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Impact of high-sensitivity next-generation sequencing, measurable residual disease and subsequent stem cell transplant in patients receiving blinatumomab for MRD-positive B-cell acute lymphoblastic leukemia

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Running title: Impact of blinatumomab as NGS MRD-directed therapy

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Blinatumomab is approved as measurable residual disease (MRD)-directed therapy in B-cell acute lymphoblastic leukemia (ALL) for MRD at a level of 10^{-3} or higher.¹ However, newer high-throughput next-generation sequencing (NGS) of immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements can now track MRD with a sensitivity of 10^{-6} or 0.0001%.^{2,3} Several studies across different clinical contexts have demonstrated that low levels of MRD detected below the sensitivity of conventional MFC or PCR increase the risk of relapse and are associated with worse long-term survival.⁴⁻⁷ While blinatumomab is routinely used in clinical practice for levels of MRD below 10^{-3} , its efficacy for lower levels of MRD detected by more sensitive NGS-based assays and the role of consolidative allogeneic hematopoietic stem cell transplantation (HSCT) are not well-established.⁸

We conducted a retrospective analysis of patients with MRD-positive B-cell ALL in complete remission who received blinatumomab for the first time and who underwent NGS MRD assessment with ClonoSEQ (Adaptive Biotechnologies Co., Seattle, WA). Patients received up to four cycles of blinatumomab at standard doses, and patients with Philadelphia chromosome (Ph)-positive ALL received a concurrent tyrosine kinase inhibitor (TKI). For this study, only IG-calibrated samples (e.g. IgH, IgK, and/or IgL) were used for MRD assessment, as previously described.⁹ NGS MRD assessment were performed both prospectively as part of routine clinical care and retrospectively from available banked samples. Patients who had no detectable residual sequences by the clonoSEQ assay after blinatumomab were considered as “NGS MRD responders”. This study was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center and was conducted in accordance with the Declaration of Helsinki.

Between July 2015 and October 2023, 100 patients were treated with blinatumomab for MRD-positive B-cell ALL, 38 of whom had post-blinatumomab NGS assessment and are the primary subject of this analysis. Baseline characteristics are summarized in **Table 1**. Twenty-six patients (68%) had Ph-negative

B-cell ALL and 12 patients (32%) had Ph-positive B-cell ALL. Among the 26 patients with Ph-negative B-cell ALL, 14 (54%) had at least 1 high-risk baseline cytomolecular feature, defined as poor-risk cytogenetics (e.g., low hypodiploidy/near triploidy, complex, or KMT2A rearrangement), TP53 mutation, and/or Ph-like B-cell ALL.

The disposition of the 38 evaluable patients who received blinatumomab for MRD is shown in **Supplemental Figure 1**. The median follow-up for the study population is 22.2 months. Overall, 25 patients (66%) achieved NGS MRD negativity following blinatumomab (**Supplemental Table 1**). Among 27 patients who were assessed for NGS MRD after the first cycle of blinatumomab, 15 (56%) achieved NGS MRD negativity. Among the 12 patients who remained NGS MRD-positive after the first cycle of blinatumomab, 3 (25%) achieved NGS MRD negativity after additional cycles of blinatumomab. The overall NGS MRD response rate was higher in patients with Ph-positive B-cell ALL than in those with Ph-negative B-cell ALL (92% [11/12 patients] versus 54% [14/26 patients]; $P=0.03$). The NGS MRD negativity rate was 69% (22/32 patients) for patients in CR1 compared and 50% (3/6 patients) for those in CR2+. There were no significant differences in NGS MRD response rates in patients with or without prior inotuzumab ozogamicin (INO) exposure, or in Ph-negative patients with standard-risk or high-risk disease.

