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RESEARCH ARTICLE

Minimal residual disease-directed immunotherapy for high-risk myelodysplastic syndrome after allogeneic hematopoietic stem cell transplantation

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Abstract **The efficacy of minimal residual disease (MRD)-directed immunotherapy, including interferon-α (IFN-α) treatment and chemotherapy plus granulocyte colony-stimulating factor-primed donor leukocyte infusion (chemo-DLI), was investigated in patients with high-risk myelodysplastic syndrome (MDS) who were MRD-positive after allogeneic hematopoietic stem cell transplantation (allo-HSCT). High-risk MDS patients who received non-T-cell-depleted allo-HSCT at the Peking University Institute of Hematology and were MRD-positive after allo-HSCT were studied ($n = 47$). The MRD-positive status was considered if leukemia-associated aberrant immune phenotypes or Wilms' tumor gene 1 expression is present in a single bone marrow sample. The cumulative incidence of the relapse and non-relapse mortality 2 years after immunotherapy were 14.5% and 21.4% ($P = 0.377$) and 9.1% and 0.0% ($P = 0.985$) for patients in the IFN-α and chemo-DLI groups, respectively. The probability of disease-free and overall survival 2 years after immunotherapy were 76.4% and 78.6% ($P = 0.891$) and 84.3% and 84.6% ($P = 0.972$) for patients in the IFN-α and chemo-DLI groups, respectively. Persistent MRD after immunotherapy was associated with poor survival. Thus, the MRD-directed immunotherapy was effective for patients with high-risk MDS who were MRD-positive after allo-HSCT, and the efficacy was comparable between chemo-DLI and IFN-α treatment.**

Keywords donor leukocyte infusion; hematopoietic stem cell transplantation; interferon-α; minimal residual disease; myelodysplastic syndrome

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective treatments for high-risk myelodysplastic syndrome (MDS); however, post-transplant relapse remains a major cause of transplant failure [1]. The impending relapse of high-risk MDS may be indicated through minimal residual disease (MRD) monitoring after allo-HSCT [2], which makes MRD-directed intervention a reasonable option for relapse prophylaxis. Hypomethylating agents (HMAs) may delay the hematologic relapse of patients with MDS or acute myeloid leukemia after allo-HSCT; however, long-term outcomes have not been satisfactory [3]. In addition, HMAs may be too expensive for patients in developing countries such as China.

MRD-directed immunotherapy is important for relapse prophylaxis after allo-HSCT [4]; moreover, chemotherapy plus granulocyte colony-stimulating factor-primed donor leukocyte infusion (chemo-DLI) is the most important immunotherapy that can significantly improve the condition of MRD-positive patients after allo-HSCT [5,6]. However, only a small (< 10) number of MDS patients with MRD have been studied [2,5,6], and the efficacy of MRD-directed chemo-DLI in patients with high-risk MDS remains unclear.

Some patients do not receive chemo-DLI because of provider or patient refusal. These patients require further

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studies to identify reasonable alternative pre-emptive interventions. Interferon-α (IFN-α) may be a feasible maintenance therapy for patients with acute myeloid leukemia [7]. In addition, IFN-α can kill leukemia cells by regulating T-cell and natural killer cell functions [8,9], induce the graft-versus-leukemia (GVL) effect, and eliminate leukemia cells in allo-HSCT recipients [10,11]. In our pilot study, MRD-directed IFN-α treatment was safe and effective for allo-HSCT recipients [12]. Thus, IFN-α may be an alternative treatment for high-risk MDS with MRD after allo-HSCT. However, small number ($n = 4$) of high-risk MDS was considered.

Thus, the efficacy of MRD-directed immunotherapy was further investigated in patients with high-risk MDS after allo-HSCT. Moreover, we wanted to compare the efficacy between MRD-directed chemo-DLI and IFN-α treatment.

Materials and methods

Patients

Consecutive patients receiving non-T-cell-depleted allo-HSCT at the Peking University Institute of Hematology were studied based on the following criteria: (1) those with high-risk or very high-risk MDS; and (2) who were positive for MRD after allo-HSCT. A total of 47 patients were enrolled from February 1, 2013 to July 31, 2016 (Fig.1), and the characteristics were comparable between the enrolled and excluded patients (data not shown). Final follow-up visits were conducted on December 20, 2017. Informed consent was obtained from all patients or their guardians. The study was conducted in accordance with the *Declaration of Helsinki*, and the protocol was approved by the Ethics Committee of Peking University People's Hospital.

Transplant regimens

Preconditioning consisted of cytarabine (Ara-C), busulfan, cyclophosphamide, and simustine. Rabbit anti-thymocyte globulin was administered to human leukocyte antigen (HLA)-haploidentical-related donor and HLA-unrelated donor groups, as detailed in the Supplementary methods [13,14]. Granulocyte colony-stimulating factor-mobilized, fresh, and unmanipulated bone marrow (BM) and peripheral blood harvests were administered to the recipients on the same day of collection. Patients received graft-versus-host disease (GVHD) prophylactic agents, including cyclosporine A, mycophenolate mofetil, and short-term methotrexate [15,16].

