Supplementary materials for

Efficacy and safety of short-course blinatumomab in patients with relapsed/refractory or MRD-positive B-cell acute lymphoblastic leukemia

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Supplementary Table 1. Comparison of effectiveness at days 12–14 between patients with ≤ 14-day and > 14-day blinatumomab duration cohorts.

Group	≤ 14-day cohort n = 55	> 14-day cohort n = 70	*p* value
Assessment at days 12–14, n	55	43	
MRD-positive patients, n	23	14	
FCM MRD-negativity, n (%)	20/23 (87.0%)	11/14 (78.6%)	0.653
R/R patients, n	32	29	
CRc, n (%)	25/32 (78.1%)	20/29 (69%)	0.603
FCM MRD-negativity, n (%)	22 /32(68.8%)	17/29 (58.6%)	0.578

CRc composite complete remission; *FCM* flow cytometry; *MRD* measurable residual disease; *R/R* relapsed or refractory.

Supplementary Table 2. Comparison of response and survival between the ≤ 14-day and > 14-day blinatumomab duration cohorts.

Group	≤ 14-day cohort	> 14-day cohort	*p* value
Total patient, n	**55**	**70**	
BM aspirate testing			
At days 12–14, n (%)	55 (100%) *	43/70 (43.9%) #	
At days 15–21, n (%)	0	38/70 (54.3%) *	
At days 22–28, n (%)	0	32/70 (45.7%) *	
R/R patients, n	**32**	**42**	
Response			
CRc	25/32 (78.1%)	34/42 (81.0%)	0.994
CR	20/32 (62.5%)	25/42 (59.5%)	> 0.99
CRh	5/32 (15.6%)	9/42 (21.4%)	> 0.99
Non-responder	7/32 (21.9%)	8/42 (19.0%)	0.994
MRD negative	22/32 (68.8%)	31/42 (73.8%)	0.827
Median DOR (m)	13.7 (2.4-25.0)	13.8 (6.7-21.0)	0.763
Survival			
Median EFS (m)	6.4 (3.8–9.9)	4.4 (3.7–11.7)	0.596
1-year EFS (%)	24.1%	28.9%	0.937
2-year EFS (%)	19.3%	28.9%	0.483
Median OS (m)	12.8 (4.8–20.7)	9.2 (4.3–13.1)	0.547
1-year OS (%)	55.0%	40.4%	0.397
2-year OS (%)	43.3%	30.9%	0.374
MRD-negative patients, n	**23**	**28**	
Response			
MRD negative	20/23 (87.0%)	24/28 (85.7%)	> 0.99
Survival			
Median EFS (m)	NR	NR	0.753
1-year EFS (%)	75.5%	70.9%	0.870
2-year EFS (%)	66.0%	53.9%	0.579
Median OS (m)	NR	26.3 (16.2–NR)	0.908
1-year OS (%)	71.2%	74.1%	0.907
2-year OS (%)	62.3%	65.6%	> 0.99

BM bone marrow; *CR* complete remission; *CRc* composite complete remission; *CRh* CR with partial hematologic recovery; *DOR* duration of response; *EFS* event-free survival; *m* month; *MRD* measurable residual disease; *NR* not reached; *OS* overall survival. Note: * the endpoint assessment; # the intermediate assessment.

Supplementary Table 3. Therapies after blinatumomab reinduction

Group	≤ 14-day cohort n = 55	> 14-day cohort n = 70
Inotuzumab ozogamicin		
Patient, n (%)	5 (9.1%)	5 (7.1%)
Cycles (n)		
1	3	3
2	2	2
Dosage regimen, n		
1 mg day1, 8, 15	7	7
Blinatumomab		
Patient, n	19 (34.5%)	18 (25.7%)
Cycle (n)		
1	11	8
2	5	6
3	0	1
4	2	1
5	1	2
Dosage regimen, n		
28 mg d1–14	34	29
28 mg d1–21	0	6
28 mg d1–28	0	2
CAR-T therapy		
Patient, n	3 (5.5%)	6 (8.6%)
Targeted CD19	2	5
Targeted CD19/CD22, n (%)	1	1
Allo-HSCT		
Patient, n	20 (36.4%)	26 (37.1%)
Intensity of conditioning		
Myeloablative, n (%)	17 (85.0%)	21 (80.8%)
Reduced intensity, n (%)	3 (15.0%)	5 (19.2%)
Conditioning regimen		
TBI-based regimen, n (%)	3 (15.0%)	6 (23.1%)
Chemotherapy regimen, n (%)	17 (85.0%)	20 (76.9%)
Donor type		
Matched sibling, n (%)	5 (25%)	5 (19.2%)
Matched unrelated donor, n (%)	1 (5%)	9 (34.6%)
Haploidentical donor, n (%)	14 (70%)	12 (46.2%)

