Supplemental Data

“Prognostic Impact of the ELN 2017 Risk classification in AML Patients Receiving Allogeneic Transplantation”

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Content

Supplemental Methods

Patients and Treatment Protocols

In the subgroup of AML patients at the age of 60 years or younger patients received induction therapy according to the AML 2002 study (#061, ClinicalTrials.gov Identifier: NCT01414231),[1] or within the PKC412 protocol (ClinicalTrials.gov Identifier: NCT00111345)[2], as part of the QuANTUM-First trial (ClinicalTrials.gov Identifier: NCT02668653) or sequential azacytidine and intensive induction chemotherapy. One patient was diagnosed with AML as a child and received treatment within the AML BFM-2014 study.[3]

Patients older than 60 years were treated according to the AML 2004 protocol (#069, ClinicalTrials.gov Identifier: NCT01497002),[4] or the OSHO #083 protocol, or received sequential azacitidine and intensive induction chemotherapy. One patient was treated with CPX-351.[5]

Allogeneic Hematopoietic Stem Cell Transplantation

For consolidation therapy, all patients received an allogeneic hematopoietic stem cell transplantation (HSCT) at the University of Leipzig. All patients received granulocyte colony stimulating factor-mobilized peripheral blood stem cells on day 0. The majority of patients (n=162; 71.1%) received non-myeloablative peripheral blood HSCT with 3x30 mg/qm Fludarabine and 2 Gy (two patients received 3 Gy) total body irradiation (TBI).[6,7] Another 49 patients (21.5%) were treated according to a myeloablative conditioning protocol (MAC; 2x60 mg/kg body weight (BW) cyclophosphamide and 12 Gy TBI).[8] Four patients received reduced intensity conditioning within the MC-FludT.14/L trial (EudraCT Number 2008-002356-18). Patient who did not achieve complete remission (CR) or CR with incomplete peripheral recovery (CRi) prior to allogeneic HSCT received sequential conditioning regimens.[9–11]

Reasons for non-myeloablative conditioning were age (patients >50 years if receiving unrelated HSCT, and patients >55 years if receiving related HSCT), acute infection (n=4), reduced renal function (n=1) and previous autologous HSCT (n=4).

Prevention of Graft *vs* Host Disease

Prevention of graft-versus-host disease (GvHD) was different according to the conditioning regimes used. All patients receiving MAC or NMA conditioning were treated with cyclosporine A (CyA), starting intravenously with 5 mg/kg BW in two daily doses from day -1. Blood levels of CyA were measured from day 0 and doses were adjusted for target levels of 200 ng/ml. Patients also received methotrexate (MTX) 15 mg intravenously on days +1, +3, +6 and +11 after transplantation. Patients with an unrelated donor additionally received in vivo T-cell depletion with thymoglobulin 2 mg/kg BW per day on days -3 to -1.

The patients receiving a reduced-intensity conditioning were treated with CyA, starting intravenously with 5 mg/kg BW in two daily doses from day -1. Blood levels of CyA were measured from day 0 and doses were adjusted for target levels of 120 - 200 ng/ml. The patients also received MTX (15 mg/m^2: day +1; 10 mg/m^2: days +3, +6, +11) with leukovorine.

Additionally, patients received mycophenolate mofetil (MMF) 3 g per day in three daily doses if receiving unrelated or 2 g per day in two daily doses if receiving related transplantation. CyA was reduced starting on day +84 or day +180 following related or unrelated transplantation, respectively, and MMF was stopped at day +28 following related and tapered from days +40 to +96 following unrelated transplantation.[12]

For all patients, immunosuppression was prolonged or extended with systemic steroids in cases of GvHD (grade >2 according to Glucksberg grading system)[13] or rapidly reduced in patients who relapsed (≥5% blasts in bone marrow).

Statistical Analyses

Associations of the assignment to the three ELN2017 risk groups with baseline clinical, demographic, and molecular features were compared using the Kruskal-Wallis test and Fisher's exact test for continuous and categorical variables, respectively.

Cumulative incidence of relapse (CIR) was calculated considering the competing risk (non-relapse mortality) using the Fine and Gray model. For CIR analyses patients with only partial remission (PR) were excluded.

Overall survival (OS) was determined from date of HSCT until death from any cause. Time-to-event analysis was then calculated using the Kaplan-Meier method and groups were compared with the log-rank test.

