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RESEARCH ARTICLE

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MRD abnormal expression predict poor outcomes for refractory or relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation

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Abstract

We retrospectively analyzed data from 197 patients with refractory or relapsed acute myeloid leukemia (r/rAML) who underwent allo-HCT between January 2013 and February 2020 in our center (patients with promyelocytic leukemia were excluded). Of all patients, 86 achieved a complete morphological remission (CR) before transplant, while 111 failed to do so (NR). In the CR group, 32 patients displayed minimal residual disease (MRD-positive). According to their immunophenotype pre-HCT, we divided the MRD-positive group and NR group into three subgroups: MRD 0+ group (without any antigen abnormal expression of CD7+, CD56+, CD38−, or HLA-DR−) 28 patients, MRD 1+ group (with one abnormal antigen expression of CD7+, CD56+, CD38−, or HLA-DR−) 63 patients, MRD 2+ group (with two or more abnormal antigens expression of CD7+, CD56+, CD38-, or HLA-DR-) 52 patients. 3-year estimates of disease-free survival (DFS) for MRD 0+, MRD 1+ and MRD 2+ patients were 59.5 ± 9.5%, 29.9 ± 6.1%, and 9.4 ± 5.1%, and 3-year estimates of overall survival (OS) were 59.5 ± 9.5%, 34.5 ± 6.3%, and 14.5 ± 10.8%, respectively. Multivariate analysis adjusted for genetic risk, blast cell level, secondary disease, age, sex, and donor relationship pre-HCT, the hazard ratios of abnormal expression of CD7+, CD56+, HLA-DR−, and $CD38^-$ were 6.69 (range 2.08–21.52; $p = 0.001$) for DFS, 2.24 (range 1.21–4.14; $p = 0.010$) for OS, and 7.18 (range 2.23–23.10; $p = 0.001$) for relapse compared with CD7−, CD56−, HLA-DR+, and CD38+ patients. Our finding suggested that abnormal expression of CD7+, CD56+, HLA-DR−, and CD38− is associated with poor outcomes, and the more number of abnormal antigens expression predict worse outcomes.

KEYWORDS
allogeneic hematopoietic stem cell transplantation, disease burden, immunophenotype, minimal residual disease, refractory or relapsed acute myeloid leukemia

1 | INTRODUCTION

Advances in chemotherapy have improved out comes for patients with acute myeloid leukemia.[1,2] However, treatment outcomes are still dismal for patients with refractory or relapsed acute myeloid leukemia(r/rAML) who are subjected to conventional chemotherapy alone.[3] We investigated the efficacy and safety of an intensive conditioning regimen that consisted of cladribine, cytarabine, and

granulocyte colony-stimulating factor plus modified busulfan combined with cytoxan for pre-transplant r/rAML patients. The outcomes improved but were still poor.[4]

Allogeneic hematopoietic cell transplantation is almost the best choice for patients with r/rAML.[5,6] However, a risk for relapse remains.[1,7] Disease status at the time of transplantation is important prognostic factor for outcome in patients with r/rAML.[8–10] Although still to be proven, aberrant antigen expression as measured by flow cytometry could have a clinical value in providing predictive biomarkers.[11] Abnormal expression of CD7 and CD56 is associated with poor outcome for AML patients.[12–14] And CD34+CD38- leukemic stem cells (LSC) greatly improve the prognostic effect of MRD detection.[15] In addition, expression of HLA-DR for prognosis of AML patients is still controversial.[16–18] To assess the prognostic significance of immunophenotype, we retrospectively analyzed data from 197 r/rAML patients who underwent allo-HCT between January 2013 and February 2020 in our center.

2 | PATIENTS AND METHODS

2.1 | Patients

A total of 227 r/rAML patients underwent allo-HCT at the Department of Hematology, Aerospace Center Hospital between January 2013 and February 2020 (patients with promyelocytic leukemia were excluded). In all, 21 patients died of pretransplant treatment and 9 patients of unrelated donors allo-HCT were removed in this study. At last, 197 r/rAML patients were enrolled in this study. Figure 1 provides the patients enrolled in this study. All participants provided informed consent for treatment with allogeneic HCT and for this retrospective study. Primary refractory disease was defined as the failure to achieve complete remission (CR, morphological blast cells <5%) after two cycles of induction chemotherapy. Relapsed refractory acute leukemia was defined as the failure to regain CR after two cycles of standard salvage chemotherapy following relapse.[19] Cytogenetic and genetic abnormalities at diagnosis were evaluated according to the 2008 World Health Organization classification.[20,21] Karyotypes were classified according to the International System for Human Cytogenetic Nomenclature.[22] Cytogenetic and molecular risk groups were stratified according to the 2017 European Leukemia Net (ELN) recommendations.[23]

