ARTICLE

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Frequency and impact of somatic co-occurring mutations on post-transplant outcomes in acute myeloid leukemia: a multicenter registry analysis on behalf of the EBMT ALWP

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Acute myeloid leukemia (AML) includes genetically defined subsets. In allogeneic hematopoietic cell transplantation (allo-HCT), the frequency and prognosis of gene-gene interactions may differ from those of patients treated with chemotherapy alone. In this study, adult patients ($N = 952$) with AML allografted between 2015 and 2023, with available next generation sequencing (NGS) at diagnosis were included. Most frequent mutations were *DNMT3A (24%), FLT3*-ITD *(21%), NPM1 (21%), RUNX1 (16%), NRAS (16%), TET2 (14%)*, and *IDH2 (12%)*. Multiple correspondence analysis identified distinct groups of co-occurring mutations. Outcome analysis was performed on 646 AML patients allografted in first complete remission (CR1). Six non-overlapping groups were constructed: 1) *TP53* mutation ($N = 47$); 2) *NPM1* mutation ($N = 129$); 3) *FLT3*-ITD and/or *DNMT3A* mutation ($N = 128$); 4) *SRSF2* and/or *ASXL1* and/or *RUNX1* mutation (SAR group) ($N = 132$); 5) *IDH1* and/or *IDH2* and/or *TET2* mutation ($N = 43$); and 6) all ten genes unmutated ($N = 167$). In multivariable analysis, *TP53* mutation, adverse karyotype, and age negatively affected leukemia-free survival (LFS) and overall survival (OS). OS was additionally negatively affected when the ten genes were unmutated. Notably, outcomes were excellent for SAR mutations (2-year LFS 76%, OS 84%), indicating allo-HCT in CR1 can overcome their adverse risk at diagnosis.

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INTRODUCTION

Acute myeloid leukemia (AML) is a highly heterogeneous hematological malignancy, characterized by a complex genomic landscape and diverse genetic subsets [1, 2]. Historically, cytogenetic abnormalities represented a major factor in prognosticating, guiding risk classification into favorable, intermediate and adverse categories based on chromosomal aberrations [3]. More recently, whole-genome sequencing unraveled the complexity of the AML genomic landscape, showing multiple infrequently mutated genes and the presence of co-occurring mutations within the same patient [2]. Moreover, multiple competing clones can be frequently encountered, playing an important role in the evolution of the disease [4].

The genomic classification of AML has evolved substantially with the identification of mutations in key regulatory pathways, including transcription factors, epigenetic modifiers, spliceosome machinery, the cohesin complex, and signaling cascades [2]. This has led to the development of more refined risk stratification models from diagnosis, such as the European LeukemiaNet (ELN) 2022 classification, which integrates specific gene aberrations to improve prognostic accuracy [5]. Among the main genetic mutations considered at diagnosis, *NPM1* status is risk-dependent on karyotype and *FLT3*-ITD co-occurrence, *FLT3*-ITD confers an intermediate-risk category, while bZIP in-frame mutated *CEBPA* is of favorable risk. Adverse-risk mutations

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encompass *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2* and *TP53* mutations [5].

The selection of patients with AML in first complete remission (CR1) for allogeneic hematopoietic cell transplantation (allo-HCT) depends on relapse risk and estimated non-relapse mortality (NRM). Current guidelines recommend allo-HCT in CR1 for those classified as intermediate- or adverse-risk, based on the ELN 2022 classification [6]. Patients with persistent measurable residual disease (MRD) are also offered allo-HCT, irrespective of genetic risk [5]. However, in the context of allo-HCT, the frequency and prognostic value of different gene-gene interactions have not been studied and may differ from that of patients treated with intensive chemotherapy alone.

In this study, leveraging data from the European Society for Blood and Marrow Transplantation (EBMT) database, we aimed to analyze the frequency and interaction of different recurrent somatic mutations in AML patients undergoing allo-HCT, with a particular focus on those transplanted in CR1. Understanding these genetic interactions may provide deeper insights into post-transplant disease biology and inform personalized therapeutic strategies.

PATIENTS AND METHODS

Study design and inclusion criteria

This is a retrospective, registry-based, multicenter study utilizing patient data collected and approved by the Acute Leukemia Working Party (ALWP) of the EBMT. The EBMT is a collaborative network comprising more than 600 transplant centers that are required to report all consecutive HCTs and subsequent follow-ups on an annual basis. Routine audits are performed to ensure data accuracy and completeness. Since January 2003, all participating transplant centers have been required to obtain written informed consent from patients prior to data registration with the EBMT, in accordance with the ethical principles outlined in the Declaration of Helsinki (1975).

For this analysis, a data collection of the NGS reports at diagnosis was performed for adult patients (≥18 years) with a diagnosis of AML who underwent allo-HCT between 2015 and 2023. Included patients had available next-generation sequencing (NGS) data at the time of diagnosis (with a margin of a maximum of 30 days from diagnosis), including a documented panel of sequenced genes from blood or bone marrow samples. Patients with a history of prior autologous HCT were excluded from the study.

In addition to NGS data, the following clinical and transplant-related variables were collected: recipient age and disease status at transplant, recipient and donor gender, karyotype, *FLT3*-ITD mutation status at diagnosis (regardless of the method of analysis), de novo versus *(*vs.) secondary AML, and time from diagnosis to transplant. Transplant-related variables included the year of transplant, Karnofsky performance status score at transplant, HCT-specific comorbidity index, conditioning regimen, graft-versus-host disease (GVHD) prophylaxis, use of post-transplant cyclophosphamide (PTCy), in vivo T cell depletion, donor type, recipient and donor cytomegalovirus status, and stem cell source. MRD status prior to transplant was also collected, recorded as either positive or negative, and based on the local institutional protocol. MRD assessment was performed using different techniques, including multiparameter flow cytometry or molecular assays, depending on test availability and the mutational profile of the disease.

Endpoints and definitions

Endpoints included leukemia-free survival (LFS), overall survival (OS), non-relapse mortality (NRM), relapse incidence (RI), and acute and chronic GVHD. All outcomes were measured from the time of allo-HCT. LFS was defined as survival without leukemia relapse or progression; patients alive without leukemia relapse or progression were censored at the time of last follow-up. OS was defined as death from any cause. NRM was defined as death without previous leukemia relapse. Conditioning intensity was measured using the transplant conditioning regimen intensity, myeloablative conditioning/reduced intensity conditioning (RIC) classification, and transplant conditioning intensity score [7, 8]. Myeloablative conditioning was defined as a regimen containing either total body irradiation with a dose greater than 6 Gy, a total dose of oral busulfan greater than 8 mg/kg, or a total dose of intravenous busulfan greater than 6.4 mg/kg. All other regimens were defined as RIC. The diagnosis and grading of acute and chronic GVHD were performed by transplant centers using standard criteria [9].

Statistical analysis

Quantitative variables are described as median, interquartile range (quartiles 1 and 3), and minimum/maximum values, while qualitative variables are reported as absolute numbers and percentages. Comparison between groups has been done using Wilcoxon tests for quantitative variables and chi-square test for qualitative variables or Fisher exact test in case of non-valid chi square test. To quantify the association of genes two-by-two, the Fisher exact test was performed, allowing to provide the exact probability of the observed contingency table under the hypothesis of independence between the two genes. The frequency of each somatic mutation was determined across the entire cohort of transplanted patients, irrespective of disease status at transplant, and reported as a percentage of patients carrying a given mutation among those with available molecular results (≥100 patients tested per mutation).

To evaluate associations among the 12 most frequently tested or mutated somatic mutations (defined as those with available mutation status in at least 800 patients and a mutation frequency of at least 5%), a multiple correspondence analysis (MCA) was performed (see Supplementary Appendix).

Post-transplant outcomes were assessed for the group of patients allografted in CR1. Follow-up was calculated using the reverse Kaplan–Meier method. The probabilities of OS and LFS were assessed using the Kaplan–Meier method, while cumulative incidence functions estimated RI and NRM in a competing-risk setting, where RI and NRM were mutually exclusive events. Acute and chronic GVHD outcomes were also analyzed using cumulative incidence functions, considering death and relapse as competing risks. Univariate comparisons were performed using the log-rank test for OS and LFS, and Gray's test for cumulative incidences. Multivariable models were performed using the Cox model to evaluate to impact of the groups of mutations adjusting on age at allo-HCT, de novo or secondary AML, ELN 2022 cytogenetic risk, donor type, myeloablative regimen and year of allo-HCT. Estimations from the models were provided as hazard ratio (HR) and their 95% confidence interval. The type-1 error rate was fixed at 0.05. All analyses were performed using R 4.1.1 (R Development Core Team, Vienna, Austria, URL:https://www.R-project.org/).

RESULTS

Frequency of somatic mutations in allografted AML patients

We collected data from 952 AML patients who underwent allo-HCT and had NGS performed at diagnosis. Patient characteristics are detailed in Supplementary Table 1. The majority had de novo AML (77%), with a median age of 55 years (range: 18–78), and 49% were male. Karyotype was normal in 416 patients (44%), abnormal in 490 (51%), and either failed or missing for (5%). Based on the ELN 2022 classification, cytogenetic abnormalities were categorized as favorable in 7%, intermediate in 68% (including 47% with diploid karyotype), and adverse in 25% of patients. At transplant, 76% were in CR1, 10% in CR2, and 12% had active disease.

