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Outcomes of children and young adults with B-cell acute lymphoblastic leukemia given blinatumomab as last consolidation treatment before allogeneic hematopoietic stem cell transplantation

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Authors' contribution

M.A. and M.M. contributed equally. M.A. and M.M. designed the study, performed the statistical analysis, interpreted data and wrote the paper; M.A., I.P., M.M., F.G collected the data; M.A., M.M., D.P., R.C., I.P., F.Q., B.E., C.R., B.L., F.B., F.D.B., M.G.C., M.B. and P.M. were involved in the clinical management of patients; R.M.P. performed human leukocyte antigen typing and unrelated donor search; G.L.P. performed graft manipulation and graft characterization; V.B. performed immune monitoring; F.G. and P.M. supported the statistical analysis and data interpretation; G.Z. contributed to data interpretation and manuscript writing; F.L. conceived and supervised the project, interpreted data and edited the manuscript.

Disclosures

M. A. served on Scientific Advisory Board for Vertex Pharmaceuticals Inc. and as Steering Committee member for Vertex Pharmaceuticals Inc., outside the submitted work. P.M. reports

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Running heads:

HSCT Outcomes in Children after Blinatumomab

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Data Sharing Statement

The data that support the findings of this study are available from the corresponding author (F.L.) on reasonable request.

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Abstract:

Blinatumomab has remarkable efficacy in patients with relapsed/refractory (r/r) or measurable residual disease (MRD)-positive B-cell acute lymphoblastic leukemia (B-ALL). In many patients, blinatumomab treatment is followed by allogeneic hematopoietic stem cell transplantation (HSCT). However, the influence of blinatumomab on HSCT outcomes in children and young adults (YA) remains to be fully elucidated. We conducted a single-center, retrospective analysis on patients given blinatumomab as last treatment before HSCT. Seventy-eight pediatric and YA patients were evaluated. With a median follow-up of 23.23 months, the 2-year disease-free (DFS) and overall survival (OS) probability were 72.2% and 89.2%, respectively, with a 2-year cumulative incidence (CI) of non-relapse mortality (NRM) of 2.6%. A trend toward improved 2-year DFS, but not OS, was noted in patients transplanted in first complete remission (CR1) (92.9%) compared to those in second or greater remission (CR2/3) (68.5%, p=0.18) due to a lower CI of relapse (0% vs. 29.9%, p=0.05). Among CR2/3 patients, those receiving the sequential combination of inotuzumab and blinatumomab had a significantly lower CI of relapse as compared to those who did not receive inotuzumab (9.5% vs. 40.4%, p=0.023). Relapse after HSCT occurred in 16 patients, all exhibiting CD19-positive blasts; 10 of them received anti-CD19 chimeric antigen receptor T-cell (CAR-T) therapy and 2 inotuzumab as salvage therapy, leading to a 2-year post-relapse OS of 52.7%. Our results indicate that HSCT following blinatumomab in children and YA with B-ALL is highly effective, being associated with low NRM and not affecting the efficacy of subsequent salvage immunotherapies, including CAR-T cells.

Main text

Introduction:

Immunotherapy has profoundly transformed the therapeutic landscape for high-risk and relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL),[1] thanks to the ability of inducing higher rates of complete remission (CR) with negative measurable residual disease (MRD) while offering an improved safety profile as compared to conventional chemotherapy. Achievement of a MRD-negative CR before allogeneic hematopoietic stem cell transplantation (HSCT) in B-ALL is key, as several previously published studies demonstrated that the risk of disease relapse after the allograft is significantly higher, and the survival lower, in patients with MRD positivity than in patients without detectable MRD.[2,3]

Blinatumomab, a first-in-class bispecific T-cell engager (BiTE) which directs CD3-positive T cells toward CD19-positive leukemia cells, is the first immunotherapeutic agent approved for treatment of both adult and pediatric patients with r/r B-ALL.[4,5] Blinatumomab has demonstrated efficacy and safety in r/r and molecularly resistant B-ALL in prospective clinical trials conducted in children, adolescents, young adults (YA) and adults.[6–8]

Notably, the benefits of blinatumomab have been further confirmed in two phase III randomized trials conducted in children and YA with high-risk and intermediate-risk first-relapse B-ALL, which were both early terminated due to improved survival, increased MRD remission, and reduced toxicity observed with blinatumomab as compared to standard intensive chemotherapy.[9,10] Longer follow-up also demonstrated a strong benefit also for overall survival (OS) in one of these 2 randomized trials and post hoc analysis clarified that the improvement in OS, event-free survival (EFS) and MRD remission rates observed with blinatumomab is independent of baseline MRD.[11,12]

A more recent study documented that the outcome of patients with standard-risk first-relapse bone marrow B-ALL is improved by the addition of blinatumomab to chemotherapy treatment[13].

Additionally, in the RIALTO expanded access study, children who did proceed to allogeneic HSCT after treatment with blinatumomab had significantly better OS as compared to those who did not,[14] documenting that, similarly to adults, allogeneic HSCT is usually required as a consolidation therapy to achieve definitive cure of r/r B-ALL.

Despite these data, the impact of blinatumomab on the outcomes of HSCT remains insufficiently explored in children and YA. As the optimal integration of immunotherapy with HSCT to maximize

survival and minimize toxicity remains a critical area for investigation,[15] in this single-center retrospective study, we assessed the outcomes of pediatric and YA patients who received blinatumomab as last therapeutic intervention prior to HSCT.

Methods:

This single-center, retrospective study, conducted at Bambino Gesù Children's Hospital in Rome, Italy, included all consecutive pediatric and YA patients diagnosed with B-ALL who received blinatumomab as last therapy before undergoing HSCT. The cut-off date for data retrieval was December 31, 2023. Primary objectives were to estimate the 2-year probability of OS, disease-free survival (DFS), as well as the cumulative incidence of relapse (CIR), and non-relapse mortality (NRM). Secondary outcomes included the cumulative incidence of acute and chronic graft-versus-host disease (aGVHD and cGVHD), neutrophil and platelet recovery, graft failure, and both infective and non-infective post-HSCT complications, along with immune reconstitution. The 2-year GVHD-free relapse-free survival (GRFS) probability was estimated, as well.

OS was defined as survival from HSCT to last follow-up or death. NRM was defined as death from non-relapse causes, considering disease relapse as a competing risk. DFS was defined as the period patients remained alive without evidence of leukemia post-HSCT.

GVHD severity was classified using established criteria: acute GVHD grades were defined according to the MAGIC criteria;[16] chronic GVHD severity was assessed using the National Institutes of Health consensus criteria.[17] GRFS was defined as the duration of survival from HSCT to the first occurrence of disease relapse, death in remission, grade III-IV acute GVHD, or moderate to severe chronic GVHD.