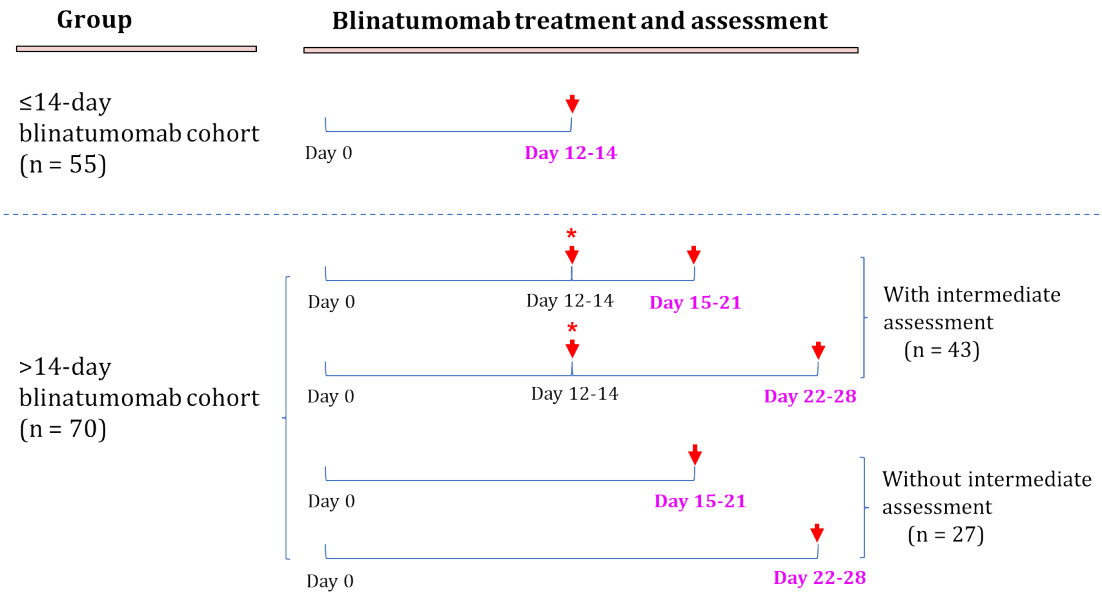
CAR-T chimeric antigen receptor T-cell; *allo-HSCT* allogeneic hematopoietic stem cell transplantation. *Inotuzumab ozogamicin was given at 1 mg on days 1, 8, 15 of each 28-day cycle. # Blinatumomab maintenance therapy was administered intravenously at 28 μg daily, with durations ranging from 14 to 28 days: in the ≤ 14-day cohort, all 19 patients received 14 days of blinatumomab maintenance, totaling 34 cumulative cycles; in the > 14-day cohort, most patients received 14-day blinatumomab maintenance (29 cumulative cycles), followed by 21 days (6 cumulative cycles), and by 28 days twice.

Supplementary Table 4. Comparison of therapy-related toxicity between the ≤ 14-day and > 14-day blinatumomab duration cohorts.

Group	≤ 14-day cohort	> 14-day cohort	*p* value
Hematologic toxicity, n (%)			
Any grade neutropenia	45/55 (81.8%)	47/70 (67.1%)	0.100
Neutropenia ≥Grade 3	34/55 (61.8%)	40/70 (58.0%)	0.730
Any grade thrombocytopenia	39/55 (70.9%)	44/70 (62.9%)	0.450
Thrombocytopenia ≥Grade 3	26/55 (47.3%)	35/70 (50.7%)	0.841
Lymphopenia			
≥Grade 3	7/55 (12.7%)	13/70 (18.6%)	0.523
Grade 4	0/55 (0.0%)	7/70 (10.0%)	0.018
Non-hematological toxicity, n (%)			
Any grade infection	16/55 (29.1%)	34/70 (48.6%)	0.043
Infection ≥Grade 3	12/55 (21.8%)	21/70 (30.0%)	0.409
Virus infection	8/55 (14.6%)	14/70 (20.0%)	0.577
Liver enzymes increase	20/55 (36.4%)	16/70 (22.9%)	0.145
Gastrointestinal reactions	10/55 (18.2%)	14/70 (20.0%)	0.978
CRS			
Any grade	43/55 (78.2%)	50/70 (71.4%)	0.514
CRS ≥Grade 1–2	43/55 (78.2%)	50/70 (71.4%)	0.514
CRS ≥Grade 3	0/55 (0.0%)	0/70 (0.0%)	1.000
ICANs	3/55 (5.5%)	4/70 (5.7%)	> 0.99

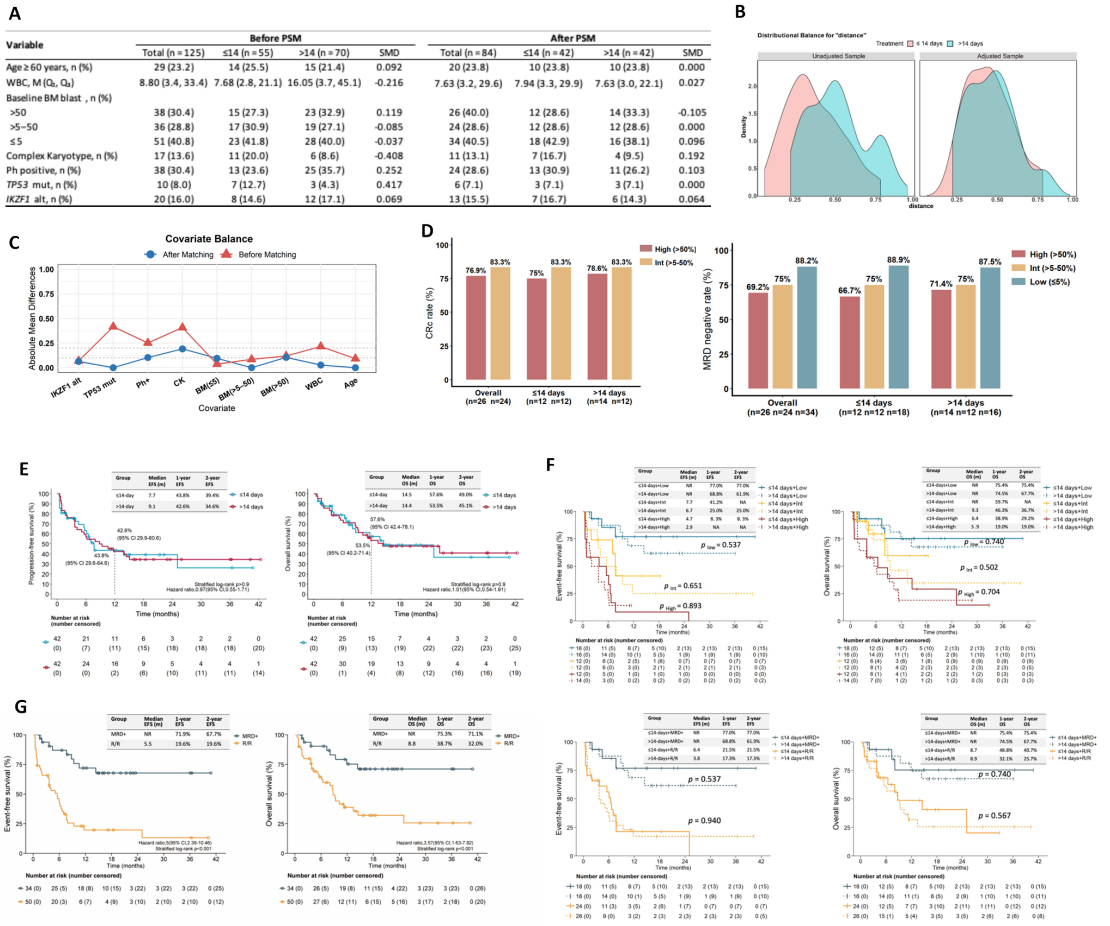
CRS cytokine release syndrome; *ICANs* immune effector cell-associated neurotoxicity syndrome.

