blinatumomab, which was administered at a higher dose of 15 μg/m^2/day (*Online Supplementary Figure S1*). Patient 5 received melphalan (20 mg/m^2) and cytarabine (1000 mg/m^2) on two consecutive days followed by the second blinatumomab cycle at 15 μg/m^2/day, which led to low MRD below quantitative range (PCR MRD load 1x10^{-6}). For consolidation he received two additional courses of blinatumomab which reduced MRD below the qualitative detection level (<1x10^{-6}). After the fourth cycle, the patient experienced a bilateral extramedullary leukemic relapse in his mastoids while retaining molecular remission in the bone marrow. Thus he received a fractionated irradiation of the viscerocranium at 18 Gray and the neurocranium at 12 Gray for local leukemia control before a reduced-intensity conditioning regimen was initiated with ATG-Fresenius rabbit (3 x 5 mg/kg BW), fludarabine (4 x 40 mg/m^2), thiotepa (10 mg/kg BW) and melphalan (2 x 70 mg/m^2). Patient 6 received vincristine (1 mg/m^2), clofarabine (3 x 40 mg/m^2), cyclophosphamide (3 x 400 mg/m^2) and etoposide (3 x 100

mg/m²) with stem cell rescue (5x10⁶/kg BW CD34⁺ cells, T-cell depleted graft) from his previous parental haploidentical donor and then a second blinatumomab cycle at increased dose of 15 μg/m²/day. Thereafter, this patient reached MRD negativity and was retransplanted from the other parental haploidentical donor. The conditioning regimen included ATG-Fresenius rabbit (3 x 5 mg/kg BW), clofarabine (4 x 50 mg/m²), thiotepa (10 mg/kg BW) and melphalan (2 x 70 mg/m²).

Three patients (Patients 7, 8 and 9) did not show any relevant response to blinatumomab and died from leukemia progression between Days 58 to 166 after blinatumomab had been initiated. Patient 8 received a second blinatumomab cycle at increased dose 15 μg/m²/day, with a dose escalation schedule up to 30 μg/m²/day for one single day without previous additional chemotherapy, and did not show any relevant clinical response. Two patients (Patients 7 and 9) were not able to receive a second cycle due to infectious complications and worsening general condition (Patient 7) or showed rapid leukemia progression during blinatumomab infusion (Patient 9). Patient 9 proceeded to a second haploidentical HSCT despite leukemia progression (> 90% blasts in BM). She received a conditioning regimen including fludarabine (3 x 40 mg/m²), thiotepa (10 mg/kg BW), melphalan (2 x 70 mg/m²) and TNI (7 Gray).

Six patients (Patients 1, 2, 3, 4, 5 and 6) achieved an M1 bone marrow. In 5 out of the 6 responders (Patients 1, 2, 3, 5 and 6), MRD conversion from positive to negative could be detected. Patient 4 had already been treated with a combination of appropriate antibiotics prior to initial blinatumomab treatment due to long-lasting infestation of multi-drug resistant *Pseudomonas* as well as ongoing local soft tissue infection with scrotal abscess formation because of multi-drug resistant *Enterococcus faecalis*. Despite a pre-existing septic complication and the obviously worsening general condition of the patient, blinatumomab treatment was started. Patient 4 achieved at least hematologic CR. There was no further investigation of MRD level in this patient because the patient died from gram-negative sepsis in aplasia during the first cycle of treatment. Four of 5 patients (Patients 1, 2, 5 and 6) with sustained molecular remission were retransplanted from another haploidentical donor after 2-4 blinatumomab cycles. Two (Patients 1 and 2) of the 4 patients retransplanted in molecular remission are in posttransplant sustained molecular remission of 675 days and 1851 days; one patient (Patient 6) died due to a subsequent (third) relapse 155 days posttransplant and 243 days post the first blinatumomab administration. One of 5 patients (Patient 3), with sustained molecular remission and with bcr/abl positive ALL, was subsequently treated with nilotinib and has been in remission for four years (1490 days).