Blinatumomab treatment details, including number of cycles and the interval between the completion of therapy and HSCT, were recorded. Remission status and MRD levels were quantified using flow-cytometry or polymerase chain reaction (PCR) with a sensitivity threshold of $1x10^{-4}$ or greater. Pre-HSCT MRD was assessed within 30 days before transplantation and was defined as negative if $<1x10^{-4}$. We considered both flow-cytometry and PCR-MRD, when both available, choosing the highest value in case of discrepancy.

The study was conducted in compliance with the Declaration of Helsinki and local ethical guidelines for retrospective studies, with patient consent waived due to the retrospective nature of the analysis.

Statistical analysis

Statistical analysis was performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA) and R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). The Kaplan-Meier method was used to estimate OS, DFS and GRFS, with the log-rank test applied to assess differences between groups. Cumulative incidences (CI) were calculated with competing risks methods, and group differences were analyzed using Gray's test. The Fisher's exact test was employed for categorical variables, while the Wilcoxon rank-sum test was used for continuous variables. Spearman's correlation coefficient was utilized for correlation analyses. For multivariable analyses of DFS and OS, Cox proportional hazards regression modeling was utilized, and the Fine-Gray sub-distribution hazard model was applied for competing risks analysis of CI. Covariate selection was focused on clinical meaningfulness. Data were sourced from electronic medical records, encompassing demographics, clinical characteristics, treatment details, and outcomes.

Results:

Study population:

Seventy-eight patients met the inclusion criteria, with a median age at HSCT of 8 years (interquartile range [IQR] 6-14; range 1-25 years). Patient characteristics, including cytogenetic/molecular profiles, are detailed in Table 1. Each patient had an indication for HSCT according to the International BFM Study Group and IntReALL Consortium risk classification, with 31 patients exhibiting at least one clinical or cytogenetic/molecular very high-risk feature (hypodiploidy; TCF3::HLF; KMT2A::AFF1; IKZF1plus deletion and poor MRD response after induction; absence of CR at Day+33 after induction therapy; very early relapse, <18 months from diagnosis, for CR2 patients).

Blinatumomab was administered for refractory disease in three patients (primary refractory disease: n=1; refractory disease post-relapse: n=2), as an MRD negativization strategy in 23 patients across various remissions (CR1: n=5; CR2: n=17; CR3: n=1), and as a consolidation strategy in 52 MRD-negative patients after reinduction/consolidation (CR1: n=7; CR2: n=41; CR3: n=4).

Among the relapsed patients, 26 patients received inotuzumab as part of the reinduction strategy prior to blinatumomab administration, and 14 of them underwent a completely chemo-free reinduction regimen composed of inotuzumab followed by blinatumomab. One patient received reinduction with dexamethasone plus ponatinib and blinatumomab for Philadelphia chromosome-positive ALL.

The number of blinatumomab cycles administered before HSCT was one (n=55) or two (n=23).

Among the 26 patients with refractory disease/positive MRD at the start of blinatumomab therapy, 20 (76.9%) achieved MRD negativity post-treatment. For the six remaining patients, the median MRD level was 2 x 10^{-4} (range 1-3.5 x 10^{-4}). All patients exhibited their best MRD response following a single blinatumomab cycle. Two of the six patients who remained MRD-positive after blinatumomab achieved a 1-log reduction in MRD, whereas MRD levels remained stably low in the remaining four subjects (1-3.5 x 10^{-4}). Median interval from blinatumomab termination to HSCT was 23 days (IQR 19-31.75).

At time of HSCT, 14 patients were in first, 59 in second, and 5 in third CR. Median follow-up duration was 23.23 months (IQR=14.5-42.8; range=3-93 months). Except for six cases, all patients received a total body irradiation (TBI)-based conditioning regimen. Main donor characteristics are detailed in Table 1. Patient transplanted from matched related and unrelated donor received unmanipulated bone marrow (BM) or peripheral blood stem cells (PBSC) grafts with conventional cyclosporin-A and short-term MTX for GvHD prophylaxis. Anti-T-lymphocyte globulin (ATLG) was administered to all recipients of unrelated donor or haploidentical donor transplants, as previously described.[18] All the 32 patients transplanted from HLA-mismatched related donors received a αβT- and B-cell–depleted (TCD) PBSC grafts without post-transplant GvHD prophylaxis.[19]

Cumulative incidence of relapse, overall and disease-free survival

Of the 78 patients, 16 (20%) experienced relapse post-HSCT, the 2-year CIR of the whole cohort being 25.2% (95% CI 15.0-36.8) [Figure 1A]. Median time to relapse from HSCT was 9 months (IQR: 5-13; range: 1-23). Nine patients had isolated bone marrow relapses, four combined bone marrow and CNS recurrence, one child an isolated CNS relapse and one patient each a relapse involving the eye and testis, or skin and pancreas). In all cases of post-transplant relapse, leukemia blasts remained CD19 positive.

Among the 16 patients experiencing relapse after HSCT, 10 subjects received chimeric antigen receptor (CAR) T-cell therapy (with 9 achieving morphological CR) and two were treated with

inotuzumab. The 2-year OS after relapse was 52.7% (95%CI 27.1-78,3), (Figure S1.). None of the 6 patients with positive pre-transplant MRD relapsed. Among them, 4 subjects had Ph+ B-ALL and received post-transplant maintenance therapy with tyrosine kinase inhibitors (TKI). A total of 9 patents died of disease recurrence.

With a median follow up of 23.2 months, the 2-year DFS and OS were 72.2% (95% CI 59.0-81.8) and 89.2% (95% CI 78.4-94.8), respectively [Figure 2A and 2B]. No additional post-transplant relapses or death in CR were reported after 2 years and the 4-year OS was 84.3% (95% CI 74.5-94.1).

No significant differences in DFS and OS were observed according to age group, conditioning regimen, MRD status at HSCT or presence of high-risk cytogenetic features. Complete univariable analysis of factors potentially affecting the probabilities of CIR, DFS and OS are shown in Table 2 and 3. Multivariable analysis did not identify any factor significantly associated with CIR, DFS and OS (Table 4).

Impact of CR status on cumulative incidence of relapse and survival outcomes

A trend toward improved 2-year DFS, but not OS, was noted in patients transplanted in CR1 (92.9% [95% CI 59.1-99.0]) compared to those in CR2/3 (68.5% [95% CI 53.8-79.4]; p=0.18) [Figure 2C].

Notably, no relapses were observed in patients transplanted in CR1. Univariable analysis showed a trend towards a lower CIR in CR1 patients as compared to those in CR2/CR3 at time of HSCT (0% [95% CI 0-0] vs. 29.9% [95% CI 17.8-42.9], p=0.05, [Figure 1B]).

Among patients transplanted in ≥CR2, those who did receive inotuzumab had a statistically significant lower CIR as compared to those who did not (9.5% [95% CI 1.5-26.6] vs. 40.4% [95% CI 23.6-56.6], p=0.023; [Figure 1C]) and exhibited a trend toward better DFS as compared to those who received only chemotherapy and blinatumomab (86.7% [95% CI 63.8-95.6] vs. 59.6 [95% CI 41.2-74]; p=0.07; [Figure 1D]).

