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NUP98::NSD1 and *FLT3/ITD* co-expression is an independent predictor of poor prognosis in pediatric AML patients

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Abstract

Objective Patients who carry *NUP98::NSD1* or *FLT3/ITD* mutations are reported to have poor prognosis. Previous studies have confidently reported that the poor outcome in younger AML patients is owning to dual *NUP98::NSD1* and *FLT3/ITD* positivity, with a high overlap for those two genetic lesions. In this study, we assessed the prognostic value of the presence of both *NUP98::NSD1* and *FLT3/ITD* in pediatric AML patients.

Methods We screened a large cohort of 885 pediatric cases from the COG-National Cancer Institute (NCI) TARGET AML cohort and found 57 AML patients with *NUP98* rearrangements.

Results The frequency of NUP98 gene fusion was 10.8% in 529 patients. *NUP98::NSD1* fusion was the most common *NUP98* rearrangement, with a frequency of 59.6%(34 of 57). *NUP98::NSD1* -positive patients who carried *FLT3/ITD* mutations had a decreased CR1 or CR2 rate than those patients carried *FLT3/ITD* mutation alone ($P=0.0001$). Moreover, patients harboring both *NUP98::NSD1* fusion and *FLT3/ITD* mutation exhibited inferior event-free survival (EFS, $P<0.001$) and overall survival (OS, $P=0.004$) than patients who were dual negative for these two genetic lesions. The presence of only *NUP98::NSD1* fusion had no significant impact on EFS or OS. We also found that cases with high *FLT3/ITD* AR levels ($>=0.5$) with or without *NUP98::NSD1* had inferior prognosis. Multivariate analysis demonstrated that the presence of both *NUP98::NSD1* and *FLT3/ITD* was an independent prognostic factors for EFS (hazard ratio: 3.2, $P=0.001$) in patients with pediatric AML. However, there was no obvious correlation with OS (hazard ratio: 1.3, $P=0.618$). Stem cell transplantation did not improve the survival rate of cases with *NUP98* fusion or *NUP98::NSD1* AML in terms of EFS or OS.

Conclusion Presence of both *NUP98::NSD1* and *FLT3/ITD* was found to be an independent factor for dismal prognosis in pediatric AML patients. Notably, lack of *FLT3/ITD* mutations in *NUP98::NSD1* -positive patients did not retain its prognostic value.

Keywords *NUP98::NSD1*, Nucleoporin gene 98, *FLT3/ITD*, Pediatric, Acute myeloid leukemia (AML)

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Introduction

As the second most common pediatric hematologic malignancy, acute myeloid leukemia (AML) is a group of diseases with various clinical presentations, genetic alterations, and responses to treatment [1]. Cytogenetic and molecular abnormalities significantly contribute to the risk stratification of pediatric acute myeloid leukemia patients and serve as crucial prognostic factors [2]. The expert consensus recommended by the 2017 European Leukemia Network supports inclusion of the nucleoporin 98 fusion gene and its associated molecular genetic abnormalities in AML risk stratification to guide treatment [3]. *NUP98* has been detected with more than 30 partner genes [4–7]in human leukemia, including the most common gene, *NUP98::NSD1* [8–10]. Patients who *carry NUP98::NSD1* gene fusions have dismal outcomes [9, 11–13]. Leukemic blasts frequently carry the *FLT3/ITD* mutation accompanying the *NUP98::NSD1* gene fusion [5, 12]. *FLT3/ITD* mutation in pediatric AML patients results in poor prognosis according to previous reports [14–18]. Though the clinical value of dual *NUP98::NSD1* and *FLT3/ITD* anomalies in younger AML patients has been described [12]. This study comprehensively analyzed the clinical significance of these two genetic lesions in pediatric AML patients younger than 18 years of age and their impact on prognosis.

Method

Data sources

The TARGET dataset contains necessary data on AML patients under 18 years of age (https://ocg.cancer.gov/programs/target/data-matrix). 885 pediatric patients were screened who were diagnosed between1996 and 2010, with the last years of follow-up until 2015. AML diagnosis and risk classification were performed based on the Children's Oncology Group (COG) guidelines [19]. AML subtypes were divided based on French-American-British (FAB) classifications. The AML treatment protocols used were AAML03P1, AAML0531, and CCG-2961. Patients in the first complete remission underwent stem cell transplantation (SCT).

Statistical evaluation

Descriptive statistics were used to summarize the clinical and biological features of the patients. For categorical variables, chi-square analysis and Fisher's exact test were adopted; for continuous variables, the nonparametric Mann-Whitney U test was adopted. The study utilized the following criteria to evaluate clinical outcomes: complete remission (CR), event-free survival (EFS), and overall survival (OS). CR is achieved when the bone marrow has less than 5% blasts at the end of induction. EFS was defined as the time between diagnosis and the first event, including induction failure, recurrence, or any cause of death. OS was defined as the time between diagnosis and death from any cause. EFS and OS were assessed and compared by the Kaplan-Meier method and the log-rank test. Multivariate Cox regression analysis was adopted to evaluate the independent impact of prognostic factors on OS and EFS. Hazard ratios (HRs) of multivariate Cox regression are presented with 95% confidence intervals. A P_value less than 0.05 indicated statistically significance. The data were evaluated with the SPSS software version 25.0 (IBM Corporation, Armonk, NY, USA).

Results

Clinical and molecular characteristics of *NUP98* fusion genes

We screened a large pediatric cohort from the COG-National Cancer Institute (NCI) TARGET AML cohort. A total of 885 AML pediatric patients were included in the TARGET database. The fusion gene status of 356 children was unknown, which was considered missing data to be excluded, so 529 patients were ultimately included as the research subjects (Supplementary Table 2 /Table 2.1). Fifty-seven had *NUP98* rearrangements, for a total frequency of 10.8%. In this study, we identified six kinds of *NUP98* fusion genes. The most common *NUP98*-fusion gene was *NUP98::NSD1* (59.6%, 34 of 57), followed by *NUP98/KMD5A* (17.5%, 10 of 57), *NUP98/HOXD13* (7%, 4 of 57), *NUP98/HMGB3*, *NUP98/HOXA9*, and *NUP98/PHF23* (5.3%, 3 of 57).

Comparison of the clinical and molecular characteristics of *NUP98*-positive and *NUP98*-negative patients revealed no significant differences in gender, median age, or median WBC count at diagnosis (Table 1). However, the *NUP98::NSD1* fusion gene patients had greater median WBC of 100.8×10^9/L than did the *NUP98::NSD1* negative patients ($P=0.002$) (Table 2). Regarding cytomorphology status, there were significant differences between the *NUP98*-positive group and the *NUP98*-negative group (50.9% vs. 2.3%, $P<0.001$) (Table 1).

Compared with *NUP98*-negative patients, *NUP98*-positive patients more often exhibited the FAB M7 phenotype (10.5% vs. 2.3%, $P=0.001$). We also found that *NUP98* fusion gene cases highly overlapped with *FLT3/ITD*-positive cases (45.6% vs. 6.1%, $P<0.001$) (Table 1) and that *NUP98::NSD1*fusion gene cases are highly overlapped with *FLT3/ITD* positivity (64.7% vs. 6.7%, $P<0.001$) (Table 2).

