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Allogeneic – Adult

Idarubicin-Intensified Hematopoietic Cell Transplantation Improves Relapse and Survival of High-Risk Acute Leukemia Patients with Minimal Residual Disease

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The optimal conditioning regimen of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for high-risk patients with minimal residual disease (MRD) remains controversial. We studied the results in 98 high-risk acute leukemia patients transplanted with idarubicin (IDA)-intensified conditioning regimens between 2012 January and 2017 January. Among these patients, 31 (31.6%) had more than 5% marrow blasts at time of transplantation and 67 patients were in morphologic remission: MRD negative status at time of conditioning was achieved in 39 patients (39.8%), whereas 28 (28.6%) remained carriers of any other positive MRD level in the bone marrow. Three-year relapse estimates of patients with MRD-positive remission was 22.0%, which was remarkably lower than patients with active disease (45.4%, $P = .027$) but approximate to that of patients in MRD-negative remission (15.5%, $P = .522$). There were no significant differences in terms of 3-year estimated overall survival (OS) and disease-free survival (DFS) between MRD-positive remission and MRD-negative remission groups (71.4% versus 79.1% [$P = .562$] and 67.9% versus 76.9% [$P = .634$], respectively). Moreover, the estimated rates of 3-year OS and DFS of patients in MRD-positive remission were significantly better than those in patients with active disease (71.4% versus 41.9% [$P = .033$] and 67.9% versus 38.7% [$P = .037$], respectively). These data indicate that IDA-intensified conditioning allo-HSCT could overcome the negative prognostic impact of MRD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents the only potentially curative therapy for high-risk patients with acute leukemia in first or subsequent complete remission (CR) [1]. Nevertheless, outcomes vary widely in acute leukemia patients who undergo allo-HSCT while in morphologic CR [2]. One key challenge to improve transplantation outcomes of high-risk acute leukemia is to accurately identify HSCT patients at high risk of relapse and to establish more effective interventions.

More recently, accumulating evidence suggests that the presence of minimal residual disease (MRD) at the time of transplantation detected using either quantitative real-time PCR (qRT-PCR) or multiparametric flow cytometry (MFC) was associated with adverse post-HSCT outcomes for those patients in morphologic CR [3-5]. Araki et al. [2] reported that the relapse rate and 3-year overall survival (OS) of acute myelocytic leukemia (AML) patients in MRD-positive morphologic CR were 67% and 26%, which was nearly parallel to the outcomes in patients with active AML. Sutton et al. [6] demonstrated that acute leukemia patients achieving bone marrow (BM) MRD negativity pre-HSCT had better outcomes (disease-free survival [DFS], 83%; OS, 92%) than those with persistent MRD pre-HSCT (DFS, 41%; OS, 64%). In this regard, all these studies of MRD had helped to identify patients who still harbor high levels of disease below the detection capabilities of morphologic analysis and have a high risk of relapse, confirming the need for developing innovative transplant strategies for those MRD-positive patients.

Relapse remains the most prominent problem in high-risk acute leukemia patients with MRD positivity [7,8]. In HLA-matched related or unrelated allo-HSCT for acute leukemia, several studies have reported a dose-dependent effect of the intensity of the conditioning regimen on disease control. In our previous studies, we demonstrated that allo-HSCT using idarubicin (IDA)-intensified conditioning regimens could reduce relapse of high-risk acute leukemia

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patients, which translated into improved survival [9-12]. One might anticipate that the intensity of the preparative regimen would be of greater importance in patients with MRD because more effective leukemic cell clearance and deeper molecular response are potentially achievable with intensified conditioning regimen for these high-risk patients. Nevertheless, to the best of our knowledge, whether treatment intensification before HSCT in efforts to reduce MRD levels diminishes the risk of relapse post-HSCT remains to be determined.

Herein, we investigated whether IDA-intensified conditioning regimens could improve the outcomes of MRD-positive CR patients, making these patients achieve more parallel outcomes with patients in MRD-negative remission but not active patients.

METHODS

Eligibility Criteria

A cohort of 98 consecutive patients with high-risk acute leukemia who underwent their first allo-HSCT using IDA-intensified conditioning regimens from January 2012 to January 2017 at the Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, were included in this retrospective study. We classified patients as high risk at diagnosis following the criteria in our previous reports [9-12]. The definition of high-risk AML were no response to induction chemotherapy, relapse within 6 months after induction or consolidation therapy, relapse within 6 months after induction therapy that could not be relieved using the original induction therapy, ≥2 relapses or relapse after auto-HSCT, unfavorable cytogenetics, or a history of preceding neoplasia and/or chemotherapy in no remission (NR). The definition of high-risk acute lymphocytic leukemia (ALL) consisted of age > 35 years, elevated WBC count (30×10^9/L for B cell lineage or 100×10^9/L for T cell lineage), beyond first CR (CR1), or high-risk cytogenetic abnormalities, determined according to the National Comprehensive Cancer Network 2013 guidelines, such as hypodiploidy, complex karyotype (≥5 chromosomal abnormalities), t(9;22) or BCR-ABL, t(v;11q23) or mixed lineage leukemia (MLL) rearrangements.

There were 51 high-risk AML and 47 high-risk ALL patients. All patients had BM examinations and MRD evaluation by qRT-PCR and/or MFC performed within 14 days of starting their preparative regimen. Patients who had ≥5% BM blasts in their pre-HSCT assessment were classified as having active acute leukemia (NR). Patients who achieved a complete or major molecular response were defined as MRD negative, and patients who carried any other positive MRD level in the BM-derived mononuclear cells were defined as MRD positive.

Table 1 summarizes the demographic and clinical characteristics of 3 groups in this study. All patients were treated on Institutional Review Board–approved protocols or standard treatment protocols and gave consent in accordance with the Declaration of Helsinki. The reference date of January 31, 2018, was used to define the end of the follow-up period.

MFC-Based Immunophenotypic Studies

Leukemia-associated immunophenotypes were considered to be leukemia cell surface antigen patterns that were either undetectable or found only in small numbers of normal BM cells. Eight-color MFC was performed as a routine clinical test on BM aspirates to obtain routine baseline assessment before allo-HSCT, using a panel of optimal combinations of antibodies to recognize residual leukemic cells in serial samples. MRD was identified by visual inspection as a population showing deviation from the normal patterns of antigen expression seen on the specific cell lineages at specific stages of maturation as compared with either normal or regenerating marrow samples [13-15]. Any level of residual disease was considered MRD positive [2,4,16]. The sensitivity of MFC-based MRD used in our center was 10^{-4}.

