Received: 26 August 2023 | Revised: 22 November 2023 | Accepted: 30 November 2023

DOI: 10.1002/ajh.27187

RESEARCH ARTICLE

Complex karyotype but not other cytogenetic abnormalities is associated with worse posttransplant survival of patients with nucleophosmin 1-mutated acute myeloid leukemia: A study from the European Society for Blood and Marrow Transplantation Acute Leukemia Working Party

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Abstract

In the 2022 European LeukemiaNet classification, patients with *nucleophosmin 1 (NPM1)*-mutated acute myeloid leukemia (AML) were classified in the adverse-risk category in the presence of high-risk cytogenetics (CG). Nonetheless, the impact of various CG aberrations on posttransplant outcomes remains to be unraveled. This registry study analyzed adult patients with *NPM1*-mutated de novo AML who underwent their first allogeneic hematopoietic cell transplantation in the first complete

remission from 2005 to 2021. A total of 3275 patients were identified, 2782 had normal karyotype, 493 had chromosomal aberrations including 160 with adverse-risk CG, 72 patients had complex karyotype (CK), and 66 monosomal karyotype (MK). Overall, 2377 (73%) patients had *FLT3*-ITD. On univariate analysis, only *FLT3*-ITD, minimal/measurable residual disease (MRD) positivity and CK, but not abnormal CG, affected posttransplant outcomes. On multivariable analysis, CK was associated with lower overall survival (OS) (hazard ratio [HR] 1.72, $p = .009$). In the subgroup of 493 patients with aberrant CG, the 2-year leukemia-free survival (LFS) and OS were around 61% and 68%, respectively. On multivariable analysis for this subgroup, CK and MRD positivity were associated with increased risk of relapse (HR 1.7, $p = .025$; and 1.99, $p = .003$ respectively) and worse LFS (HR 1.62, $p = .018$; and 1.64, $p = .011$ respectively) while *FLT3*-ITD, MK, or other CG abnormalities had no significant effect. Importantly, CK negatively affected OS (HR 1.91, $p = .002$). In the first complete remission transplant setting, CK was found as the only cytogenetic risk factor for worse outcomes in *NPM1*-mutated AML. Nevertheless, even for this subgroup, a significant proportion of patients can achieve long-term posttransplant survival.

1 | INTRODUCTION

Acute myeloid leukemia (AML) with *nucleophosmin 1 (NPM1)* gene mutation has been identified as a distinct entity in the European LeukemiaNet (ELN) genetic classification, with a favorable prognosis for patients with normal cytogenetics and absent/low allelic ratio of internal tandem duplication (ITD) of the *fms-related tyrosine kinase 3 (FLT3)* gene.[1–5] The impact of various cytogenetic (CG) aberrations in this setting remains controversial.[6,7] Two previous cohorts including 355[7] and 95[8] patients, respectively, reported no significant impact on overall survival (OS) related to abnormal karyotype in *NPM1*-mutated AML, although the latter suggested inferior event-free survival (EFS) in these patients. A pooled analysis of individual patient data from several study group registries or individual centers in Europe, Australia, and the United States identified around 18% AML patients as having abnormal CG among more than 2400 *NPM1*-mutated patients.[9] This pooled analysis elucidated the significant impact of adverse CG aberrations on survival in *NPM1*-mutated AML patients (5-year OS of around 19.5%). Recently, the updated ELN 2022 reclassified *NPM1*-mutated AML with poor-risk CG abnormalities as adverse-risk.[10] Allogeneic hematopoietic stem cell transplant (alloHSCT) in this setting has been associated with a significantly lower risk of death (hazard ratio [HR], 0.27; 95% CI, 0.09 to 0.82).[9] Fu et al.[11] have also recently reported worse outcomes for patients with *NPM1*-mutated AML and associated karyotype abnormalities, with a 39% 5-year OS and 33% EFS rate, respectively, with longer survival in patients who underwent alloHSCT in the first complete remission (CR1).

Limited data are available regarding cytogenetic abnormalities in *NPM1*-mutated AML, especially those receiving an alloHSCT, as well as posttransplant outcomes in these patients. The optimal conditioning and donor type also remain questionable. We thus aimed at evaluating through the European Society for Blood and Marrow Transplantation (EBMT) registry, the landscape of concomitant CG aberrations in *NPM1*-mutated AML receiving an alloHSCT in CR1.

2 | MATERIALS AND METHODS

2.1 | Study design

This is a retrospective, registry-based, multicenter analysis of patients' data provided and approved by the Acute Leukemia Working Party (ALWP) of the EBMT, which is a voluntary working group of more than 600 transplant centers that are required to report all consecutive HCTs and follow-ups once a year. Audits are routinely performed to determine the accuracy of the data. Since January 2003, all transplant centers have been required to obtain written informed consent prior to data registration with the EBMT, following the guidelines of the Declaration of Helsinki, 1975.