Prognosis of NUP98 fusion genes carriers

We initially evaluated the impact of *NUP98* fusion gene positivity on prognosis. In terms of treatment response (Table 1), 80.1% (378 of 472)of *NUP98*-negative patients achieved a favorable CR in course 1 which was greater than the 59.6% (34 of 57) *NUP98*-positive patients

Table 1 Characteristics of study population according to *NUP98* gene fusion status

	All patient	NUP98+	NUP98-	*P* -value
Total	529	57	472	
Protocol				
AAML03P1, N (%)	55	5 (8.8%)	50 (10.6%)	0.670
AAML0531, N (%)	438	47 (82.4%)	391 (82.8%)	0.942
CCG-2961, N (%)	36	5 (8.8%)	31 (6.6%)	0.533
Gender				0.169
Male, N (%)	270	34 (59.6%)	236 (50%)	
Female, N (%)	259	23 (40.4%)	236 (50%)	
Median age at diagnosis (years, range)	8.9 (0-17.9)	9.8 (1.0-17.6)	8.5 (0-17.9)	0.293
Median WBC ×10^9/L (range)	40.5 (0.2–610)	53.5 (1.1-447.3)	39 (0.8–610)	0.160
FAB type, N (%)				
M0	8	1 (1.8%)	7 (1.5%)	0.874
M1	36	8 (14.0%)	28 (5.9%)	0.028
M2	139	11 (19.3%)	128 (27.1%)	0.205
M4	143	14 (24.6%)	129 (27.3%)	0.657
M5	117	7 (12.3%)	110 (23.3%)	0.058
M6	4	4 (7.0%)	0 (0%)	< 0.001
M7	17	6 (10.5%)	11 (2.3%)	0.001
Treatment response				
CR status at the end of course1, N (%)	412	34 (59.6%)	378 (80.1%)	< 0.001
CR status at the end of course2, N (%)	462	38 (66.7%)	424 (89.8%)	< 0.001
HSCT in CR1	51	11 (19.3%)	40 (8.5%)	0.009
FLT3/ITD				
Positive, N (%)	55	26 (45.6%)	29 (6.1%)	< 0.001
Negative, N (%)	471	31 (54.4%)	440 (84.7%)	< 0.001
WT1 mutation				
Positive, N (%)	32	15 (26.3%)	17 (3.6%)	< 0.001
Negative, N (%)	487	42 (73.7%)	445 (94.3%)	< 0.001
*NPM1*mutation				
Positive, N (%)	8	3 (5.3%)	5 (1.1%)	0.014
Negative, N (%)	511	54 (94.7%)	457 (96.8%)	0.412
Cytogenetic status				
Normal, N%	40	29 (50.9%)	11 (2.3%)	< 0.001
Abnormal, N%	479	22 (38.6%)	457 (96.8%)	< 0.001
Risk group				
Low	242	4 (7.0%)	238 (50.4%)	< 0.001
Standard	238	30 (52.6%)	208 (44.1%)	0.402
high	41	19 (33.3%)	22 (4.7%)	< 0.001

Data for FAB classification, *FLT3/ITD*, *NPM1*, *WT1* mutation, cytogenetic status and risk group were missing for some patients

Table 2 Characteristics of study population according to *NUP98::NSD1* gene fusion status

	NUP98::NSD1 +	NUP98::NSD1 -	*P* -value
Total, N (%)	34	495	
Protocol			
AAML03P1	2(5.9%)	53(10.7%)	0.373
AAML0531	29(85.3%)	409(82.6%)	0.690
CCG-2961	3(8.8%)	33(6.7%)	0.629
Gender			
Male, N (%)	24(70.6%)	246(49.7%)	0.018
Female, N (%)	10(29.4%)	249(50.3%)	0.018
Median age at diagnosis (years, range)	10.3(1.2–17.6)	8.5 (0-17.9)	0.169
Median WBC ×10^9/L (range)	100.8(1.1-447.3)	36 (0.8–610)	0.002
FAB type, N (%)			
M0	0	8(1.6%)	0.455
M1	3(8.8%)	33(6.7%)	0.629
M2	7(20.6%)	132(26.7%)	0.436
M4	10(29.4%)	133(26.9%)	0.747
M5	4(11.8%)	113(22.8%)	0.133
M6	2(5.9%)	2(0.4%)	< 0.001
M7	3(8.8%)	14(2.8%)	0.055
Treatment response			
CR status at the end of course1 N (%)	15(44.1%)	397(80.2%)	< 0.001
CR status at the end of course2, N (%)	17(50.5%)	445(89.9%)	< 0.001
HSCT in CR1	7(20.6%)	44(8.9%)	0.025
FLT3/ITD			
Positive, N (%)	22(64.7%)	33(6.7%)	< 0.001
Negative, N (%)	12(35.3%)	459(92.7%)	< 0.001
WT1 mutation			
Positive, N (%)	13(38.2%)	19(3.8%)	< 0.001
Negative, N (%)	21(61.7%)	466(94.1%)	< 0.001
NPM1 mutation			
Positive, N (%)	0(0.0%)	8(1.6%)	0.455
Negative, N (%)	34(100%)	477(96.4%)	0.258
Cytogenetic status			
Normal, N%	19(55.9%)	21(4.2%)	< 0.001
Abnormal, N%	10(29.4%)	469(94.7%)	< 0.001
Risk group			
Low	1(2.9%)	241(48.7%)	< 0.001
Standard	14(41.2%)	224(45.3%)	0.644
high	16(47.1%)	25(5.1%)	< 0.001

Data for FAB classification, *FLT3/ITD*, *NPM1*, *WT1* mutation, cytogenetic status and risk group were missing for some patients

($P<0.001$). After the second course of induction therapy, CR was achieved in 38 of 57 (66.7%) patients with *NUP98* gene fusion and in 424 (89.8%) of 472 patients without *NUP98* gene fusion ($P<0.001$).

A total of 885 AML pediatric patients were included in the TARGET database, and 57 patients with *NUP98* fusion gene positivity were analyzed in this study. The

fusion gene status of 356 children was unknown, which was considered missing data; thus, 529 patients were included as the research subjects (Supplementary Table 2 /Table 2.1). Survivors were followed up for a median of 5.6 years among all 529 patients with and without the *NUP98* fusion gene positivity. As shown in Fig. 1(A), patients harboring a *NUP98* fusion gene had an inferior EFS (32.4%) compared with wild-type patients (49.2%, $P=0.002$). Figure 1(B) illustrated a trend toward worse OS in *NUP98* fusion gene patients(48.1%)than in *NUP98* wild-type patients(67.0%, $P=0.006$).

Stratification analysis of *FLT3/ITD* and *NUP98* fusion genes

NUP98 fusion gene patients had a high frequency of 45.6% (26 of 57) in cooperating genetic alterations with *FLT3/ITD* mutation. In the 55 patients with positive FLT3/ITD mutation, the CR rate 1 in patient who carried an *NUP98* gene fusion was lower at 34.6% (9 of 26) compared to the rate of 79.3% (23 of 29) observed in patients without *NUP98* gene fusion ($P=0.0008$), as well the CR rate 2 in dual positive of *NUP98* gene fusion and *FLT3/ITD* was 42.3%(11/26)compared with cases without *NUP98* gene fusion 89.7% (26 of 29) ($P=0.0002$)(Supplementary Table 1).