PCR-Based Molecular Studies

PCR methods for detection of fusion gene transcripts became an important MRD tool in acute leukemia because of its age-related high frequency [13,17]. The fusion genes including *WT1*, *AML1/ETO*, *CBFβ/MYH11*,

Table 1
Pretransplant Demographic and Clinical Characteristics of Study Cohort, Stratified by Disease Status

Characteristics	MRD Negative	MRD Positive	NR	*P*
No. of patients	39	28	31	
Median age, yr (range)	28 (10-57)	28.5 (15-55)	30 (10-50)	.753
Gender				
Male	28 (71.8)	16 (57.1)	23 (74.2)	.312
Female	11 (28.2)	12 (42.9)	8 (25.8)	
Diagnosis				.862
AML	19 (48.7)	15 (53.6)	17 (54.8)	
ALL	20 (51.3)	13 (46.4)	14 (45.2)	
Disease state				—
CR1	33 (84.6)	16 (57.1)	—	
CR2	6 (15.4)	8 (28.6)	—	
CR3	0 (0)	4 (14.3)	—	
Median duration from diagnosis to HSCT, mo (range)	6.0 (2-14)	5.5 (3-13)	5.0 (2-12)	.475
Donor/recipient related HSCT	31 (79.5)	21 (75.0)	23 (74.2)	.882
HLA matching: 6/6	8 (20.5)	3 (10.7)	3 (9.7)	
HLA matching: 5/6	3 (7.7)	1 (3.6)	2 (6.5)	
HLA matching: 4/6	14 (35.9)	12 (42.9)	15 (48.4)	
HLA matching: 3/6	6 (15.4)	5 (17.9)	3 (9.7)	
Donor/recipient unrelated HSCT	8 (20.5)	7 (25.0)	8 (25.8)	.909
HLA matching: 10/10	5 (12.8)	3 (10.7)	6 (19.4)	
HLA matching: 9/10	2 (5.1)	3 (10.7)	1 (3.2)	
HLA matching: ≤8/10	1 (2.6)	1 (3.6)	1 (3.2)	
ABO match				.894
Mismatched	16 (41.0)	13 (46.4)	14 (45.2)	
Matched	23 (59.0)	15 (53.6)	17 (54.8)	
CMV serostatus				.590
Positive/positive	10 (25.6)	7 (25.0)	4 (12.9)	
Positive/negative	7 (17.9)	2 (7.1)	6 (19.4)	
Negative/positive	5 (12.8)	3 (10.7)	6 (19.4)	
Negative/negative	17 (43.6)	16 (57.1)	15 (48.4)	
Median nucleated cells, $\times 10^8$/kg (range)	14.37 (9.23-39.12)	17.80 (6.50-38.03)	15.06 (7.26-38.35)	.647
Median $CD34^+$ cells, $\times 10^6$/kg (range)	6.40 (2.05-14.95)	7.40 (2.65-11.79)	6.68 (2.36-11.15)	.436
Median follow-up for survivors, mo (range)	29 (12-67)	30 (12-71)	30 (12-70)	.571

Values are n (%) unless otherwise defined.

MLL-PTD, *MLL-AF9*, *MLL/AF4*, *BCR/ABL*, and *E2A/PBX1* in isolated BM mononuclear cells were detected using qRT-PCR following the Europe Against Cancer program recommendation with a sensitivity of 10^{-5}. The absolute copy numbers of fusion gene transcripts were standardized according to the expression of the housekeeping gene *ABL*. Also, any level of fusion genes was considered MRD positive [18]. The sensitivity of PCR-based MRD used in our center was 10^{-5} to 10^{-6}.

Conditioning Regimens

The IDA-BUCy2 regimen provided for high-risk AML was as follows: IDA administered by continuous i.v. injection (15 mg/m^2/day from days −11 to −9), followed by i.v. injection of busulfan (BU) (3.2 mg/kg/day in divided doses, from days −6 to −4), and i.v. injection of cyclophosphamide (Cy) (1.8 g/m^2/day from days −3 to −2). For high-risk ALL patients a conditioning regimen of IDA-intensified total body irradiation (TBI)-Cy were given as follows: TBI of 8 Gy was administered in a single fraction from a linear accelerator with partial shielding of the lungs (4 Gy) on day −8 and IDA of 15 mg/m^2/day was administered by continuous i.v. injection for more than 20 hours from days −6 to −5, followed by i.v. injection of Cy (60 mg/kg/day) over 2 hours on days −3 to −2. For a donor–recipient HLA 4/6-matched setting, patients additionally received antithymocyte globulin (Thymoglobulin; Sanofi Aventis, Paris, France) at a total dose of 6 mg/kg (3 mg/kg/day on days −1 to 0). For HLA 3/6-matched transplant, a total dose of 9 mg/kg antithymocyte globulin (3 mg/kg/day on days −4 to −2) was administrated.

HLA Typing and Stem Cell Source

For HLA-identical transplantation donor–recipient pairs were typed at the HLA-A, -B, and -DRB1 loci at our institute. To determine HLA-A and HLA-B status, low-resolution DNA techniques were used. High-resolution techniques were used for HLA-DRB1 typing. For unrelated donor transplantation donor–recipient pairs were typed at the HLA-A, -B, -C, -DRB1, and -DQB1 using high-resolution techniques. Each patient with haploidentical HSCT received stem cells from a family member who shared 1 HLA haplotype with the patient but differed to varying degrees for the HLA-A, -B, and -DR antigens of the haplotype, which were not shared. Donors were primed with recombinant human granulocyte colony-stimulating factor (rhG-CSF) 8 to 10 μg/kg per day injected subcutaneously to mobilize BM and/or peripheral blood. For HLA-identical sibling unrelated donor and HLA 4/6-matched transplantation, unmanipulated rhG-CSF–primed peripheral blood stem cells were infused into the recipient on the day of collection. For the donor–recipient HLA 3/6-matched setting, both rhG-CSF–primed BM (harvested on day 0, 4 days after G-CSF) and rhG-CSF–primed peripheral blood stem cells (harvested on days 1 and 2, 5 to 6 days after G-CSF) were harvested and infused into the recipients on the day of collection without manipulation.