Taken together, 3 of 9 patients are alive in enduring molecular remission with a median follow up of 1490 days (range 675-1851 days). The Kaplan-Meier survival estimation of event-free survival probability for all of our patients is 30% (Figure 1), while for responders this is 44%. Six of 9 patients died. Four patients died from leukemia progression (Patients 7 and 8) or relapse after re-haploidentical HSCT (Patients 6 and 9). Two patients (Patients 4 and 5) died from gram-negative sepsis. Patient 4 died from pre-existing infectious complication and Patient 5 died from late posttransplant infectious complication 655 days after blinatumomab had been initiated, i.e. 428 days post third allogeneic HSCT with no association to blinatumomab treatment.

Four patients (Patients 1, 5, 6 and 9) received additional prophylactic immunotherapy using an FC-optimized CD19 antibody after blinatumomab treatment had been finished.[35]

Immune response and recovery

We grouped the patients according to their response to blinatumomab treatment. The first group comprised patients who responded directly when blinatumomab was started, achieving complete remission. We defined these patients as primary responding patients or primary responders (Patients 1, 2, 3 and 4). The second group comprised patients who were originally refractory to blinatu-

Table 1. Patients' characteristics.

	All patients n=9	Patient ID
Diagnosis, n.		
cALL	6	2, 4-8
cALL, bcr/abl positive	1	3
pre-B-ALL	2	1, 9
Age[a], years (range) 10.4 (4.3 - 18.5)		
Sex, n.		
Male	5	1-5
Female	4	6-9
Site of relapse, n.		
Isolated bone marrow	7	1, 2, 5-9
Combined bone marrow and CNS	2	3, 4
Status prior to blinatumomab therapy, n		
1st relapse	1	8
2nd relapse	5	1, 2, 6, 7, 9
3rd relapse	2	3, 5
4th relapse	1	4
Pre-treatment overview		
Previous SCTs, n.		
1 x MUD	3	1, 2, 7
1 x Haploidentical	3	6, 8, 9
1 x MSD, 1 x MUD	1	5
1 x CB, 1 x Haploidentical	1	4
1 x MSD, 1 x MUD, 1 x Haploidentical	1	3
Previous therapy of relapse, n.		
None	4	1, 5, 6, 9
Hydroxyurea	1	7
Nilotinib, CNS irradiation i.th. therapy (MTX/AraC/Pred)	1	3
AraC/VCR, AraC/ Mel, i.th. therapy (MTX/AraC/Pred)	1	8
Clof/CP/VP-16, 2x Amsacrine/VP16/Pred + AraC i.th., Mel/AraC/Dexa + AraC i.th.	1	2
Vindesin/Pred, Flud/AraC, VCR/MTX/Dexa/L-Asp	1	4

Median (range); cALL: common acute lymphoblastic leukemia; CNS: central nervous system; SCT: stem cell transplantation; MUD: matched unrelated donor; MSD: matched sibling donor; CB: cord blood; Gy: Gray; MTX: methotrexate; AraC: cytarabine; Pred: prednisone; i.th.: intrathecal; VCR: vincristine; Mel: melphalan; Clof: clofarabine; CP: cyclophosphamide; VP-16: etoposide; Flud: fludarabine; Dexa: dexamethasone; L-Asp: L-asparaginase. Chemotherapy cycles: Patient 7: hydroxyurea 30 mg/kg/day x 6; Patient 3: nilotinib 2 x 200 mg/kg/day, CNS irradiation at 18 Gy; Patient 8: AraC 75 mg/m²/day x 4 + VCR 1.5 mg/m², AraC 75 mg/m2 x 4 + melphalan 10 mg/m²; Patient 2: Clof 40 mg/m2 x 3 + CP 400 mg/m² x 3,+ VP-16 100 mg/m² x 3; amsacrine 100 mg/m² x 2,+ VP-16 500 mg/m² x 2; Mel 20 mg/m² + AraC 2000 mg/m² x 4 + Dexa 20 mg/m²; Patient 4: vindesin 3 mg/m²; Flud 30 mg/m² x 5, AraC 2000 mg/m² x 5; VCR 1.5 mg/m² + MTX 1 g/m² + Dexa 20 mg/m² + L-Asp 10,000 U/m² + AraC 30 mg i.th.

momab, but who responded to a modified regimen including chemotherapy followed by blinatumomab (Patients 5 and 6). These patients were defined as secondary responding patients or secondary responders. Patients who did not respond were defined as non-responding patients or non-responders (Patients 7, 8 and 9).