We included in this analysis, adult patients (≥18 years) with de novo AML, *NPM1*-mutated, with known CG and *FLT3*-ITD status, who received their first alloHSCT in CR1, between 2005–2021, from either a matched sibling donor (MSD), matched or mismatched unrelated donor (UD), or haploidentical donor. The stem cell source was granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood (PB) or bone marrow (BM). We excluded patients with favorable CG (18 patients) and those with ex vivo T cell depletion (TCD).

Variables collected included recipient age at transplant, recipient and donor gender, *FLT3*-ITD status, karyotype, and cytogenetic risk group at diagnosis; time from diagnosis to transplant, year of transplant, measurable residual disease (MRD) status at transplant,

TABLE 1 Patient characteristics.

Variable	Total ($n = 3275$)	Normal CG ($n = 2782$)	Abnormal CG ($n = 493$)	*p*-Value
Patient age (years)	53.9	53.7	54.6	
Min–max	(18.1–82.5)	(18.1–82.5)	(18.4–78.7)	.64
[IQR]	[45.1–61.5]	[45.3–61.3]	[44–62.5]	
Gender (%)				
Male	1502 (46)	1242 (44.8%)	260 (52.7)	.001
Female	1764 (54)	1531 (55.2%)	233 (47.3)	
Missing	9	9	0	
FLT3-ITD (%)				
Negative	898 (27)	686 (25)	212 (43)	<.0001
Positive	2377 (73)	2096 (75)	281 (57)	
CG risk group (%)				
Intermediate	3115 (95)	2782 (100)	333 (67.5)	NA
Adverse	160 (5)	NA	160 (32.5)	
Type of chromosomal abnormalities seen in >20 patients or associated with adverse risk (*) CG (%)		NA		NA
Trisomy 8	141 (4.3)		141 (28.6)	
CK*	72 (2.2)		72 (14.6)	
MK*	66 (2)		66 (13.4)	
Del(9)	40 (1.2)		40 (8.1)	
Trisomy 4	33 (1)		33 (6.7)	
Del(7)*	29 (0.9)		29 (5.9)	
Del(Y)	27 (0.8)		27 (5.5)	
Del(5)*	22 (0.7)		22 (4.5)	
11q23*	15 (0.5)		15 (3)	
Abn3q26*	11 (0.4)		11 (2.2)	
Del(17p)*	8 (0.2)		8 (1.6)	
t(9;22)*	5 (0.2)		5 (1)	
t(6;11)*	3 (0.1)		3 (0.6)	
t(6;9)*	3 (0.1)		3 (0.6)	
t(11;19)*	1 (0.03)		1 (0.2)	
t(10;11)*	1 (0.03)		1 (0.2)	
Median diagnosis to HCT (months)	5	5	4.7	.0005
(min–max)	(0.1–101)	(1–101)	(0.1–30.6)	
[IQR]	[3.9–6.3]	[4–6.4]	[3.7–5.9]	
KPS				
<90	703 (22.7)	596 (22.6)	107 (23)	.85
≥90	2399 (77.3)	2041 (77.4)	358 (77)	
Missing	173	145	28	
HT-CI				
0	1367 (54.2)	1164 (54.4)	203 (53.1)	.9
1 or 2	624 (24.7)	527 (24.6)	97 (25.4)	
≥3	531 (21.1)	449 (21)	82 (21.5)	
Missing	753	642	111	
MRD preHCT				
Negative	1370 (57.6)	1186 (57.8)	184 (56.4)	.64

(Continues)

TABLE 1 (Continued)

Variable	Total ($n = 3275$)	Normal CG ($n = 2782$)	Abnormal CG ($n = 493$)	*p*-Value
Positive	1007 (42.4)	865 (42.2)	142 (43. 6)	
Missing	898	731	167	
Recipient CMV (%)				
Negative	1061 (32.6)	884 (32)	177 (36.3)	.064
Positive	2189 (67.4)	1878 (68)	311 (63.7)	
Missing	25	20	5	

Abbreviations: 11q23: t(v;11q23) excluding t(9;11); Abn3q26: 3q26/EVI1 rearrangement; CG, cytogenetics; CK, complex karyotype; CMV, cytomegalovirus; del(17p): del (17p) or monosomy 17; del(5): monosomy 5 or del(5q); del7: monosomy 7 or del(7q); *FLT3*-ITD, FMS-like tyrosine kinase-3 internal tandem duplication; HCT, hematopoietic cell transplant; HT-CI, hematopoietic cell transplantation specific comorbidity index; IQR, interquartile range; KPS, Karnofsky performance scale; max, maximum; min, minimum; MK, monosomal karyotype; MRD, measurable residual disease.

Karnofsky performance status (KPS) score at transplant, HCT comorbidity index (HCT-CI); in addition to transplant-related factors including conditioning regimen, graft-versus-host disease (GVHD) prophylaxis, the use of posttransplant cyclophosphamide (PTCy), in vivo TCD, donor type, the recipient and donor cytomegalovirus (CMV) status, and stem cell source (BM or PB).