Among CR2/CR3 patients given inotuzumab and blinatumomab, no differences in relapse rate and survival outcomes were observed among those who received also chemotherapy and those who did not (data not shown). Additionally, among patients in CR2 at HSCT, those who had experienced a relapse less than 18 months from initial B-ALL diagnosis had a trend towards higher CIR (43.6% [95% CI 20.2-65.0] vs. 26% [95% CI 11.3-43.5], p=0.21).

Incidence of acute and chronic GvHD and impact on survival outcomes

The CI of grade II-IV aGVHD and cGVHD were 12.8% (95%CI 6.5-21.3) and 13% (95%CI 6.2-21.8) respectively, with 3 cases of moderate to severe cGVHD (CI=4.6%; 95%CI 1.5-13.6) (Figure S2).

Patients who received 2 or more cycles of blinatumomab had a significantly higher CI of aGVHD and a trend towards increased CI of cGVHD compared to those who received only 1 cycle. The CI for aGVHD was 11.0% (95% CI 4.4-20.9) for those receiving 1 cycle and 47.8% (95% CI 26.2-66.6) for those receiving 2 cycles ($p<0.01$). For cGVHD, the CI was 8.0% (95% CI 2.5-17.7) and 25.6% (95% CI 8.4-47.4) for patients receiving 1 or 2 cycles of blinatumomab, respectively ($p=0.06$). Multivariable analysis confirmed a significant association between the number of cycles of blinatumomab and the development of aGVHD (HR=3.5 [95% CI, 1.6-7.64], $p=0.001$), but not cGVHD (Table S1 and S2). In univariable analysis, patients who did develop grade II-IV aGVHD demonstrated an inferior 2-year OS compared to those who did not [60% (95%CI 7.6-90.4) versus 91.7% (95%CI 81.0-96.5); $p=0.04$], without differences in DFS (Table 3). The two-year GRFS of the whole cohort was 68.4% (95% CI 55.7-78.2).

Toxicities, infections and non-relapse mortality

We did not record cases of sinusoidal obstruction syndrome (SOS) or transplantation-associated thrombotic microangiopathy (TMA). SOS prophylaxis was performed with ursodeoxycholic acid (UDCA) alone, as per internal policy. Details on clinically significant infections (i.e. those necessitating therapeutic intervention) are detailed in Table S3. The CI of NRM was 2.6% (95%CI 0-9.9) [Figure 1D]; in particular, two patients died for transplant-related causes, one because of idiopathic pneumonia in CR1 and one of disseminated adenovirus infection in CR3 after a TCD haploidentical HSCT.

Engraftment and Immune reconstitution

One patient experienced primary graft failure following an haploidentical HSCT and was successfully re-transplanted with a TCD graft from the other haploidentical parent. No secondary graft failure was reported. Ninety percent of subjects were independent from intravenous immunoglobulin (IVIG) replacement therapy at 48 months post-transplantation, with a median interval to independence of IVIG replacement therapy of 4.03 months (95% CI 2.43-5.83). Additional details on engraftment and immune reconstitution are reported in Table S4.

AB0 incompatibility

Among the 78 patients, 18 exhibited major ABO incompatibility with the donor. Anti-A/B antibody screening was performed in 14 of these patients, while the remaining 4 received TCD peripheral blood stem cells. Among those screened, six patients did not have detectable anti-A/B antibodies, and the remaining eight had low natural isohemagglutinin titers (range 1:1 to 1:8), and did not require any specific intervention at time of transplantation. No adverse reactions attributable to the infusion of an ABO incompatible graft were documented.

Discussion:

Our study demonstrates, in the largest pediatric cohort reported so far, that blinatumomab is a safe and effective strategy for bridging children and YA with B-ALL to HSCT. In our cohort, pre-transplant blinatumomab treatment led 76.9% of resistant/MRD-positive patients to complete MRD negativity, a prognostic factor of utmost importance for relapse prevention in pediatric ALL.[20] These results align with findings by Locatelli et al., who reported MRD negativity in 90% of subjects,[9] and Brown *et al.*, with a 75% MRD negativity rate post-blinatumomab treatment.[10] Most patients in this study received blinatumomab as a consolidation strategy after already achieving an MRD-negative status with previous chemotherapy or immunotherapy approaches, and all maintained MRD negativity after treatment. Ultimately, 92.3% of patients undergoing HSCT were MRD-negative, further corroborating the role of blinatumomab in obtaining and maintaining MRD-negativity, allowing optimal disease control before allogeneic HSCT.[3,11,21]

The remaining six patients who did not achieve MRD-negativization exhibited extremely low MRD levels, in all cases below 3.5×10^{-4}, which did not adversely impact their outcome. Indeed, none of these relapsed. This finding is in contrast with several other reports showing that positive pre-transplant MRD levels increase the relapse risk in ALL and may be due to the limited number of patients transplanted with MRD-positive disease.[2,15,20] In addition, as four of the six patients with positive MRD before HSCT had Ph+ B-ALL, the benefit of post-transplant maintenance therapy with TKI cannot be excluded.

Our 2-year OS and DFS compare favorably with those reported by the Berlin-Frankfurt-Munster (BFM) group[22,23] and are superimposable to those obtained in the For Omitting Radiation Under Majority Age (FORUM) trial in in B-ALL patients (2-year OS: 84%; 2-year DFS: 71%).[24] However, the FORUM study included a higher proportion of patients in CR1 (56%) compared to our cohort (18%).

We observed a remarkable 2-year OS and DFS of 92.9% in patients transplanted in CR1, without relapses. These unprecedented results, although obtained in a limited number of patients, further support the incorporation of blinatumomab in the frontline treatment of high-risk pediatric B-ALL, particularly in that narrow cohort of children eligible for HSCT in CR1. Patients transplanted in CR2/3 exhibited a trend towards a reduced DFS (68.5% vs. 92.9%), due to an increased relapse risk. The remission status at transplant (i.e., CR1 *vs* CR2) is a known prognostic factor for relapse that maintain its predictive value also in blinatumomab-bridged patients.[22–24]

Noteworthy, cytogenetic/molecular alterations had no impact on disease recurrence after transplant, thereby confirming the agnostic nature of therapeutic effect of blinatumomab with respect to B-ALL genetic abnormalities.[14,25] We were not able to dissect the impact of other mutational event occurring at relapse, such as those involving TP53, which have been associated with poor outcomes after both conventional treatments and immunotherapy,[26,27] because this information was available only for a minority of patients.

