Clinical characteristics and therapeutic determinants of *RUNX1::RUNX1T1* differ from those of *CBFB::MYH11* acute myeloid leukemia

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Abstract

Core binding factor acute myeloid leukemia (CBF-AML) includes *RUNX1::RUNX1T1* and *CBFB::MYH11 AML*. To investigate whether they should be regarded as distinct entities and treated separately, we retrospectively analyzed 536 patients with CBF-AML aged 60 years or younger. For *CBFB::MYH11* AML, no outcome differences were observed between standard-dose (SD) and intermediate-dose (ID) cytarabine induction, with 5-year overall survival (OS) and relapse-free survival (RFS) at 86.4% *versus* 85.3% (P=0.99) and 74.1% *versus* 68.4% (P=0.93), respectively. However, ID induction improved the outcomes of *RUNX1::RUNX1T1* AML, with 5-year OS and RFS rates of 77.7% *versus* 60.3% (P<0.001) and 71.4% *versus* 54.1% (P<0.001), respectively. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) improved *CBFB::MYH11* patients' RFS but not OS for both the entire *CBFB::MYH11* cohort and patients with a measurable residual disease (MRD) <3-log reduction after two courses of chemotherapy. Allo-HSCT improved survival of *RUNX1::RUNX1T1* patients with MRD <3-log reduction when receiving SD induction, with 5-year OS and RFS rates for transplantation *versus* non-transplantation of 71.9% *versus* 43.6% (P=0.023) and 72.9 % *versus* 35.0% (P=0.001), while conferring no additional benefits for those with ID regimen, with a 5-year OS and RFS of 35.6% *versus* 71.2% (P=0.73) and 37.4% *versus* 60.7% (P=0.71), respectively. Overall, our study suggests that *RUNX1::RUNX1T1* and *CBFB::MYH11* AML are different entities and should be treated with distinct strategies.

Introduction

Core binding factor acute myeloid leukemia (CBF-AML) is a common type of leukemia, accounting for approximately 15% of *de novo* adult AML.[1,2] It is characterized by the formation of *RUNX1::RUNX1T1* or *CBFB::MYH11* fusion genes, corresponding to t(8;21) or inv(16) chromosomal rearrangements. The two chromosomal rearrangements disrupt the subunits encoding the CBF complex, thereby impairing normal hematopoiesis and leading to maturation arrest.[3]

Despite being classified as distinct entities in current AML classification systems, *RUNX1::RUNX1T1* and *CBFB::MYH11*-positive AML are frequently grouped and investigated collectively in clinical research due to their overlapping pathogenic mechanisms and similarly favorable prognostic profiles.[4] These two types of AML share similar treatment regimens and strategies. The '7+3' regimen (7 days of continuous infusion cytarabine plus 3 days of anthracycline) with or without gemtuzumab ozogamicin (GO) serving as the standard induction regimen is recommended by current guidelines for both genetic groups.[5,6] However, patients with *RUNX1::RUNX1T1* or *CBFB::MYH11* AML seem to display notable differences in several biological features. For instance, the mutation profiles revealed that *RUNX1::RUNX1T1* patients frequently harbor mutations in

epigenetic regulators, chromatin modulators, or cohesion proteins, whereas *CBFB::MYH11* AML is more commonly associated with kinase mutations.[7-9] Although both *RUNX1::RUNX1T1* and *CBFB::MYH11* AML are categorized as favorable risk according to European LeukemiaNet (ELN) risk stratification,[5] their outcomes diverge significantly. Overall, patients with *CBFB::MYH11* have a lower risk of relapse and longer survival compared to those with *RUNX1::RUNX1T1*.[10,11] Hence, the question arises as to whether patients with *RUNX1::RUNX1T1* and *CBFB::MYH11* AML should be regarded as distinct entities based not only on biological differences, but also on therapeutic determinants. To answer this question, we conducted a retrospective analysis of 370 patients with *RUNX1::RUNX1T1* and 166 with *CBFB::MYH11* at our center with the aim of elucidating the differential clinical characteristics and therapeutic determinants of these two AML subtypes.

Methods

Patients

In total, 3,144 consecutive patients with newly diagnosed AML at our center between January 2011 and January 2024 were retrospectively assessed in this study. Enrolled patients had to meet the following criteria: (i) onset age not exceeding 60 years, (ii) CBF-AML, and (iii) receiving intensive inductions. This study represents an expanded and updated cohort of patients with CBF-AML, building on our previously published work.[12] The study was approved by the Blood Diseases Hospital Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Written informed consent for treatment and biological sample collection was obtained from all patients.

Treatments

All patients received intensive induction therapy with a combination of standard-dose (SD) (100 mg/m^2/day 1-7 as a 12-hour [h] intravenous [iv] infusion) or intermediate-dose (ID) cytarabine (100 mg/m^2/day 1-4 as a 12-h iv infusion and 1 mg/m^2/day every 12 h as a 3-h iv infusion on days 5-7) plus anthracycline ± homoharringtonine (HHT), followed by three to four courses of consolidation regimens with high or intermediate doses of cytarabine after complete remission (CR). The treatment regimen was consistent with the therapeutic protocols previously reported for AML patients in our center.[13,14] Intrathecal injections were administered for routine prophylaxis of central nervous system leukemia. The regimen typically included cytarabine (40-50 mg/dose), methotrexate (5-15 mg/dose), and dexamethasone (5-10 mg/dose), with two doses administered during induction and consolidation phases. Hematopoietic stem cell transplantation (HSCT) was recommended for patients with a measurable residual disease (MRD) <3-log reduction after two courses of chemotherapy. In our study, 86 patients underwent HSCT during first complete remission (CR1). Of these, 69 (80.2%) had MRD-based indications for transplantation, while 17 (19.8%) opted for HSCT despite achieving >3-log MRD reduction post-induction.

Next-generation sequencing

Next-generation sequencing (NGS) was performed on a custom targeted gene panel of 267 genes closely related to hematological neoplasms through the Illumina platform with a sensitivity of 1%. Total DNA was extracted from the bone marrow mononuclear cells. Using ribosomal RNA depletion for library preparation, high-throughput sequencing was performed at a depth of ranging from 1,000X to 2,000X. The visualization of mutations was performed by complexheatmap R package.[15] NGS was conducted on 331 patients, representing 61.8% of the total cohort.

