Long-term relapse-free survival in a phase 2 study of blinatumomab for the treatment of patients with minimal residual disease in B-lineage acute lymphoblastic leukemia

In adults with acute lymphoblastic leukemia (ALL), advances in chemotherapy have improved hematologic response rates to greater than 80% and relapses rates to below 50%. Leukemic cells not detected by conventional morphological methods may persist or reappear after chemotherapy as minimal residual disease (MRD), defined as at least 10^{-4} (0.01%) leukemic cells detected by quantitative polymerase chain reaction. Patients aged 15 to 55 years with MRD after consolidation have a 5-year relapse-free survival (RFS) rate of 25%, compared with a rate of 67% among those without MRD.[1] Allogeneic hematopoietic stem cell transplantation (HSCT) in patients with MRD improves the 5-year RFS to 44% (*versus* 11% without allogeneic HSCT), but fewer than 50% of patients with MRD undergo allogeneic HSCT, often because of rapidly occurring relapse.[1]

Blinatumomab, a bispecific T-cell engager (BiTE®) antibody construct with dual specificity for CD19 and CD3,[2] demonstrated efficacy in the treatment of relapsed or refractory B-lineage ALL in two phase 2 studies, with response rates of 43% to 69% and median RFS of 5.9 to 7.6 months.[3,4] A randomized phase 3 study showed significantly improved overall survival with blinatumomab compared to standard-of-care chemotherapy in adults with relapsed or refractory ALL.[5]

The data summarized here represent the final analysis of 5-year RFS in a single-arm phase 2 study in adult patients with B-lineage ALL who had persistent MRD or MRD relapse after intensive chemotherapy. The full study methods were described previously.[6] Patients aged ≥18 years with B-lineage ALL in hematologic complete remission (CR) with quantifiable MRD of $\geq 10^{-4}$ after consolidation I of first-line therapy using GMALL protocols were enrolled between January 2008, and August 2009. Each patient received up to four cycles of initial treatment with blinatumomab and up to three additional cycles if hematologic relapse had not occurred. Each cycle included open-label blinatumomab 15 μg/m^2/day by continuous intravenous infusion over 4 weeks, followed by a 2-week treatment-free interval. The primary endpoint was MRD response within the first four cycles, defined as *BCR-ABL* or *MLL-AF4* below the detection limit or individual rearrangements of immunoglobulin or T-cell receptor genes $<10^{-4}$. An institutional review board or independent ethics committee approved the protocol for each study center.

In the primary analysis, after all patients had completed blinatumomab treatment, the rate of MRD response was 80% (16 of 20 evaluable patients) and all MRD responses had occurred in the first cycle.[6] Patients completed follow-up visits for RFS assessment for up to 5 years. In the first follow-up analysis after a median follow-up of 33 months, 12 of 20 evaluable patients (60%) were still in remission.[7] The final analysis reported here was conducted after the last patient completed the last study visit, with a median follow-up of 50.8 months. Ten patients (50%) were still in remission 5 years after the start of blinatumomab treatment.

Per study protocol, patients could receive allogeneic HSCT any time after the first cycle of blinatumomab treatment. Nine patients proceeded to transplantation:

Table 1. Baseline characteristics and treatment outcome by duration of RFS.

	RFS ≥5 years (n=10*)	RFS <5 years (n=10**)
Sex, n (%)		
Male	3 (30)	5 (50)
Female	7 (70)	5 (50)
Age, years, median (range)	48.5 (23-77)	54.5 (20-72)
Prior consolidation II, n (%)	4 (40)	7 (70)
Disease status, n (%)		
Molecular relapse in CR1	0 (0)	4 (40)
Molecular relapse in CR2+	0 (0)	1 (10)
Molecular failure in CR1	10 (100)	5 (50)
Molecular failure in CR2+	0 (0)	0 (0)
MRD status, n (%)		
MRD $<10^{-3}$	3 (30)	1 (10)
MRD $\geq 10^{-3}$	7 (70)	9 (90)
Method for MRD evaluation, n (%)***		
Ig/TCR rearrangements PCR	7 (70)	9 (90)
BCR-ABL translocations PCR	2 (20)	3 (30)
MLL-AF4 translocations PCR	1 (10)	1 (10)
Number of blinatumomab cycles, median (range)	4 (1-7)	3.5 (2-5)
Complete MRD response after 1 cycle, n (%)	7 (70)	9 (90)
Allogeneic HSCT after blinatumomab treatment, n (%)	5 (50)	4 (40)