2.2 | Definitions and objectives

Cytogenetics was reported by full karyotype for 33% of patients, most often using the International System for Human Cytogenomic Nomenclature (ISCN) guidelines.[12] In 67% of patients, CG abnormalities were reported by answering the presence or absence for each specific abnormality asked in the EBMT registry. Cytogenetic subgroups were classified according to the ELN 2017[3] but considering only cytogenetic features. Complex karyotype (CK) was defined as having three or more abnormalities. Monosomal karyotype (MK), which was defined as presence of two or more distinct monosomies (excluding loss of X or Y), or one single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding core-binding factor AML), was also included in the adverse risk group. Myeloablative conditioning (MAC) was defined as a regimen containing either total body irradiation with a dose greater than 6 Gy, a total dose of oral busulfan (Bu) greater than 8 mg/kg, or a total dose of intravenous Bu greater than 6.4 mg/kg.

The primary objective of this study was to evaluate the association of the various concurrent CG aberrations with posttransplant outcomes for patients with *NPM1*-mutated AML. Non-relapse mortality (NRM) was used to define death with no evidence of disease relapse. The probability of leukemia-free survival (LFS) and OS was defined as survival with no evidence of disease relapse or progression, and time to death from any cause, respectively. GVHD-free, relapse-free survival (GRFS) is defined as the time being alive with no grade ≥III GVHD, extensive chronic GVHD, or disease relapse. The diagnosis and grading of acute[13] and chronic GVHD[14] were performed by transplant centers using standard criteria.

2.3 | Statistical analysis

Patient-, disease-, and transplant-related variables were compared between groups using the chi-square or Fisher statistic for categorical and the Mann–Whitney test for continuous variables. All outcomes were measured from the time of transplant. NRM was calculated using cumulative incidence curves in a competing risk setting. Death and relapse were considered competing events. When assessing the cumulative incidence of acute and chronic GVHD, we considered relapse and death as competing events. LFS, OS, and GRFS were evaluated using the Kaplan–Meier method, the log-rank test was used for univariate comparisons. As planned initially, analyses were done both in the entire population and in the subgroup of patients with aberrant CG. Univariate analyses were performed using the log-rank test for LFS, OS, and GRFS, and Gray's test for CI estimates. A Cox's proportional hazards model was used for all endpoints in multivariate analyses. In order to test for a center effect, we introduced a random effect or frailty for each center into the model. All tests were two-sided with the type I error at 0.05. All analyses were performed using SPSS 27.0 (SPSS Inc, Chicago, IL, USA) and R 4.1.1 (R Development Core Team, Vienne, Austria, URL: https://www.R-project.org/). Follow-up was calculated using the reverse Kaplan–Meier method, and propotional hazards assumptions were checked using the Grambsch–Therneau residual-based test.

3 | RESULTS

3.1 | Patient and transplant characteristics

We identified a total of 3275 patients with de novo *NPM1* mutated AML (54% female; median age 54 years, range 18–83) allografted between 2005 and 2021 in CR1 from an MSD (32%), UD (57%), or haploidentical donor (11%) (Tables 1 and 2). *FLT3*-ITD was detected in 2377 patients (73%). Overall, 2782 patients (85%) had normal karyotype and 493 patients (15%) had aberrant CG. The most common CG aberrations identified included trisomy 8 (141 patients), CK (72 patients), and MK (66 patients). Adverse-risk CG was noted in

TABLE 2 Donor and transplant characteristics.

Variable	Total ($n = 3275$)	Normal CG ($n = 2782$)	Abnormal CG ($n = 493$)	p-Value
Follow-up (months)				
Median	35.4	34.7	37.1	.23
Quartiles	[33.3–36.3]	[32–36.1]	[35–45.6]	
Transplant year				
Median	2017	2017	2017	.52
(Min–Max)	(2005–2021)	(2005–2021)	(2005–2021)	
Type of donor (%)				
MSD	1058 (32)	892 (32)	166 (34)	.29
UD	1867 (57)	1583 (57)	284 (58)	
Haplo	350 (11)	307 (11)	43 (9)	
Donor gender (%)				
Male	2064 (63.4)	1755 (63.5)	309 (63.1)	.85
Female	1190 (36.6)	1009 (36.5)	181 (36.9)	
Missing	21	18	3	
Female to male combination (%)				
No	2806 (86.2)	2392 (86.5)	414 (84.1)	.16
Yes	451 (13.8)	373 (13.5)	78 (15.9)	
Missing	18	17	1	
Cell source (%)				
PB	2907 (88.8)	2469 (88.7)	438 (88.8)	.95
BM	368 (11.2)	313 (11.3)	55 (11.2)	
Donor CMV (%)				
Negative	1488 (45.9)	1254 (45.6)	234 (47.9)	.35
Positive	1753 (54.1)	1498 (54.4)	255 (52.1)	
Missing	34	30	4	
Conditioning (%)				
MAC	1673 (51.3)	1434 (51.8)	239 (48.6)	.18
RIC	1586 (48.7)	1333 (48.2)	253 (51.4)	
Missing	16	15	1	
In vivo TCD (%)				
No	1236 (38)	1061 (38.4)	175 (35.9)	
Yes	2014 (62)	1702 (61.6)	312 (64.1)	
Missing	25	19	6	
PTCy (%)				
No	2729 (84.5)	2308 (84)	421 (86.8)	.12
Yes	502 (15.5)	438 (16)	64 (13.2)	
Missing	44	36	8	

