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blood advances

Pretransplant MRD does not seem to affect survival in *NPM1*-mutated AML undergoing allogeneic stem cell transplantation

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Key Points

- Pre–allo-SCT MRD positivity and dynamics in patients with *NPM1*^{mut} AML do not significantly affect posttransplant survival or relapse rates.
- Maintenance therapy significantly improves leukemia-free survival after allo-SCT in *NPM1*^{mut} AML.

Whether patients with acute myeloid leukemia (AML) harboring nucleophosmin mutations (*NPM1*^{mut}) and measurable residual disease (MRD) should undergo allogeneic stem cell transplantation (allo-SCT) in complete remission (CR) remains debatable. This study assessed whether bone marrow (BM) *NPM1*^{mut} MRD, detected via quantitative reverse transcription polymerase chain reaction (qRT-PCR) with 10^{-5} sensitivity, influences allo-SCT benefit. Data from 4 German transplantation centers included 174 patients with AML *NPM1*^{mut} who underwent first allo-SCT between 2011 and 2022. Among 122 patients transplanted in CR, pre–allo-SCT MRD was positive in 54%. After allo-SCT, BM MRD negativity increased from 65% (day +30) to 73% (day +100), with *FMS-like tyrosine kinase 3*–internal tandem duplication and ELN risk profile affecting MRD conversion at day +30. No significant difference in leukemia-free survival (LFS) or overall survival (OS) was observed based on pretransplant MRD (3-year LFS MRD positive [MRD$^+$], 60% vs MRD negative [MRD$^-$], 74%; hazard ratio [HR], 1.5; $P = .28$; 3-year OS MRD$^+$, 68% vs MRD$^-$, 78%; HR, 1.42; $P = .39$). MRD persistence and molecular relapse outcomes did not differ ($P = .8$). Adverse molecular risk (HR, 4.69; $P = .003$) and relapsed/refractory disease (HR, 2.83/3.59; $P = .005/0.001$) predicted poor prognosis, while posttransplant maintenance improved survival (HR, 0.48; $P = .06$). Our findings suggest that in patients with *NPM1*^{mut} AML MRD positivity at transplant, as assessed by qRT-PCR do not experience worse posttransplant outcomes.

Introduction

The distinct biological and clinical features of acute myeloid leukemias (AMLs) with nucleophosmin-1 (*NPM1*^{mut}) gene mutations position them as an individual molecular subgroup within the World Health Organization classification.[1,2] Despite their classification as largely favorable or intermediate-risk disease according to European LeukemiaNet (ELN) criteria, allogeneic stem cell transplantation (allo-SCT) is recommended in relapse, for primary refractory disease, or as consolidation therapy in first complete remission (CR1), especially when *FMS-like tyrosine kinase 3* (*FLT3*)–internal tandem

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The full-text version of this article contains a data supplement.

duplication (ITD) mutation co-occurs.[3-5] However, the potential benefit of allo-SCT in patients with *NPM1*^{mut} AML, characterized by low *FLT3*-ITD allelic burden or poor measurable residual disease (MRD) response, remains a subject of debate.[6-9]

The impact of pretransplant MRD on the outcome of allo-SCT has widely been reported, indicating a decline in prognosis upon transplantation if positive.[10-13] In acute lymphoblastic leukemia and chronic myeloid leukemia, MRD has been established to guide treatment decisions such as transplant indication[14,15]; however, in AML, handling and impact of MRD positivity before allo-SCT remains uncertain.

Most studies in AML have used flow cytometry (FCM) for MRD assessment. Yet, the reported effect might vary according to the genetic and immunophenotypic diversity of the disease, transplant setting, and MRD technology used. The recommended method for MRD assessment in patients harboring a *NPM1*^{mut} is quantitative reverse transcription polymerase chain reaction (qRT-PCR), which affords a sensitivity up to 10^{-5}.[16] Several studies have demonstrated a good correlation between *NPM1*^{mut} MRD measured in the peripheral blood (PB) and bone marrow (BM) at different time points during treatment and outcome, mainly focusing on the decision whether to proceed to allo-SCT when MRD is negative after induction.[16-18] Thus far, delaying allo-SCT to attain MRD negativity is not recommended.[4,19,20] However, tailoring conditioning therapy, donor selection, and graft-versus-host disease (GVHD) prophylaxis in allo-SCT may help counteract the adverse effects associated with MRD positivity.[21]

Here, we aim to determine the prognostic impact of *NPM1*^{mut} presence before allo-SCT on outcomes.

Patients and methods

Eligibility criteria and data collection

Data for this retrospective multicenter study were retrieved from members of the "Arbeitsgemeinschaft Knochenmark- und Stammzell-Transplantation" (Bone Marrow and Blood Cell Transplant Working Party), Munich, Germany, a nonprofit scientific society representing 4 transplant centers in the Munich area: University Hospital of the Ludwig Maximilian University Munich, School of Medicine of the Technical University Munich, University Medical Center Augsburg, and Munich Clinic Schwabing.

All adult patients with newly diagnosed *NPM1*^{mut} AML who received a first allo-SCT between January 2011 and January 2022 were included in the study and retrospectively analyzed to assess the impact of MRD, measured within 4 weeks before allo-SCT. Patients with exclusive extramedullary involvement were excluded. Conditioning regimens and GVHD prophylaxis strategies were administered according to the discretion of the treating physician. There were no restrictions regarding donor type or disease status at time of allo-SCT. MRD monitoring had to be performed using qRT-PCR, excluding patients who had MRD assessed and monitored by next-generation sequencing (NGS) or FCM. A total of 174 patients had BM or PB samples analyzed through qRT-PCR. Based on their morphological and molecular remission status before start of conditioning, patients were categorized into those with active disease, those in CR with presence of *NPM1*^{mut} (CR MRD positive [MRD^{+}]), and those in CR without presence of *NPM1*^{mut} (CR MRD negative [MRD^{-}]) for further analysis (see Figure 1).

This study received approval from the Ludwig Maximilian University ethics committee (no. 23-0774) and was conducted in compliance with German legislation and the Declaration of Helsinki. All procedures involving human participants were in accordance with the institutional ethical standards and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Quantification of *NPM1* transcript levels by qRT-PCR

MRD was measured by either institutional assays or in third-party laboratories. BM with or without concordant PB *NPM1* mutational status was assessed serially, that is, at first diagnosis, after induction therapy and within 4 weeks before allo-SCT by qRT-PCR of RNA as previously described[22] at a sensitivity level of 10^{-5}. After allo-SCT, samples were regularly examined at day 28, 60, 100, and 180, and at 1 year via BM aspiration with/without PB. Thereafter PB continued to be analyzed for the reappearance of *NPM1* MRD at 2 to 6 months intervals.

