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ORIGINAL ARTICLE

Comparative value of post-remission treatment in cytogenetically normal AML subclassified by *NPM1* and *FLT3*-ITD allelic ratio

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Post-remission treatment (PRT) in patients with cytogenetically normal (CN) acute myeloid leukemia (AML) in first complete remission (CR1) is debated. We studied 521 patients with CN-AML in CR1, for whom mutational status of *NPM1* and *FLT3*-ITD was available, including the *FLT3*-ITD allelic ratio. PRT consisted of reduced intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (alloHSCT) ($n = 68$), myeloablative conditioning (MAC) alloHSCT ($n = 137$), autologous hematopoietic stem cell transplantation (autoHSCT) ($n = 168$) or chemotherapy ($n = 148$). Favorable overall survival (OS) was found for patients with mutated *NPM1* without *FLT3*-ITD (71 ± 4%). Outcome in patients with a high *FLT3*-ITD allelic ratio appeared to be very poor with OS and relapse-free survival (RFS) of 23 ± 8% and 12 ± 6%, respectively. Patients with wild-type *NPM1* without *FLT3*-ITD or with a low allelic burden of *FLT3*-ITD were considered as intermediate-risk group because of similar OS and RFS at 5 years, in which PRT by RIC alloHSCT resulted in better OS and RFS as compared with chemotherapy (hazard ratio (HR) 0.56, $P = 0.022$ and HR 0.50, $P = 0.004$, respectively) or autoHSCT (HR 0.60, $P = 0.046$ and HR 0.60, $P = 0.043$, respectively). The lowest cumulative incidence of relapse (23 ± 4%) was observed following MAC alloHSCT. These results suggest that alloHSCT may be preferred in patients with molecularly intermediate-risk CN-AML, while the choice of conditioning type may be personalized according to risk for non-relapse mortality.

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INTRODUCTION

Acute myeloid leukemia (AML) is a cytogenetically and molecularly heterogeneous disease. Cytogenetically normal AML (CN-AML) is the largest cytogenetic subgroup (40–50% of AML patients),[1] which currently can be further refined based on molecular markers. Mutations in nucleophosmin-1 (*NPM1*) and fms-like tyrosine kinase 3 internal tandem duplications (*FLT3*-ITD) are found in, respectively, 50% and 30% of patients with CN-AML.[2] Molecular diagnostic analyses provide additional prognostic information that may be used for a risk-adapted treatment approach.[3–6] *FLT3*-ITD, particularly *FLT3*-ITD with a high-mutant to wild-type ratio, is associated with an unfavorable prognosis, whereas *NPM1* mutations in the absence of *FLT3*-ITD are associated with a relatively favorable outcome.[2,3,7–11] Patients who obtain a first complete remission (CR1) are subsequently treated with post-remission treatment (PRT), including an additional cycle of chemotherapy, high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (autoHSCT) or allogeneic HSCT (alloHSCT) following either myeloablative conditioning (MAC) or reduced intensity conditioning (RIC). PRT in patients with CN-AML CR1 is a subject of continued debate, especially taking molecular markers into account.[12–19] AlloHSCT is generally not associated with better survival in patients with *NPM1* mutations without *FLT3*-ITD, whereas the role of autoHSCT and alloHSCT in patients with *FLT3*-ITD is not definitely settled.[3,9,10,12,19–21] In addition, large comparative studies of PRT including autoHSCT are lacking in molecularly defined subgroups. In the present study, we addressed the impact of *NPM1* and *FLT3*-ITD including the *FLT3*-ITD allelic ratio on the outcome in patients with CN-AML, treated upfront within four prospective, consecutive HOVON-SAKK and EORTC studies. Second, we compared the outcome of PRT with alloHSCT and autoHSCT vs chemotherapy by time-dependent

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analysis in patients with AML in CR1, according to molecularly defined subgroups.

PATIENTS AND METHODS

Patients

A total number of 521 patients with newly diagnosed CN-AML were included, treated between 1995 and 2010 and who obtained CR1 after one or two induction cycles of chemotherapy. Patient data were derived from two cohorts including consecutive, prospective HOVON-SAKK phase III trials (AML29, AML42/42A and AML92; $n = 399$),[22–24] and a prospective EORTC phase III trial (AML12; $n = 122$).[25] Patients were excluded if molecular information was not available or if *EVI1* overexpression was present. Figure 1 shows the total number of patients enrolled in different trials and reasons why patients were excluded in the present analysis. The ratio of *FLT3*-ITD mutant to wild-type, defined by *FLT3*-ITD divided by *FLT3*-ITD plus *FLT3* wild-type, was available for 86% of the patients with *FLT3*-ITD AML. A predefined cutoff of >0.50 was applied to define subgroups with a low or high allelic ratio of *FLT3*-ITD. Patients were considered as having a low allelic ratio in case the ratio was not available in order to define a mere poor-risk group. Details of the molecular analysis are provided in the Supplementary Appendix. All studies were approved by the ethics committees of participating institutions and were conducted in accordance with the Declaration of Helsinki. All participants had given written informed consent. A detailed description of the inclusion and exclusion criteria of the studies can be found in the Supplementary Appendix.

