REGULAR ARTICLE

blood advances

Early detection of *WT1* measurable residual disease identifies high-risk patients, independent of transplantation in AML

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Key Points

- Postinduction *WT1* measurable residual disease is associated with shorter survival and higher risk of relapse in younger patients with AML.
- Postinduction *WT1* residual disease is an independent prognostic factor in patients eligible for allogeneic stem cell transplantation.

WT1 overexpression is frequently identified in acute myeloid leukemia (AML) and has been reported to be a potential marker for monitoring measurable residual disease (MRD). We evaluated the use of postinduction *WT1* MRD level as a prognostic factor, as well as the interaction between postinduction *WT1* MRD response and the effect of allogeneic stem cell transplantation (allo-SCT) in the first complete remission (CR). In the ALFA-0702 trial, patients with AML, aged 18 to 59, had a prospective quantification of *WT1* MRD. The occurrence of a *WT1* MRD ratio >2.5% in bone marrow or >0.5% in peripheral blood was defined as MRD^{high}, and ratios below these thresholds were defined as MRD^{low}. The prognostic value of MRD after induction chemotherapy was assessed in 314 patients in first CR by comparing the risk of relapse, the relapse-free survival (RFS), and the overall survival (OS). Interaction between MRD response and the allo-SCT effect was evaluated in patients by comparing the influence of allo-SCT on the outcomes of patients with MRD^{high} with those with MRD^{low}. The results showed that patients with MRD^{high} after induction had a higher risk of relapse and a shorter RFS and OS. The MRD response remained of strong prognostic value in the subset of 225 patients with intermediate-/unfavorable-risk AML who were eligible for allo-SCT, because patients with MRD^{high} had a significantly higher risk of relapse resulting in worse RFS and OS. The effect of allo-SCT was higher in patients with MRD^{low} than in those with MRD^{high}, but not significantly different. The early *WT1* MRD response highlights a population of high-risk patients in need of additional therapy.

Introduction

Seventy to 80% of patients with acute myeloid leukemia (AML) achieve complete remission (CR) after front-line induction chemotherapy. However, more than 50% of them eventually relapse, and overall survival (OS) is less than 30%. Currently, the risk classification of patients on the basis of cytogenetics and

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

molecular markers at diagnosis[1] is the main criterion that guides the decision for allogeneic stem cell transplantation (allo-SCT) in younger adult patients in the first CR. However, the prognosis remains heterogeneous, especially for patients in the intermediate group, and relapse may occur before or even after allo-SCT. Detection of measurable residual disease (MRD) is a posttherapeutic tool that helps refine the prognosis of patients at the individual level. The prognostic value of MRD detected by polymerase chain reaction (PCR) or multiparameter flow cytometry (MFC) is well established, and relapse is more likely in patients with detectable MRD.[2,3] However, there is still a lack of standardization, and no common molecular marker is available for all genetic AML subsets.

Quantitative real time-quantitative PCR (qRT-PCR) is a robust technique for quantifying MRD in AML. A fusion gene derived from chromosomal translocations such as mutations in *PML-RARA*, *RUNX1-RUNXT1*, *CBFB-MYH11*, and *NPM1* are sensitive targets for MRD detection with qRT-PCR. However, these markers are absent in 40% of patients aged 15 to 60 years and in nearly 70% of patients aged >70 years.[3] For patients lacking these markers, overexpression of Wilms' tumor gene 1 (*WT1*) is a potential alternative target. *WT1* overexpression is identified in 70% to 90% of patients with AML[4,5] and has been reported as a potential marker for MRD detection in the postremission and the posttransplant settings.[6-8] *WT1* overexpression can be detected in peripheral blood (PB) and in bone marrow (BM) samples with a standardized and reproducible method of qRT-PCR with specific primers and probes described by Cilloni and colleagues in a study of the European LeukemiaNet (ELN) published in 2009.[9] Nevertheless, in contrast to leukemia-specific markers, *WT1* is expressed by normal hematopoietic progenitor cells, which confers relatively limited sensitivity and specificity to *WT1* MRD assessment. Therefore, the significance of *WT1* MRD response as a prognostic and/or treatment-stratifying factor remains under debate.

In the present work, we assessed the value of postremission *WT1* MRD as a prognostic marker on outcomes in a series of younger adults with AML in the first CR who were treated in the prospective, randomized, multicenter ALFA-0702 trial. We also evaluated the interaction between *WT1* MRD response and the effect of allo-SCT in the first CR. The ALFA-0702 study is registered on https://clinicaltrials.gov/ as NCT00932412.

Methods

Patients and treatment

In the ALFA-0702 study, 713 patients aged 18 to 59 years with newly diagnosed de novo AML were enrolled in 33 French centers from April 2009 through August 2013.[10] Patients with acute promyelocytic leukemia, core binding factor AML, and Philadelphia chromosome–positive AML were excluded. All patients received time-sequential induction chemotherapy with daunorubicin (60 mg/m^2 per day on days 1 to 3; 35 mg/m^2 per day on days 8 and 9), cytarabine (500 mg/m^2 per day continuous IV infusion on days 1 to 3 and 1 g/m^2 per 12 hours on days 8 to 10), and granulocyte colony stimulating factor (G-CSF) priming (5 μg/kg per day on days 1 to 10). An optional second induction course with idarubicin (12 mg/m^2 per day on days 1 to 3) and cytarabine (3 g/m^2 per 12 hours on days 1, 3, 5, and 7) was permitted in patients who did not achieve CR after the first course. Patients in CR with intermediate- or unfavorable-risk AML without a sibling or fully 10/10 HLA-matched unrelated donor for allo-SCT stem cell transplantation were randomly assigned for high-dose cytarabine (HDAC) or clofarabine+cytarabine (CLARA) consolidation chemotherapy. HDAC consisted of cytarabine 3 g/m^2, per 12 hours on days 1, 3, and 5 with G-CSF priming, and CLARA consisted of clofarabine 30 mg/m^{2}per day on days 2 to 6, cytarabine 1 g/m^2 per day on days 1 to 5 with G-CSF priming. The ALFA-0702 study protocol was approved in December 2008 by the Institutional Review Board of the French Regulatory Agency and the Ethics Committee Sud-Est IV. All patients gave informed consent for both treatment and genetic analysis before inclusion, according to the Declaration of Helsinki. The median follow-up time was 50 months. All authors had access to primary clinical trial data.