Graft-versus-Host Disease Prophylaxis

A graft-versus-host disease (GVHD) prophylaxis regimen consisting of cyclosporine A (CsA) and short-term methotrexate was given for HLA-identical sibling transplantation. Mycophenolate mofetil (7.5 mg/kg, orally twice daily) and anti-CD25 MoAb (basiliximab) were additionally given to the patients unrelated to their donors. All haploidentical HSCT recipients received a combination of CsA, short-term methotrexate, mycophenolate mofetil, and basiliximab for GVHD prophylaxis. Intravenous CsA was started (2.5 mg/kg/day) on day −1 with target trough levels of 150 to 250 ng/mL for at least 1 month and continued until patients were able to take CsA orally. CsA was progressively tapered by 5% weekly and discontinued after around 3 to 4 months in cases with no evidence of GVHD. MTX was administered i.v. at dosages of 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. Mycophenolate mofetil was administered from day −9, which was tapered to half until day +60 and was discontinued thereafter based on the presence or absence of severe GVHD, infectious diseases, and relapse risk. Basiliximab was given i.v. at a dose of 20 mg by 30-minute i.v. infusion on day 0 (2 hours before graft infusion) and day +4. The clinical diagnosis (with biopsy confirmation if clinically appropriate) of acute GVHD (aGVHD) was established according to consensus criteria [19]. Chronic GVHD (cGVHD) was assessed using published criteria [20].

Hematopoietic Engraftment and Transplant-Related Toxicity

The definition of hematopoietic engraftment was similar to that reported previously [9-12]. Chimerism was typically evaluated in recipient BM or whole peripheral blood without separation usually on days +30, +90, +180, +270, and +360 after transplantation. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Supportive Care

Institutional standard antimicrobial prophylaxis covering fungal and bacterial agents were administered in all patients [9-12]. Cytomegalovirus (CMV) monitoring was performed once a week using a RT Taqman CMV DNA PCR until day 100 after allo-HSCT, then once every 2 weeks until day 180, followed by once every month until 1 year after HSCT. Preemptive ganciclovir therapy or foscarnet was administered to patients who had 2 consecutive positive tests for CMV DNA and was continued until the CMV DNA monitoring was negative on 2 occasions. BM aspiration for morphologic and cytogenetic analysis using flow cytometry and qRT-PCR was scheduled for 1, 3, 6, 9, and 12 months and then every 3 months during months 12 to 24 post-HSCT. When hematologic or cytogenetic relapse was diagnosed after HSCT, the relapse was treated with a trial phase of immunosuppressant withdrawal followed by therapeutic donor lymphocyte infusion.

Statistical Analysis

Comparisons among groups were compared using the Wilcoxon rank-sum test for the continuous variables and Fisher's exact test for the categorical variables. Death was the competing risk for neutrophil and platelet engraftments, aGVHD and cGVHD, and relapse. Transplant-related mortality (TRM) was defined as early death and death occurring in CR without previous relapse and was considered a competing risk for relapse, whereas relapse was considered a competing risk for TRM. Estimates of OS and DFS were calculated by the Kaplan-Meier method. Comparisons of OS and DFS among different groups were evaluated using the log-rank test. The potential prognostic factors for OS, DFS, and cumulative incidence of relapse were evaluated using Cox proportional hazards regression with a backward-stepwise model selection approach.

All P values were 2-sided, and $P < .05$ was considered as significant. Data analyses were primarily conducted with SPSS software (SPSS Inc., Chicago, IL), whereas graphs were drawn with Graphpad Prism 5.0 (Graphpad Software, Inc., San Diego, CA).

RESULTS

Patient Characteristics

The characteristics of the study population, donors, and transplants stratified by disease status are summarized in Table 1. The median follow-up for all patients was 20 months (range, 2 to 71), whereas for survivors it was 30 months (range, 12 to 71). There was no statistically significant difference among patients in MRD-negative remission, in MRD-positive remission, and with active disease. In the MRD-negative group there were 33 patients in CR1, 6 in CR2, and 0 in third CR (CR3). There were 16 cases in CR1, 8 in CR2, and 4 in CR3 in MRD-positive group. There was significant difference between 2 groups with regards to the disease state before allo-HSCT ($P = .01$). Seventy-five patients received donor–recipient HLA-related HSCT, whereas 23 patients underwent HLA-unrelated HSCT. Among HLA-related HSCT, there were 14 HLA 6/6-matched donors, 6 HLA 5/6-matched donors, 41 HLA 4/6-matched donors, and 14 HLA 3/6-matched donors. Among HLA-unrelated HSCT there were 14 HLA 10/10-matched donors, 6 HLA 9/10-matched donors, and 3 HLA ≤8/10-matched donors. Although the level of HLA typing varied among donor types, there were no significant differences among the 3 groups. The median doses of infused nucleated and CD34$^+$ cells for the whole study were 17.35×10^8/kg (range, 6.50 to 39.12) and 7.49×10^6/kg (range, 2.05 to 14.95), respectively.

Engraftment and Regimen Toxicities

All except 1 patient achieved sustained, full-donor chimerism. The patient who failed to achieve sustained, full-donor chimerism was identified by heterosomes using fluorescein in situ hybridization in recipient BM cells without separation. The neutrophil engraftments occurred at 13 days (range, 8 to 22) for MRD-negative remission, at 12 days (range, 11 to 24) for MRD-positive remission, and at 13 days (range, 9 to 21) for patients with active disease. The time to neutrophil engraftment was comparable among the 4 groups ($P = .328$). Time to engraftment of platelets did not differ among the 3 groups (MRD-negative remission, 15 days [range, 11 to 29]; MRD-positive remission, 14 days [range, 11 to 26]; NR group, 13 days [range, 9 to 25]; $P = .450$).

Most cases only experienced mild to moderate fever during the neutropenic period among the 3 groups (64.1%, 60.7%, and 58.1%; $P = .874$). Toxicities involving the gastrointestinal tract