Treatment protocols

Treatment in the HOVON-SAKK AML29, AML42/42A and AML92 studies involved a maximum of two remission induction cycles consisting of an anthracycline with cytarabine chemotherapy, as previously described.[22–24] Induction chemotherapy was followed by three types of PRT in patients in CR1 according to a predefined strategy as outlined in the study protocols, including either a third cycle of chemotherapy with mitoxantrone and etoposide, high-dose chemotherapy with busulfan and cyclophosphamide followed by autoHSCT, or alloHSCT following either MAC or RIC. These different therapeutic modalities were applied according a risk-adapted strategy as previously described.[22–24,26,27] Induction treatment in the EORTC AML12 study consisted of a combination of anthracycline, etoposide and cytarabine-based chemotherapy.[25] All patients in the EORTC AML12 study received PRT with at least one cycle of chemotherapy after obtaining CR1 followed by continued PRT with either autoHSCT or alloHSCT. The preferred type of PRT in patients below the age of 50 years with an available donor was alloHSCT, whereas in patients above the age of 50 years or patients lacking a donor autoHSCT was performed as the preferred PRT.[25] Conditioning with either RIC or MAC was performed based on center's choice.

Transplantation protocols

Patients received either a MAC or a RIC regimen followed by the infusion of donor cells. RIC alloHSCT was introduced in patients below 60 years as from 2001, whereby the indication for RIC or MAC was selectively determined by age and consistently adhered to by the individual center throughout the HOVON AML42/42A and AML92 studies. While some centers maintained their policy of MAC alloHSCT for all patients up to the age of 60, a number of centers changed their policy by setting the age limit for MAC at <40 and RIC for patients of 40 years and beyond. The MAC regimen contained high-dose cyclophosphamide with total body irradiation (TBI) in 61 out of 81 (84%) HOVON patients, whereas the remainder received busulfan with cyclophosphamide. RIC regimens varied, but the vast majority consisted of 2.0 gray total body irradiation preceded

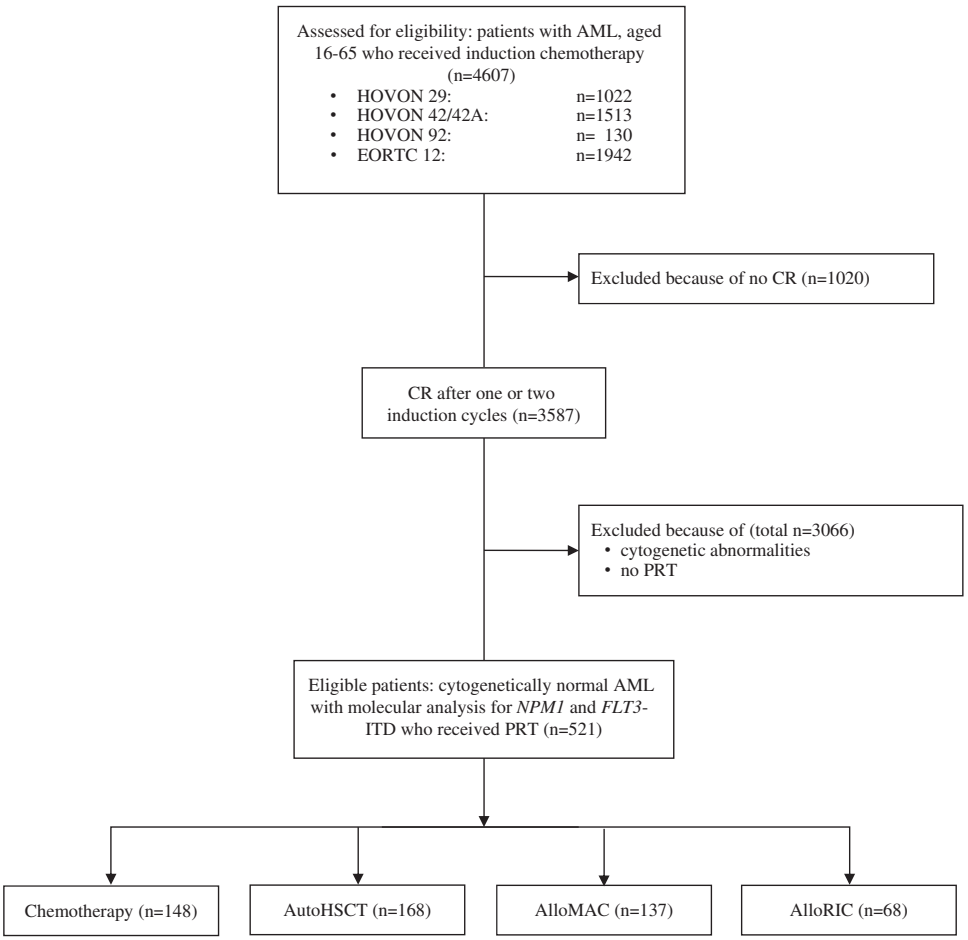

Figure 1. Patient flow chart. Patients with AML, included in EORTC and HOVON-SAKK trials, who were eligible for the present analysis with CN-AML in CR1 with available molecular analysis who received PRT.