Definitions

Genetic aberrations were classified according to the ELN guidelines.[3,4,23] Cytogenetic risk was defined according to the Medical Research Council classification.[24] Molecular relapse was diagnosed if there were 2 consecutive positive samples showing increasing transcript levels in a patient who had previously tested MRD negative in a technically adequate sample, or an increase of $\geq$1 $\log_{10}$ between 2 samples collected from the same tissue, consistent with ELN guidelines.[7] Relapse was defined as BM blast counts of >5%, extramedullary manifestation, or the recurrence of leukemic blasts in the PB. Refractory disease was defined as primary induction failure with patients not achieving CR after receiving 1 course of induction therapy or relapse, refractory to standard treatment. CR with incomplete remission was defined according to standard criteria: <5% blasts, incomplete recovery of neutrophils (<1000/μL) and/or platelets (<100 000/μL). Acute GVHD (aGVHD) and chronic GVHD were scored according to published criteria.[25,26] Maintenance therapy included donor lymphocyte infusions (DLI), tyrosine kinase inhibitors, or azacytidine. Maintenance therapy was restricted to patients who were alive and in remission by day 100 after transplant.

End point definitions and statistical analysis

Medical records were retrospectively analyzed for demographic data, patient and disease characteristics, treatment regimens, treatment response, and MRD status at predefined time points. Patient characteristics were compared by using a Kruskal-Wallis test for quantitative variables, and the χ^2 or Fisher exact tests for categorical variables. All end points were measured from the time of transplantation. The primary study end point was leukemia-free survival (LFS). Secondary end points were relapse incidence, nonrelapse mortality (NRM), and overall survival (OS). Probabilities of OS and LFS were estimated by using the Kaplan-Meier method and compared by using the log-rank test. Estimates of NRM, relapse incidence, aGVHD, and chronic GVHD were calculated by using cumulative incidence function (CIF) to accommodate competing risks and were compared by using the Gray test. A

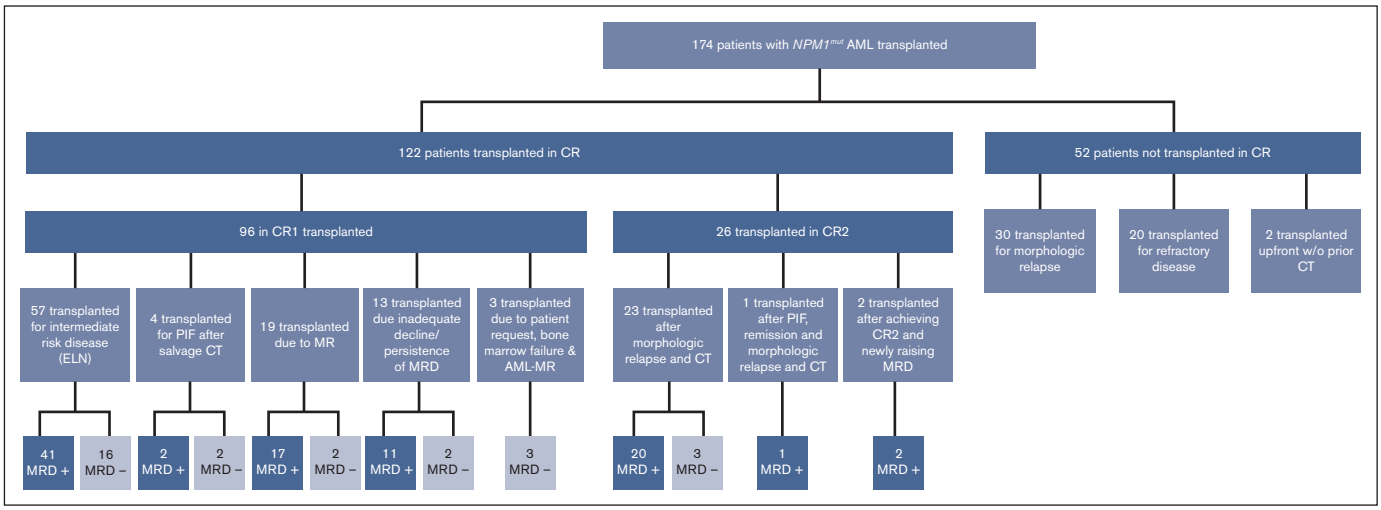


Figure 1. CONSORT diagram showing the number of patients depending on their remission status. CR defined as <5% blasts in bone marrow. AML-MR, acute myeloid leukemia-myelodysplasia related; CT, chemotherapy; MR, molecular relapse; PIF, primary induction failure.

multivariate regression was performed with all variables found to be significant on univariate analysis and further using a stepwise model selection. Results were expressed as a hazard ratio (HR) with a 95% confidence interval (CI). Tests were 2-sided and *P* values < .05 were considered significant. All statistical analyses were performed using R Project software, version 3.4.4.

Results

Patient, donor, and transplant characteristics

We identified 174 patients who underwent their first allo-SCT because of *NPM1*^{mut} AML between 2011 and 2022. The median age of the entire cohort was 56 years (range, 21-75) and Eastern Cooperative Oncology Group performance status ranged between 0 and 2. Upon diagnosis, most patients presented with cytogenetically normal AML (88%) and *FLT3*-ITD gene variants as the most prevalent comutation (54%). Most patients (n = 122; 70%) underwent transplantation in CR, with 94 of these being MRD^{+} and 28 MRD^{-} at time of transplantation. The remaining 52 patients (30%) began conditioning with active disease (Figure 1; Table 1). For the purpose of the study, we have categorized the patient cohort based on their remission status. Table 1 outlines the comparison of patient, disease, and transplant characteristics according to remission status and presents additional clinical and molecular data.

The patient distribution was well balanced across groups. Although the number of chemotherapy cycles before allo-SCT did not differ significantly, patients who received transplantation with active disease were less likely to have received FLT3 inhibition before allo-SCT. Reduced-intensity conditioning (RIC) was the predominant regimen (146 patients), with sequential conditioning being significantly more frequent in patients with active disease (*P* < .001). Fully HLA-matched transplantation was performed in 123 patients, of whom 83 underwent matched-unrelated donor transplantation and 40 underwent sibling donor transplantation. In all of them, anti–thymocyte globulin (ATG) was used as GVHD prophylaxis. Patients with active disease predominately underwent HLA-haploidentical SCT receiving posttransplantation cyclophosphamide (PTCy) as GVHD prophylaxis. In mismatched unrelated donor transplantation, GVHD prophylaxis was performed using either ATG (n = 13) or PTCy (n = 3).