As shown in Fig. 2 and Supplementary Table 3, with respect to the *FLT3/ITD* mutation, patients with NUP98-positive and *FLT3/ITD* mutation had the worst prognosis with a 5-year OS of 41.4±10.3% and a 5-year EFS of 13.5±7.0% compared with patients with wild-type *FLT3/ITD* without the NUP98 gene fusion patients (OS 68.0±2.3%, $P=0.002$; EFS 50.3±2.4%, $P<0.001$) (Supplementary Fig. 1A, B). According to the *NUP98* gene fusion status and *FLT3/ITD* status, the survival curves were grouped into 4 subgroups (Fig. 2). In *FLT3/ITD* wild-type cases, *NUP98*-positive patients did not significantly affect 5-year OS (54.0±9.1% *NUP98*+vs. 68.0±2.3% *NUP98*-; $P=0.188$) or EFS (48.0±9.0% *NUP98*+vs. 50.3±2.4% *NUP98*-; $P=0.862$) (Supplementary Fig. 1.1A, B).

According to the *NUP98* gene fusion status, *FLT3/ITD*-positive patients had a negative impact on the 5-year EFS rate (13.5±7.0% *NUP98*+vs. 31.0±8.6% *NUP98*-; $P=0.008$) but not on the 5-year OS rate (41.4±10.3% *NUP98*+vs. 55.0±9.3% *NUP98*-; $P=0.133$) (Supplementary Fig. 1.2A, B). A tending negative impact was shown by FLT3/ITD positive patients on 5-year EFS (31.0±8.6% FLT3/ITD positive vs. 50.3±2.4% FLT3/ITD wild-type; $P=0.056$) in NUP98 negative patients, but not on 5-year OS (55.0±9.3%FLT3/ITD positive vs. 68.0±2.3% FLT3/ITD wild-type; $P=0.242$) (Supplementary Fig. 1.3A, B).

Stratification evaluation of *FLT3/ITD* in *NUP98::NSD1* patients

Among 26 patients positive for both *NUP98* gene fusion and *FLT3/ITD*, 84.6% (22 of 26) of patients harbored *NUP98::NSD1* and *FLT3/ITD*. The prognostic effect of *NUP98::NSD1* in patients who carried *FLT3/ITD* was then evaluated. The CR rate after the first course of induction therapy in those with both *NUP98::NSD1* and *FLT3/ITD* was 27.3% (6 of 22) compared with 78.8% (26 of 33) for *FLT3/ITD* mutation patients without *NUP98::NSD1* ($P=0.0001$).The CR rate after second course of induction therapy in patients carrying *NUP98::NSD1* and *FLT3/ITD* was 36.4% (8 of 22) compared with 87.9%(29

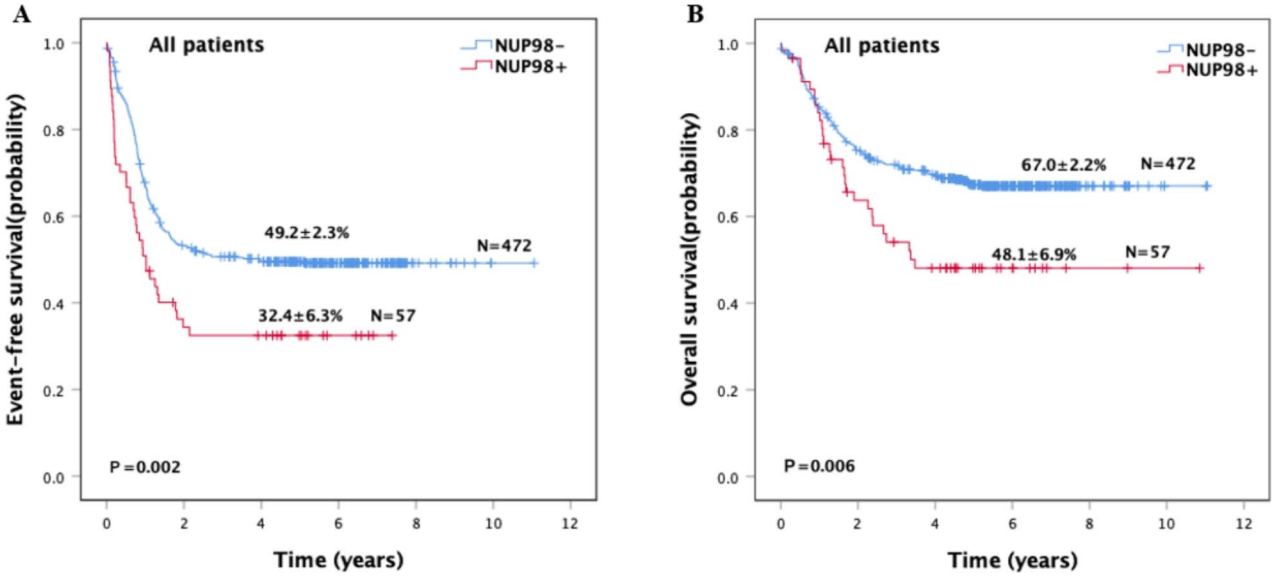

Fig. 1 Survival curves of pediatric AML patients with and without *NUP98* gene fusion. **A** Probability of EFS for all patients with and without *NUP98* gene fusion. **B** Probability of OS for all patients with and without *NUP98* gene fusion

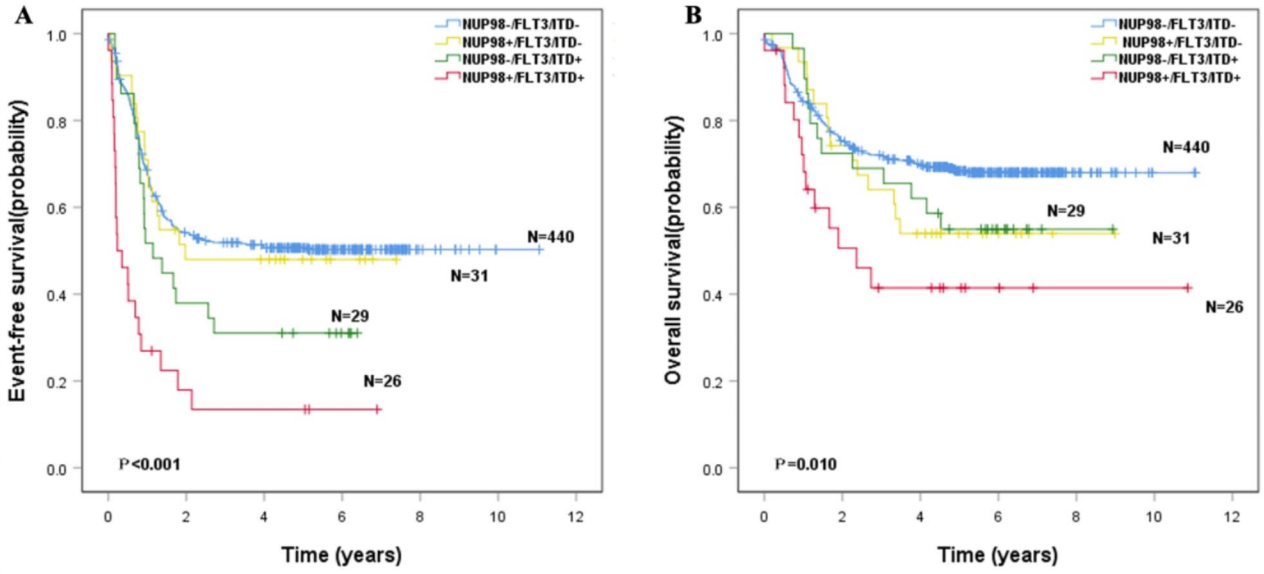

Fig. 2 Survival curves of pediatric AML patients according to combined *NUP98* gene fusion and *FLT3/ITD* status. **A** Probability of EFS for AML patients. **B** Probability of OS for AML patients. Pairwise comparison data provided in Supplementary Figs. 1-1.3 and Supplementary Table 3

of 33)for patients with *FLT3/ITD* mutation but without *NUP98::NSD1* (P=0.0001) (Supplementary Table 1).