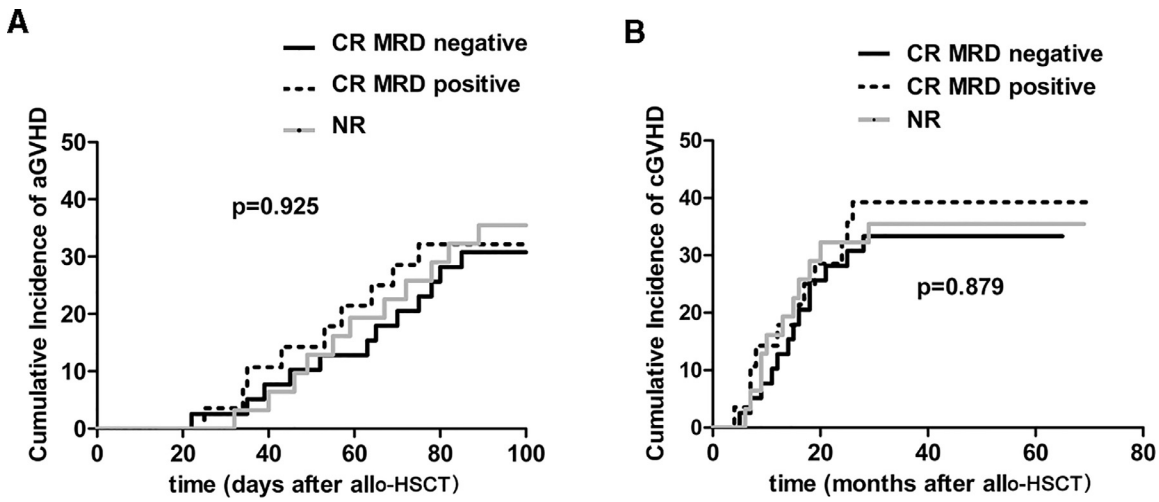

Figure 1. Estimates of (A) aGVHD and (B) cGVHD after intensified conditioning allo-HSCT for acute leukemia patients, shown individually for patients in MRD-negative and MRD-positive morphologic remission as well as those with active acute leukemia.

were commonly observed, including mild nausea and vomiting (41.0%, 35.7%, and 38.7%; $P = .908$) and diarrhea (23.1%, 28.6%, and 32.3%; $P = .687$). Oropharyngeal mucositis was more often observed in those patients receiving IDA-intensified conditioning regimens. The cumulative incidences of oropharyngeal mucositis in the 3 groups were 61.5%, 57.1%, and 64.5%, respectively ($P = .844$). Reversible elevations of liver enzymes were commonly seen in the 3 groups (53.8%, 46.4%, and 48.4%; $P = .816$). However, only 1 patient with high-risk AML in the MRD-negative group receiving HLA-identical allo-HSCT suffered mild hepatic veno-occlusive disease that was relieved by early intervention. Reversible elevations of creatinine and/or urea nitrogen were also often observed in all groups (43.6%, 39.3%, and 35.5%; $P = .787$). No cardiotoxicity was recorded except that 4 cases in the MRD-positive group and 2 in the NR group had grades 1 to 2 hypertension. Pulmonary toxicity and transplant-related thrombotic microangiopathy were not observed in any patients.

Acute and Chronic GVHD

As shown in Figure 1A, grades I to IV aGVHD occurred at a median time of 64 days (range, 22 to 85), 53 days (range, 25 to 75), and 59 days (range, 32 to 89) in MRD-negative, MRD-positive, and NR groups after intensified conditioning allo-HSCT, with 100-day cumulative incidences of 30.8%, 32.1%, and 35.5%, respectively ($P = .925$). The 100-day cumulative incidences of grades II to IV aGVHD in the 3 groups were 26.8%, 25.3%, and 28.7%, respectively ($P = .878$). The 100-day cumulative incidence of grades III to IV aGVHD was comparable among the 3 groups (MRD-negative, 15.3%; MRD-positive, 18.5%; NR, 17.5%; $P = .782$).

As shown in Figure 1B, the cumulative incidences of cGVHD for patients in MRD-negative, MRD-positive, and NR groups were 33.3%, 39.3%, and 35.5%, respectively ($P = .879$). The cumulative incidences of cGVHD were not statistically significantly different among the 3 groups (limited cGVHD, $P = .316$; extensive cGVHD, $P = .567$).

Relapse

As illustrated in Figure 2A, the 3-year estimated cumulative incidence of relapse in the 3 groups was 15.5%, 22.0%, and 45.4%, respectively. The relapse rate of NR patients was remarkably higher than that in MRD-negative and MRD-positive remission patients ($P = .007$ and $P = .027$). However, there was no significant difference between patients in MRD-negative and MRD-positive remission ($P = .522$). In the MRD-negative group hematologic relapse occurred in 4 patients, cytogenetic relapse in 1 patient, and extramedullary relapse in 1 patient. In the MRD-positive group there were hematologic relapse in 3 patients, cytogenetic relapse in 1 patient, and extramedullary relapse in 2 patients. However, 9 hematologic relapses, 3 cytogenetic relapses, and 2 extramedullary relapses occurred in patients with active disease. Additionally, the NR group had significantly higher relapse-related mortality when compared with MRD-negative and MRD-positive groups ($P = .001$ and $P = .031$).

As for high-risk AML patients, the 3-year estimated cumulative incidences of relapse after allo-HSCT were 10.5% in the MRD-negative group, 20.0% in the MRD-positive group, and 41.2% in NR patients, separately. The relapse rate of NR patients was remarkably higher than that in MRD-negative and MRD-positive patients ($P = .003$ and $P = .019$; Figure 3A). It was noteworthy that there was no significant difference between AML patients in MRD-negative and MRD-positive groups ($P = .446$). In the high-risk ALL group 4 patients (20.0%) in the MRD-negative group, 3 (23.1%) in the MRD-positive group, and 7 (50.0%) in the NR group exhibited disease recurrence after transplantation. The 3-year estimated cumulative incidence of relapse in NR patients was remarkably higher than that in MRD-negative patients and MRD-positive patients ($P = .021$ and $P = .034$; Figure 4A). No significant differences existed between MRD-negative and MRD-positive groups ($P = .802$).

TRM and Cause of Death

As for the estimated cumulative incidences of 3-year TRM, there was no significant difference among MRD-negative remission, MRD-positive remission, and NR groups (10.6%, 17.9%, and 24.7%; $P = .364$; Figure 2B). The estimated cumulative incidences of TRM at 3 years among the 3 groups were also not significant regardless of high-risk AML (10.5%, 13.3%, and 16.4%; $P = .653$; Figure 3B) or ALL (10.1%, 23.1%, and 21.4%; $P = .577$; Figure 4B).