Primary outcomes: OS and LFS

With a median follow-up of 53 months (range, 4-137) among survivors, the estimated probabilities of OS and LFS at 3 years were 57% (95% CI, 0.50-0.65) and 50% (95% CI, 0.43-0.59), respectively (supplemental Figure 1A,C). Patients who received transplantation in remission had significantly superior OS and LFS (68%, 96% CI, 0.60-0.78 and 61%, 95% CI, 0.53-0.71) compared with those who received transplantation with active disease (31%, 95% CI, 0.21-0.48 and 26 %, 95% CI, 0.17-0.42; *P* < .0001; supplemental Figure 1B,D). Among patients in remission, no difference in OS and LFS was detected based on pre-SCT MRD status (OS: CR MRD^{+} 66%; 95% CI, 0.56-0.76; CR MRD^{-} 78%, 95% CI, 0.64-0.95; not significant [ns]; LFS: CR MRD^{+} 57%; 95% CI, 0.48-0.69; CR MRD^{-} 74%; 95% CI, 0.59-0.93; ns; Figure 2A-B). First-line transplantation in CR1 was performed in 96 patients, of whom 71 had detectable *NPM1*^{mut} transcript (CR1 MRD^{+}) at time of allo-SCT. Presence of *NPM1*^{mut} in this subgroup did not affect survival after allo-SCT (3-year OS: CR1 MRD^{+} 73%; 95% CI, 0.65-0.97; CR MRD^{-} 80%; 95% CI, 0.63-0.86, *P* = .56; 3-year LFS: CR1 MRD^{+} 64%; 95% CI, 0.53-0.78; CR1 MRD^{-} 80%; 95% CI, 0.65-0.97; *P* = .28; supplemental Figure 2). Patients who received transplantation in second CR (CR2; n = 26) exhibited significantly poorer outcomes (CR2: 3-year OS, 48%; HR, 0.42; 95% CI, 1.22-4.54; *P* = .008; 3-year LFS: 41%, HR, 0.45; 95% CI, 1.21-4.11; *P* = .008), which were comparable with those with active disease (3-year OS, 31%; LFS, 26%). Because of the small size of this subgroup, stratification according to MRD status was not analyzed.

In univariate analysis for OS, complex aberrant karyotype, adverse risk per ELN 2010 and 2017, Medical Research Council adverse risk, and relapsed/refractory disease were associated with shorter survival (Figure 2E; supplemental Table 1). Conversely,

Table 1. Patient, donor, and transplant characteristics

Characteristics	Evaluable, n	n (%)				Overall *P* value	P value MRD$^+$ vs MRD$^-$
		All	Non-CR	Hematologic CR			
				MRD$^+$	MRD$^-$		
Total patients		174 (100)	52	94	28		
Age, median (range), y	174	56 (21-75)	58 (23-73)	54 (21-75)	57 (21-69)	0.46	0.72
Sex							
Male	174	74 (43)	21 (40)	38 (40)	15 (54)	0.43	0.31
Female		100 (57)	31 (60)	56 (60)	13 (46)		
Diagnosis							
AML, de novo	174	137 (79)	39 (75)	76 (81)	22 (79)	0.71	1.0
AML-MR		37 (21)	13 (25)	18 (19)	6 (21)		
NPM1 mutation type							
A	174	129 (78)	38 (76)	71 (78)	20 (80)	0.74	0.23
B		12 (7)	3 (6)	8 (9)	1 (4)		
D		12 (7)	3 (6)	6 (7)	3 (12)		
Not classified		21 (12)	8 (15)	9 (10)	4 (14)		
Comutations							
FLT3-ITD	171	93 (54)	29 (57)	49 (53)	15 (56)	0.88	0.97
Allelic ratio, >0.5	154	55 (36)	10 (23)	35 (41)	10 (40)	0.059	0.74
FLT3-TKD	162	17 (10)	7 (14)	9 (10)	1 (4)	0.41	0.70
biCEBPA	101	2 (2)	1 (3)	0 (0)	1 (9)	0.13	0.17
IDH1	105	11 (10)	4 (13)	4 (7)	3 (18)	0.37	0.19
IDH2	105	16 (15)	1 (3)	13 (22)	2 (12)	0.056	0.50
DNMT3A	100	47 (47)	19 (59)	23 (41)	5 (42)	0.24	1.0
TP53	146	1 (0.7)	0 (0)	1 (1)	0 (0)	0.69	1.0
Karyotype							
CN-AML	165	146 (88)	40 (80)	82 (92)	24 (92)	0.26	0.83
Complex-aberrant		6 (4)	3 (6)	2 (2)	1 (4)		
Other		13 (8)	7 (14)	5 (6)	1 (4)		
ELN 2010							
Favorable	164	67 (41)	19 (38)	39 (44)	9 (36)	0.32	0.43
Intermediate 1		80 (49)	22 (44)	43 (48)	15 (60)		
Intermediate 2		12 (7)	7 (14)	5 (6)	0 (0)		
Adverse		5 (3)	2 (4)	2 (2)	1 (4)		
ELN 2017							
Favorable	156	91 (58)	31 (67)	48 (55)	12 (52)	0.32	0.74
Intermediate		59 (38)	12 (26)	37 (43)	10 (43)		
Adverse		6 (4)	3 (7)	2 (2)	1 (4)		
MRC classification							
Favorable	165	0 (0)	0 (0)	0 (0)	0 (0)	0.52	0.54
Intermediate		159 (96)	47 (94)	87 (98)	25 (96)		
Adverse		6 (4)	3 (6)	2 (2)	1 (4)		
Induction chemotherapy							
7 + 3	174	79 (45)	17 (33)	47 (50)	15 (54)	0.008	0.71
7 + 3 + midostaurin		38 (22)	8 (15)	22 (23)	8 (29)		

BW, body weight; CMV, cytomegalovirus; CN, cytogenetically normal; CRi, CR with incomplete remission; FLAMSA, chemotherapy regimen consisting of fludarabine, amsacrine, and cytarabine; GO, gemtuzumab ozogamicin; HCT-CI, hematopoietic cell transplantation–specific comorbidity index; kg, kilogram; IDH, isocitrate dehydrogenase; MAC, myeloablative conditioning; MR, myelodysplasia-related changes; MRC, Medical Research Council; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; NC, nucleated cells; NPM1, nucleophosmin; PBSCs, peripheral blood stem cells, PIF, primary induction failure; RIC, reduced intensity conditioning; s-HAM, sequential high-dose cytarabine plus mitoxantrone; SIB-D, sibling donor; TCD, T-cell depletion; TKD, tyrosine kinase domain; 7+3, combination of cytarabine with daunorubicin.