by fludarabine ($n = 51$, 93%). MAC alloHSCT in the EORTC study preferably consisted of high-dose cyclophosphamide with total body irradiation and alternatively busulfan with high-dose cyclophosphamide. The most frequently used RIC regimen in the EORTC study was busulfan combined with fludarabin. A calcineurin inhibitor (either ciclosporin or tacrolimus) plus mycophenolate mofetil or methotrexate was given as prophylaxis for graft vs host disease.

End points

The primary end point of the study was overall survival (OS), according to the type of PRT received. OS and relapse-free survival (RFS) were measured from the date of starting the first PRT. OS was based on death from any cause, and patients were censored at the date of last contact if alive. The events for RFS were death in CR1, designated as non-relapse mortality (NRM) or hematological relapse. The cumulative risks of relapse and NRM over time were calculated as competing risks with actuarial methods, where patients alive in continuing CR1 were censored at the date of last contact.

Statistical methods

A time-dependent analysis of PRT was performed as described previously,[27,28] by applying multivariable Cox regression with time-dependent covariates autoHSCT and alloHSCT following MAC or RIC. The multivariable analysis is conceptually similar to a Mantel–Byar analysis,[29] but more general as it allows for the adjustment of other factors. A number of patients received PRT with chemotherapy ($n = 28$) first before they proceeded to alloHSCT. In both the multivariable analysis and the estimation of survival curves, these patients were counted as at risk in the chemotherapy group from start of PRT until alloHSCT and after that as at risk in the MAC or RIC alloHSCT group. Multivariable Cox regression analysis for OS, RFS, relapse and NRM was applied, stratified by study cohort with adjustment for age, sex, white blood cell count at diagnosis and late CR (after cycle II instead of I). Outcome estimates are at 5 years unless explicitly stated otherwise. All *P*-values were based on log-likelihood ratio tests, except when explicitly stated otherwise. Log-likelihood ratio tests were also used to test for interactions. The proportional hazard assumption was tested on the basis of Schoenfeld residuals.[29,30] *P*-values were not adjusted for multiple testing. All analyses were done with Stata Statistical Software: Release 13.1 (2013, Stata Corporation, College Station, TX, USA).

RESULTS

Patients

A total of 521 patients with CN-AML proceeded to PRT with either chemotherapy ($n = 148$), autoHSCT ($n = 168$) or alloHSCT following MAC ($n = 137$) or RIC ($n = 68$). Patient characteristics are presented in Table 1. Recipients of MAC alloHSCT were younger as compared with the other types of PRT. Patients with wild-type *NPM1* received RIC alloHSCT more frequently as compared with chemotherapy and autoHSCT. More allografted patients obtained a relatively late CR1 (achieved after two cycles of induction chemotherapy). In addition, time from remission to PRT was longer for recipients of autoHSCT, and RIC alloHSCT was performed more frequently in the recent years. The median follow up of patients still alive was 77 months and differed between patients receiving chemotherapy (100 months), autoHSCT (70 months), MAC alloHSCT (79 months) and RIC alloHSCT (72 months). Patient's characteristics by the different study cohorts are presented in Supplementary Table 1. Due to different study protocols, time from CR1 to PRT was significantly longer for patients treated by the EORTC. All patients treated by the EORTC received PRT with chemotherapy followed by final PRT with either autoHSCT or alloHSCT with RIC or MAC.

Treatment outcome

OS and RFS of all patients were 53 ± 2% and 47 ± 2%, respectively, at 5 years from the start of PRT. Outcome by molecular subgroups demonstrated distinct favorable and poor-risk subgroups (Figure 2). Outcome of patients with mutated *NPM1* was clearly determined by the absence or presence of *FLT3*-ITD with OS of 71 ± 4% and 39 ± 4%, respectively. In contrast, OS of patients with *FLT3*-ITD appeared to be not influenced by *NPM1* mutational status ($NPM1^{mut}$ 39 ± 4%, $NPM1^{wt}$ 39 ± 8%), but by the ratio of mutant to wild-type *FLT3*-ITD (low ratio 42 ± 3%, high ratio 23 ± 8%). Patients with mutated *NPM1* without *FLT3*-ITD had a favorable outcome with OS and RFS of 71 ± 4% and 65 ± 4%, respectively. In contrast, AML patients with a high *FLT3*-ITD mutant to wild-type ratio appeared to exhibit a very poor outcome with OS and RFS of 23 ± 8% and 12 ± 6%, respectively. A large group of AML patients, designated as molecular intermediate risk, with either a low *FLT3*-ITD ratio (mutant or wild-type *NPM1*) or wild-type *NPM1* without *FLT3*-ITD showed fairly similar OS and RFS estimating about 45% and 40%, respectively, allowing us to consider these three subgroups as one intermediate-risk group.