MRD monitoring

Patients were monitored for MRD post-transplantation according to leukemia-associated aberrant immune phenotypes (LAIPs) and Wilms' tumor gene 1 (*WT1*) expression. LAIPs were detected through multicolor flow cytometry. LAIP positivity was considered when $> 0.01\%$ of cells have a LAIP in post-transplantation BM samples

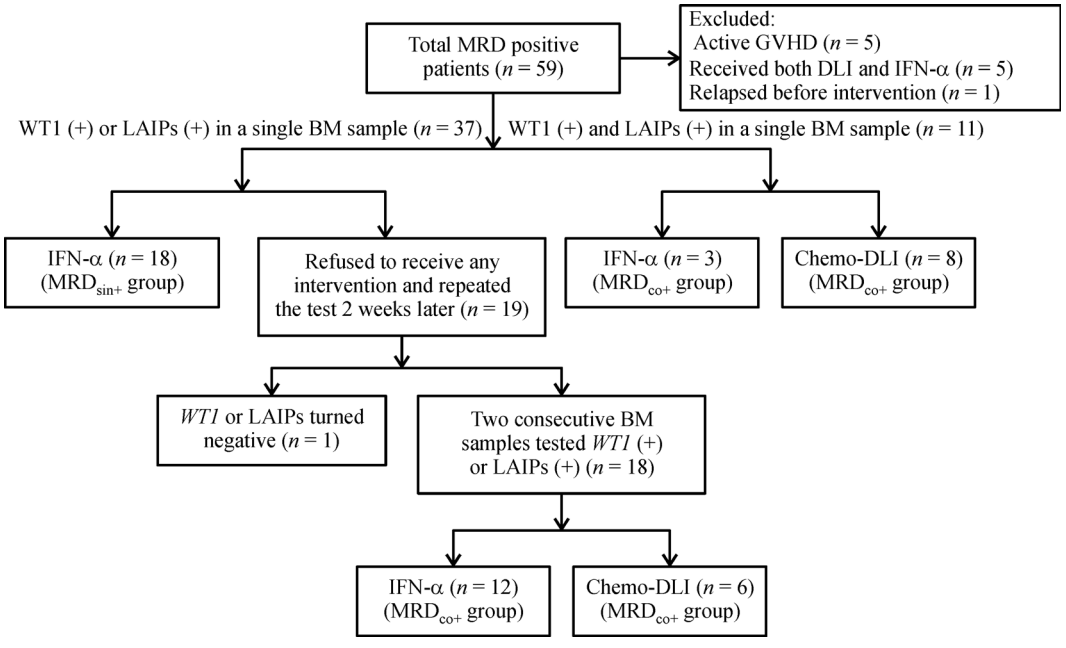

Fig. 1 Diagram of patients enrolled. MRD-directed chemo-DLI was the first choice of patients in the MRD_{co+} group and those who did not receive chemo-DLI because of patient or provider refusal to receive MRD-directed IFN-α treatment.

[17]. The expression of *WT1* was determined using TaqMan-based reverse transcription-polymerase chain reaction of diagnostic specimens. The *WT1* transcript level above 0.60% was considered positive [18]. Routine MRD monitoring was performed after 1, 2, 3, 4.5, 6, 9, and 12 months post-transplantation and at 6-month intervals thereafter. A patient was considered MRD-positive when a single BM sample was positive for LAIPs or *WT1* expression [19]. High-level MRD was considered for *WT1* transcript levels $\geqslant$ 1.0% and/or LAIP positivity of $\geqslant$ 0.1% in post-transplantation BM samples; low-level MRD was considered for the remaining patients.

MRD-directed immunotherapy protocol

Patients who tested positive for LAIPs or *WT1* expression in a single BM sample were defined as MRD_{sin+}. Patients who tested positive for LAIPs or *WT1* expression in two consecutive BM samples within a 2-week interval or those who tested positive for both LAIPs and *WT1* expression in a single BM sample were defined as MRD_{co+}. Chemo-DLI is efficient [6], but the role of IFN-α treatment has remained undefined in MRD_{co+} patients. Hence, MRD-directed chemo-DLI was the first choice for patients in the MRD_{co+} group, and those who could not receive DLI received IFN-α treatment.

A total of 18 MRD_{sin+} patients received IFN-α treatment. The tests for patients who did not agree to receive any intervention were repeated 2 weeks after the first positive results for *WT1* or LAIPs ($n = 19$). If two consecutive BM samples tested positive for LAIPs or *WT1* expression within a 2-week interval, patients received MRD-directed immunotherapy, and those who did not receive DLI due to patient ($n = 10$) or provider ($n = 2$) refusal received IFN-α treatment. Patients who tested positive for both *WT1* expression and LAIPs in a single BM sample received IFN-α treatment if they did not receive DLI because of patient refusal ($n = 3$). Thus, a total of 18, 15, and 14 patients were categorized as MRD_{sin+}, MRD_{co+} receiving IFN-α treatment, and MRD_{co+} receiving chemo-DLI groups, respectively (Fig.1). Furthermore, MRD status was monitored 1, 2, 3, 4.5, 6, 9, and 12 months after MRD-directed immunotherapy and at a 6-month interval thereafter.

Patients with active GVHD, active infections, severe myelosuppression, organ failure, or relapse were excluded from MRD-directed IFN-α treatment. Recombinant human IFN-α-2b injections (Anferon; Tianjin Hualida Biotechnology Co., Ltd., Tianjin, China) were subcutaneously administered for 6 cycles (twice or thrice weekly every 4 weeks) at 3 million units (MU) for patients over 16 years and at 3 MU/m^2 for those under 16 years (capped by 3 MU). Prolonged treatment with IFN-α was permitted per patient request. IFN-α was suspended in all patients with severe GVHD, severe infection, grade $\geqslant$ 3 toxicity, relapse, or non-relapse mortality (NRM).