Cytogenetic analysis

Cytogenetic R-banding analysis was performed on diagnostic BM samples by using standard methods. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature recommendations and classified within favorable, intermediate, and unfavorable groups.[1]

Gene mutations analysis

FLT3-internal tandem duplication (*FLT3*-ITD),[11] mutations of *FLT3* tyrosine kinase domain (*FLT3*-TKD [tyrosine kinase domain]; *FLT3*D835/I836),[11] *NPM1* (exon 11),[12] and *CEBPA*,[13] were assessed centrally on genomic DNA, as previously described. In *FLT3*-ITD specimens, the allelic ratio was quantified by GeneScan-based fragment-length analysis and expressed as ITD/wild-type allele ratio.

ALFA risk classification

In the ALFA-0702 trial, patients were classified according to the ALFA risk classification detailed in supplemental Table 1. Genetic risk groups were retrospectively centrally reviewed, and patients were also classified according to the 2017 European LeukemiaNet (ELN) recommendations.[1]

Quantification of *WT1* expression levels

The quantification of *WT1* transcripts was performed on an ABI Prism 7900 platform with the standardized ELN qRT-PCR assay. *WT1* mRNA levels were normalized to the *ABL* control gene by using the respective commercially available plasmid standards (ipsogen WT1 ProfileQuant Kit CE; Qiagen). Results were expressed as the ratio *WT1* copy number/*ABL* copy number × 100. Based on the results of Cilloni and colleagues, who analyzed a large series of samples from patients with AML to determine standardized method and cutoff for *WT1* detection,[9] the upper limit of normal was defined as 2.5% in BM samples or as 0.5% in PB samples, and we decided that *WT1* was overexpressed at AML diagnosis when the *WT1* mRNA level was ≥10-fold the upper limit of normal (ie, 25% in BM samples and 5% in PB samples). The occurrence of a *WT1* MRD ratio >2.5% in BM or >0.5% in PB was defined in this study as MRD with a high ratio (MRD^{high}), whereas a ratio less than these thresholds was defined as MRD with a low ratio (MRD^{low}). Testing for *WT1* and evaluation for CR were based on the same BM and PB samples. All samples were prospectively collected at the date of the evaluation of the response to induction chemotherapy. As recommended in the ALFA-0702 protocol, response to induction

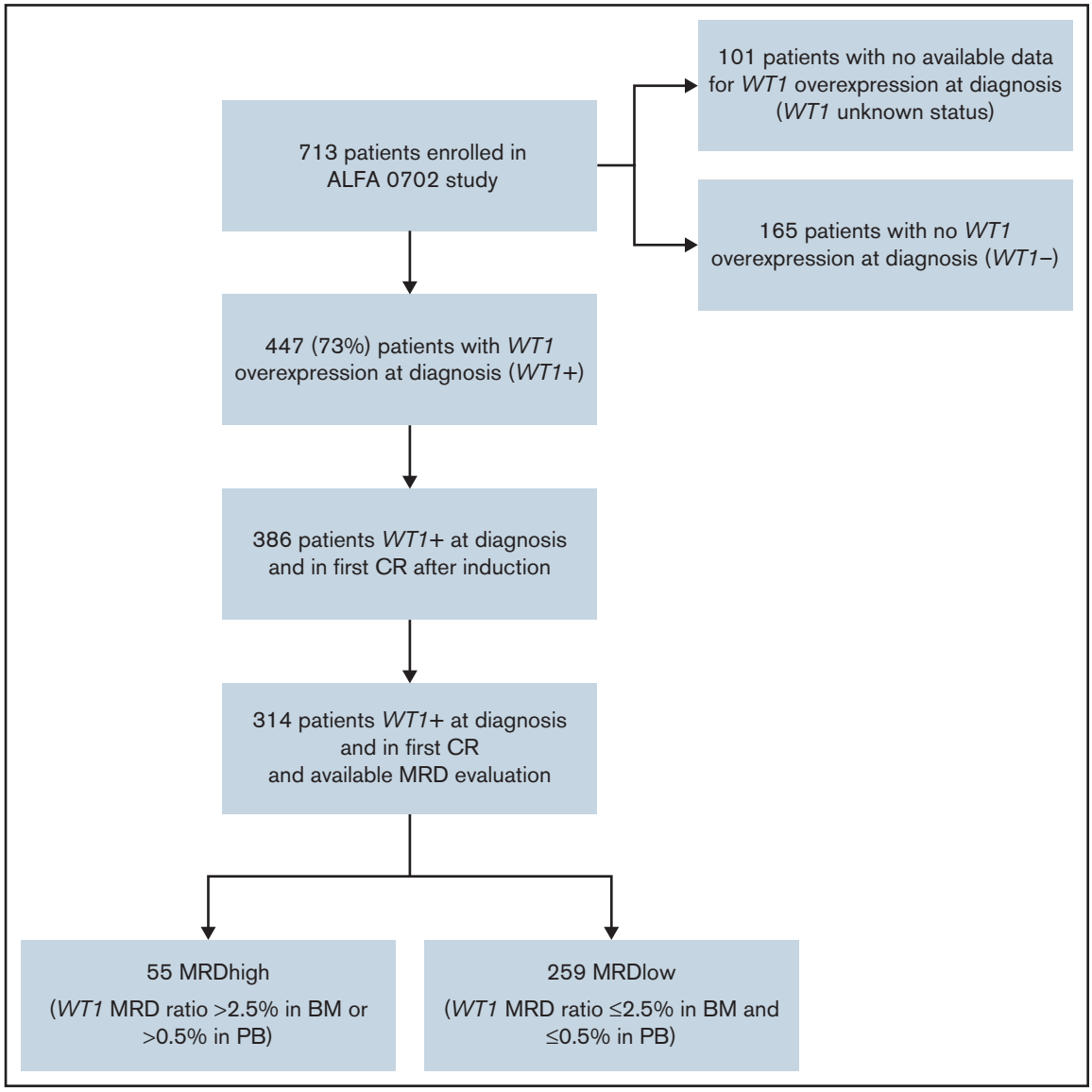

Figure 1. Flowchart. CR, complete remission.

chemotherapy was evaluated between days 28 and 45 after chemotherapy.