Table 1. Patient characteristics

	Post-remission treatment			
	Chemotherapy (N = *148*)	*AutoHSCT* (N = *168*)	*AlloMAC* (N = *137*)	*AlloRIC* (N = *68*)
Sex				
Male	72 49%	87 52%	67 49%	36 53%
Female	76 51%	81 48%	70 51%	32 47%
Age (years)				
Median	50	48	44	54
Range	18–60	16–61	16–59	37–60
WBC at diagnosis				
Median	34	28	26	11
Range	0.8–400	0.8–278	0.6–291	0.9–182
NPM1				
Mutated	95 64%	96 57%	72 53%	30 44%
Wild-type	53 36%	72 43%	65 47%	38 56%
FLT3-ITD				
Not present	94 64%	116 69%	92 67%	44 65%
Low ratio	39 26%	48 29%	37 27%	20 29%
High ratio	15 10%	4 2%	8 6%	4 6%
CR reached after				
Cycle 1 (early CR)	126 85%	155 92%	97 71%	49 72%
Cycle 2 (late CR)	22 15%	13 8%	40 29%	19 28%
Time from CR to PRT (months)				
Median	2.1	2.6	2.4	2.3
IQ range	1.4–2.7	2.0–2.9	1.0–2.9	1.2–2.8
Year of PRT				
< 2005	104 70%	86 51%	76 55%	20 29%
$\geqslant 2005$	44 30%	82 49%	61 45%	48 71%

Abbreviations: AlloMAC, allogeneic hematopoietic stem cell transplantation following myeloablative conditioning; AlloRIC, alloHSCT following reduced intensity conditioning; AutoHSCT, autologous hematopoietic stem cell transplantation; CR, complete remission; *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication; IQ, interquartile range; *NPM1*, nucleophosmin-1; PRT, post-remission treatment; WBC, white blood cell count.

Outcome by PRT in molecular subgroups

Favorable risk (NPM1 mutant without FLT3-ITD AML). Patients with mutated *NPM1* without *FLT3*-ITD shared similar OS following chemotherapy, autoHSCT, MAC alloHSCT or RIC alloHSCT (68 ± 7% and 71 ± 6%, 74 ± 7% or 67 ± 14%, respectively, $P = 0.94$, Figure 3a, Table 2). Although autoHSCT or alloHSCT following either MAC or

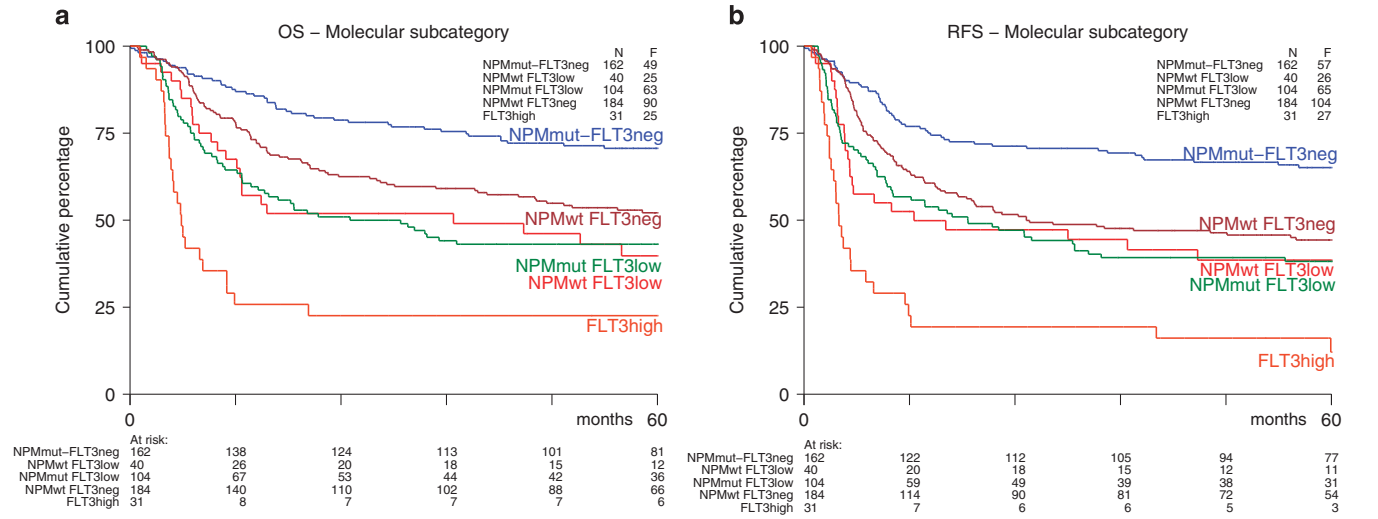

Figure 2. OS and RFS by molecular subcategory. Kaplan–Meier estimates of OS (**a**) and (**b**) by molecular subcategory of patients with CN-AML in first complete remission from start of post-remission treatment. F, number of failures (i.e., death whatever the cause); *FLT3*high, high allelic ratio of *FLT3*-ITD; *FLT3*low, low allelic ratio of *FLT3*-ITD; *FLT3*neg, no *FLT3*-ITD; N, number of patients.