The median number of sequenced genes included in the NGS panel was 40 (interquartile range (IQR) 23–48), with 6% of patients having fewer than 20 genes analyzed and 7% having more than 55. The most frequently detected mutations were *DNMT3A (24%), FLT3-ITD (21%), NPM1 (21%), RUNX1 (16%), NRAS (16%), TET2 (14%), IDH2 (12%), ASXL1 (11%), IDH1 (10%), SRSF2 (10%), KRAS (8%), WT1 (8%), NF1 (7%), TP53 (7%), PTPN11* (7%), *CEBPA (7%), STAG2 (7%), FLT3-TKD (6%), BCOR (5%) and BCORL1 (5%)* (Fig. 1a). The median number of somatic mutations per patient was 2 (IQR: 1–4), with 8% having no detected mutation and 28% harboring four or more. Similarly, the median number of mutated genes per patient was 2 (IQR: 1–3), with 23% having four or more mutated genes (Fig. 1b, Supplementary Table 1).

Among the 716 patients allografted in CR1, mutation frequencies were relatively consistent, with the most frequently detected being *FLT3*-ITD *(23%), DNMT3A (23%), NPM1 (20%), RUNX1 (17%), TET2 (14%), IDH2 (13%), NRAS (12%), SRSF2 (11%), ASXL1 (10%),*

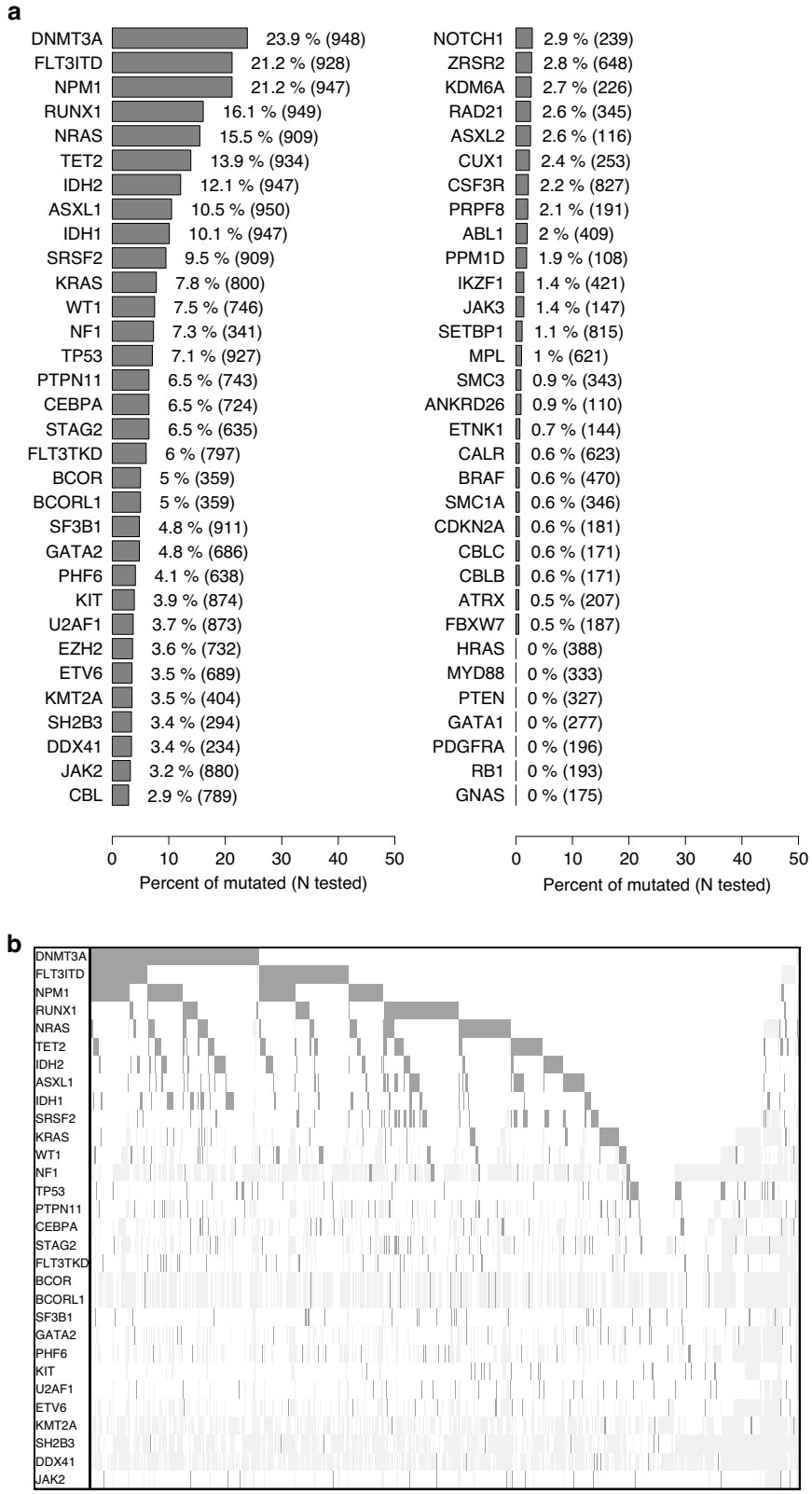

Fig. 1 Somatic mutations in allografted AML patients. a Frequency of somatic mutations in the entire cohort of 952 AML patients undergoing allo-HCT. The bars represent the percentage of patients harboring each mutation among those with available molecular data for the respective gene (between parenthesis). **b** Distribution of the number of detected somatic mutations per patient in the entire cohort. Each column represents a single patient, with the blue color indicating the presence of specific mutations.

