

# Gemtuzumab Ozogamicin in *NPM1*-Mutated Acute Myeloid Leukemia: Early Results From the Prospective Randomized AMLSG 09-09 Phase III Study

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## abstract

**PURPOSE** High CD33 expression in acute myeloid leukemia (AML) with mutated *NPM1* provides a rationale for the evaluation of gemtuzumab ozogamicin (GO) in this AML entity. We conducted a randomized trial to evaluate GO in combination with intensive induction and consolidation therapy in *NPM1*-mutated AML.

**PATIENTS AND METHODS** Between May 2010 and September 2017, patients  $\geq 18$  years old and considered eligible for intensive therapy were randomly assigned up front for induction therapy with idarubicin, cytarabine, etoposide, and all-*trans*-retinoic acid with or without GO. The early ( $P = .02$ ) primary end point of event-free survival (EFS) was evaluated 6 months after completion of patient recruitment.

**RESULTS** Five hundred eighty-eight patients were randomly assigned (standard arm,  $n = 296$ ; GO arm,  $n = 292$ ). EFS in the GO arm was not significantly different compared with that in the standard arm (hazard ratio, 0.83; 95% CI, 0.65 to 1.04;  $P = .10$ ). The early death rate during induction therapy was 10.3% in the GO arm and 5.7% in the standard arm ( $P = .05$ ). Causes of death in both arms were mainly infections. The cumulative incidence of relapse (CIR) in patients achieving a complete remission (CR) or CR with incomplete hematologic recovery (CRI) was significantly reduced in the GO arm compared with the standard arm ( $P = .005$ ), with no difference in the cumulative incidence of death ( $P = .80$ ). Subgroup analysis revealed a significant beneficial effect of GO in female, younger ( $\leq 70$  years), and *FLT3* internal tandem duplication–negative patients with respect to EFS and CIR.

**CONCLUSION** The trial did not meet its early primary end point of EFS, mainly as a result of a higher early death rate in the GO arm. However, in patients achieving CR/CRI after induction therapy, significantly fewer relapses occurred in the GO compared with the standard arm.

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## ASSOCIATED CONTENT

## Appendix

## Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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## INTRODUCTION

Mutations in the nucleophosmin-1 (*NPM1*) gene occur in approximately 20%-33% of adult patients with acute myeloid leukemia (AML)<sup>1-4</sup> and are present in all age groups.<sup>4,5</sup> AML with mutated *NPM1* has been included in the 2008 WHO classification as an AML disease entity.<sup>6</sup> In the pivotal study describing *NPM1* mutations in AML,<sup>1</sup> an association between high CD33 expression and the mutation was reported and confirmed in subsequent work.<sup>7,8</sup> Furthermore, AML with mutated *NPM1* in the absence of an *FLT3* internal tandem duplication (ITD) has been reported to be associated with a favorable prognosis<sup>2</sup> and grouped

in the low-risk group of current guidelines.<sup>9,10</sup> Even in older patients, mutated *NPM1* is associated with a favorable prognosis, and these patients may particularly benefit from intensive induction chemotherapy.<sup>3,11-14</sup> Beyond chemotherapy sensitivity, AML with mutated *NPM1* may benefit from the addition of all-*trans*-retinoic acid (ATRA) to induction and consolidation therapy.<sup>3,15</sup> Recently, gemtuzumab ozogamicin (GO) was approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of newly diagnosed CD33-positive AML in combination with induction and consolidation therapy.<sup>16,17</sup> Of note, subset analysis of the pivotal ALFA 0701 study

indicated that AML with mutated *NPM1*, in particular, may benefit from GO with regard to event-free survival (EFS) and overall survival (OS).<sup>17</sup> The aim of this prospective, randomized, open-label, multicenter, phase III trial (AMLSSG 09-09) was to determine the effect of GO added to induction and first consolidation therapy in adult patients with *NPM1*-mutated AML.

## PATIENTS AND METHODS

### Patients

Screening for *NPM1* mutations was performed in patients with newly diagnosed AML within the AMLSSG BiO study.<sup>4</sup> Between May 2010 and September 2017, 1,619 adult patients age  $\geq 18$  years screened positive for mutated *NPM1*, and 600 patients (37%) were enrolled in the AMLSSG 09-09 study. Two hundred thirty patients exhibiting mutated *NPM1* in combination with *FLT3*-ITD participated in the AMLSSG 16-10 study, which was open for accrual between June 2012 and May 2016<sup>18</sup>; 144 patients exhibiting mutated *NPM1* not eligible for intensive chemotherapy were enrolled in the AMLSSG 15-10 trial (ClinicalTrials.gov identifier: [NCT01237808](https://clinicaltrials.gov/ct2/show/study/NCT01237808)). Details of inclusion and exclusion criteria are available in the Appendix (online only). The protocol was approved by the lead ethics review committee and registered at [clinicaltrialsregister.eu](https://clinicaltrialsregister.eu) (EudraCT No.: 2009-011889-28) and [ClinicalTrials.gov](https://clinicaltrials.gov) (ClinicalTrials.gov identifier: [NCT00893399](https://clinicaltrials.gov/ct2/show/study/NCT00893399)).

### Genetic Analyses

Details of genetic analysis are available in the Appendix.

### Study Design

**Induction therapy.** From May 2010 to September 2017, patients were randomly assigned (1:1) to receive induction chemotherapy without GO (standard arm) or with GO (3 mg/m<sup>2</sup> intravenously [IV] on day 1; GO arm). Induction therapy consisted of 2 cycles idarubicin (12 mg/m<sup>2</sup> IV on days 1, 3, and 5 [in induction cycle 2 and for patients > 60 years old, reduced to days 1 and 3]), cytarabine (100 mg/m<sup>2</sup> continuously IV on days 1 to 7), and etoposide (100 mg/m<sup>2</sup> IV on days 1 to 3 [in induction cycle 2 and for patients > 60 years old, reduced to days 1 and 3]; ICE) plus ATRA (45 mg/m<sup>2</sup> orally [PO] on days 6 to 8 and 15 mg/m<sup>2</sup> PO on days 9 to 21) with or without GO.<sup>15</sup> Etoposide was reduced from 3 to 2 days for all patients in induction cycle 2 by amendment in April 2011 as a result of prolonged hematologic recovery in both arms. Patients with refractory disease after the first induction cycle went off study treatment and entered observational follow-up.

**Consolidation therapy.** Patients who achieved a complete remission (CR) or CR with incomplete hematologic recovery (CRi) after induction therapy received consolidation therapy. Patients were assigned to 3 cycles of high-dose cytarabine plus ATRA, with cytarabine (age 18-60 years, 3 g/m<sup>2</sup> every 12 hours on days 1 to 3; age > 60 years, 1 g/m<sup>2</sup> every 12 hours on days 1 to 3),<sup>19</sup> ATRA (15 mg/m<sup>2</sup>/d PO

on days 4 to 21), and pegfilgrastim (6 mg subcutaneously) on day 8 in both study arms. Patients in the GO arm received GO (3 mg/m<sup>2</sup> IV on day 1) in first consolidation therapy.

### Definition of Response Criteria, Survival End Points, and Hematologic Recovery

Events for EFS defined according to the protocol were no CR or CRi within 60 days, relapse from CR or CRi, or death from any cause; patients not known to have any of these events were censored at the date they were last examined. The definition of EFS was modified for this analysis according to the European LeukemiaNet (ELN) recommendations,<sup>9</sup> replacing the criterion of no CR or CRi within 60 days with no CR or CRi after induction therapy. Cumulative incidence of relapse (CIR) and cumulative incidence of death (CID) were defined according to ELN 2017 recommendations.<sup>9</sup> Definitions of response criteria and hematologic recovery are available in the Appendix.