Definition of Clinical Endpoints

Complete remission (CR) was defined as bone marrow blasts <5%, absence of circulating blasts and blasts with Auer rods, absence of extramedullary disease, absolute neutrophils ≥1.0 x10^9/L; platelet count ≥100 x10^9/L.[14] CRi was defined as all CR criteria except for residual neutropenia (<1.0 x100 x10^9/L) or thrombocytopenia (<100 x10^9/L).[14] PR was defined as decrease of bone marrow blasts to 5% to 25% and a decrease of blast percentage of at least 50% compared to pre-treatment bone marrow.[14]

Multivariate Analyses

We constructed multivariate proportional hazard models for CIR and OS to evaluate the impact of the allocation to the three ELN2017 risk groups by forward adjusting for other variables.[15] In addition to the ELN2017 risk classification the following variables were considered for multivariate analyses: hemoglobin at diagnosis, platelet count at diagnosis, white blood count at diagnosis, blasts percentage in peripheral blood and bone marrow at diagnosis, disease origin (*de novo vs* secondary), age at HSCT, disease status at transplantation (CR *vs* no CR), number of chemotherapy cycles prior to HSCT, HLA match (antigen match *vs* mismatch), HLA donor type (related *vs* unrelated) and sex of donor and recipient (female into male *vs* all others). Of these, variables significant at α=0.20 in univariate analyses were considered for multivariate analyses (displayed as Forest plots, Supplemental Figure S7). The final model was chosen after forward adjusting for these variables based on the Bayesian information criterion (BIC).

EVI1 expression

The quantification of *EVI1* and *18S* expression was performed by RT-qPCR using the Taqman gene expression assays (Applied Biosystems, Carlsbad, CA) Hs00602795_m1 (*EVI1*), and Hs99999901_s1 (*18S*) following the manufacturer's protocols. The to *18S* normalized expression of *EVI1* in the cell line SKOV3 was used to define *EVI1* positive expressers, who were defined as patients with an expression higher than 0.1 relative to the *EVI1* expression of SKOV3.[16]

Supplemental Results

Survival Entire Cohort and Distinct Subgroups

In 234 AML patients receiving HSCT we observed a CIR of 33.3% (95% confidence interval 29.6% – 39.9%), and an OS of 57.3% (95% confidence interval 51.1% – 64.4%) two years after HSCT (Supplemental Figure S1).

Comparable to the entire cohort, the assignment to the three genetic risk groups according to the ELN2017 classification significantly associated with CIR (P<0.001) and OS (P=0.03; Supplemental Figure S2) in patients receiving HSCT in CR1. Altogether 57 favorable, 22 intermediate, and 58 adverse risk patients received allogeneic HSCT in CR1.

When we analyzed patients receiving allogeneic HSCT in CR without detectable measurable residual disease (MRD), we observed that adverse risk patients still had significantly higher CIR than patients classified as intermediate or favorable (P<0.001), however OS only differed by trend between the three genetic risk groups (P=0.09; Supplemental Figure S3).

When we analyzed patients ≥ 60 years at diagnosis the ELN2017 classification retained its prognostic impact on CIR (P<0.001) and OS (P=0.03; Supplemental Figure S4) in our cohort of AML patients receiving allogeneic HSCT.

Outcome Analysis for Patients with Monosomal Karyotype

Presence of a monosomal karyotype was newly introduced in the ELN2017 classification to define AML patients with adverse genetic risk.[14] We observed significantly higher CIR (77.3% *vs* 48.4% two years after allogeneic HSCT; P=0.02) and shorter OS (27.5% *vs* 56.8% two years after allogeneic HSCT; P=0.01) for patients with monosomal karyotype compared to the other adverse risk patients (Supplemental Figure S6).

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Supplemental Tables

Table S1. Distribution of genetic lesion and allocation to the three ELN2017 genetic risk groups in 234 AML patients undergoing allogeneic hematopoietic stem cell transplantation.