*Other causes comprised: secondary AML (n = 3); BM failure after induction treatment (n = 1); and patient request in a patient with favorable risk profile according to ELN.

Table 1 (continued)

Characteristics	Evaluable, n	n (%)				Overall *P* value	P value MRD$^+$ vs MRD$^-$
		All	Non-CR	Hematologic CR			
				MRD$^+$	MRD$^-$		
7 + 3 + GO		4 (2)	3 (6)	1 (1)	0 (0)		
s-HAM		34 (20)	17 (33)	13 (14)	4 (14)		
Other		17 (10)	5 (10)	11 (12)	1 (3)		
None		2 (1)	2 (4)	0 (0)	0 (0)		
No. of chemotherapy cycles							
Median (range)	174	3 (0-25)	3 (0-8)	3 (1-25)	3 (1-6)	0.75	0.61
FLT3-inhibitor before allo-SCT							
Yes	108	42 (39)	10 (9)	24 (22)	8 (28)	0.6	0.85
No		66 (61)	24 (22)	34 (31)	8 (28)		
Status at allo-SCT							
CR1/CRi1	174	96 (56)	0 (0)	71 (76)	25 (89)	< .0001	0.19
CR2/CRi2		26 (14)	0 (0)	23 (24)	3 (11)		
Active disease		52 (30)	52 (100)	0 (0)	0 (0)		
Indication for allo-SCT							
Risk profile (ELN)	174	57 (33)	0 (0)	41 (44)	16 (57)	< .0001	0.01
PIF		25 (14)	20 (38)	3 (2)	2 (7)		
Hematologic relapse		53 (30)	30 (58)	20 (22)	3 (11)		
Molecular relapse		21 (12)	0 (0)	19 (20)	2 (7)		
Inadequate molecular response		13 (8)	0 (0)	11 (12)	2 (7)		
Other*		5 (3)	2 (4)	0 (0)	3 (11)		
HCT-CI score							
0	174	61 (35)	12 (23)	41 (44)	8 (29)	0.069	0.21
1-2		61 (35)	24 (46)	25 (27)	12 (43)		
≥3		52 (30)	16 (31)	28 (30)	8 (29)		
Donor type							
SIB-D	174	40 (23)	11 (21)	18 (19)	11 (39)	0.12	0.06
MUD		83 (48)	21 (40)	50 (53)	12 (43)		
MMUD		16 (9)	7 (13)	6 (6)	3 (11)		
Haploidentical		35 (20)	13 (25)	20 (21)	2 (7)		
Donor age, median (range), y		37 (15-69)	36 (20-61)	36 (15-69)	40 (20-63)	0.16	0.05
Donor sex							
Male	174	132 (76)	38 (73)	73 (78)	21 (75)	0.82	0.97
Female		42 (24)	14 (27)	21 (22)	7 (25)		
Donor sex match							
Patient male/donor female	174	16 (9)	5 (10)	8 (9)	3 (11)	0.80	0.38
Patient female/donor male		74 (43)	22 (42)	43 (46)	9 (32)		
Match		84 (48)	25 (48)	43 (46)	16 (57)		
ABO blood group match	174	79 (45)	17 (33)	48 (51)	14 (50)	0.089	1.0
CMV match		124 (71)	39 (75)	72 (77)	13 (46)	0.0049	0.005
CMV-negative in positive	174	37 (21)	12 (23)	16 (17)	9 (32)	0.21	0.28
CMV-positive in negative		13 (7)	1 (2)	6 (6)	6 (21)	0.01	0.03

BW, body weight; CMV, cytomegalovirus; CN, cytogenetically normal; CRi, CR with incomplete remission; FLAMSA, chemotherapy regimen consisting of fludarabine, amsacrine, and cytarabine; GO, gemtuzumab ozogamicin; HCT-CI, hematopoietic cell transplantation–specific comorbidity index; kg, kilogram; IDH, isocitrate dehydrogenase; MAC, myeloablative conditioning; MR, myelodysplasia-related changes; MRC, Medical Research Council; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; NC, nucleated cells; NPM1, nucleophosmin; PBSCs, peripheral blood stem cells, PIF, primary induction failure; RIC, reduced intensity conditioning; s-HAM, sequential high-dose cytarabine plus mitoxantrone; SIB-D, sibling donor; TCD, T-cell depletion; TKD, tyrosine kinase domain; 7+3, combination of cytarabine with daunorubicin.

*Other causes comprised: secondary AML (n = 3); BM failure after induction treatment (n = 1); and patient request in a patient with favorable risk profile according to ELN.

Table 1 (continued)

Characteristics	Evaluable, n	n (%)				Overall *P* value	P value MRD$^+$ vs MRD$^-$
		All	Non-CR	Hematologic CR			
				MRD$^+$	MRD$^-$		
Cytoreduction prior conditioning							
FLAMSA	174	81 (47)	32 (62)	37 (39)	12 (43)	0.00021	0.47
Other		17 (10)	10 (19)	7 (7)	0 (0)		
None		76 (44)	10 (19)	50 (53)	16 (57)		
Conditioning intensity							
MAC	174	28 (16)	8 (15)	18 (19)	2 (7)	0.31	0.16
RIC		146 (84)	44 (85)	76 (81)	26 (93)		
Stem cell source							
PBSCs	174	158 (91)	44 (85)	87 (93)	27 (96)	0.15	0.68
BM		16 (9)	8 (15)	7 (7)	1 (4)		
Median cell dose (range)							
NC × 10^8/kg BW	174	2.66 (1.75-5.1)	2.66 (1.75-3.4)	2.70 (2.4-3.4)	2.2	0.56	0.25
CD34$^+$ × 10^6/kg BW		7.3 (1.4-22.87)	6.85 (3.6-22.87)	7.31 (1.4-13.36)	7.3 (4-16)	0.76	0.68
In vivo TCD							
ATG	174	136 (78)	38 (73)	73 (78)	25 (89)	0.05	0.28
PTCy		38 (22)	14 (27)	21 (22)	3 (11)		

BW, body weight; CMV, cytomegalovirus; CN, cytogenetically normal; CRi, CR with incomplete remission; FLAMSA, chemotherapy regimen consisting of fludarabine, amsacrine, and cytarabine; GO, gemtuzumab ozogamicin; HCT-CI, hematopoietic cell transplantation–specific comorbidity index; kg, kilogram; IDH, isocitrate dehydrogenase; MAC, myeloablative conditioning; MR, myelodysplasia-related changes; MRC, Medical Research Council; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; NC, nucleated cells; NPM1, nucleophosmin; PBSCs, peripheral blood stem cells, PIF, primary induction failure; RIC, reduced intensity conditioning; s-HAM, sequential high-dose cytarabine plus mitoxantrone; SIB-D, sibling donor; TCD, T-cell depletion; TKD, tyrosine kinase domain; 7+3, combination of cytarabine with daunorubicin.