2.2 | Multiparametric flow cytometry detection of MRD

Multiparametric flow cytometry detection of MRD positive was defined as any measurable disease (≥20 events) and leukemia cells detectable above a threshold (0.1%).[24] Eight-color flow cytometry was performed on bone marrow aspirates before and after allo-HCT as described.[25,26] The panel consisted one tube as follows: CD7FITC, CD56PE, CD34Percp, CD117PE/Cyanine7, CD33APC, HLA-DRAPC/Cyanine7, CD38Brilliant Violet 421, and CD45V500 according to the Euroflow AML/MDS panel,[27] and some antibody conjugates were adjusted in combination according to our situation. CD56PE, CD34Percp, CD33APC, IntraSure™ Kit and FACS™ Lysing Solution were obtained from Becton-Dickinson, CD7 FITC was obtained from Beckman-Coulter. CD45V500 was obtained from BD Biosciences, and other antibodies were obtained from Biolegend. Monitoring frequency was according to the 2018 Chinese expert consensus[28] 500,000 to 1 million events (excluding all CD45-negative cells and debris) per tube were acquired with a DX Flex (Beckman-Coulter) flow cytometer. The abnormal population was quantified as a percent of total CD45+ white cell events, and data compensation and analysis were performed with Kaluza2.1. We used the "different-from-normal" approach, which was based on the characteristic antigenic patterns that differed sufficiently from patterns expected in normal or regenerating marrow, even when present at low levels.[11,29]

2.3 | Statistical analyses

Kaplan-Meier analyses were used to estimate disease-free survival (DFS) and overall survival (OS), and log-rank tests were used to compare survival outcomes. Probabilities of relapse and nonrelapse mortality (NRM) were summarized by using cumulative incidence estimates. Categorical patient characteristics were compared between MRD-, MRD+ (0.1% ≦ FCM blast cells < 5.0%) and NR (morphological blast cells ≥ 5%) groups using Pearson's x-square tests. Continuous characteristics were compared with two-sample Student *t* tests. Cox regression for multivariable analyses was performed to assess the independent effects of the following factors: age (≦25 vs. >25), AML status (de novo vs. secondary), genetic risk group (favorable *vs* intermediate/unfavorable), disease status (CR vs. NR), MRD status (MRD− vs. MRD+), blast cell level (<20% vs. ≥20%), and immunophenotype (MRD 0+ vs. MRD 1+/MRD 2+). All *p*-values were two-sided and $p < 0.05$ was considered statistically significant. Statistical analyses were performed with Statistics 22 (SPSS Inc) and GraphPad Prism 8 (GraphPad Software Inc).

3 | RESULTS

3.1 | Patient characteristics

Patients ($n = 197$) with refractory or relapsed acute myeloid leukemia who underwent hematopoietic cell transplantation (HCT) were enrolled in this study. The inclusion condition was clinically classified refractory or relapsed AML and regular monitoring of bone marrow MRD by flow cytometry before and after allogenic HCT. The median follow-up time was 37 ± 1.54 months, and in pre-HCT, 111 (56.3%) patients were not in complete remission (NR) and 86 patients were CR, including 54 MRD-negative and 32 MRD-positive (0.2%–4.8%). Post-HCT 103 (52.3%) patients died, of which 58 (56.3%) died of relapse. Ninety-four (47.7%) patients were alive and 82 (87.2%) of these patients remained in remission. Table 1 summarizes the characteristics of the study population; genetic risk (determined at diagnosis) was stratified according to 2017 ELN criteria.[30]