Adolescents and YA, a group in which worse survival outcomes have historically been reported,[28] exhibited a remarkable 78% DFS, without difference with patients below 12 years of age. This favorable findings may be attributed both to the lower toxicity associated with immunotherapy and the specific efficacy of blinatumomab against molecular subtypes of ALL, such as Ph-positive translocations, prevalent in this age subgroup.[28,29]

Noteworthy, we observed significantly lower CIR, and a trend towards better DFS, in relapsed patients receiving the combination of inotuzumab and blinatumomab before transplantation as compared to those who did not, suggesting that combined targeting of both CD22 and CD19 before transplantation is a highly attractive strategy to achieve deep and sustained CR.

NRM of our cohort was remarkably low (2.6%), despite the fact that most patients had been extensively pretreated patients, this result being likely due to the ability of blinatumomab of inducing deep MRD responses before transplant, while minimizing toxicity. A recent report observed a remarkably low NRM in adult patients with ALL treated with blinatumomab before HSCT.[30] Low NRM rates were recently reported also in a cohort of children with ALL who received pre-transplant blinatumomab bridging therapy, compared to a contemporary cohort of subjects who received chemotherapy alone before HSCT, although these differences were not significant due to the small sample size of patients in both groups.[31] Our study provides additional support to the excellent safety profile of the combination of blinatumomab and allogeneic HSCT in the pediatric setting.

No cases of SOS was reported in our cohort, including the 26 patients who received inotuzumab before HSCT, this contrasting with the SOS incidence of 13% to 52% observed in patients who underwent allogeneic HSCT in the INO-VATE study and other studies investigating inotuzumab in pediatric r/r ALL.[32–34] The absence of SOS in our cohort, in which conventional UDCA prophylaxis was employed, could be explained by the longer interval between inotuzumab administration and initiation of conditioning regimen, facilitated by bridging treatment with blinatumomab.[34] Our data support the adoption of a sequential strategy of targeted therapies for relapsed B-ALL patients in which inotuzumab is administered before blinatumomab, with or without interspersed chemotherapy, to achieve optimal efficacy and mitigate the SOS risk. In addition, the 15 relapsed patients whose induction/consolidation strategy did not include chemotherapy in our study had outcomes comparable to those given chemotherapeutic approaches, thereby suggesting that the combination of inotuzumab and blinatumomab alone can be considered a suitable treatment alternative to spare toxicity.

In our cohort, the administration of 2 blinatumomab cycles was an independent risk factor for development of aGVHD, a finding in contrast with the experience of adult patients[35] which warrants further investigation. Although obtained from a retrospective and heterogeneous cohort, this observation suggests that, for those patients achieving MRD-negativity after one single cycle, a second cycle has limited benefit and may be associated with an increased GVHD rate after transplantation.

Given the powerful B-cell depleting action of blinatumomab, we also evaluated isohemagglutinin titers in the setting of major ABO incompatibility, which has been associated, albeit not in all studies, with inferior outcomes when BM grafts are employed.[36,37] ABO incompatibility in HSCT occurs in 20-50% of allogeneic transplants, with major ABO incompatibility (where the recipient has pre-formed antibodies against donor red cells) being found in around 10-20% of cases.[38] This type of mismatch is concerning, as it can result in complications such as acute hemolysis (occurring in 5-10% of major ABO mismatches), delayed red cell engraftment (15-25%), or pure red cell aplasia, which has been reported in up to 20-30% of cases.[39,40] Standard pre-transplant management for major ABO incompatibility typically involves strategies such as plasma exchange in the recipient or red cell depletion of the donor graft, especially when pre-transplant titers are high.[39,40] These interventions, however, might lead to significant loss of stem cells within the graft, or may not always be entirely effective in preventing post-transplant complications.[39] Consistently with blinatumomab's mechanism of action, we observed extremely low or absent

isohemagglutinin titers before transplant in patients with major ABO incompatibility, which led to successful and uneventful infusions of BM grafts without any impact on transplant outcomes.

In all cases of disease recurrence, our patients' leukemic blasts were found to be CD19-positive, indicating that blinatumomab treatment prior to HSCT did not hinder subsequent salvage therapy with anti-CD19 CAR-T cells. Concerns that CD19 modulation by blinatumomab could compromise outcomes with anti-CD19 CAR-T cells led to the exclusion of previously exposed patients from the global registration trial of tisagenlecleucel, the first approved CAR-T cell therapy.[41,42] However, findings from the recent CAR-Multicenter Analysis (CAR-MA) study suggest that prior blinatumomab exposure does not independently predict poorer outcomes,[43] and our results in the post-transplant setting are consistent with and provide further support to this observation. The potential to rescue patient with CAR-T cells in the post-transplant period is particularly appealing, especially considering that manufacturing issues related to low lymphocyte counts or suboptimal T-cell fitness in this setting can be overcome by generating CAR-T cells from allogeneic donors, as recently demonstrated.[46]

Our study has some limitations, including its retrospective, single-center nature and relatively small sample size. These factors, combined with the heterogeneity in patient characteristics, may limit the generalizability of the results. Despite these limitations, our study underscores the feasibility and safety of pre-transplant blinatumomab in children and YA with B- ALL and supports its beneficial effect on reducing NRM, preventing disease recurrence and ultimately improving survival after HSCT. Results obtained in CR1 patients are particularly attractive and support the incorporation of blinatumomab bridging in all subjects with a transplant indication after first-line therapy. For CR2/3 patients, sequential targeting of CD22 and CD19 with inotuzumab and blinatumomab before HSCT offers excellent DFS probabilities. Lastly, in the event of post-transplant relapse, the combination of blinatumomab and HSCT does not impede the possibility of achieving sustained CR with subsequent salvage immunotherapy, including anti-CD19 CAR-T cells.

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Tables:

Table 1. Patient, donor and transplant characteristics (N=78)

	Number/median	% (range)
Sex		
Male	47	60.3
Female	31	39.7
Age at transplantation, y	8	1-25
Year of transplantation		
2016-2019	16	20.5
2020-2023	62	79.5
Donor recipient sex mismatch	16	20.5
Disease status at HSCT		
CR1	14	17.9
CR2	59	75.6
CR3	5	6.5
Cytogenetics		
Complex karyotype	1	1.3
Constitutional trisomy 21	1	1.3
Hyperdiploidy	1	1.3
Hypodiploidy	2	2.5
IKZF1^{plus}	4	5.1
JAK2-r	2	2.5
KMT2A-r	9	11.5
t(4;11)/KMT2A::AFF1	5	6.4
N.A.	7	8.9
Normal	31	39.6
t(9;22)/BCR::ABL	9	11.5
Ph-like	2	2.5
t(12;21)/ETV6::RUNX1	7	8.9
t(1;19)TCF3::PBX1	1	1.3
t(17;19)/TCF3::HLF	1	1.3
High-risk clinical and treatment-response features	31	39.7
IKZF1^{plus} and poor MRD response after induction	4	5.1
No CR at Day 33 without cytogenetic abnormalities	1	1.3
Very early relapse (<18 months from diagnosis) for CR2 patients	20	25.6
Donor type		
MMFD donor	32	41.0
MMU donor (9/10 HLA match)	7	9.0
MS donor	13	16.7
MU donor	26	33.3
AB0 group compatibility		
Major/bidirectional mismatch	18	23.1

Other	60	76.9
MRD pre HSCT		
Positive	6	7.6
Negative	72	92.4
Conditioning regimen		
TBI based	72	92.3
TBI-VP16	43	55.1
TBI-TT-FLU	23	29.5
TBI-TT-MEL	6	7.7
Busulfan based	6	7.7

Y, years; HSCT, allogeneic hematopoietic stem cell transplantation; CR, complete remission; N.A., not available; MMFD, mismatched family donor; MMU, mismatched unrelated; MS, matched sibling; MU, matched unrelated; MRD, minimal residual disease; TBI, total body irradiation; TT, Thiotepa; VP16, etoposide; Flu, fludarabine; MEL, melphalan.