*Includes one patient who completed the study in remission, with RFS of 59.7 months (4.97 years). **One patient was censored after 43 days (1.4 months) because of withdrawal of consent. ***Patients could have both rearrangements and translocations. HSCT: hematopoietic stem cell transplantation; CR1: first hematologic complete remission; CR2+: second or greater hematologic CR; Ig: immunoglobulin; MRD: minimal residual disease; PCR: polymerase chain reaction; RFS: relapse-free survival; TCR: T-cell receptor.

seven with and two without MRD responses. Kaplan-Meier analyses of RFS for all 20 patients, with or without censoring for allogeneic HSCT, are provided in Figure 1A. Overall, five of nine transplanted patients and five of 11 not transplanted after blinatumomab remained in continuous CR 5 years after starting blinatumomab treatment. The patients' characteristics by allogeneic HSCT use are provided in *Online Supplementary Table S1*. Serum immunoglobulin levels were available at baseline and at the end of follow-up for six patients (*Online Supplementary Table S2*).[8]

Kaplan-Meier analyses of RFS for 15 patients with Philadelphia chromosome (Ph)-negative ALL are provided in Figure 1B. Four of six patients with Ph-negative ALL who did not receive a transplant or any other subsequent therapy were in hematologic remission 5 years after starting blinatumomab treatment.

The patients' characteristics at baseline are summarized for patients with and without documented RFS ≥5 years in Table 1. Nineteen of 20 patients were treated in first hematologic CR. Five patients had Ph-positive ALL (*BCR-ABL* translocations) and two had *MLL-AF4* translocations.

All ten patients with RFS ≥5 years had molecular failure. Among ten patients with RFS <5 years, five had molecular relapses and five had molecular failure. In an interim follow-up analysis from the confirmatory phase 2 BLAST study of blinatumomab in 116 patients with MRD-positive ALL, patients in first CR had significantly higher rates of RFS at 18 months than patients in second or subsequent CR.[9] Kaplan-Meier analyses of RFS for 15 patients with molecular failure in first CR in the study reported herein are provided in Figure 1C. Only one patient was treated in second CR, so it was not possible to compare outcomes between patients in first and second CR. Data from both studies indicate that blinatumomab may have a curative potential in patients with MRD-positive ALL in first CR.

In this study, efficient T-cell activation and expansion was observed in 19 of 20 patients, irrespective of MRD status after blinatumomab treatment,[10] and T-cell expansion was similar between patients with or without RFS ≥5 years (*Online Supplementary Figure S1*). Patients with and without RFS ≥5 years showed comparable serum concentrations of cytokines during the first week of cycle 1 (*data not shown*). Although there was a visually discernible trend towards increased T-cell expansion with higher MRD level, no correlation was identified (R^2 = 0.048) in this small population of patients (*Online Supplementary Figure S2*). In another phase 2 study of patients with relapsed or refractory B-lineage ALL who received blinatumomab, patients with an overall survival ≥30 months and MRD response had a markedly increased T-cell expansion, compared with patients who had an overall survival <30 months and persistent MRD.[11] Patients with relapsed or refractory disease have a larger activation matrix (i.e., tumor load) for T cells, possibly leading to more pronounced T-cell expansion in

Table 2. Individual baseline characteristics and outcomes, by duration of RFS.