Non-responders (Patients 7, 8 and 9) and secondary responders (Patients 5 and 6) in the non-responding first cycle did not display any significant changes in the absolute counts and composition of mononuclear cells (e.g. $CD3^+$, $CD19^+$ counts) during blinatumomab administration. On the contrary, in the responding cycles, mainly the first responding cycle of the patients (Patients 1, 2, 3, 4, 5 and 6) led to significant changes in the absolute counts and composition of mononuclear cells, in particular T cells and leukemic blasts. The most evident changes occurred within the first few days of the first responding cycle with a substantial increase in T-cell count and in a parallel fall in the number of $CD19^+$ blasts (Figure 2). The subsequent cycles showed stable counts of $CD3^+$ T cells ($CD4^+$ and $CD8^+$) as well as other cells of the immune compartment with further depletion and efficacious suppression of all $CD19^+$ cells, including blasts and non-malignant B cells.

We grouped the cycles of treatment with blinatumomab into non-responding, responding and maintenance cycles. Non-responding cycles were cycles with no reduction or

Table 2. Overview and outcome.

N.	Number of cycles and dosages (μg/m²/day)	Prior leukemic load	Primary response	Secondary response	Best response, post treatment MRD level	Detection method	Further treatment	Outcome
1	2 (5)	97%	yes	no	MRD negative	PCR, FC	haplo SCT	molecular remission (d675)
2	1 (15)	3.10%	yes	no	MRD negative	PCR	haplo SCT	molecular remission (d1851)
3	4 (15)	0.40%	yes	no	MRD negative	PCR, FC	nilotinib	molecular remission (d1490)
4	1 (15)	70%	yes	no	<1%	microscope	none	died from gram-negative sepsis (d17)
5	4 (5, 15, 15, 15)	70%, 30%	no	yes	MRD negative	PCR	haplo SCT	died from gram-negative sepsis (d655)
6	2 (5, 15)	80%, 11%	no	yes	MRD negative	PCR, FC	haplo SCT	died from relapse (d398)
7	1 (5)	7%	no	no	>90%	FC	palliative care	died from progression (d58)
8	2 (15, 15, 30)	11%	no	no	6.70%	FC	palliative care	died from progression (d166)
9	1 (5, 15)	51%	no	no	>90% blast load	FC	haplo SCT	died from relapse (d265)

Outcome (days) is calculated from the first day of blinatumomab administration to the last day of follow up or date of death. Minimal residual disease (MRD) negative defined as (<0.01% or <10E-4). PCR: polymerase chain reaction; FC: flow cytometry; d: days.

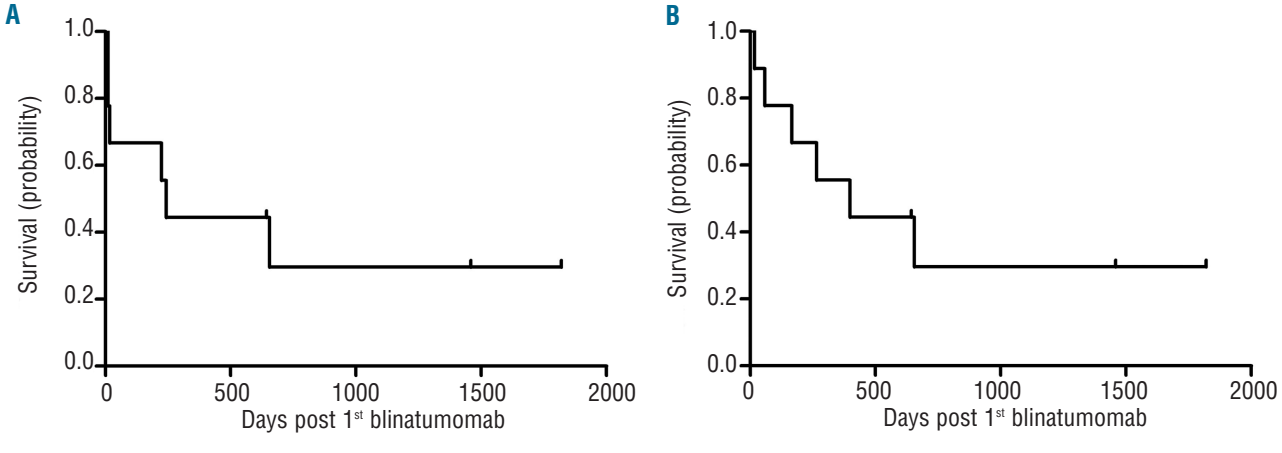

Figure 1. Probability of event-free survival (A) and overall-survival (B) (in days) after the first day of blinatumomab application in all 9 patients. Median follow up for overall survival 398 days; event-free survival 243 days (range for both 17 to 1819 days).

increase in blast load in the bone marrow. Responding cycles were defined as reduction of blast load of less than 5% in the bone marrow and a minimum 2 log-fold reduction in blasts. Maintenance cycles were defined as blinatumomab cycles that preserved the patient's current MRD status.