As shown in Fig. 3(A), in all 529 patients, patients harboring *NUP98::NSD1* had an inferior EFS (26.1±7.6%) compared with patients who were *NUP98::NSD1* -negative (48.9±2.3%, P<0.001). Additionally, *NUP98::NSD1* patients displayed a trend toward worse OS(50.1±8.9%) compared to *NUP98::NSD1* negative patients (66.0±2.2%, P=0.029) (Fig. 3(B)). In analysis restricted to 57 *NUP98* fusion genes patients, there were no significant differences in OS between *NUP98::NSD1*(+) and *NUP98::NSD1*(-) patients, with EFS being poor in both (26.1±7.6% vs. 41.9±10.5%, P=0.026) (Fig. 3(C, D)).

When examining only patients who were *NUP98::NSD1* positive and harbored *FLT3/ITD* mutation, we found unfavorable 5-year OS (41.7±11.0%) and 5-year EFS (13.6±7.3%) outcomes compared with those of *FLT3/ITD* wild-type cases without *NUP98::NSD1* (OS 67.1±2.3%, P=0.004; EFS 50.1±2.4%, P<0.001) (Fig. 4B/ Supplementary Fig. 2A, B). Survival curves were generated based on *NUP98::NSD1* status and *FLT3/ITD* status (Fig. 4(A, B) and Supplementary Table 4), revealing that *NUP98::NSD1* patients had no impact on 5-year EFS (50.0±14.4% *NUP98::NSD1*+vs. 50.1±2.4%*NUP98::NSD1* -; P=0.705) or OS (65.6±14.0% *NUP98::NSD1*+vs. 67.1±2.3% *NUP98::NSD1* -; P=0.900) in *FLT3/ITD*-negative patients (Supplementary Fig. 2.1A, B). FLT3/ITD-positivity had a negative impact on 5-year EFS (29.0±8.0% FLT3/ITD-positive vs. 50.1±2.4% FLT3/ITD-wild-type; P=0.025) in NUP98::NSD1-negative patients but not on 5-year OS (53.0±8.9% FLT3/ITD-positive vs. 67.1±2.3% FLT3/ITD-wild-type; P=0.190) (Supplementary Fig. 2.2A, B).

Overall, *FLT3/ITD* mutation patients had a negative impact on 5-year EFS (13.6±7.3% *NUP98::NSD1*+vs. 29.0±8.0% *NUP98::NSD1* -; P=0.005) but not on 5-year OS (41.7±11.0% *NUP98::NSD1*+vs. 53.0±8.9% *NUP98::NSD1* -; P=0.147) according to *NUP98::NSD1* status (Supplementary Fig. 2.3A, B).

Stratification evaluation of the thresholds *FLT3/ITD* AR and *NUP98::NSD1*

As illustrated in Fig. 5(A, B), patients harboring *NUP98::NSD1* gene fusion with a high *FLT3/ITD* AR had poor prognosis with a 5-year OS of 33.3±13.6%, however, there was no significant difference compared with the 55.6±16.6% in patients with low *FLT3/ITD* AR. The reason may be the small sample size.

In analysis restricted to high *FLT3/ITD* AR, patients had poor 5-year EFS prognosis, regardless of the presence or absence of *NUP98::NSD1* (23.1±11.7% *NUP98::NSD1*+vs. 26.5±9.9% *NUP98::NSD1* -, P=0.214). Figure 5(C, D) indicated that patients with high *FLT3/ITD* AR and *NUP98::NSD1* gene fusion positivity had a shorter 5-year OS of 33.3±13.6%, which was not significantly different from the 55.5±11.1% of patients without *NUP98::NSD1* fusion.

As illustrated in Fig. 5(E, F), for patients with low *FLT3/ITD* AR, *NUP98::NSD1* had no impact on prognosis, with a 5-year OS of 55.6±16.6% compared with 48.6±14.8% for patients without *NUP98::NSD1* (P=0.796).