Abbreviations: BM, bone marrow; CG, cytogenetics; CMV, cytomegalovirus; Haplo, haploidentical donor; IQR, interquartile range; MAC, myeloablative conditioning; Max, maximum; Min, minimum; MSD, matched sibling donor; PB, peripheral blood; PTCy, post transplant cyclophosphamide; RIC, reduced intensity conditioning; TCD, T-cell depletion; UD, unrelated donor.

160 patients (Table 1). Only eight patients had del(17p). At transplant, 1007 patients (42.4%) were MRD positive, 1370 were MRD negative, while MRD status was missing for 898 patients. HCT-CI was zero in 1367 patients (54.2% of those with available data). The conditioning regimen was MAC in 51.3% of patients, and 88.8% received a PB stem cell harvest. Approximately 60% of patients received in vivo TCD, while PTCy was given to 15.5% of patients. The majority (67.4%) of patients as well as donors (54.1%) were CMV positive.

TABLE 3 Multivariable analysis for the total cohort and for the subgroup with abnormal cytogenetics.

	Relapse		NRM		LFS		OS		GRFS	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Total cohort										
CK	1.53 (0.97–2.41)	.067	1.34 (0.63–2.86)	.45	**1.47 (0.99–2.17)**	**.055**	**1.72 (1.14–2.6)**	**.009**	1.33 (0.94–1.87)	.11
CG abnl vs. NK	1.13 (0.91–1.41)	.28	0.77 (0.55–1.06)	.11	1 (0.84–1.2)	.98	0.98 (0.8–1.2)	.87	1.07 (0.92–1.25)	.39
FLT3-ITD	**1.54 (1.27–1.86)**	**<.001**	1.09 (0.86–1.39)	.47	**1.37 (1.18–1.59)**	**<.001**	**1.27 (1.07–1.49)**	**.005**	**1.22 (1.07–1.38)**	**.002**
MRD negative (reference)	**1**		1		**1**		**1**		**1**	
MRD positive	**2.38 (1.98–2.87)**	**<.001**	1.09 (0.85–1.41)	.49	**1.85 (1.59–2.14)**	**<.001**	**1.72 (1.46–2.04)**	**<.001**	**1.56 (1.37–1.78)**	**<.001**
MRD missing	**1.26 (1.02–1.55)**	**.028**	1.08 (0.85–1.38)	.53	**1.19 (1.01–1.39)**	**.037**	**1.12 (0.94–1.34)**	**.2**	**1.14 (0.99–1.3)**	**.072**
Age	0.95 (0.88–1.02)	.18	1.36 (1.21–1.51)	<.0001	**1.07 (1.01–1.13)**	**.039**	**1.18 (1.1–1.27)**	**<.0001**	1.03 (0.98–1.08)	.26
Time diagnosis to CR1	0.97 (0.95–1)	.023	0.98 (0.95–1.01)	.11	**0.97 (0.96–0.99)**	**.007**	**0.97 (0.94–0.99)**	**.003**	0.99 (0.98–1.01)	.36
KPS ≥90	1.02 (0.85–1.24)	.81	**0.73 (0.59–0.92)**	**.007**	0.91 (0.78–1.05)	.18	**0.85 (0.72–1)**	**.048**	0.92 (0.81–1.05)	.22
Donor MSD reference	1		1		1		1		1	
UD	0.99 (0.82–1.18)	.88	**1.69 (1.29–2.19)**	**.0001**	**1.17 (1.01–1.36)**	**.037**	**1.32 (1.12–1.55)**	**.001**	1.08 (0.95–1.23)	.23
Haplo donor	0.73 (0.53–1)	.051	**2.44 (1.69–3.5)**	**<.0001**	1.15 (0.91–1.46)	.23	**1.32 (1.02–1.72)**	**.038**	0.89 (0.72–1.11)	.28
Female to male	0.93 (0.74–1.18)	.55	**1.51 (1.15–1.99)**	**.003**	1.12 (0.93–1.33)	.23	**1.21 (0.99–1.46)**	**.057**	1.14 (0.98–1.33)	.086
RIC	1.13 (0.94–1.35)	.19	1.15 (0.91–1.46)	.23	1.14 (0.99–1.31)	.076	1.13 (0.96–1.33)	.14	1.02 (0.9–1.16)	.74
Patient CMV pos	0.9 (0.76–1.07)	.25	**1.3 (1.02–1.66)**	**.032**	1.03 (0.9–1.19)	.67	1.13 (0.96–1.32)	.13	1.02 (0.91–1.16)	.71
Donor CMV pos	0.96 (0.81–1.13)	.64	0.92 (0.74–1.14)	.43	0.94 (0.83–1.08)	.38	0.95 (0.82–1.1)	.46	1.02 (0.91–1.14)	.76
Year of transplant	0.99 (0.96–1.01)	.24	**0.97 (0.94–1)**	**.035**	0.98 (0.96–1)	.043	0.97 (0.95–0.99)	.009	0.98 (0.96–0.99)	.009
Cell source (PB vs. BM)	1.07 (0.82–1.39)	.64	0.96 (0.69–1.33)	.81	1.02 (0.83–1.26)	.82	1.04 (0.82–1.31)	.76	**1.32 (1.09–1.59)**	**.004**
TCD	1.02 (0.85–1.23)	.84	1.03 (0.81–1.3)	.83	1.02 (0.88–1.18)	.81	0.98 (0.83–1.16)	.8	**0.8 (0.71–0.91)**	**<.001**
Patients with abnormal CG										
CK	**1.7 (1.07–2.7)**	**.025**	1.34 (0.56–3.2)	.51	**1.62 (1.09–2.4)**	**.018**	**1.91 (1.26–2.9)**	**.002**	1.45 (1.02–2.05)	.037
FLT3-ITD	1.21 (0.83–1.75)	.32	1.85 (0.94–3.65)	.076	1.31 (0.96–1.8)	.093	1.23 (0.87–1.73)	.24	1.11 (0.85–1.45)	.43
MRD negative	1		1		1		1		1	
MRD positive	1.99 (1.26–3.15)	.003	1.03 (0.45–2.33)	.95	1.64 (1.12–2.4)	.011	1.48 (0.98–2.24)	.065	1.51 (1.09–2.09)	.013
MRD missing	0.99 (0.62–1.59)	.98	0.65 (0.29–1.42)	.28	0.9 (0.62–1.32)	.6	0.85 (0.56–1.28)	.43	0.95 (0.69–1.31)	.75
Patient age (per 10 years)	0.99 (0.84–1.17)	.93	**1.66 (1.17–2.34)**	**.004**	1.09 (0.95–1.27)	.22	1.14 (0.98–1.33)	.095	1.02 (0.91–1.16)	.69
Time diag to CR1 (month)	1.02 (0.97–1.07)	.53	0.98 (0.88–1.09)	.7	1.02 (0.97–1.06)	.48	1.02 (0.98–1.08)	.33	1.01 (0.97–1.05)	.58
KPS ≥90	0.92 (0.58–1.44)	.71	0.96 (0.46–2.02)	.91	0.91 (0.63–1.32)	.63	0.9 (0.6–1.35)	.61	0.9 (0.65–1.24)	.51
MSD reference	1		1		1		1		1	
UD	1.27 (0.82–1.96)	.28	**2.29 (1.03–5.09)**	**.042**	1.44 (1–2.1)	.053	**1.6 (1.07–2.4)**	**.023**	1.14 (0.84–1.55)	.39