Statistical analysis

Qualitative variables are presented as counts and percentages and quantitative variables as median and interquartile range (IQR). Comparison between patients with and without *WT1* MRD after induction chemotherapy was performed using Fisher's exact test for qualitative variables and the Wilcoxon rank sum test for quantitative variables. In addition to CR, CR without platelet recovery and CR with incomplete hematologic recovery were included. Overall survival (OS) was defined as the time between the date of CR achievement and death. Relapse-free survival (RFS) was defined as the time between the date that CR was achieved and the date of first relapse or death without relapse. Data were not censored at the time of allo-SCT. OS and RFS were estimated and plotted using the Kaplan-Meier estimate. Cumulative incidence of relapse (CIR) was estimated within a competing-risk framework, with death without relapse as a competing end point. The median follow-up time was estimated by using reverse Kaplan-Meier. The prognostic value of postinduction *WT1* MRD and several prognostic covariates was assessed on risk of relapse, risk of relapse or death without relapse, and risk of death and by fitting Cox cause-specific proportional hazard models and Cox proportional hazard models are 2 different type of regression models that results in 2 different types of effect size, namely the Cause-specific Hazard ratio (CSHR) and the Hazard Ratio (HR). The independent prognostic value of MRD was assessed by fitting multivariable Cox models for all 3 outcomes adjusted for classic prognostic variables in AML. Interactions between the prognostic effect of *WT1* MRD and other covariates on the 3 outcomes were searched for by adding an interaction term and testing for its significance in Cox models. Results are displayed using a Forest plot.

The impact of allo-SCT on risk of relapse or death without relapse, cause-specific risk of relapse, and risk of death was assessed in the

Table 1. Characteristics of patients studied for *WT1* MRD and comparison of patients with and those without *WT1* MRD assessment after induction chemotherapy

	WT1 MRD available (n = 314)	*WT1* MRD missing (n = 72)	*P*
Male, n (%)	165 (52.5)	40 (55.6)	.695
Median age, y [IQR]	46 [36-54]	48 [40-54.5]	.114
Median WBC count, 10^9/L [IQR]	9.8 [2.71-31.25]	10.75 [3.58-49.73]	.3
ALFA risk classification, n (%)*			.0436
Favorable	79 (26)	27 (39.7)	
Intermediate	131 (43.1)	28 (41.2)	
Unfavorable	94 (30 .9)	13 (19.1)	
ELN 2017 risk classification, n (%)†			.115
Favorable	126 (42.4)	37 (55.2)	
Intermediate	87 (29.3)	18 (26.9)	
Unfavorable	84 (28.3)	12 (17.9)	
NPM1 mutation, n (%)‡	130 (42.2)	42 (60)	.0079
FLT3-ITD, n (%)§	87 (28.2)	20 (28.6)	.99
FLT3-TKD, n (%)||	21 (6.9)	10 (14.3)	.0531
Induction cycle to reach CR, n (%)			.192
1	290 (92.4)	70 (97.2)	
2	24 (7.6)	2 (2.8)	
Postremission randomization, n (%)			.0018
HDAC	62 (19.7)	4 (5.6)	
CLARA	62 (19.7)	10 (13.9)	
Not randomized	190 (60.5)	58 (80.6)	

*Data were not available for 14 patients.
†Data were not available for 22 patients.
‡Data were not available for 8 patients.
§Data were not available for 8 patients.
||Data were not available for 10 patients.

subgroup of patients with intermediate-/unfavorable-risk AML only, because patients with favorable-risk AML according to ALFA risk classification were not eligible for allo-SCT in first CR. In these analyses, allo-SCT was regarded as a time-dependent covariate, and Simon-Makuch methodology was displayed to show its effect on RFS.

All statistical analyses were performed with R software (version 3.5.0). All *P*-values were 2-sided, with $P < .05$ denoting statistical significance.

Results

Patient characteristics

Among the 713 patients enrolled in the ALFA-0702 trial, 447 had *WT1* overexpression at AML diagnosis, 165 had a *WT1*/100*ABL* ratio under the prespecified thresholds, and 101 were not evaluated for *WT1* quantification. In our cohort, 73% (447 of 612) of evaluable patients had an overexpression of *WT1* at diagnosis. After induction chemotherapy, 386 of 447 patients (86.3%) achieved CR. Three hundred sixty patients received 1 induction cycle of chemotherapy, and 26 patients received 2 induction cycles to reach CR. Postinduction *WT1* MRD evaluation was available in 314 of 386 patients (81.3%; Figure 1). Baseline characteristics of these 314 patients are indicated in Table 1. A comparison of the 314 patients with an available *WT1* MRD and the 72 patients with no available *WT1* MRD after induction showed that patients with no available *WT1* MRD are more often in the favorable-risk group and had *NPM1* mutations more often (Table 1).

At diagnosis, *WT1/ABL* ratio was assessed in BM for 106 patients with a median ratio of 69.2% and in PB for 208 patients with a median ratio of 57.4%. Postinduction *WT1* MRD status was assessed only in PB in 45 patients, only in BM in 38 patients, and in both in 231 patients. Among the 231 patients with MRD assessed in both samples, we observed only 20 discrepancies (9%). All of those patients were considered to have MRD with a high ratio; thus, a patient was considered MRD^{high} if at least 1 sample was above the prespecified thresholds and MRD^{low} if both samples where below the thresholds.