ELN2017 risk group	Genetic aberration	n (%)
favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	13 (14.0)
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	15 (16.1)
	mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}	62 (66.7)
	biallelic mutated *CEBPA*	3 (3.2)
intermediate	mutated *NPM1* and *FLT3*-ITD^{high}	3 (10.0)
	wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}	14 (46.7)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*	2 (6.7)
	cytogenetic abnormalities not classified as favorable or adverse	11 (36.7)
	trisomy 8	6 (54.5)
	trisomy 11	2 (18.2)
	other genetic aberrations	3 (27.3)
adverse	t(6;9)(p23;q34.1); *DEK-NUP214*	1 (0.4)
	t(v;11q23.3); *KMT2A* rearranged	13 (5.6)
	t(9;22)(q34.1;q11.2); *BCR-ABL1*	2 (0.9)
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*	9 (3.9)
	-5 or del(5q)	32 (13.7)
	-7	34 (14.6)
	-17/abn(17p)	8 (3.4)
	complex karyotype	50 (21.5)
	monosomal karyotype	33 (14.2)
	wild-type *NPM1* and *FLT3*-ITD^{high}	9 (3.9)
	mutated *RUNX1*	15 (6.4)
	mutated *ASXL1*	14 (6.0)
	mutated *TP53*	13 (5.6)

Table S2. Clinical characteristics of adverse risk AML patients receiving allogeneic transplantation according to the *TP53* mutational status.

Characteristics	all n=51	*TP53*wt n=39	*TP53*mut n=12	*P*
Age at diagnosis, years				
Median	63.6	64.1	62.4	0.66
Range	46.6 – 74.8	49.6 – 74.8	46.6 – 74.4	
Sex, n (%)				
Female	28 (54.9)	20 (51.3)	8 (66.7)	0.51
Male	23 (45.1)	19 (48.7)	4 (33.3)	
WBC at diagnosis, $x10^9$/l				
Median	4.7	4.7	4.1	0.26
Range	0.7 – 385	0.8 – 385	0.7 – 47.9	
Platelets at diagnosis, $x10^9$/l				
Median	63	68.5	38	0.21
Range	3 – 305	3 – 305	13 – 145	
Hemoglobin at diagnosis, g/dl				
Median	9.0	9.1	8.5	0.25
Range	5.6 – 14.9	5.6 – 14.9	5.8 – 10.0	
Peripheral blasts at diagnosis, %				
Median	20	23	12.5	0.03
Range	0 – 97	2 – 97	0 – 36	
Bone marrow blasts at diagnosis, %				
Median	44	55	24	<0.001
Range	3 – 95	24 – 95	3 – 50	
Complex karyotype, n (%)				
Absent	34 (68.0)	31 (81.6)	3 (25.0)	<0.001
Present	16 (32.0)	7 (18.4)	9 (75.0)	
Monosomal karyotype, n (%)				
Absent	41 (82.0)	36 (94.7)	5 (41.7)	<0.001
Present	9 (18.0)	2 (5.3)	7 (58.3)	
Remission status at HSCT, n (%)				
CR	36 (70.6)	28 (71.8)	8 (66.7)	0.57
CRi	13 (25.5)	10 (25.6)	3 (25.0)	
PR	2 (3.9)	1 (2.6)	1 (8.3)	
Chemotherapy before HSCT, n (%)				
1 cycle	13 (26.5)	10 (27.0)	3 (25.0)	0.69
2 cycles	31 (63.3)	24 (64.9)	7 (58.3)	
3 cycles	5 (10.2)	3 (8.1)	2 (16.7)	

Abbreviations: ELN2017, 2017 European LeukemiaNet genetic risk classification; WBC, white blood count; HSCT, hematopoietic stem cell transplantation; CR, complete remission; CRi, complete remission with incomplete peripheral recovery; PR, partial response.

[a]*P*-Values are from Fisher's exact or Kruskal-Wallis test and compare *TP53* mutated *vs TP53* wild-type patients.