### Sample Size Planning and Statistical Analysis

Originally, the study was planned to show an improvement in EFS of 15% after 2 years from 37% to 52% (hazard ratio [HR], 0.66; 2-sided log-rank test;  $\alpha = .05$ ; power, 80%; dropout rate, 5%), resulting in a number of 179 required events and a corresponding sample size of 276 patients. Given the dose reduction in induction and consolidation chemotherapy for patients older than 60 years, randomization and subsequent analysis were stratified for this age cutoff. In 2013, the study was amended based on data from the ALFA 0701 trial published in 2012,<sup>17</sup> with OS as primary end point and an interim futility analysis after 276 patients were randomly assigned (October 31, 2013) based on conditional power (cutoff for continuation, > 30%; observed, 39.7%). Corresponding sample size planning for an OS improvement of 10% from 40% to 50% after 4 years as clinically meaningful estimated a number of 405 required events and hence a sample size of 588 patients ( $\alpha = .05$ ; power, 80%; dropout rate, 5%). Sequential testing was implemented using the fallback procedure of Wiens<sup>20</sup> of EFS at 6 months at a significance level of 2% and OS at 4 years at a significance level of 3% after inclusion of the last patient. Hence, EFS was tested at a significance level of 2% 6 months after the last patient was randomly assigned. If the null hypothesis for EFS could be rejected at this time point, OS was tested at a significance level of 5% 4 years after the last patient was randomly assigned; otherwise, it was tested at a significance level of 3%. Here, we report on the EFS evaluation 6 months after the last patient was randomly assigned.

Pairwise comparisons were performed using the Mann-Whitney or Kruskal-Wallis test for continuous variables and the Fisher's exact test for categorical variables. Univariable and multivariable logistic regression models were applied to investigate the influence of covariates on response to induction therapy. The primary end point of this analysis was

EFS; exploratory end points were CIR, CID, and toxicity. To evaluate the primary objective, a stratified log-rank test was performed with a significance level of 2% and age as a stratification factor. For CIR and CID, equality of cumulative incidences between the treatment groups was evaluated using Gray's test<sup>21</sup> stratified by age. Effect estimates for EFS are given by HRs plus corresponding 95% CIs. The median duration of follow-up was calculated using the reverse Kaplan-Meier estimate,<sup>22</sup> and the Kaplan-Meier method was used to estimate the distributions of EFS.<sup>23</sup> Estimation of CIR and CID was done using the Aalen-Johansen estimator.<sup>24</sup>

In addition to the univariable test, exploratory analyses were performed to investigate whether the treatment effect differed for subgroups. To this aim, a multivariable Cox proportional hazards model and a cause-specific hazards models were fitted including the following covariates: age, sex, WBC count, type of AML (de novo v therapy-related or secondary AML), normal karyotype, *FLT3*-ITD, *FLT3* tyrosine kinase domain, and *DNMT3A*. Modified covariates<sup>25</sup> were used to model interaction effects of the covariates of interest and treatment without having to consider main effects. The subgroup results of (cause-specific) proportional hazards models are summarized in forest plots.

For all hypotheses that were not tested using the fallback procedure of Wiens,<sup>20</sup>  $P < .05$  was considered statistically significant. All statistical analyses were performed with the statistical software environment R, version 3.5.1, using the R packages rms, version 5.1-2; prodlim, version 2018.04.18; survival, version 2.43-3; riskRegression, version 2018.10.03; and cmprsk, version 2.2-7.<sup>26</sup>

## RESULTS

### Patients and Baseline Characteristics

Of 600 patients enrolled, 12 patients (2%) were excluded from the analysis, 6 as a result of violation of inclusion or exclusion criteria and 6 as a result of early (within 5 days) withdrawal of informed consent (Fig 1). Of 588 patients, 296 were randomly assigned to the standard arm, and 292 were assigned to the GO arm. Table 1 lists patient demographics and presenting laboratory and genetic characteristics according to treatment arms, which were well balanced except for hemoglobin, which had significantly higher values in the standard arm compared with the GO arm. Median age was 58.7 years (range, 18.4-82.3 years). Before initiation of first induction therapy, 3 patients died, 2 in the GO arm and 1 in the standard arm.

### Induction Therapy

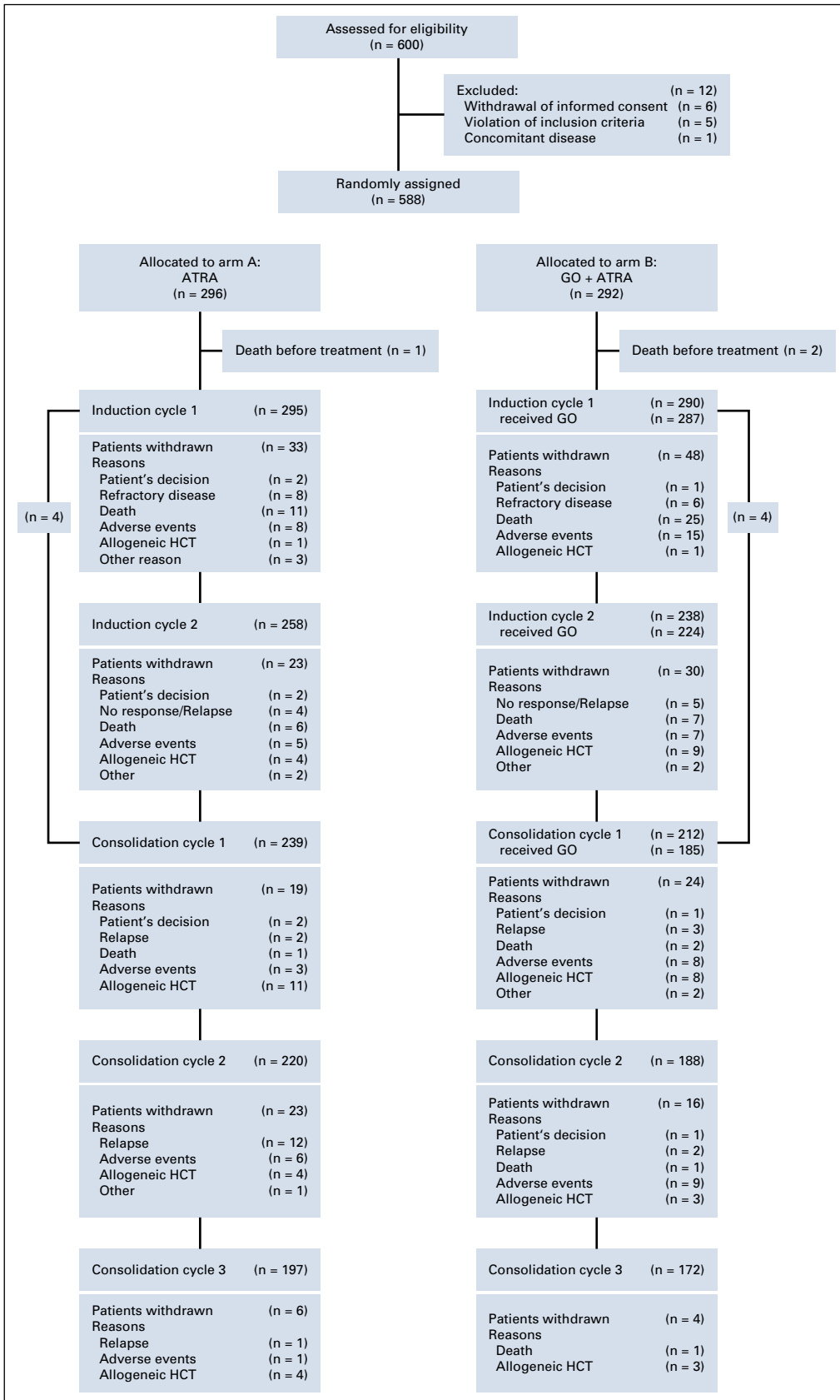
First induction therapy was started in 295 and 290 patients in the standard arm and GO arm, respectively. GO was not added to induction therapy in 3 patients in the GO arm. The rates of observed adverse events categorized according to Common Terminology Criteria for Adverse Events during first induction therapy (Table 2) were in the expected range