Measurable residual disease detection by quantitative polymerase chain reaction

Fresh bone marrow samples were collected, and total RNA was isolated using an RNA extraction kit following the manufacturer's protocol. Extracted RNA was analyzed by real-time quantitative polymerase chain reaction (qPCR) for *RUNX1::RUNX1T1* and *CBFB::MYH11* fusion transcripts on an ABI QuanStudio 5. Values were expressed as a percentage of the fusion transcript to normalizing *ABL1*. The sensitivity of detection was 1 in 100,000. The patients underwent fusion transcript testing at diagnosis and after each course. The MRD level was calculated as the log reduction in transcript levels between diagnosis and two cycles of chemotherapy. In total, 512 patients underwent serial MRD assessments, with 359 (97.0%) patients in the *RUNX1::RUNX1T1* group and 153 (92.2%) patients in the *CBFB::MYH11* group.

Statistical analysis

Descriptive statistics were used to describe patients' baseline characteristics, and data comparisons were conducted using the χ^2 test or Mann-Whitney U test. Cumulative incidence of relapse (CIR) was measured from the date of CR until the date of relapse or last follow-up. CIR was compared by Fine-Gray test. Overall survival (OS) was defined as the interval from the date of diagnosis to death from any cause or last follow-up. Relapse-free survival (RFS) was defined as the time from achieving complete remission (CR) to the first relapse, death, or the date of the last follow-up. OS and RFS were evaluated by Kaplan-Meier method and compared by the Log-rank test. Landmark analysis was used to avoid bias introduced by patients who experienced early relapse or death before HSCT when analyzing the effect of HSCT. The landmark day was set as the median time from CR to HSCT. Statistical significance was set at P<0.05. This study applied R version 4.2.0 for graphing and statistical analysis.

Results

Clinical characteristics

A total of 536 patients with newly diagnosed CBF-AML were retrospectively enrolled and treated between January 2011 and January 2024 at our center. Figure 1 depicts the detailed screening process. Among the 536 patients, 303 (56.5%) were male and 233 (43.5%) were female, with a median age of onset at 35 years (range, 14-60 years). According to the World Health Organization (WHO) 2022 classification,[16] 370 (69.0%) cases were categorized as AML with *RUNX1::RUNX1T1* and 166 (31.0%) as AML with *CBFB::MYH11*. Patients with *RUNX1::RUNX1T1* presented with an earlier age of onset and lower white blood cell counts (WBC), with a median

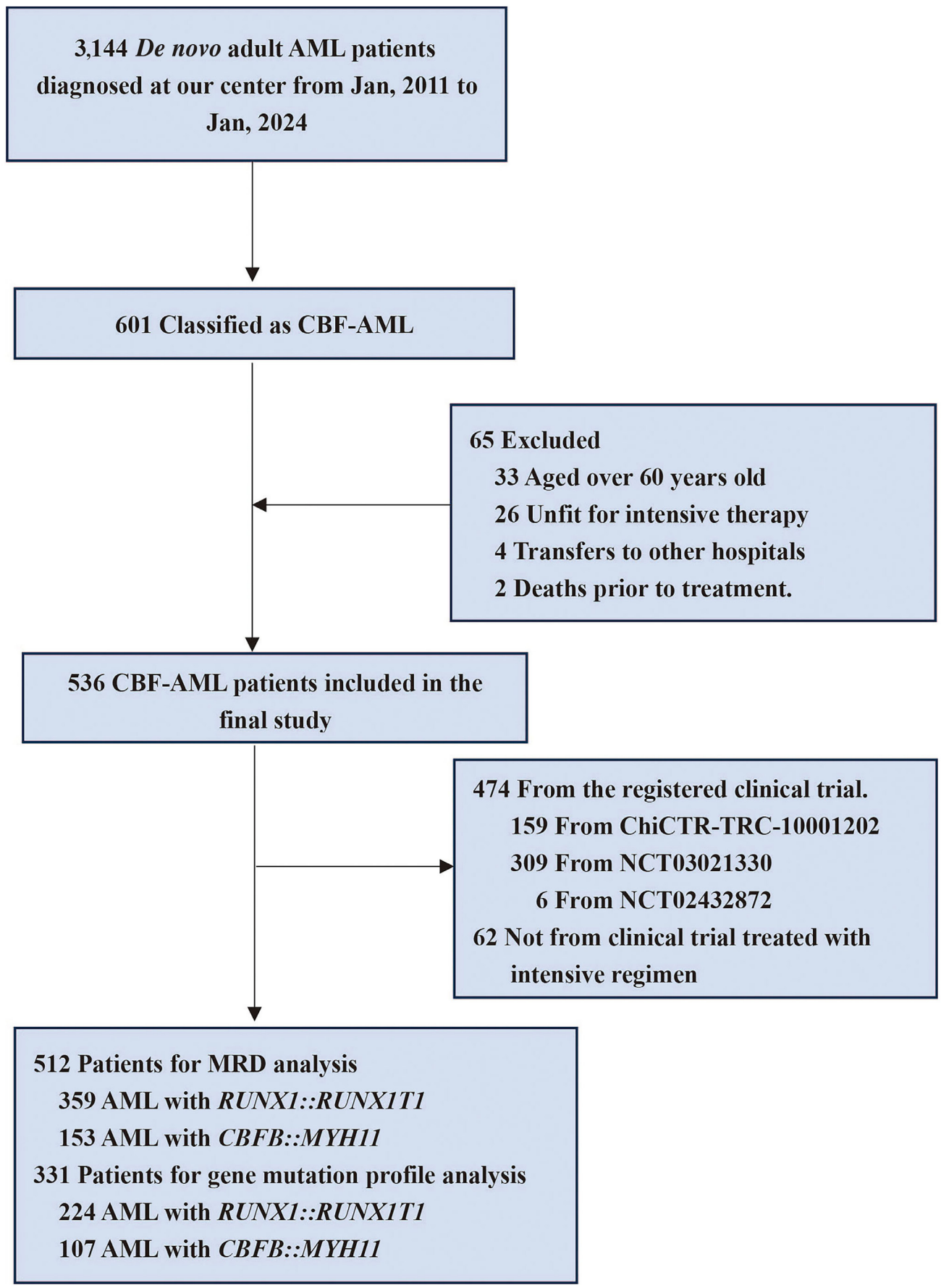

Figure 1. Study flow diagram. ChiCTR-TRC-10001202: a phase III study on optimizing treatment based on risk stratification for acute myeloid leukemia; *clincaltrials gov. Identifier: NCT03021330*: efficacy of intermediate-dose cytarabine induction regimen in adult acute myeloid leukemia (AML); *clincaltrials gov. Identifier: NCT02432872:* treatment of older adult acute myeloid leukemia patients aged 55 to 65 years. CBF-AML: core-binding factor acute myeloid leukemia; MRD: measurable residual disease.

age of 34 years *versus* 39.5 years (*P*<0.001) and a median WBC count of 8.5 *versus* 30.3 (*P*<0.001) than patients with *CBFB::MYH11*, respectively. All patients in the cohort were treated with intensive chemotherapy, with 185 (50.0%) of *RUNX1::RUNX1T1* and 99 (59.6%) of *CBFB::MYH11* undergoing induction with SD cytarabine, and 185 (50.0%) and 67 (40.4%) receiving induction with ID cytarabine, respectively. Additionally, 60 (16.2%) patients with *RUNX1::RUNX1T1* and 26 (15.7%) with *CBFB::MYH11* underwent HSCT in CR1. The detailed clinical characteristics of the two groups were presented in Table 1.