*Other causes comprised: secondary AML (n = 3); BM failure after induction treatment (n = 1); and patient request in a patient with favorable risk profile according to ELN.

maintenance treatment after allo-SCT with sorafenib (n = 17), donor lymphocyte infusion (DLI; n = 12), or other modalities (n = 9) such as hypomethylating agents or isocitrate dehydrogenase inhibitors positively influenced OS (HR, 0.42; 95% CI, 0.19-0.92; $P = .031$). In multivariable analysis, only complex aberrant karyotype, relapsed/refractory disease, and indication other (upfront transplantation, bone marrow failure) significantly influenced OS (Table 2). Univariate analysis of LFS similarly found that relapsed/refractory disease negatively affected survival, whereas maintenance therapy was linked to prolonged LFS (supplemental Table 1), a finding that was confirmed in multivariable analysis (Table 2). Additionally, patients who received transplantation from a mismatched unrelated donor had lower OS and LFS compared with those receiving grafts from other donor types, although this effect did not reach significance in the multivariable model.

Secondary outcomes: relapse, NRM, GVHD, and engraftment

The 3-year CIF of relapse (CIR) was significantly higher in patients with active disease (n = 52; CIR, 49%) compared with those in CR (n = 122; CIR, 12%); $P < .0001$; supplemental Figure 1F). No difference was observed when analysis was restricted to patients in CR according to MRD status (1-year CIR: CR MRD$^+$ [n = 94], 7% vs CR MRD$^-$ [n = 28], 4%; $P = .42$; Figure 2D). The same applies if the analysis was limited to patients in CR1 (n = 96, 1-year CIR: CR MRD$^+$ [n = 71], 8% vs CR MRD$^-$ [n = 25], 4%; $P = .36$). Baseline factors associated with a higher CIR were the presence of adverse risk as per ELN 2010 and refractory disease (Figure 2F; supplemental Table 2).

The estimated CIF of NRM for the entire cohort at 1 year was 17% (supplemental Figure 1E). No significant differences were observed between groups based on their remission status (1-year NRM: active disease (n = 52) 20% vs CR (n = 122) 15%; $P = .74$) or MRD status (1-year NRM: CR MRD$^+$ (n = 94) 17% vs CR MRD$^-$ (n = 28) 11%; $P = .69$; Figure 2C). Infections were the leading cause of death. Details on the causes of death are summarized in supplemental Table 3.

Dynamics of *NPM1^{mut}* transcript levels during treatment

At initial diagnosis a total of 125 BM samples were available for *NPM1^{mut}* transcript quantification. After induction therapy, 159 samples were analyzed deriving from either BM (n = 135) or PB (n = 24). Before transplantation, 194 samples were collected (paired BM/PB samples in 25 patients). Median transcript levels were 390 copies per Abelson murine leukemia viral oncogene homolog 1 (ABL1) copies at initial diagnosis, 0.63 copies per ABL1 copies in the BM and 0.42 copies per ABL1 copies in the PB after induction therapy, and 0.42 copies per ABL1 copies in the BM and 0.07 copies per ABL1 copies in the PB within 2 weeks before transplantation (Figure 3). No significant differences were found in median values between sample sources. Notably, patients entering conditioning in CR MRD$^-$ displayed the highest median baseline transcript levels at diagnosis and experienced the most