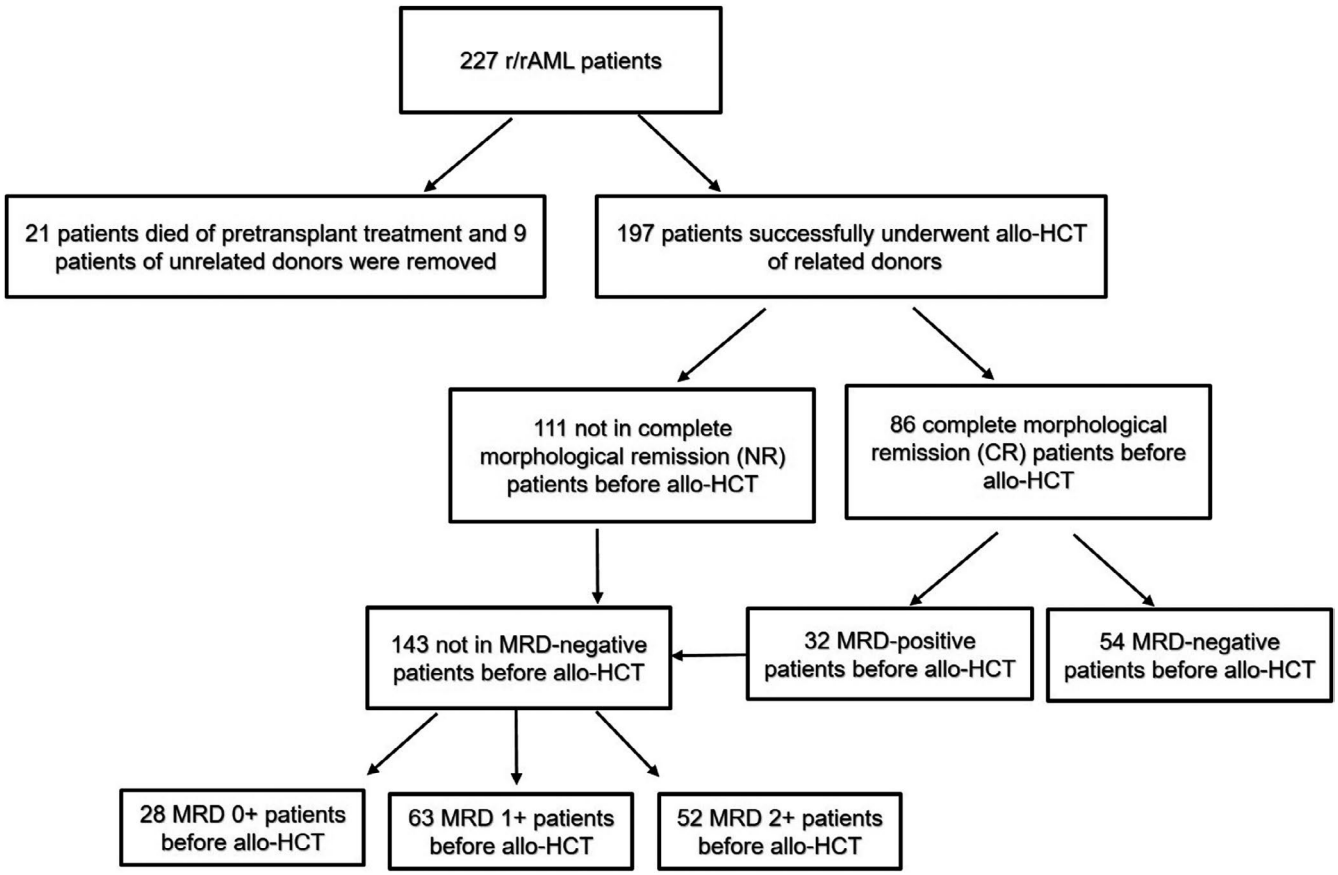

FIGURE 1 Patients enrolled in this study. MRD 0+, without any antigen abnormal expression of CD7+, CD56+, CD38-, or HLA-DR−; MRD 1+, with one abnormal antigen expression of CD7+, CD56+, CD38−, or HLA-DR−; MRD 2+, with two or more abnormal antigens expression of CD7+, CD56+, CD38−, or HLA-DR−