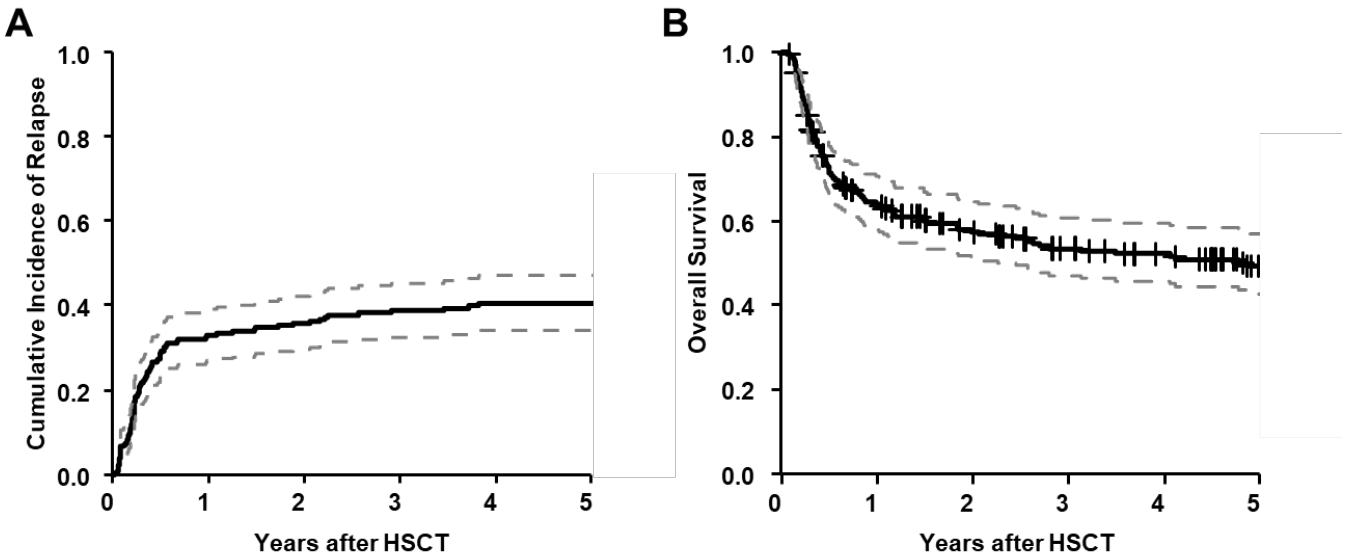
Table S3. Clinical characteristics of AML patients receiving allogeneic transplantation in CR1 according to the ELN2017 genetic risk group.

Characteristics	all n=137	ELN2017 favorable n=57	ELN2017 intermediate n=22	ELN2017 adverse n=58	*P*
Age at diagnosis, years					
Median	59.6	53.5	60.8	60.7	0.37
Range	19.1 – 74.8	22.2 – 73.7	19.1 – 72.2	23.3 – 74.8	
Sex, n (%)					
Female	65 (47.4)	27 (47.4)	9 (40.9)	29 (50.0)	0.78
Male	72 (52.6)	30 (52.6)	13 (59.1)	29 (50.0)	
WBC at diagnosis, $x10^9$/l					
Median	8.1	13.2	2.3	6.3	0.03
Range	0.7 – 385	1.2 – 324	0.7 – 117	0.9 – 385	
Platelets at diagnosis, $x10^9$/l					
Median	68	75	78	62	0.61
Range	5 – 268	7 – 238	10 – 268	5 – 218	
Hemoglobin at diagnosis, g/dl					
Median	8.3	8.4	9.3	8.0	0.37
Range	4.5 – 14.7	4.5 – 14.7	5.2 – 13.4	5.3 – 13.4	
Peripheral blasts at diagnosis, %					
Median	21.5	29	17	18	0.82
Range	0 – 97	2 – 97	2 – 96	0 – 97	
Bone marrow blasts at diagnosis, %					
Median	52.2	52.5	50	56.5	0.93
Range	10 – 95	20 – 95	21 – 95	10 – 95	
DNMT3A mutation at diagnosis, n (%)					
Absent	71 (82.6)	26 (74.3)	18 (90.0)	27 (87.1)	0.29
Present	15 (17.4)	9 (25.7)	2 (10.0)	4 (12.9)	
FLT3-TKD mutation at diagnosis, n (%)					
Absent	117 (89.3)	47 (87.0)	21 (95.5)	49 (89.1)	0.64
Present	14 (10.7)	7 (13.0)	1 (4.5)	6 (10.9)	
JAK2 mutation at diagnosis, n (%)					
Absent	56 (88.9)	16 (100.0)	15 (71.4)	25 (96.2)	0.008
Present	7 (11.1)	0 (0.0)	6 (28.6)	1 (3.8)	
EVI1 expression at diagnosis, n (%)					
Absent	63 (81.8)	33 (100.0)	9 (64.3)	22 (71.0)	<0.001
Present	14 (18.2)	0 (0.0)	5 (35.7)	9 (29.0)	
Chemotherapy before HSCT, n (%)					
1 cycle	10 (9.9)	2 (5.1)	1 (4.8)	7 (17.1)	0.29
2 cycles	70 (69.3)	26 (66.7)	16 (76.2)	28 (68.3)	
≥3 cycles	21 (20.8)	11 (28.2)	4 (19.0)	6 (14.6)	

Abbreviations: ELN2017, 2017 European LeukemiaNet genetic risk classification; WBC, white blood count; HSCT, hematopoietic stem cell transplantation; CR, complete remission; CRi, complete remission with incomplete peripheral recovery; PR, partial remission; *DNMT3A*, *DNA methyltransferase 3 alpha*; *FLT3*, *fms related receptor tyrosine kinase 3*; TKD, tyrosine kinase domain; *JAK2*, *Janus kinase 2*; *EVI1*, *ecotropic viral integration site 1*.