Table 2. Cumulative incidence of relapse: univariable analysis

Outcome	2 years CIR			
Recipient sex	No. of patients	Probability	95% CI	p value
Male	47	0.224	(0.108-0.366)	0.754
Female	31	0.289	(0.119-0.486)	
Recipient age group (years)				
0-3	6	0.167	(0.004-0.556)	0.451
4-12	48	0.309	(0.166-0.463)	
>12	24	0.174	(0.036-0.398)	
Major AB0 incompatibility				
Yes	18	0.271	(0.071-0.527)	0.887
No	60	0.250	(0.134-0.384)	
Disease phase at HSCT				
CR1	14	0.000	(0.000-0.000)	**0.049**
CR2	59	0.328	(0.196-0.467)	
CR3	5	0.000	(0.000-0.000)	
Conditioning regimen				
TBI-based	72	0.262	(0.151-0.385)	0.746
Chemo-based	6	0.167	(0.004-0.556)	
HR cytogenetics*				
Yes	36	0.181	(0.058-0.358)	0.196
No	42	0.318	(0.166-0.481)	
Disease status pre blinatumomab				
No CR	3	0.333	(0.001-0.832)	0.382
CR 1	13	0.115	(0.005-0.420)	
CR2	57	0.301	(0.171-0.442)	
CR3	5	0.000	(0.000-0.000)	
Lymphocytes at blinatumomab				
>720	37	0.259	(0.116-0.428)	0.691
<720	41	0.242	(0.109-0.402)	
B-cell aplasia at blinatumomab				
Yes	34	0.234	(0.100-0.401)	0.144
No	22	0.059	(0.003-0.242)	
Inotuzumab before blinatumomab (CR2 or greater patients only)				
No	38	0.404	(0.236-0.566)	**0.023**
Yes	26	0.095	(0.015-0.266)	
Chemotherapy free induction/consolidation treatment				
No	63	0.287	(0.161-0.425)	0.304
Yes	15	0.133	(0.020-0.355)	
MRD at HSCT				
Positive	6	0.000	(0.000-0.000)	0.184
Negative	72	0.275	(0.164-0.398)	
Type of transplantation				
TCD	32	0.320	(0.165-0.486)	0.114
Unmanipulated	46	0.217	(0.079-0.398)	
Days from stop of blinatumomab to HSCT				
>23	37	0.276	(0.133-0.440)	0.507
<23	41	0.231	(0.094-0.402)	
Acute GVHD				
Yes	17	0.278	(0.080-0.523)	0.506
No	61	0.239	(0.129-0.368)	
Grade II-IV acute GVHD				
Yes	13	0.386	(0.103-0.671)	0.0982
No	65	0.221	(0.119-0.343)	
Chronic GvHD				
Yes	9	0.619	(0.048-0.927)	0.267
No	69	0.228	(0.126-0.347)	
Very early relapse† (CR2 patients only)				
Yes	20	0.436	(0.202-0.650)	0.211
No	39	0.260	(0.113-0.435)	

HSCT, hematopoietic stem cell transplantation; CR, complete remission; TCD, T-cell depleted; MRD, minimal residual disease; TBI, total body irradiation; NE, not estimable; GVHD, graft-versus-host disease.

* KMT2A rearrangements; Philadelphia or Philadelphia-like chromosome; hypodiploidy; constitutional trisomy of chromosome 21; TCF3-Rearranged.