TABLE 3 (Continued)

	Relapse		NRM		LFS		OS		GRFS	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Haplo	0.6 (0.22–1.59)	.3	**3.48 (1.04–11.62)**	**.043**	0.99 (0.51–1.95)	.99	1.06 (0.51–2.22)	.88	0.59 (0.32–1.08)	.089
Female to male	1.02 (0.6–1.72)	.94	**2.89 (1.36–6.14)**	**.006**	1.37 (0.92–2.05)	.12	1.17 (0.75–1.84)	.49	1.17 (0.82–1.68)	.39
RIC	0.8 (0.51–1.26)	.33	1.08 (0.48–2.44)	.85	0.87 (0.59–1.27)	.47	0.92 (0.61–1.38)	.69	0.82 (0.6–1.12)	.22
Patient CMV pos	1.12 (0.75–1.67)	.58	1.29 (0.61–2.7)	.5	1.15 (0.82–1.62)	.42	1.07 (0.74–1.55)	.73	0.97 (0.72–1.3)	.83
Donor CMV pos	**0.63 (0.42–0.94)**	**.023**	1.35 (0.66–2.74)	.41	0.79 (0.57–1.1)	.16	0.87 (0.61–1.24)	.45	0.91 (0.69–1.21)	.53
Year of HCT	0.98 (0.92–1.03)	.43	0.93 (0.84–1.04)	.2	0.97 (0.93–1.02)	.23	0.98 (0.93–1.03)	.39	0.98 (0.94–1.02)	.32
Cell source PB vs. BM	0.8 (0.43–1.48)	.47	1.07 (0.36–3.2)	.91	0.9 (0.53–1.51)	.69	0.97 (0.55–1.73)	.92	1.18 (0.74–1.86)	.49
In vivo TCD	1.03 (0.66–1.59)	.91	0.52 (0.24–1.1)	.086	0.82 (0.57–1.18)	.28	0.76 (0.51–1.12)	.16	**0.7 (0.52–0.95)**	**.021**

Note: Bold values are statistically significant.