RIC reduced relapse more strongly, RFS appeared not statistically significantly different as compared with chemotherapy (66 ± 6%, 71 ± 7% or 67 ± 14 vs 58 ± 7%, respectively, $P = 0.78$, Figure 3b, Table 2 and Supplementary Table 2). Limiting the analysis to strictly favorably risk patients with an early CR (after one cycle of induction chemotherapy) did not show any differences in OS or RFS.

Intermediate risk (NPM1 wild-type without FLT3-ITD or low FLT3-ITD allelic ratio). Recipients of RIC alloHSCT showed significantly better OS as compared with chemotherapy (63 ± 7% vs 39 ± 6%, respectively, $P = 0.046$). AutoHSCT and MAC alloHSCT had similar OS, which was not significantly different as compared with chemotherapy or RIC alloHSCT. RFS was improved by RIC alloHSCT as compared with chemotherapy (59 ± 7% vs 30 ± 5%, respectively, $P = 0.008$, Figure 3d). AutoHSCT and MAC alloHSCT reduced relapse more strongly as compared with chemotherapy, but RFS was not significantly different (40 ± 5%, 44 ± 5% vs 30 ± 5, respectively, $P = 0.20$, Figure 3d, Table 2 and Supplementary Table 2). These results remained similar in patients with an early CR with improved OS and RFS by RIC alloHSCT as compared with chemotherapy.

Poor risk (FLT3-ITD high-mutant to wild-type ratio). OS and RFS in patients with a *FLT3*-ITD mutant to wild-type ratio of >0.50 are very poor (Supplementary Figures 1A and B). Numbers of patients were low hampering a reliable comparison of the different types of PRT.

Multivariable analysis in molecularly intermediate-risk patients

Table 3 shows the results of the multivariable analysis with adjustment for type of PRT, sex, age, white blood cell count below or above 100 and late CR. OS and RFS were better by RIC alloHSCT as compared with chemotherapy (hazard ratio (HR) 0.56, $P = 0.022$ and HR 0.50, $P = 0.004$, respectively) and autoHSCT (HR 0.60, $P = 0.046$ and HR 0.60, $P = 0.043$, respectively), whereas NRM was not significantly different comparing RIC alloHSCT with chemotherapy or autoHSCT (HR 2.54, $P = 0.16$ and HR 1.58, $P = 0.42$, respectively). Although no significant differences were found comparing autoHSCT and chemotherapy, the risk of relapse after autoHSCT was reduced with a HR of 0.71, $P = 0.087$. RFS was improved comparing MAC alloHSCT with chemotherapy (HR 0.67, $P = 0.048$), with a strongly decreased risk of relapse (HR 0.20, $P < 0.001$) and counterbalancing increased risk of NRM following MAC alloHSCT (HR 9.14, $P < 0.001$). OS and RFS following autoHSCT or MAC alloHSCT yielded similar results with an reduced risk of relapse following MAC alloHSCT as compared with autoHSCT (HR 0.29, $P < 0.001$), but increased the risk of NRM (HR 5.70, $P < 0.001$). Furthermore, increasing age exhibited a significant HR for worse OS. In addition, late CR was associated with a significantly increased HR for OS, RFS and relapse as compared with CR after one cycle of induction chemotherapy. Of note, time from CR1 to start of PRT and year of treatment (before and after 2005) were added as factors to the model but showed no significant effects on OS, RFS, relapse or NRM. In addition, a sensitivity analysis of only patients receiving PRT after 2005 showed similar results for PRT on all outcome parameters.

DISCUSSION

The preferred type of PRT in patients with CN-AML in CR1 continues to be debated. Molecular diagnostics provide additional prognostic information to further stratify patients with CN-AML in CR1. Here, we demonstrate that type of PRT does not differentially affect outcome in the favorable group of patients with mutated *NPM1* without *FLT3*-ITD. Outcome in patients with a high allelic ratio of *FLT3*-ITD appeared very poor, with low patient numbers hampering a comparison by type of PRT. In contrast, outcome by type of PRT appeared to differ in a larger intermediate group, characterized by *FLT3*-ITD with a low allelic ratio and wild-type *NPM1* without *FLT3*-ITD AML. RIC alloHSCT appeared associated with significantly better OS and RFS as compared with chemotherapeutic PRT, whereas MAC alloHSCT and autoHSCT yielded similar OS, which did not significantly differ from PRT by chemotherapy.

