Supplemental files S3.1-7 to NILG AML 02/06 final study report

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S3.7.2. Kaplan-Meier survival analyses according to random 1 arm (ICE or sHD induction course 1) and random 2 arm (BU-CY autotransplantation [ASCT] or repetitive blood stem cell-supported HD courses [A20]). (A) overall survival according to random 2 arm for patients receiving ICE chemotherapy at random 1; (B) overall survival according to random 2 arm for patients receiving sHD chemotherapy at random 1.

S3.8. Comprative toxicity analysis in random 2 patients (BU-CY autotransplantation [ASCT] or repetitive blood stem cell-supported HD courses [A20]).

S3.9. Landmark analysis of OS in patients that achieved early or late CR (following course 1 or course 2, respectively). Data are cumulative and landmark time was fixed at 90 days, that is the median time from randomization to late CR evaluation: (A) all patients, (B) patients at high risk, and (C) patients at standard risk, according to study definitions.

S3.10. Kaplan-Meier estimates of OS and RFS, according to randomization arm, in patients aged ≤60 years with *de novo* AML, patients with SR or HR AML and patients with favorable genetic risk AML, according to the ELN 2010 stratification. (A) Patients aged ≤60 years with *de novo* AML, (B) unselected patients with (*left*) standard-risk or (*right*) high-risk AML, (C) patients aged ≤60 years with *de novo* AML and with (*left*) standard-risk or (*right*) high-risk AML, and (D) patients with genetic risk subsets according to the ELN 2010 stratification.

S3.1. Results of the NILG AML 01/00 trial, which generated the study hypothesis for the NILG AML 02/06 trial.

The results of the phase II trial, NILG-AML 01/00 (Clinical.Trials.gov identifier: NCT00400673), demonstrated the value of sequential high-dose salvage in patients refractory to ICE, regardless of the clinico-cytogenetic risk group. These results confirmed that patients entering complete remission (CR) during course 1 (early CR) had better outcomes than patients entering CR during course 2 (late CR). The results also demonstrated the safety and feasibility of dose-dense, multiple high-dose cytarabine courses, supported by re-infusion of a limited amount of autologous blood stem cells.

Study design. This prospective trial design included a two-step, response-oriented CR induction. *Course 1*: patients received standard ICE chemotherapy (idarubicin 12 mg/m^2/d on days 1-3, etoposide 100 mg/m^2/d on days 1-5, cytarabine 100 mg/m^2/bd on days 1-7, and G-CSF starting on day 8). *Course 2*: Patients refractory to standard ICE chemotherapy received a sequential high-dose schedule (sHD: idarubicin 17.5 mg/m^2/d on days 1 and 8, cytarabine 3 g/m^2/bd on days 2, 3, 9, and 10, G-CSF starting on day 11). *Post-remission therapy*: Patients were allocated to different treatments, based on the risk of relapse. The high-risk (HR) group received allogeneic stem cell transplantation (HSCT). The standard-risk (SR) group received up to three high-dose cytarabine courses, supported by autologous blood stem cells (1-2 $\times$ 10^6/kg CD34$^+$ cells), after each course.

Risk class. 581 patients with a median age of 52 years (range 19-68 years) formed the study group. 180 patients (30.9%) had secondary AML and/or were older than 60 years. Patients were stratified as SR or HR, according to cytogenetics (i.e., favorable, unfavorable) and additional risk factors, in cases of intermediate/normal/unknown cytogenetics. Patients were considered HR, when any of the following were true: WBC count >50$\times$10^9/L, FAB class M0/6/7, hepato/splenomegaly, myelodysplasia (MDS)-related/secondary AML, *FLT3*- internal tandem duplication (ITD), or late CR. Cytogenetic risk groups were: favorable, 8.5% (n=50); intermediate/normal, 59% (n=342; [SR=123; HR=219]); unfavorable, 20.5% (n=120); and unknown, 12% (n=69 [SR=24; HR=45]).