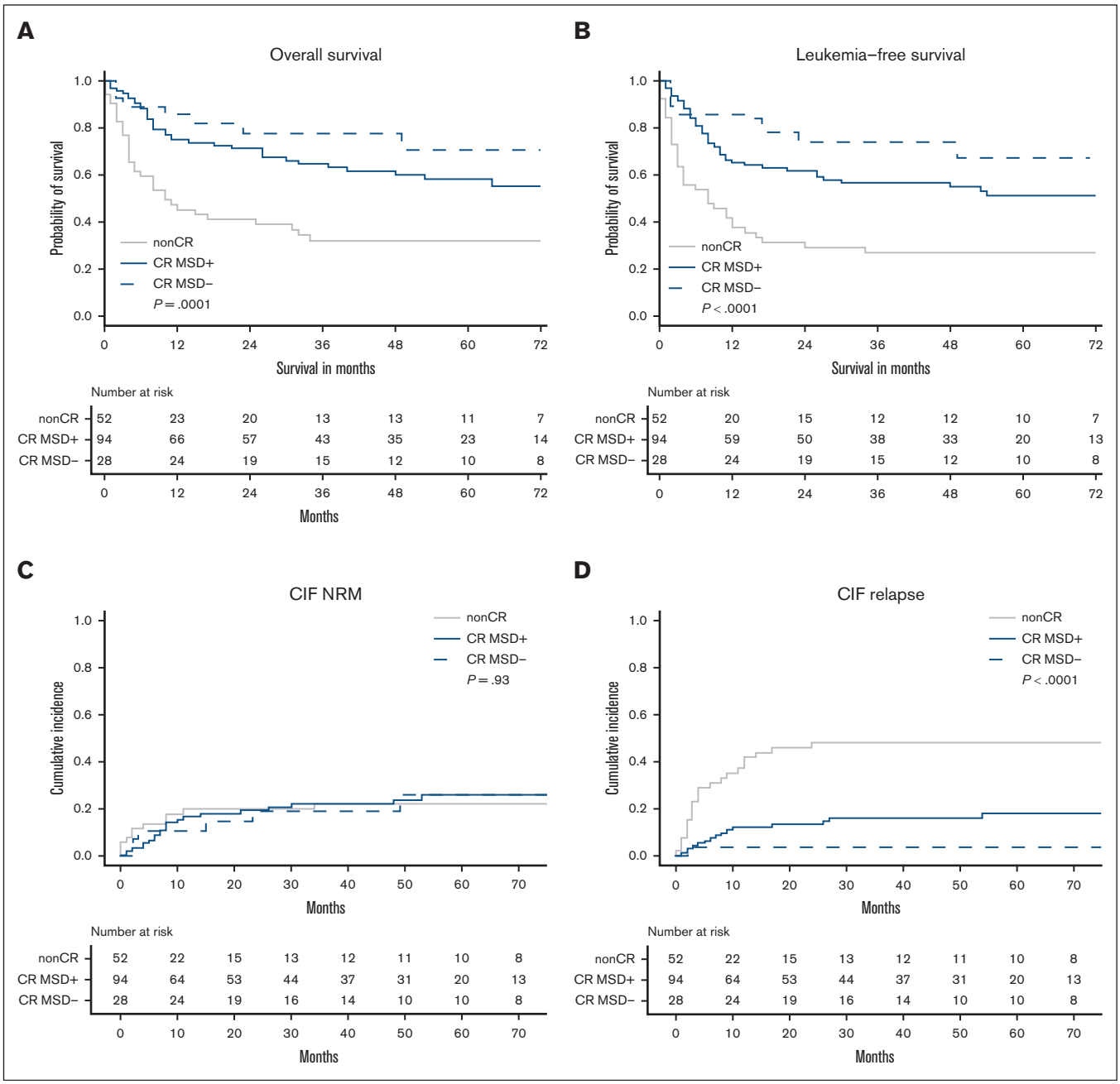


Figure 2. Impact of pre–allo-SCT remission status on clinical outcomes. Kaplan-Meier curves of (A) OS and (B) LFS. Cumulative incidences (CIs) of (C) NRM and (D) relapse. Forest plot showing the HRs of (E) OS and (F) relapse, estimated by univariate regression outcome analysis. CIF, cumulative incidence function; HCT-CI, hematopoietic cell transplantation–specific comorbidity index; MAC, myeloablative conditioning; MMUD, mismatched unrelated donor; MRC, Medical Research Council; MSD, matched sibling donor; NRM, nonrelapse mortality; PIF, primary induction failure.

substantial reduction (7 $\log_{10}$ units) before allo-SCT (Figure 3). Comutations in this group did not substantially differ in comparison to the other remission groups. Notably, only a minority (10%) in our patient cohort achieved complete MRD clearance after induction therapy, eventually requiring transplantation because of molecular relapse (n = 3), hematologic relapse (n = 5), ELN intermediate/high risk classification (n = 7), and other (n = 2; patient request, 1; secondary AML, 1).

Impact of MRD dynamics and clearance on outcome in patients in CR

CR MRD^{+} before allo-SCT was observed in 94 patients, with 19 showing newly rising transcript levels indicating molecular relapse, whereas the remaining 75 had stable or decreasing MRD levels (Figure 1). *NPM1*^{mut} MRD dynamics, whether decreasing or increasing transcript levels according to molecular persistence or

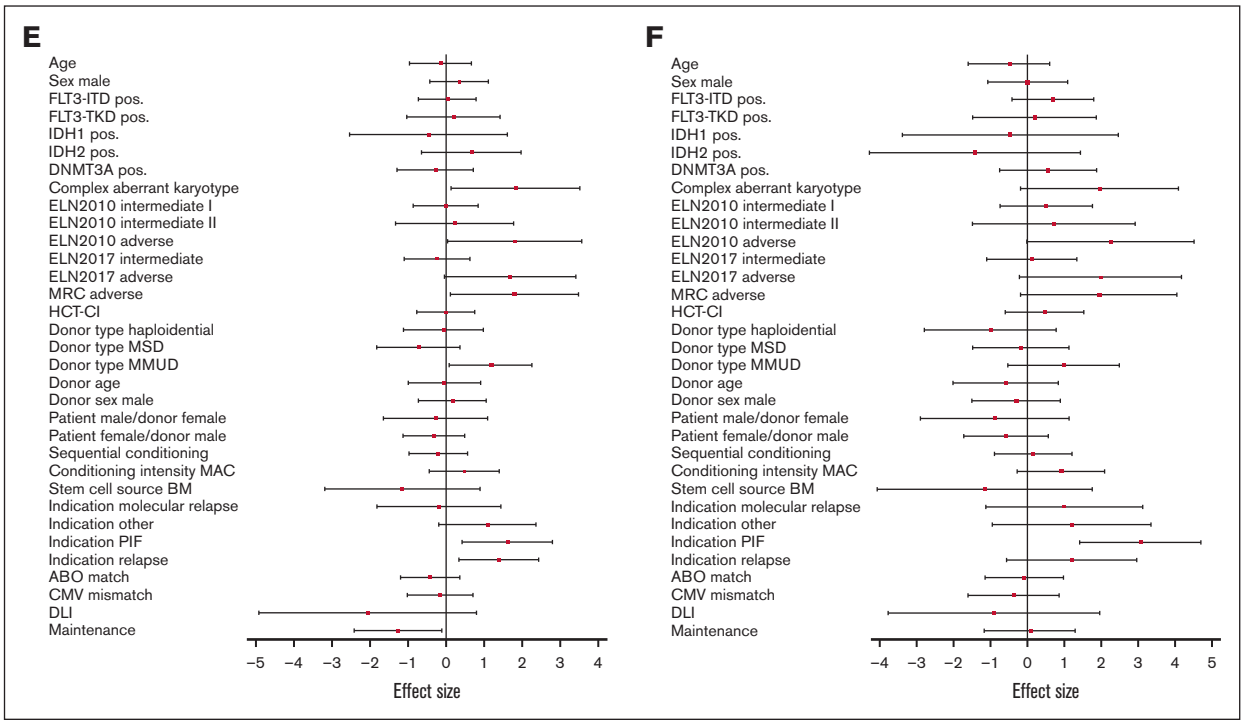


Figure 2 (continued)

molecular relapse did not affect survival (3-year OS: molecular persistence 68% vs molecular relapse 65%; HR, 1.13; 95% CI, 0.4-3.27; $P = .81$; 3-year LFS: MP 60% vs molecular relapse 60%; HR, 1.09; 95% CI, 0.42-2.8; $P = .87$).

Molecular clearance at day +30 after allo-SCT occurred in 73 of 94 (78%) patients who had evidence of NPM1^{mut} MRD before allo-SCT, either in the context of relapse (n = 13) or CR MRD^{+} (n = 60). An additional 18 patients achieved MRD negativity at day +100, 3 at day +180, and another 4 experienced MRD conversion only within the first year. Overall, 4 patients converted without intervention. This reflects in a cumulative rate of BM MRD negativity after allo-SCT increasing from 65% at day +30% to 73% by day +100. Presence of *FLT3*-ITD^{mut} at first diagnosis and ELN risk classification affected the odds of achieving MRD conversion at day +30 after allo-SCT (*FLT3*-ITD: OR, 0.25; $\chi^2 = 6.6$; $P = .01$). Neither FLT3 inhibitor pretreatment, conditioning intensity, conditioning modality, nor donor platform influenced MRD conversion at day +30. Occurrence of aGVHD did not have any influence on MRD conversion until day +100. Neither did the use of ATG or PTCy as GVHD prophylaxis.