Patients with active GVHD, active infections, organ failure, or relapse were excluded from MRD-directed chemo-DLI treatment, with a detailed protocol in the Supplementary methods [5,6,20,21].

Diagnosis and treatment of GVHD after MRD-directed IFN-α treatment

GVHD after MRD-directed immunotherapy was diagnosed [22,23] and treated [24,25] according to international criteria (Supplementary methods).

Definitions and assessments

MDS patients were categorized according to the WHO Prognostic Scoring System (WPSS) [26] which can predict the post-HSCT outcomes [27]. Therapeutic toxicities, rated according to the WHO criteria, were monitored every 1–2 weeks after IFN-α treatment, and GVHD was excluded as an adverse event. Relapse was defined as morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary samples or by the recurrence and sustained presence of pre-transplantation chromosomal abnormalities. MRD patients were not classified as showing relapse. NRM was defined as death without disease progression or relapse. Disease-free survival (DFS) was defined as the survival period with continuous CR. Overall survival (OS) events were defined as death from any cause.

Statistical analysis

Mann–Whitney *U*-test was used to compare continuous variables, and χ^2 and Fisher's exact tests were used for categorical variables. The Kaplan–Meier method was used to estimate the probability of survival. Competing risk analyses were performed to calculate the cumulative incidence of GVHD, relapse, and NRM [28]. Landmark analysis was performed to assess the effects of MRD-directed immunotherapy on each outcome by using the post-HSCT day as the landmark day.

Potential prognostic factors for clinical outcomes after MRD-directed immunotherapy were evaluated by multivariate analysis by using Cox proportional hazards regression with a forward-stepwise model selection approach. The regression model considered the risk level according to the WPSS, MRD type (MRD_{sin+} vs. MRD_{co+}) and level (high- vs. low-level MRD) before immunotherapy, type of immunotherapy (IFN-α vs. chemo-DLI), and MRD status after immunotherapy (positive vs. negative). The level of significance was set at $P < 0.05$. Data analyses were primarily conducted using the SPSS software (SPSS Inc., Chicago, IL, USA), while the R software package (version 2.6.1; http://www.r-project.org) was used for competing risk analysis.

Results

Patient characteristics

Table 1 summarizes the characteristics of the 47 patients, which all had full donor chimerism. Fourteen patients received MRD-directed chemo-DLI. The median doses of mononuclear, $CD3^+$, and $CD34^+$ cells were 1.0 (0.8–1.8) $\times 10^8$, 3.7 (2.6–7.6) $\times 10^7$, and 0.3 (0.1–0.6) $\times 10^6$/kg, respectively. Thirty-three patients received MRD-directed IFN-α treatment, the median cycles of IFN-α treatment was 3 (range, 1–13 cycles), and the reasons for discontinuing IFN-α treatment included complete treatment ($n = 11$), occurrence of severe GVHD ($n = 15$), relapse ($n = 4$), or grade $\geqslant$ 3 toxicity (hematologic: $n = 1$; infectious: $n = 2$).

GVHD after MRD-directed immunotherapy

Thirteen patients developed acute GVHD (aGVHD) after IFN-α treatment, and grades I, II, and III aGVHD were observed in 1, 8, and 4 patients, respectively. The median duration from immunotherapy to the occurrence of aGVHD was 14 (5–91) days. A patient developed grade II aGVHD 18 days after chemo-DLI. The cumulative incidence of grade I–II aGVHD and grade III aGVHD 2 years after immunotherapy was 27.3% versus 7.1% ($P = 0.129$) and 12.1% versus 0.0% ($P = 0.179$) in the IFN-α and chemo-DLI groups, respectively.

Fourteen patients experienced chronic GVHD (cGVHD) after IFN-α treatment; mild, moderate, and severe cGVHD were observed in 3, 5, and 6 patients, respectively. Six patients experienced cGVHD after chemo-DLI; moderate and severe cGVHD were observed in 4 and 2 patients, respectively. The median duration from immunotherapy to the occurrence of cGVHD was 59 (7–519) and 95 (72–117) days in the IFN-α and chemo-DLI groups, respectively ($P = 0.274$). The cumulative incidence of mild to moderate cGVHD and severe cGVHD 2 years after immunotherapy was 30.6% versus 36.4% ($P = 0.401$) and 18.2% versus 18.2% ($P = 0.799$) in the IFN-α and chemo-DLI groups, respectively.

MRD status and relapse after MRD-directed immunotherapy

Four and three patients showed relapse after IFN-α treatment and chemo-DLI, respectively, with the median duration from the beginning of IFN-α treatment and chemo-DLI to the relapse of 333 (200–468) and 56 (37–64) days, respectively. The cumulative incidence of relapse (CIR) 2 years after immunotherapy was 16.4% among all patients, which was comparable in patients with MRD_{sin+}, MRD_{co+} receiving IFN-α treatment, and MRD_{co+} receiving chemo-DLI (Fig. 2A).