Gene mutation profiles and cytogenetic abnormalities

Genetic mutation spectrum revealed that both subtypes of AML were frequently associated with kinase-related gene mutations, such as *KIT*, *RAS*, and *FLT3*. In contrast, genes involved in epigenetic regulation, such as *ASXL1*, *ASXL2*, *TET2*, and *ZBTB7A*, were more likely to be found in patients with *RUNX1::RUNX1T1* compared to those with *CBFB::MYH11* (*Online Supplementary Figure S1*). We then investigated whether gene mutations in a sample size of more than ten patients affected patients' survival. Univariate analysis revealed that, for patients with *RUNX1::RUNX1T1*, the presence of *KIT* and *TET2* mutations adversely correlated with OS and RFS, with hazard ratio (HR) for OS of 2.81 (95% confidence interval [CI]: 1.48-5.33; *P*=0.002) and 3.80 (95% CI: 1.59-9.09; *P*=0.003), and for RFS at 2.86 (95% CI: 1.69-4.82; *P*<0.001) and 3.57 (95% CI: 1.75-7.29; *P*<0.001), respectively. While NRAS mutations were generally indicative of better OS and RFS, with HR=0.31 (95% CI: 0.11-0.88; *P*=0.027) for OS and 0.38 (95% CI: 0.17-0.83; *P*=0.015) for RFS. In contrast, these genetic aberrations did not exert a statistically significant impact on the survival of patients with *CBFB::MYH11* (*Online Supplementary Table S1*). *Online Supplementary Figure S2* presented the survival curves for patients with and without the specified gene mutations in *RUNX1::RUNX1T1* AML. Other gene mutations have not been further explored due to insufficient sample sizes.

We next compared the cytogenetic profiles between the two CBF-AML subtypes. As summarized in Table 1, loss of sex chromosomes (52.9%) was the most prevalent abnormality in *RUNX1::RUNX1T1* patients, followed by deletion of chromosome 9 (5.8%). In *CBFB::MYH11* patients, the predominant cytogenetic abnormalities were trisomy 22 (23.3%) and trisomy 8 (8.8%). To evaluate their prognostic significance, we conducted survival analyses focusing on these recurrent chromosomal alterations. In *RUNX1::RUNX1T1* patients, neither sex chromosome loss nor chromosome 9 deletion significantly impacted survival outcomes. Patients with sex chromosome loss showed comparable OS (5-year OS: 72.4±3.8% *vs*. 67.1±4.1%; *P*=0.35) and RFS (5-year RFS: 66.2±3.8% *vs*. 60.8±4.2%; *P*=0.61) to those without this abnormality. Similarly, deletion of chromosome 9 did not significantly affect outcomes, with 5-year OS and RFS at

Table 1. Clinical characteristics of the patients diagnosed as core binding factor acute myeloid leukemia.

Characteristics, N (%)	*RUNX1::RUNX1T1* N=370	*CBFB::MYH11* N=166	*P*
Sex			0.433
Male	205 (55.4)	98 (59.0)	
Female	165 (44.6)	68 (41.0)	
Age at diagnosis, years			<0.001
Median (range)	34 (14-60)	39.5 (14-59)	
14-17	45 (12.2)	9 (5.4)	
18-40	196 (53.0)	74 (44.6)	
40-60	129 (34.9)	83 (50.0)	
WBC $\times 10^9$/L, median (range)	8.5 (0.94-138.8)	30.3 (1.3-268.99)	<0.001
Cytogenetics#			<0.001
Trisomy 8	3 (0.8)	14 (8.8)	
Trisomy 9	0	1 (0.6)	
Trisomy 21	1 (0.3)	3 (1.9)	
Trisomy 22	2 (0.6)	37 (23.3)	
Loss of sex chromsome	190 (52.9)	1 (0.6)	
Deletion of chromosome 9	21 (5.8)	0	
Gene mutations			<0.001
KIT	108 (48.2)	39 (36.4)	
NRAS	46 (20.5)	57 (53.3)	
FLT3	35 (15.6)	31 (29.0)	
ASXL2	28 (15.2)	1 (1.2)	
ZBTB7A	14 (12.3)	1 (1.4)	
ASXL1	27 (12.1)	1 (0.9)	
KRAS	14 (6.3)	28 (26.2)	
TET2	14 (6.3)	0	
KIT mutations			<0.001
Exon 17: D816	46 (20.5)	21 (19.6)	
Exon 17: N822	59 (26.3)	5 (4.7)	
Exon 17: D820	7 (3.1)	0	
Exon 8: D419	16 (7.1)	18 (16.8)	
Other exons	4 (1.8)	1 (0.9)	
Induction regimen			0.039
SD cytarabine	185 (50.0)	99 (59.6)	
ID cytarabine	185 (50.0)	67 (40.4)	
HSCT in CR1	60 (16.2)	26 (15.7)	0.872
CR within 1 cycle	322 (87.0)	157 (94.6)	0.009
CR within 2 cycles	353 (95.4)	160 (96.4)	0.605
MRD reduction >3-log*	226 (63.0)	43 (28.1)	<0.001
PCR-undetectable MRD*	66 (18.4)	11 (7.2)	0.001

#A total of 359 patients with *RUNX1::RUNX1T1* and 159 patients with *CBFB::MYH11* underwent conventional karyotype analysis. *Determined after 2 cycles of induction therapy. The polymerase chain reaction (PCR) undetectable measurable residual disease (MRD) patients represent a subset of those achieving a >3-log MRD. CBF-AML: core binding factor acute myelogenous leukemia; WBC: white blood cell; CR1: the first complete remission; SD: standard dose of cytarabine; ID: intermediate dose of cytarabine; HSCT: hematopoietic stem cell transplantation.