† <18 months from initial diagnosis

Table 3. Disease free survival and overall survival: univariable analysis

Outcome	2 years DFS					2 years OS			
Recipient sex	No. of patients	Events	Probability	95% CI	p value	Events	Probability	95% CI	p value
Male	47	10	0.754	(0.498-0.864)	0.67	6	0.863	(0.695-0.942)	0.63
Female	31	8	0.678	(0.447-0.830)		3	0.935	(0.766-0.983)	
Recipient age group									
0-3	6	2	0.667	(0.195-0.904)	0.69	1	0.833	(0.273-0.975)	0.87
4-12	48	12	0.691	(0.513-0.815)		5	0.930	(0.798-0.977)	
>12	24	4	0.784	(0.504-0.918)		3	0.795	(0.459-0.935)	
Major AB0 incompatibility									
Yes	18	5	0.673	(0.366-0.856)	0.60	3	0.825	(0.549-0.940)	0.46
No	60	13	0.733	(0.579-0.838)		6	0.914	(0.781-0.968)	
Disease phase at HSCT									
CR1	14	1	0.929	(0.591-0.990)	0.39	1	0.929	(0.591-0.990)	0.85
CR2	59	16	0.672	(0.514-0.788)		7	0.894	(0.760-0.955)	
CR3	5	1	0.800	(0.204-0.969)		1	0.800	(0.204-0.969)	
Risk classification at HSCT									
Low	14	1	0.929	(0.591-0.990)	0.18	1	0.929	(0.763-0.948)	0.77
Intermediate	64	17	0.684	(0.537-0.794)		8	0.887	(0.591-0.990)	
high	0	NA							
Conditioning regimen									
TBI-based	72	16	0.725	(0.584-0.825)	0.53	8	0.896	(0.779-0.953)	0.78
Chemo-based	6	2	0.667	(0.195-0.904)		1	0.833	(0.273-0.975)	
HR cytogenetics *									
Yes	42	11	0.682	(0.494-0.813)	0.56	4	0.868	(0.675-0.951)	0.97
No	36	7	0.764	(0.550-0.885)		5	0.913	(0.753-0.951)	
Disease status pre blinatumomab									
No CR	3	1	0.667	(0.054-0.945)	0.95	1	0.667	(0.054-0.945)	0.36
CR 1	13	2	0.808	(0.410-0.950)		2	0.738	(0.245-0.937)	
CR 2	57	14	0.699	(0.538-0.813)		5	0.938	(0.818-0.980)	
CR 3	5	1	0.800	(0.204-0.969)		1	0.800	(0.204-0.969)	
Lymphocytes at blinatumomab									
>720	37	8	0.741	(0.541-0.864)	0.86	4	0.897	(0.714-0.966)	0.992
<720	41	10	0.710	(0.521-0.835)		5	0.891	(0.731-0.959)	
B cell aplasia at blinatumomab									
Yes	34	9	0.707	(0.509-0.837)	0.06	4	0.860	(0.662-0.946)	0.48
No	22	1	0.941	(0.650-0.991)		1	1	(NE- NE)	
Inotuzumab before blinatumomab (CR2 or greater patients only)									
No	38	14	0.596	(0.412-0.740)	0.07	6	0.882	(0.714-0.954)	0.65
Yes	26	3	0.867	(0.638-0.956)		2	0.911	(0.684-0.977)	
Chemotherapy free induction/consolidation treatment									
No	63	15	0.698	(0.540-0.810)	0.59	7	0.898	(0.766-0.957)	0.94
Yes	15	3	0.800	(0.500-0.931)		2	0.867	(0.564-0.965)	
MRD at HSCT									
Positive	6	0	1	(NE- NE)	0.15	0	1.000	(NE- NE)	0.30
Negative	72	18	0.714	(0.574-0.815)		9	0.881	(0.762-0.942)	
Type of donor									
MR donor	13	2	0.692	(0.163-0.928)	0.31	13	0.857	(0.334-0.979)	0.85
MU donor	26	5	0.733	(0.465-0.881)		3	0.893	(0.617-0.974)	
MMU donor	7	0	1.000	(NE- NE)		0	1.000	(NE- NE)	
MMR donor	32	11	0.649	(0.456-0.788)		5	0.873	(0.696-0.950)	
Days from stop of blinatumomab to HSCT									
>23	37	10	0.697	(0.505-0.826)	0.5	4	0.915	(0.760-0.972)	0.76
<23	41	8	0.745	(0.542-0.868)		5	0.868	(0.680-0.950)	
Acute GVHD									
Yes	17	4	0.722	(0.417-0.886)	0.76	3	0.774	(0.315-0.945)	0.23
No	61	14	0.728	(0.580-0.832)		6	0.911	(0.797-0.962)	
Grade II-IV acute GVHD									
Yes	13	4	0.614	(0.266-0.835)	0.18	3	0.600	(0.076-0.904)	**0.04**
No	65	13	0.770	(0.632-0.861)		6	0.917	(0.810-0.965)	
cGvHD									

Yes	9	2	0.762	(0.332-0.935)	0.91	2	0.833	(0.273-0.975)	0.12
No	69	15	0.743	(0.607-0.839)		7	0.922	(0.821-0.967)	
Very early relapse† (CR2 patients only)									
Yes	20	8	0.564	(0.313-0.754)	0.21	2	0.944	(0.666-0.992)	038
No	39	8	0.740	(0.532-0.866)		5	0.874	(0.693-0.952)	

HSCT, hematopoietic stem cell transplantation; CR, complete remission; MMR, mismatched related; MMU, mismatched unrelated; MR, matched related; MU, matched unrelate; MRD, minimal residual disease; TBI, total body irradiation; NE, not estimable.

* KMT2A rearrangements; Philadelphia or Philadelphia-like chromosome; hypodiploidy; constitutional trisomy of chromosome 21; TCF3-Rearranged.

† <18 months from initial diagnosis

Table 4. Cumulative incidence of relapse, disease free survival and overall survival: multivariable analysis

	Cumulative incidence of relapse			Disease free survival			Overall survival		
	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value
Age group	0.67	0.21 - 2.10	0.50	0.71	0.25 - 1.98	0.51	1.29	0.30 - 5.19	0.72
CR number before HSCT	1.38	0.62 - 3.07	0.18	1.62	0.48 - 5.40	0.43	1.31	0.26- 6.57	0.74
Unmanipulated vs. TCD haploidentical HSCT	0.47	0.17- 1.28	0.14	0.48	0.18 - 1.26	0.14	0.71	0.18 - 2.84	0.64
High risk cytogenetics*	0.58	0.16- 2.09	0.41	0.93	0.34 - 2.58	0.89	1.02	0.24 - 4.35	0.97
TBI based conditioning	1.84	0.19-17.34	0.59	0.81	0.14 - 4.72	0.81	0.56	0.05-7.13	0.66

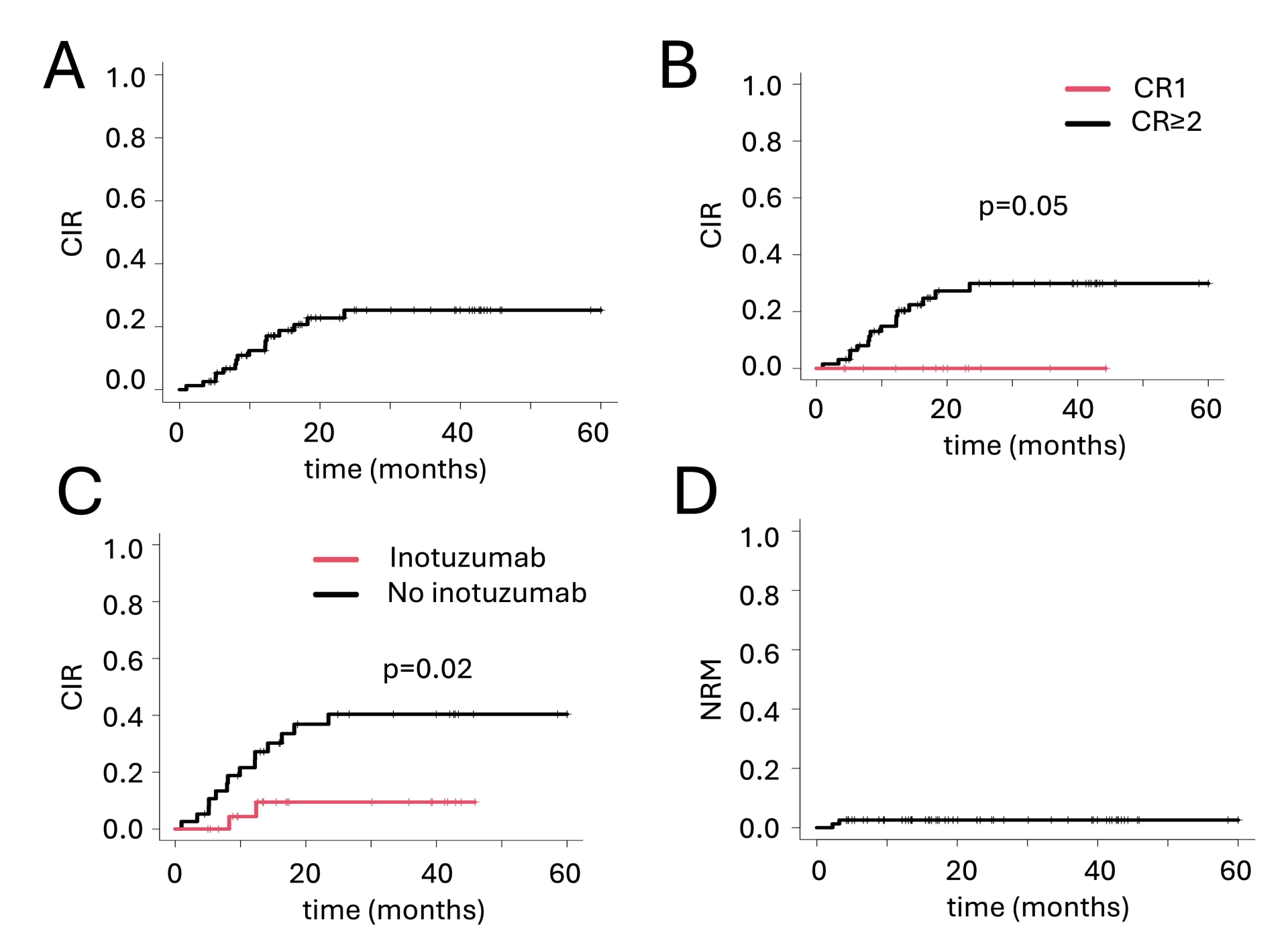
CI, confidence interval; CR, complete remission; HSCT, hematopoietic stem cell transplantation; TCD, T-cell depleted; TBI, total body irradiation.