A total of 16 (48.5%), 5 (15.2%), 3 (9.0%), and 5 (15.2%) patients were MRD-negative 1, 2, 3, or > 3 months after MRD-directed IFN-α treatment, respectively, while 6 (42.9%) and 1 (7.1%) patients were MRD-negative 1 or > 3 months after chemo-DLI, respectively. The cumulative incidence of turning MRD negative 3 months and 2 years after immunotherapy was showed in Fig. 3A and Fig. 3B. The CIR 2 years after immunotherapy of patients who turned MRD-negative and those with persistent MRD was 12.9% and 27.3%, respectively ($P = 0.137$, Fig. 2B).

In the multivariate analysis, no variable was significantly associated with increased relapse, but the persistent MRD after immunotherapy seemed to increase the risk of relapse (Table 2).

Toxicities after MRD-directed immunotherapy

Grade $\geqslant$ 3 hematologic toxicity was experienced by a patient in the IFN-α group for 14 days, and 14 in the chemo-DLI group for a median duration of 11 (3–40) days. Two (6.1%) and five (35.7%) patients suffered grade $\geqslant$ 3 non-hematologic toxicities in the IFN-α and chemo-DLI groups, respectively.

NRM after MRD-directed immunotherapy

Three patients died from NRM (infection: $n = 2$; GVHD: $n = 1$) after IFN-α treatment, with a median time from the beginning of IFN-α treatment to NRM of 130 (range, 77–164) days. A patient died because of severe pneumonia 750 days after chemo-DLI. The cumulative incidence of NRM 2 years after immunotherapy was 6.4% among all patients, which was comparable among those who were MRD_{sin+}, MRD_{co+} receiving IFN-α treatment, and MRD_{co+} receiving chemo-DLI (Fig. 4A). The cumulative incidence of NRM 2 years after immunotherapy in patients who turned MRD-negative and with persistent MRD was 2.8% and 18.2%, respectively ($P = 0.067$, Fig. 4B). In the multivariate analysis, the elimination of MRD after immunotherapy significantly decreased the risk of NRM (Table 2).

DFS and OS after MRD-directed immunotherapy

The probability of DFS 2 years after immunotherapy was 77.3% among all patients, which was comparable among those who were MRD_{sin+}, MRD_{co+} receiving IFN-α treatment, and MRD_{co+} receiving chemo-DLI (Fig. 5A), while that in patients who turned MRD-negative and with persistent MRD was 84.3% and 54.5%, respectively ($P = 0.006$, Fig. 5B).

The probability of OS 2 years after immunotherapy was 84.5% among all patients, which was comparable among those who were MRD_{sin+}, MRD_{co+} receiving IFN-α treatment, and MRD_{co+} receiving chemo-DLI (Fig. 6A),

Table 1 Patient characteristics between the IFN-α and chemo-DLI group

Characteristics	IFN-α group ($n = 33$)	Chemo-DLI group ($n = 14$)	P value
Median age at allo-HSCT, years (range)	41 (11–61)	42 (10–58)	0.981
Median time from allo-HSCT to intervention, days (range)	192 (39–1016)	194 (84–1552)	0.625
Sex, male/female, *n*	22/11	9/5	1.000
WHO classification, *n* (%)			
RCMD	5 (15.1)	1 (7.1)	0.708
RAEB-1	13 (39.4)	4 (28.6)	
RAEB-2	15 (45.5)	9 (64.3)	
Cytogenetic risk, *n* (%)			
Good	1 (3.0)	1 (7.2)	0.094
Intermediate	28 (84.8)	8 (57.1)	
Poor	4 (12.1)	5 (35.7)	
Severe anemia, *n* (%)[a]	19 (57.6)	5 (35.7)	0.212
WPSS risk, *n* (%)			
High	26 (78.8)	6 (42.9)	0.037
Very high	7 (21.2)	8 (57.1)	
Chemotherapy prior to HSCT, *n* (%)	9 (27.3)	6 (42.9)	0.324
Disease status at transplantation, *n* (%)			
CR	7 (21.2)	2 (14.3)	0.704
Non-CR	26 (78.8)	12 (85.7)	
Donor type			
HLA-identical sibling donor	11 (33.3)	7 (50.0)	0.464
HLA-haploidentical related donor	19 (57.6)	7 (50.0)	
HLA-unrelated donor	3 (9.1)	0 (0.0)	
Number of HLA-A, HLA-B, HLA-DR mismatches, *n* (%)			
0	13 (39.4)	7 (50.0)	0.661
1	4 (12.1)	0 (0.0)	
2	1 (3.0)	0 (0.0)	
3	15 (45.5)	7 (50.0)	
MRD status before immunotherapy, *n* (%)			
MRD_{sin+}[b]	18 (54.5)	0 (0.0)	<0.001
WT1 positive once	17 (51.5)	0 (0.0)	
LAIPs positive once	1 (3.0)	0 (0.0)	
MRD_{co+}, two BM samples	12 (36.4)	6 (42.9)	
WT1 positive twice	12 (36.4)	4 (28.6)	
LAIPs positive twice	0 (0.0)	2 (14.3)	
MRD_{co+}, one BM sample			
WT1 positive and LAIPs positive simultaneously	3 (9.1)	8 (57.1)	
MRD level before immunotherapy, *n* (%)[c]			
Low level	11 (33.3)	3 (21.4)	0.503
High level	22 (66.7)	11 (78.6)	
Discontinuing immunosuppression before immunotherapy, *n* (%)	19 (57.6)	9 (64.3)	0.753
Median duration of follow-up after immunotherapy, days (range)	559 (77–1410)	498 (94–1685)	0.675

allo-HSCT, allogeneic hematopoietic stem cell transplantation; chemo-DLI, chemotherapy plus granulocyte colony-stimulating factor-primed donor leukocyte infusion; CR, complete remission; HLA, human leukocyte antigen; IFN-α, interferon-α; LAIPs, leukemia-associated immunophenotypic patterns; MDS, myelodysplastic syndrome; MRD, minimal residual disease; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia; WPSS, WHO classification-based Prognostic Scoring System; *WT1*, Wilms' tumor gene 1.