and severity of intensive induction chemotherapy<sup>15</sup> and not different between the 2 treatment arms. After first induction therapy, 276 patients (93.2%) and 257 patients (88.0%) responded, including CR, CRi, and partial remission, and a second induction therapy was started in 258 and 239 patients in the standard arm and GO arm, respectively. GO was not applied in the second cycle in 15 patients mainly because of toxicity observed during the first induction cycle ( $n = 8$ ). Overall response to induction therapy in the standard and GO arms was CR/CRi in 262 patients (88.5%) and 249 patients (85.3%), refractory disease in 16 patients (5.4%) and 12 patients (4.1%), and death during induction therapy in 17 patients (5.7%) and 30 patients (10.3%), respectively. There was no difference between the arms in CR/CRi rate ( $P = .27$ ), but a higher death rate ( $P = .05$ ) was observed in the GO arm. In particular, more patients  $> 70$  years old died during induction therapy in the GO arm (20.4%,  $n = 10$ ) compared with the standard arm (4%,  $n = 2$ ; Appendix Table A1, online only).

WBC recovery was faster in the GO arm after first induction therapy (median recovery, 23 days in GO arm v 24 days in standard arm;  $P = .003$ ) and similar after second induction therapy (median recovery, 21 days in GO arm v 20 days in standard arm;  $P = .51$ ). No difference in platelet recovery was noted after the first induction therapy (median recovery, 24 days in GO arm v 23 days in standard arm;  $P = .18$ ). In contrast, platelet recovery was significantly prolonged after second induction therapy (median recovery, 25 days in GO arm v 20 days in standard arm;  $P < .001$ ). In a Cox model stratified for dosage of etoposide (3 days v 2 days), factors associated with prolonged platelet recovery after second induction therapy were GO treatment (HR, 0.63;  $P < .001$ ), age  $> 60$  years (HR, 0.72;  $P = .002$ ), female sex (HR, 0.62;  $P < .001$ ), and prolonged platelet recovery after induction cycle 1 (HR, 0.58;  $P = .03$ ).

### Consolidation Therapy

For patients in CR/CRi after induction therapy, 3 cycles of consolidation therapy were intended. One, 2, and 3 cycles of consolidation therapy were applied in 19, 23, and 197 patients in the standard arm (total cycles,  $n = 656$ ) and in 23, 16, and 172 patients in the GO arm (total cycles,  $n = 571$ ), respectively. A higher number of consolidation therapy cycles was applied in the standard arm (83%, 656 of 786 cycles) compared with the GO arm (76%, 571 of 747 cycles). In the GO arm, 211 patients received first consolidation therapy. In 26 patients, GO was not added to first consolidation again mainly as a result of toxicity ( $n = 15$ ) in previous treatment cycles. As with second induction therapy, platelet recovery in the GO arm after first consolidation therapy was significantly prolonged compared with the standard arm (median recovery, 23 v 19 days, respectively;  $P < .001$ ). Allogeneic hematopoietic cell transplantation was performed in first CR/CRi in 48 patients (24 in each treatment arm) after first ( $n = 2$ ) and second



**FIG 1.** CONSORT dia-gram. ATRA, all-*trans*-retinoic acid; GO, gemtuzumab ozogamicin; HCT, hematopoietic cell transplantation.

**TABLE 1.** Patient and Disease Characteristics According to Randomization

Characteristic	All Patients (N = 588)	Standard Arm (n = 296)	GO Arm (n = 292)	P
Median age, years (range)	58.7 (18.4-82.3)	58.7 (20.9-80.2)	58.6 (18.4-82.3)	.95
Male sex, No. (%)	273 (46.4)	144 (48.6)	129 (44.2)	.28
WBC, × 10 <sup>9</sup> /L				.37
Median (range)	19.1 (0.6-295.8)	20.8 (0.6-295.8)	17 (0.6-278.9)	
Missing, No.	0	0	0	
Hemoglobin, g/dL				.05
Median (range)	9.1 (4.3-19.3)	9.4 (4.3-19.3)	9.1 (4.4-15)	
Missing, No.	1		0	
Platelets, × 10 <sup>9</sup> /L				.76
Median (range)	73 (3-660)	69.5 (3-660)	74 (7-404)	
Missing, No.	1	0	1	
Bone marrow blasts, % <sup>a</sup>				.57
Median (range)	73.5 (0-100)	71.5 (3-100)	75 (0-100)	
Missing, No.	20	10	10	
Peripheral blood blasts, % <sup>a</sup>				.93
Median (range)	26 (0-99)	25.5 (0-99)	26 (0-99)	
Missing, No.	27	12	15	
LDH, U/L				.27
Median (range)	437 (86-9,670)	439 (86-5,650)	424 (137-9,670)	
Missing, No.	3	2	1	
AML type, No. (%)				.54
De novo AML	524 (89.3)	265 (89.8)	259 (88.7)	
s-AML	48 (8.2)	21 (7.1)	27 (9.2)	
t-AML	15 (2.5)	9 (3.0)	6 (2.0)	
Missing	1	1	0	
Cytogenetics, No. (%)				.52
Normal karyotype	474 (87.1)	237 (88.1)	237 (86.2)	
Missing	44	27	17	
Mutated <i>DNMT3A</i> , No. (%)	181 (45.5)	84 (41.6)	97 (49.5)	.13
Missing	190	94	96	
<i>FLT3</i> -ITD, No. (%)	99 (16.8)	49 (16.6)	50 (17.1)	.52
Missing	0	0	0	
<i>FLT3</i> -TKD, No. (%)	76 (12.9)	34 (11.5)	42 (14.4)	.33
Missing	0	0	0	
ELN 2010, No. (%)				.51
Favorable	393 (74.6)	198 (75.9)	195 (73.3)	
Intermediate 1/2	128 (24.3)	60 (23)	68 (25.6)	
Adverse	6 (1.1)	3 (1.1)	3 (1.1)	

Abbreviations: AML, acute myeloid leukemia; *DNMT3A*, DNA methyltransferase 3A; ELN, European LeukemiaNet; *FLT3*-ITD, FMS-like tyrosine kinase 3 gene internal tandem duplication; *FLT3*-TKD, FMS-like tyrosine kinase 3 gene tyrosine kinase domain mutation; GO, gemtuzumab ozogamicin; LDH, lactate dehydrogenase; s-AML, AML after previous myelodysplastic syndrome or myeloproliferative neoplasm; t-AML, therapy-related AML.

<sup>a</sup>In case of bone marrow blasts < 20%, diagnosis of AML was established based on extramedullary disease or peripheral blood blasts > 20%.