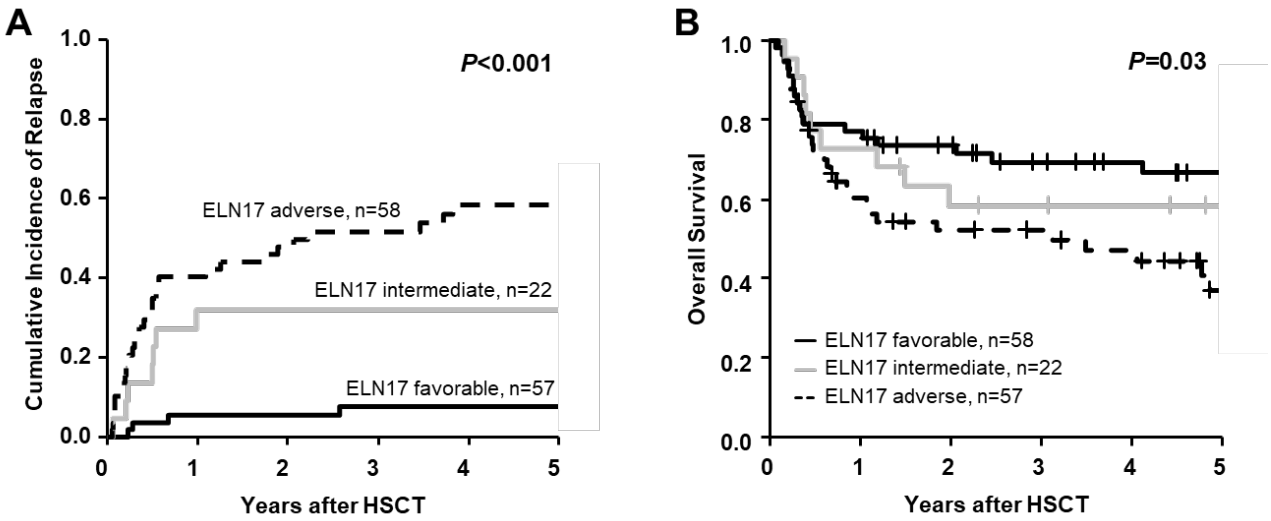
[a]*P*-Values are from Fisher's exact or Kruskal-Wallis test and compare the three ELN2017 genetic risk groups.