3.2 | Relationship between disease burden, MRD, and survival

The 3-year estimates of DFS for MRD-negative and MRD-positive and not in complete remission (NR) patients were 75.7 ± 6.6%, 43.0 ± 9.3% and 24.8 ± 4.3%, respectively, MRD− vs. MRD+ (*p* 0.000), MRD+ vs. NR (*p* 0.006). The 3-year estimates of OS were 80.9 ± 5.9%, 51.8 ± 9.5% and 31.6 ± 4.8%, MRD− vs. MRD+ (*p* 0.004), MRD+ vs. NR (*p* 0.010). The estimates of relapse at 3 years were 16.7 ± 5.9%, 53.1 ± 9.3% and 73.9 ± 4.3%, MRD− vs. MRD+ (*p* 0.000), MRD+ vs. NR (*p* 0.000). The 3-year estimates of NRM were 11.1 ± 5.2%, 25.0 ± 9.7% and 27.9 ± 5.7%, respectively, MRD− vs. MRD+ (*p* 0.040), MRD+versus NR (*p* 0.233; Figure 2).

3.3 | Relationship between immunophenotype and survival

We divided the MRD+ group and NR group into three subgroups according to their abnormal expression of CD7, CD56, CD38, and HLA-DR: MRD 0+ group (without any antigen abnormal expression of CD7+, CD56+, CD38−, or HLA-DR−) 28 patients, MRD 1+ group (with one abnormal antigen expression of CD7+, CD56+, CD38−, or HLA-DR−) 63 patients (divided into four small subgroups: CD7+, CD56+, CD38- and the HLA-DR− subgroup), MRD 2+ group (with two or more abnormal antigens expression of CD7+, CD56+, CD38−, or HLA-DR−) 52 patients. 3-year estimates of DFS for MRD 0+, MRD 1+ and MRD 2+ patients were 59.5 ± 9.5%, 29.9 ± 6.1% and 9.4 ± 5.1%, MRD 0+ vs. MRD 1+(*p* 0.003), MRD 1+ vs. MRD 2+(*p* 0.038); and 3-year estimates of OS were 59.5 ± 9.5%, 34.5 ± 6.3% and 14.5 ± 10.8%, respectively, MRD 0+ vs. MRD 1+(*p* 0.016), MRD 1+ vs. MRD 2+(*p* 0.336). The estimates of relapse at 3 years were 40.5 ± 9.5%, 72.0 ± 6.1% and 94.4 ± 4.7%, MRD 0+ vs. MRD 1+ (*p* 0.006), MRD 1+ vs. MRD 2+ (*p* 0.033). The 3-year estimates of NRM were 11.1 ± 5.2%, 25.0 ± 9.7% and 27.9 ± 5.7%, respectively, MRD− vs. MRD+ (*p* 0.745), MRD+ vs. NR (*p* 0.688). The median disease-free survival was 9.00 ± 2.25 months (95%CI, 4.58–13.42), 5.00 ± 0.72 months (95%CI, 3.59–6.41); and the median overall survival time was 12.00 ± 2.24 months (95%CI, 7.62–16.38), 9.00 ± 1.80 months (95%CI, 5.47–12.53) for the MRD 1+, MRD 2+groups, respectively (Figure 3).

3.4 | Multivariable analyses

In the MRD-positive and NR groups, the unadjusted univariate analyses of blast cell level (<20% vs. ≥20%), immunophenotype (MRD 0+ vs. MRD 1+/MRD 2+), genetic risk group (favorable/

TABLE 1 Pre-transplantation demographic and clinical characteristics of study population ($N = 197$)

	MRD- ($n = 54$)		MRD+ ($n = 32$)		NR ($n = 111$)			
Parameter	**No.**	**%**	**No.**	**%**	**No.**	**%**	p^*	p^{**}
Age								
Median	27.69		28.50		32.66		0.801	0.422
Range	2–54		4–60		2–62			
Sex								
Male	30	55.56	19	59.38	62	55.86	0.453	0.875
Female	24	44.44	13	40.62	49	44.14		
Genetics risk								
Favorable	16	29.63	6	18.75	22	19.82	0.040	0.455
Intermediate	24	44.44	9	28.13	40	36.04		
Unfavorable	14	25.93	17	53.12	49	44.14		
r/rAML status								
De novo	51	94.44	25	78.12	96	86.49	0.028	0.693
Secondary	3	5.56	7	21.88	15	13.51		
Donor relationship								
Haploidentical	29	53.70	18	56.25	54	48.65	0.568	0.355
Other related	25	46.30	14	43.75	57	51.35		
NRM	6	11.11	8	25.00	31	27.93	0.085	0.053
Disease burden								
0.1–5.0	0	0	32	100	0	0		0.000
5.0–20	0	0	0	0	27	24.32		
≥20	0	0	0	0	84	75.68		
MRD phenotype								
MRD 0+	0	0	14	43.75	14	12.61		0.001
MRD 1+	0	0	10	31.25	53	47.75		
MRD 2+	0	0	8	25.00	44	39.64		