Abbreviations: BM, bone marrow; CG abnl, abnormal cytogenetics; CG, cytogenetics; CI, confidence interval; CK, complex karyotype; CMV, cytomegalovirus; CR1, first complete remission; FLT3-ITD, FMS-like tyrosine kinase-3 internal tandem duplication; GRFS, GVHD-free and relapse-free survival; haplo, haploidentical donor; HCT, hematopoietic cell transplantation; HR, hazard ratio; KPS, Karnofsky performance status; LFS, leukemia-free survival; mo, months; MSD, matched sibling donor; NK, normal karyotype; NRM, non-relapse mortality; OS, overall survival; PB, peripheral blood; pos, positive; RIC, reduced intensity conditioning; TCD, T cell depletion; UD, unrelated donor; y, year.

3.2 | Transplant outcomes and their prognostic factors

The median follow-up for alive patients was 35.4 months (interquartile range [IQR] 33.3–36.3), during which a total of 961 patients died (48.3% due to disease relapse). Patients with a normal karyotype had a 2-year relapse rate of 23.3%, NRM of 13.3%, LFS of 63.4%, OS of 71%, and GRFS of 51.1%. Conversely, these numbers for patients with aberrant CG were respectively 27.1%, 11.5%, 61.4%, 68.1%, and 48.2%. For patients with normal CG, acute GVHD grade II–IV was noted in 23.4% while chronic GVHD was seen in 36.3%, as compared to 25.8% and 36.1%, respectively for those with aberrant CG. No statistically significant difference was noted for all these outcomes when comparing normal to aberrant CG (Table S2).

On univariate analysis, factors associated with a significantly increased risk of relapse included the presence of CK (44% vs. 23.5%; $p = .001$), *FLT3*-ITD (25.8% vs. 18.8%; $p = .001$), and MRD positivity at transplant (42.2% vs. 18.1%; $p = .009$), while del(Y) was associated with a lower relapse risk (3.7% vs. 24.1%, $p = .02$). None of the evaluated factors had a significant impact on NRM, while worse LFS, OS, and GRFS was noted in patients with CK (44.9% vs. 63.5%; $p = .006$, 51% vs. 71%; $p = .001$, and 34.6% vs. 51%; $p = .008$, respectively), those with *FLT3*-ITD (61% vs. 68.6%; $p = .001$, 68.9% vs. 75%; $p = .011$, and 49.1% vs. 54.7%; $p = .012$, respectively), as well as those with MRD positivity at transplant (39.6% vs. 69.4%; $p = .004$, 49% vs. 75.8%; $p = .008$, and 35.4% vs. 55.2%; $p = .025$, respectively). On multivariable analysis (Table 3), CK as well as *FLT3*-ITD were associated with a worse OS with HR of 1.72 (95% CI 1.14–2.6) and 1.27 (95% CI 1.07–1.49), respectively. Additionally, MRD positivity at transplant was associated with worse OS with HR 1.72 (95% CI 1.46–2.04). When analyzed by subgroup by univariate analysis according to *FLT3*-ITD status, the impact of CK was maintained for those with *FLT3* wild-type: 2-year LFS 39.6% versus 70.5%; $p = .006$, and 2-year OS 49% versus 75.3%, $p = .012$. Similarly for patients with *FLT3*-ITD, CK was associated with a higher risk of relapse (45.7% vs. 26.5%, $p = .021$), and worse OS (52.7% vs. 67.4%; $p = .047$) with no significant effect on other outcomes.

3.3 | Transplant outcomes and their prognostic factors for patients with aberrant CG

For the 493 patients with aberrant CG, the median age was 54.6 years (range: 18.4–78.7), 52.7% were males and *FLT3*-ITD was observed in 281 patients (57%). A total of 164 (33.2%) patients died during follow-up, and the most common cause of death was disease relapse ($n = 89$), followed by GVHD ($n = 35$). No significant difference was observed when comparing outcomes of patients with intermediate versus adverse-risk CG, the 2-year NRM, LFS, and GRFS for the latter were 11%, 58.8%, and 44.7%, respectively.

On univariate analysis for patients with CG aberrations, CK affected posttransplant outcomes (Figure 1) and was associated with increased risk of relapse (44% vs. 24.3%; $p = .003$), worse 2-year LFS