Achievement of NGS MRD negativity after blinatumomab was associated with excellent long-term outcomes (**Figure 1**). The median RFS of NGS MRD responders and those who remained NGS MRD-positive at any level following blinatumomab (hereafter referred to as “non-responders”) were both not reached, and the 2-year RFS rates were 73% and 52%, respectively ($P=0.15$). The median OS of NGS MRD responders and non-responders was not reached and 25 months, respectively, and the 2-year OS rates were 91% and 48%, respectively ($P=0.02$). In patients with Ph-negative B-cell ALL, achievement of NGS

MRD negativity after blinatumomab was prognostic in standard-risk but not in high-risk disease (**Supplemental Figure 2A-D**). Among the 14 high-risk patients, the median RFS of NGS MRD responders and non-responders were both not reached, and the 2-year RFS rates were 51% and 86%, respectively ($P=0.29$). The median OS of both high-risk NGS MRD responders and non-responders were not reached, and the 2-year OS rates were both 86% ($P=0.91$). Among the 12 patients with standard-risk disease, the median RFS of NGS MRD responders and non-responders were not reached and 11.9 months, respectively, and the 2-year RFS rates were 100% and 20%, respectively ($P=0.02$). The median OS of standard-risk NGS MRD responders and non-responders were not reached and 12.3 months, respectively, and the 2-year OS rates were 100% and 20%, respectively ($P=0.04$).

Relapse rates were higher in NGS MRD responders with prior INO exposure compared to NGS MRD responders who did not receive INO previously (**Supplemental Figure 2E-F**). The median RFS of NGS MRD responders with and without prior INO exposure was 17.2 months and not reached, respectively, and the 2-year RFS rates were 43% and 100%, respectively ($P=0.006$), and the 2-year OS rates were 43% and 79%, respectively ($P=0.10$). Outcomes in Ph-negative B-ALL and Ph-positive B-ALL subgroups are shown in **Supplemental Figure 2G-J**. The median RFS of NGS MRD responders and non-responders in Ph-negative B-ALL were both not reached, and the 2-year RFS rates were 67% and 57%, respectively ($P=0.39$). The median OS of NGS MRD responders and non-responders in Ph-negative B-ALL were both not reached, and the 2-year OS rates were 91% and 56%, respectively ($P=0.11$). There was only 1 NGS MRD non-responder in the Ph-positive B-ALL subgroup.

Two NGS MRD responders were bridged to transplant. Among 7 patients who did not achieve NGS MRD negativity with blinatumomab and who subsequently underwent HSCT in MRD-positive CR, the median time from start of blinatumomab therapy to HSCT was 2.8 months (range, 1.8-4.1 months). We

performed a landmark analysis to analyze the impact of HSCT on patients who did not achieve NGS MRD negativity with blinatumomab. The median RFS of HSCT and no HSCT in non-responding patients was not reached and 13.5 months, respectively, and the 2-year RFS rates were 71% and 27%, respectively (P=0.20; **Figure 2A**). The median OS of HSCT and no HSCT in non-responding patients was not reached and 25 months, respectively, and the 2-year OS rates were 71% and 27%, respectively (P=0.24; **Figure 2B**). The median OS of NGS MRD responders who did not undergo HSCT and non-responders who underwent HSCT were both not reached, and the 2-year OS rates were 95% and 71%, respectively (P=0.14).

In this study, we showed that adults with B-cell ALL who received blinatumomab for any level of MRD and achieved NGS MRD negativity had excellent outcomes, despite most of these patients not undergoing allogeneic HSCT following blinatumomab, with a 2-year RFS rate of 73% and a 2-year OS rate of 91%. In contrast, patients who did not achieve MRD negativity after blinatumomab had poor outcomes, with a 2-year RFS rate of 52% and a 2-year OS rate of 48%. Post-blinatumomab HSCT in MRD-positive remission appeared to improve outcomes for these patients with suboptimal response to blinatumomab. Overall, these data demonstrate that high-sensitivity MRD as assessed by NGS improve prognostication in B-cell ALL and can identify patients who are predicted to have robust, long-term responses to blinatumomab. Lack of NGS MRD response to blinatumomab may also identify patients who may benefit from additional therapies, including consideration of HSCT.

The optimal consolidative approach for patients who achieve MRD negativity after blinatumomab is controversial. In the BLAST trial, the benefit of HSCT consolidation after achieving MRD negativity was unclear.¹⁰ While patients who underwent HSCT consolidation had lower rates of relapse, the potential survival benefit of HSCT was diminished by the introduction of transplant-related mortality; furthermore,