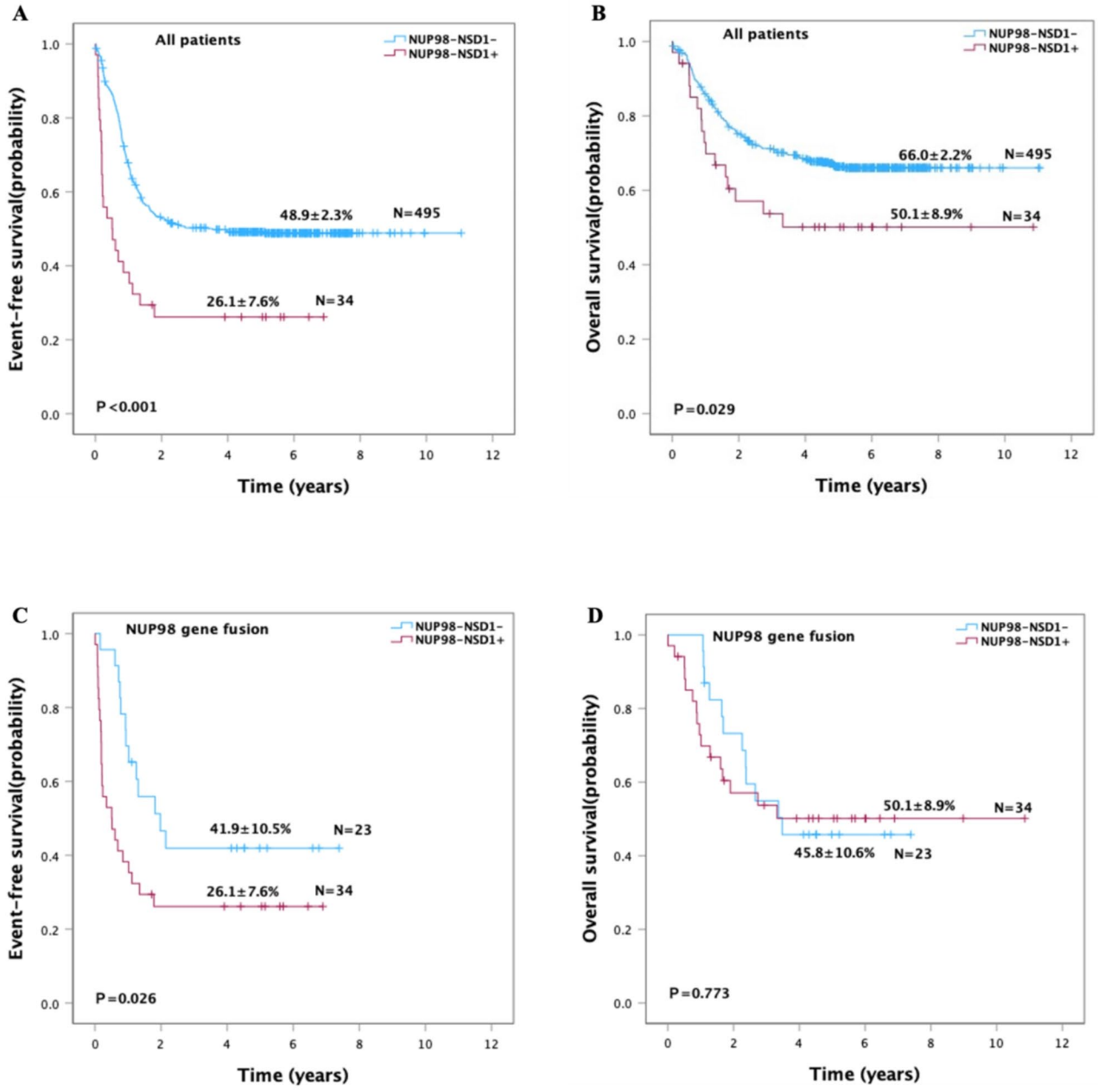

Fig. 3 Survival curves of pediatric AML patients with and without *NUP98::NSD1*. **A** Probability of EFS for all patients with and without *NUP98::NSD1*. **B** Probability of OS for all patients with and without *NUP98::NSD1*. **C** Probability of EFS for *NUP98* positive patients with and without *NSD1* gene fusion. **D** Probability of OS for *NUP98* positive patients with and without *NSD1* gene fusion

The impact of SCT in pediatric AML patients carrying *NUP98* gene fusion

Among the 57 *NUP98*-positive cases, only 11 patients received allogeneic SCT, and data were available for 40 patients. Evaluation of the effect of SCT on *NUP98* gene fusion patients revealed that the therapy did not have a significant effect on the clinical outcome with respect to 5-year EFS (36.4±14.5% SCT vs. 43.0±9.5% no SCT, $P=0.962$) or OS (50.5±15.8% SCT vs. 53.1±9.6% no SCT, $P=0.923$) rates when compared with chemotherapy (no SCT) Fig. 6(A, B).

The survival curses depicted In Fig. 6(C, D), demonstrated that SCT had no significant effect on the 5-year EFS (42.9±18.7% of patients with SCT vs. 40.0±14.6% of patients with non-SCT, $P=0.717$) or OS (68.6±18.6% of patients with SCT vs. 65.6±14.0% of patients with non-SCT, $P=0.802$) compared to chemotherapy (no SCT) in *NUP98::NSD1* patients.

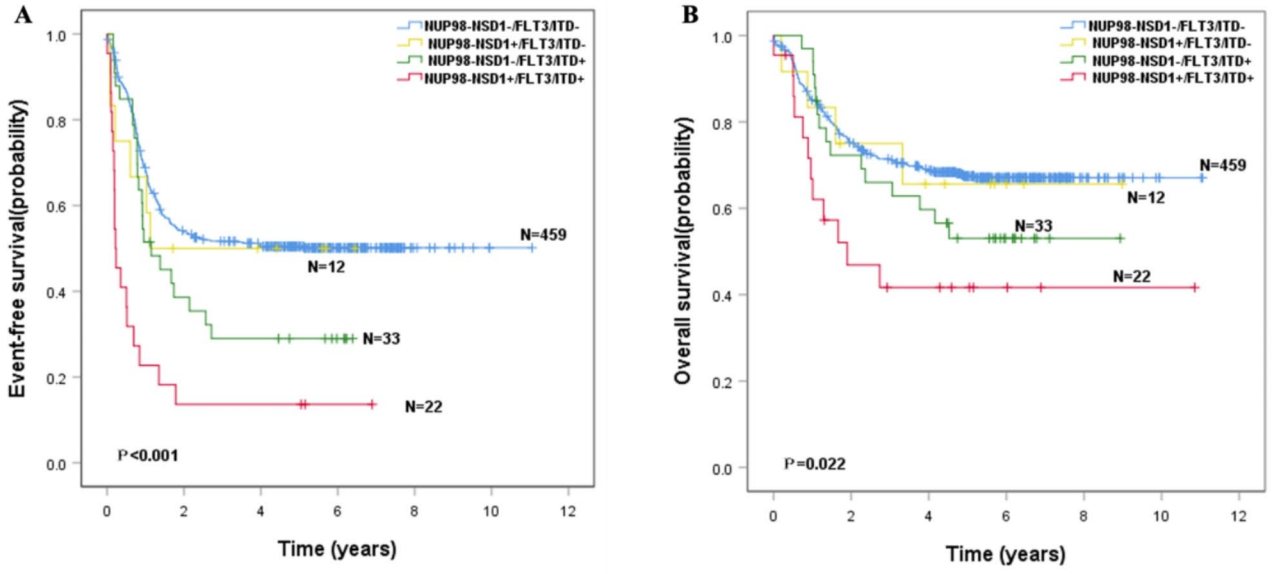

Fig. 4 Survival curves of pediatric AML patients according to combined *NUP98::NSD1* gene fusion and *FLT3/ITD* status. **A** Probability of EFS for AML patients. **B** Probability of OS for AML patients. Pairwise comparison data provided in Supplementary Figs. 2-2.3 and Supplementary Table 4

Multivariate analysis of prognostic factors for overall and event-free survival in pediatric AML patients

Multivariable Cox regression analysis including *NUP98::NSD1+/FLT3/ITD+*, *NUP98::NSD1+/FLT3/ITD-*, *NUP98::NSD1-/FLT3/ITD+*, *NPMI* status, *WT1 status*, age>10years, WBC, and risk group was performed to determine whether *NUP98::NSD1+/FLT3/ITD+*is an independent prognostic factor and concluded that *NUP98::NSD1+/FLT3/ITD+*is an independent prognostic factor for poor EFS (HR=3.2, 95% CI: 1.6–6.5, *P*=0.001), though it did not have a significant impact on OS. Regardless of *NUP98::NSD1+/ FLT3/ITD* -or *NUP98::NSD1-/ FLT3/ITD+*status, EFS and OS were not affected. As expected, we showed *WT1*mutation was significantly associated with inferior EFS and OS. Moreover, a WBC count>=100×10^9/L, but not OS, had a significant negative influence on EFS. Conversely, neither *NPM1* mutation nor risk group had significant impact on EFS or OS (Table 3).

Discussion

Cytogenetic and molecular abnormalities play significant roles in the risk stratification of pediatric acute myeloid leukemia patients and are important prognostic factors [2]. Previous studies have shown that expression of the *NUP98* fusion genes in hematopoietic cell lines results in poor prognosis [6, 7, 11, 20, 21]. The frequency of the *NUP98* fusion gene presence in pediatric AML was reported to be 10.8% in this large cohort, which is consistent with previous studies [22]. Several prior reports have shown that *NUP98*-positive patients have higher WBC counts, more frequent FAB-M4/M5 phenotypes, and more *FLT3/ITD* mutations, especially among those with *NUP98::NSD1* gene fusion patients [5, 9], where we found similar results. However, we observed that *NUP98*-positive patients tended to have a normal cytogenetic status. In this study, patients harboring the *NUP98* fusion gene had a lower CR rate. Furthermore, our analysis demonstrates that the presence of the *NUP98* fusion gene leads to poorer EFS and OS outcomes. Considering that the presence of *NUP98* fusion gene has a high overlap with *FTL3/ITD* mutation, we aimed to investigate whether expression of *FTL3/ITD* in patients with *NUP98*-fusion proteins is required for poor prognosis. Patients with *NUP98* gene fusion co-expression and *FLT3/ITD* mutation had an inferior EFS of 13.5% compared with the 31.0% in patients with *FLT3/ITD* mutation alone. Moreover, we found that patients harboring *NUP98* fusion gene only had favorable outcome for EFS of 48.0%, and similar to dual negative of *FLT3/ITD* and *NUP98* gene fusion of EFS 50.3%. Based on our findings, the dismal prognosis of *NUP98*-positive patients might be due to the interaction of the *FLT3/ITD* mutation.