Abbreviations: MRD 0+, without any antigen abnormal expression of CD7+, CD56+, CD38−, or HLA-DR−; MRD 1+, with one abnormal antigen expression of CD7+, CD56+, CD38−, or HLA-DR−; MRD 2+, with two or more abnormal antigens expression of CD7+, CD56+, CD38−, or HLA-DR−; MRD−, MRD-negative; MRD+, MRD-positive; NR, not in complete remission; NRM, non-relapse mortality; r/rAML, refractory or relapsed acute myeloid leukemia; Genetic risk was stratified according to 2017 ELN criteria.

*For the comparison MRD− vs. MRD+.

**For the comparison CR (MRD− and MRD+) vs. NR.

intermediate vs. unfavorable), r/rAML status (de novo vs. secondary), age (≦25 vs. >25), sex and donor relationship (haploidentical vs. other related) pre-HCT for DFS, OS, relapse and NRM is summarized in Table 2. In the final multivariate model only MRD immunophenotype was significant at $p < 0.05$ for DFS, OS and relapse, the hazard ratios of immunophenotype MRD 1+/MRD 2+ vs. MRD 0+ were 6.69(range 2.08–21.52; $p = 0.001$) for DFS, 2.24(range 1.21–4.14; $p = 0.010$) for OS, and 7.18(range 2.23–23.10; $p = 0.001$) for relapse.

4 | DISCUSSION

This retrospective analysis supports three major conclusions. First, allo-HCT is the best choice for r/rAML patients even not in CR status, our 3-year estimates of DFS and OS were 24.8 ± 4.3% and 31.6 ± 4.8% respectively. This result is better than previous studies[5] because we treated r/rAML patients with an intensive conditioning regimen consisting of CLAG plus modified BuCy prior to allo-HCT.[4] Besides, we chose cord-haplo HCT, which involves the usual haplo-HCT procedure plus a low-dose UCB infusion.[19] Second, not in CR status and MRD-positive were associated with poor outcome compared with CR status and MRD-negative patients. This result was similar to previous studies.[29,31,32] Third, abnormal expression of CD7+, CD56+, HLA-DR− and CD38− was associated with decreased DFS (HR, 6.69; 95%CI, 2.08–21.52; $p = 0.001$) and OS (HR, 2.24; 95%CI, 1.21–4.14; $p = 0.010$), and increased relapse (HR, 7.18; 95%CI, 2.23–23.10; $p = 0.001$) relative to CD7−, CD56−, HLA-DR+ and CD38+ patients pre-HCT.

It was difficult to achieve MRD-negative for r/rAML patients pre-HCT even with an intensive treatment regimen.[4,33] In our