As shown in Table 2, the major single cause of death was recurrence of disease. Four patients (10.3%) in MRD-negative remission, 3 patients (10.7%) in MRD-positive remission, and 11 patients (35.5%) in NR died of relapse of primary disease. Four patients (10.3%) in MRD-negative remission died from TRM, including severe infection (n = 2), aGVHD (n = 1), and

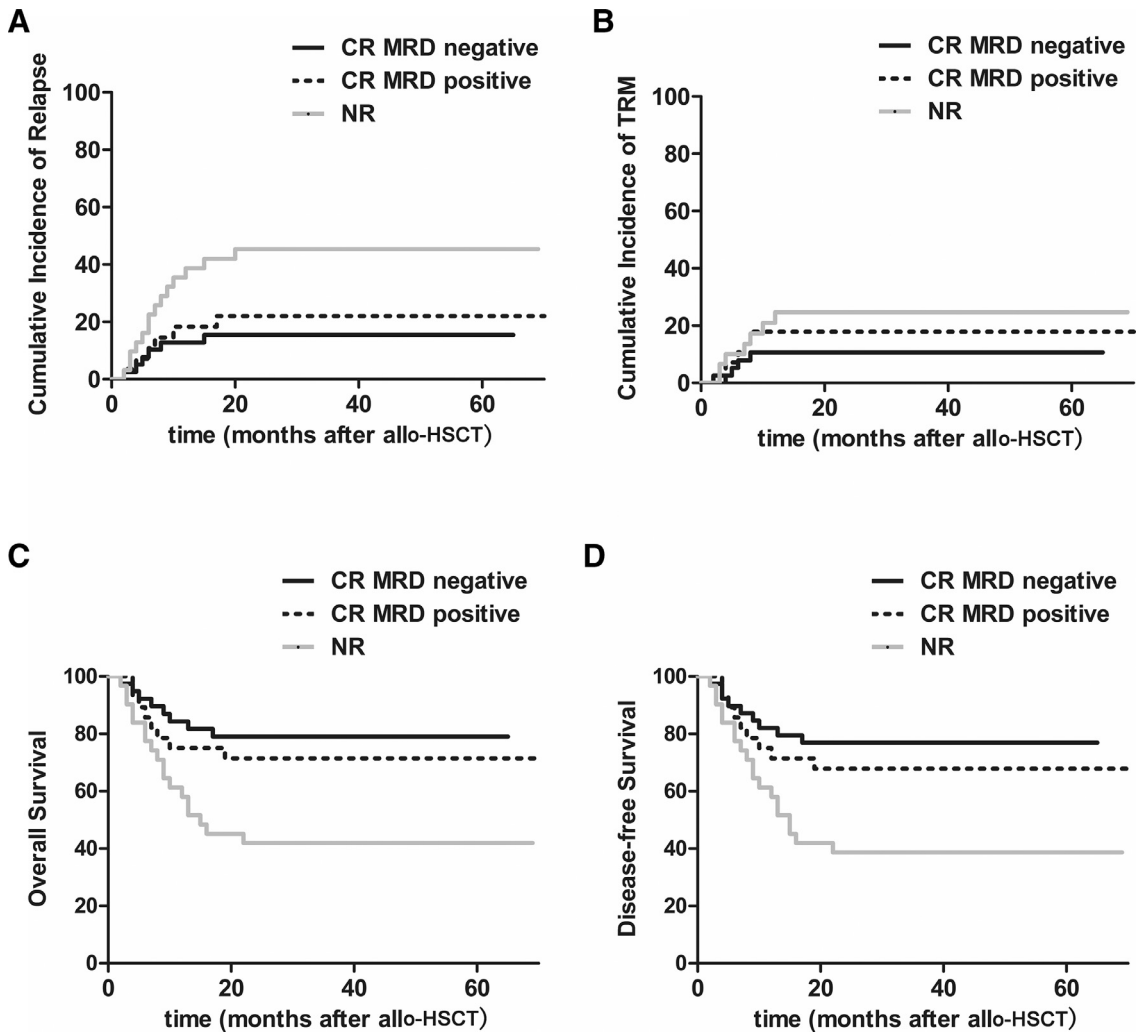

Figure 2. Association between pretransplant disease status and outcome for patients with acute leukemia after IDA-intensified conditioning HSCT. Cumulative incidence of (A) relapse and (B) TRM and estimates of (C) OS and (D) DFS after IDA-intensified conditioning HSCT for patients with acute leukemia, shown individually for patients in MRD-negative and MRD-positive morphologic remission as well as those with active acute leukemia.

cGVHD (n = 1). Five patients (17.9%) in MRD-positive remission died from TRM, including severe infection (n = 1), aGVHD (n = 2), cGVHD (n = 1), and other (n = 1). In the NR group 7 patients (22.6%) died of TRM, including severe infection (n = 2), aGVHD (n = 2), cGVHD (n = 1), rejection (n = 1), and other (n = 1).

OS and DFS

The median follow-up time after allo-HSCT for survivors at last contact was 29 months (range, 12 to 67) for patients in MRD-negative remission, 30 months (range, 12 to 71) for patients in MRD-positive remission, and 30 months (range, 12 to 70) for patients with active disease. The estimated cumulative probabilities of 3-year OS and DFS in the 3 groups were 79.1%, 71.4%, and 41.9% and 76.9%, 67.9%, and 38.7%, respectively. The probabilities of estimated 3-year OS and DFS of NR patients was significantly impaired when compared with MRD-negative remission patients and MRD-positive remission patients (3-year OS, $P = .002$ and $P = .033$; 3-year DFS, $P = .003$ and $P = .037$; Figure 2C,D). In particular, the survival of MRD-positive remission patients was comparable with MRD-negative remission patients (3-year OS, $P = .464$; 3-year DFS, $P = .426$).

As for high-risk AML patients, the estimated cumulative probabilities of 3-year OS after allo-HSCT were 83.3% in MRD-negative remission, 73.3% in MRD-positive remission, and 47.1% in NR patients, respectively. The estimated cumulative probabilities of 3-year DFS were 78.9%, 68.8%, and 41.2%, respectively. The estimated 3-year OS and DFS of NR patients was remarkably inferior when compared with that in MRD-negative remission and MRD-positive remission patients (3-year OS, $P = .022$ and $P = .035$; 3-year DFS, $P = .026$ and $P = .039$; Figure 3C,D).

In the high-risk ALL group the estimated cumulative probabilities of 3-year OS were 75.0% in MRD-negative remission, 69.2% in MRD-positive remission, and 38.9% in NR patients, respectively. The estimated cumulative probabilities of 3-year DFS were 71.4%, 64.3%, and 37.5% in the 3 groups. The estimated 3-year OS and DFS of MRD-negative remission and MRD-positive remission patients was remarkably higher than that in NR patients (3-year OS, $P = .035$ and $P = .041$; 3-year OS, $P = .032$ and $P = .038$; Figure 4C,D). There were no significant differences between MRD-negative remission and MRD-positive remission patients in terms of estimated 3-year OS and DFS regardless of high-risk AML or ALL (Figures 3C,D and 4C,D).