Analysis of prognostic factors on outcomes in the whole patient cohort

Baseline factors associated with a higher risk of relapse, a worse RFS, and a worse OS were age, white blood cell (WBC) count, unfavorable-risk AML according to both ALFA and ELN2017 risk classification, absence of *NPM1* mutation (except for OS, not statistically significant), presence of *FLT3*-ITD mutation and MRD with a high ratio. The number of cycles to reach CR had no significant effect on risk of relapse, RFS, and OS in our cohort. *FLT3*-TKD

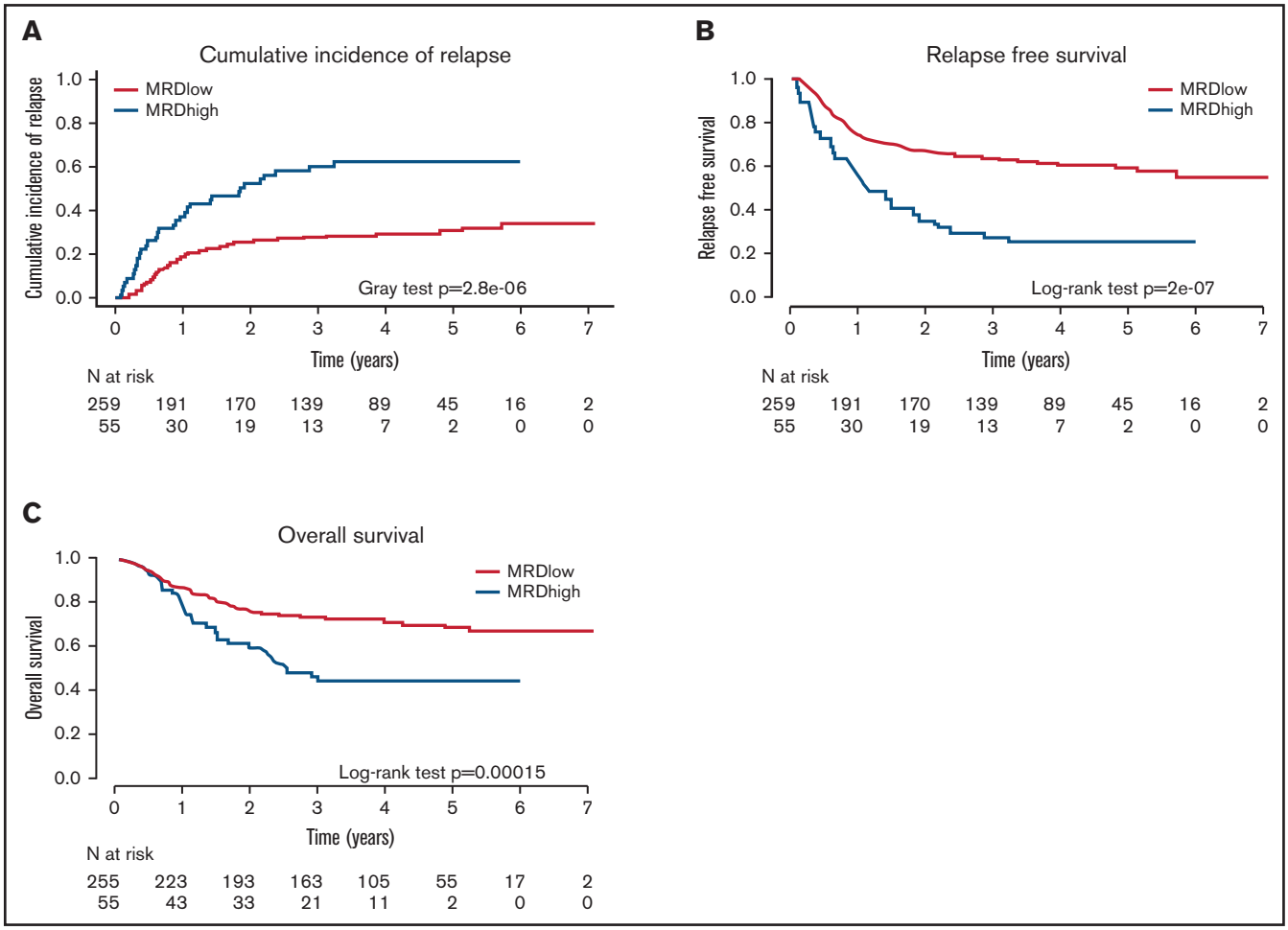

Figure 2. CIR, RFS, and OS according to *WT1* MRD status in the whole cohort when not censured at time of allo-SCT. CIR (A), RFS (B), and OS (C). Blue line: MRD^{high}, patients with *WT1*/100*ABL* >2.5% in BM or >0.5% in PB. Red line: MRD^{low}, patients with *WT1*/100*ABL* <2.5% in BM or <0.5% in PB.

mutation had no prognostic impact in our cohort. Univariable analyses for relapse, RFS, and OS are detailed in supplemental Table 2.

Prognostic impact of postinduction *WT1* MRD on risk of relapse and survival in the whole cohort

Among 314 patients with available postinduction MRD, 259 were MRD^{low} and 55 were MRD^{high}. At this early time point, MRD was predictive of subsequent relapse: 4-year CIR was 29% in patients with MRD^{low} vs 61% in patients with MRD^{high} (cause-specific hazard ratio [CSHR] 2.82; 95% confidence interval [CI], 1.87-4.25; $P < .0001$; supplemental Table 2; Figure 2A). When adjusted for age, WBC count, and ALFA risk classification, MRD^{high} remained independently associated with risk of relapse (CSHR, 2.2; 95% CI, 1.44-3.36; $P = .0003$; Table 2). The effect of MRD was unchanged when the patients were classified according to the ELN2017 risk classification (supplemental Table 3). Results for CIR according to *WT1* MRD status in the whole cohort, when censured at the time of allo-SCT, are shown in supplemental Figure 1A.

A higher risk of relapse of patients with MRD^{high} resulted in shorter survival. At 4 years, RFS was 60% for MRD^{low} vs 26% for MRD^{high} (HR, 2.56; 95% CI, 1.77-3.70; $P < .0001$, supplemental Table 2; Figure 2B), and OS was 71% vs 44% for MRD^{low} and MRD^{high}, respectively (HR, 2.22; 95% CI, 1.45-3.40; $P = .0005$; supplemental Table 2; Figure 2C). When adjusted for age, WBC count, and ALFA risk classification, MRD^{high} remained independently associated with worse RFS (HR, 2.05; 95% CI, 1.40-3.00; $P = .0002$) and worse OS (HR, 1.7; 95% CI, 1.10-2.63; $P = .018$, Table 2). The effect of MRD was unchanged when patients were classified according to the ELN2017 risk classification, except for OS, which was not statistically significant (supplemental Table 3). Results for RFS and OS, according to *WT1* MRD status in the whole cohort when censured at the time of allo-SCT, are shown in supplemental Figure 1B-C.