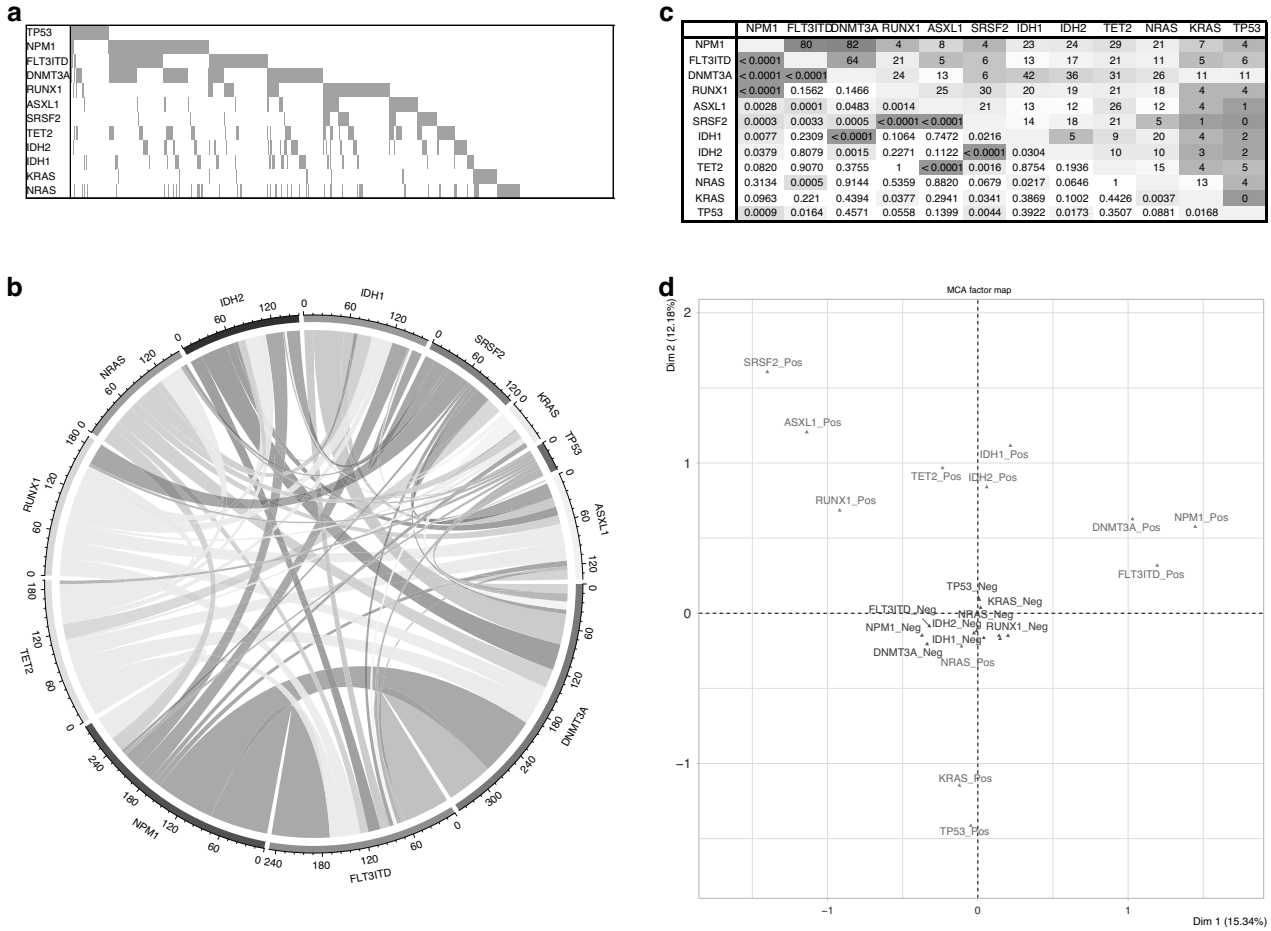

Fig. 2 Association of somatic mutations in allografted AML patients. a Waterfall plot illustrating the distribution of co-occurring mutations in the most frequently mutated genes (more than 5% positivity and selected genes tested for at least 800 patients) across individual patients ($n = 753$). Each column represents a single patient, with the blue color indicating the presence of specific mutations. **b** Circos plot showing the pairwise co-occurrence frequency of the most frequent somatic mutations. The width of connecting lines reflects the frequency of co-occurrence between each mutation pair. **c** Network diagram of positive and negative interactions among the most frequent somatic mutations. Blue shading indicates frequent co-occurrence, red shading indicates mutual exclusivity, and gold shading reflects the statistical significance (p value of the exact Fisher test) of the interaction. **d** Multiple Correspondence Analysis. Associations between the 12 most frequent somatic mutations.

IDH1 (10%), KRAS (7%), WT1 (7%), STAG2 (7%), TP53 (7%), CEBPA (6%), NF1 (6%), PTPN11 (5%), *FLT3-TKD (5%), SF3B1 (5%) and PHF6 (5%)* (Supplementary Fig. 1). The median number of somatic mutations per patient remained 2 (IQR: 1–4), with 9% having no detected mutation and 26% carrying four or more, while the median number of mutated genes per patient was also 2 (IQR: 1–3), with 21% having four or more (Supplementary Table 2).

Association of frequently mutated genes

We analyzed the co-occurrence and interactions of the most frequently mutated genes, present in more than 5% of cases and tested in at least 800 patients. The twelve selected genes included *NPM1*, *FLT3*-ITD, *DNMT3A*, *RUNX1*, *ASXL1*, *SRSF2*, *TET2*, *IDH1*, *IDH2*, *KRAS*, *NRAS* and *TP53* in 753 patients. The interactions between these genes are illustrated in Fig. 2, with a waterfall plot (Fig. 2a) showing the co-occurrence of mutations in individual patients, a circos plot (Fig. 2b) depicting the frequency of co-occurrence between mutation pairs, and a diagram (Fig. 2c) displaying positive (co-occurring) and negative (mutually exclusive) associations. *NPM1* mutations were positively associated with *FLT3*-ITD and *DNMT3A* ($p < 0.0001$ each), and, to a lesser extent, with *IDH1* ($p = 0.007$) and *IDH2* ($p = 0.037$), but negatively associated with *RUNX1* ($p < 0.0001$), *ASXL1* ($p = 0.002$), *SRSF2* ($p = 0.0003$), and *TP53* ($p = 0.0009$). *FLT3*-ITD was also positively linked to *DNMT3A* ($p < 0.0001$) but negatively linked to *ASXL1* ($p = 0.0001$), *SRSF2* ($p = 0.0033$), *NRAS* ($p = 0.0005$), and *TP53* ($p = 0.0164$). *DNMT3A* was also positively associated with *IDH1* ($p < 0.0001$) and *IDH2* ($p = 0.0015$), yet negatively associated with *ASXL1* ($p = 0.048$) and *SRSF2* ($p = 0.0005$). *RUNX1* mutations were also positively associated with *ASXL1* ($p = 0.0014$) and *SRSF2* ($p < 0.0001$) mutations, but negatively associated with *KRAS* ($p = 0.037$). *ASXL1* mutation was also positively associated with *SRSF2* ($p < 0.0001$) and *TET2* ($p < 0.0001$), while *SRSF2* was positively linked to *IDH1* ($p = 0.021$), *IDH2* ($p < 0.0001$), and *TET2* ($p = 0.0016$) mutations, but negatively associated with *KRAS* ($p = 0.034$) and *TP53* ($p = 0.0044$). *IDH1* was also positively associated with *NRAS* ($p = 0.02$) but negatively associated with *IDH2* ($p = 0.03$), while *IDH2* was negatively associated with *TP53* ($p = 0.017$). Lastly, *NRAS* was negatively associated with *KRAS* ($p = 0.0037$), and *KRAS* was negatively associated with *TP53* ($p = 0.016$) (Fig. 2c).

MCA identified three distinct groups of co-occurring mutations: the first group included *DNMT3A*, *NPM1*, and *FLT3-ITD*; the second consisted of *ASXL1*, *SRSF2*, and *RUNX1*, and the third comprised *IDH1, IDH2, and TET2* (Fig. 2d). Additionally, *TP53* and *KRAS* mutations occupied the same position due to their common

pattern of being predominantly associated with the absence of other somatic mutations; however, they were not found together in the same patients (Fig. 2c, d). Finally, *NRAS* did not present a specific co-occurrence pattern.

Effect of individual somatic mutations on post-transplant outcomes in CR1

Outcome analysis was performed on a subset of 646 AML patients allografted in CR1 with available follow-up data and molecular results for the ten genes identified through MCA: *DNMT3A, NPM1, FLT3*-ITD*, ASXL1, SRSF2, RUNX1, IDH1, IDH2, TET2, and TP53*. Patient characteristics are described in Table 1. Most patients had de novo AML (75%), while 25% had secondary AML, with a median age of 55 years (range: 19–75 years). The majority received primarily reduced intensity conditioning (57%) and peripheral blood stem cells (95%) from matched sibling (32%), matched unrelated (35%), and haploidentical (18%) donors. Based on the ELN 2022 classification, 4% had favorable-risk cytogenetics, 70% intermediate-risk, and 26% adverse-risk. At the time of transplant, MRD was positive in 129 patients (35%), and negative in 238 (65%), while 279 patients had no MRD data available. After a median follow-up of 3.1 years, the 2-year RI, NRM, LFS and OS for the whole cohort were 21%, 13%, 66%, and 73%, respectively.

We then evaluated the impact of individual somatic mutations on 2-year post-transplant outcomes (Table 2). *NPM1* mutation was associated with improved outcomes, showing higher 2-year LFS (74% vs 63%; $p = 0.02$) and OS (82% vs 71%; $p = 0.009$). Conversely, *TP53* mutation was linked to higher 2-year RI (42 vs 20%; $p < 0.001$) and lower 2-year LFS (35% vs 68%; $p < 0.001$) and reduced 2-year OS (47% vs 76%; $p < 0.001$). *FLT3*-ITD mutation was associated with lower NRM (10% vs 15%; $p = 0.04$) and improved OS (80% vs 71%; $p = 0.01$). *SRSF2* mutation was associated with a slight increase in 2-year RI (26% vs 21%; $p = 0.05$). Meanwhile, *IDH2* mutation was linked to lower 2-year RI (13% vs 23%; $p = 0.02$) and improved LFS (76% vs 64%; $p = 0.02$) and OS (83% vs 72%; $p = 0.02$). The effects of other individual mutations on post-transplant outcomes are detailed in Table 2.

Effect of groups of somatic mutations on post-transplant outcomes in CR1

As stated before, the MCA identified three distinct groups of co-occurring mutations: *NPM1/FLT3*-ITD*/DNMT3A*, *SRSF2/ASXL1/RUNX1*, and *IDH1/IDH2/TET2* (Fig. 2d). Because of the known impact of *NPM1*, the first group was split into two subgroups according to the presence or absence of *NPM1* mutation. In addition, we added a group of *TP53* mutation for its known negative impact and because in the MCA, *TP53* mutations were predominantly associated with the absence of other somatic mutations. Therefore, six non-overlapping groups were constructed: Group 1 comprised patients with *TP53* mutation regardless of other co-mutations ($N = 47$; 7%); Group 2 comprised patients with *NPM1* mutation and wild type *TP53* regardless of other co-mutations ($N = 129$; 20%); Group 3 comprised patients with *FLT3*-ITD and/or *DNMT3A* mutation, without meeting the criteria for previous groups ($N = 128$; 20%); Group 4 comprised patients with *RUNX1* and/or *ASXL1* and/or *SRSF2* mutation (SAR group) without meeting the criteria for previous groups ($N = 132$; 20%); Group 5 comprised patients with *IDH1* and/or *IDH2* and/or *TET2* mutation without meeting the criteria for previous groups ($N = 43$; 7%); and Group 6 comprised patients with all ten genes unmutated ($N = 167$; 26%). These groups differed significantly in median age (60, 53, 57, 59, 55 and 47 years, respectively; $p < 0.001$), the frequency of secondary AML (51%, 14%, 19%, 36%, 12% and 25%, respectively; $p < 0.001$), and the frequency of adverse cytogenetics (64%, 8%, 20%, 25%, 23% and 37%, respectively) (Table 1).