The *FLT3*-ITD is an important molecular determinant of AML risk classification and outcome.[4,5,31] Here, not only *FLT3*-ITD itself, but especially the mutant to wild-type ratio strongly affected outcome with poor outcome for patients with a high allelic ratio. Based on these and previous results, the *FLT3*-ITD allelic ratio should be included in AML risk classifications and PRT decision-making.[7–10,19,32] PRT has not extensively been studied in

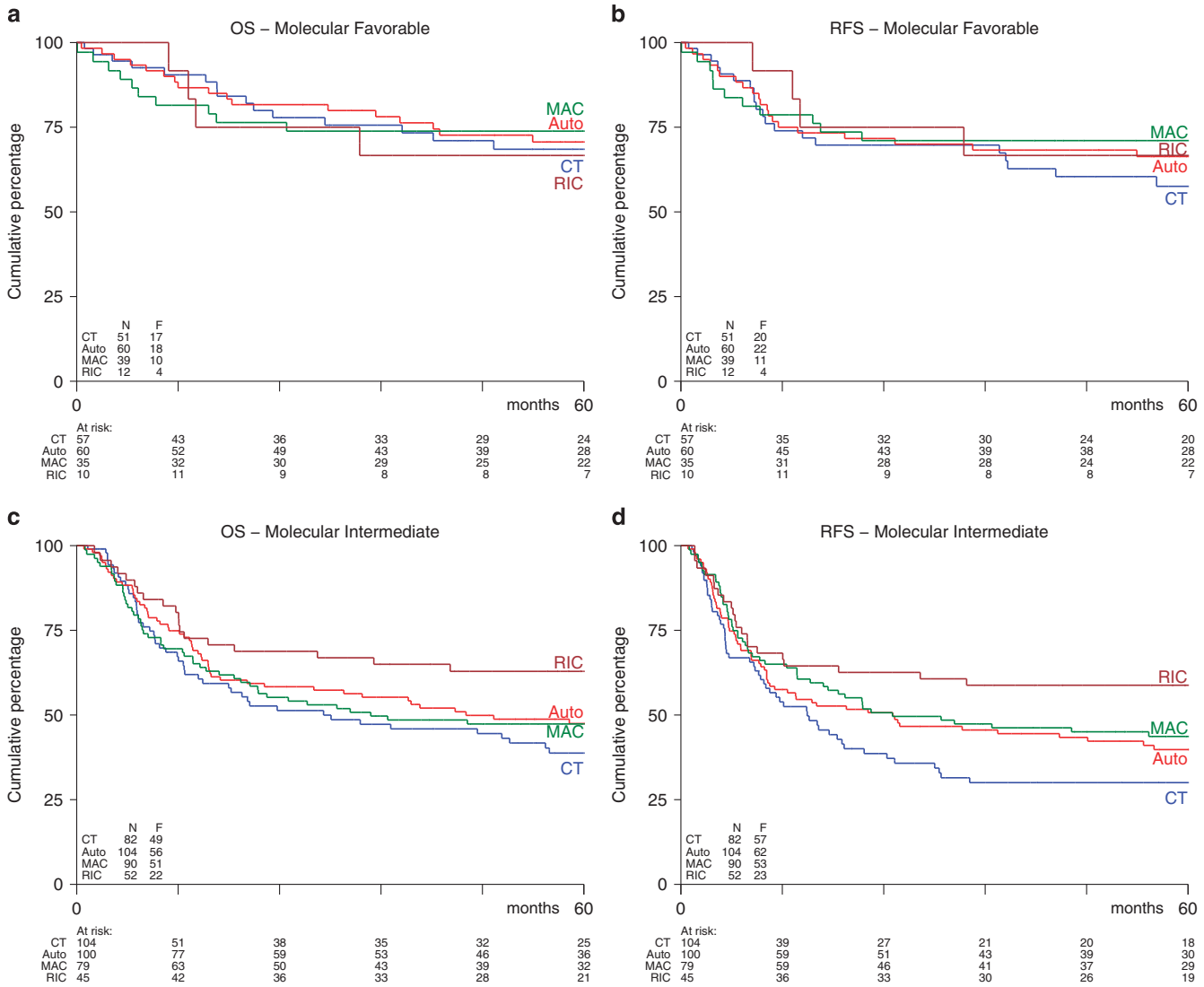

Figure 3. OS and RFS in molecular subcategories by post-remission treatment. OS and RFS in molecularly favorable risk (**a**) and (**b**) and molecularly intermediate-risk (**c**) and (**d**) patients with CN-AML in first complete remission from start of post-remission treatment. Molecularly favorable includes patients with mutated *NPM1* without *FLT3*-ITD, and molecularly intermediate includes patients with wild-type *NPM1* without *FLT3*-ITD or patients with a low allelic ratio of *FLT3*-ITD. Of note, numbers of patients at risk (indicated below the *x* axis) differ from the patient numbers (indicated in Table 1 and within the figure) because of the time-dependent nature of this analysis, which allows for time to transplantation by switching patients at the time of allograft in CR1 to the transplantation curve. Auto, autologous hematopoietic stem cell transplantation; Cox LR, cox likelihood ratio; CT, chemotherapy; F, number of failures (i.e., death whatever the cause); MAC, myeloablative conditioning; N, number of patients; RIC, reduced intensity conditioning hematopoeitic stem cell transplantation.