Consistent with the findings of a previous study [22], *NUP98::NSD1* gene fusion was the most common *NUP98* rearrangement in pediatric AML patients, occurring in 59.6% of 57 cases. Additionally, *NUP98::NSD1* gene fusion and *FLT3/ITD* mutation are potential key factors for dismal prognosis of AML patients [11]. Notably, patients who are positive for both *FLT3/ITD* and *NUP98::NSD1* experience poor outcomes [23]. This study unequivocally demonstrated that patients carried *FLT3/*

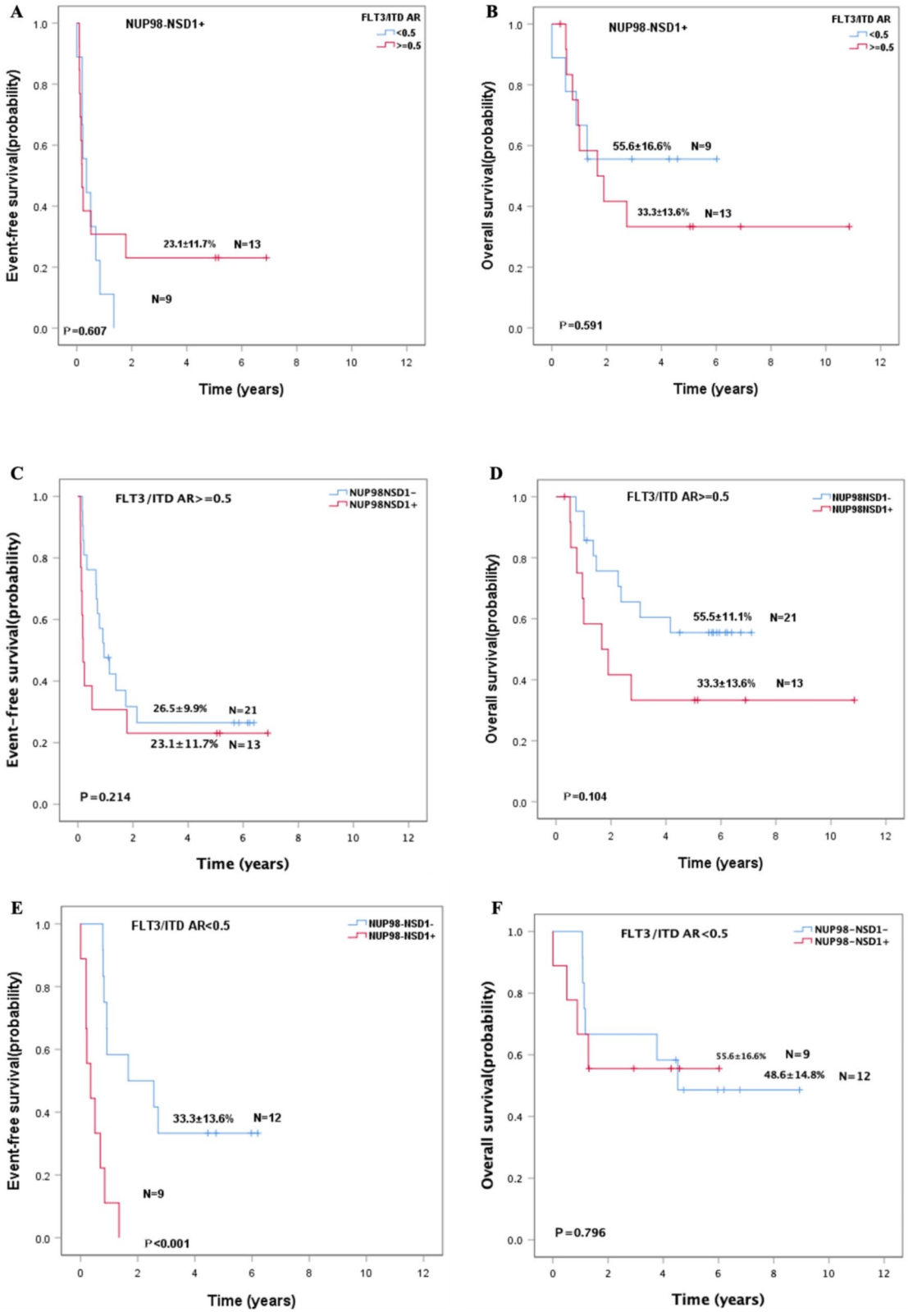

Fig. 5 Survival curves of pediatric AML patients according to combined *NUP98::NSD1* gene fusion and *FLT3/ITD* status. **A** Probability of EFS for *NUP98::NSD1* gene fusion with *FLT3/ITD* AR status. **B** Probability of OS for *NUP98::NSD1* gene fusion with *FLT3/ITD* AR status. **C** Probability of EFS for high *FLT3/ITD* AR with or without *NUP98::NSD1* gene fusion. **D** Probability of OS for high *FLT3/ITD* AR with or without *NUP98::NSD1* gene fusion. **E** Probability of EFS for low *FLT3/ITD* AR with or without *NUP98::NSD1* gene fusion. **F** Probability of OS for low *FLT3/ITD* AR with or without *NUP98::NSD1* gene fusion