Finally, we evaluated the prognostic impact of postinduction *WT1* MRD in specific patient subgroups (Figure 3). There was no statistically significant interaction between MRD and the other covariates for relapse, RFS, and OS (Figure 3A-C, respectively) except for age, with the negative effect of MRD^{high} being less important in patients >50 years of age.

Prognostic impact of postinduction *WT1* MRD in patients eligible for allo-SCT

We determined the prognostic impact of postinduction *WT1* MRD outcomes in patients with intermediate-/unfavorable-risk AML who

Table 2. Prognostic factors on relapse, RFS, and OS in multivariate analyses in the whole cohort and AML risk according to ALFA classification

	Risk of relapse			RFS			OS		
	CSHR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
WT1 MRD			.0003			.0002			.018
Low ratio	1.0	–		1.0	–		1.0	–	
High ratio	2.2	1.44-3.36		2.05	1.4-3		1.7	1.1-2.63	
Age effect for 1-y increase	1.03	1.01-1.05	.0032	1.03	1.01-1.04	.0015	1.04	1.02-1.06	.0002
WBC effect for 1 × 10^9/L increase	1.0	1-1.01	.0011	1.0	1-1.01	.0018	1.01	1-1.01	.0002
ALFA risk classification			<.0001			<.0001			<.0001
Favorable	1	–	1		–		1	–	
Intermediate	3.23	1.69-6.14		2.53	1.5-4.25		2.99	1.54-5.83	
Unfavorable	4.5	2.28-8.9		3.4	1.96-5.9		4.79	2.39-9.57	

WT1 MRD high ratio: *WT1*/100*ABL* >2.5% in BM or >0.5% in PB; *WT1* MRD low ratio: patients with *WT1*/100*ABL* <2.5% in BM or <0.5% in PB.

are eligible for allo-SCT and the interaction between postinduction WT1 MRD and allo-SCT by analyzing the impact in a subset of 225 patients. In the ALFA-0702 trial, the decision to perform allo-SCT was based on the risk defined by ALFA risk classification; thus, we performed univariate and multivariate analyses with ALFA risk classification only.

In this subgroup, 177 patients were MRD^{low} and 48 patients were MRD^{high}. allo-SCT was performed in 142 patients (115 MRD^{low} and 27 MRD^{high}), but not in the 83 remaining patients (62 MRD^{low} and 21 MRD^{high}). In univariate analyses, patients with MRD^{high} had a higher risk of relapse (CSHR, 2.28; 95% CI, 1.47-3.52; $P = .0005$; supplemental Table 4), worse RFS (HR, 2.12; 95% CI, 1.43-3.14; $P = .0004$; supplemental Table 4), and worse OS (HR, 1.76; 95% CI, 1.12-2.76; $P = .0184$; supplemental Table 4). When adjusted for age, WBC count, and ALFA risk classification, *WT1* MRD with a high ratio after induction chemotherapy was still associated with a higher risk of relapse (CSHR, 2.38; 95% CI, 1.53-3.71; $P = .0001$; Table 3) and shorter RFS and OS (RFS, HR, 2.16; 95% CI, 1.45-3.22; $P = .0002$; OS, HR, 1.72; 95% CI, 1.08-2.73; $P = .022$; Table 3). Despite a higher effect of allo-SCT in patients with MRD^{low}, the interaction between MRD status and the effect of allo-SCT was not statistically significant for RFS (HR, 0.38; 95% CI, 0.24-0.60, for patients with MRD^{low} vs HR, 0.52; 95% CI, 0.26-1.11, for patients with MRD^{high}; $P = .48$; Figure 4), OS (HR, 0.48; 95% CI, 0.29-0.79, for patents with MRD^{low} vs HR, 0.88; 95% CI, 0.41-1.93, for patients with MRD^{high}; $P = .12$) or for risk of relapse (HR, 0.23; 95% CI, 0.13-0.39, for patients with MRD^{low} vs HR, 0.39; 95% CI, 0.17-0.88 for patients with MRD^{high}; $P = .32$). This result means that the beneficial effect of allo-SCT was similar in postinduction patients with *WT1* MRD^{high} and MRD^{low} and also that the prognostic effect of postinduction *WT1* MRD^{high} persisted even after allo-SCT.

Discussion

In AML, the detection of patients at high risk of relapse is a major issue. We evaluated the effect of postinduction MRD assessed by *WT1/ABL* ratio measured with a standardized method of qRT-PCR[9] on outcomes and the interaction between MRD status and allo-SCT in a cohort of younger patients prospectively enrolled in the ALFA-0702 trial. Our results show that *WT1* MRD^{high} after induction chemotherapy was significantly associated with higher risk of relapse and shorter survival, independent of pretherapeutic prognostic factors, such as age, WBC count, and AML risk classification. Several studies have shown a correlation between detectable *WT1* MRD and clinical outcomes.[4,9,14-18] Although comparison between these studies is hindered by a lack of standardization in sample source, time-point evaluation, and thresholds of positivity, most of the studies showed that *WT1* MRD predicts relapse and survival. There are some limits to our study. One of them is the absence of a universally accepted threshold for *WT1* positivity that led us to choose the cutoff values proposed by Cilloni and colleagues.[9] Another is the missing data for *WT1/ABL* ratio in 19% of patients in first CR and is informative for this marker at diagnosis. However, our results confirm the prognostic value of *WT1* MRD monitoring using a standardized method of qRT-PCR and prespecified thresholds in the evaluable population.

Because we found that *WT1* MRD was predictive of relapse and survival in the whole cohort, we investigated the relevance of *WT1* MRD in patients eligible for allo-SCT. In this population, we showed that MRD^{high} after induction was still an independent prognostic factor. Other studies reported worse outcomes in patients undergoing allo-SCT when *WT1* MRD was detectable before transplantation.[15,18-22] In our study, we showed that early detection of *WT1* MRD predicts outcomes independent of allo-SCT and allows for early identification of patients with a high risk of relapse. This observation may help in selecting patients who could benefit from more intensive treatment before allo-SCT (for example, with the addition of a targeted therapy or new agents). Immunomodulation after allo-SCT, with rapid decrease of immunosuppression and/or donor lymphocyte infusion planning are also therapeutic options, as well as maintenance therapy.