30% of patients who did not undergo HSCT remained in remission at 5-year follow-up, suggesting that durable remissions without consolidative HSCT are possible in some patients.¹¹ Emerging data suggests that early achievement of NGS MRD negativity after frontline therapy is associated with excellent outcomes, even in high-risk patients. Here, we show that NGS MRD negativity following blinatumomab was associated with superior survival outcomes across different B-cell ALL subgroups. The most durable responses were observed in responding patients with standard-risk Ph-negative B-cell ALL (2-year RFS: 100%), suggesting that blinatumomab as MRD-directed therapy could be potentially curative for many patients, if deep MRD negativity is achieved. In contrast, we observed that patients with high-risk Ph-negative B-cell ALL who achieved NGS MRD negativity after blinatumomab had a 2-year RFS of only 51%, suggesting that blinatumomab may not be curative for many of these patients, even after a deep NGS MRD-negative response. Thus, our findings suggest that baseline cytomolecular risk should be combined with NGS MRD response after blinatumomab to aid in risk stratification and to inform selection of consolidative therapy.

Patients who did not achieve NGS MRD negativity after blinatumomab had poor outcomes, although allogeneic HSCT seemed to improve survival in these patients. Our data suggest that patients who do not achieve NGS MRD negativity with blinatumomab should undergo alternative, MRD-directed therapies. While we observed improved outcomes with HSCT, it is possible that outcomes would be further improved with other MRD-directed therapies, either as definitive therapy or followed by subsequent HSCT.^{12,13} For patients planned for allogeneic HSCT, efforts should be made to first eradicate MRD (when feasible), as detectable MRD prior to HSCT has been associated with poor outcomes in several studies, even if only low levels of MRD (e.g. 10⁻⁴ to 10⁻⁶) are present.⁴

There are several limitations to our study. One limitation is the heterogeneity of our study population with regards to prior therapy exposure (e.g. 21 [55%] patients received INO prior to blinatumomab, 6 [16%] patients were treated with blinatumomab at CR2+, and 7 of 32 [21%] patients in CR1 received a low-intensity induction therapy) and disease subtype (12 [32%] patients with Ph-positive B-ALL concurrently treated with TKI). These differences in disease biology temper our ability draw definitive conclusions on the impact of blinatumomab alone on MRD. Furthermore, due to the retrospective nature of our study, there was no standardization in deciding which patients should undergo HSCT consolidation. Prospective studies are needed to determine how NGS MRD response should inform decisions about HSCT in remission.

In conclusion, our study shows that patients who receive blinatumomab for MRD-positive B-cell ALL and achieve deep levels of MRD-negative remission as assessed by NGS have superior survival outcomes and can have excellent, long-term remissions without allogeneic HSCT. In contrast, patients who do not achieve NGS MRD negativity with blinatumomab have poor outcomes and may benefit from allogeneic HSCT or alternative MRD-directed approaches. These findings highlight the importance of high-sensitivity NGS MRD assessment in evaluating the effectiveness of B-cell ALL therapy and in informing consolidative strategies in these patients.

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Table 1. Baseline characteristics at time of blinatumomab initiation

Patient characteristic (N=38)	N (%) or median [range]
Age, years	34 [18-72]
Age ≥60 years	4 (11)
ECOG performance status	
0	12 (32)
1	24 (63)
2	2 (5)
WBC at diagnosis x 10⁹/L	5.8 [1.9-53.8]
History of CNS/EM disease	2 (5)
Disease subtype	
Ph-negative	26 (68)
Ph-positive	12 (32)
Karyotype	
Ph-positive	12 (32)
Diploid	5 (13)
Complex	1 (2)
Low hypodiploidy / near triploidy	1 (2)
<i>KMT2Ar</i>	4 (11)
High hyperdiploidy	1 (2)
Other	12 (32)
Insufficient metaphases	2 (5)
Ph-like ALL	6/26 (23)
<i>TP53</i> mutation	3 (8)
High-risk Ph-negative B-ALL*	14/26 (54)
Frontline therapy (CR1 only)	
Hyper-CVAD-based	22/32 (69)
Mini-CVD-based	7/32 (21)
Pediatric-inspired high intensity	2/32 (9)
Prior inotuzumab ozogamicin exposure	21 (55)