Table 2. Outcome by post-remission treatment in CN-AML patients subclassified by *NPM1* and *FLT3*-ITD mutational status

Molecular subgroup	*Outcome at 5 years (%) by post-remission treatment*											
	Chemotherapy			*AutoHSCT*			*AlloMAC*			*AlloRIC*		
	No.	*OS*	*RFS*	*No.*	*OS*	*RFS*	*No.*	*OS*	*RFS*	*No.*	*OS*	*RFS*
Favorable (*NPM1*^{mut} without *FLT3*-ITD) ($n=162$)	51	68±7	58±7	60	71±6	66±6	39	74±7	71±7	12	67±14	67±14
Intermediate ($n=328$)	82	39±6	30±5	104	47±5	40±5	90	47±5	44±5	52	63±7	59±7
NPM1^{wt} without *FLT3*-ITD ($n=184$)	43	43±8	27±7	56	48±7	40±7	53	54±7	52±7	32	65±9	59±9
NPM1^{mut} *FLT3*-ITD mut to wt ratio <0.50 ($n=104$)	30	44±9	42±9	33	45±9	35±8	27	41±9	37±9	14	42±13	42±13
NPM1^{wt} *FLT3*-ITD mut to wt ratio <0.50 ($n=40$)	9	0±11	0±0	15	49±14	50±14	10	30±14	20±13	6	100±0	100±0
Poor (*FLT3*-ITD mut to wt ratio >0.50) ($n=31$)	15	20±10	7±6	4	50±25	25±22	8	13±12	13±12	4	25±22	25±22

Abbreviations: AlloMAC, allogeneic hematopoietic stem cell transplantation following myeloablative conditioning; AlloRIC, alloHSCT following reduced intensity conditioning; AutoHSCT, autologous hematopoietic stem cell transplantation; CN-AML, cytogenetically normal-acute myeloid leukemia; *FLT3*-ITD, fms-like tyrosine kinase 3 internal tandem duplication; mut, mutant; *NPM1*, nucleophosmin-1; OS, overall survival; RFS, relapse-free survival, wt, wild-type.

Table 3. Multivariable analysis in molecularly intermediate-risk patients[a]

	OS			*RFS*			*Relapse*			*NRM*		
	HR[a]	*95% CI*	P-*value*	*HR*[a]	*95% CI*	P-*value*	*HR*[a]	*95% CI*	P-*value*	*HR*[a]	*95% CI*	P-*value*
Post-remission treatment												
Auto vs CT	0.93	0.63–1.38	0.72	0.83	0.57–1.20	0.32	0.71	0.48–1.05	0.087	1.60	0.40–6.48	0.50
MAC alloHSCT vs CT	0.86	0.57–1.30	0.48	0.67	0.45–1.00	0.048	0.20	0.12–0.35	<0.001	9.14	2.74–30.42	<0.001
RIC alloHSCT vs CT	0.56	0.34–0.93	0.022	0.50	0.31–0.82	0.004	0.35	0.20–0.62	<0.001	2.54	0.65–9.95	0.16
MAC alloHSCT vs Auto	0.93	0.62–1.38	0.72	0.81	0.55–1.19	0.29	0.29	0.16–0.50	<0.001	5.70	2.33–13.89	<0.001
RIC alloHSCT vs Auto	0.60	0.36–1.00	0.046	0.60	0.37–1.00	0.043	0.49	0.27–0.89	0.014	1.58	0.51–4.88	0.42
Sex (female vs male)	0.99	0.73–1.34	0.94	0.96	0.72–1.29	0.80	1.00	0.71–1.39	0.99	0.81	0.44–1.48	0.48
Age[b]	1.19	1.03–1.37	0.014	1.08	0.95–1.23	0.26	1.07	0.92–1.24	0.37	1.17	0.86–1.60	0.29
WBC at diagnosis (>100 vs $\leqslant 100$)	1.43	0.96–2.14	0.086	1.34	0.90–1.99	0.16	1.93	1.23–3.02	0.006	0.49	0.19–1.25	0.10
CR (late vs early)	1.55	1.09–2.20	0.019	1.51	1.07–2.12	0.022	1.81	1.21–2.70	0.006	1.21	0.63–2.33	0.57

Abbreviations: Allo, allogeneic hematopoietic stem cell transplantation; Auto, autologous hematopoietic stem cell transplantation; CI, confidence interval; CR, complete remission; CT, chemotherapy; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; MAC, myeloablative conditioning; NRM, non-relapse mortality (with event death in first CR and censored at relapse); OS, overall survival (with event death whatever the cause); RFS, relapse-free survival (with event death in first complete CR or relapse); Relapse (with time as RFS and with event relapse and censored at death in first CR); RIC, reduced intensity conditioning; WBC, white blood cell count. [a]The HRs are the estimates of the effect of covariates for each outcome parameter, adjusted for sex, age, CR (late vs early), WBC at diagnosis below or above 100 and the type of post-remission treatment. [b]Linear with estimates of 10 years difference.

patients with AML, with a high allelic burden of *FLT3*-ITD, but improved outcome following alloHSCT has been suggested in patients with a *FLT3*-ITD allelic ratio of >0.50.[10,19,32] In our study, the few surviving patients with a high allelic burden of *FLT3*-ITD were recipients of an alloHSCT in either CR1 or CR2, which compares well with recent results by Ho *et al.*,[19] suggesting improved outcome by alloHSCT.