82.1±9.4% *versus* 69.1±2.9% (*P*=0.29) and 73.8±10.2% *versus* 62.1±3.0% (*P*=0.38) (*Online Supplementary Figure S3A-D*). Among *CBFB::MYH11* patients, trisomy 8 showed a trend toward better RFS at 100% *versus* 69.0±5.3% (*P*=0.082), while OS remained comparable between groups at 100% *versus* 85.7±3.4% (*P*=0.21) (*Online Supplementary Figure S4A, B*). Trisomy 22 status did not significantly influence survival outcomes, with similar OS (91.7±5.7% *vs*. 85.3±3.7%; *P*=0.23) and RFS (70.1±5.7% *vs*. 76.5±9.9%; *P*=0.31) observed regardless of its presence (*Online Supplementary Figure S4C, D*). Other chromosomal abnormalities were not further analyzed due to limited sample size.

Response and outcomes

Among the 536 patients, 513 (95.7%) achieved CR within two cycles of induction, including 353 (95.4%) with *RUNX1::RUNX1T1* and 160 (96.4%) with *CBFB::MYH11*. A total of 512 in these 513 CR patients had informative MRD testing results. Of these, 226 (63.0%) in *RUNX1::RUNX1T1* and 43 (28.1%) in *CBFB::MYH11* achieved a >3-log MRD reduction, which included 66 (18.4%) and 11 (7.2%) patients, respectively, who demonstrated PCR-undetectable MRD. In the *RUNX1::RUNX1T1* group, 131 patients (72.4%) in the ID cytarabine induction achieved a >3-log MRD reduction, compared to 95 patients (53.4%) in the SD group, while in the *CBFB::MYH11* group, 23 patients (35.9%) in the ID group achieved this reduction *versus* 20 patients (22.5%) in the SD group. With a median follow-up of survivors of 50 months, the estimated 5-year OS and RFS were 70.0±2.8% *versus* 86.1±3.3% (*P*=0.003) and 63.7±2.8% *versus* 71.1±4.9% (*P*=0.010) for *RUNX1::RUNX1T1* and *CBFB::MYH11* patients, respectively (Figure 2A, B). The 5-year OS and RFS censored at HSCT were 71.2±2.9% *versus* 85.7± 3.6% (*P*=0.010) and 65.7±2.9% *versus* 66.7±5.8% (*P*=0.042), respectively (*Online Supplementary Figure S5*). We then performed a multivariate analysis incorporating age, sex, WBC, induction regimen, CR1 HSCT, and gene mutations in *KIT*, *TET2*, and *NRAS*. The results indicated that for patients with *RUNX1::RUNX1T1*, age, induction regimen, and mutations in *KIT*, *TET2*, and *NRAS* served as independent prognostic factors for OS, but not for patients with *CBFB::MYH11* (Table 2).

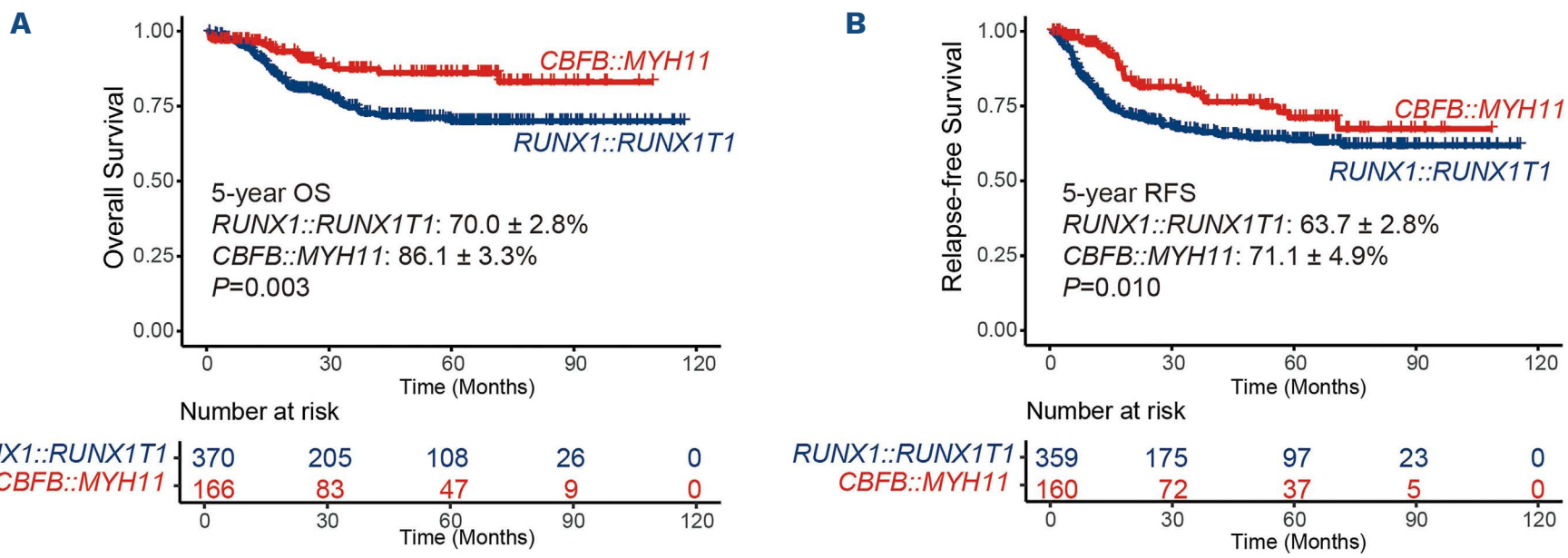

Figure 2. Outcomes of patients with *RUNX1::RUNX1T1* and *CBFB::MYH11*. (A) Overall survival (OS) and (B) relapse-free survival (RFS). The red line represents patients with *CBFB::MYH11*, whereas the blue line represents those with *RUNX1::RUNX1T1*.

Table 2. Multivariate analysis results for overall survival of patients with core binding factor acute myeloid leukemia.

	RUNX1::RUNX1T1		*CBFB::MYH11*	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Age	1.029 (1.002-1.057)	0.037	1.028 (0.971-1.088)	0.344
WBC	1.004 (0.986-1.023)	0.65	0.996 (0.981-1.011)	0.597
Female	1.202 (0.654-2.211)	0.554	0.961 (0.26-3.558)	0.952
ID induction	0.471 (0.253-0.876)	0.017	0.798 (0.214-2.973)	0.737
CR1 HSCT	0.818 (0.368-1.817)	0.622	1.542 (0.139-17.121)	0.724
KIT^{mut}	2.813 (1.479-5.353)	0.002	0.384 (0.077-1.908)	0.242
NRAS^{mut}	0.262 (0.091-0.75)	0.013	-	-
TET2^{mut}	4.619 (1.733-12.313)	0.002	-	-

WBC: white blood cell; ID: intermediate-dose induction; CR1: first complete remission; HSCT: hematopoietic stem cell transplantation. *KIT*^{mut}: *KIT* mutation; *NRAS*^{mut}: *NRAS* mutation; *TET2*^{mut}: *TET2* mutation; HR: hazard ratio; CI: confidence interval.