Table 2. Multivariable analysis

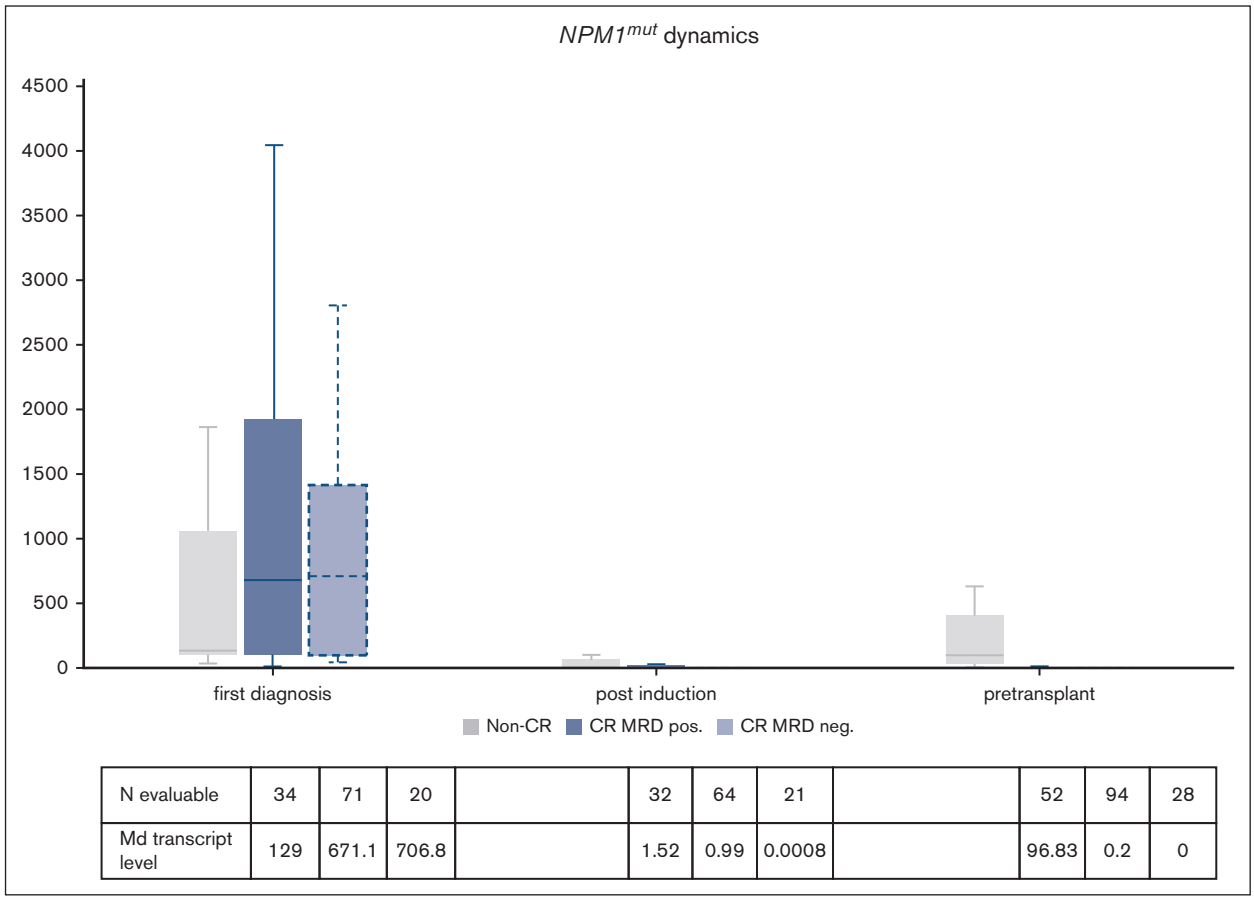
Variable	HR	95% CI	*P* value
Model 1: OS, stepwise model selection			
Karyotype complex-aberrant	6.00	1.68-21.46	.0059
Karyotype other	1.08	0.42-2.78	.88
Indication molecular relapse	0.66	0.14-3.04	.59
Indication other	2.46	0.91-6.62	.076
Indication PIF	3.60	1.48-8.77	.0047
Indication relapse	2.82	1.27-6.25	.011
Model 2: LFS, stepwise model selection			
Karyotype complex-aberrant	3.93	1.13-13.60	.031
Karyotype other	1.93	0.82-4.50	.13
Maintenance	0.42	0.19-0.95	.037

PIF, primary induction failure.

Discussion

This study investigates the impact of pretransplant *NPM1*^{mut} MRD on treatment response after allo-SCT in patients with AML, using an RNA-based qRT-PCR assay with a sensitivity of 10^{-5}. Our main finding indicates that pretransplant *NPM1*^{mut} MRD status does not significantly affect survival in patients who received transplantation in CR. This supports the current recommendation to proceed with allo-SCT regardless of tumor burden.[4] Our results align with data from the HOVON-SAKK-132 trial, which demonstrated that allo-SCT in CR1 can overcome the poor prognosis associated with *NPM1*^{mut} MRD positivity, detected via qRT-PCR.[18]

Several studies have highlighted the predictive value of *NPM1*^{mut} qRT-PCR MRD, especially when assessed after 2 cycles of induction therapy.[16-18] Patients with persisting *NPM1*^{mut} transcript in qRT-PCR were identified as an unfavorable subgroup, with elevated risk of relapse, irrespective of *FLT3*-ITD mutational status.[6] In line with these findings, 90% of patients in our cohort, who ultimately proceeded to transplantation, did not achieve MRD negativity after induction therapy. Patients who started conditioning in molecular remission exhibited the highest baseline transcript levels in the BM at diagnosis and experienced the most substantial reduction (7 $\log_{10}$ units) before transplantation. This might likely reflect a higher initial blast burden with elevated proliferation



N evaluable	34	71	20		32	64	21		52	94	28
Md transcript level	129	671.1	706.8		1.52	0.99	0.0008		96.83	0.2	0

Figure 3. Box plots depicting BM *NPM1*^{mut} kinetics before allo-SCT according to remission status. Nonremission patients in gray, patients in MRD^{+} CR in blue, and patients in MRD^{-} CR in light blue. *NPM1*^{mut} transcript levels are reported as the normalized values of *NPM1*^{mut} copy number per ABL1 copy number.

activity, rendering these patients more responsive to treatment. Similarly, the Acute Leukemia French Association was able to show that patients who did not achieve a 4-log reduction of *NPM1*^{mut} in the PB were at higher risk of relapse.[6]

To date, studies on the management of MRD^{+} CR in AML before allo-SCT have yielded conflicting results. Some studies suggest the necessity of achieving MRD negativity because of the inherent survival disadvantage of MRD positivity.[11,13,27] However, the results and comparisons between these studies should be approached with caution because of the lack of standardized and harmonized assays for MRD determination. For example, the prospective Figaro trial[11] uses FCM-based quantification and includes various AML subgroups, whereas Schwind et al used NGS-based MRD monitoring.