		Baseline characteristics						MRD response			RFS			
Patient N.	Sex	Age (y)	Ph status	MolF/ MolR	CR1/ CR2+	MRD	Dose Incr.	Response	Duration (mo.)	Time to HSCT (mo.)	Duration (mo.)	≥5 y	Type of event	CD19-positive relapse
1	F	42	Ph–	MolF	CR1	≥10^{-3}	No	Yes	0.5*	–	1.4**	No	–	–
2	F	62	Ph–	MolR	CR1	≥10^{-3}	No	No	–	–	3.2	No	Hematologic	No
3	F	67	Ph+	MolF	CR1	≥10^{-3}	No	Yes	3.3	–	4.2	No	Extramedullary	Yes
4	F	72	Ph+	MolR	CR1	<10^{-3}	No	Yes	2.8	–	5.1	No	Hematologic	No
5	M	62	Ph–	MolF	CR1	≥10^{-3}	No	Yes	5.6	–	6.5	No	Extramedullary	Yes
6	M	20	Ph–	MolR	CR1	≥10^{-3}	No	Yes	1.4*	2.5	12.4	No	Hematologic	Unknown
7	M	47	Ph–	MolR	CR2+	≥10^{-3}	No	Yes	1.6*	2.8	19.1	No	Death in remission	–
8	F	37	Ph–	MolF	CR1	≥10^{-3}	No	Yes	1.4*	2.7	31.0	No	Hematologic	Unknown
9	M	69	Ph+	MolR	CR1	≥10^{-3}	No	Yes	7.3	–	44.3	No	Hematologic	Yes
10	M	28	Ph–	MolF	CR1	≥10^{-3}	No	Yes	14.4	18.7	50.8	No	Hematologic	Yes
11	F	31	Ph–	MolF	CR1	<10^{-3}	No	Yes	0.5*	1.9	59.5*	Yes	–	–
12	M	40	Ph+	MolF	CR1	≥10^{-3}	No	No	–	3.1	61.9*	Yes	–	–
13	F	63	Ph–	MolF	CR1	≥10^{-3}	No	Yes	62.1*	–	62.9*	Yes	–	–
14	F	34	Ph–	MolF	CR1	<10^{-3}	Yes	No	–	5.6	63.4*	Yes	–	–
15	F	68	Ph–	MolF	CR1	≥10^{-3}	No	Yes	46.7*	–	63.8*	Yes	–	–
16	F	77	Ph–	MolF	CR1	≥10^{-3}	No	Yes	29.9*	–	64.3*	Yes	–	–
17	F	23	Ph–	MolF	CR1	≥10^{-3}	No	Yes	4.2*	5.8	64.4*	Yes	–	–
18	F	57	Ph–	MolF	CR1	≥10^{-3}	No	Yes	64.2*	–	65.0*	Yes	–	–
19	M	31	Ph–	MolF	CR1	≥10^{-3}	Yes	Yes	2.9*	4.4	65.8*	Yes	–	–
20	M	65	Ph+	MolF	CR1	<10^{-3}	Yes	No	–	–	70.1*	Yes	–	–

*Censored at the end of follow-up. **Patient was censored after 43 days (1.4 months) because of withdrawal of consent. –: not applicable; HSCT: hematopoietic stem cell transplantation; CR1: first hematologic complete remission; CR2+: second or greater hematologic CR; extramedullary: extramedullary relapse; F: female; hematologic: hematologic relapse; incr.: increased M: male; mo.: months; MolF: molecularly refractory; MolR: molecular relapse; MRD: minimal residual disease; N.: number; Ph–: Philadelphia chromosome–negative disease; Ph+: Philadelphia chromosome–positive disease; RFS: relapse-free survival; y: year.

patients with long-term overall survival. Importantly, the present study did not require a lower initial dose of blinatumomab in cycle 1, potentially overcoming limitations in efficient T-cell activation with stepwise dosing.

Detailed data for each of the 20 patients are provided in Table 2. Seven of ten patients with RFS ≥5 years achieved MRD response during blinatumomab treatment that was ongoing at the time of the final MRD assessment. The other three patients with RFS ≥5 years did not achieve MRD response during blinatumomab treatment, but all three had low MRD levels at baseline ($<1.5x10^{-3}$). One had Ph-positive ALL, did not undergo allogeneic HSCT, and had increasing MRD that was controlled by administration of the tyrosine kinase inhibitor dasatinib during and after blinatumomab treatment. The other two had low MRD that was maintained during blinatumomab treatment (one with Ph-negative ALL and one with Ph-positive ALL who also received the tyrosine kinase inhibitor imatinib), and both underwent allogeneic HSCT after blinatumomab. Overall, five patients had Ph-positive ALL, of whom two were in remission at final analysis (one with and one without allogeneic HSCT).

Among ten patients who did not have documented RFS ≥5 years, six had hematologic relapse (two $CD19^-$, two $CD19^+$, and two with unknown CD19 status), two had extramedullary relapse (both $CD19^+$), and one withdrew consent (censored in the RFS analysis) while still in remission (previously reported at 2.6 months;[7] subsequent documentation showed the patient withdrew consent at 1.4 months). One patient who underwent allogeneic HSCT died of graft-*versus*-host disease, sepsis, and multiorgan failure while in remission 19.1 months after starting blinatumomab treatment. The six hematologic relapses occurred 3.2, 5.1, 12.4, 31.0, 44.3, and 50.8 months after starting blinatumomab treatment. The patient who had a hematologic relapse at 50.8 months had an initial MRD response to blinatumomab but experienced MRD relapse during follow-up. After MRD relapse, the patient received one cycle of blinatumomab retreatment, then allogeneic HSCT. The patient did not achieve another MRD response before extramedullary relapses in the brain and testis 4.2 and 6.5 months after blinatumomab retreatment.