Supplementary Fig. 1. Schematic diagram of patient grouping and assessment timing.



This flowchart shows the study design and categorization, as follows: In the ≤ 14-day cohort (n = 55), patients received blinatumomab for 12–14 days, and bone marrow (BM) aspiration was performed on the day of discontinuation (days 12–14) for the endpoint assessment. In the > 14-day cohort (n = 70), patients received blinatumomab for > 14 days (15–28 days) and underwent an endpoint assessment on the last day of treatment (at days 15–28). Among these, 43 patients underwent an intermediate evaluation at day 14 (red arrow and red star) to monitor early response. Note: The red arrow indicates the time point for BM aspiration; purple text indicates the duration of blinatumomab treatment.

Supplementary Fig. 2. Propensity score matching analysis on the entire cohort and verification.



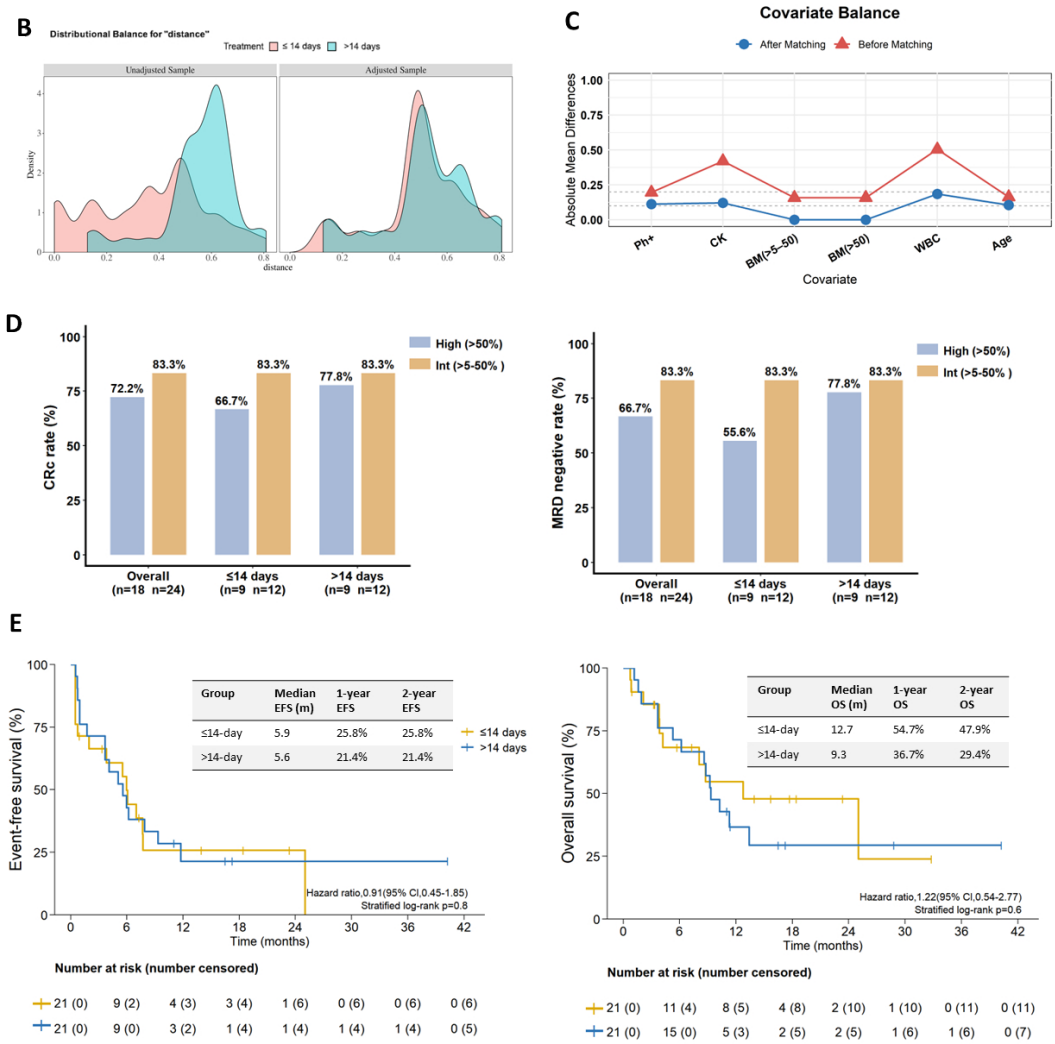
A Comparison of key clinical variables between the ≤ 14-day and > 14-day groups before and after propensity score matching (PSM) (n = 42 per group). **B** The propensity score density plots illustrate the distributional balance of the estimated propensity scores before (Unadjusted) and after (Adjusted) matching. The overlapping areas indicate improved common support between the two groups. **C** Love plot displays the absolute standardized mean differences (SMD) for covariates. All matched covariates had SMDs < 0.2, indicating a good balance. **D** Comparison of CRc and MRD-negative rates between the ≤ 14-day and > 14-day cohorts in the matched cohort. Comparison of EFS and OS in the entire cohort (**E**) or in subgroups stratified by leukemia burden (**F**) or disease status (**G**) in the matched cohort. *BM* bone marrow; *CRc* composite complete remission; *EFS* event-free survival; *MRD* measurable residual disease; *NR* not reached; *OS* overall