Dillon et al were among the first to focus on *NPM1*^{mut} AML, attempting to establish an qRT-PCR–based MRD threshold to distinguish between low and high MRD levels for predicting outcomes, and found that MRD positivity had a negative prognostic impact. In contrast, our study dichotomized MRD status into 2 groups: any detectable level vs absence. This methodological difference, alongside with the timing of MRD assessment before allo-SCT (30 days in our study vs 60 days), the biological heterogeneity (ie, 52% FLT3-ITD comutations in our study vs 32%) and the use of maintenance strategies, might explain why we could not endorse the negative impact of *NPM1*^{mut} MRD positivity on outcomes.

Currently, the impact of baseline and modifiable pre–allo-SCT factors on the potential of MRD conversion and outcome have been a matter of ongoing debate. In this context, we studied the effect of specific conditioning regimens on the potential of MRD conversion and outcome. There are conflicting results regarding whether intensifying conditioning regimens, such as through sequential therapy or myeloablative conditioning, can improve posttransplant outcomes by effectively eliminating MRD.[11-13] In our analysis, we did not observe any survival or disease control benefits related to the intensity of conditioning. Both sequential RIC and myeloablative conditioning yielded similar results. Notably, none of our patients received nonmyeloablative conditioning. This might explain the contrasting findings of Schwind et al, who reported significantly poorer disease control in patients with *NPM1*^{mut} MRD positivity, 70% of whom underwent nonmyeloablative conditioning before allo-SCT.[27] These observations lead us to the assumption that patients with *NPM1*^{mut} MRD positivity may benefit from at least RIC. Although, it is important to note that the MRD assay used by Schwind et al is NGS DNA based, which offers a 100- to 1000-fold lower sensitivity. Considering only patients in MRD^{+} CR, we found that *FLT3*-ITD mutation and ELN risk classification affected the odds of achieving MRD negativity. Interestingly, patients carrying a *FLT3*-ITD mutation, were less likely to achieve MRD conversion at day +30 after allo-SCT, probably because of the lack of specific kinase inhibition during induction and consolidation

therapy in the early 2010s. Although achieving MRD negativity at day +30 significantly improved survival, *FLT3*-ITD^{mut} did not translate into a survival disadvantage in our cohort, most likely because of the stringent use of posttransplant maintenance therapies including sorafenib and DLIs.

Although molecular failure after induction therapy has been proposed as the best independent tool to predict relapse, MRD assessment during follow-up also allows the identification of patients at risk for hematologic relapse by 2 to 4 months in advance. Comparing patients in CR with MRD persistence with patients with newly raising transcripts in terms of molecular relapse, we did not find any difference in OS and LFS once having received transplantation. This could warrant the decision to closely monitor MRD in patients with low copies of *NPM1*^{mut} transcript and delay transplant indication to molecular relapse. Yet, it is essential to prevent an overt relapse as in line with previous research we found relapsed/refractory and active disease to be associated with significantly poorer OS and LFS in univariate as well as multivariable analysis. However, transplant organization may be time consuming, thus strategies aimed at preventing disease progression in the peritransplant setting, such as use of HMA plus venetoclax or an extended use of menin inhibitors are required.

Most of our patients who received transplantation with active hematologic relapse (n = 52) were treated with sequential conditioning (n = 42; 81%), with no prior attempt to achieve CR. Hereby, overall toxicity as measured by CIF of NRM showed no significant difference to patients who received transplantation in remission. These results align with the results of the recently published large randomized phase 3 ASAP trial, which demonstrated similar survival rates and NRM among patients with active disease (3-year LFS, 59%; 3-year OS, 46%), regardless of whether they proceeded directly to allo-SCT or underwent intensive remission induction before it.[28] Whether these observations are to keep up with novel treatment strategies such as combination therapy with *BCL2* or *FLT3* inhibition or novel therapeutic agents such as the menin inhibitors remains to be seen.

NPM1^{mut} have been described as immunogenic.[29,30] Consistent with this finding, Dillon et al observed a strong association between the use of T-cell depletion and adverse outcome.[13] All of our patients underwent in vivo T-cell depletion with either ATG or PTCy, so we could not investigate this finding. To augment the putative graft-versus-leukemia effect on posttransplant outcome, we studied the impact of DLI and sorafenib maintenance treatment. Interestingly, we found a significant improvement in disease control resulting in a survival benefit in our cohort. Novel immunotherapeutic treatment strategies such as *NPM1*-specific T cells might enter the clinical routine and be an attractive option in combination to allo-SCT.[31]

Because of the dynamic nature of AML therapy, conclusions drawn from retrospective analyses inherently have limited relevance to current and future treatment approaches. Further, the restricted sample size of the patient groups in subanalysis, as well as the lack of an unbiased control patient set consolidated with chemotherapy alone may have affected data interpretation in patients in CR. However, in contrast to register analysis, our study provides an accurate and detailed comparison in a multicenter setting with similar local standards. Randomized studies to investigate these approaches to align and synchronize novel therapeutic modalities with allogeneic regimens are urgently required.

In conclusion, our findings suggest that *NPM1*^{mut} molecular failure or relapse is a crucial indication for immediate allo-SCT, without further attempts to attain MRD negativity. Survival outcomes among patients in CR undergoing allo-SCT in the MRD^{+} CR context mirror those of patients with MRD^{-} CR and notably surpass those of patients experiencing hematologic relapse as well as of those achieving CR2. As an immunogenic mutation that responds to DLI, we do encourage the use of this maintenance treatments. Future efforts should prioritize the enhancement of T-cell immunity through advanced immunotherapeutic strategies to achieve durable disease control. Moreover, greater emphasis should be placed on fully harnessing the immunotherapeutic potential of allo-SCT as a versatile platform for innovative treatments.

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Authorship

Contribution: A.F. and J.T designed the study, analyzed the data, and wrote the manuscript; V.J. performed and oversaw the statistical analysis; K.H, E.S., K.K., S.B., S.H., H.D., K.H.M., K.S., M.H., A.H., and M.V. were involved in collecting clinical data; M.R.-T., A.D., K.H.M., K.S., and T.H. were involved in analyzing the provided samples; and C.S., T.H., and J.T. supervised the study and contributed to the preparation of the manuscript.

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