Disease status	
CR1	32 (84)
CR2	4 (11)
CR3	1 (2)
CR4	1 (2)
TKI (Ph-positive B-cell ALL only)	
Ponatinib	12/12 (100)
MRD status at blinatumomab start[†]	
NGS+/MFC-	15 (39)
<0.0001%	8 (21)
≥0.0001% to <0.001%	4 (11)
≥0.001%	3 (8)
MFC+	23 (61)
<0.01%	6 (16)
≥0.01% to <0.1%	6 (16)
≥0.1% to <1%	7 (18)
≥1%	4 (11)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; WBC, white blood cell; CNS, central nervous system; EM, extramedullary; Ph, Philadelphia chromosome; CR, complete remission; TKI, tyrosine-kinase inhibitor; NGS, next generation sequencing; MRD, measurable residual disease; MFC, multiparameter flow cytometry

*High-risk defined as poor-risk cytogenetics (e.g., low hypodiploidy/near triploidy, complex, or *KMT2A* rearrangement), *TP53* mutation, and/or Ph-like B-cell ALL

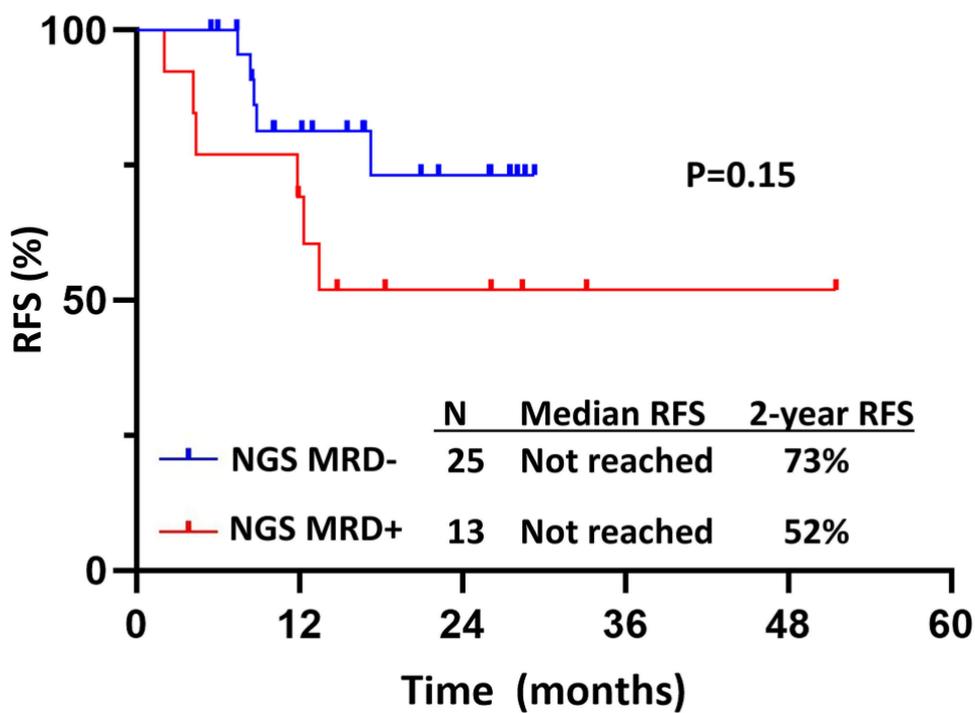
[†]NGS MRD assessment performed using ClonoSEQ MRD Assay (Adaptive Biotechnologies Co., Seattle, WA). MFC assessment MFC MRD assessment performed using 6-color multiparameter flow cytometry

Figure 1. Impact of NGS MRD negativity after blinatumomab. **A)** Relapse-free survival and **B)** Overall survival