Impact of induction regimen

We explored the effects of the induction regimen on the patients' outcomes. For *RUNX1::RUNX1T1* patients, the ID regimen demonstrated a significant advantage over the SD regimen, with 5-year OS and RFS rates of 77.7±3.4% *versus* 60.3±4.5% (*P*<0.001) and 71.4±3.5% *versus* 54.1±4.6% (*P*<0.001), respectively. In contrast, for *CBFB::MYH11* patients, no significant differences in survival were observed between the two inductions, with 5-year OS and RFS at 85.3±5.0% *versus* 86.4±4.4% (*P*=0.99) and 68.4±7.7% *versus* 74.1± 5.8% (*P*=0.93) (Figure 3), respectively. Although all patients received high-intensity induction, variations in anthracycline doses and HHT use existed across trials. To eliminate potential biases arising from these variations, we conducted separate analyses within two primary clinical trials. For *RUNX1::RUNX1T1* patients, both clinical trials demonstrated that the ID group had better OS and RFS compared to the SD group (*Online Supplementary Figure S6A-D*), while *CBFB::MYH11* patients exhibited no significant survival differences between ID and SD groups (*Online Supplementary Figure S7A-D*). Next, we investigated the effect of MRD levels on the outcomes of the two patient groups. It was found that patients with a <3-log MRD reduction in *RUNX1::RUNX1T1* had significantly poorer outcomes than those with a >3-log MRD reduction, with 5-year CIR, OS, and RFS rates of 46.2±4.9% *versus* 26.5±3.2% (*P*<0.001),

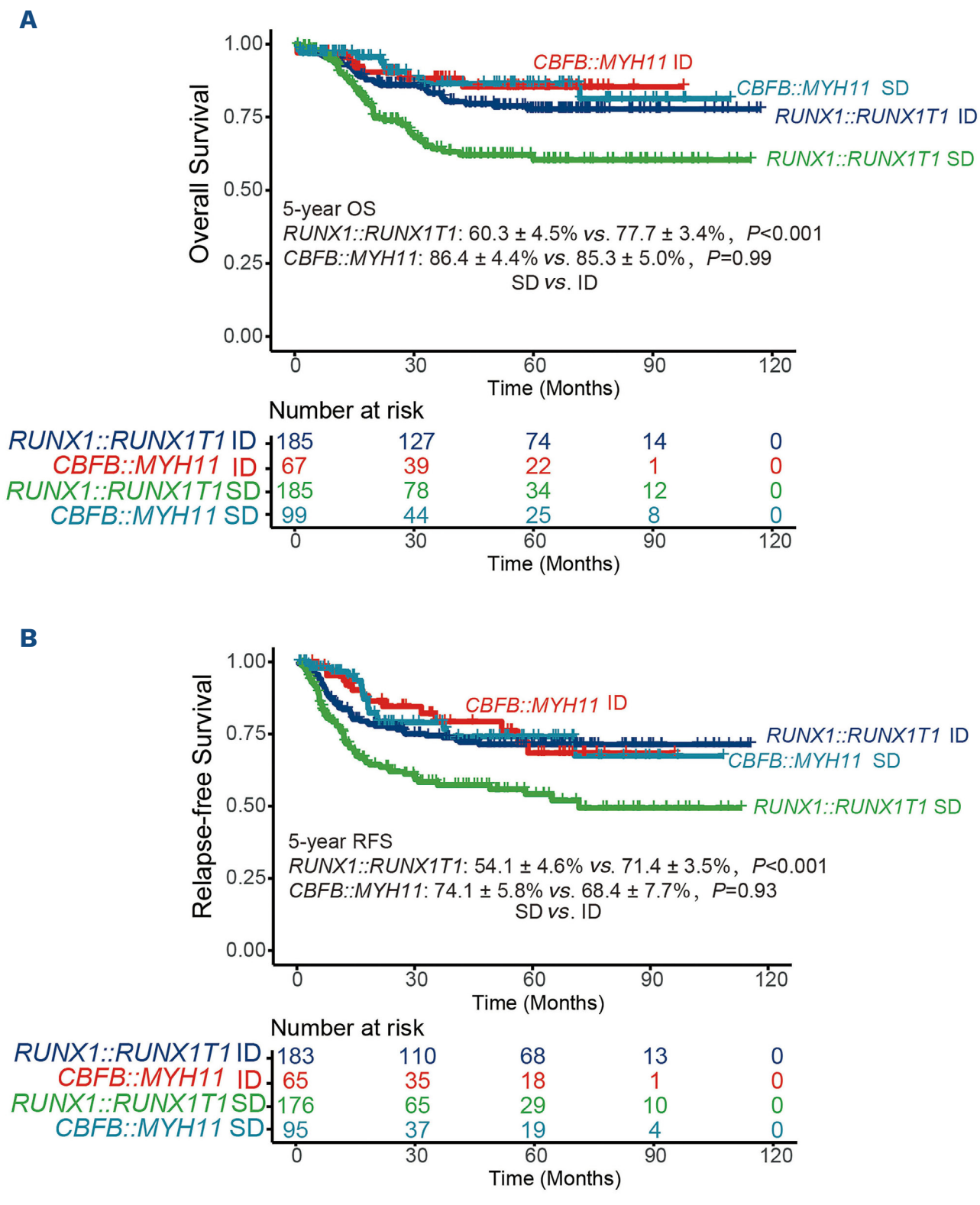

Figure 3. Outcomes of patients with *RUNX1::RUNX1T1* and *CBFB::MYH11* in different induction regimens groups. (A) Overall survival (OS) and (B) relapse-free survival (RFS). The blue line represents *RUNX1::RUNX1T1* patients receiving intermediate-dose (ID) induction, while the green line represents standard-dose (SD) regimen. The red line represents *CBFB::MYH11* patients with the ID regimen, while sky blue represents the SD regimen.