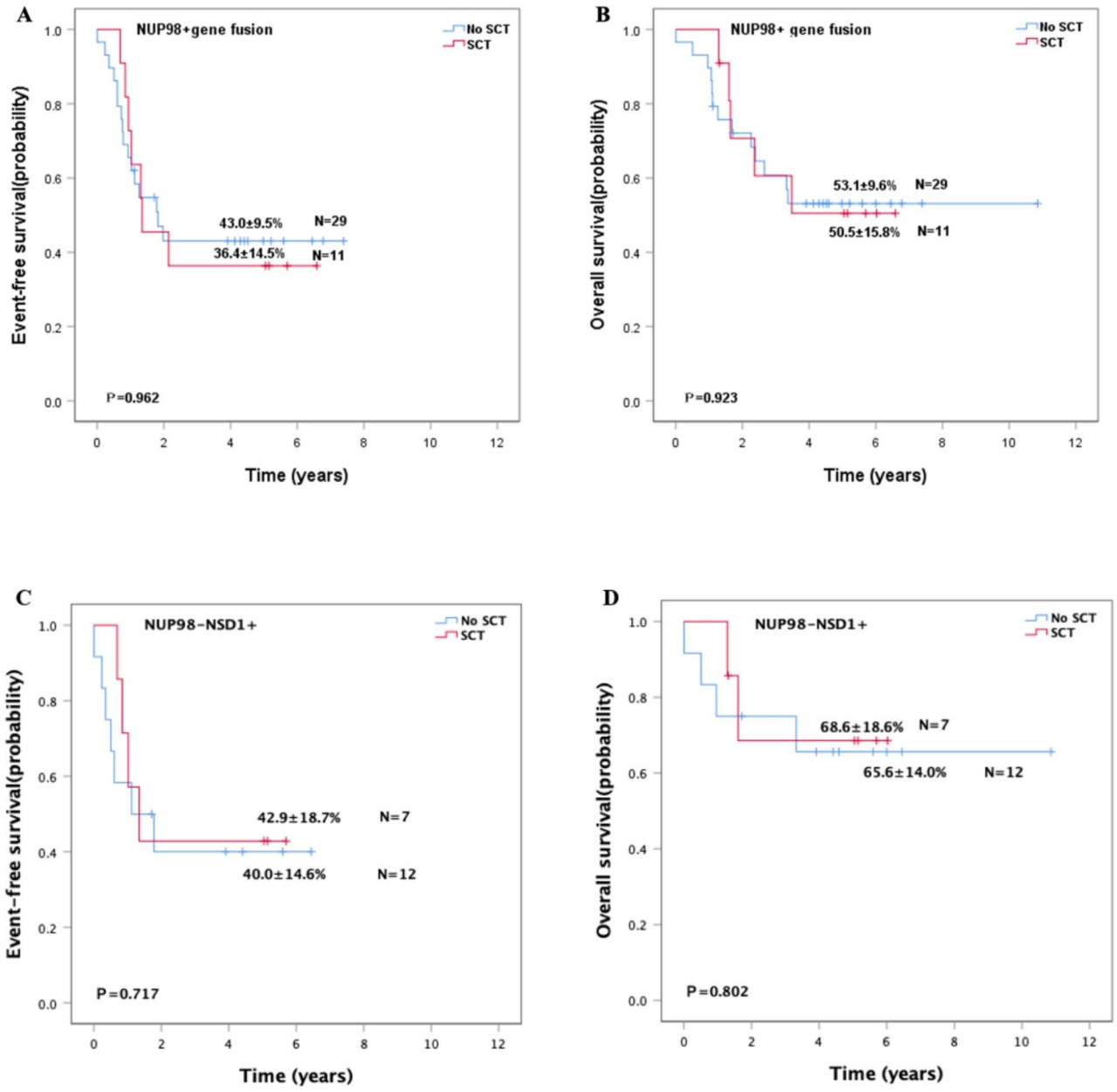

Fig. 6 Survival curves of pediatric AML patients according to *NUP98* gene fusion and stem cell transplantation (SCT) status. **A** Probability of EFS for *NUP98* gene fusion patients according to SCT status. **B** Probability of OS for *NUP98* gene fusion patients according to SCT status. **C** Probability of EFS for *NUP98::NSD1* patients according to SCT status. **D** Probability of OS for *NUP98::NSD1* patients according to SCT status

ITD mutations and *NUP98::NSD1* had significantly worse outcomes, with a 5-year OS of 41.7% and a 5-year EFS of 13.6%, than *NUP98::NSD1* -negative patients with wild-type *FLT3/ITD* (OS, $P=0.004$; EFS, $P<0.001$). These outcomes also worse than those of patients with *FLT3/ITD* positivity alone, who had a 5-year EFS of 29.0%, but 5-year OS was not affected. Patients positive for *NUP98::NSD1* alone have a favorable outcome, similar to those who are negative for both *NUP98::NSD1* and *FLT3/ITD*. This study demonstrated that patients who carry both *NUP98::NSD1* gene fusion and *FLT3/ITD* mutation have poor outcomes.

In conclusion, this study indicated that outcome may not be significantly impacted by the presence of only *NUP98::NSD1* or *NUP98* gene fusion in the absence of *FLT3/ITD* mutation.

Compared with *FLT3/ITD* positive, a high *FLT3/ITD* AR status predicted poor prognosis. A previous study reported that the negative impact of *FLT3/ITD* even with high AR might not be as severe as previously published

Table 3 Multivariate analysis of prognostic factors for overall and event-free survival in 529 pediatric AML patients

Variables	Overall survival(OS)		Event-free survival(EFS)	
	HR(95%CI)	*P* value	HR(95% CI)	*P* value
NUP98::NSD1 (+)/FLT3/ITD(+)	1.3(0.5,3.2)	0.618	3.2(1.6,6.5)	0.001
NUP98::NSD1 (-)/FLT3/ITD(+)	1.1(0.5,2.5)	0.824	1.5(0.8,2.9)	0.170
NUP98::NSD1 (+)/FLT3/ITD(-)	0.8(0.2,2.4)	0.641	1.0(0.4,2.5)	0.971
Age > 10years	1.1(0.8,1.6)	0.420	0.9(0.7,1.2)	0.662
high risk group	1.2(0.5,2.7)	0.712	0.9(0.5,1.8)	0.840
NMP1 mutation	1.0(0.3,3.2)	0.990	1.0(0.4,2.7)	0.991
WT1 mutation	1.8(1.0,3.2)	0.040	1.7(1.1,2.8)	0.020
WBC > = 100 × 10^9/L	1.5(1.1,2.2)	0.013	1.6(1.2,2.1)	0.001

in the absence of *NUP98::NSD1* fusion [24]. In this study, we observed that *NUP98::NSD1* gene fusion cases concomitant high *FLT3/ITD* AR led to worse OS, though this was not statistically significant compared with those low *FLT3/ITD* AR cases. However, high *FLT3/ITD* AR cases led to worse survival for EFS of 23.1% and 26.5% with or without *NUP98::NSD1* gene fusion, respectively.

Several prior reports have shown that patients who carry both *NUP98::NSD1* gene fusion and *FLT3/ITD* mutation and who undergo SCT have an improved prognosis compared with those who receive chemotherapy alone [12, 25]. In this study, SCT did not improve the EFS or OS of pediatric patients with *NUP98* gene fusion or *NUP98::NSD1*.

This study is the first to analyze the prognostic significance of *NUP98* gene fusion or *NUP98::NSD1* in *FLT3/ITD* pediatric AML patients. These findings clearly show that patients who carry both *FLT3/ITD* and *NUP98::NSD1* have poor outcomes. Co-expression of *FLT3/ITD* and *NUP98::NSD1* was identified as an independent factor for dismal outcomes, while positivity for *NUP98::NSD1* alone was not a significant prognostic factor. The presence of *NUP98::NSD1* gene fusion and *FLT3/ITD* mutation could identify a high risk subgroup. Moreover, those two genetic lesions with the presence of high *FLT3/ITD* AR appears to lead to worse prognosis, with a low EFS rate of 23.1% and OS rate of 33.3%. A larger sample is needed for further study. Multivariable analysis showed that *WT1* mutation was significantly associated with inferior EFS and OS, and *NPM1* mutation had no significant impact on EFS or OS. We did not further analyze the interactions among *FLT3-ITDs*, *WT1* and *NUP98*, which is a limitation of our study. To further clarify the impact of these 2 genetic lesions on the prognosis of pediatric AML patients, *WT1* mutations, *NPM1* mutations, or more cytogenetic and molecular abnormalities with highly overlapping expression levels should be included in subsequent studies to confirm the predictors with variable effects on the prognosis. Based on prior studies, we cannot conclude that underwent SCT benefits patients with these 2 genetic lesions. Therefore, quickly detecting genetic variants early and using *FLT3-ITD* inhibitors as soon as possible may improve the prognosis of these children.

In summary, presence of both *NUP98::NSD1* and *FLT3/ITD* was found to be an independent factor for dismal prognosis in pediatric AML patients. Notably, lack of *FLT3/ITD* mutations in *NUP98::NSD1* -positive patients did not retain its prognostic value.

Abbreviations

AML	Acute Myeloid Leukemia
SCT	Stem Cell Transplantation
CR	Complete Remission
EFS	Event-Free Survival
OS	Overall Survival
FAB	French-American-British

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-05007-3.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

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Author contributions

L.H.X, X.G.Y and J.W.W participated in project design, analysis, interpretation, and manuscript drafting. J.W.W, Y.L obtained, assembled data analyzed and interpreted the data. All authors contributed to the article and approved the submitted version.