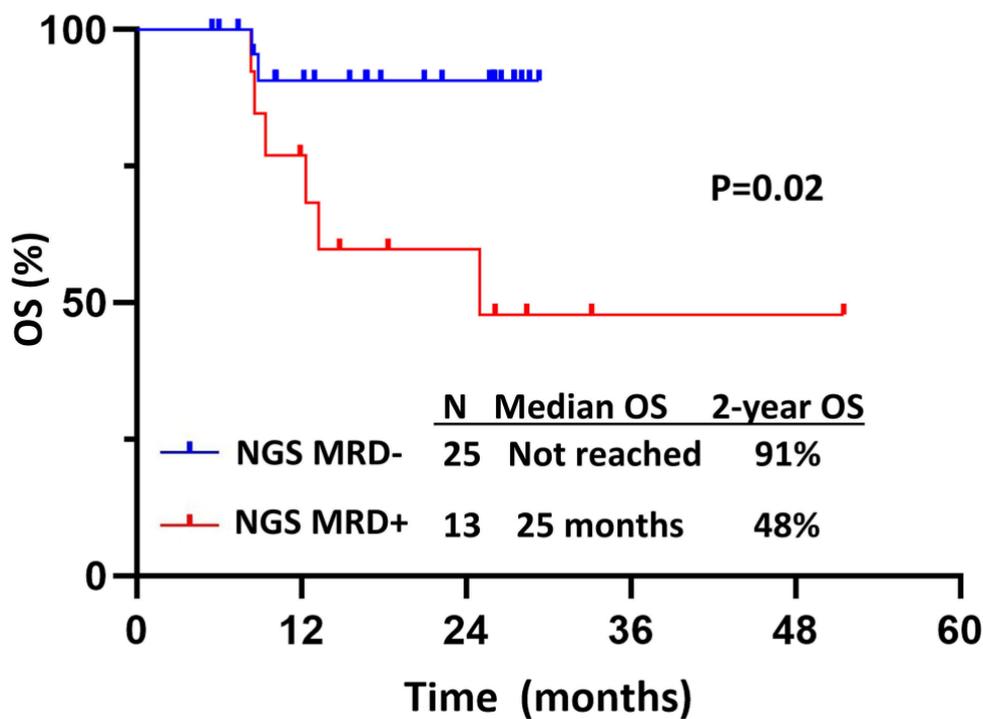
Figure 2. Impact of NGS MRD response in patients receiving blinatumomab, stratified by consolidative allogeneic HSCT. **A)** Relapse-free survival and **B)** Overall survival

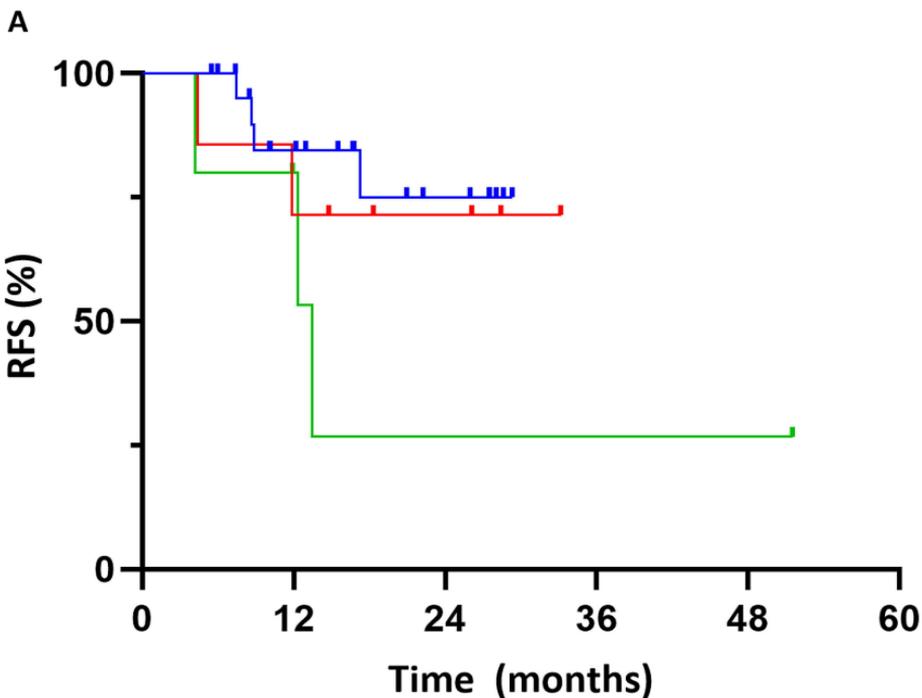
*Two NGS MRD responders were bridged to transplant