The comparison of absolute T-cell count in the peripheral blood (n=12; *P*=0.69) in non-responding cycles (median CD3$^+$ 179/μl) *versus* responding cycles (median CD3$^+$ 194/μl) and percentage as well as absolute blast count in the bone marrow in non-responding cycles (median blast load 31%; median absolute blast count 2150/μl) *versus* responding cycles (median blast load 21.5%; median absolute blast count 1240/μl) prior to the start of blinatumomab revealed no big differences (*Online Supplementary Figure S2*).

Regarding Patients 5 and 6 who showed secondary response, we observed no change in the T-cell count comparing the first non-responding and the second responding cycle in Patient 6 but a nearly 1-log-fold decrease in T-cell count in Patient 5 in the responding cycle. Furthermore, there was an approximate 1-log-fold reduction in blasts in Patient 6 but a mere 50% reduction of blasts in Patient 5 achieved by the additional chemotherapy administered between the non-responding and the responding cycle. Both Patients 5 and 6 showed an obviously higher T-cell-to-blast ratio in the responding cycle.

The time interval from HSCT to the first day of blinatumomab treatment had no impact on response, when comparing the time interval in days of non-responders (median 260 days) and responders (median 282 days) (*Online Supplementary Figure S3*). Neither did time off immunosuppression have any impact on response, when comparing non-responders (median 22 days) and responders (median 23 days) to the first day of blinatumomab administration (*Online Supplementary Figure S4*).

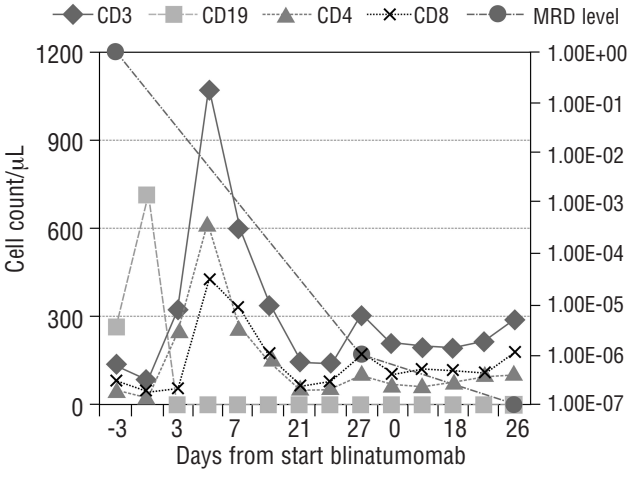

Figure 2. Pharmacodynamics of blinatumomab on peripheral blood mononuclear cells. The course of B and T cells in the peripheral blood of patient (Patient 1). When blinatumomab was started the blast load was 97% in the bone marrow. After the 1st cycle of treatment, the patient reached the MRD level of 1x10^6 and after the 2nd cycle there was no longer any detectable MRD (<1x10^6). The figure shows the effect of blinatumomab on the absolute counts of CD3$^+$, CD4$^+$, CD8$^+$ and CD19$^+$ cells as well as the course of the MRD level (PCR) displayed on the secondary axis.

Toxicity

The most common toxicities independent of cause were decreased neutrophil and lymphocyte count, anemia and low platelet count, as well as increase of acute-phase proteins and pyrexia (Table 3). Three patients were temporarily affected by central ataxia, tremor and somnolence (Patients 2, 5 and 8) that resolved completely within days without any specific treatment under continuous blinatumomab administration. Grade 3/4 toxicities were elevation of bilirubin in 3 patients (Patients 4, 5 and 8) and two seizures, one with apnea and consecutive syncope in Patient 5. The first generalized cerebral seizure occurred with apnea (minimal oxygen saturation level 80%) on Day 7 of the second blinatumomab cycle, which was the first responding cycle in this patient. Intravenous lorazepam terminated the seizure within two minutes. Antiepileptic prophylaxis with levetiracetam was started at 40 mg/kg BW and dexamethasone 15 mg/m^2 was applied for one

Table 3. Adverse events independent from cause.