survival; *R/R* relapsed or refractory; *Ph* Philadelphia chromosome; *WBC* white blood cell.

Supplementary Fig. 3. Propensity score matching analysis on the R/R ALL cohort and verification.

A

Variable	Before PSM				After PSM			
	Total (n = 74)	≤14 (n = 32)	>14 (n = 42)	SMD	Total (n = 42)	≤14 (n = 21)	>14 (n = 21)	SMD
Age ≥ 60 years, n (%)	18 (24.32)	9 (28.12)	9 (21.43)	-0.163	11 (26.19)	5 (23.81)	6 (28.57)	0.105
WBC, M (Q_1, Q_3)	8.80 (2.6, 31.4)	4.4 (1.7, 13.0)	19.4 (4.1, 54.3)	0.505	8.0 (1.8, 18.8)	4.8 (1.4, 17.5)	9.2 (3.0, 29.4)	0.185
Baseline BM blast , n (%)								
>50	38 (51.35)	15 (46.88)	23 (54.76)	0.158	18 (42.86)	9 (42.86)	9 (42.86)	0.000
>5–50	36 (48.65)	17 (53.12)	19 (45.24)	-0.158	24 (57.14)	12 (57.14)	12 (57.14)	0.000
Complex Karyotype, n (%)	11 (14.86)	7 (21.88)	4 (9.52)	0.421	7 (16.67)	4 (19.05)	3 (14.29)	0.121
Ph positive, n (%)	20 (27.03)	7 (21.88)	13 (30.95)	-0.196	11 (26.19)	6 (28.57)	5 (23.81)	0.112



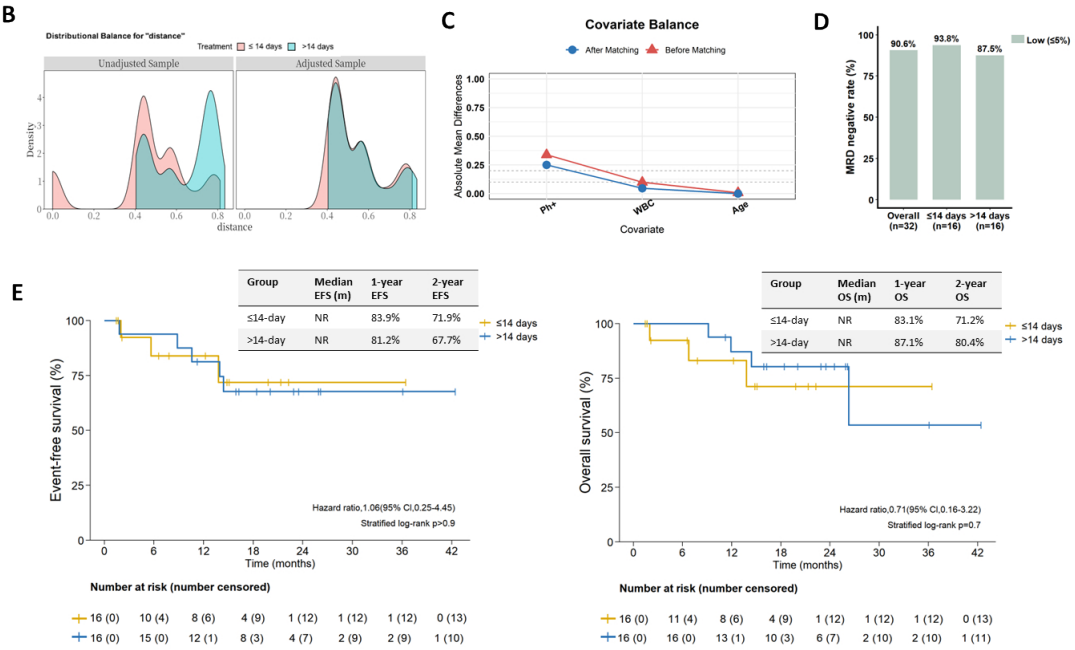
A Comparison of key clinical variables between the ≤ 14-day and > 14-day groups before and after propensity score matching (PSM) (n = 21 per group). **B** The propensity score density plots illustrate the distributional balance of the estimated propensity scores before (Unadjusted) and after (Adjusted) matching. The overlapping areas indicate improved common support between the two groups. **C** Love plot displays the absolute standardized mean differences (SMD)

for covariates. **D** Comparison of CRc and MRD negativity between the ≤ 14-day and > 14-day cohorts in the matched R/R B-ALL cohort. **E** Comparison of EFS and OS between the two cohorts in the matched R/R B-ALL cohort. *BM* bone marrow; *CRc* composite complete remission; *EFS* event-free survival; *MRD* measurable residual disease; *OS* overall survival; *Ph* Philadelphia chromosome; *WBC* white blood cell.

Supplementary Fig. 4. Propensity score matching analysis on the MRD-positive ALL cohort and verification.