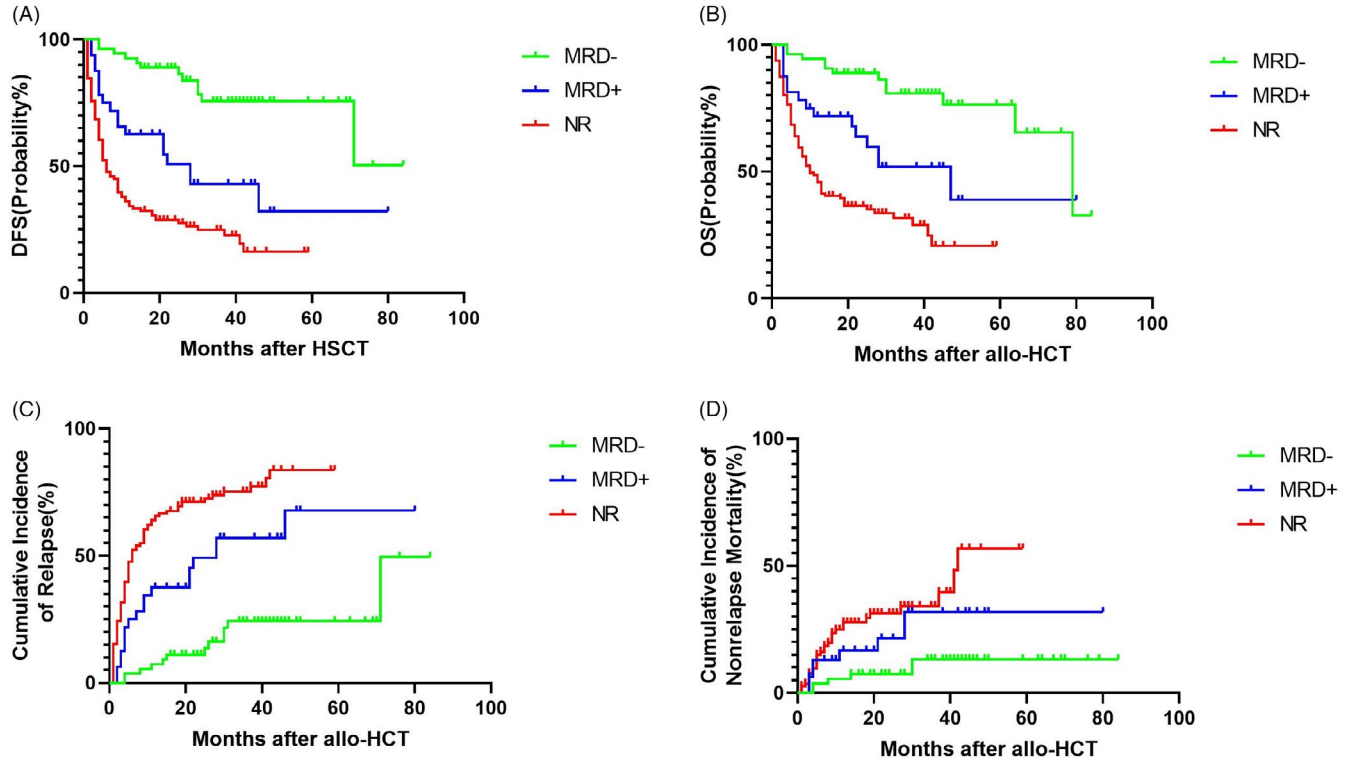

FIGURE 2 Effects of disease burden and MRD pre-HCT on outcomes in patients with refractory or relapsed acute myeloid leukemia. Kaplan-Meier survival analysis of probability of (A) disease-free and (B) overall survival. Cumulative incidence of (C) relapse and (D) non-relapse mortality. MRD−, MRD-negative; MRD+, MRD-positive. NR, not in complete remission