CR induction results. Following ICE, the CR rate was 82% in the SR group and 68% in the HR group (*P*=.002). 129 patients had resistant AML (22%). 95 patients were refractory to ICE (73.6%; SR=22, HR=73) and received sHD (course 2). In this group, the CR rates were 64% among those with SR and 55% among those with HR (*P*=.46). The response rate was rather homogeneous across different cytogenetic risk subsets (Table S3.1.1).

Table S3.1.1: Risk classes for patients that received ICE (Course 1) or ICE followed by sHD (Course 2)

Risk class		**Course 1 (ICE)**		**Course 2 (sHD)**	
Cytogenetics	Other factors*	Patients, N	Refractory, N (%)	Patients, N	CR, N (%)
Favorable	No	30	3 (10)	2	2 (100)
	Yes	20	1 (5)	1	1 (100)
Normal	No	94	13 (14)	9	5 (55.5)
	Yes	178	33 (18.5)	22	15 (68)
Intermediate	No	53	12 (23)	10	6 (60)
	Yes	86	23 (27)	17	7 (41)
Unfavorable	No	42	13 (31)	8	5 (62.5)
	Yes	78	31 (40)	26	13 (50)

*WBC count >50×10^9/L, FAB class M0/6/7, hepato/splenomegaly, MDS-related/secondary AML, *FLT3*/ITD mutation

Long-term outcome. The 5-year overall survival (OS) varied significantly in both SR and HR groups, according to whether CR was achieved after course 1 or course 2. The 5-year OS rates after course 1 vs. course 2 were: 56% vs. 39% in the SR group (*P*=.004), and 40% vs. 17% in the HR group (*P*<.0001; Figure S3.1.1). The best outcome was observed in the SR group of early responders aged <60 years with *de novo* AML.

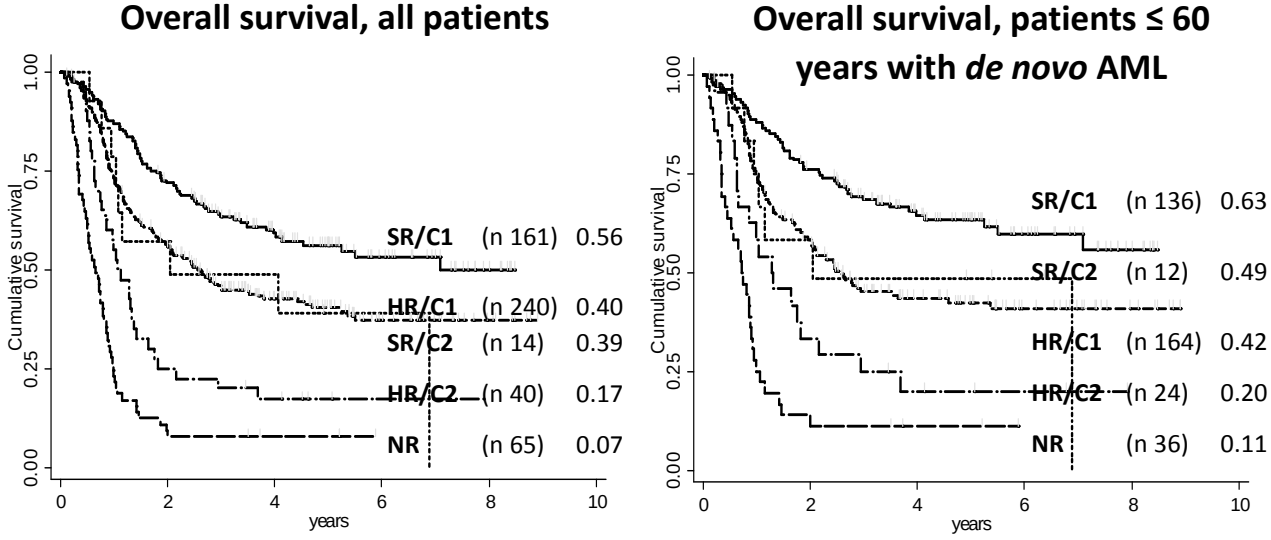


Figure S3.1.1: Kaplan-Meier plots show 5-year overall survival in standard-risk (SR) and high-risk (HR) groups, according to whether complete remission was achieved after course 1 (C1) or after course 2 (C2). Overall survival is shown for (*left*) all patients and (*right*) patients aged <60 years with *de novo* AML. NR, non-responders