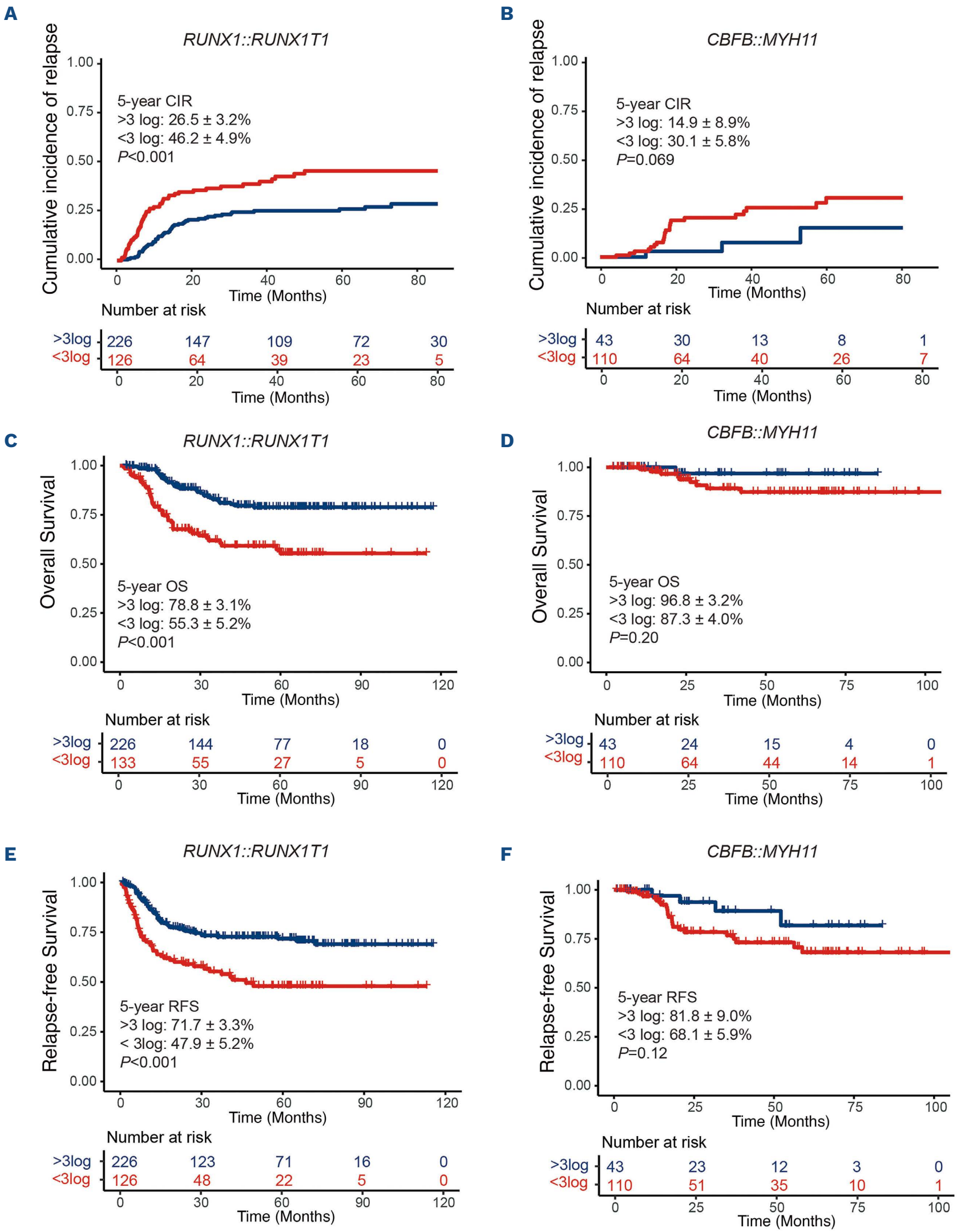

Figure 4. Outcomes of patients with *RUNX1::RUNX1T1* (A, C, and E) and *CBFB::MYH11* (B, D, and F) in the different measurable residual disease groups. (A and B) Cumulative incidence of relapse (CIR); (C and D) overall survival (OS); and (E and F) are relapse-free survival (RFS). The red line represents patients with a <3-log measurable residual disease (MRD) reduction, while the blue line represents patients with a >3-log MRD.

55.3±5.2% *versus* 78.8±3.1% (*P*<0.001), and 47.9± 5.2% *versus* 71.7±3.3% (*P*<0.001) (Figure 4A, C and E), respectively. For *CBFB::MYH11* AML, patients with a <3-log MRD reduction demonstrated a trend associated with higher CIR with 5-year CIR rates of 30.1±5.8% *versus* 14.9±8.9% (*P*=0.069); however, they shared similar OS and RFS with that of their counterparts, 5-year OS rates of 87.3±4.0% *versus* 96.8±3.2% (*P*=0.20), and 5-year RFS rates of 68.1±5.9% *versus* 81.8±9.0% (*P*=0.12) (Figure 4B, D and F). For *CBFB::MYH11* AML, we did not find significantly different OS and RFS, even with a 2-log threshold (*Online Supplementary Figure S8)*. We could not further decrease the threshold due to the limited sample size in the low-level MRD group.

Impact of hematopoietic stem cell transplantation in first complete remission

We further investigated the role of HSCT in CBF-AML patients. To avoid bias introduced by patients who experienced early relapse and death, a landmark analysis was performed. The landmark day was set at 7.2 months. HSCT improved *CBFB::MYH11* patients' RFS but not OS, with 5-year RFS at 91.6± 5.7% with HSCT, while 65.8±5.8% without HSCT (*P*=0.037), and 5-year OS at 91.6±5.7% *versus* 88.1±3.6%, respectively (*P*=0.69) (*Online Supplementary Figure S9*). When comparing survival of patients censored for HSCT, patients underwent HSCT still had improved 5-year RFS at 91.6±5.7%, while 66.7± 5.8% in patients censored for HSCT (*P*=0.045), while no significant difference in OS with 5-year OS at 91.6±5.7% and 85.7±3.6% (*P*=0.46), respectively (*Online Supplementary Figure S10A, B*). However, for all *RUNX1::RUNX1T1* patients, HSCT did not confer a benefit, as the OS and RFS of patients with HSCT were comparable to those of non-HSCT or censored for HSCT (*Online Supplementary Figured S9* and *S10C, D*). We then sought to determine whether HSCT could improve the outcomes in patients with high MRD levels. For patients with a <3-log MRD reduction, those who underwent HSCT demonstrated significantly improved RFS compared to non-transplanted patients, with the *RUNX1::RUNX1T1* group showing 51.6±12.1% *versus* 46.3 ±6.0% (*P*=0.025) and the *CBFB::MYH11* group showing 95.0±4.9% *versus* 60.9±7.0%, respectively (*P*=0.027). However, both groups only had a non-significant trend toward better OS, with rates of 52.6±16.8% *versus* 56.6± 6.1% (*P*=0.13) and 95.0±4.9% *versus* 85.3±4.9% (*P*=0.45), respectively. In *RUNX1::RUNX1T1* patients with a >3-log MRD reduction, the transplantation group exhibited comparable OS and RFS to the non-transplantation group (*P*=0.56 and *P*=0.98, respectively) (*Online Supplementary Figure S11*). For *CBFB::MYH11* patients with a >3-log MRD reduction, we could not compare the effects of HSCT on outcomes because only four patients with a >3-log MRD reduction received HSCT.