[a]Severe anemia was defined as hemoglobin < 90 g/L in male or < 80 g/L in female.

[b]Patients who tested positive for LAIPs or *WT1* expression in a single BM sample were defined as MRD_{sin+}. Patients who tested positive for LAIPs or *WT1* expression in two consecutive BM samples within a 2-week interval or those who tested positive for both LAIPs and *WT1* expression in a single BM sample were defined as MRD_{co+}.

[c]High-level MRD was considered at *WT1* transcript levels $\geqslant 1.0\%$ and/or LAIP positivity in $\geqslant 0.1\%$ of cells with LAIPs in post-transplantation BM samples; otherwise, low-level MRD was considered.

Statistical significance was set at $P < 0.05$.

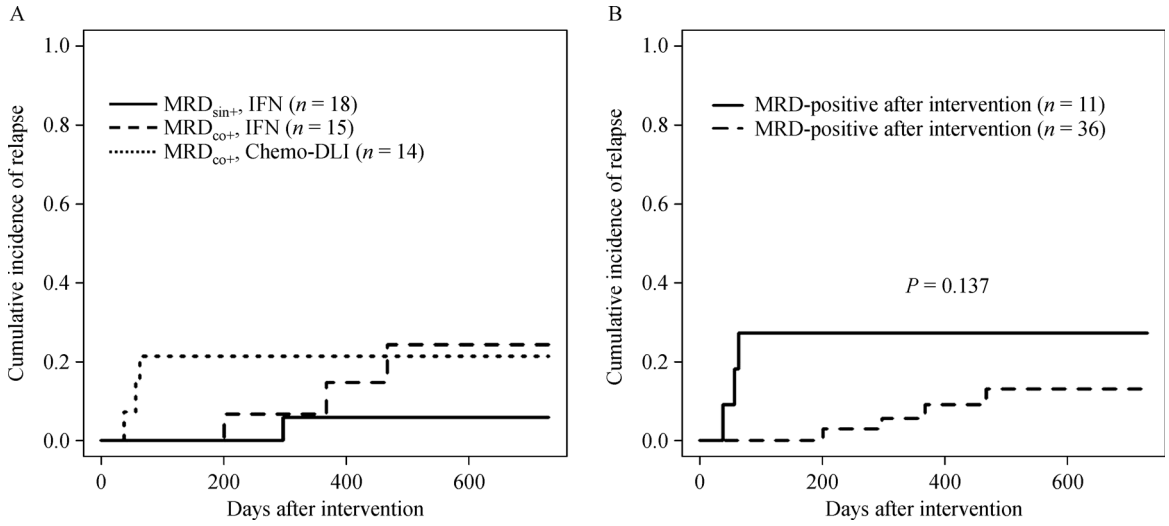

Fig. 2 Cumulative incidence of relapse 2 years after MRD-directed immunotherapy according to (A) MRD status prior to immunotherapy: MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving IFN-α treatment: 5.9% vs. 24.4%, $P = 0.241$; MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 5.9% vs. 21.4%, $P = 0.174$; MRD_{co+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 24.4% vs. 21.4%, $P = 0.833$; and (B) MRD status after immunotherapy (12.9% vs. 27.3%, $P = 0.137$).