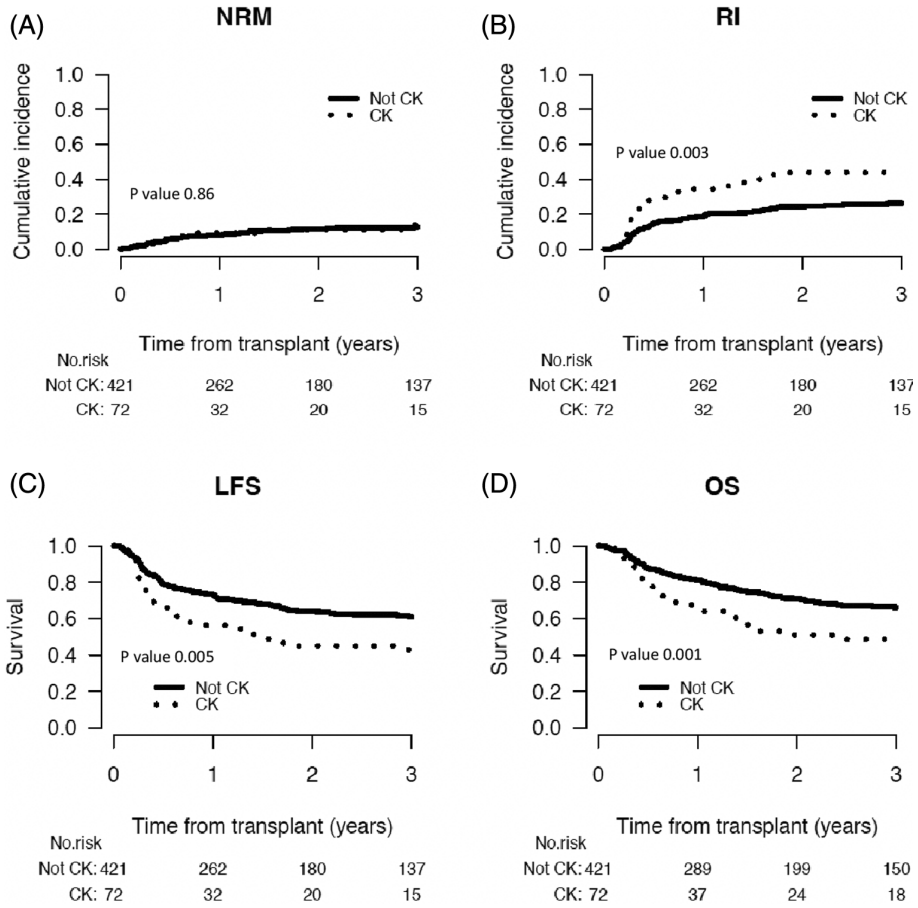

FIGURE 1 Posttransplant outcomes in patients with nucleophosmin 1-mutated acute myeloid leukemia and cytogenetic abnormalities according to the presence or absence of complex karyotype. (A) Non-relapse mortality; (B) relapse risk; (C) leukemia-free survival; (D) overall survival.

(44.9% vs. 64.1%; $p = .005$), OS (51% vs. 71%; $p = .001$), as well as GRFS (34.6% vs. 50.5%; $p = .018$). Additionally, MRD positivity at transplant was associated with increased risk of relapse (37.1% vs. 20%; $p = .001$), worse LFS (52.5% vs. 65.4%; $p = .005$), OS (60.1% vs. 71.5%; $p = .036$), as well as GRFS (37.7% vs. 54.1%; p .001). In addition, del(Y) was associated with a significantly lower risk of relapse (3.7% vs. 28.4%; $p = .008$). All other CG abnormalities including MK (Table S1), as well as *FLT3*-ITD status did not significantly affect outcomes. For MK, the 2-year OS was 67.6% (compared to 68.2% for those without MK; $p = .97$).

On multivariable analysis for patients with CG abnormalities (Table 3), CK was associated with a significant effect on posttransplant outcomes including relapse risk with an HR of 1.7 (95% CI 1.07–2.7), LFS with an HR of 1.62 (95% CI 1.09–2.4), and OS with an HR of 1.91 (95% CI 1.26–2.9). No interaction was observed between CK and *FLT3*-ITD. Additionally, MRD positivity at transplant was associated with higher risk for relapse with HR 1.99 (95% CI 1.26–3.15), and worse LFS with HR 1.64 (95% CI 1.12–2.4). Of note, in vivo TCD was associated with an improved GRFS with an HR of 0.7 (95% CI 0.52–0.95).

4 | DISCUSSION

NPM1-mutated AML has been associated with favorable outcomes in the setting of normal karyotype and absence of *FLT3*-ITD.[15] Nonetheless, much is yet to be unraveled regarding the landscape of CG aberrations in *NPM1*-mutated AML and the effect of each of these abnormalities on posttransplant outcomes. AlloHSCT has been associated with prolonged OS when offered to patients with normal- and intermediate-risk CG,[16] however, there have been continuing concerns regarding its role for patients with adverse-risk CG.