	Grade I/II	Grade III/IV	Grade V
Blood and lymphatic system disorders, n.			
Febrile neutropenia	4	3	0
Anemia	0	8	0
Infections and infestations, n.			
Bacterial infection	8	0	0
Pancreatitis	0	1	0
Sepsis	0	0	1*
Nervous system disorders, n.			
Syncope/convolusion	0	1	0
Seizure	0	1	0
Headache	5	0	0
Temporary disorientation	1	0	0
Somnolence	2	0	0
Ataxia	3	0	0
Tremor	3	0	0
Peripheral paresthesia	1	0	0
Gastrointestinal disorders, n.			
Nausea / vomiting	0	1	0
Skin and subcutaneous tissue disorders, n.			
Local erythema	1	0	0
Renal and urinary disorders, n.			
Edema	7	2	0
Elevation of creatinine	1	0	0
Investigations, n.			
Bilirubin	1	3	0
ASAT/ALAT	7	2	0
Platelet count decreased	0	6	0
Cardiac disorders, n.			
Heart failure during sepsis	0	1	0
Vascular disorders, n.			
Hypertension	4	0	0
Immune system disorders, n.			
Cytokine release syndrome	1	2	0

*Total n. patients n=9. Treatment-emergent adverse events, regardless of causality from the start of therapy to Day 30 post administration. *Patient 4 started blinatumomab treatment in pre-septical clinical state.*

week. Three days later, on Day 10 of the second cycle, a second generalized seizure occurred (minimal oxygen saturation level 88%) and was resolved, again with lorazepam. Levetiracetam was increased to 70 mg/kgBW. No further seizures were observed. Anticonvulsive treatment successfully ensured continuation of blinatumomab administration. Elevation of transaminases and bilirubin was not accompanied by any impairment of liver function in terms of protein synthesis or detoxification. The pharmacodynamic effect of blinatumomab was accompanied by clinical features of cytokine release such as fever, low blood pressure and fatigue. At the beginning of the first cycle, in the presence of T cells and blasts, all patients experienced transient fever except for Patient 3 who had low blast load (MRD level) and Patient 7 who showed no response to blinatumomab treatment. Patient 1 developed only mild hypotension and was not treated with either additional fluid substitution or with any medication. Fluid substitution due to hypotension caused by blinatumomab treatment was necessary only in Patient 5 for two days when the second blinatumomab cycle was started, which was the first responding cycle of this patient. There was no need for catecholamine treatment or transfer to the intensive care unit. A grade 5 treatment-emergent adverse event occurred in Patient 4 who died from pre-existing *Pseudomonas* sepsis with hypotension and need for catecholamines during blinatumomab administration. Patient 8 suffered from pre-existing pancreatitis during blinatumomab administration that resolved during blinatumomab treatment. There were no deaths due to graft-*versus*-host disease (GvHD) or related to blinatumomab.

Discussion

The current report intends to demonstrate that, in addition to conventional treatment in refractory and post-HSCT relapsed pediatric ALL, an immunotherapeutical approach with blinatumomab is feasible and might increase long-term leukemia-free survival.

In adult B-precursor ALL, Topp *et al*. demonstrated that blinatumomab is very potent at inducing molecular remission in chemorefractory patients.[31] The last follow up of this patient cohort with refractory disease, and therefore the very poor prognosis, indicate significantly increased long-term relapse-free survival after induction and consolidation of remission by blinatumomab used as a single agent with or without subsequent HSCT.[36]

The salvage treatment of posttransplant relapsed B-precursor ALL using blinatumomab as a single agent in our cohort of pediatric patients was administered on a compassionate use basis in accordance with previous treatment in adult CD19$^+$ malignancies, including non-Hodgkin lymphomas and B-ALL.[30,31,36] In the first year post-transplant there is little tolerance for chemotherapy, and hardly any chance for cure without proceeding to another transplant, which is only beneficial in a low or preferably negative MRD state.[7,8,10,12,37] We have shown that blinatumomab administration was feasible and had impressive antileukemic activity against highly chemorefractory disease in our pediatric patients, as has already been shown for adult B-ALL.[31,36] Six of 9 patients achieved complete remission after blinatumomab treatment irrespective of leukemia burden prior to the start of therapy. There was good primary response with the induction of complete MRD remission by blinatumomab. It is remarkable that in 2 patients with primary non-response, additional chemotherapy treatment after initial blinatumomab courses resulted in a secondary response. The synergic effect of conventional chemotherapy to reduce blast load and then reattempt blinatumomab treatment might be a reasonable therapeutical strategy in non-responders. In patients with poor bone marrow function, the use of stem cell boosts from the previous donor might enable the patient to receive such additional chemotherapy and may contribute to relevant T-cell response.