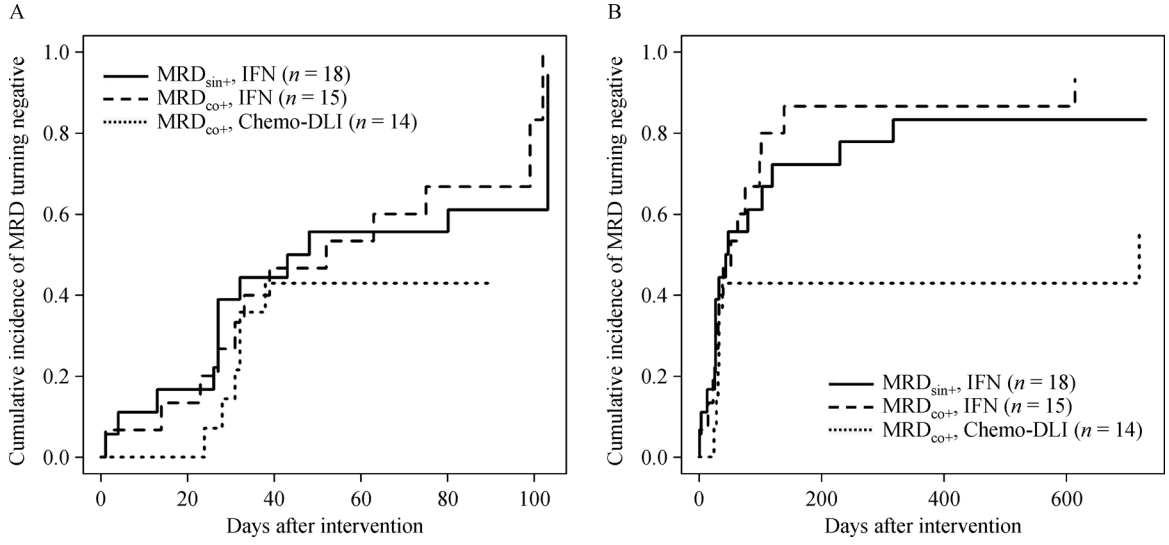

Fig. 3 Cumulative incidence of MRD-negative patients after immunotherapy. (A) 3 months: MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving IFN-α treatment: 61.1% vs. 66.7%, $P = 0.611$; MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 61.1% vs. 42.9%, $P = 0.278$; MRD_{co+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 66.7% vs. 42.9%, $P = 0.260$; (B) 2 years: MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving IFN-α treatment: 83.3% vs. 86.7%, $P = 0.624$; MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 83.3% vs. 54.8%, $P = 0.064$; MRD_{co+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 86.7% vs. 54.8%, $P = 0.027$.

while that in patients who turned MRD-negative and with persistent MRD was 91.2% and 60.6%, respectively (P = 0.006, Fig. 6B).

In the multivariate analysis, the MRD negativity after immunotherapy was significantly associated with increased survival (Table 2).

Discussion

MDS patients who received MRD-directed immunotherapy had a 2-year CIR of 16.4%, and the 2-year probability of DFS and OS after immunotherapy was 77.3% and 84.5%, respectively. To our knowledge, this study provides

Table 2 Multivariate analysis of risk factors for the 2-year clinical outcomes after MRD-directed immunotherapy

Outcome	HR (95% CI)	*P*
Treatment failure as defined by OS		
Intervention type		
IFN-α treatment	1	0.389
Chemo-DLI	0.38 (0.04–3.39)	
Other significant factors		
MRD status after intervention		
Positive	1	
Negative	0.12 (0.02–0.61)	0.011
Treatment failure as defined by DFS		
Intervention type		
IFN-α treatment	1	0.388
Chemo-DLI	0.52 (0.12–2.29)	
Other significant factors		
MRD status after intervention		
Positive	1	
Negative	0.21 (0.06–0.72)	0.014
Relapse		
Intervention type		
IFN-α treatment	1	0.519
Chemo-DLI	0.52 (0.07–3.87)	
Non-relapse mortality		
Intervention type		
IFN-α treatment	1	0.241
Chemo-DLI	6.24 (0.29–133.69)	
Other significant factors		
MRD status after intervention		
Positive	1	
Negative	0.06 (0.01–0.65)	0.021

Chemo-DLI, chemotherapy plus granulocyte colony-stimulating factor-primed donor leukocyte infusion; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; IFN-α, interferon-α; MRD, minimal residual disease; OS, overall survival.
Statistical significance was set at $P < 0.05$.

an opportunity to explore the currently undefined role of MRD-directed immunotherapy in patients with high-risk MDS after allo-HSCT. In addition, this is the first study that compares chemo-DLI with IFN-α treatment in a disease-specific population of patients with high-risk MDS.

The relapse and DFS rates 2 years after MRD-directed immunotherapy were 16.4% and 77.3%, respectively. In MRD_{sin+} patients who received MRD-directed IFN-α treatment, the relapse and DFS rates were 5.9% and 83.0%, respectively. These results are similar to those of MDS patients who were MRD-negative after allo-HSCT in our previous studies (relapse: 8.3%; DFS: 82.9%) [2]. The GVL effect was the major mechanism involved in clearing MRD in both IFN-α treatment and DLI. In addition, IFN-α could inhibit blast cell growth in patients with acute leukemia [29,30]. Thus, MRD-directed immunotherapy was effective for patients with high-risk MDS after allo-HSCT.

cGVHD is closely related to the GVL effect [31,32], and the cGVHD after MRD-directed immunotherapy is critical for clearing MRD in patients with acute leukemia [5,12]. Its occurrence was comparable between the IFN-α and chemo-DLI groups, which suggests their similar ability for inducing GVL effect in patients with MDS. By contrast, among the patients who received IFN-α treatment, the relapse and DFS rates of MRD_{co+} patients were 24.4% and 68.9%, respectively, which are lower than those of MRD_{sin+} patients. Thus, MRD-directed IFN-α treatment may be more appropriate for MRD_{sin+} patients.

Several studies reported the efficacy of MRD-directed IFN-α treatment in patients with acute leukemia [4]. The CIR and DFS rates in patients with acute leukemia receiving IFN-α treatment after allo-HSCT were 11.5%–27.3% and 68.2%–82.4%, respectively [12,33]. In the present study, the CIR and DFS rates were 14.5% and 76.4% in the IFN-α group. Thus, the efficacy of MRD-directed IFN-α treatment was comparable between patients with acute leukemia and high-risk MDS.