Interestingly, we found no interaction between *WT1* MRD status and allo-SCT, whereas in a prior study of the ALFA group, Balsat et al found that a 4-log decrease in postinduction *NPM1* MRD was a strong prognostic factor in patients with mutations in patients with *NPM1* treated in the ALFA-0702 study and observed that those

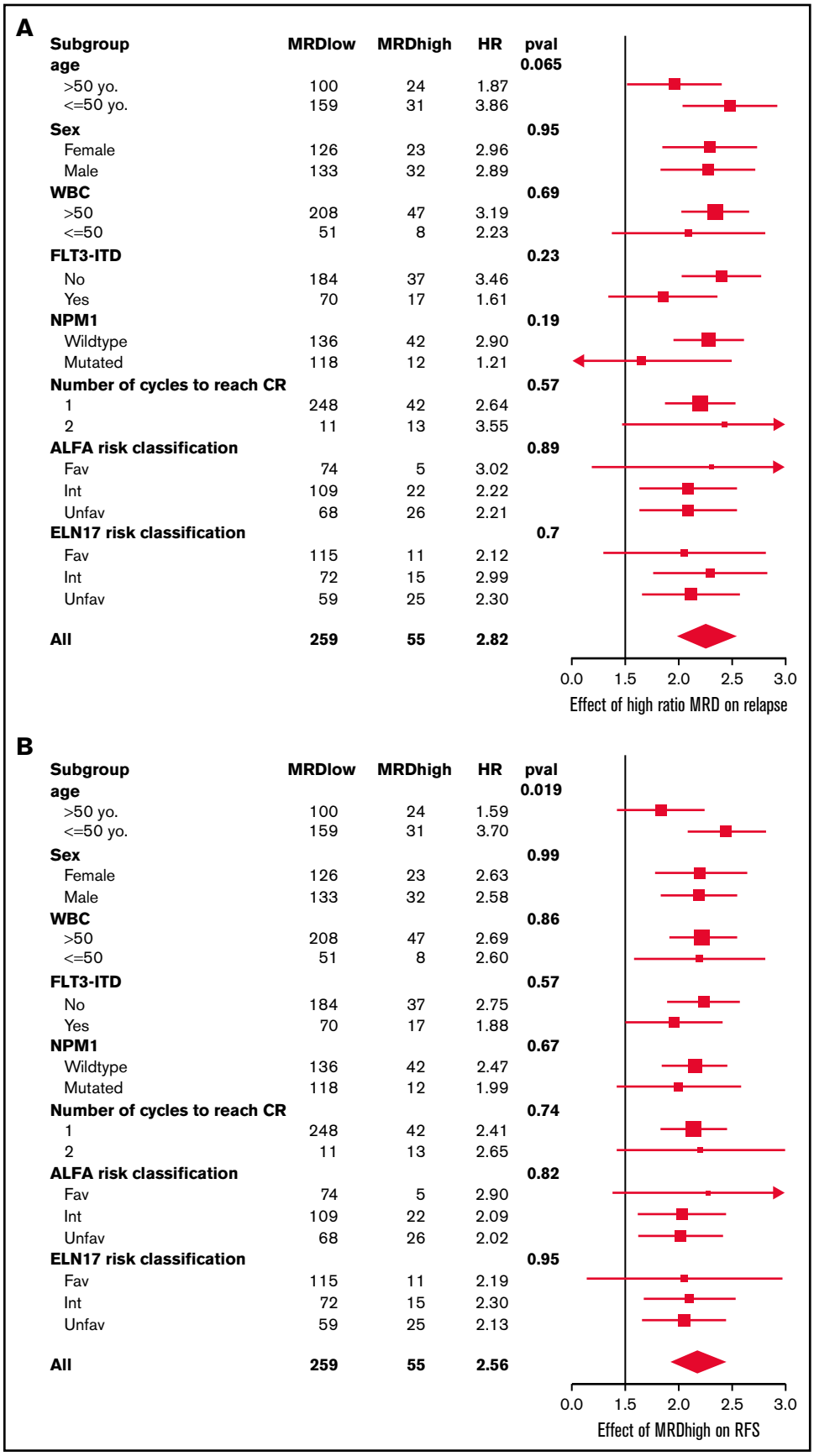
A
Subgroup MRDlow MRDhigh HR pval
age 0.065
>50 yo. 100 24 1.87
<=50 yo. 159 31 3.86
Sex 0.95
Female 126 23 2.96
Male 133 32 2.89
WBC 0.69
>50 208 47 3.19
<=50 51 8 2.23
FLT3-ITD 0.23
No 184 37 3.46
Yes 70 17 1.61
NPM1 0.19
Wildtype 136 42 2.90
Mutated 118 12 1.21
Number of cycles to reach CR 0.57
1 248 42 2.64
2 11 13 3.55
ALFA risk classification 0.89
Fav 74 5 3.02
Int 109 22 2.22
Unfav 68 26 2.21
ELN17 risk classification 0.7
Fav 115 11 2.12
Int 72 15 2.99
Unfav 59 25 2.30
All 259 55 2.82
0.0 1.5 2.0 2.5 3.0
Effect of high ratio MRD on relapse
B
Subgroup MRDlow MRDhigh HR pval
age 0.019
>50 yo. 100 24 1.59
<=50 yo. 159 31 3.70
Sex 0.99
Female 126 23 2.63
Male 133 32 2.58
WBC 0.86
>50 208 47 2.69
<=50 51 8 2.60
FLT3-ITD 0.57
No 184 37 2.75
Yes 70 17 1.88
NPM1 0.67
Wildtype 136 42 2.47
Mutated 118 12 1.99
Number of cycles to reach CR 0.74
1 248 42 2.41
2 11 13 2.65
ALFA risk classification 0.82
Fav 74 5 2.90
Int 109 22 2.09
Unfav 68 26 2.02
ELN17 risk classification 0.95
Fav 115 11 2.19
Int 72 15 2.30
Unfav 59 25 2.13
All 259 55 2.56
0.0 1.5 2.0 2.5 3.0
Effect of MRDhigh on RFS

Figure 3.