Studies evaluating PRT by alloHSCT in patients with *FLT3*-ITD irrespective of the allelic ratio reported different results. While a study from the French GOELAMS study group reported improved outcome by alloHSCT,[33] a recent prospective-matched pair study failed to show such a survival benefit.[34] The evaluation of all *FLT3*-ITD patients, including an unknown number of patients with a high allelic ratio, may have impacted on those results, questioning the comparability of those and other studies, focusing on *FLT3*-ITD. We combined patients with a low *FLT3*-ITD allelic ratio (irrespective of *NPM1* mutations) and patients with wild-type *NPM1* without *FLT3*-ITD into an intermediate-risk group because of similar OS and RFS in these subgroups. In that molecularly intermediate-risk group, OS and RFS were significantly better following RIC alloHSCT as compared with chemotherapy, which was confirmed by multivariable analysis stratified by study cohort and following adjustment for covariates. Of note, with a median follow up of 72 months, NRM was low and a graft-vs-leukemia effect was preserved as evidenced by a HR of 0.35 for relapse as compared with chemotherapy. Although MAC alloHSCT showed an even stronger HR of 0.20, the anti-leukemic activity was counterbalanced by a significantly higher NRM (HR 9.14). Although a number of studies have shown a higher relapse rate following RIC alloHSCT as compared with MAC alloHSCT,[35–41] the net effect in terms of OS and RFS in well-defined and sufficiently sized subcategories of AML CR1 patients is still underreported. Here, we show that the balance of a preserved graft-vs-leukemia and a low NRM eventually resulted in favorable outcome in molecularly intermediate-risk AML CR1 recipients, who proceeded to RIC alloHSCT. MAC alloHSCT and autoHSCT yielded similar outcomes in that intermediate-risk category of patients. Most comparative PRT studies in molecular subgroups compare alloHSCT with chemotherapy, but lack a group of autoHSCT recipients. Here, a large subgroup of recipients of an autograft was also included. Although autoHSCT was not significantly associated with improved outcome as compared with chemotherapy or MAC alloHSCT, autoHSCT may provide a valuable alternative PRT in these subgroups, especially in patients lacking a well-matched donor or in patients at higher risk for NRM determined by risk scores.[42–44] In addition, the incorporation of minimal residual disease status assessed by flow cytometry[45,46] or molecular analysis[47] may add to that decision-making by the preferred application of autoHSCT in minimal residual disease negative, molecularly intermediate-risk patients in CR1. Of note, while RFS following autografting estimated 40% in the intermediate-risk group, OS was 47%, indicating that a considerable number of relapsing patients may be rescued by an allograft in CR2, as previously reported in AML patients.[48–50]

Combining results from two cooperative groups may implicate limitations. Although the induction chemotherapeutic regimens varied among the different study groups, all patients received cytarabine-/anthracycline-based chemotherapy, obtained a hematological CR1 within two cycles of induction chemotherapy, and outcome was not significantly different among the different study groups. In addition, differences in PRT approach among the study groups may have resulted in selection bias, although that bias is presumably similar among the three molecularly defined groups in the analysis, which were not differentially approached by the study groups. The analysis presented did not prospectively compare RIC and MAC regimens prior alloHSCT, which withholds us from conclusions in that regard. Given the significant lower NRM associated with RIC, as shown in many studies, the presentation of RIC alloHSCT and MAC alloHSCT as two distinct categories is, however, warranted. The latter notion is supported by results of the prospective randomized US study, showing different outcomes following either conditioning type.[51] Although MAC alloHSCT is associated with a significantly stronger anti-leukemic effect, its counterbalancing effect on NRM need to be taken into account, especially in older patients with comorbidities. Therefore, as advocated before,[5] we prefer to apply either treatment modality in a personalized fashion, tailored by risk factors, predicting NRM.[52]

Collectively, these results suggest that RIC alloHSCT may provide better survival than chemotherapeutic PRT in patients with CN-AML with wild-type *NPM1* without *FLT3*-ITD or *FLT3*-ITD with a low allelic burden. AutoHSCT may be applied if not eligible, if no well-matched donor is available in CR1 or in case of absence of minimal residual disease. Although MAC alloHSCT is associated with the strongest anti-leukemic effect, our results suggest that it might preferentially be applied in patients with an acceptable risk for complications and NRM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

JV, FEMitH, GH and JJC contributed to the study design; all authors provided study materials or patients; all authors were involved in collection and assembly of clinical data; JV, FEMitH, GH and JJC were involved in analysing and interpreting the data and writing this report; and all authors reviewed and approved the final version of the manuscript.

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