The 2-year NRM rate after MRD-directed immunotherapy was only 6.4% in the present study, which was similar to that of patients with MRD-negative MDS after allo-HSCT (8.8%) [2], MRD-directed chemo-DLI and IFN-α treatment ranged from 4.4%–14.4% [5,6,12,34] and 4.3%–4.5% [12,33], respectively. Severe aGVHD can cause NRM after post-HSCT immunotherapy. In this study, the incidence of severe aGVHD was 0.0% and 12.1% in the chemo-DLI and IFN-α groups, respectively ($P = 0.179$), which is similar to that of the MRD-directed chemo-DLI (4.0%–8.2%) [5,6,12] and IFN-α treatment (4.5%–5.7%) [12,33] in our previous studies. Thus, MRD-directed chemo-DLI and IFN-α treatment after allo-HSCT were safe for patients with high-risk MDS.

HMAs are safe in allo-HSCT recipients [35,36] and may be an effective treatment option for MDS patients with relapse after allo-HSCT, particularly in patients with low disease burden [37,38]. However, MRD-directed HMA treatment may only delay the hematologic relapse [3,39]. In the RELAZA trial, although 16 out of 20 patients (80%) responded to pre-emptive azacitidine treatment, 65% experienced hematologic relapse [3]. In the present study, the 2-year CIR after MRD-directed immunotherapy was only 16.4%. However, patients with persistent MRD after immunotherapy had poor clinical outcomes, and other methods that destroy leukemia cells through different mechanisms could be considered. Thus, the efficacy of HMA in the patients who did not respond to MRD-directed immunotherapy remains to be explored.

The first limitation of this retrospective study is the use of a relatively small number of patients, which could only explain the problem to some extent. Second, the observa-

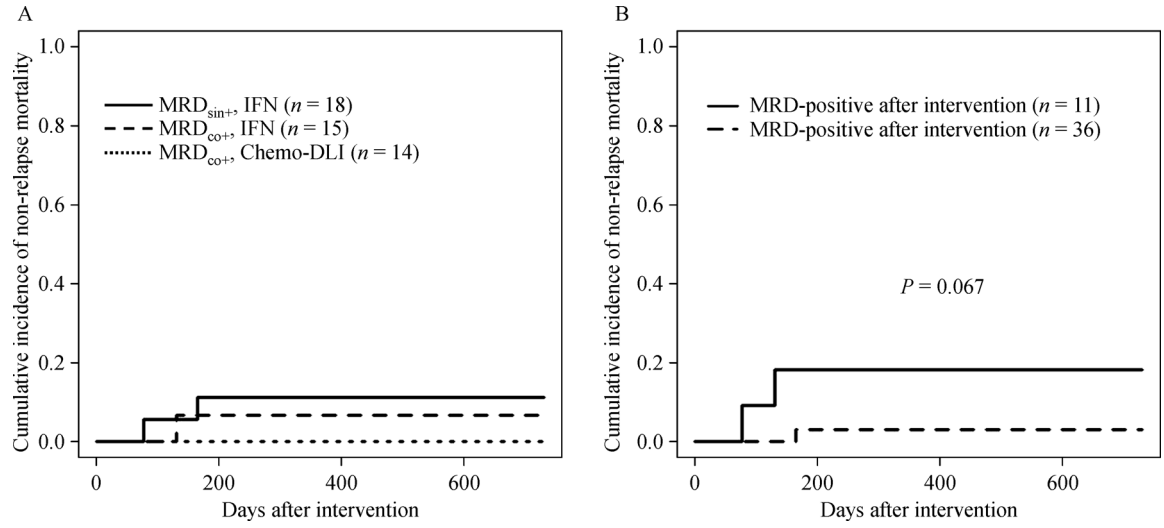

Fig. 4 Cumulative incidence of non-relapse mortality 2 years after MRD-directed immunotherapy according to (A) MRD status prior to immunotherapy: MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving IFN-α treatment: 11.1% vs. 6.7%, $P = 0.664$; MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving Chemo-DLI: 11.1% vs. 0.0%, $P = 0.206$; MRD_{co+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 6.7% vs. 0.0%, $P = 0.335$; and (B) MRD status after immunotherapy (2.8% vs. 18.2%, $P = 0.067$).