Supplemental Figures



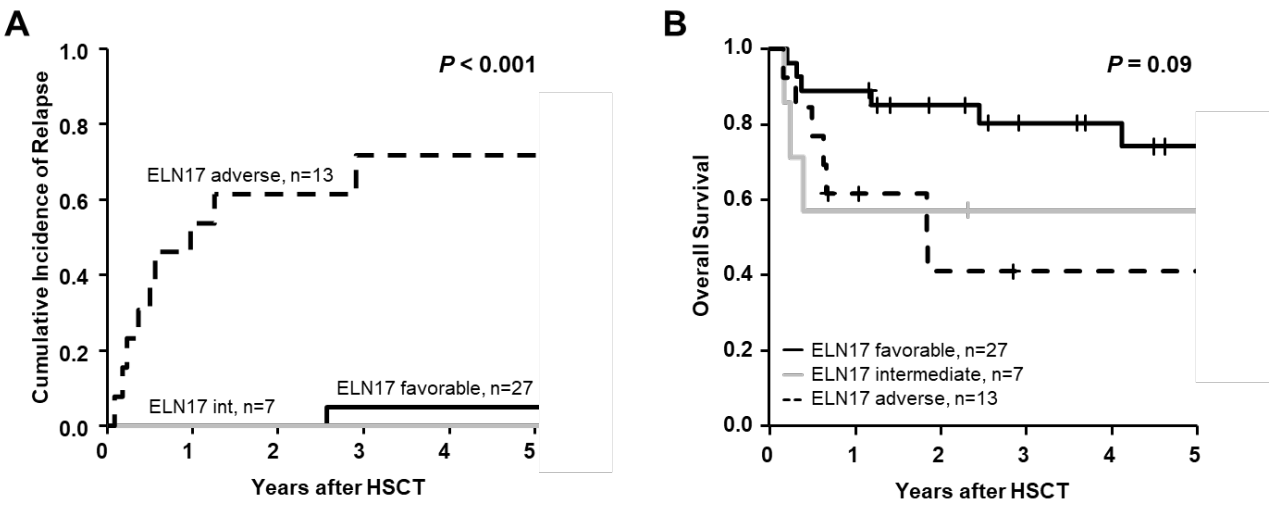
Supplemental Figure S1

Cumulative incidence of relapse (A) and overall survival (B) or the analyzed 234 AML patients receiving allogeneic HSCT.



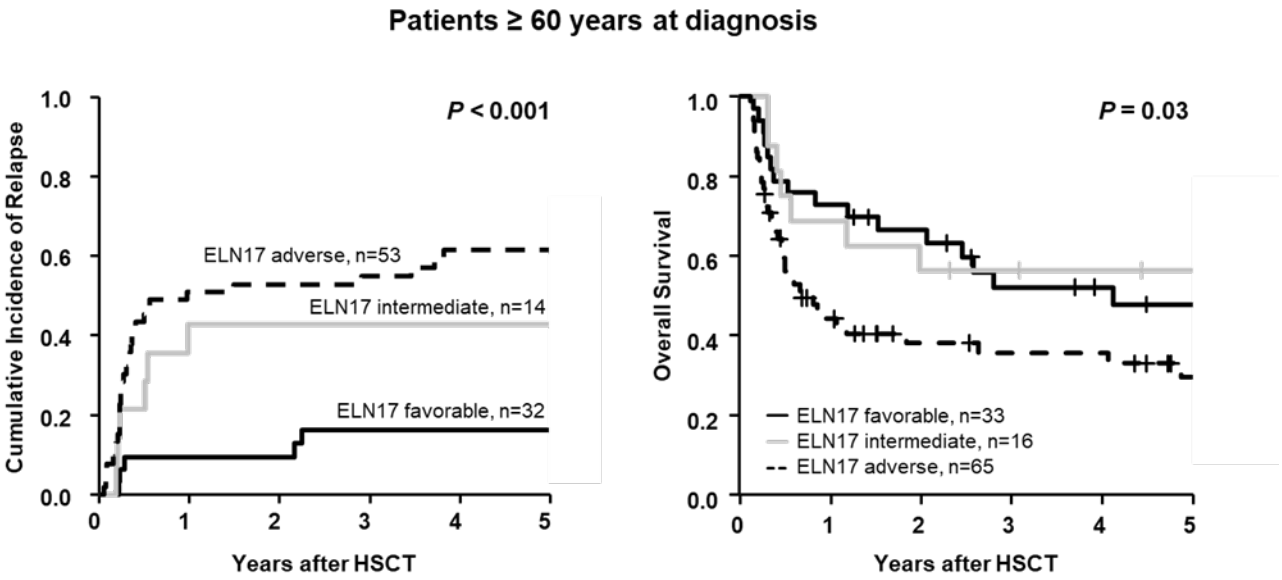
Supplemental Figure S2

Prognostic impact of allocation to the three ELN2017 genetic risk groups in patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission on cumulative incidence of relapse (A) and overall survival (B).