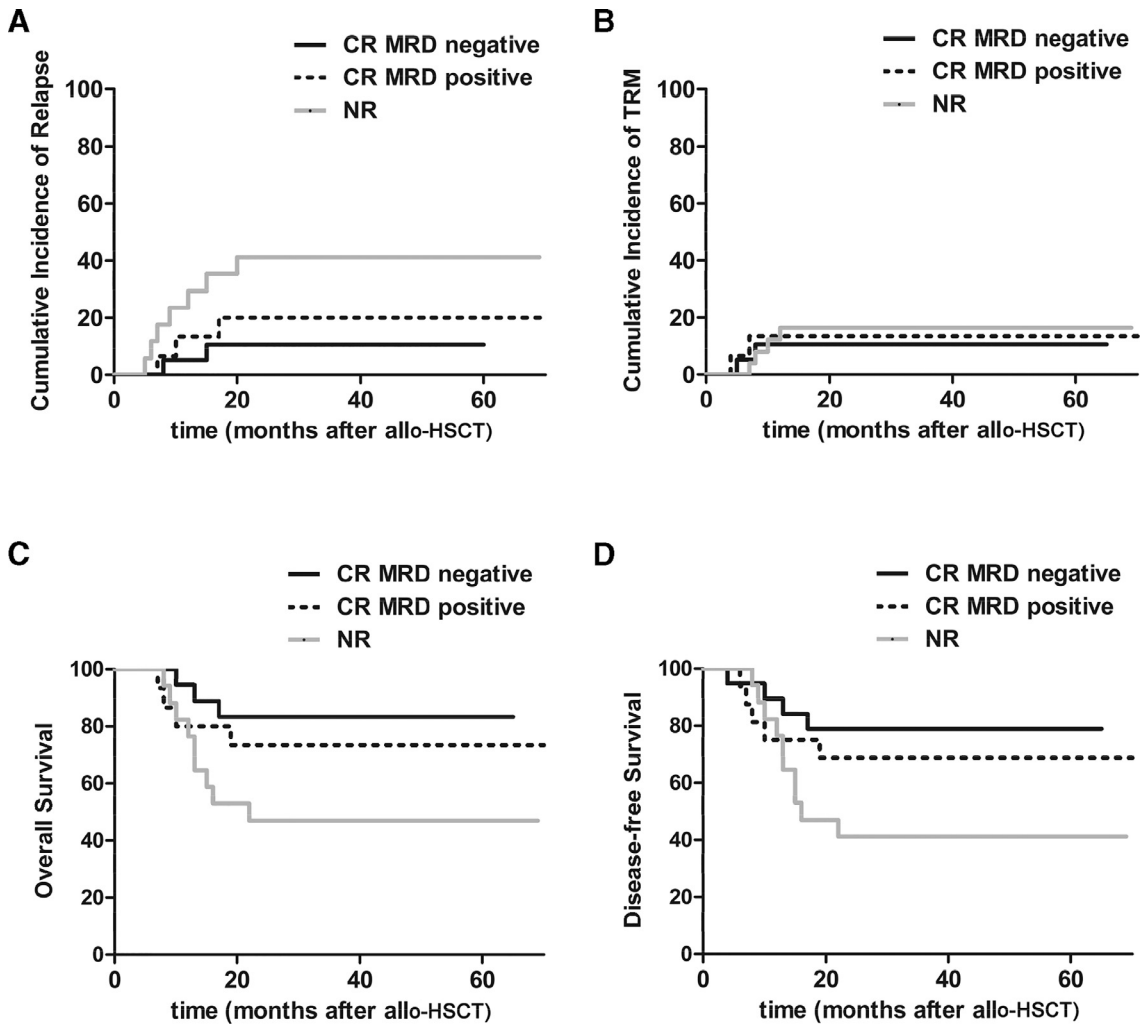

Figure 3. Association between pretransplant disease status and outcome for patients with high-risk AML after IDA-intensified conditioning HSCT. Cumulative incidence of (A) relapse and (B) TRM and estimates of (C) OS and (D) progression-free survival after IDA-intensified conditioning HSCT for patients with AML, shown individually for patients in MRD-negative and MRD-positive morphologic remission as well as those with active AML.

Multivariate Analysis

As shown in Table 3, for all high-risk acute leukemia patients, those who were in NR at the time of allo-HSCT had a remarkably higher relapse and worse 3-year OS and DFS ($P = .005$, $P = .014$, and $P = .015$). Patients who experienced limited cGVHD had a significantly lower relapse and better 3-year OS and DFS ($P = .038$, $P = .037$, and $P = .045$). The occurrence of grades III to IV aGVHD was found to be associated with worse OS and DFS ($P = .026$ and $P = .045$) but not relapse. In addition, multivariate analysis failed to show significant differences in relapse, OS, and DFS between high-risk AML and ALL. There were no remarkable differences between haploidentical and nonhaploidentical groups in terms of relapse rate, OS, and DFS. Other factors were not found to be associated with relapse, OS, and DFS.

Haploidentical HSCT versus Nonhaploidentical HSCT

Fifty-five patients were transplanted from haploidentical donors, whereas 43 patients received transplantation from nonhaploidentical donors. We then compared the relapse rate, OS, and DFS between haploidentical and nonhaploidentical groups. As illustrated in Figure 5A, the 3-year estimated cumulative incidences of relapse in the haploidentical and nonhaploidentical groups were 22.6% and 30.1%, respectively ($P = .402$). The 3-year rates of OS and DFS in the 2 groups were 68.6% and 66.2% and 60.1% and 58.8%, respectively ($P = .367$ and $P = .318$; Figure 5B,C).There were no remarkable differences between the 2 groups in terms of relapse rate, OS, and DFS. Therefore, we did not find that the use of haploidentical donors had any advantage on outcomes due to a possible enhanced graft-versus-leukemia effect.

DISCUSSION

The purpose of this retrospective study was to explore the efficacies of IDA-intensified conditioning regimens for high-risk acute leukemia patients with positive MRD that was considered as a powerful prognostic factor independent of any other traditional risk factor (age, WBC count, immunophenotype, genetics/cytogenetics). Findings from the current study demonstrated that our IDA-intensified allo-HSCT could provide better disease control for high-risk acute leukemia patients with MRD positivity (3-year OS, 71.4%; 3-year DFS, 67.9%), making these patients achieve results more parallel with MRD negativity and significantly better than patients with active disease.