Given the finding that the ID induction regimen can improve outcomes for patients with *RUNX1::RUNX1T1* compared to the SD regimen, we further investigated the benefits of HSCT in patients receiving different regimens with residual disease. As shown in Figure 5A-D, for patients who received SD induction, HSCT did provide additional benefits for those with a <3-log MRD reduction, with OS and RFS rates of 71.9±11.5% *versus* 43.6±9.1% (*P*=0.023) and 72.9±11.8% *versus* 35.0±8.2% (*P*=0.001) (Figure 5A, B), respectively. For patients with a >3-log MRD reduction, we could not investigate the benefits of HSCT because only five patients underwent HSCT (Figure 5C, D). Interestingly, among patients receiving ID induction, HSCT did not further improve survival for patients with a <3-log MRD reduction; the 5-year OS and RFS for those undergoing transplantation *versus* non-transplantation were 35.6±26.2% and 37.4±16.4%, 71.2±8.2% and 60.7±8.7%, respectively (*P*=0.73 and *P*=0.71) (Figure 5E, F). Only 15 *RUNX1::RUNX1T1* patients receiving ID induction therapy demonstrated a <2-log MRD reduction. HSCT seemed to improve OS and RFS compared to chemotherapy, although no statistical differences were observed (*Online Supplementary Figure S12*).

Discussion

In this study, we found that ID cytarabine induction significantly improved outcomes for patients with *RUNX1::RUNX1T1*, but not for patients with *CBFB::MYH11*. HSCT improved *CBFB::MYH11* patients' RFS but not OS for both the entire *CBFB::MYH11* cohort and patients with a <3-log MRD reduction after two courses of chemotherapy. For patients with *RUNX1::RUNX1T1* who received SD induction, HSCT did provide additional benefits for those with a <3-log MRD reduction after two courses of chemotherapy, while for those treated with ID cytarabine, HSCT did not confer additional benefits.

Although both *RUNX1::RUNX1T1* and *CBFB::MYH11* AML affect the function of the CBF complex, *CBFB::MYH11* AML carries a better prognosis.[11] Consistent with previous studies, our research confirmed that, despite similar CR rates of over 90% after two induction cycles in both subtypes, the risk of relapse was lower and OS was better in patients with *CBFB::MYH11* compared to those with *RUNX1::RUNX1T1*. Our study revealed an intriguing paradox: *CBFB::MYH11* patients showed slower initial MRD clearance but superior outcomes compared to *RUNX1::RUNX1T1* patients. Serial MRD data showed that while 63.0%, 71.3%, and 78.9% of *RUNX1::RUNX1T1* patients achieved a >3-log reduction after two, three and four cycles of chemotherapy, respectively, *CBFB::MYH11* patients showed more gradual but sustained clearance (28.1%, 46.3%, and 63.6%). This differential MRD kinetics may provide mechanistic insights into the superior clinical outcomes observed in *CBFB::MYH11* patients. The sustained MRD reduction in this group likely reflects more effective eradication of leukemic clones, whereas the rapid but potentially incomplete clearance in *RUNX1::RUNX1T1* patients may predispose to disease recurrence. Further-