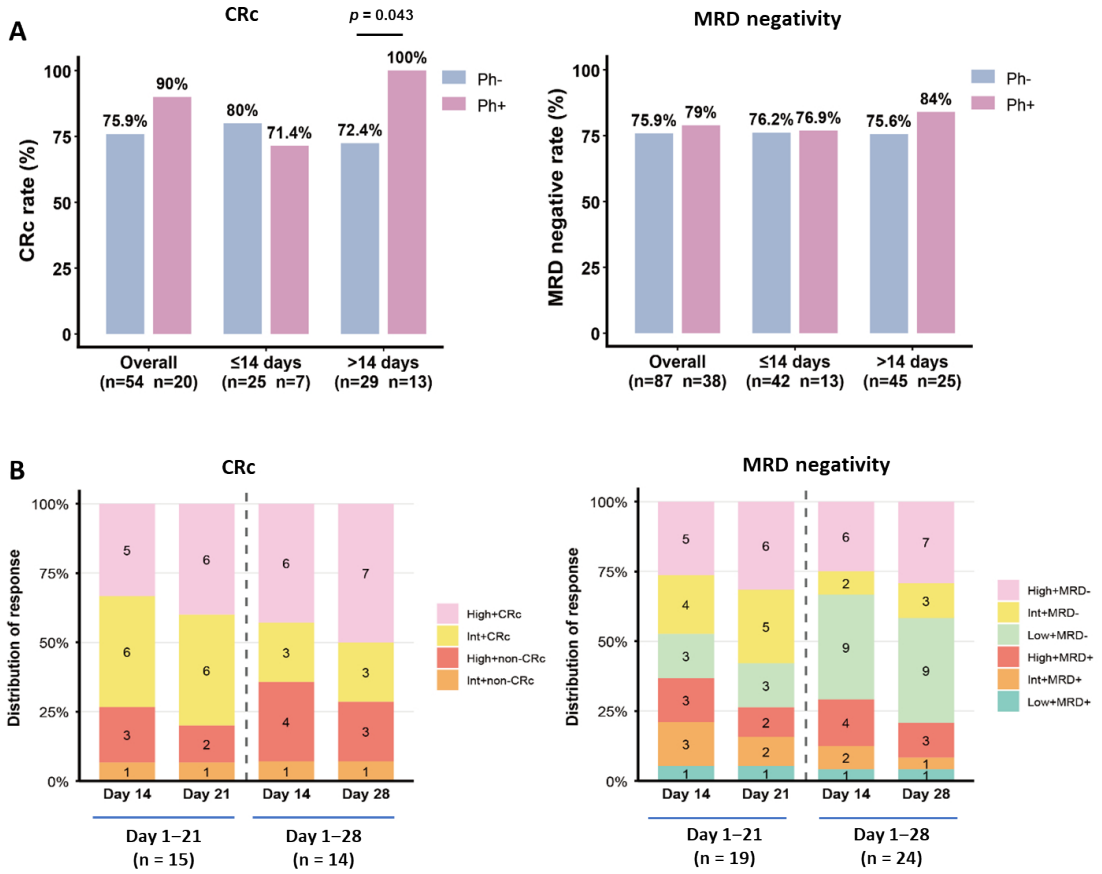
A

Variable	Before PSM				After PSM			
	Total (n = 51)	≤14 (n = 23)	>14 (n = 28)	SMD	Total (n = 32)	≤14 (n = 16)	>14 (n = 16)	SMD
Age ≥ 60 years, n (%)	11 (21.6)	5 (21.7)	6 (21.4)	-0.008	6(18.8)	3(18.8)	3(18.8)	0.000
WBC, M (Q_1, Q_3)	9.3 (4.4, 34.5)	13.2 (6.8, 40.4)	7.0 (3.7, 32.8)	-0.099	7.1 (4.2, 25.5)	13.1 (6.8, 31.5)	5.5 (3.4, 7.2)	0.047
Ph positive, n (%)	18 (35.3)	6 (26.1)	12 (42.8)	0.339	14 (43.7)	6 (37.5)	8 (50.0)	0.250



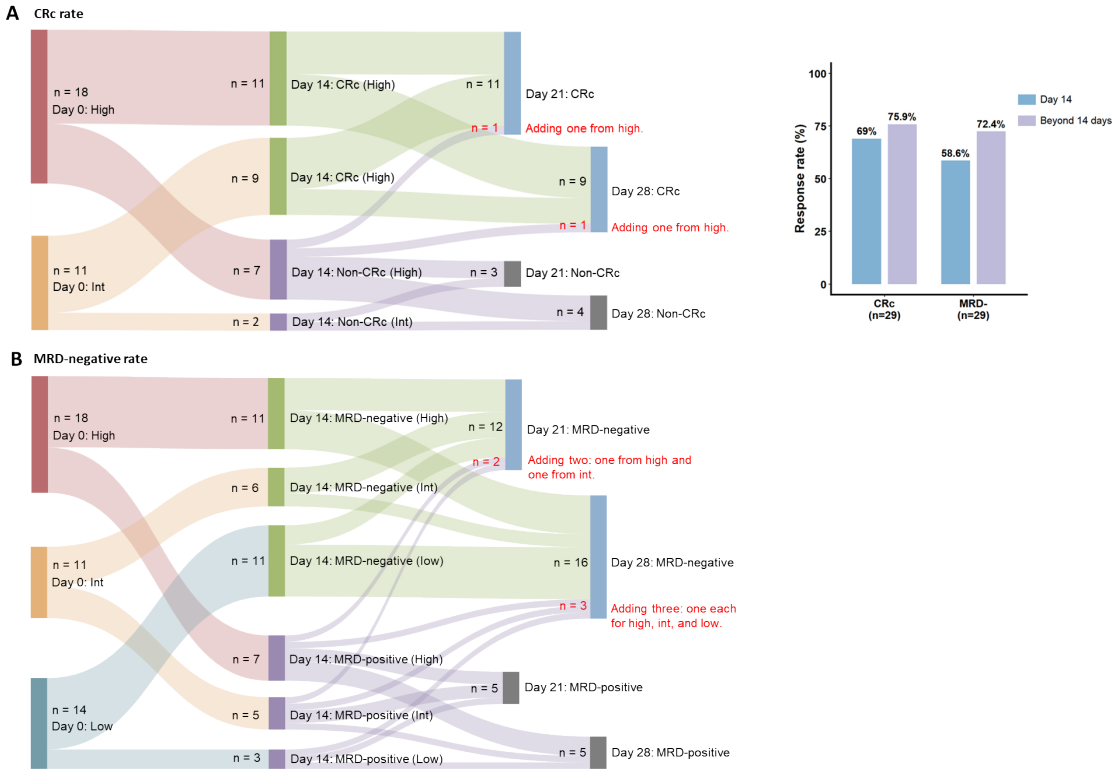
A Comparison of key clinical variables between the ≤ 14-day and > 14-day groups before and after propensity score matching (PSM) (n = 16 per group). **B** The propensity score density plots illustrate the distributional balance of the estimated propensity scores before (Unadjusted) and after (Adjusted) matching. The overlapping areas indicate improved common support between the two groups. **C** Love plot displays the absolute standardized mean differences (SMD) for covariates. **D** Comparison of MRD negativity between the ≤ 14-day and > 14-day cohorts in the matched MRD-positive ALL cohort. **E** Comparison of EFS and OS between the two cohorts in the matched MRD-positive ALL cohort. *EFS* event-free survival; *MRD* measurable residual disease; *NR* not reached; *OS* overall survival; *Ph* Philadelphia chromosome; *WBC* white blood cell.