* KMT2A rearrangements; Philadelphia or Philadelphia-like chromosome; hypodiploidy; constitutional trisomy of chromosome 21; TCF3-rearranged B-ALL.

Figure legends

Figure 1. **Cumulative Incidence Analyses for Relapse and Non-Relapse Mortality in the Cohort** (A) cumulative incidence of relapse (CIR) of the whole cohort. (B) CIR according to disease status at hematopoietic stem cell transplantation (complete remission 1 [red line] vs. complete remission ≥2 [black line]). (C) CIR according to use of inotuzumab in the induction/consolidation treatment of relapse, complete remission≥2 patients only (no inotuzumab [black line] vs. inotuzumab [red line]). (D) cumulative incidence of non-relapse mortality (NRM) of the whole cohort.

Figure 2. **Survival analysis of the Cohort** (A) overall survival (OS) of the whole cohort. (B) disease-free survival (DFS) of the whole cohort. (C) DFS according to complete remission number at hematopoietic stem cell transplantation (complete remission 1 [red line] vs. complete remission ≥2 [black line]). (D) DFS according to use of inotuzumab in the induction/consolidation treatment of relapse, complete remission ≥2 patients only (no inotuzumab [black line] vs. inotuzumab [red line]).


A
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0.8
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0.2
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CIR
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20
40
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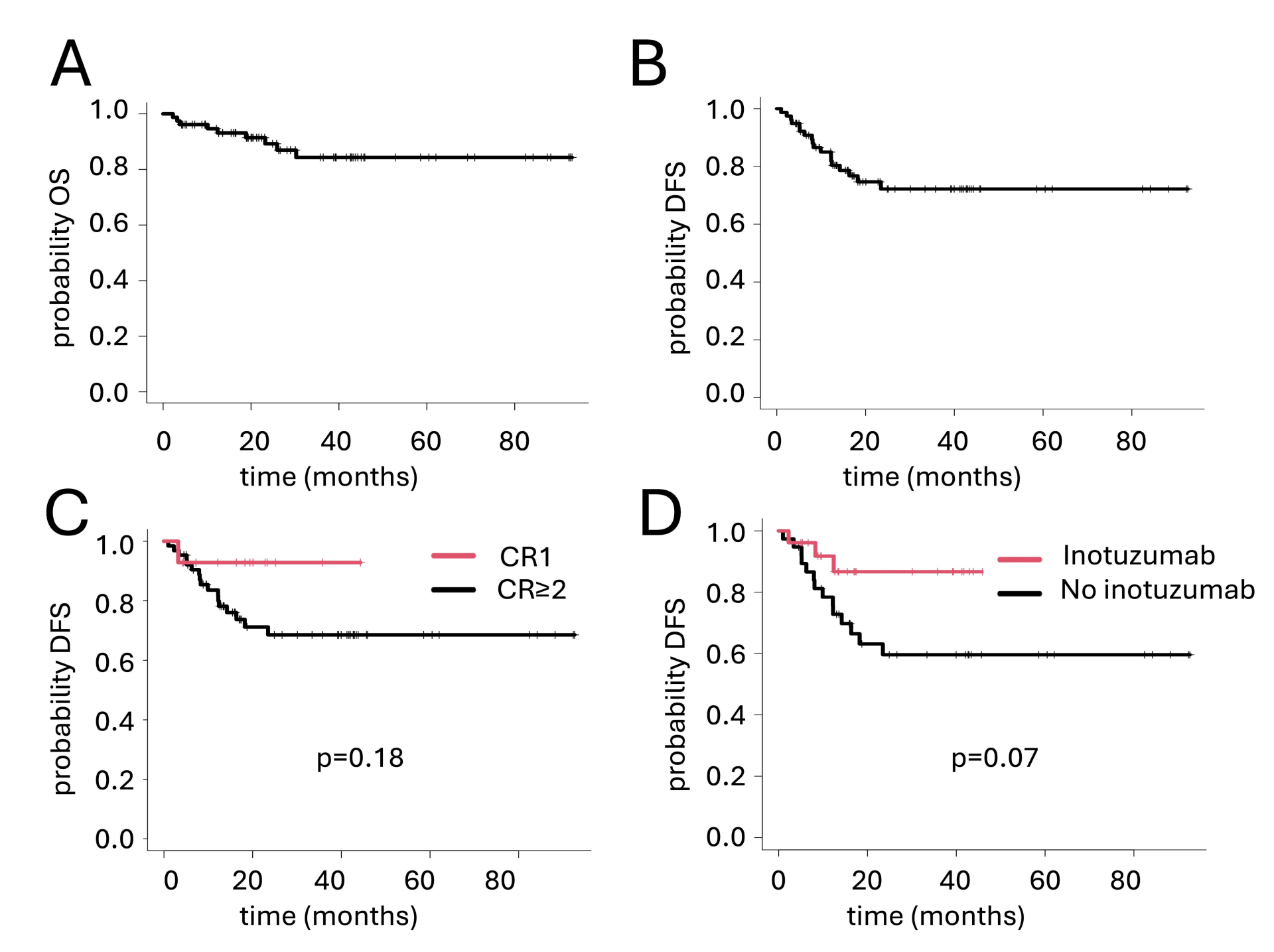
time (months)
B
CR1
CR≥2
p=0.05
C
Inotuzumab
No inotuzumab
p=0.02
D
NRM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0.0
CR1
CR≥2
p=0.18
0
20
40
60
80
time (months)
D
probability DFS
1.0
0.8
0.6
0.4
0.2
0.0
Inotuzumab
No inotuzumab
p=0.07
0
20
40
60
80
time (months)