Post-transplant relapse remains a major cause of treatment failure, and further therapeutic options are limited [21]. The use of MRD as a biomarker for the intrinsic resistance of the leukemia to therapy has come of age in acute leukemia regardless of during

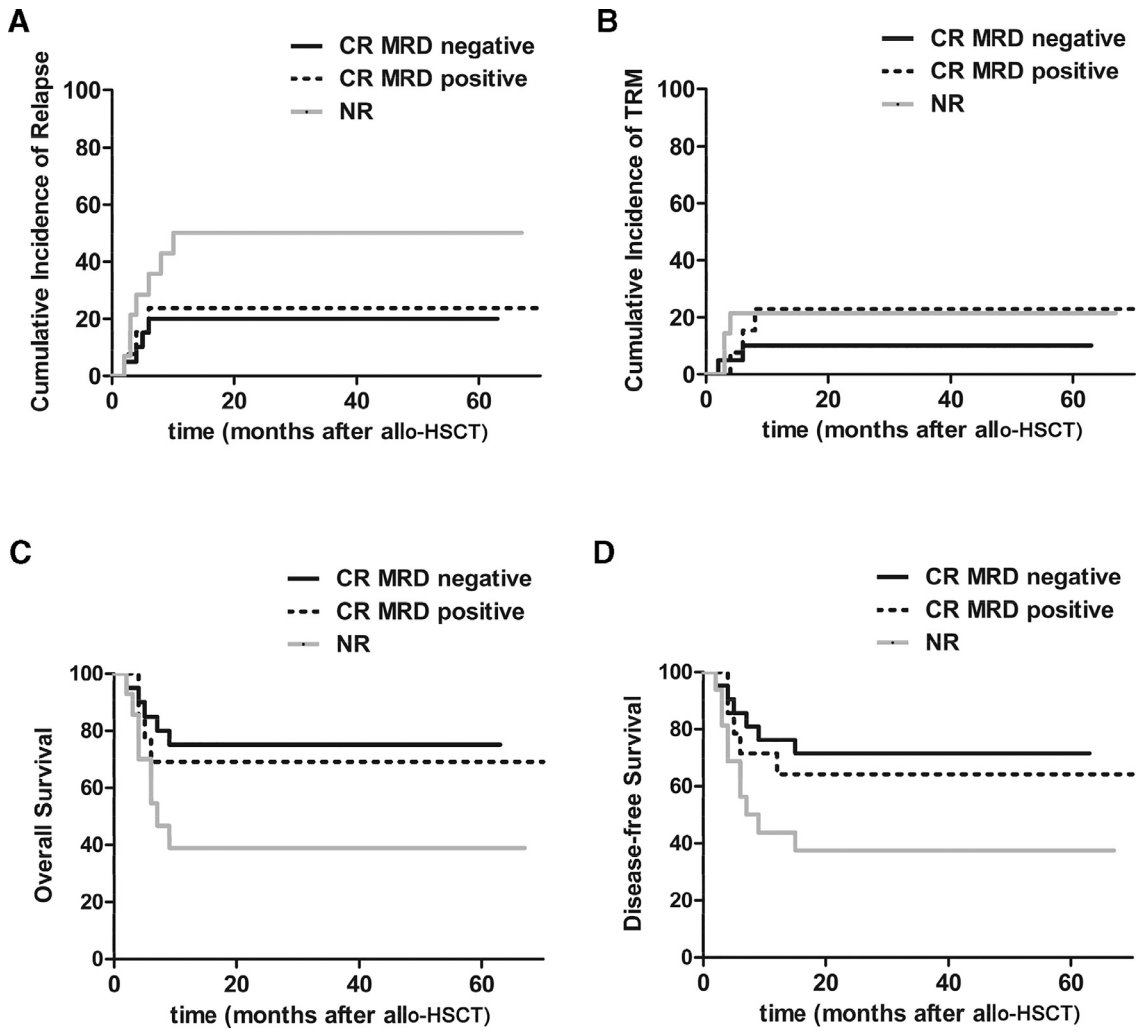

Figure 4. Association between pretransplant disease status and outcome for patients with high-risk ALL after IDA-intensified conditioning HSCT. Cumulative incidence of (A) relapse and (B) TRM and estimates of (C) OS and (D) progression-free survival after IDA-intensified conditioning HSCT for patients with ALL, shown individually for patients in MRD-negative and MRD-positive morphologic remission as well as those with active ALL.

Table 2
Primary Causes of Death in the Study Cohort, Shown Individually for Patients in MRD-Negative and MRD-Positive Morphologic Remission and in Those with Active Acute Leukemia

	MRD Negative	MRD Positive	NR	*P*
No. of patients	39	28	31	
Relapse	4 (10.3)	3 (10.7)	11 (35.5)	.012
TRM	4 (10.3)	5 (17.9)	7 (22.6)	.401
Rejection	0	0	1 (3.2)	.602
aGVHD	1 (2.6)	2 (7.1)	2 (6.5)	.730
cGVHD	1 (2.6)	1 (3.6)	1 (3.2)	.971
Infection	2 (5.1)	1 (3.6)	1 (3.2)	.911
Other	0	0	1 (3.2)	.602

Values are n (%).

conventional chemotherapy or allo-HSCT. The presence of MRD is usually associated with an increased risk of disease recurrence and worse outcome [22]. Relative to MRD-negative patients, MRD-positive patients in CR1 undergoing allo-HSCT had a significantly worse outcome with a 3-year cumulative incidence of relapse that approximated 60% to 76%, resulting in an estimated survival of approximately 20% to 30%, with MRD being the dominant risk factor for adverse outcomes [8,23,24]. Because these outcomes with current approaches to allo-HSCT for MRD-positive CR patients are unsatisfactory, it is appealing to consider treatment intensifications during induction/postremission therapy and/or before transplantation in an attempt to induce an MRD negative state [25].

Conversion to MRD negativity using additional courses of chemotherapies is a reasonable immediate therapeutic goal; however, the administration of further chemotherapy might lead to organ toxicity or life-threatening infections that delay or prevent planned transplant procedure, even at a risk of losing the opportunity to undergo allo-HSCT for these high-risk patients [26]. There is also a possibility that the disease progresses while increasing chemotherapy regimens. Buccisano et al. [27] analyzed the kinetics of MRD reduction during the different phases of therapy for adult AML patients. Among 92 patients, only 10 AML patients were MRD positive after induction and rendered MRD negative after consolidation chemotherapy, 26 patients with MRD after induction therapy remained positive even after consolidation therapy, and 9 patients who were MRD negative converted to MRD positivity after consolidation chemotherapy. A large analysis of Center for International Blood and Marrow Transplant Research data on 524 ALL patients demonstrated that chemotherapy provided no

Table 3
Multivariate Analysis of Factors Associated with Outcomes of High-Risk Acute Leukemia Patients after IDA-Intensified Conditioning Allo-HSCT