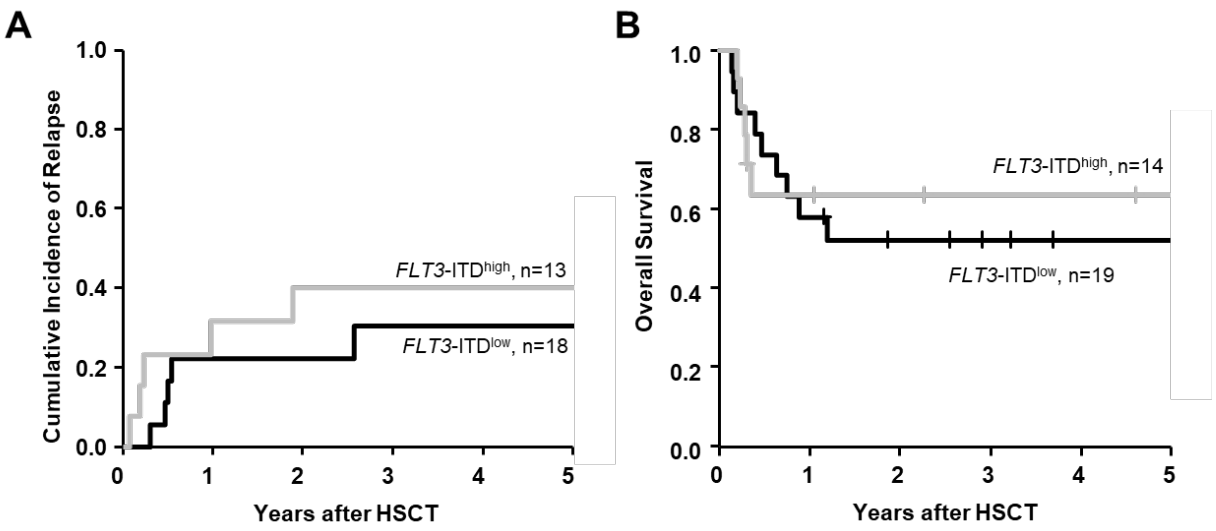
Supplemental Figure S3

The allocation to the three ELN2017 genetic risk groups retained its prognostic impact on cumulative incidence of relapse (A) and overall survival by trend (B) in AML patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) in complete remission without detectable measurable residual disease.



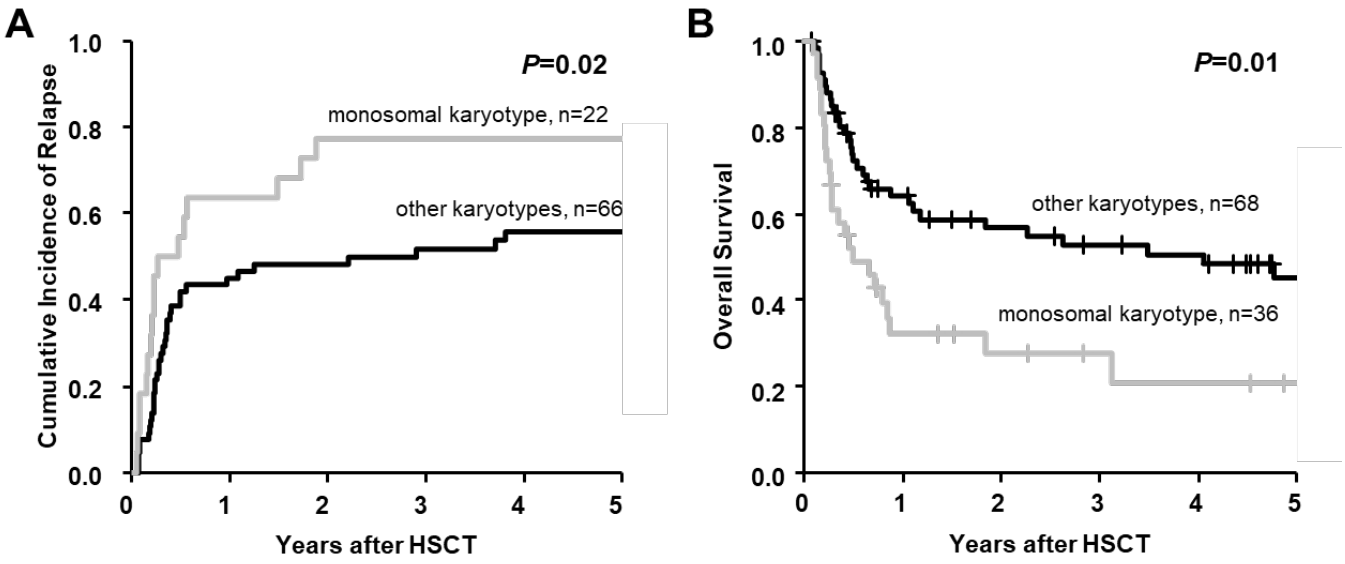
Supplemental Figure S4

The ELN2017 genetic risk classification retained its prognostic impact on cumulative incidence of relapse (A) and overall survival (B) in older AML patients (≥ 60 years at diagnosis) receiving allogeneic hematopoietic stem cell transplantation (HSCT).