Supplementary Fig. 5. Distribution of treatment responses stratified by blinatumomab duration and Philadelphia chromosome in the entire cohort.



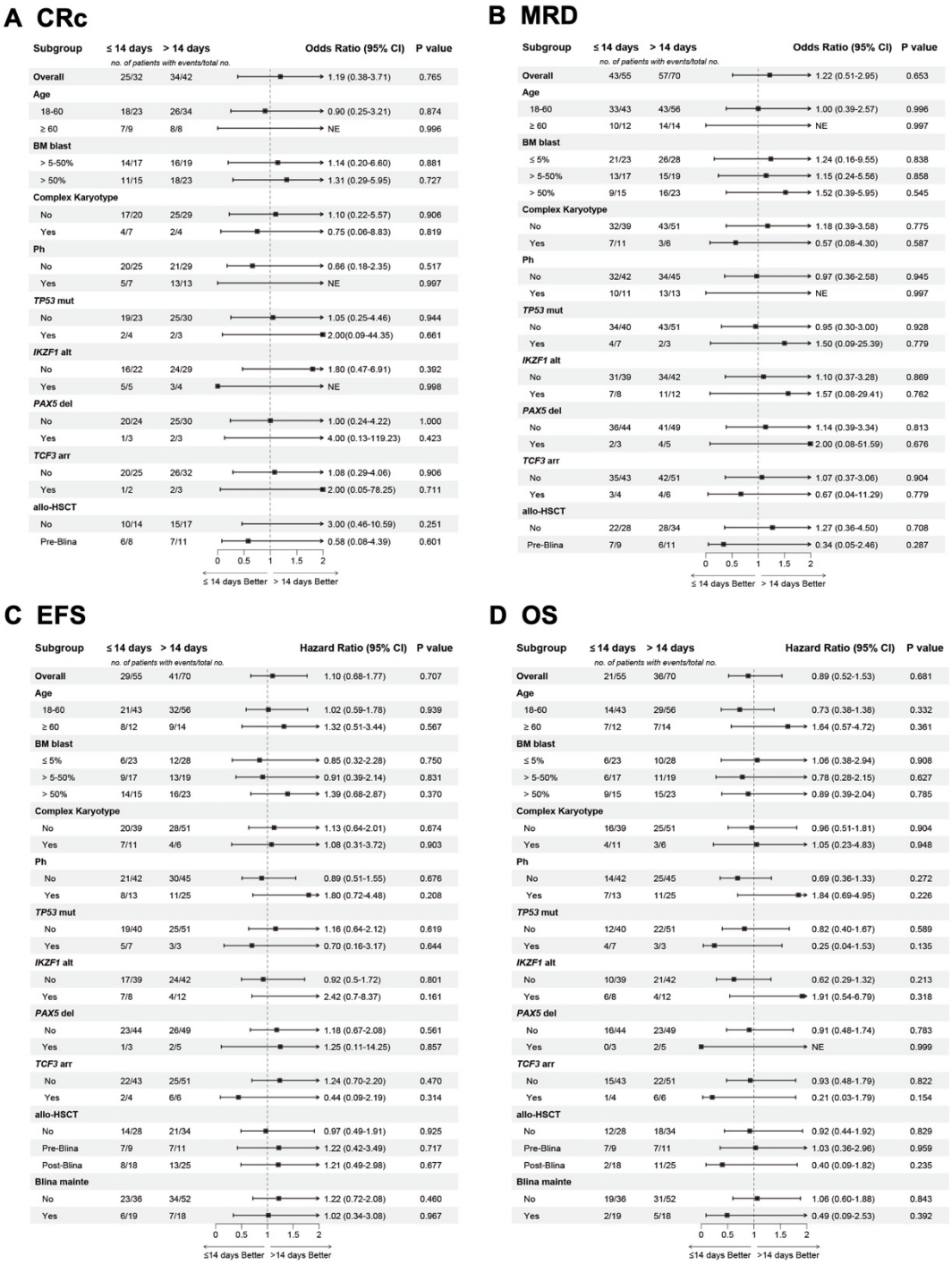
A CRc and MRD-negativity rates stratified by blinatumomab duration and Philadelphia chromosome. **B** The stacked bar charts illustrate the distribution of CRc and MRD status categorized by pretreatment leukemia burden on days 14/21 or days 14/28 of treatment. *CRc* composite complete remission; *High* high leukemia burden*; Int* intermediate leukemia burden*; Low* low leukemia burden; *MRD* measurable residual disease; *Ph* Philadelphia chromosome.