Moreover, the groups of somatic mutations significantly impacted the 2-year RI (42%, 17%, 21%, 18%, 13% and 24%, respectively; $p = 0.005$), LFS (35%, 74%, 68%, 71%, 71% and 61%, respectively; $p < 0.001$) and OS (47%, 82%, 74%, 81%, 76% and 67%, respectively; $p < 0.001$) whereas NRM was not significantly affected (Fig. 3).

Multivariable analysis

In the multivariable analysis (Table 3), compared to the Group 2 (*NPM1* mutation and wild-type *TP53*), RI, LFS, and OS were negatively affected by Group1 (*TP53* mutation; hazard ratio [HR] 2.6, 2.9, and 3.05, respectively, all $p < 0.001$) and OS was additionally negatively affected by Group 6 (all ten genes unmutated; HR 1.73, $p = 0.02$). NRM, LFS and OS were negatively affected by older age (HR 1.1, $p < 0.001$; 1.04, $p = 0.02$ and 1.05; $p = 0.02$, respectively). Finally, adverse karyotype negatively affected RI, LFS, and OS (HR 1.93, 1.64 and 1.64, respectively, all $p < 0.001$).

Characteristics and post-transplant outcomes of patients with *NPM1* mutation

We then analyzed the characteristics, distribution and impact of karyotype, *FLT3*-ITD, and MRD status in 129 *NPM1* mutated AML patients allografted in CR1 (Group 2) ($N = 4$). The patients' characteristics and associated mutations are described in Supplementary Table 3. Karyotype was intermediate in 108 (92%) patients, predominantly normal (96 (74%) patients), and adverse in 9 (8%) patients. MRD was positive in 47 (43%) patients, negative in 62 (57%) patients and missing in 20 patients. *FLT3*-ITD was present in 79 (61%) patients and negative in 48 (37%) patients. Patients with *FLT3*-ITD were transplanted earlier and were less likely to be MRD positive at transplant (33% versus 61%). In univariate analysis (Supplementary Table 4), karyotype did not significantly affect post-transplant survival. We compared survival outcomes based on pre-transplant MRD positivity (2-year LFS: 67% vs. 79%; 2-year OS: 75% vs. 84%) and *FLT3*-ITD status (2-year LFS: 70% vs. 78%; 2-year OS: 81% vs. 82%), though these differences did not reach statistical significance.

Effect of *SRSF2* and/or *ASXL1* and/or *RUNX1* mutation on post-transplant outcomes

Given the unexpectedly favorable outcomes of Group 4, we further analyzed the characteristics, distribution and impact of SAR mutations in the whole cohort of allografted AML patients in CR1, including those with *FLT3*-ITD, *NPM1*, *DNMT3A*, and *TP53* mutations. The patients' characteristics and associated mutations are described in Supplementary Table 5. According to ELN 2022, karyotype was favorable in 7 (4%) patients, intermediate in 131 (73%, normal in 47%) and adverse in 41 (23%) patients. The number of SAR mutations was 1 for 125 (66%) patients, 2 in 46 (24%) patients and 3 in 18 (10%) patients. Pre-transplant MRD was positive in 28 (28%) patients and negative in 72 (72%) patients (89 missing). The 2-year LFS and OS were 69% and 78% for all 189 SAR patients, and were non different to those of the 132 SAR patients belonging to Group 4. The remaining 57 SAR patients included six patients with concomitant *TP53* mutations (Group 1), 12 with concomitant *NPM1* mutations (Group 2), and 39 with concomitant *FLT3*-ITD and/or *DNMT3A* mutations (Group 3). In univariate analysis (Supplementary Table 6), post-transplant outcomes were not significantly affected by karyotype, pre-transplant MRD status and the number of SAR mutations. SAR mutations were associated with favorable post-transplant outcomes in our cohort, suggesting that their traditionally adverse-risk classification at diagnosis may not fully apply in the transplant setting.

Characteristics and post-transplant outcomes of patients with *TP53* mutation

The 2-yr LFS of 35% and 2-yr OS of 47% in *TP53* mutant patients are quite encouraging. We therefore analyzed the characteristics, distribution and impact of karyotype, type of AML, and *TP53*

Table 1. Patient characteristics of the cohort of AML patients allografted in CR1 with available data on follow-up and for the ten genes derived from the MCA results (*DNMT3A, NPM1, FLT3*-ITD, *ASXL1, SRSF2, RUNX1, IDH1, IDH2, TET2 and TP53*).

Variables	Modalities	All patients	Decuple wild type	IDH/ TET2	RUNX1 ASXL1 SRSF2	FLT3ITD DNMT3A	NPM1	TP53	P
		***N* = 646**	**167**	**43**	**132**	**128**	**129**	**47**	
Age at HCT	Median (range), years	55 (19–75)	47 (19–75)	55 (20–74)	60 (20–74)	57 (19–73)	53 (20–75)	60 (22–72)	<0.001
Year of HCT	Median (range)	2019 (2013–23)	2019 (2013–23)	2019 (2016–23)	2019 (2015–23)	2019 (2015–23)	2019 (2015–22)	2020 (2015–22)	0.1
Patient Sex	Male, *N* (%)	318 (49)	93 (56)	20 (47)	77 (58)	56 (44)	52 (40)	20 (43)	
Time to HCT	Median (range), mo.	4.7 (1–27)	4.5 (2–23)	5 (3–14)	4.4 (2–23)	4.5 (1–9)	5.1 (3–27)	5 (3–17)	0.001
MRD	Positive, *N* (%)	129 (35)	26 (35)	3 (16)	19 (30)	27 (34)	47 (43)	7 (30)	0.21
	Missing	279	93	24	69	49	20	24	
AML type	de novo, *N* (%)	486 (75)	125 (75)	38 (88)	85 (64)	104 (81)	111 (86)	23 (49)	<0.001
	Sec AML	160 (25)	42 (25)	5 (12)	47 (36)	24 (19)	18 (14)	24 (51)	
Cytogenetic ELN2022	Favorable	25 (4)	17 (11)	0 (0)	6 (5)	1 (1)	1 (1)	0 (0)	Not done
	Intermediate	421 (70)	84 (53)	31 (78)	88 (70)	94 (79)	108 (92)	16 (36)	
	Adverse	160 (26)	58 (36)	9 (22)	31 (25)	24 (20)	9 (7)	29 (64)	
	Missing	40	8	3	7	9	11	2	
Number of genes tested	Median (range)	40 (8–141)	39 (21–141)	40 (21–96)	40 (15–141)	40 (11–96)	36 (8–78)	40 (12–55)	0.12
Number of mutations	Median (range)	2 (0–12)	1 (0–7)	2 (1–8)	3 (1–10)	3 (0–9)	3 (1–10)	2 (1–12)	<0.001
Donor type	MSD	223 (34)	57 (34)	15 (35)	44 (34)	49 (38)	39 (31)	19 (40)	Not done
	Haplo	119 (18)	31 (19)	7 (16)	27 (21)	22 (17)	23 (18)	9 (19)	
	MUD	223 (35)	56 (33)	16 (37)	46 (35)	39 (30)	53 (41)	13 (28)	
	Others	80 (13)	23 (14)	5 (12)	14 (10)	18 (15)	14 (10)	6 (13)	
Source of cells	PB	611 (95)	156 (93)	40 (93)	129 (98)	120 (94)	122 (95)	44 (94)	Not done
	BM	30 (4)	8 (5)	3 (7)	2 (2)	8 (6)	5 (4)	3 (6)	
	Others	5 (1)	3 (2)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	
Myeloablativeconditioning	Yes	271 (43)	85 (53)	15 (39)	51 (39)	53 (42)	55 (43)	12 (26)	
	Missing	13	5	4	2	1	1	0	

N = 646 patients.
HCT Hematopoietic cell transplantation, *mo.* months, *MRD* measurable residual disease, *AML* Acute myeloid leukemia, *sec* secondary, *ELN* European LeukemiaNet, *MSD* Matched related donor, *Haplo* haploidentical donor, *MUD* matched unrelated donor, *PB* peripheral blood, *BM* bone marrow.

Table 2. Impact of individual somatic mutations on 2-year post-transplant outcomes for AML patients allografted in CR1.