A

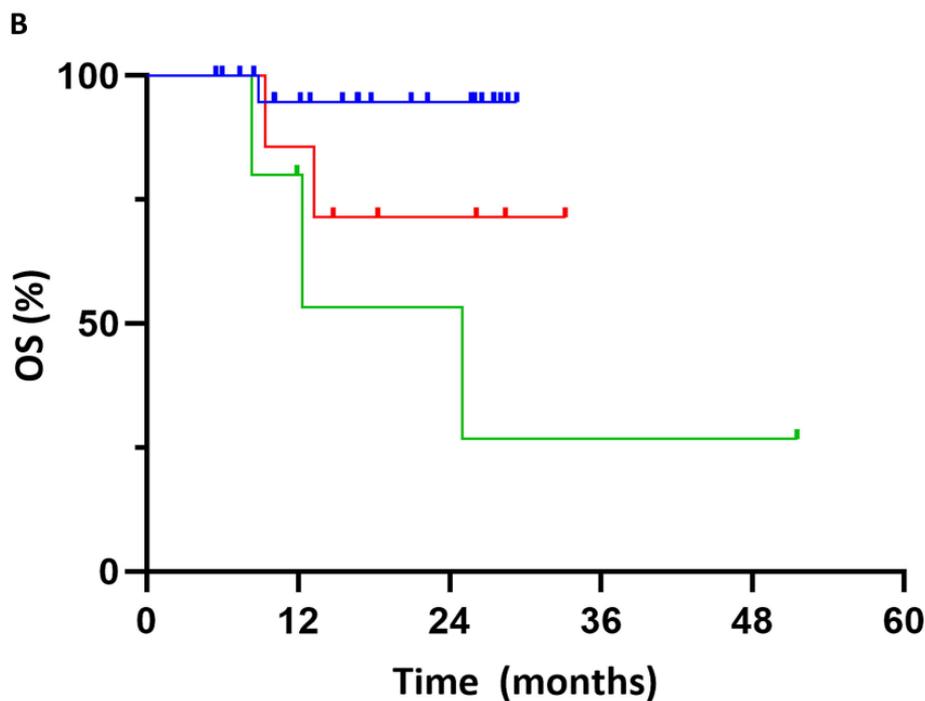


B





	<u>N</u>	<u>Median RFS</u>	<u>2-year RFS</u>
—+ NGS MRD-/No HSCT*	23	Not reached	75%
—+ NGS MRD+/HSCT	7	Not reached	71%
—+ NGS MRD+/No HSCT	5	13.5 months	27%



	<u>N</u>	<u>Median OS</u>	<u>2-year OS</u>
—+ NGS MRD-/No HSCT*	23	Not reached	95%
—+ NGS MRD+/HSCT	7	Not reached	71%
—+ NGS MRD+/No HSCT	5	25 months	27%

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Supplementary Information

Supplemental Table 1. NGS MRD response rates NGS MRD response rates

Supplemental Figure 1. Disposition of patients who received blinatumomab for MRD

Supplemental Figure 2. Impact of NGS MRD negativity after blinatumomab in different subgroups