Here, we report the largest cohort of patients with *NPM1*-mutated AML and CG aberrations receiving an alloHSCT. The most common chromosomal aberrations noted were trisomy 8 ($n = 141$), CK ($n = 72$), and MK ($n = 66$). Our analysis showed no significant difference in post alloHSCT outcomes when comparing normal to abnormal karyotype, or intermediate- to adverse-risk CG. Conversely, in previous studies on *NPM1*-mutated AML, CG aberrations were associated with worse outcomes from diagnosis, including OS and EFS (5-year OS 38.9 ± 12.9% vs. 59.8 ± 7.2%; $p = .037$, and 5-year EFS 33.3 ± 12.2% vs. 50.1 ± 7.7%; $p = .043$), with improved outcomes noted in a small number of patients receiving alloHSCT ($n = 4$).[11]

Importantly, we show that CK but not other adverse-risk CG is associated with significantly worse posttransplant outcomes. Nevertheless, even for patients with *NPM1*-mutated AML and abnormal CG, including those with CK, alloHSCT appears to provide long-term survival (2-year OS 71% and 51%, respectively). Data from the ALWP of the EBMT and the MD Anderson Cancer Center, including 1342 AML patients with CK has shown a

2-year LFS of 31.3%, OS of 36.8%, and GRFS of 19.8%.[17] This indicates again the importance of incorporating both CG and molecular characteristics for the optimal risk classification of patients with AML.[18,19]

In our analysis, in the setting of *NPM1*-mutated AML, MK was not associated with a significant effect on posttransplant outcomes, which is in contrast to results from a retrospective analysis of 465 adult patients with AML receiving an alloHSCT where the presence of MK resulted in an increased risk of relapse, and a worse OS (HR 2.6; $p = .001$),[20] although this latter analysis was not limited to *NPM1*-mutated AML which is a characteristic of our cohort. MK has been identified as an independent negative prognostic factor for patients with AML across various treatment strategies.[21–23] The confirmation of the prognostic impact of high-risk CG aberrations such as CK is specifically helpful in identifying patients at high risk of posttransplant relapse, who may benefit from posttransplant maintenance and preemptive treatments to further improve these patients' outcomes.[24,25] Recent data has also shown the prognostic impact of cytogenetics even in the era of molecular classification of AML with its targeted therapies,[26] as well as the MRD directed management approaches.[27] This analysis again shows the significance of disease status at transplant (MRD positivity has significant impact on almost all posttransplant outcomes). Addtionally, our data indicate a significant impact of the type of donor on posttransplant outcomes where both haploidentical and unrelated donor were associated with significantly higher NRM (HR 2.44, 95% CI 1.69–3.5 and HR 1.69; 95% CI 1.29–2.19, respectively), and worse OS (HR 1.32; 95% CI 0.038 and HR 1.32; 95% CI 1.12–1.55, respectively).

Limitations of this study include its retrospective nature, which might have led to slection bias, in addition to the lack of a comparator group which limits the ability to identify CK as a predictive factor of outcomes in the transplant setting based on this analysis solely, or to clearly conclude a direct beneficial impact of transplantation for these patients. However, these data confirm the prognostic impact of CK, encourage the consideration of HCT for these patients, and suggest the need for furture studies in that setting. Other limitations include the small number of patients with certain CG aberrations. Specifically, our cohort included only eight patients with del (17p), while TP53 mutation was present in 18 patients including 14 patients with CK, absent in 304 patients and missing in around 90% of the patients, and thus additional studies are needed to evaluate the significance of these adverse-risk abnormalities in the setting of *NPM1*-mutated AML. The presence of multiple abnormalities in some patients also indicates the need to specifically analyze the interaction between such abnormalities and their impact on posttransplant outcomes. An additional limitation is that cytogenetics was reported by full karyotype for 33% of the patients, most often using ISCN guidelines. In 67% of the patients, cytogenetics abnormalities were reported by answering the presence or the absence for each specific abnormality asked in the EBMT registry, complex, and monosomal karyotype (Yes/No). In addition, at the time of this analysis, t(8;16) was not routinely collected in the registry.

5 | CONCLUSION

With the recent ELN 2022 reclassification of AML with adverse- and high-risk CG, our data indicate that in the transplant setting, only CK but not other adverse-risk CG is associated with worse outcomes. These results indicate that alloHSCT may overcome the poor prognosis of adverse-risk CG including MK in patients with *NPM1*-mutated AML. Nevertheless, even in patients with CK, transplant appears to provide long-term survival for a significant proportion of patients and thus should be considered for such patients in CR1. Future studies should aim to compare transplant to alternative consolidation treatments in an effort to clearly identify its role for these patients.

ACKNOWLEDGMENTS

We thank the contributing centers of the EBMT registry for providing their patients' data, as well as the ALWP-EBMT staff for help with data management. We also thank the patients who provided data.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data analyzed in this study were provided and approved by the Acute Leukemia Working Party (ALWP) of the EBMT. All relevant data are provided within the article and the Appendix. The relevant working party of the EBMT will review requests from qualified external researchers for data from the EBMT studies in a responsible manner that includes protecting patient privacy, assurance of data security and integrity, and furthering scientific and medical innovation. Additional details on data sharing criteria and process for requesting access should be sent to ebmt.do-paris@ebmt.org. Individual patient data will not be shared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Moukalled N, Labopin M, Versluis J, et al. Complex karyotype but not other cytogenetic abnormalities is associated with worse posttransplant survival of patients with nucleophosmin 1-mutated acute myeloid leukemia: A study from the European Society for Blood and Marrow Transplantation Acute Leukemia Working Party. *Am J Hematol*. 2024;1-10. doi:10.1002/ajh.27187