Variable	Modality	2-year OS % [95% CI]	2-year LFS % [95% CI]	2-year RI % [95% CI]	2-year NRM % [95% CI]
NPM1	Negative	71 [66.6–74.9]	63.2 [58.7–67.4]	22.3 [18.6–26.1]	14.5 [11.5–17.8]
	Positive	82.4 [74.5–88.1]	73.9 [65.3–80.7]	17.6 [11.4–24.8]	8.5 [4.5–14.2]
	P value	0.009	0.02	0.15	0.14
TP53	Negative	75.5 [71.7–78.9]	68 [63.9–71.7]	19.6 [16.3–23]	12.4 [9.9–15.3]
	Positive	46.8 [31.8–60.5]	34.6 [21.3–48.4]	41.5 [26.9–55.5]	23.8 [12.6–37]
	P value	<0.0001	<0.0001	0.0002	0.09
DNMT3A	Negative	74.1 [69.8–77.9]	65.3 [60.7–69.5]	22 [18.3–26]	12.6 [9.8–15.8]
	Positive	70.9 [62.4–77.8]	66 [57.4–73.2]	18.6 [12.6–25.6]	15.4 [10–21.8]
	P value	0.99	0.82	0.33	0.46
FLT3-ITD	Negative	71.4 [66.9–75.4]	63.9 [59.3–68.2]	21.5 [17.8–25.5]	14.5 [11.5–17.9]
	Positive	79.8 [72.2–85.5]	70.2 [61.9–77]	20.3 [14.1–27.3]	9.5 [5.5–15]
	Missing *N* (%)	4 (1%)	4 (1%)	4 (1%)	4 (1%)
	P value	0.01	0.06	0.69	0.04
RUNX1	Negative	73.3 [69.1–77]	65.1 [60.7–69.2]	21.8 [18.2–25.6]	13.1 [10.3–16.2]
	Positive	73.8 [64.2–81.1]	66.8 [57–74.9]	18.8 [12–26.8]	14.4 [8.6–21.6]
	Missing *N* (%)	1 (0%)	1 (0%)	1 (0%)	1 (0%)
	P value	0.95	0.96	0.61	0.59
ASXL1	Negative	72.3 [68.3–75.9]	64.8 [60.6–68.7]	21.7 [18.3–25.3]	13.5 [10.8–16.5]
	Positive	83.5 [71.5–90.8]	71.1 [57.5–81]	17.6 [9–28.7]	11.3 [4.9–20.6]
	P value	0.39	0.82	0.78	0.9
SRSF2	Negative	73.3 [69.2–76.9]	65.8 [61.5–69.7]	21.6 [18.1–25.2]	12.6 [10–15.6]
	Positive	77 [64.1–85.7]	64.8 [51.1–75.5]	17.5 [8.9–28.5]	17.7 [9.4–28.2]
	Missing *N* (%)	16 (2%)	16 (2%)	16 (2%)	16 (2%)
	P value	0.84	0.69	0.43	0.17
IDH2	Negative	71.9 [67.7–75.6]	63.9 [59.6–67.8]	22.5 [19–26.2]	13.6 [10.9–16.7]
	Positive	83.2 [72.8–89.9]	75.7 [64.4–83.8]	13.2 [6.7–21.9]	11.2 [5.4–19.2]
	Missing *N* (%)	1 (0%)	1 (0%)	1 (0%)	1 (0%)
	P value	0.02	0.02	0.02	0.65
IDH1	Negative	74.3 [70.3–77.8]	66 [61.8–69.8]	20.7 [17.4–24.3]	13.3 [10.6–16.2]
	Positive	65.4 [51–76.5]	60.3 [45.9–72]	25.6 [14.7–37.9]	14.1 [6.5–24.6]
	Missing *N* (%)	2 (0%)	2 (0%)	2 (0%)	2 (0%)
	P value	0.11	0.28	0.72	0.27
TET2	Negative	72.7 [68.6–76.3]	65.4 [61.1–69.4]	21.6 [18.1–25.2]	13 [10.3–16.1]
	Positive	77.1 [66.3–84.8]	64.4 [52.7–73.8]	20.4 [12.2–30.1]	15.2 [8.5–23.7]
	Missing *N* (%)	3 (0%)	3 (0%)	3 (0%)	3 (0%)
	P value	0.86	0.94	0.41	0.38

OS overall survival, *LFS* leukemia-free survival, *RI* Relapse Incidence, *NRM* non-relapse mortality.

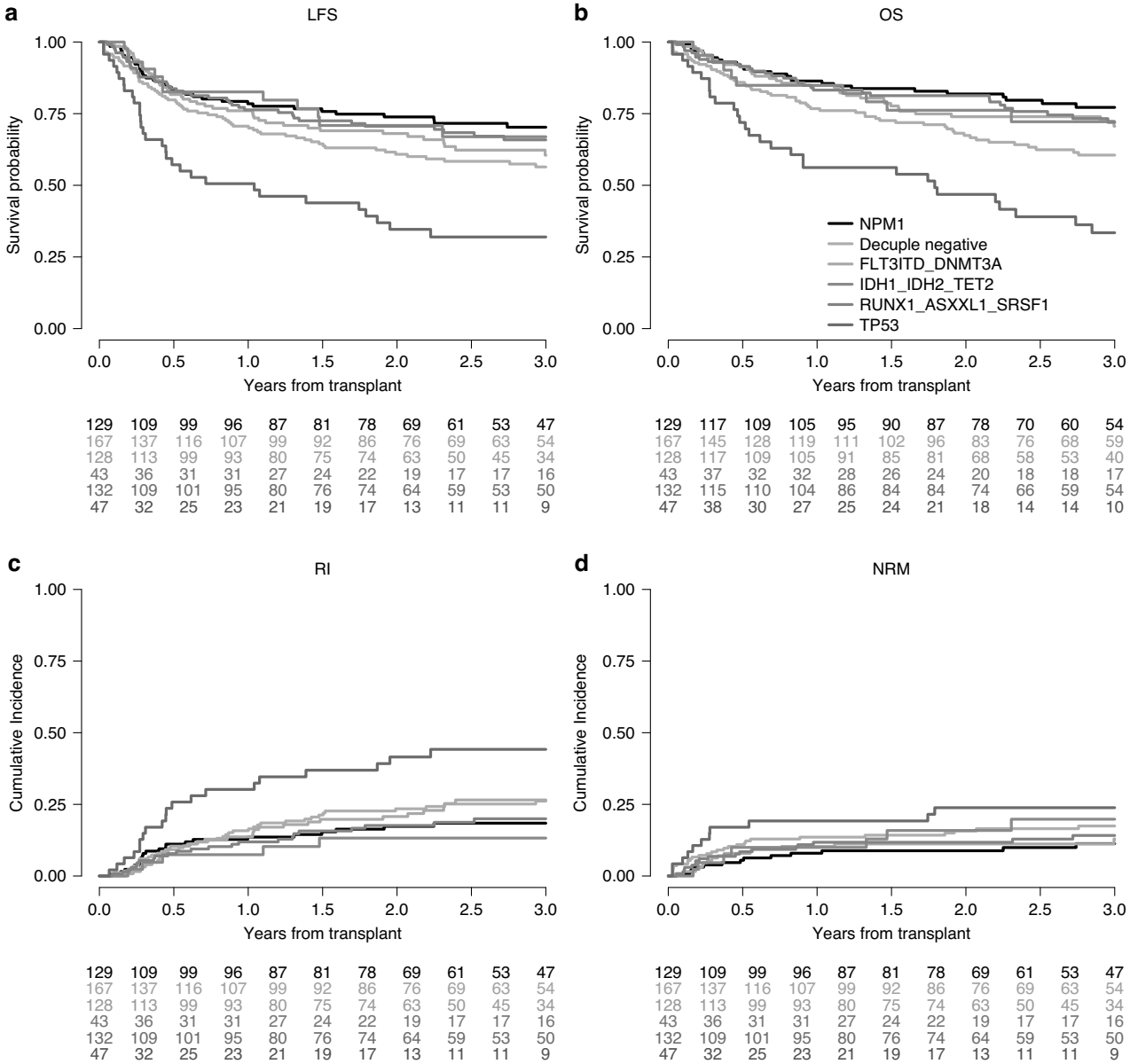

Fig. 3 Impact of somatic mutation groups on post-transplant outcomes for patients allografted in CR1. Kaplan–Meier curves illustrating the effect of the six somatic mutation groups on post-transplant outcomes. **a** Leukemia-free survival (LFS), **b** Overall survival (OS), **c** Relapse incidence (RI) at 2 years, **d** Non-relapse mortality (NRM).

variant allele frequency (VAF) in 47 *TP53* mutated AML patients (23 de novo and 24 secondary) allografted in CR1. The patients' characteristics and associated mutations are described in Supplementary Table 7. According to ELN2022, karyotype was intermediate in 16 (36%) patients and adverse in 29 (64%) patients including 17 (38%) patients with chromosome 17 abnormalities (missing, $N = 2$). Median VAF was 45.5 (IQR 31.2–50.8), missing VAF in 5 patients. In univariate analysis (Supplementary Table 8), outcomes of secondary vs de novo AML (2-year LFS: 31% vs. 39%; 2-year OS: 34% vs. 60%), were not significantly different. Conversely, adverse karyotype negatively affected post-transplant survival compared to intermediate karyotype (2-year LFS 22% vs 49%, $p = 0.03$; 2-year OS 34% vs 61%, $p = 0.03$). Finally, *TP53* mutation VAF above median (45–94) significantly increased the incidence of relapse (2-year RI 55% vs 24%, $p = 0.02$) but did not significantly affect post-transplant survival (2-year LFS 21% vs 47%; 2-year OS 36% vs 52%) in this small cohort. It is important to note that for 21 patients with *TP53* mutation VAF below median, karyotype was intermediate in 10 patients and adverse in 10 patients whereas for 21 patients with *TP53* mutation VAF above median, karyotype was intermediate in 4 patients and adverse in 16 patients. These results suggest better post-transplant outcomes for AML patients with *TP53* mutation in the absence of adverse karyotype or high *TP53* VAF.