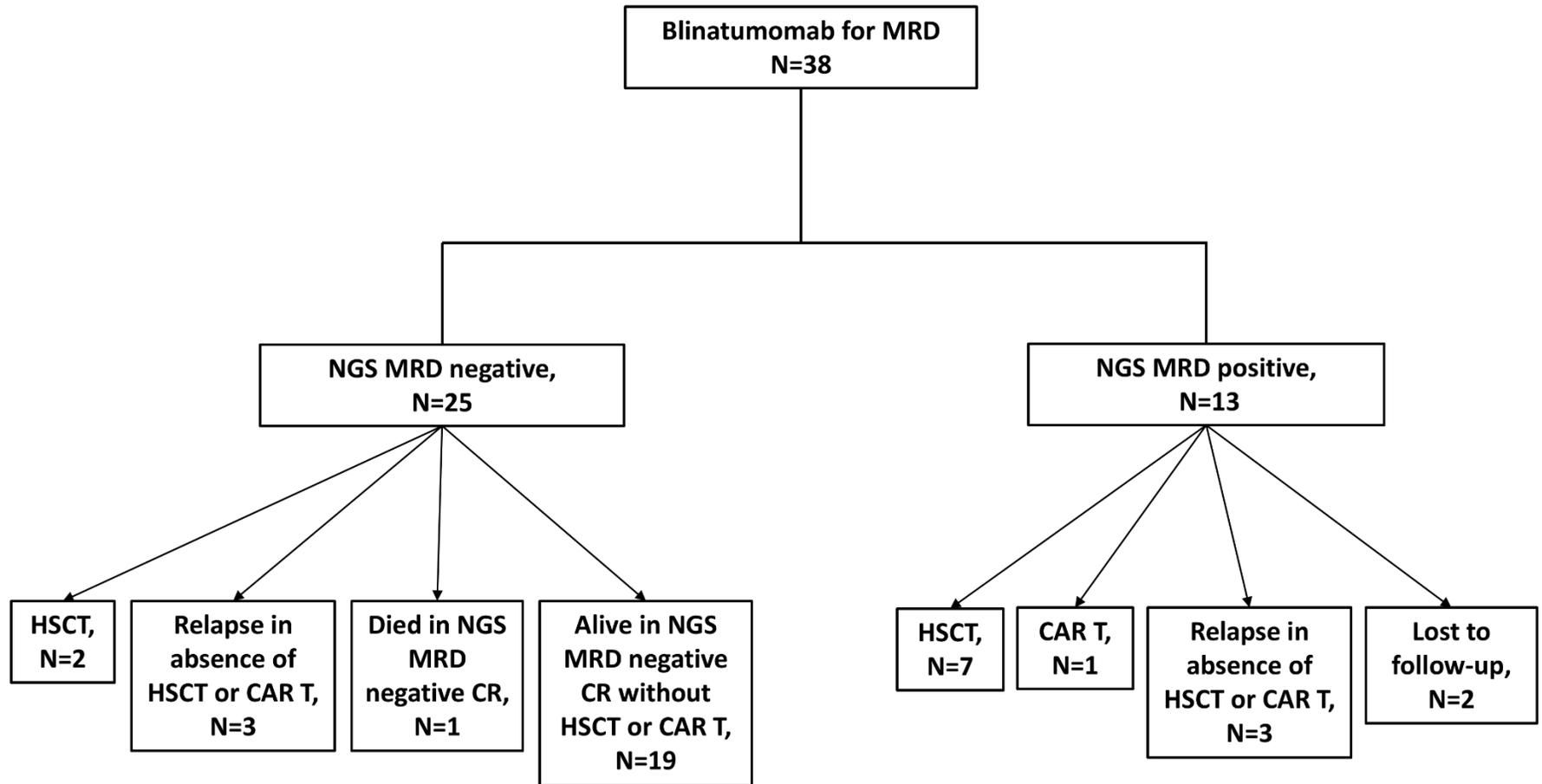
Supplemental Table 1. NGS MRD response rates

Response N (%)	Ph-negative (N=26)	Ph-positive (N=12)	Total cohort (N=38)	P*
NGS MRD negativity (overall)	14/26 (54)	11/12 (92)	25/38 (66)	
NGS MRD negativity (after 1 cycle)	8/18 (44)	7/9 (78)	15/27 (56)	
MFC+	8/15 (53)	7/8 (88)	15/23 (65)	>0.99
MFC-/NGS+	6/11 (55)	4/4 (100)	10/15 (67)	
MRD $\geq 10^{-3}$	3/8 (38)	3/3 (100)	6/11 (55)	0.46
MRD $< 10^{-3}$	11/18 (61)	8/9 (89)	19/27 (70)	
CR1	12/21 (57)	10/11 (91)	22/32 (69)	0.39
CR2 or later	2/5 (40)	1/1 (100)	3/6 (50)	
No prior inotuzumab exposure	4/7 (57)	9/10 (90)	13/17 (77)	0.31
Prior inotuzumab exposure	10/19 (53)	2/2 (100)	12/21 (57)	
Standard-risk disease	7/12 (58)	-	7/12 (58)	0.71
High-risk disease	7/14 (50)	-	7/14 (50)	

*P-value refers to comparisons in total cohort

Abbreviations: NGS, next generation sequencing; MRD, measurable residual disease; Ph-, Philadelphia chromosome negative; Ph+, Philadelphia chromosome positive; MFC, multiparameter flow cytometry; CR1, first remission

Supplemental Figure 1. Disposition of patients who received blinatumomab for MRD



Supplemental Figure 2. Impact of NGS MRD negativity after blinatumomab in different subgroups. **(A, B)** RFS and OS of NGS MRD responders vs non-responders in high-risk Ph-negative B-cell ALL. **(C, D)** RFS and OS of NGS MRD responders vs non-responders in standard-risk, Ph-negative B-cell ALL. **(E, F)** RFS and OS of NGS MRD responders with and without prior inotuzumab exposure. **(G, H)** RFS and OS of NGS MRD responders vs non-responders in Ph-negative B-ALL. **(I, J)** RFS and OS of NGS MRD responders vs non-responders in Ph-positive B-ALL