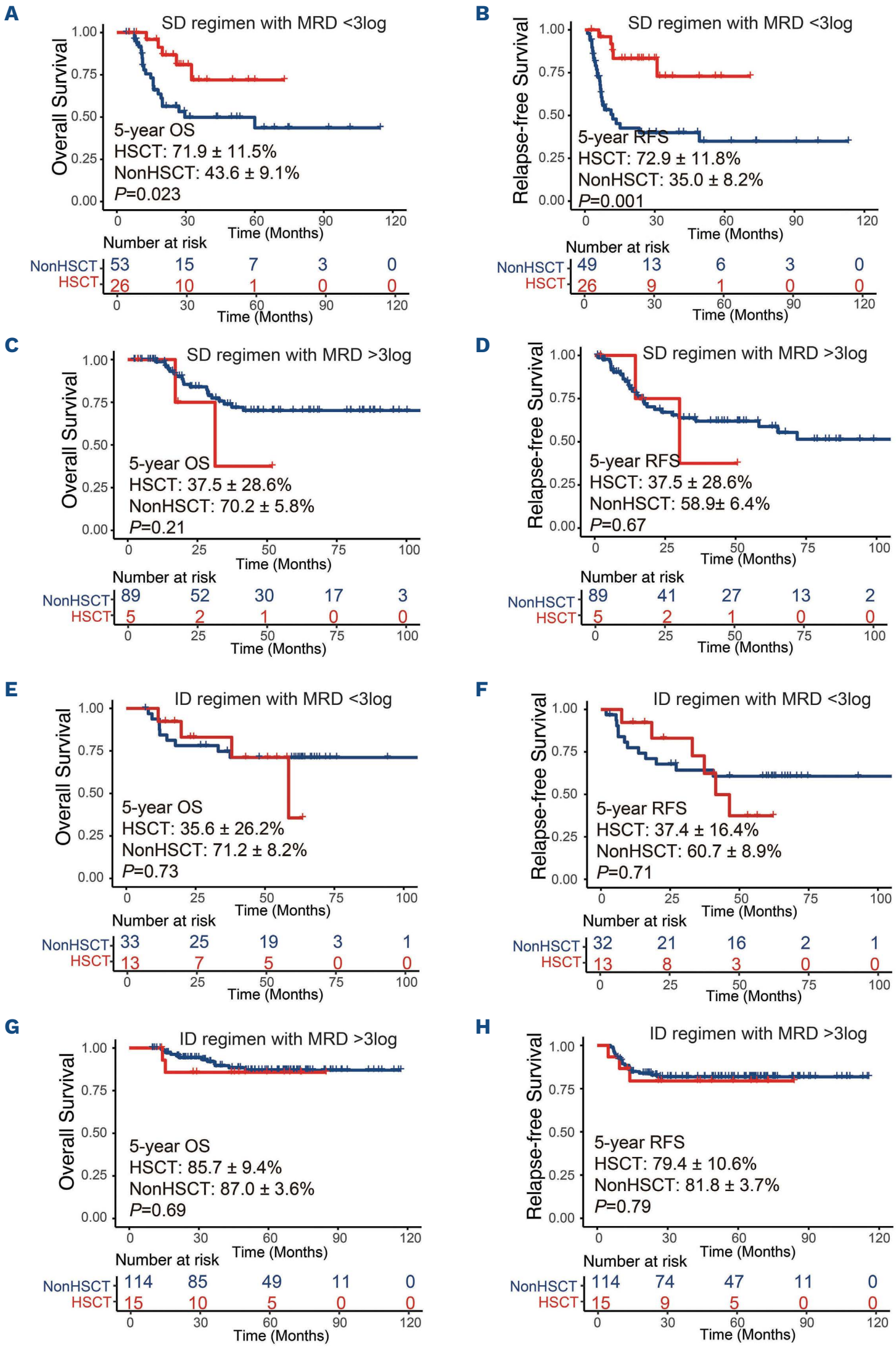

Figure 5. Outcomes of *RUNX1::RUNX1T1* patients with or without hematopoietic stem cell transplantation in different induction regimens. (A-D) Standard-dose regimen (SD); (E-H) intermediate-dose regimen (ID) and measurable residual disease (MRD) levels: (A, B) and (E, F) <3-log; (C, D) and (G, H) >3-log. The red line represents patients who underwent hematopoietic stem cell transplantation (HSCT), whereas the blue line represents patients who did not undergo HSCT. OS: overall survival; RFS: relapse-free survival.

more, the higher prevalence of adverse-risk mutations in *RUNX1::RUNX1T1* patients, may compound this effect by conferring intrinsic treatment resistance.

The optimal induction dose of cytarabine for AML has been the focus of extensive research, with studies yielding varying conclusions.[17] While Willemze *et al*. found that high-dose cytarabine during induction produced higher remission and survival rates than SD cytarabine, especially in patients younger than age 46 years,[18] Löwenberg *et al*. demonstrated that high-dose cytarabine resulted in excessive toxic effects without therapeutic benefits in patients aged 18 to 60 years.[19] Similarly, another study showed no significant benefit of ID cytarabine over SD cytarabine.[20] In our study, we observed that ID cytarabine induction was associated with improved MRD response rates and survival outcomes in patients with *RUNX1::RUNX1T1*, while the benefit was less pronounced in *CBFB::MYH11* patients. These findings align with the notion that the efficacy of cytarabine dose intensification may depend on the genetic context. However, the conflicting results across studies underscore the need for further research to identify the patient subgroups most likely to benefit from dose escalation.

MRD is a well-established prognostic factor of AML.[21] Previous studies have shown that a single threshold for assigning acute lymphoblastic leukemia (ALL) patients to an MRD risk group failed to reflect the relapse risk of the different genetic subtypes.[22] Similarly, in this study, we found that similar MRD levels had distinct prognostic implications for *RUNX1::RUNX1T1* and *CBFB::MYH11* subtypes. Specifically, a less than 3-log MRD reduction of correlated with an increased risk of relapse and decreased OS for patients with *RUNX1::RUNX1T1*, while it did not negatively affect the survival of *CBFB::MYH11* patients. The ELN MRD guidelines emphasize the importance of tailored MRD monitoring strategies, recommending distinct time points for MRD assessment based on genetic subgroups.[21] Our findings align with these recommendations, particularly in underscoring the need for genetic context when interpreting MRD results. However, the optimal timing and frequency of MRD assessments may differ between these subgroups, as reflected in the ELN guidelines. Future risk assessment algorithms for CBF-AML should incorporate both MRD levels and genetic background to more accurately identify patient relapse risk. Additionally, future studies should explore the integration of serial MRD monitoring into treatment protocols, ensuring that MRD dynamics are captured comprehensively to refine risk stratification and therapeutic strategies.

Although HSCT during CR1 has demonstrated success in reducing relapse risk and improving survival in AML patients with certain risk factors, it is not routinely recommended for CBF AML. However, for CBF-AML patients with an less than 3-log MRD reduction after two induction cycles, the ELN guidelines recommend HSCT due to their higher relapse risk.[21,23-25] Our study also confirmed that HSCT benefits *CBFB::MYH11* AML patients with a <3-log MRD reduction. However, for *RUNX1::RUNX1T1* patients, the impact of HSCT was influenced by the induction regimen. Among patients treated with SD cytarabine, HSCT did provide additional benefits for those with a <3-log MRD reduction after two courses of chemotherapy, while for patients receiving ID regimens, the same MRD threshold could not reliably determine the necessity for transplantation. Our previous study also suggested that AML patients with flow cytometry-detected MRD positivity in different risk groups have different criteria for transplantation stratification.[13] Therefore, we propose that different types of AML patients treated with varying regimens may require distinct MRD thresholds to guide transplantation decisions.

Mutations in kinase-related genes are the most common molecular genetic abnormalities in patients with CBF-AML.[7] In the present study, we have corroborated previous findings that KIT mutations, particularly those in exon 17 (*Online Supplementary Figures S13* and *S14*), correlate with poor prognosis and NRAS mutations with better outcomes in *RUNX1::RUNX1T1* patients.[11,26-29] Additionally, we have identified *TET2* mutations as a prognostic marker indicative of poor outcomes in patients with *RUNX1::RUNX1T1*. Previous literature has reported an association between *TET2* mutations and advanced age.[30,31] However, our data may not provide evidence for older age in patients with *TET2* mutations, likely due to limitations in age distribution and insufficient sample size. Further investigations involving larger, age-diverse cohorts are needed to validate our conclusions. Previous studies have found *ZBTB7A* mutations in 13 of 56 and four of 41 *RUNX1::RUNX1T1* AML, respectively.[32,33] In this study, we reported *ZBTB7A* mutations in 14 of 114 *RUNX1::RUNX1T1* AML cases, with a mutation frequency of approximately 12%. The presence or absence of this mutation did not appear to significantly affect the patient survival.

Our study has several limitations. First, it was a retrospective, single-center study. While this design enables consistent data collection and analysis, the findings should be generalized cautiously to broader populations or different clinical settings. Second, our findings were based on data from patients aged 60 years or younger who received intensive chemotherapy. Whether these results apply to older patients or those undergoing lower-intensity therapy remains to be investigated. Additionally, the unavailability of GO in our country precluded a comparison of ID induction regimens with those containing GO. Finally, the relatively small sample size of patients with targeted DNA sequencing limited the granularity of our mutational spectrum analysis.

Disclosures

No conflicts of interest to disclose.

Contributions

HW and JW participated in concept design. MY participated in data analysis, drafting and revising the manuscript. WW,

YW and GZ collected and organized the clinical data. SQ, BL, and YM interpreted the results. All authors have read and approved the final manuscript.

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Data-sharing statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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