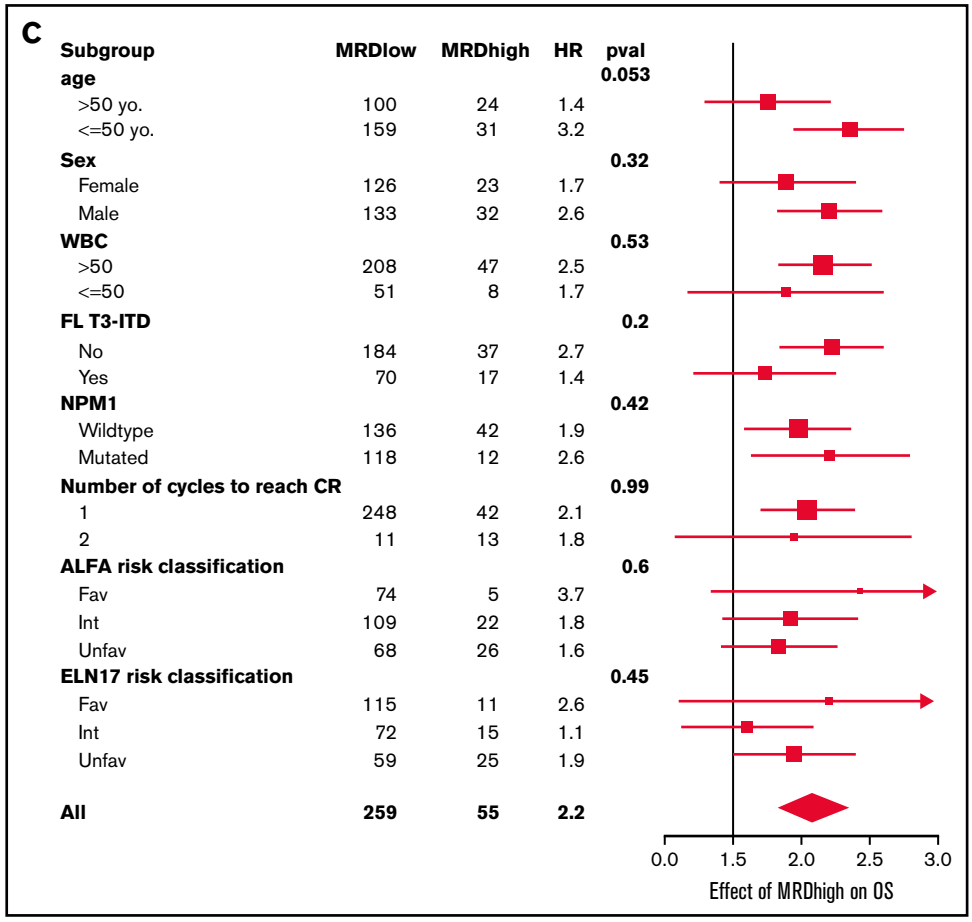

Figure 3. (Continued) Forest plot of the effect of MRD on relapse, RFS, and OS in the whole cohort for various subgroups. Relapse (A), RFS (B), and OS (C). MRD^{high}: patients with *WT1*/100*ABL* >2.5% in BM or >0.5% in PB. MRD^{low}, patients with *WT1*/100*ABL* <2.5% in BM or <0.5% in PB. HR, hazard ratio. WBC count, 10^9/L. *P*val: *P*-value; Yo, years old; Fav, favorable; Int, intermediate; Unfav, unfavorable. For *NPM1* and *FLT3*-ITD mutations: 0, not mutated, and 1, mutated.

patients did not benefit from allo-SCT.[23] Our study may not be powerful enough to show such an interaction, but this discrepancy could also be explained by the difference in sensitivity of these 2 markers. Because of the background of normal bone marrow cells, quantification of MRD by *WT1* cannot reach the sensitivity and the specificity of leukemia-specific markers, such as mutated *NPM1*.[24]

Table 3. Prognostic factors of relapse, RFS, and OS in multivariate analyses in patients with intermediate/unfavorable AML risk

	Risk of relapse			RFS			OS		
	CSHR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
***WT1* MRD**			.0001			.0002			.022
Low ratio	1.00			1.00	–		1.00	–	
High ratio	2.38	1.53-3.71		2.16	1.45-3.22		1.72	1.08-2.73	
Allo-SCT time-dependent			<.0001			<.0001			.021
No	1.00	–		1.00	–		1.00	–	
Yes	0.25	0.16-0.4	0.40	0.27-0.61	0.6	0.39-0.93			
Age effect for 1-y increase	1.03	1-1.05	.014	1.03	1.01-1.04	.0051	1.04	1.01-1.06	.0007
WBC effect for 1 × 10^9/L increase	1.01	1-1.01	<.0001	1.01	1-1.01	<.0001	1.01	1-1.01	<.0001
ALFA risk classification			.0061			.011			.0077
Intermediate	1.00	–		1.00	–		1.00	–	
Unfavorable	1.86	1.19-2.9		1.66	1.12-2.45		1.81	1.17-2.81	

AML risk according to ALFA classification supplemental Table 1. *WT1* MRD high ratio, patients with WT1/100*ABL* >2.5% in BM or >0.5% in PB. *WT1* MRD low ratio, patients with *WT1*/100*ABL* <2.5% in BM or <0.5% in PB.