Adverse events during the primary study and interim follow-up analysis were described previously.[6,7] Two new grade 3 or 4 adverse events were reported in this final analysis. Investigators did not consider either event to be related to blinatumomab treatment. One patient with RFS of 12.4 months had a grade 3 pulmonary hemorrhage during follow-up which resolved the following day. One patient had metastatic recurrence of breast cancer during follow-up which the investigator reported as an adverse event.

Most patients with molecular failure or molecular

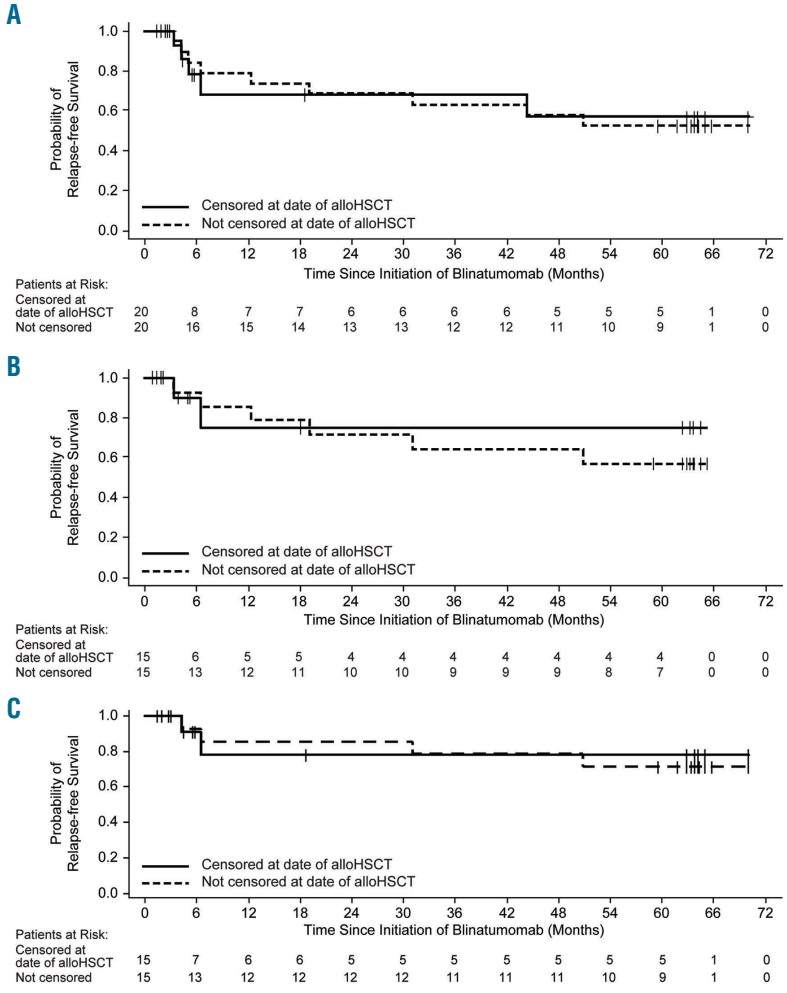

Figure 1. Kaplan-Meier analyses of hematologic relapse-free survival after blinatumomab therapy, with or without censoring at the date of allogeneic hematopoietic stem cell transplantation (alloHSCT). (A) All evaluable patients (n=20). (B) Patients with Philadelphia chromosome–negative disease (n=15). (C) Patients with molecular failure in first hematologic remission (CR1; n=15).

relapse of ALL experience hematologic relapse within 5 years after chemotherapy. In this final analysis of a phase 2 study, half of the patients with B-precursor ALL and MRD who received blinatumomab achieved long-term remission through 5 years of follow-up, further supporting the results of the study's primary analysis and interim follow-up analysis (*Online Supplementary Figure S3*).[6,7] Among nine transplanted patients, one transplant-related death due to graft-*versus*-host disease was reported. To our knowledge, this was the first study conducted with an immunotherapy for ALL that focused on patients with MRD-positive disease. Long-term RFS included patients with Ph-positive or MLL-AF4–positive disease, but the sample sizes were small. In conclusion, patients with MRD-positive ALL can achieve long-term RFS after blinatumomab treatment with or without subsequent allogeneic HSCT. These findings warrant further investigation of early administration of blinatumomab in adults with ALL, hematologic remission, and MRD to prevent relapse, instead of later administration at the time of hematologic relapse.

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