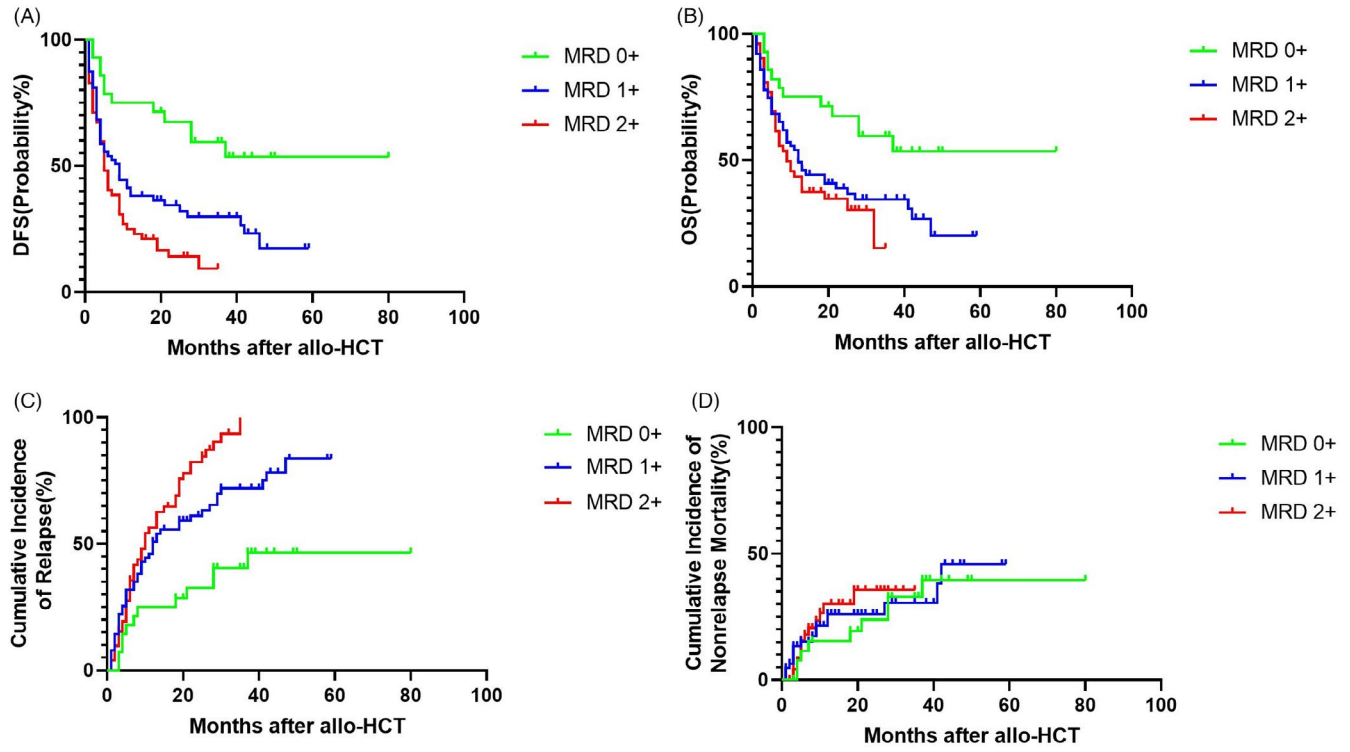

FIGURE 3 Effects of immunophenotype pre-HCT on outcomes in patients with refractory or relapsed acute myeloid leukemia. Kaplan-Meier survival analysis of probability of (A) disease-free and (B) overall survival. Cumulative incidence of (C) relapse and (D) non-relapse mortality

TABLE 2 Univariate Analyses for DFS, OS, Relapse and NRM in MRD+ and NR patients (*p*)

Factor	DFS	OS	Relapse	NRM
Blast cell level				
<20% (*n* = 59)	0.006	0.303	0.008	0.236
≥20% (84)				
Immunophenotype				
MRD 0+ (*n* = 28)	0.000	0.004	0.000	0.598
MRD 1+/MRD 2+ (*n* = 115)				
Genetic risk group				
Favorable/intermediate (*n* = 77)	0.223	0.070	0.112	0.128
Unfavorable (*n* = 66)				
r/rAML status				
De novo (*n* = 121)	0.713	0.163	0.940	0.041
Secondary (*n* = 22)				
Age				
≦25 (*n* = 51)	0.612	0.024	0.384	0.005
>25 (*n* = 92)				
Sex				
Female (*n* = 62)	0.275	0.598	0.148	0.631
Male (*n* = 81)				
Donor relationship				
Haploidentical (*n* = 69)	0.220	0.656	0.195	0.927
Other related (*n* = 74)				

Abbreviations: MRD 0+, without any antigen abnormal expression of CD7+, CD56+, CD38−, or HLA-DR−; MRD 1+/MRD 2+, with any antigen abnormal expression of CD7+, CD56+,CD38− or HLA-DR-; r/rAML, refractory or relapsed acute myeloid leukemia; Genetics risk was stratified according to 2017 ELN criteria. Other related, human leukocyte antigen matched more than half.

study, only 54 patients (27.41%) achieved MRD-negative, whereas 143 patients (72.59%) had varying degrees of disease burden (0.2%–97%). To identify the correlation of immunophenotype and outcomes, we divided the MRD+ group and NR group into three subgroups according to their abnormal expression of CD7, CD56, CD38, and HLA-DR: MRD 0+, MRD 1+, and MRD 2+, our result shown 3-year estimates of DFS for MRD 0+, MRD 1+ and MRD 2+ patients were 59.5 ± 9.5%, 29.9 ± 6.1% and 9.4 ± 5.1%, MRD 0+ vs. MRD 1+(*p* 0.003), MRD 1+ vs. MRD 2+(*p* 0.038); and the estimates of relapse at 3 years were 40.5 ± 9.5%, 72.0 ± 6.1% and 94.4 ± 4.7%, MRD 0+ vs. MRD 1+ (*p* 0.006), MRD 1+ vs. MRD 2+ (*p* 0.033). This result suggested that not only abnormal expression of CD7+, CD56+, HLA-DR−, and CD38− is associated with poor outcomes, but also the more number of abnormal antigens expression predict the worse outcomes.