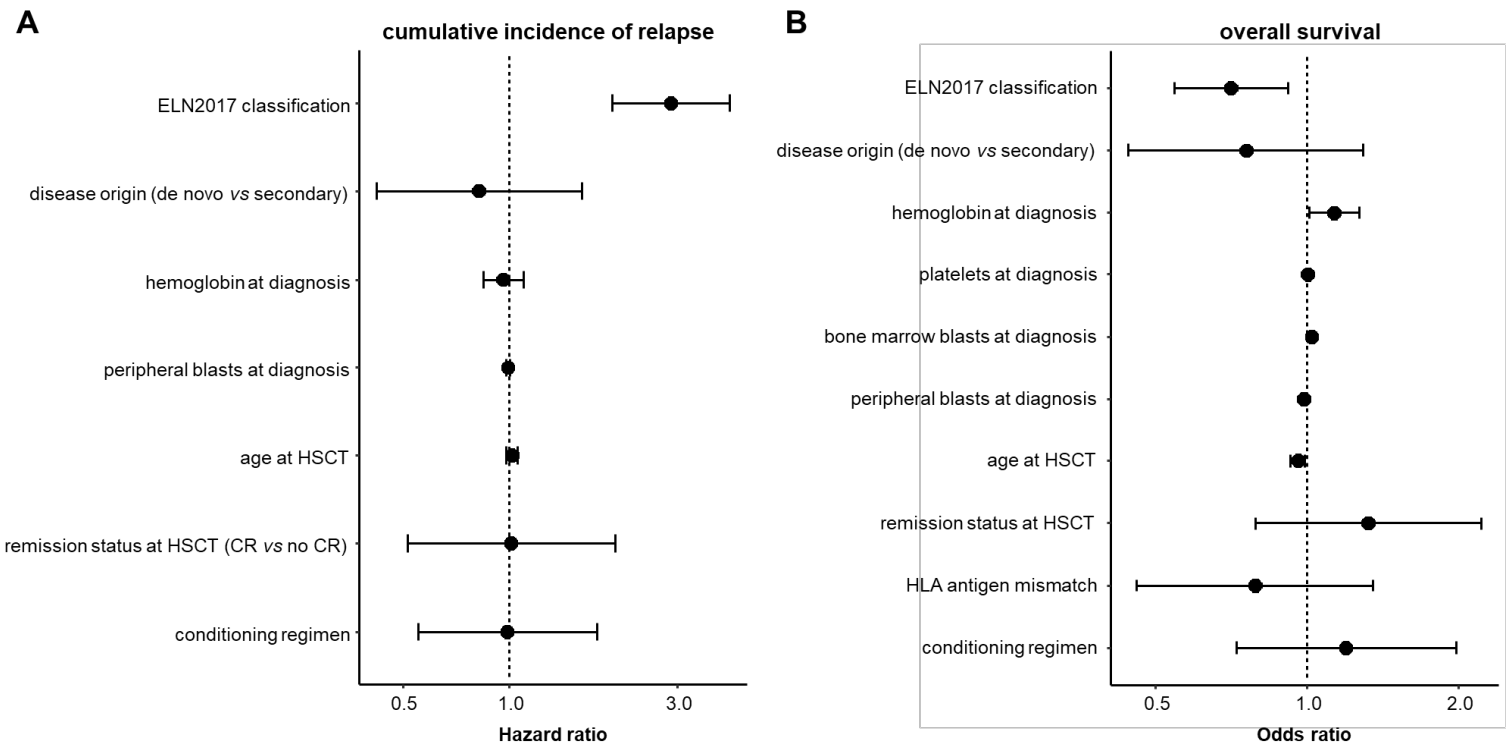
Supplemental Figure S5

Impact of the allelic ratio in AML patients harboring *FLT3* internal tandem duplication (ITD) on cumulative incidence of relapse (A) and overall survival (B). Patients with an *FLT3*-ITD allelic ratio ≥0.5 were defined as *FLT3*-ITD^{high}, while patients with an allelic ratio <0.5 were labeled *FLT3*-ITD^{low}. All patients received an allogeneic hematopoietic stem cell transplantation (HSCT).



Supplemental Figure S6

Prognostic impact of presence of a monosomal karyotype in AML patients assigned to the ELN2017 adverse risk group on cumulative incidence of relapse (A) and overall survival (B). All patients received an allogeneic hematopoietic stem cell transplantation (HSCT).



Supplemental Figure S7

All variables considered for multivariate analysis of cumulative incidence of relapse (A) and overall survival (B) based on $\alpha \leq 0.20$ in univariate analyses.