Supplementary Fig. 6. Dynamics of CRc and MRD status during blinatumomab treatment from day 14 to beyond 14 days.



A Sankey diagram illustrated the evolution of CRc from baseline (Day 0) to intermediate assessment (Day 14) and endpoint assessment (Day 21 or 28), stratified by pretreatment leukemia burden. **B** Sankey diagram illustrated the evolution of MRD status from Day 0 to Day 14 and Day 21 or 28. The inset bar charts compare the responses between the intermediate (Day 14) and endpoint assessments (Beyond 14 days). *CRc* composite complete remission; *High* high leukemia burden*; Int* intermediate leukemia burden*; Low* low leukemia burden; *MRD* measurable residual disease; *Non-CRc* non-composite complete remission.

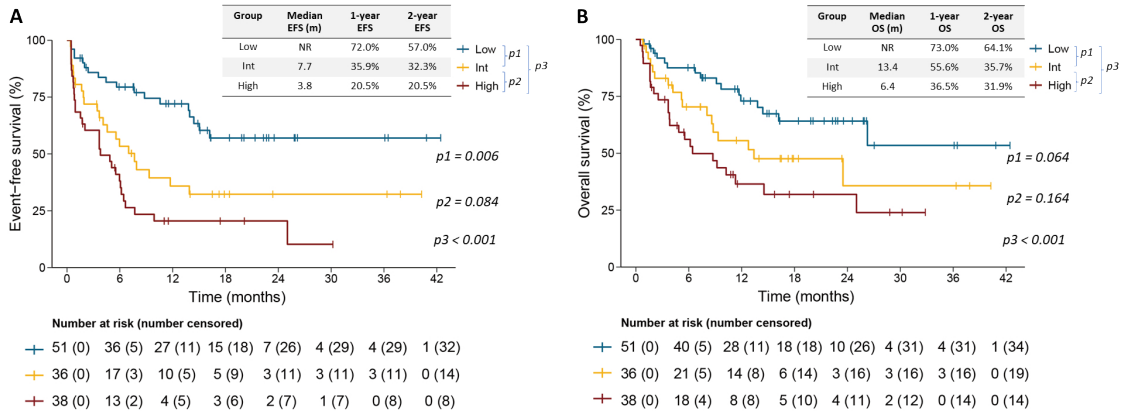
Supplementary Fig. 7. Subgroup analyses of treatment response and survival outcomes in the ≤ 14-day and > 14-day blinatumomab duration cohorts.



Forest plots displaying (**A, B**) ORs for CRc and MRD-negativity rates and (**C, D**) HRs for EFS and OS in prespecified subgroups comparing the ≤ 14-day and > 14-

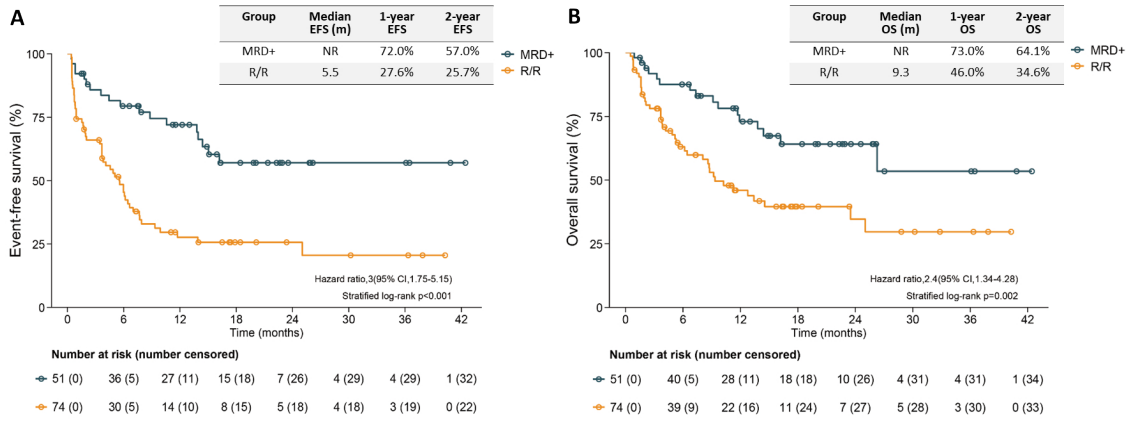
day cohorts. Squares represent point estimates, and horizontal lines indicate 95% CIs. ORs or HRs < 1 favor the ≤ 14-day group (arrows). *alt* alteration; *arr* rearrangement; *Blina* blinatumomab; *BM* bone marrow; *CI* confidence interval; *CRc* composite complete remission; *del* deletion; *EFS* event-free survival; *HR* Hazard Ratio; *HSCT* allogeneic hematopoietic stem cell transplantation; *m* month; *MRD* measurable residual disease; *mut* mutation; *NE* not estimable; *OR* Odds Ratio; *OS* overall survival; *Ph* Philadelphia chromosome; *Post-blina* after blinatumomab; *Pre-blina* before blinatumomab; *R/R* relapsed or refractory.

Supplementary Fig. 8. EFS and OS stratified by leukemia burden prior to blinatumomab, uncensored for allo-HSCT.