**TABLE 2.** AEs According to CTCAE (version 3.0) Category Occurring During First Induction Therapy According to Treatment With or Without GO

AE Reported	Standard Arm			GO Arm			<i>P</i>	
	All Grades (n = 275)	Grade 1/2	Grade 3-5	All Grades (n = 274)	Grade 1/2	Grade 3-5	All Grades	Grade 3-5
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)		
Blood/bone marrow	235 (85)	4 (1.5)	231 (83.5)	228 (83)	3 (1)	225 (82)	.48	.57
GI	222 (81)	140 (51)	82 (30)	232 (85)	134 (49)	98 (36)	.26	.15
Infection	202 (73)	41 (14)	161 (59)	217 (79)	43 (15)	174 (64)	.13	.26
Constitutional symptoms	173 (81)	147 (72)	26 (9)	190 (85)	156 (73)	34 (12)	.13	.28
Metabolic/laboratory	158 (57)	70 (25)	88 (32)	173 (63)	90 (33)	83 (30)	.19	.71
Pain	144 (52)	118 (43)	26 (9)	154 (56)	126 (46)	28 (10)	.39	.78
Dermatology/skin	123 (45)	103 (38)	20 (7)	116 (42)	104 (38)	12 (4)	.61	.20
Renal/genitourinary	110 (40)	95 (35)	15 (5)	102 (37)	88 (32)	14 (5)	.54	.99
Hemorrhage/bleeding	81 (29)	60 (21)	21 (8)	98 (36)	76 (28)	22 (8)	.12	.88
Pulmonary/upper respiratory	78 (28)	56 (20)	22 (8)	85 (31)	63 (23)	22 (8)	.71	.99
Neurology	75 (27)	67 (24)	8 (3)	70 (26)	61 (22)	9 (4)	.70	NA
Cardiac general	55 (20)	37 (13)	18 (7)	66 (24)	48 (18)	18 (6)	.26	NA
Allergy/immunology	27 (10)	23 (8.5)	4 (1.5)	40 (15)	31 (11)	9 (4)	.09	NA
Coagulation	31 (11)	25 (9.5)	4 (1.5)	23 (8)	21 (7)	2 (1)	.32	NA
Cardiac arrhythmia	29 (11)	23 (8)	6 (3)	21 (8)	15 (5.5)	6 (2.5)	.30	NA
Vascular	17 (7)	13 (5.5)	4 (1.5)	18 (7)	17 (6.5)	1 (0.5)	.86	NA
Ocular/visual	17 (6)	16 (6.5)	1 (0.5)	15 (5)	14 (4.5)	1 (0.5)	.86	NA
Musculoskeletal/soft tissue	16 (6)			8 (3)			.14	NA

Abbreviations: AE, adverse event; CTCAE, Common Toxicity Criteria of Adverse Events; GO, gemtuzumab ozogamicin; NA, not applicable.

induction therapy (n = 13) and after first (n = 19), second (n = 7), and third (n = 7) consolidation therapy.

### Survival Analysis

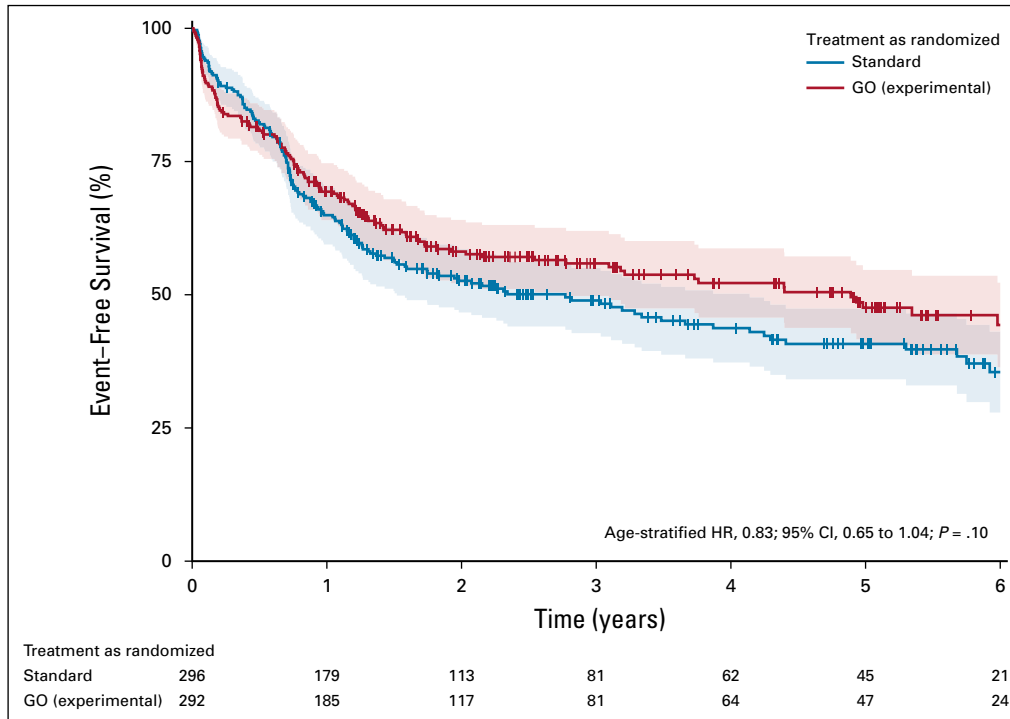
Data were locked as of September 28, 2018. The median follow-up time was 40.0 months (95% CI, 35.5 to 47.0). Median EFS time and 2-year EFS rate defined according to ELN 2017 recommendations were 39.4 months (95% CI, 27.1 to 59.2 months) and 55.3% (95% CI, 51.3% to 59.7%), respectively. Of 588 randomly assigned patients, 523 achieved a first CR/CRi; of these, 178 experienced relapse, and 48 died in CR/CRi.

Univariable survival analyses (age stratified:  $\leq v > 60$  years) on an intent-to-treat basis revealed no significant differences in EFS defined according to 2017 ELN criteria (HR, 0.83; 95% CI, 0.65 to 1.04;  $P = .10$ ; Fig 2) and according to protocol (HR, 0.81; 95% CI, 0.65 to 1.01;  $P = .05$ ). The 2-year EFS rates were 52.6% (95% CI, 47.0% to 58.9%) and 58.1% (95% CI, 52.5% to 64.4%) in the standard and GO arms, respectively.

In patients achieving CR/CRi within the protocol, CIR was significantly higher in the standard arm compared with the GO arm ( $P = .005$ , age stratified; Fig 3; Appendix Fig A1, online only), with CIR rates at 2 years of 36.9% (95% CI, 30.8% to 43.0%) and 25.5% (95% CI, 19.7% to 31.2%),

respectively. No difference was observed for CID ( $P = .80$ ), with rates at 2 years of 7.1% (95% CI, 3.9% to 10.3%) and 8.3% (95% CI, 1.8% to 11.8%) in the standard and GO arms, respectively.

Subset analysis revealed substantial heterogeneity for the end points of EFS and CIR. In this analysis, CD33 expression was not included because it was not systematically assessed within the study. There was a strong interaction between sex and treatment arm ( $P = .03$ ). Female patients had significantly improved EFS (HR, 0.67; 95% CI, 0.49 to 0.92) with the addition of GO (Fig 4; Appendix Fig A2, online only), whereas this was not seen in male patients (HR for EFS, 1.08; 95% CI, 0.77 to 1.51). Similar results were observed for CIR (Appendix Fig A1). Furthermore, the interaction between *FLT3*-ITD and treatment arm was significant ( $P = .002$ ) in that patients with concurrent presence of *FLT3*-ITD did not benefit from the addition of GO in terms of EFS (HR, 1.53; 95% CI, 0.95 to 2.48), whereas patients without *FLT3*-ITD did benefit (HR, 0.72; 95% CI, 0.56 to 0.95; Fig 4; Appendix Fig A3, online only). Finally, older patients (age > 70 years) did not benefit from GO with regard to EFS (HR, 1.22; 95% CI, 0.76 to 1.95) and CIR (HR, 1.01; 95% CI, 0.55 to 1.86). In contrast, an explorative post hoc analysis in younger patients (age  $\leq 70$  years) revealed significantly improved EFS defined