Covariates	Hazard Ratio	95% Confidence Interval	*P*
Relapse			
Disease status (NR vs. CR)	2.606	1.341-5.089	.005
ALL vs. AML	1.456	.751-2.765	.261
Limited cGVHD	.218	.081-.938	.038
HLA related vs. unrelated	.814	.394-2.369	.855
Haploidentical vs. nonhaploidentical	.715	.326-1.568	.327
OS			
Disease status (NR vs. CR)	2.762	1.232-6.193	.014
ALL vs. AML	1.309	.788-2.174	.298
aGVHD grades III-IV	2.479	1.212-3.984	.026
Limited cGVHD	.129	.021-.943	.037
HLA related vs. unrelated	1.086	.436-2.468	.856
Haploidentical vs. nonhaploidentical	.729	.367-1.449	.486
DFS			
Disease status (NR vs. CR)	2.696	1.584-4.292	.015
ALL vs. AML	1.425	.879-2.312	.151
aGVHD grades III-IV	2.438	1.233-3.906	.045
Limited cGVHD	.179	.047-.816	.023
HLA related vs. unrelated	.867	.387-1.956	.724
Haploidentical vs. nonhaploidentical	.737	.373-1.457	.370

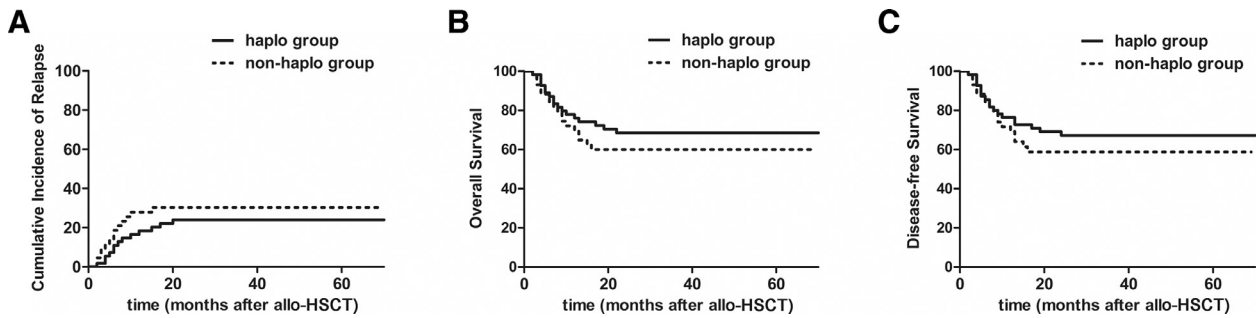

Figure 5. Comparison between patients receiving IDA-intensified conditioning haploidentical HSCT and nonhaploidentical HSCT. Cumulative incidence of (A) relapse and estimates of (B) OS and (C) progression-free survival after IDA-intensified conditioning HSCT for patients with acute leukemia, shown individually for patients receiving haploidentical and nonhaploidentical HSCT.

apparent advantage for CR1 patients with an immediately available donor considered for a prompt myeloablative allo-HSCT [28]. Moreover, additional intensive chemotherapy might not always reduce the leukemia burden because of enhanced resilience of leukemia cells that survive the multiagent regimens [29]. Based on these findings we recommended that the decision regarding firm allocation to allo-HSCT conditioning with intensified regimens might be beneficial for these high-risk patients with an MRD-positive CR situation.

Given that deeper control of leukemia pretransplant is crucial for preventing relapse post-transplant in high-risk acute leukemia, the intensification of conditioning regimens has been studied to offer stronger antitumor activity for these MRD-positive high-risk diseases in the area of allo-HSCT. Findings from at least 1 study raise the possibility that intensive conditioning may overcome adverse prognosis associated with an MRD postremission status, but such an effect is still controversial [30,31]. Zheng et al. [32] reported that intensified myeloablative conditioning with BUCy2 or TBI-Cy plus high-dose cytarabine followed by unrelated cord blood transplantation might benefit AML patients in morphologic CR1 or CR2 who have detectable MRD. There were no apparent differences in 3-year OS (68.9% in MRD-negative group and 57.9% in MRD-positive group, $P = .31$) and 3-year DFS (62.5% in MRD-negative group and 52.7% in MRD-positive group, $P = .42$). However, Walter et al [4] found that 3-year relapse estimates were 28% and 57% for MRD-negative and MRD-positive patients receiving nonmyeloablative HSCT and 22% and 63% for these patients receiving myeloablative HSCT, separately. Similarly, the survival of MRD-positive patients was not improved accompanied by the strengthening of the preparative regimens, with 3-year OS estimates of 41% for nonmyeloablative HSCT patients and 25% for myeloablative HSCT patients. In the current study by using IDA-intensified allo-HSCT, the relapse rate of MRD-positive patients was 22.0%, and the 3-year OS and DFS for these high-risk patients was as high as 71.4% and 67.9%, respectively, which was remarkably better than the aforementioned studies and almost consistent with the outcomes of our MRD-negative patients, suggesting that our intensified allo-HSCT system is highly effective for these high-risk MRD-positive patients.

An increase in the frequency of regimen-related toxicities has been a barrier after intensified conditioning regimens [33,34]. We observed a high frequency of severe mucositis and febrile episodes before engraftment in the present study, but they were transient and managed by supportive treatment. The 3-year rates of TRM among MRD-negative remission, MRD-positive remission, and NR groups were 10.6%, 17.9% and 24.7%. This compares favorably with myeloablative conditioning allo-HSCT, which is associated with TRM rates of around 40% and with those of 28% to 33% in the sequential FLAMSA strategy (fludarabine (4×30 mg/m^2), amsacrine (4×100 mg/m^2), and Ara-C (4×2 g/m^2)) [35-37]. These data provide rationale that IDA-intensified conditioning would

potentiate the antileukemic effects of conditioning while retaining sufficient tolerability.

This study had several limitations. First, this was a retrospective study with a relatively small number of patients, which may influence the accuracy of our findings. Second, the groups were extremely heterogeneous, which makes generalizations difficult. Third, the techniques of MRD analysis were not uniform in this study. Therefore, a prospective controlled and randomized study should be designed to determine the effects of intensified conditioning regimen on the clinical outcomes in MRD-positive acute leukemia patients.

In summary, our report suggests that our novel IDA-intensified myeloablative conditioning could control disease relapse, surmount the poor prognosis generated by MRD positivity pretransplant, and improve long-term survival for these patients. In addition, it could be successfully used in high-risk patients with persistent positive MRD.

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