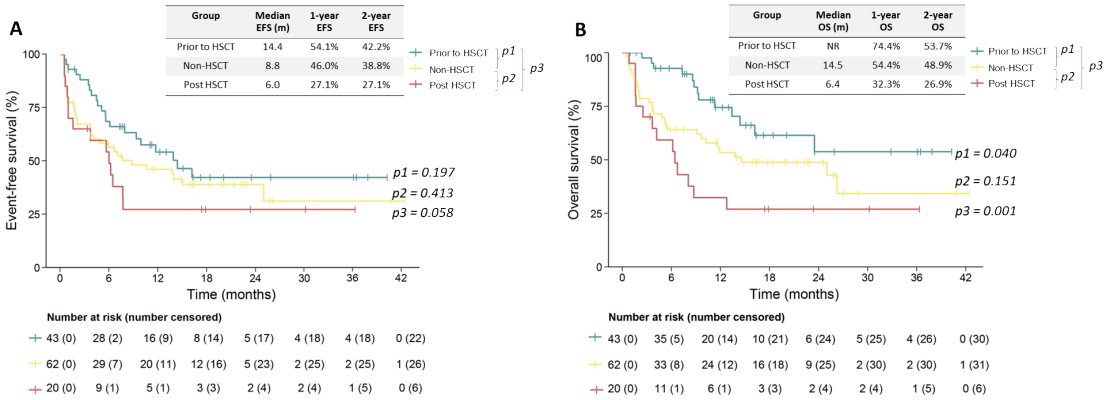
A EFS stratified by pretreatment leukemia burden (e.g., Low, Intermediate, and High) prior to blinatumomab. **B** OS was stratified by leukemia burden (e.g., Low, Intermediate, and High) prior to blinatumomab. Pairwise comparisons are indicated as follows: *p*1, Low versus Intermediate; *p*2, Intermediate versus High; *p*3, Low versus High. *allo-HSCT* allogeneic hematopoietic stem cell transplantation; *EFS* event-free survival; *High* high leukemia burden*; Int* intermediate leukemia burden*; Low* low leukemia burden; *m* month; *NR* not reached; *OS* overall survival.

Supplementary Fig. 9. EFS and OS stratified by disease status prior to blinatumomab, uncensored for allo-HSCT.



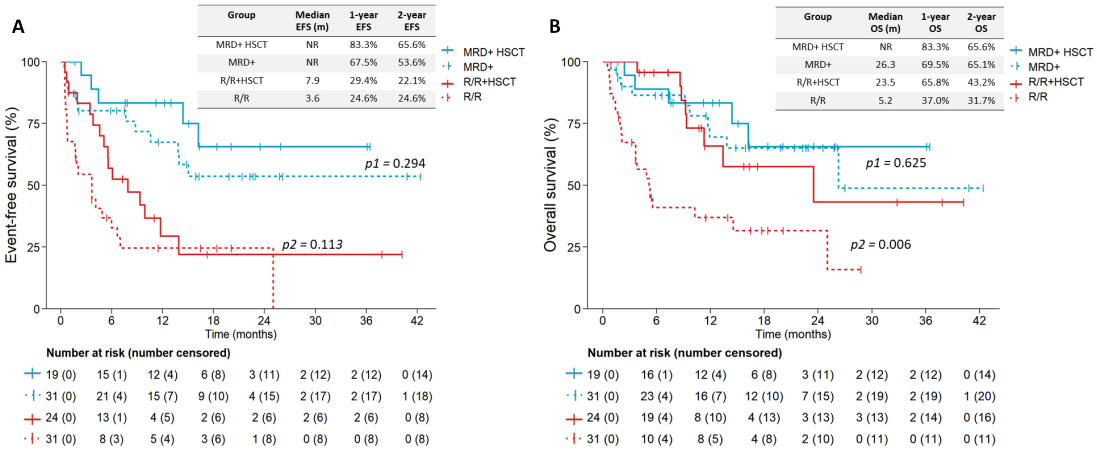
A EFS stratified by disease status (e.g., MRD-positive or R/R) prior to blinatumomab. **B** OS stratified by disease status (e.g., MRD-positive or R/R) prior to blinatumomab. *allo-HSCT* allogeneic hematopoietic stem cell transplantation; *EFS* event-free survival; *m* month; *MRD+* measurable residual disease positivity; *NR* not reached; *OS* overall survival; *R/R* relapsed or refractory.

Supplementary Fig. 10. EFS and OS stratified by the timing of allo-HSCT relative to blinatumomab therapy.



A EFS stratified by the timing of allo-HSCT and blinatumomab therapy. **B** OS stratified by the timing of allo-HSCT and blinatumomab therapy. Pairwise comparisons: *p*1, Prior to HSCT versus Non-HSCT; *p*2, Non-HSCT versus Post HSCT; *p*3, Prior to HSCT versus Post HSCT. *allo-HSCT or HSCT* allogeneic hematopoietic stem cell transplantation; *EFS* event-free survival; *m* month; *Non-HSCT* without allo-HSCT after blinatumomab therapy; *NR* not reached; *OS* overall survival; *Post HSCT* after allo-HSCT; *Prior to HSCT* before allo-HSCT.

Supplementary Fig. 11. EFS and OS stratified by disease status prior to blinatumomab and the timing of allo-HSCT.



A EFS stratified by disease status (e.g., MRD-positive or R/R) prior to blinatumomab and allo-HSCT. **B** OS stratified by disease status (e.g., MRD-positive or R/R) prior to blinatumomab and allo-HSCT. Pairwise comparisons: *p*1 compares patients with versus without bridging allo-HSCT within the MRD-positive cohort; *p*2 compares patients with versus without bridging allo-HSCT within the R/R cohort. *allo-HSCT or HSCT* allogeneic hematopoietic stem cell transplantation; *EFS* event-free survival; *MRD+* measurable residual disease positivity; *NA* not available; *NR* not reached; *OS* overall survival; *R/R* relapsed or refractory.