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Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent for publication

The studies involving human participants were reviewed and approved by TARGET Publications Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Consent for publication

Not applicable.

Clinical trial information

COG studies AAML0531, AAML03P1, and CCG2961 were registered at the United States clinical trial registry and www.clinicaltrials.gov as #NCT00372593

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Competing interests
The authors declare no competing interests.

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References

1. Tseng S, Lee ME, Lin PC. A review of Childhood Acute myeloid leukemia: diagnosis and Novel Treatment. Pharmaceuticals (Basel) 2023, 16(11).
2. de Rooij JD, Zwaan CM, van den Heuvel-Eibrink M. Pediatric AML: from Biology to Clinical Management. J Clin Med. 2015;4(1):127–49.
3. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
4. Gough SM, Slape CI, Aplan PD. NUP98 gene fusions and hematopoietic malignancies: common themes and new biologic insights. Blood. 2011;118(24):6247–57.
5. Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Pratcorona M, Abbas S, Kuipers JE, van Galen JF, Beverloo HB, Sonneveld E, Kaspers GJ, et al. NUP98-NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. Blood. 2011;118(13):3645–56.
6. Marceau-Renaut A, Duployez N, Ducourneau B, Labopin M, Petit A, Rousseau A, Geffroy S, Bucci M, Cuccuini W, Fenneteau O, et al. Molecular profiling defines distinct prognostic subgroups in Childhood AML: a Report from the French ELAM02 Study Group. Hemasphere. 2018;2(1):e31.
7. Michmerhuizen NL, Klco JM, Mullighan CG. Mechanistic insights and potential therapeutic approaches for NUP98-rearranged hematologic malignancies. Blood. 2020;136(20):2275–89.
8. Miyamura T, Moritake H, Nakayama H, Tanaka S, Tomizawa D, Shiba N, Saito AM, Tawa A, Shimada A, Iwamoto S, et al. Clinical and biological features of paediatric acute myeloid leukaemia (AML) with primary induction failure in the Japanese Paediatric Leukaemia/Lymphoma Study Group AML-05 study. Br J Haematol. 2019;185(2):284–8.
9. Struski S, Lagarde S, Bories P, Puiseux C, Prade N, Cuccuini W, Pages MP, Bidet A, Gervais C, Lafage-Pochitaloff M, et al. NUP98 is rearranged in 3.8% of pediatric AML forming a clinical and molecular homogenous group with a poor prognosis. Leukemia. 2017;31(3):565–72.
10. Jaju RJ, Fidler C, Haas OA, Strickson AJ, Watkins F, Clark K, Cross NC, Cheng JF, Aplan PD, Kearney L, et al. A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in de novo childhood acute myeloid leukemia. Blood. 2001;98(4):1264–7.
11. Shiba N, Ichikawa H, Taki T, Park MJ, Jo A, Mitani S, Kobayashi T, Shimada A, Sotomatsu M, Arakawa H, et al. NUP98-NSD1 gene fusion and its related gene expression signature are strongly associated with a poor prognosis in pediatric acute myeloid leukemia. Genes Chromosomes Cancer. 2013;52(7):683–93.
12. Ostronoff F, Othus M, Gerbing RB, Loken MR, Raimondi SC, Hirsch BA, Lange BJ, Petersdorf S, Radich J, Appelbaum FR, et al. NUP98-NSD1 and FLT3/ITD coexpression is more prevalent in younger AML patients and leads to induction failure: a COG and SWOG report. Blood. 2014;124(15):2400–7.
13. Niktoreh N, Walter C, Zimmermann M, von Neuhoff C, von Neuhoff N, Rasche M, Waack K, Creutzig U, Hanenberg H, Reinhardt D. Mutated WT1, FLT3-ITD, and NUP98-NSD1 Fusion in various combinations define a Poor Prognostic Group in Pediatric Acute myeloid leukemia. J Oncol. 2019;2019:1609128.
14. Meshinchi S, Woods WG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK, Bernstein ID, Radich JP. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood. 2001;97(1):89–94.
15. Zwaan CM, Meshinchi S, Radich JP, Veerman AJ, Huismans DR, Munske L, Podleschny M, Hählen K, Pieters R, Zimmermann M, et al. FLT3 internal tandem duplication in 234 children with acute myeloid leukemia: prognostic significance and relation to cellular drug resistance. Blood. 2003;102(7):2387–94.
16. Staffas A, Kanduri M, Hovland R, Rosenquist R, Ommen HB, Abrahamsson J, Forestier E, Jahnukainen K, Jónsson ÓG, Zeller B, et al. Presence of FLT3-ITD and high BAALC expression are independent prognostic markers in childhood acute myeloid leukemia. Blood. 2011;118(22):5905–13.
17. Shimada A, Taki T, Tabuchi K, Taketani T, Hanada R, Tawa A, Tsuchida M, Horibe K, Tsukimoto I, Hayashi Y. Tandem duplications of MLL and FLT3 are correlated with poor prognoses in pediatric acute myeloid leukemia: a study of the Japanese childhood AML Cooperative Study Group. Pediatr Blood Cancer. 2008;50(2):264–9.
18. Patnaik MM. The importance of FLT3 mutational analysis in acute myeloid leukemia. Leuk Lymphoma. 2018;59(10):2273–86.
19. Gamis AS, Alonzo TA, Meshinchi S, Sung L, Gerbing RB, Raimondi SC, Hirsch BA, Kahwash SB, Heerema-McKenney A, Winter L, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. J Clin Oncol. 2014;32(27):3021–32.
20. Tosić N, Stojiljković M, Colović N, Colović M, Pavlović S. Acute myeloid leukemia with NUP98-HOXC13 fusion and FLT3 internal tandem duplication mutation: case report and literature review. Cancer Genet Cytogenet. 2009;193(2):98–103.
21. Matsukawa T, Aplan PD. Clinical and molecular consequences of fusion genes in myeloid malignancies. Stem Cells. 2020;38(11):1366–74.
22. Bertrums EJM, Smith JL, Harmon L, Ries RE, Wang YJ, Alonzo TA, Menssen AJ, Chisholm KM, Leonti AR, Tarlock K, et al. Comprehensive molecular and clinical characterization of NUP98 fusions in pediatric acute myeloid leukemia. Haematologica. 2023;108(8):2044–58.
23. Bolouri H, Farrar JE, Triche T Jr., Ries RE, Lim EL, Alonzo TA, Ma Y, Moore R, Mungall AJ, Marra MA, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med. 2018;24(1):103–12.
24. Schlenk RF, Kayser S, Bullinger L, Kobbe G, Casper J, Ringhoffer M, Held G, Brossart P, Lübbert M, Salih HR, et al. Differential impact of allelic ratio and insertion site in FLT3-ITD-positive AML with respect to allogeneic transplantation. Blood. 2014;124(23):3441–9.
25. Akiki S, Dyer SA, Grimwade D, Ivey A, Abou-Zeid N, Borrow J, Jeffries S, Caddick J, Newell H, Begum S, et al. NUP98-NSD1 fusion in association with FLT3-ITD mutation identifies a prognostically relevant subgroup of pediatric acute myeloid leukemia patients suitable for monitoring by real time quantitative PCR. Genes Chromosomes Cancer. 2013;52(11):1053–64.

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