Supplementary

Table S1: multivariable analysis for CI of aGVHD (Fine and Gray regression)

Variable	Hazard Ratio	Lower 95% CI	Upper 95% CI	p-value
Age Group	0.4685	0.2041	1.075	0.0740
CR number at HSCT	1.2200	0.4490	3.314	0.7000
TCD vs. unmanipulated	2.3810	0.6694	8.467	0.1800
Number of blinatumomab Cycles	3.4990	1.6020	7.640	0.0017

CR, complete remission; TCD, T-cell deplated

Table S2: multivariable analysis for CI of cGVHD (Fine and Gray regression)

Variable	Hazard Ratio	Lower 95% CI	Upper 95% CI	p-value
Age Group	16.1400	2.3260	112.000	0.0049
CR number at HSCT	0.7013	0.3320	1.481	0.3500
TCD vs. unmanipulated	4.4100	0.5417	35.900	0.1700
Number of blinatumomab cycles	2.3010	0.7661	6.913	0.1400

CR, complete remission; TCD, T-cell depleted

Table S3. Details on clinically significant infections

Type of infection/reactivation	Number of cases (percentage)
Cytomegalovirus reactivation	19 (24)
Epstein-Barr virus reactivation	6 (7.7)
Adenovirus reactivation	10 (12.8)
Varicella-Zoster virus reactivation	4 (5.1)
Respiratory viruses infection	29 (37.2)
SARS-CoV2	9 (11.5)
Systemic bacterial infection	15 (19.2)
Invasive aspergillosis	2 (2.6)

Table S4. Details on enfgraftment and immune reconstitution

ENGRAFTMENT				
Time to neutrophil engraftment* (median, interquartile range)			17 days (14-19)	
Time to platelet engraftment‡ (median, interquartile range)			16 days (11-20)	
IMMUNE RECONSTITUTION	Timepoint			
	1 month	3 months	6 months	12 months
Total lymphocytes	400 (70-2100)	510 (190-1870)	1100 (180-5170)	1760 (460-5640)
$CD3^+$ T cells/μL	182.74 (1.56-1646.73)	205 (12.98-1527.8)	459 (87-2595.34)	938 (275.3-3102)
$CD4^+$ T cells/μL	33.54 (0.32-520)	67.12 (0-457.46)	165.62 (15.4-1045)	417 (86.5-1186)
$CD8^+$ T cells/μL	56.64 (0.02-1467.5)	98 (0.59-1167.7)	203 (17-1559.8)	308 (92.7-1309)
$\alpha\beta^+$ T cells/μL	110 (0.34-1594)	163.3 (2.34-1490)	368.5 (58.9-2125)	764 (251-2404)
$\gamma\delta^+$ T cells/μL	33.93 (0.54-364)	31.2 (2.96-401.4)	54.72 (1.1-430.8)	87.5 (9.01-1305)
$CD3^-CD56^+$ NK cells/μL	178.5 (0.29-759.75)	215.6 (41.5-1209.5)	208 (49.6-892.5)	216 (41.5-1209)
Treg/μL	0.46 (0-39.44)	3.6 (0.12-28.27)	13.78 (1.5-46.1)	24.9 (9.98-93.7)
$CD19^+$ B cells/μL	0.39 (0-81)	26.7 (0-247)	136 (0-1576.8)	437 (64.3-1725)
IgA (g/L)	0.07 (0-0.76)	0.145 (0-0.67)	0.34 (0-1.34)	0.50 (0-2.35)
IgM (g/L)	0.095 (0-1.53)	0.22 (0-1.96)	0.51 (0-2.82)	0.56 (0-3.40)
Independence from IgG replacement therapy (%)	11.9	44.7	65.7	86.1

*Neutrophil engraftment: first day with a neutrophil count $\geq 0.5\times10^9$/L for three consecutive days;
‡Platelet engraftment: first day of achieving an unsupported platelet count $\geq 20\times10^9$/L for at least 10 days

Figure S1. Overall survival in the 16 patients who relapsed after HSCT.

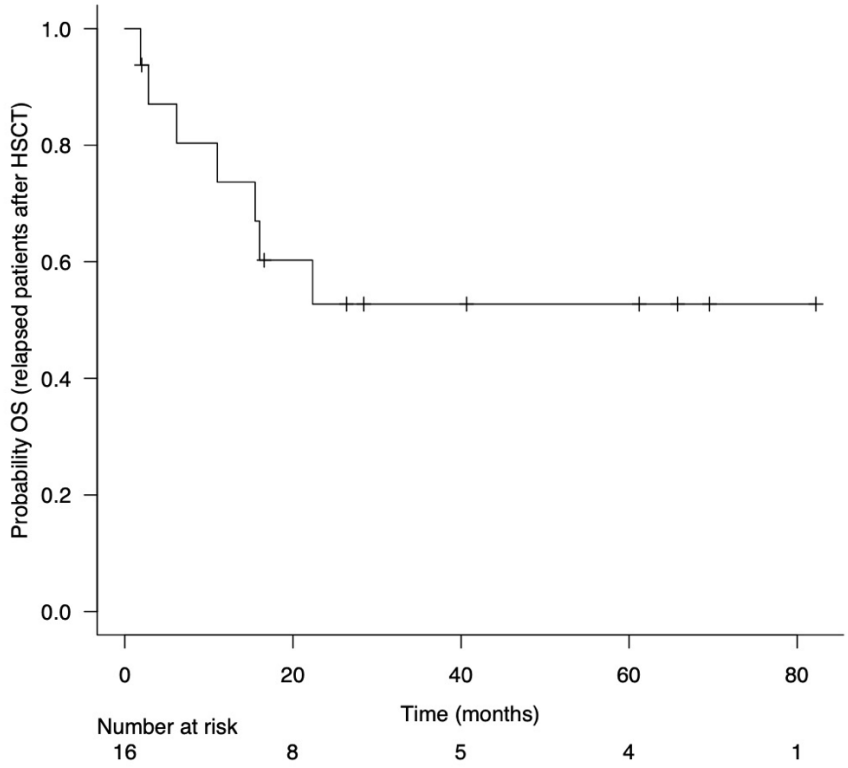

Figure S2. (A) Cumulative incidence of grade II-IV acute GVHD. (B) cumulative incidence of acute GVHD according to graft manipulation (T cell depleted haploidentical donors [black line] vs. unmanipulated allograft [red line]). (C) cumulative incidence of all-grades chronic GVHD. (D) cumulative incidence of all chronic GVHD according to graft manipulation (T cell depleted haploidentical donors [black line] vs. unmanipulated allograft [red line]).