In 2 patients of our cohort (Patients 5 and 6) we observed, via flow cytometric analyses of T cells in the peripheral blood and CD19$^+$ blasts in the bone marrow as well as in the peripheral blood, that effector-to-target ratio could play a more crucial role in treatment success than absolute T-cell count (T cells/μl) in the peripheral blood and absolute blast count in the bone marrow. In general, in responding cycles, absolute CD3$^+$ cell count in the peripheral blood was no higher than in non-responding cycles (P=0.7) (*Online Supplementary Figure S5*). Yet in the responding cycles of Patients 5 and 6, the T-cell-to-blast ratio was remarkably shifted in favor of T cells by the additional chemotherapy given after the first non-responding cycle. The T-cell-to-blast ratio in the non-responding cycle was below 0.2, whereas in the responding cycle it was over 10 (Patient 6) and over 100 (Patient 5). All blinatumomab cycles with a T-cell-(PB)-to-blast(BM) ratio over 7 either showed a complete MRD response or maintenance of complete remission (*Online Supplementary Figure S5*).

Based on this observation, it might be helpful to achieve a favorable T-cell-to-blast ratio by any means of action in order to increase the response rate in refractory patients. Further investigation of peripheral blood and bone marrow is warranted to find out if T-cell count, T-cell-to-blast ratio and functional T-cell analyses will help to predict probability of response.

Patient 3 with Philadelphia chromosome-positive ALL, who was previously reported by Handgretinger *et al*.,[32] is an example of the successful treatment of relapse (combined CNS and BM) post third HSCT with long-term leukemia-free survival, without having proceeded to another HSCT due to high pre-treatment organ toxicity. Molecular remission was induced by blinatumomab and then was consolidated and maintained by the tyrosine kinase inhibitor nilotinib. However, pediatric posttransplant relapsed patients who again achieve low MRD or remission through blinatumomab treatment are in need of a second HSCT to facilitate long-term survival. Second allogeneic HSCT can cure approximately 35% of patients who achieve complete remission. Therefore, blinatumomab can facilitate long-term survival by inducing complete remission for subsequent HSCT.[6] At a median observation time of 398 days, the event-free survival in our cohort of patients is 30%. Three of 9 patients remain in ongoing complete remission. Thus, the combination of chemotherapy- and blinatumomab-induced molecular remission, followed by subsequent HSCT may be considered a new treatment strategy that can induce long-term leukemia-free survival in posttransplant relapsed B-ALL in childhood.

In adult patients, blinatumomab administration was well tolerated.[36] In our pediatric patient cohort treated with blinatumomab at a dose of 5 and 15 μg/m^2/day, pre-

dominant dysfunction of hematopoiesis and infectious complications were pre-existent and based on the primary disease. Side-effects including seizure and hypotension could be managed satisfactorily in all of our patients and consequently blinatumomab therapy was not terminated because of treatment-emergent adverse events in any of them. Cytokine release syndrome was not a major issue in our patients. It has been shown in one case of severe clinical cytokine release syndrome that tocilizumab treatment can ameliorate symptoms and clinical signs rapidly after discontinuation of blinatumomab.[38]

In conclusion, blinatumomab is a novel and efficacious treatment for pediatric patients who suffer from post-transplant relapsed B-lineage precursor ALL. The experience of this treatment for compassionate use is in line with the convincing data acquired in adult patients.[31,36] For posttransplant relapsed and primary refractory CD19-expressing childhood precursor ALL of the B-lineage in general, blinatumomab might come to the forefront. Systematic data for pediatric patients will be provided by the ongoing blinatumomab trial.[39]

Acknowledgments

This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB685), from the BMBF and from the Reinhold Beitlich-Stiftung to PL.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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