Previous studies have shown immunophenotype abnormalities are useful for MRD detection and quantification with the aim of providing prognostic information.[34,35] Chang et al.[36] found that, in normal karyotype AML, CD7 was expressed in 37% of patients, and CD7 was associated with shorter DFS. In our study, 48 patients expressed CD7 in 143 not in MRD-negative patients pre-HCT (34%), and survival analysis shown CD7+ patients associated with the worst prognosis compared with CD56+, HLA-DR-, and CD38- patients in the MRD 1+ group (Figure A1 in Appendix 1). Yang et al.[37] reported that CD56 expression in AML correlated with reduced DFS and OS, including inpatients who underwent transplantation. However, a recent study shown CD56 expressed in different cohorts predicted distinct outcomes, the 5-year DFS were 69%, 39%, and 19%, respectively in *t*(8;21), 11q23, and high-intensity expression of CD56 patients. Their results remind us that we should consider immunophenotype combined with genotype. Our data shown 42 patients expressed CD56 in 143 not in MRD-negative r/rAML patients (29%), and we need more cases of CD56+ patients to clear the outcome in different genotype groups. In addition, the activity of leukemic stem cells (LSC) in AML is thought to reside within the stem cell CD34+CD38-compartment, and experimental data indicate that LSC are more resistant to chemotherapy than the more mature CD34+CD38+ progeny.[19,37] Besides, AML relapse was associated with the dysregulation of pathways that may influence immune function, including down-regulation of MHC class II genes (HLA-DPA1, HLA-DPB1, HLA-DQB1, and HLA-DRB1) involved in antigen presentation.[38] Our study first time combine CD7, CD56, CD38, and HLA-DR together as our flow cytometry r/rAML MRD panel, and the result shown the more number of abnormal antigens expression predict the worse outcomes. The abnormal expression of CD7+,、CD56+,、HLA-DR−, and CD38− may be used for r/rAML prognosis stratification, and we need more data combine with genotype to confirm this point.

Our study had some limitations. First, our flow cytometry r/rAML MRD panel may not have covered all the blast cells, a condition that would result in low sensitivity. Second, the disappearance of characteristic antigen profiles at relapse may cause false negative cases. Third, the number of patients in each of MRD subgroup was small, and we need more data to verify the abnormal expression of CD7+, CD56+, HLA-DR−, and CD38− for prognosis. Lastly, our analysis was a case-control study, thus, there may been some bias in patient selection.

Cytogenetic and molecular markers have been used to define risk groups, however, most patients lack risk-associated molecular markers. Multiparametric flow cytometry detection of MRD has permitted the stratification of patients who lack genetic abnormalities associated with outcomes.[29]

Our data suggested that the abnormal expression of CD7+, CD56+, HLA-DR−, and CD38− is associated with poor DFS, poor OS, and high relapse; and the more number of abnormal antigens expression predict worse outcomes.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare at https://doi.org/10.1002/JCLA.23974.

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APPENDIX 1

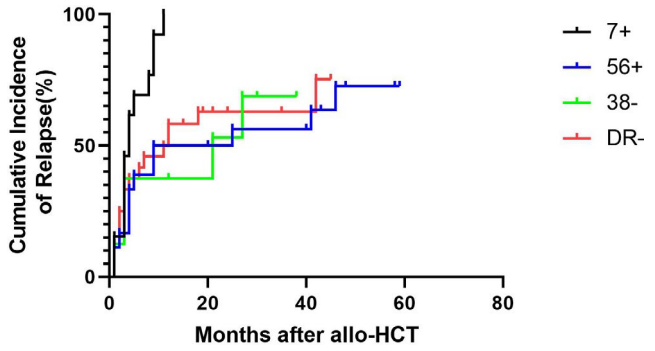

Figure A1 Effects of immunophenotype pre-HCT on outcome in 63 MRD 1+ r/rAML patients