Characteristics and post-transplant outcomes of patients lacking mutations in the 10 most frequently mutated genes

Finally, patients in the decuple-negative group, those lacking mutations in the ten most frequently mutated genes, exhibited inferior survival compared to other groups without *TP53* mutations, with a 2-year OS of 67%. This finding is notable given the absence of well-characterized driver mutations. We therefore analyzed the characteristics, distribution and impact of karyotype, type of AML, and pre-transplant MRD status in 167 decuple-negative AML patients (125 (75%) de novo and 42 (25%) secondary) allografted in CR1. The patients' characteristics and

Table 3. Multivariable analysis.

Variable	Modality	LFS		OS		RI		NRM	
		HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Group of mutations	NPM1	1		1		1		1	
	SRSF2/ASXL1/ RUNX1	1.16 (0.7–1.8)	0.51	1.13 (0.7–1.9)	0.62	1.11 (0.6–2)	0.72	1.17 (0.6–2.3)	0.66
	FLT3-ITD /DNMT3A	1.2 (0.8–1.9)	0.42	1.2 (0.7–2)	0.46	1.24 (0.7–2.2)	0.44	1.06 (0.5–2.2)	0.88
	IDH/TET2	1.05 (0.6–2)	0.87	1.17 (0.6–2.3)	0.66	0.8 (0.3–2)	0.63	1.48 (0.6–3.7)	0.4
	Decuple negative	1.49 (1–2.3)	0.06	1.73 (1.1–2.7)	0.02	1.29 (0.8–2.2)	0.35	1.8 (0.9–3.4)	0.08
	TP53	2.6 (1.6–4.3)	<0.001	2.90 (1.7–5)	<0.001	3.05 (1.6–5.8)	<0.001	2.07 (0.9–4.8)	0.09
Age at HCT (3 y increment)		1.04 (1–1.08)	0.02	1.05 (1–1.09)	0.02	1 (0.96–1)	0.88	1.11 (1.1–1.9)	<0.001
Type of AML	de novo	1		1		1			
	Sec AML	0.88 (0.7–1.2)	0.42	0.92 (0.7–1.3)	0.62	0.78 (0.5–1.2)	0.23	1.02 (0.6–1.6)	0.92
ELN 2022 cytogenetic	Fav/Int	1		1		1			
	Adverse	1.64 (1.2–2.2)	<0.001	1.64 (1.2–2.2)	<0.001	1.93 (1.4–2.8)	<0.001	1.28 (0.8–2)	0.29
Donor type	Matched relative	1		1		1			
	Mismatched relative	0.9 (0.6–1.3)	0.6	0.88 (0.6–1.3)	0.55	0.8 (0.5–1.3)	0.39	0.98 (0.6–1.7)	0.95
	Unrelated	0.96 (0.7–1.3)	0.76	0.9 (0.7–1.2)	0.51	1 (0.7–1.5)	1	0.87 (0.6–1.4)	0.54
Myelo-ablative regimen	No	1		1		1			
	Yes	1.03 (0.8–1.4)	0.85	0.99 (0.7–1.4)	0.97	1.03 (0.7–1.5)	0.87	1 (0.6–1.6)	1
Year of HCT (by 5 y increment)		0.98 (0.7–1.4)	0.93	1.13 (0.7–1.7)	0.54	0.93 (0.6–1.5)	0.77	0.98 (0.6–1.7)	0.94

LFS leukemia-free survival, *OS* overall survival, *RI* Relapse Incidence, *NRM* non-relapse mortality, *HR* Hazard ratio, *CI* Confidence interval, *HCT* hematopoietic cell transplantation, *y* year, *AML* acute myeloid leukemia, *Sec* secondary, *Fav* favorable, *Int* intermediate.

associated mutations are described in Supplementary Table 9. According to ELN2022, karyotype was favorable in 17 (11%) patients, intermediate in 84 (53%) and adverse in 58 (36%) patients. Pre-transplant MRD was positive in 26 (35%) patients, negative in 48 (65%) patients and missing in 93 patients. In univariate analysis (Supplementary Table 10), age negatively affected NRM, whereas pre-transplant MRD positivity numerically decreased survival. Adverse karyotype significantly increased relapse (2-year RI 35% compared to 12% for favorable and 18% for intermediate, $p = 0.02$) and accordingly numerically reduced survival. Surprisingly, in that group, post-transplant outcomes were better in secondary AML (2-year LFS 77% vs 56%, $p = 0.03$; 2-year OS 78% vs 64%, $p = 0.02$).

DISCUSSION

In this large, transplant-focused cohort of 952 AML patients with available diagnostic NGS data, we were able to delineate distinct groups of mutations with different prognostic implications, highlighting potential limitations in the applicability of the ELN 2022 risk classification in the allogeneic transplant setting [5]. Unlike most prior studies that evaluate the impact of individual mutations in isolation, our analysis uniquely assesses the effect of grouped mutational profiles, offering a more integrated view of molecular risk stratification, specifically in the context of allo-HCT [10–14]. Despite a highly selected population undergoing allo-HCT, where the majority of patients had de novo AML with mainly diploid intermediate-risk cytogenetics, and transplanted in first remission, we observed that the most frequently mutated genes included *DNMT3A*, *FLT3*-ITD, and *NPM1*, consistent with prior genomic studies that analyzed mutation frequency at diagnosis [2, 15]. Notably, 23% of patients harbored four or more somatic mutations, with a median of two mutations per patient, highlighting the complex clonal architecture at diagnosis, even among those achieving remission prior to allo-HCT. This genetic heterogeneity was further explored through co-mutation analysis of the 12 commonly altered genes, revealing distinct co-occurrence and exclusivity patterns which are biologically plausible: for example, *NPM1* was strongly associated with *FLT3*-ITD and *DNMT3A*, a well-established mutational triad, and was less likely to occur with adverse-risk genes like *TP53*, *RUNX1*, ASXL1 and *SRSF2*. In contrast, mutations in epigenetic and splicing regulators (*ASXL1*, *SRSF2*, *RUNX1*) formed a separate cluster, frequently co-mutating with one another but largely excluding *FLT3*-ITD and *NPM1* [16–18]. This multidimensional clustering by MCA supports the presence of biologically coherent subgroups that may inform prognostic modeling. Six genetically defined subgroups were constructed based on recurrent co-mutation patterns and the established prognostic roles of *TP53* and *NPM1*. These subgroups had different clinical and biological features, including age, secondary AML, cytogenetics, and MRD status. Importantly, they showed significantly different post-transplant survival outcomes. Basically, there

were three prognostic categories according to somatic mutations: *TP53* mutation (poor risk), no mutation (intermediate risk), and others (good risk). As expected, *TP53*-mutated patients (Group 1) had the worst outcomes among all groups, with a 2-year RI of 42% and OS of 47%, but notably superior to previously reported benchmarks [19–23]. A meta-analysis reported a pooled 2-year OS of 30% for 297 *TP53*-mutated AML patients undergoing allo-HCT [24]. The relatively improved outcomes in our *TP53* cohort may reflect the fact that only 45% of patients with mutant *TP53* harbored either 17p chromosomal abnormalities or complex karyotype, both of which are strongly linked to poor outcomes. In a recent large cohort from the EBMT, *TP53*-mutated AML patients lacking both 17p abnormalities and complex karyotype had a markedly improved 2-year OS of 65.2% [25]. Overall, our results suggest excellent post-transplant outcomes for AML patients with TP53 mutation in the absence of adverse karyotype or high *TP53* VAF. However, the prognosis observed in our study may not represent the entire *TP53*-mutant population at diagnoses, since a significant proportion of *TP53*-mutated patients may fail to achieve remission or proceed to transplant.

Conversely, *NPM1*-mutated patients with *TP53* wild-type status (Group 2) had excellent outcomes, with a 2-year OS and LFS of 82% and 74%, respectively, in line with prior data demonstrating sensitivity of this subgroup to intensive chemotherapy and allo-HCT [26, 27]. In this group, outcomes were not significantly affected by karyotype, pre-transplant MRD status or the presence of *FLT3*-ITD, suggesting that other factors may contributed to post-transplant prognosis in this subgroup.

Notably, *FLT3*-ITD mutation was paradoxically linked to reduced NRM and improved 2-year OS (80%) in our cohort, suggesting that *FLT3*-ITD mutant AML may be transitioning toward a more favorable-risk category when managed with a total therapy approach, incorporating allo-HCT in CR1 along with pre- and post-transplant FLT3 inhibitor therapies [28–34].