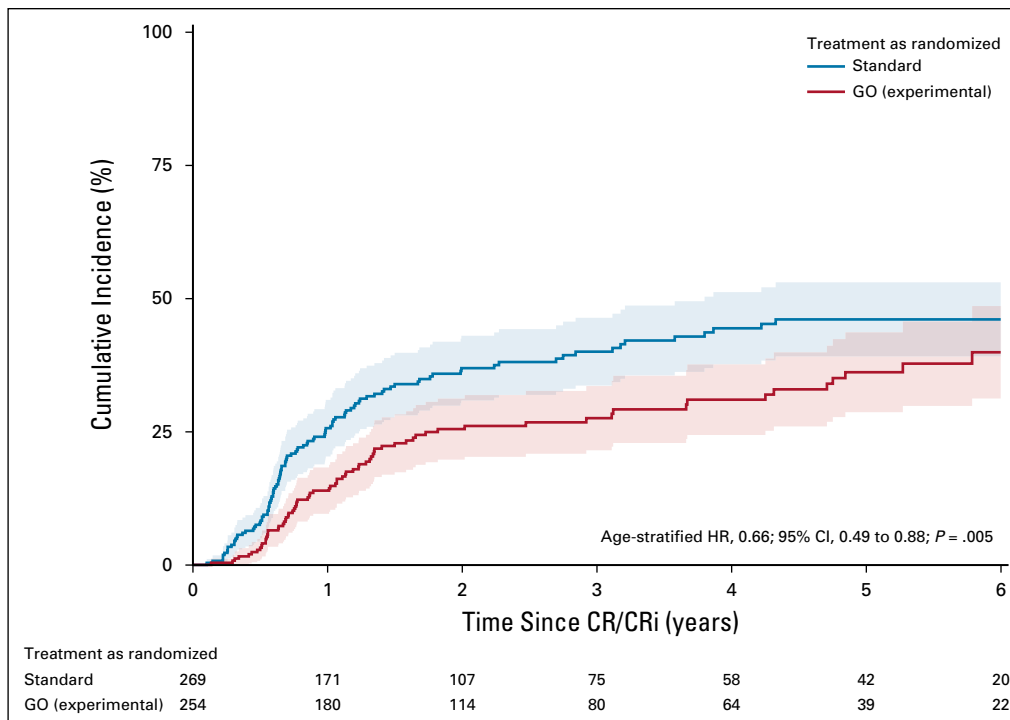
**FIG 2.** Kaplan-Meier plot illustrating event-free survival defined according to European LeukemiaNet 2017 recommendations in the gemtuzumab ozogamicin (GO) arm and standard arm as randomized (age-stratified hazard ratio [HR], 0.83; 95% CI, 0.65 to 1.04;  $P = .10$ ). Overall, 289 events were observed (standard arm,  $n = 157$ ; GO arm,  $n = 132$ ).

according to 2017 ELN recommendations ( $P = .05$ ) and according to protocol ( $P = .02$ ) with the addition of GO (Appendix Fig A4, online only).

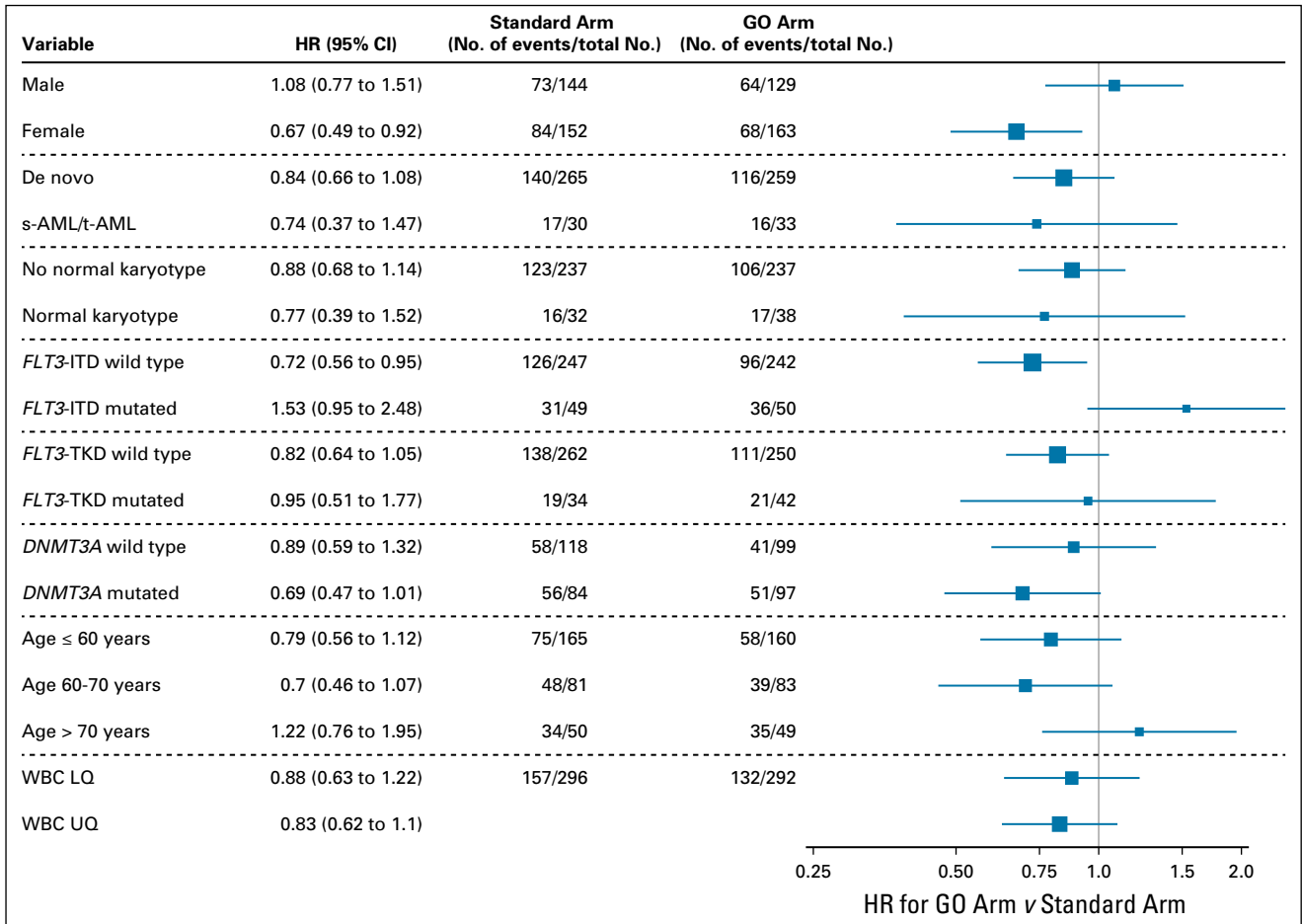
**DISCUSSION**

After the approval of GO by the FDA and EMA for patients with CD33-positive AML, our randomized trial is, to our

knowledge, the first to focus on the distinct WHO 2016 disease entity of AML with mutated *NPM1*. Consistent with previous reports, GO did not improve the response rate after induction therapy.<sup>27</sup> On the contrary, we observed a higher death rate in the GO arm. A higher death rate was also observed in a previous trial, in which GO was added to induction therapy at day 4 in a dosage of 6 mg/m<sup>2</sup> in adult



**FIG 3.** Cumulative incidence plot illustrating cumulative incidence of relapse (CIR) in the gemtuzumab ozogamicin (GO) arm and standard arm as randomized (age-stratified hazard ratio [HR], 0.66; 95% CI, 0.49 to 0.88;  $P = .005$ ). CR, complete remission; CRi, complete remission with incomplete hematologic recovery.