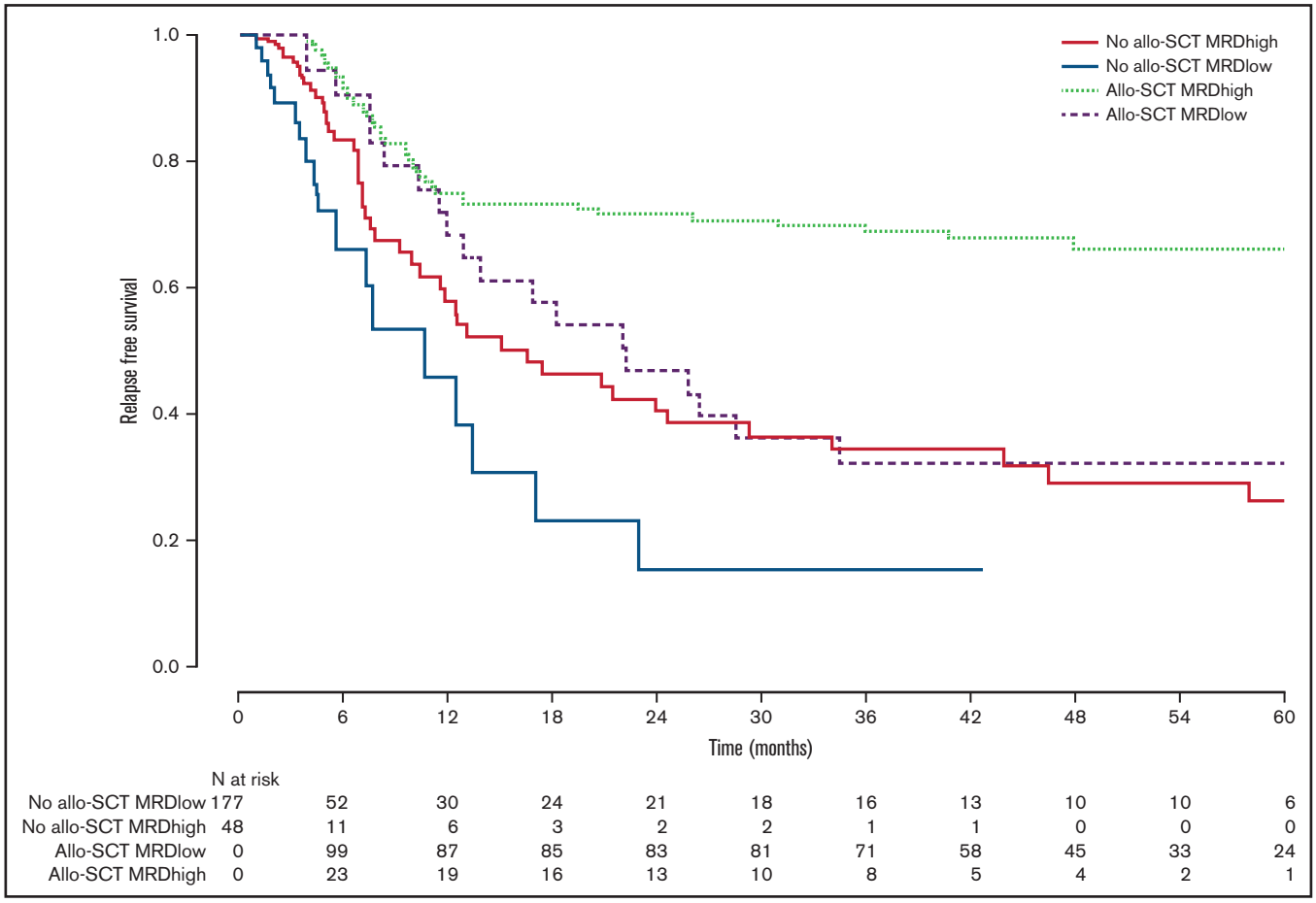

Figure 4. Simon-Makuch plots for RFS according to *WT1* MRD and postremission therapy (allo-SCT or no allo-SCT) in patients with intermediate-/unfavorable-risk AML. MRD^{high}, patients with *WT1*/100*ABL* >2.5% in BM or >0.5% in PB. MRD^{low}, patients with *WT1*/100*ABL* <2.5% in BM or <0.5% in PB.

Our results do not support avoiding allo-SCT in patients with *WT1* MRD^{low}.

Our results of MRD monitoring according to *WT1* ratio are close to those reported with MRD quantified by MFC. The persistence of a detectable leukemia–associated immunophenotype after induction[25,26] is also associated with a high risk of relapse, and the sensitivity of MFC is equivalent to that of *WT1* detection by qRT-PCR. Malagola et al reported results using both methods to detect MRD in a small cohort of patients with AML <70 years of age and found that both had predictive value for RFS.[27] Although detection of *NPM1* mutations for MRD is sensitive enough to identify a population of good responders who do not benefit from allo-SCT, *WT1* MRD s identification of poor responders with a high risk of relapse. The observation that patients with *WT1* MRD^{high} after induction have a poor prognosis despite allo-SCT raises questions about their complete remission status. A recent study showed that survival outcomes of patients in morphological partial response are similar to those from patients with a positive MFC-MRD after induction chemotherapy in young adults with AML.[28] A study in myelodysplastic syndrome supported the value of *WT1* level transcripts as a surrogate marker of tumor burden and showed that patients with high *WT1* levels during stable disease had a higher disease progression rate and a more frequent AML evolution.[29] A recent study found an important decrease in *WT1* levels by using gemtuzumab ozogamicin in addition to chemotherapy in first-line treatment and highlighted the debulking potential of this regimen.[30] Taken together, these results support that the persistence of *WT1* MRD after induction chemotherapy could be a surrogate marker of partial remission.

In 2017, the ELN panel defined a new response criterion based on MRD, which is "CR with negative MRD." The panel recommended the preferential use of MFC or fusion transcripts/*NPM1* mutation to detect MRD but did not mention the *WT1* ratio.[31] MFC is a valuable tool to assess MRD in AML; however, fresh samples, a trained biologist, and standardization of the method are necessary. Detection of *WT1* can be performed on frozen samples with a robust qRT-PCR method in a time frame compatible with clinical practice. Our results support the potential of *WT1* as a surrogate marker of CR, given that we showed the strong prognostic value of *WT1* MRD on outcomes. The *WT1* ratio may be useful to identify patients more accurately who have high risk of relapse after induction than cytomorphological BM assessment.

In summary, our data support that early detection of the *WT1* MRD ratio is an important tool for MRD monitoring, especially in patients who lack a more sensitive marker, and could influence the treatment strategy in young adults with AML.

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Authorship

Contribution: Juliette Lambert, Jerome Lambert, H.D., C.P., and N.D. designed the study; Juliette Lambert, X.T., J.-B.M., C.R., E.R., A.P., C.B., S.C., K.-C.L., N.B., P.R., and H.D. collected and analyzed the clinical data; A.M.-R., A.R., E.C., S.H., C.T., C.P., and N.D. collected and analyzed the biological data; Juliette Lambert and Jerome Lambert performed the data analyses; Juliette Lambert, Jerome Lambert, and H.D. wrote the manuscript; and all authors approved the manuscript and the submission.

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