Interestingly, beyond the negative prognostic role of *TP53* mutation, there was no significant difference in post-transplant outcomes in CR1 between the other groups of somatic mutations. Notably, patients classified as group 4, harboring *SRSF2*, *ASXL1*, and/or *RUNX1* mutations (SAR mutations), in the absence of *TP53*, *FLT3*-ITD, *DNMT3A*, or *NPM1*, had unexpectedly favorable post-transplant outcomes, with a 2-year OS of 81% and LFS of 71%. These results persisted despite the group's older median age, higher frequency of secondary AML, and enrichment for adverse cytogenetics. Historically, SAR mutations are considered adverse-risk in the ELN classification [5, 35], based on their poor response to chemotherapy and association with clonal hematopoiesis and secondary AML [16–18, 36]. However, our data suggest that in the transplant setting, their adverse impact may be abrogated, possibly through enhanced sensitivity to graft-versus-leukemia effects. When extended to the entire CR1 cohort with SAR mutations, regardless of additional co-mutations, we confirmed a similarly favorable outcome profile (2-year OS 78%, LFS 70%). One possible explanation for the favorable outcomes observed in this group is that patients with these mutations typically exhibit low response rates to conventional chemotherapy, often less than 50% [16–18]. Therefore, those who achieved CR1 and proceeded to allo-HCT may represent a biologically selected group with inherently better disease control or chemosensitivity, translating into superior post-transplant outcomes. Additionally, the lack of an adverse prognostic impact of our SAR group may be dominated by the *RUNX1*-associated risk, as a *RUNX1*-only mutation was present in 38% of patients in this subgroup. The existing literature shows that patients harboring isolated *RUNX1* mutations without co-occurring mutations in the four most frequently mutated splicing genes (*SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*) have outcomes comparable to the intermediate-risk group, with a reported 5-year OS of 44% [37]. In contrast, the co-occurrence of *RUNX1* and *SRSF2* or *ASXL1* and *SRSF2* mutations, both of which have been independently associated with significantly inferior survival in prior studies, accounted for only 8% and 11% of our cohort, respectively [2, 16, 37]. This finding may partially explain the unexpectedly favorable outcomes observed in our SAR group.

Finally, patients in the decuple-negative group, those lacking mutations in the ten most frequently mutated genes, exhibited inferior survival compared to other groups without *TP53* mutations, with a 2-year OS of 67%. This finding is notable given the absence of well-characterized driver mutations. Interestingly, a substantial proportion (40%) of these patients harbored either *KRAS*, *NRAS*, or *PTPN11* mutations, genes often associated with proliferation signaling, chemoresistance, and suboptimal post-transplant outcomes [11, 38–40]. Furthermore, 37% of them had adverse cytogenetics, a rate significantly higher than that observed in the other molecular subgroups (8%-25%) excluding those with *TP53* mutations. These findings may have contributed to their poorer prognosis post-transplant.

This study has several limitations due to its retrospective nature. The absence of centralized NGS testing across centers likely resulted in variations in NGS platforms, library preparation protocols, and data analysis pipelines. Moreover, there were some missing genetic data, as many genes were not systematically tested across the cohort. VAF data were also unavailable for the majority of the genes tested, limiting the ability to assess the clonal burden at diagnosis and its potential prognostic value. Most importantly, it is critical to mention that included patients were primarily from centers performing NGS at diagnosis, many of which were high-expertise or high-volume centers. MRD data pre-transplant were also missing in 43% of the overall cohort. However, this was not uniform as in cases with *NPM1* mutations, MRD data were missing in only 16%, while in patients with decuple-negative, MRD was missing in 55% of cases, suggesting that MRD data availability was dependent on the mutational profile where MRD techniques actually exist. Finally, the lack of data on the induction and consolidation treatments received before transplant, and the lack of a control group of non-transplanted patients, preclude the direct assessment of the benefit of allo-HCT versus chemotherapy alone for the different groups of mutational profiles. Finally, we lacked information on pre-transplant NGS testing, including data on mutation clearance. This is particularly relevant for high-risk mutations such as *TP53*, where emerging evidence suggests that pre-transplant mutation clearance may be associated with improved outcomes. The absence of these data limits our ability to fully assess the dynamic prognostic value of certain mutations.

CONCLUSION

NGS at diagnosis can be extremely useful in risk stratification of AML patients undergoing allo-HCT, potentially allowing adequate post-transplant interventions. Surprisingly, despite their older age and higher frequency of secondary AML and adverse cytogenetics, the excellent outcomes (2-year LFS 71%, OS 81%) were observed for patients harboring *SRSF2* and/or *ASXL1* and/or *RUNX1* in the absence of *FLT3*-ITD or *NPM1*, *DNMT3A* and *TP53* mutation, indicating that allo-HCT in CR1 can overcome the adverse-risk associated with these somatic mutations at diagnosis. Together, these results argue for a revised transplant-specific risk model with better post-transplant prognostication. In particular, the data challenge the assumption that *FLT3*-ITD and SAR mutations uniformly confer adverse prognosis, and raise the possibility that certain mutational contexts, historically deemed high-risk, may derive substantial benefit from allo-HCT. Importantly, our study evaluates the impact of mutations specifically in patients who underwent allo-HCT, and therefore does not capture the full clinical impact of these mutations at diagnosis, particularly for patients who did not reach transplant, including those with chemo-resistant disease or early treatment failure. Thus, while our

data challenge the assumption that mutations such as *FLT3*-ITD or SAR mutations uniformly confer poor prognosis, these conclusions apply only to the transplant setting. Prospective studies are warranted to validate these observations and explore the mechanistic basis of transplant sensitivity in these subgroups.

DATA AVAILABILITY

The data analyzed in this study were provided and approved by the ALWP of the EBMT. All relevant data supporting the findings of this study are available within the Article and the Supplementary Material. Requests for access to EBMT study data from qualified external researchers will be reviewed by the relevant working party in accordance with EBMT's data-sharing policies. This process ensures the protection of patient privacy, maintains data security and integrity, and promotes scientific and medical innovation. Additional details on data-sharing criteria and the request process can be obtained by contacting ebmt.do-paris@ebmt.org. Individual patient-level data will not be shared.