**FIG 4.** Forest plot of cause-specific proportional hazards models for event-free survival. *DNMT3A*, DNA methyltransferase 3A; *FLT3*, FMS-like tyrosine kinase 3 gene; GO, gemtuzumab ozogamicin; HR, hazard ratio; ITD, internal tandem duplication; LQ, lower quartile; s-AML, secondary acute myeloid leukemia after previous myelodysplastic syndrome or myeloproliferative neoplasm; t-AML, therapy-related acute myeloid leukemia; TKD, tyrosine kinase domain; UQ, upper quartile.

patients up to the age of 60 years.<sup>28</sup> On the basis of a meta-analysis, GO dosages > 3 mg/m<sup>2</sup> per application were associated with a higher early mortality rate.<sup>27</sup> Although we adhered to the 3 mg/m<sup>2</sup> dosage without capping the dose at 5 mg, we observed a mortality rate of up to 20% in patients older than 70 years. In addition, we combined GO with the intensive ATRA-ICE regimen, which may have contributed further to an unfavorable safety evaluation.

By design of our study, EFS as early end point was analyzed 6 months after the last patient was randomly assigned. The trial failed to show a significant improvement in EFS, although the 2-year EFS was numerically improved from 52.6% (95% CI, 47.0% to 58.9%) to 58.1% (95% CI, 52.5% to 64.4%). Reasons for this lack of benefit are, among others, the higher early mortality rate in the GO arm in older patients and the better outcome overall in both arms compared with the available historical data at the time of sample size planning with an estimated 2-year EFS of 37%. An explorative post hoc analysis of EFS in younger

patients (age ≤ 70 years) revealed improved EFS defined according to 2017 ELN recommendations ( $P = .05$ ), indicating a better risk-benefit profile of GO in younger patients.

CIR was significantly reduced by the addition of GO. This observation is in line with previous reports<sup>17,27</sup> in the total trial population as well as in subset analysis focusing on AML with mutated *NPM1*.<sup>17</sup> On the basis of subgroup effect estimates, we identified good efficacy in female patients with an HR for CIR of 0.49 (95% CI, 0.32 to 0.74) and an HR for EFS of 0.67 (95% CI, 0.49 to 0.92), whereas in male patients, no significant effect was present for CIR (HR, 0.91; 95% CI, 0.59 to 1.41) or EFS (HR, 1.08; 95% CI, 0.77 to 1.51). This observation is in contrast to the ALFA 0701 study using fractionated dosing and the meta-analysis in which comparable effects were reported for both sexes.<sup>17,27</sup> Of note, similar sex-specific differences were also noted in a large randomized study in older patients.<sup>29</sup> In this study, patients were randomly assigned to GO 6 mg/m<sup>2</sup> on day 1 and



3 mg/m<sup>2</sup> on day 8 or to best supportive therapy. The improvement in OS was unequally distributed in women (HR, 0.53; 95% CI, 0.35 to 0.79) and men (HR, 0.90; 95% CI, 0.63 to 1.28). Whether these sex-specific differences are a result of differences in antibody-drug conjugate clearance in men and women previously reported for rituximab<sup>30</sup> remains elusive. Another subset of patients who did not benefit from the addition of GO with respect to relapse risk reduction were patients older than age 70 years. This may be attributed to age-specific factors, independent of prognostic factors such as the *NPM1* genotype<sup>31</sup> and the intensive induction therapy regimen (ATRA-ICE) used in this study.

Consistent with previous reports,<sup>16,17</sup> we observed a substantial prolongation of platelet recovery after repeated GO exposure in second induction and first consolidation therapy, leading to omission of GO in 15 and 26 patients, respectively. In contrast, no difference between treatment arms in adverse events and hematologic recovery in first induction therapy was noticed. On the background of lack of efficacy of GO in postremission therapy,<sup>32</sup> this argues for restriction of GO to first induction therapy. In summary, our study failed to show a significant benefit in EFS by the addition of GO to intensive therapy in patients with *NPM1*-mutated AML. There was a significant reduction in CIR with GO, providing evidence for antileukemic activity of the antibody-drug conjugate.

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**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST****Gemtuzumab Ozogamicin in *NPM1*-Mutated Acute Myeloid Leukemia: Early Results From the Prospective Randomized AMLSG 09-09 Phase III Study**

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## APPENDIX

**Inclusion and Exclusion Criteria**

Diagnoses included de novo acute myeloid leukemia (AML), secondary AML with a preceding history of myelodysplastic syndrome or myeloproliferative neoplasms, and therapy-related AML. Patients with acute promyelocytic leukemia and core-binding factor AML; patients with concomitant renal (creatinine  $> 1.5 \times$  upper normal serum level), liver (AST or ALT  $> 2.5 \times$  upper normal serum level), or cardiac dysfunction (New York Heart Association class III or IV); and patients with uncontrolled infection, primary coagulation disturbance, or Eastern Cooperative Oncology Group performance status  $> 2$  were excluded. Written informed consent was obtained from all patients.

**Definition of Response Criteria and Hematologic Recovery**

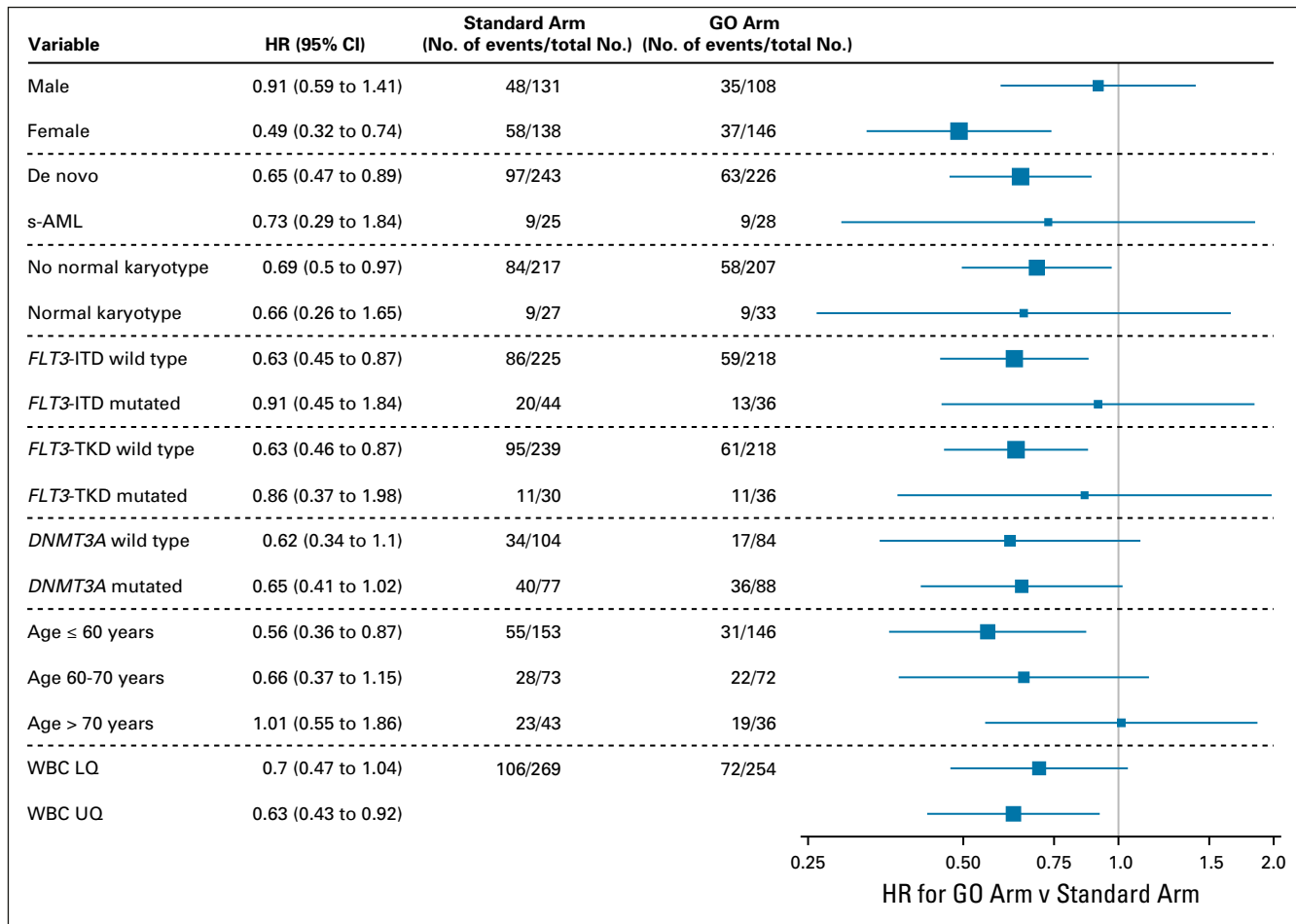
In accordance with standard criteria, complete remission (CR) was defined as  $< 5\%$  bone marrow blasts, an absolute neutrophil count of  $\geq 1.0 \times 10^9/L$ , a platelet count of  $\geq 100 \times 10^9/L$ , no blasts in the peripheral blood, and no extramedullary leukemia. CR with incomplete hematologic recovery was defined as CR except for residual neutropenia (neutrophils  $< 1.0 \times 10^9/L$ ) or thrombocytopenia (platelets  $< 100 \times 10^9/L$ ).<sup>9</sup> Partial remission was defined by all hematologic criteria of CR, decrease of bone marrow blast percentage to 5%-25%,