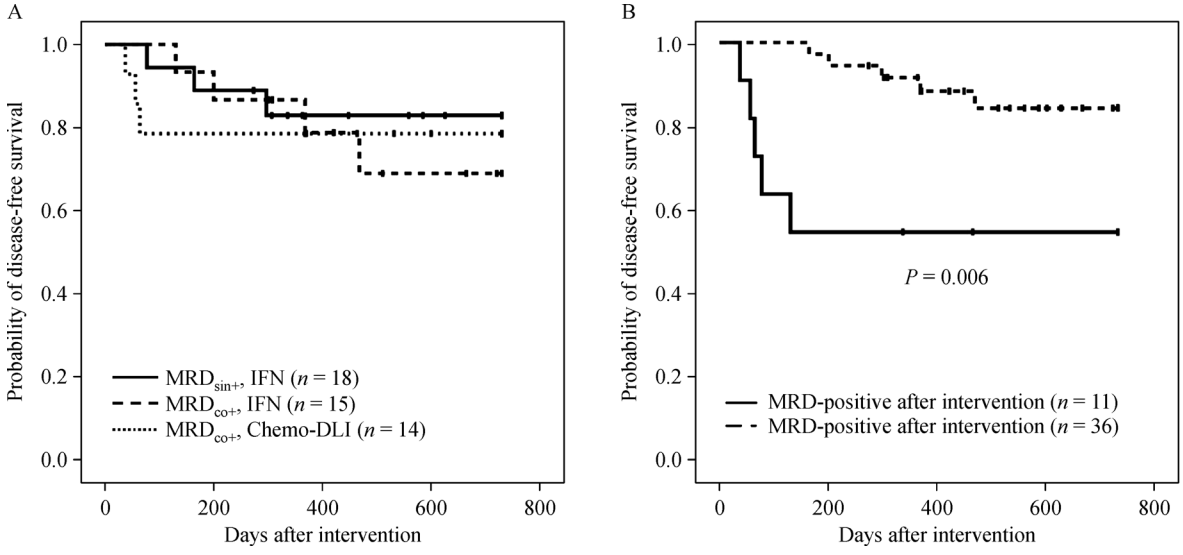

Fig. 5 Probability of disease-free survival 2 years after MRD-directed immunotherapy according to (A) MRD status prior to immunotherapy: MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving IFN-α treatment: 83.0% vs. 68.9%, $P = 0.570$; MRD_{sin+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 83.0% vs. 78.6%, $P = 0.649$; MRD_{co+} receiving IFN-α treatment vs. MRD_{co+} receiving chemo-DLI: 68.9% vs. 78.6%, $P = 0.847$; and (B) MRD status after immunotherapy (84.3% vs. 54.5%, $P = 0.006$).

tion period was relatively short, and our extension study could further evaluate the long-term clinical outcomes after MRD-directed immunotherapy in patients with high-risk MDS after receiving allo-HSCT. Lastly, patients with active GVHD and infections, severe myelosuppression, or organ failure were not administered with MRD-directed immunotherapy, and pre-emptive intervention methods should be further studied.

In summary, MRD-directed immunotherapy is effective for patients with high-risk MDS after allo-HSCT. Additionally, the efficacy was comparable between the chemo-DLI and IFN-α treatment. In the future, large-scale, randomized trials should be conducted to further compare the efficacy among IFN-α, chemo-DLI, and HMA

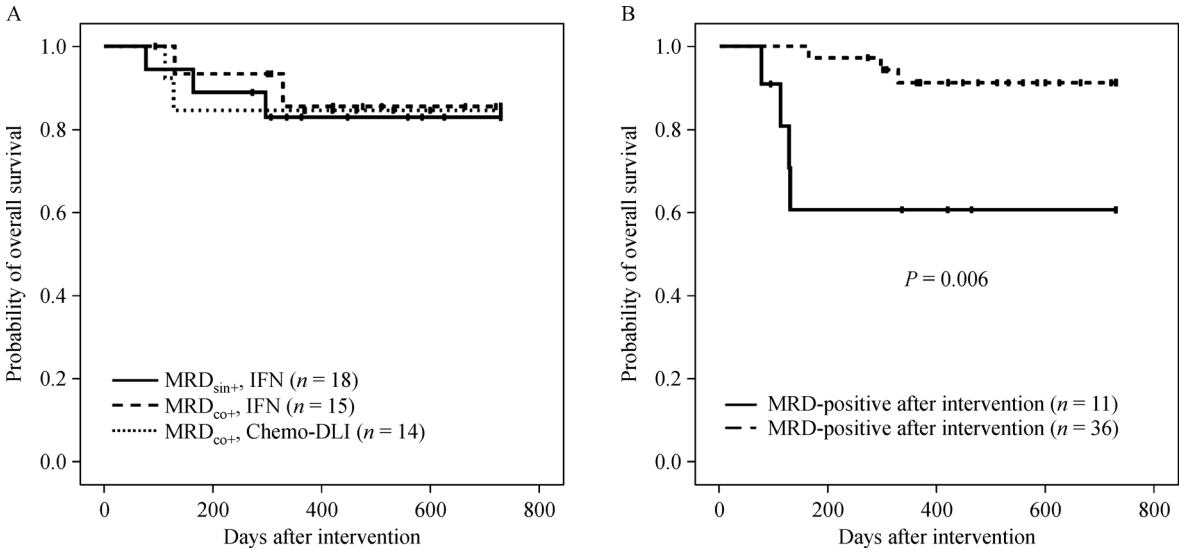

Fig. 6 Probability of OS 2 years after MRD-directed immunotherapy according to (A) MRD status prior to immunotherapy: MRD$_{sin+}$ receiving IFN-α treatment vs. MRD$_{co+}$ receiving IFN-α treatment: 83.0% vs. 85.6%, P = 0.762; MRD$_{sin+}$ receiving IFN-α treatment vs. MRD$_{co+}$ receiving chemo-DLI: 83.0% vs. 84.6%, P = 0.927; MRD$_{co+}$ receiving IFN-α treatment vs. MRD$_{co+}$ receiving chemo-DLI: 85.6% vs. 84.6%, P = 0.849; and (B) MRD status after immunotherapy (91.2% vs. 60.6%, P = 0.006).

treatments in MRD-positive patients after allo-HSCT and to identify the most effective intervention strategy.

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Compliance with ethics guidelines

Xiaodong Mo, Xiaohui Zhang, Lanping Xu, Yu Wang, Chenhua Yan, Huan Chen, Yuhong Chen, Wei Han, Fengrong Wang, Jingzhi Wang, Kaiyan Liu, and Xiaojun Huang declare no potential financial conflict of interest related to this manuscript. Informed consent was obtained from all patients or their guardians. The study was conducted in accordance with the *Declaration of Helsinki*. The study protocol was approved by the Ethics Committee of Peking University People's Hospital.

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