REFERENCES

1. DiNardo CD, Erba HP, Freeman SD, Wei AH. Acute myeloid leukaemia. Lancet. 2023;401:2073–86. https://doi.org/10.1016/s0140-6736(23)00108-3.
2. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374:2209–21. https://doi.org/10.1056/NEJMoa1516192.
3. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116:354–65. https://doi.org/10.1182/blood-2009-11-254441.
4. Romer-Seibert JS, Meyer SE. Genetic heterogeneity and clonal evolution in acute myeloid leukemia. Current Opin Hematol. 2021;28:64–70. https://doi.org/10.1097/moh.0000000000000626.
5. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140:1345–77. https://doi.org/10.1182/blood.2022016867.
6. Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. Blood. 2016;127:62–70. https://doi.org/10.1182/blood-2015-07-604546.
7. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biology Blood Marrow Transplant. 2009;15:1628–33. https://doi.org/10.1016/j.bbmt.2009.07.004.
8. Spyridonidis A, Labopin M, Savani BN, Niittyvuopio R, Blaise D, Craddock C, et al. Redefining and measuring transplant conditioning intensity in current era: a study in acute myeloid leukemia patients. Bone Marrow Transpl. 2020;55:1114–25. https://doi.org/10.1038/s41409-020-0803-y.
9. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304. https://doi.org/10.1097/00007890-197410000-00001.
10. Luskin MR, Carroll M, Lieberman D, Morrissette JJD, Zhao J, Crisalli L, et al. Clinical utility of next-generation sequencing for oncogenic mutations in patients with acute myeloid leukemia undergoing allogeneic stem cell transplantation. Biology Blood Marrow Transplant. 2016;22:1961–7. https://doi.org/10.1016/j.bbmt.2016.07.018.
11. Yoshizato T, Nannya Y, Atsuta Y, Shiozawa Y, Iijima-Yamashita Y, Yoshida K, et al. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. Blood. 2017;129:2347–58. https://doi.org/10.1182/blood-2016-12-754796.
12. Ahn JS, Kim HJ, Kim YK, Lee SS, Jung SH, Yang DH, et al. DNMT3A R882 mutation with FLT3-ITD positivity is an extremely poor prognostic factor in patients with normal-karyotype acute myeloid leukemia after allogeneic hematopoietic cell transplantation. Biology Blood Marrow Transplant. 2016;22:61–70. https://doi.org/10.1016/j.bbmt.2015.07.030.
13. Mohty R, Bazarbachi AH, Labopin M, Esteve J, Kröger N, Cornelissen JJ, et al. Isocitrate dehydrogenase (IDH) 1 and 2 mutations predict better outcome in patients with acute myeloid leukemia undergoing allogeneic hematopoietic cell transplantation: a study of the ALWP of the EBMT. Bone Marrow Transpl. 2024;59:1534–41. https://doi.org/10.1038/s41409-024-02384-2.
14. Abou Dalle I, Galimard J-E, Poire X, Huynh A, Wagner Drouet E, Burns D, et al. The impact of DNMT3A mutation on survival of AML patients receiving allogeneic hematopoietic cell transplantation in first remission depends on the karyotype and co-occurring mutations: on Behalf of the EBMT Acute Leukemia Working Party. Blood. 2023;142:658. https://doi.org/10.1182/blood-2023-179531.
15. Patel JP, Gönen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366:1079–89. https://doi.org/10.1056/NEJMoa1112304.
16. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. Leukemia. 2016;30:2160–8. https://doi.org/10.1038/leu.2016.126.
17. Mendler JH, Maharry K, Radmacher MD, Mrózek K, Becker H, Metzeler KH, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with cytogenetically normal acute myeloid leukemia and with distinct gene and MicroRNA expression signatures. J Clin Oncol. 2012;30:3109–18. https://doi.org/10.1200/jco.2011.40.6652.
18. Paschka P, Schlenk RF, Gaidzik VI, Herzig JK, Aulitzky T, Bullinger L, et al. ASXL1 mutations in younger adult patients with acute myeloid leukemia: a study by the German-Austrian Acute Myeloid Leukemia Study Group. Haematologica. 2015;100:324–30. https://doi.org/10.3324/haematol.2014.114157.
19. Weinberg OK, Siddon A, Madanat YF, Gagan J, Arber DA, Dal Cin P, et al. TP53 mutation defines a unique subgroup within complex karyotype de novo and therapy-related MDS/AML. Blood Adv. 2022;6:2847–53. https://doi.org/10.1182/bloodadvances.2021006239.
20. Middeke JM, Herold S, Rücker-Braun E, Berdel WE, Stelljes M, Kaufmann M, et al. TP53 mutation in patients with high-risk acute myeloid leukaemia treated with allogeneic haematopoietic stem cell transplantation. Brit J Haematol. 2016;172:914–22. https://doi.org/10.1111/bjh.13912.
21. Zhao D, Zarif M, Zhou Q, Capo-Chichi JM, Schuh A, Minden MD, et al. TP53 mutations in AML patients are associated with dismal clinical outcome irrespective of frontline induction regimen and allogeneic hematopoietic cell transplantation. Cancers. 2023;15:3210 https://doi.org/10.3390/cancers15123210.
22. Murdock HM, Kim HT, Denlinger N, Vachhani P, Hambley B, Manning BS, et al. Impact of diagnostic genetics on remission MRD and transplantation outcomes in older patients with AML. Blood. 2022;139:3546–57. https://doi.org/10.1182/blood.2021014520.
23. Nawas MT, Kosuri S. Utility or futility? A contemporary approach to allogeneic hematopoietic cell transplantation for TP53-mutated MDS/AML. Blood Adv. 2024;8:553–61. https://doi.org/10.1182/bloodadvances.2023010417.
24. Shahzad M, Tariq E, Chaudhary SG, Anwar I, Iqbal Q, Fatima H, et al. Outcomes with allogeneic hematopoietic stem cell transplantation in TP53-mutated acute myeloid leukemia: a systematic review and meta-analysis. Leukemia lymphoma. 2022;63:3409–17. https://doi.org/10.1080/10428194.2022.2123228.
25. Loke J, Labopin M, Craddock C, Cornelissen JJ, Labussière-Wallet H, Wagner-Drouet EM, et al. Additional cytogenetic features determine outcome in patients allografted for TP53 mutant acute myeloid leukemia. Cancer. 2022;128:2922–31. https://doi.org/10.1002/cncr.34268.
26. Nagler A, Labopin M, Salmenniemi U, Wu D, Blaise D, Rambaldi A, et al. Trends in allogeneic transplantation for favorable risk acute myeloid leukemia in first remission: a longitudinal study of >15 years from the ALWP of the EBMT. Bone Marrow Transpl. 2024;59:1563–76. https://doi.org/10.1038/s41409-024-02379-z.
27. Othman J, Potter N, Ivey A, Tazi Y, Papaemmanuil E, Jovanovic J, et al. Molecular, clinical, and therapeutic determinants of outcome in NPM1-mutated AML. Blood. 2024;144:714–28. https://doi.org/10.1182/blood.2024024310.
28. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med. 2017;377:454–64. https://doi.org/10.1056/NEJMoa1614359.
29. Bazarbachi A, Labopin M, Battipaglia G, Djabali A, Forcade E, Arcese W, et al. Allogeneic stem cell transplantation for FLT3-mutated acute myeloid leukemia: in vivo T-cell depletion and posttransplant sorafenib maintenance improve survival. A retrospective acute leukemia Working Party-European Society for Blood and Marrow Transplant Study. Clin Hematol Int. 2019;1:58–74. https://doi.org/10.2991/chi.d.190310.001.
30. Burchert A, Bug G, Fritz LV, Finke J, Stelljes M, Röllig C, et al. Sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with FLT3-internal tandem duplication mutation (SORMAIN). J Clin Oncol. 2020;38:2993–3002. https://doi.org/10.1200/jco.19.03345.
31. Abou Dalle I, El Cheikh J, Bazarbachi A. Pharmacologic strategies for post-transplant maintenance in acute myeloid leukemia: it is time to consider!. Cancers. 2022;14:1490 https://doi.org/10.3390/cancers14061490.
32. Stone RM, Yin J, Mandrekar SJ, Benner A, Saadati M, Galinsky IA, et al. 10 year follow-up of CALGB 10603/ratify: midostaurin versus placebo plus intensive chemotherapy in newly diagnosed FLT3 mutant acute myeloid leukemia patients aged 18-60 years. Blood. 2024;144:218. https://doi.org/10.1182/blood-2024-201058.
33. Bazarbachi A, Labopin M, Gedde-Dahl T, Remenyi P, Forcade E, Kröger N, et al. Improved posttransplant outcomes in recent years for AML patients with FLT3-ITD

and wild-type NPM1: a report from the EBMT Acute Leukemia Working Party. Clinical Cancer Res. 2023;29:4441–8. https://doi.org/10.1158/1078-0432.ccr-23-0954.
34. Pandya BJ, Burns LJ, Wang T, Xie B, Touya M, Spalding J, et al. Clinical outcomes and treatment patterns in adults with FLT3-ITD(mut+) acute myeloid leukemia undergoing allogeneic hemopoietic cell transplantation in the United States and Canada. Transplant Cell Ther. 2024;30:683.e681–683.e613. https://doi.org/10.1016/j.jtct.2024.04.016.
35. Gardin C, Pautas C, Fournier E, Itzykson R, Lemasle E, Bourhis JH, et al. Added prognostic value of secondary AML-like gene mutations in ELN intermediate-risk older AML: ALFA-1200 study results. Blood Adv. 2020;4:1942–9. https://doi.org/10.1182/bloodadvances.2019001349.
36. Lindsley RC, Mar BG, Mazzola E, Grauman PV, Shareef S, Allen SL, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood. 2015;125:1367–76. https://doi.org/10.1182/blood-2014-11-610543.
37. van der Werf I, Wojtuszkiewicz A, Meggendorfer M, Hutter S, Baer C, Heymans M, et al. Splicing factor gene mutations in acute myeloid leukemia offer additive value if incorporated in current risk classification. Blood Adv. 2021;5:3254–65. https://doi.org/10.1182/bloodadvances.2021004556.
38. Alawieh D, Cysique-Foinlan L, Willekens C, Renneville A. RAS mutations in myeloid malignancies: revisiting old questions with novel insights and therapeutic perspectives. Blood Cancer J. 2024;14:72. https://doi.org/10.1038/s41408-024-01054-2.
39. Fobare S, Kohlschmidt J, Ozer HG, Mrózek K, Nicolet D, Mims AS, et al. Molecular, clinical, and prognostic implications of PTPN11 mutations in acute myeloid leukemia. Blood Adv. 2022;6:1371–80. https://doi.org/10.1182/bloodadvances.2021006242.
40. Heuser M, Gabdoulline R, Löffeld P, Dobbernack V, Kreimeyer H, Pankratz M, et al. Individual outcome prediction for myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia from MDS after allogeneic hematopoietic cell transplantation. Ann Hematol. 2017;96:1361–72. https://doi.org/10.1007/s00277-017-3027-5.

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AUTHOR CONTRIBUTIONS

ABa proposed the study, interpreted the data, and wrote the manuscript. J-EG and MMo participated in study design, data interpretation, and manuscript editing. J-EG performed the statistical analysis. IAD and MLa contributed to data interpretation and manuscript revision. JS, HH, JM, CS, BL, LG, JMa, MI-R, AK, M-PG-H, GB, J-MR, AG, CSc, MK, XP, PC, MJC, FB, CC, EB, AN, and FC collected and updated patient data, and critically reviewed and approved the manuscript. All authors reviewed the final version, approved the data presented, and agreed with the conclusions of the work.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This is a retrospective, registry-based, multicenter study utilizing patient data collected and approved by the Acute Leukemia Working Party (ALWP) of the EBMT. The EBMT is a collaborative network comprising more than 600 transplant centers that are required to report all consecutive HCTs and subsequent follow-ups on an annual basis. Routine audits are performed to ensure data accuracy and completeness. Since January 2003, all participating transplant centers have been required to obtain written informed consent from patients prior to data registration with the EBMT, in accordance with the ethical principles outlined in the Declaration of Helsinki (1975).

ADDITIONAL INFORMATION

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