and decrease of pretreatment bone marrow blast percentage by at least 50% and no extramedullary leukemia.<sup>9</sup> Relapse was defined as  $> 5\%$  bone marrow blasts unrelated to recovery from the preceding course of chemotherapy or new extramedullary leukemia in patients with previously documented CR.

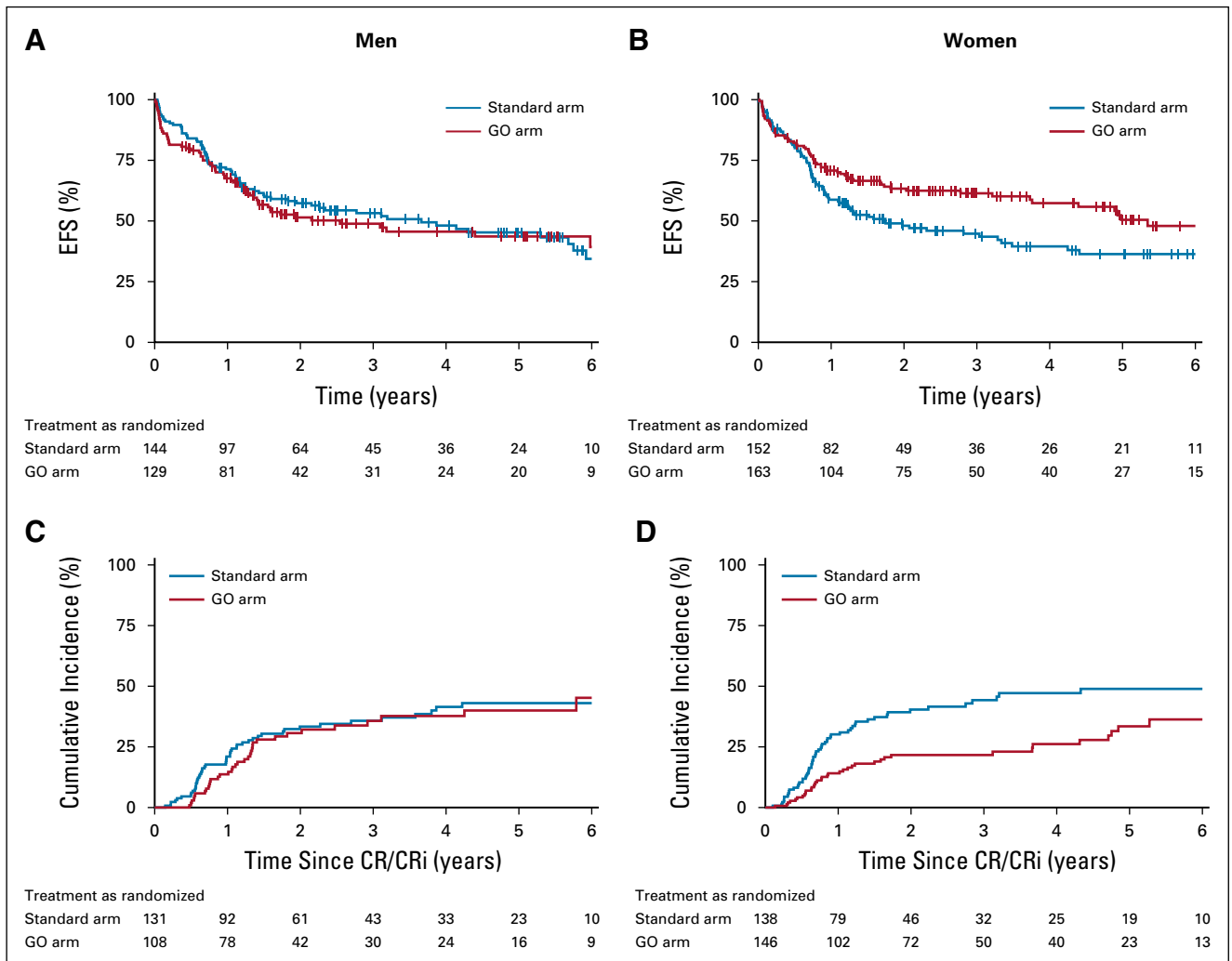
Times to WBC, neutrophil, and platelet recovery were measured from the first day of chemotherapy of each cycle until the first day with unsupported values  $\geq 1$ ,  $\geq 0.5$ , and  $\geq 20 \times 10^9/L$  for WBC, neutrophils, and platelets, respectively. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0.

**Genetic Analyses**

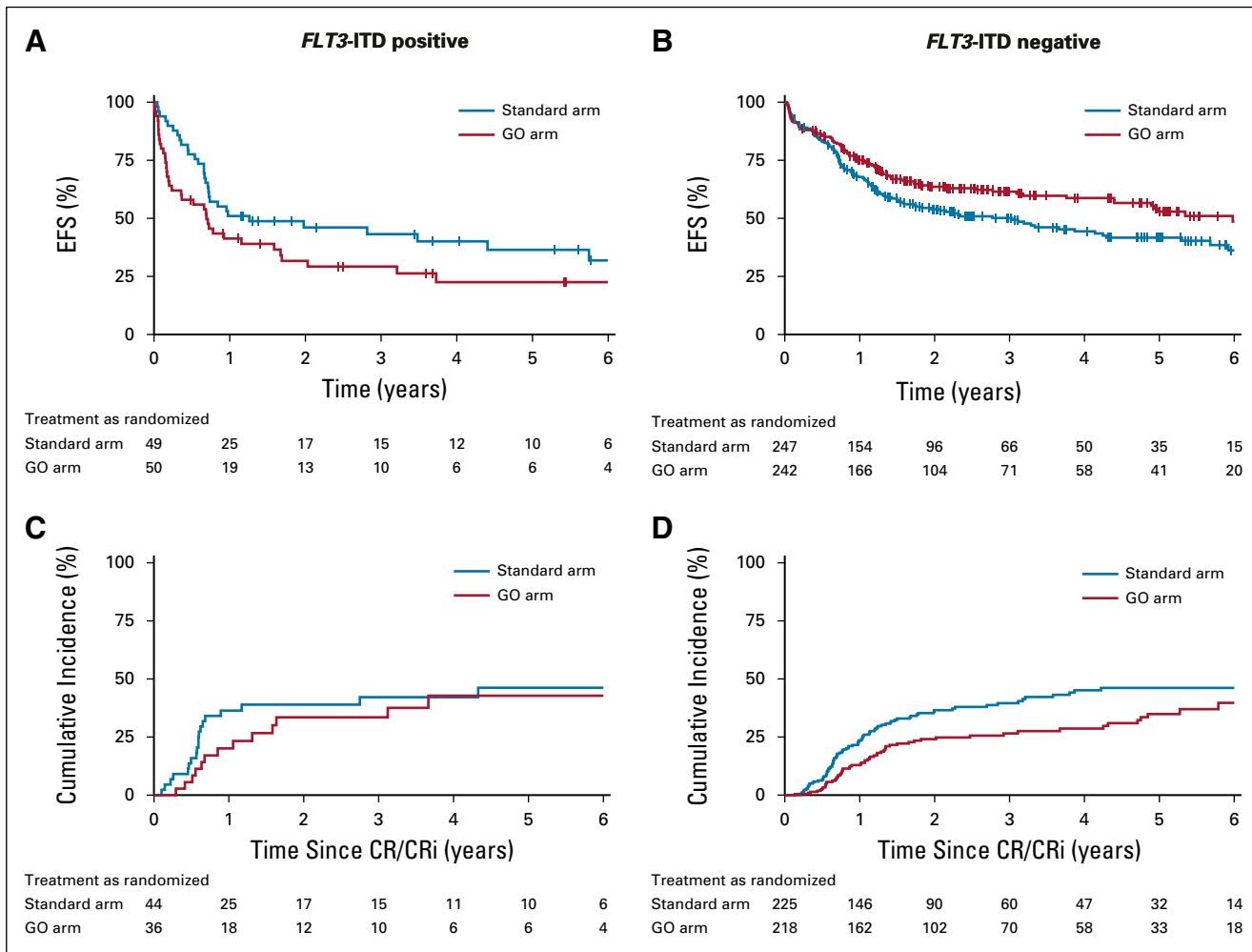
Screening for *NPM1* mutations was performed in 2 reference laboratories at Ulm University Hospital and Hannover Medical School.<sup>2</sup> Leukemia samples were also analyzed for *FLT3* internal tandem duplication, mutations in *FLT3* tyrosine kinase domain at codons D835 and I836, and mutations in *DNMT3A*, as previously described (Gaidzik VI, et al: Blood 121:4769-4777, 2013).<sup>2,3</sup> Karyotypes were designated according to the International System for Human Cytogenetic Nomenclature (Mitelman F: Basel, Switzerland, Karger, 1995).



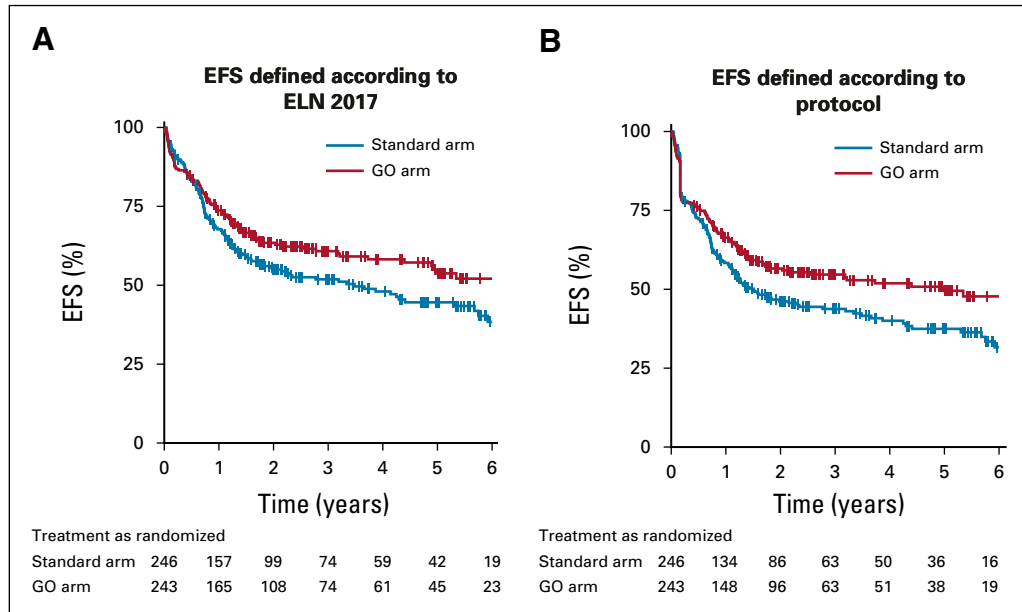
**FIG A1.** Forest plot of cause-specific proportional hazards models for cumulative incidence of relapse. *DNMT3A*, DNA methyltransferase 3A; *FLT3*, FMS-like tyrosine kinase 3 gene; GO, gemtuzumab ozogamicin; HR, hazard ratio; ITD, internal tandem duplication; LQ, lower quartile; s-AML, secondary acute myeloid leukemia after previous myelodysplastic syndrome or myeloproliferative neoplasm; t-AML, therapy-related acute myeloid leukemia; TKD, tyrosine kinase domain; UQ, upper quartile.



**FIG A2.** Kaplan-Meier plots illustrating (A and B) event-free survival (EFS) defined according to European LeukemiaNet 2017 recommendations and (C and D) cumulative incidence plots according to sex in the GO arm and standard arm as randomized. CR, complete remission; CRi, complete remission with incomplete hematologic recovery.



**FIG A3.** Kaplan-Meier plots illustrating (A and B) event-free survival (EFS) defined according to European LeukemiaNet 2017 recommendations and (C and D) cumulative incidence plots according to *FLT3* internal tandem duplication (ITD) status in the GO arm and standard arm as randomized. CR, complete remission; CRi, complete remission with incomplete hematologic recovery.



**FIG A4.** Kaplan-Meier plots illustrating event-free survival (EFS) defined (A) according to 2017 European LeukemiaNet (ELN) recommendations and (B) according to protocol in younger patients ( $\leq 70$  years old) in the GO arm and standard arm as randomized.



**TABLE A1.** Response to Induction Therapy

Response	No. of Patients (%)	
	Standard Arm	GO Arm
All patients <sup>a</sup>	296	292
CR	163 (55.1)	134 (45.9)
CRi	100 (33.8)	116 (39.7)
RD	16 (5.4)	12 (4.1)
Deaths	17 (5.7)	30 (10.3)
Deaths according to age group		
Age 18-60 years		
All patients	164	159
Death before Tx	0	2 (1.3)
Death after Tx	6 (3.7)	9 (5.7)
Age > 60-70 years		
All patients	81	83
Death before Tx	0	0
Death after Tx	9 (11.1)	9 (10.8)
Age > 70 years		
All patients	50	49
Death before Tx	1 (2.0)	0
Death after Tx	1 (2.0)	10 (20.4)

Abbreviations: CR, complete remission; CRi, complete remission with incomplete hematologic recovery; GO, gemtuzumab ozogamicin; RD, refractory disease; Tx, intensive chemotherapy.

<sup>a</sup>Two patients had missing response assessment after second induction cycle as a result of prior study discontinuation (standard arm) or nonevaluable response (GO arm). Both achieved CRi after first induction cycle and were counted as CRi for this analysis.