Autologous blood stem cell-supported high-dose consolidation. Patients underwent mobilization of CD34+ blood stem cells (≥2 ×10^6/kg) after intermediate-dose cytarabine (1g/m^2/bd on days 1-4) and G-CSF. Patients at SR were consolidated with 2-3 high-dose courses (cytarabine 2g/m^2/bd on days 1-5, idarubicin 8 mg/m^2/d on days 1 and 2) supported by 1-2 ×10^6/kg CD34+ cells on day 5,

plus G-CSF, to accelerate neutrophil recovery, reduce the incidence of infectious complications, and maintain short treatment intervals. In addition, considering SR patients and HR patients unable to proceed to HSCT, this treatment was administered to 200 total patients in CR, for a total of 576 courses, with few aplastic deaths (n=3, 0.5%; Figure S3.1.2).

Overall trial results. 5-year OS and disease-free survival (DFS) rates are reported for different disease categories and risk classes, according to the final post-remission therapy (Figure S3.1.2).

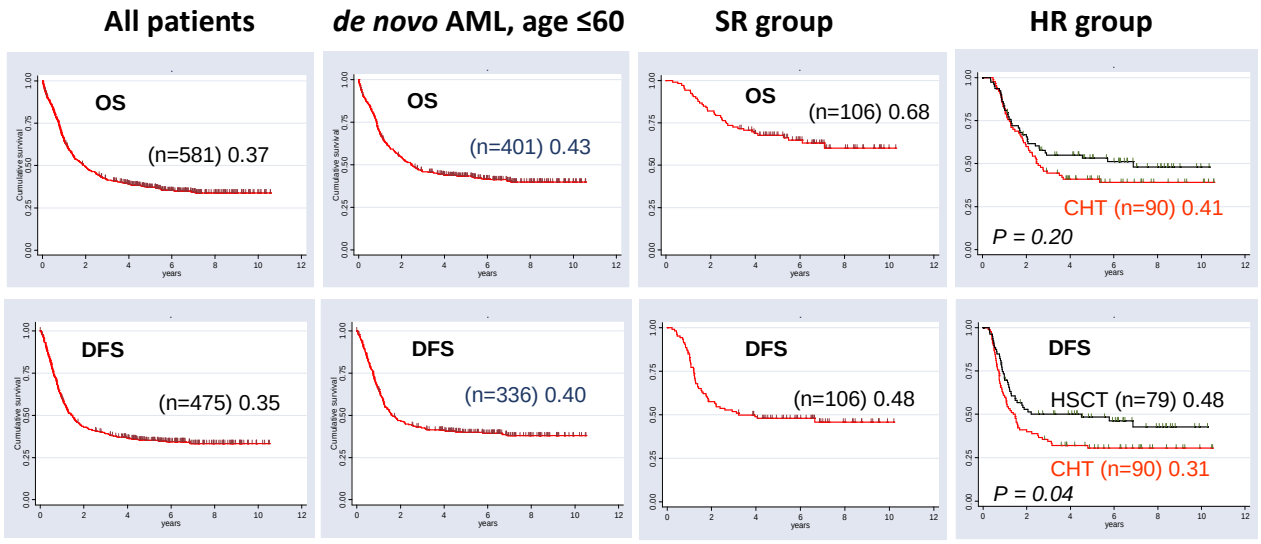
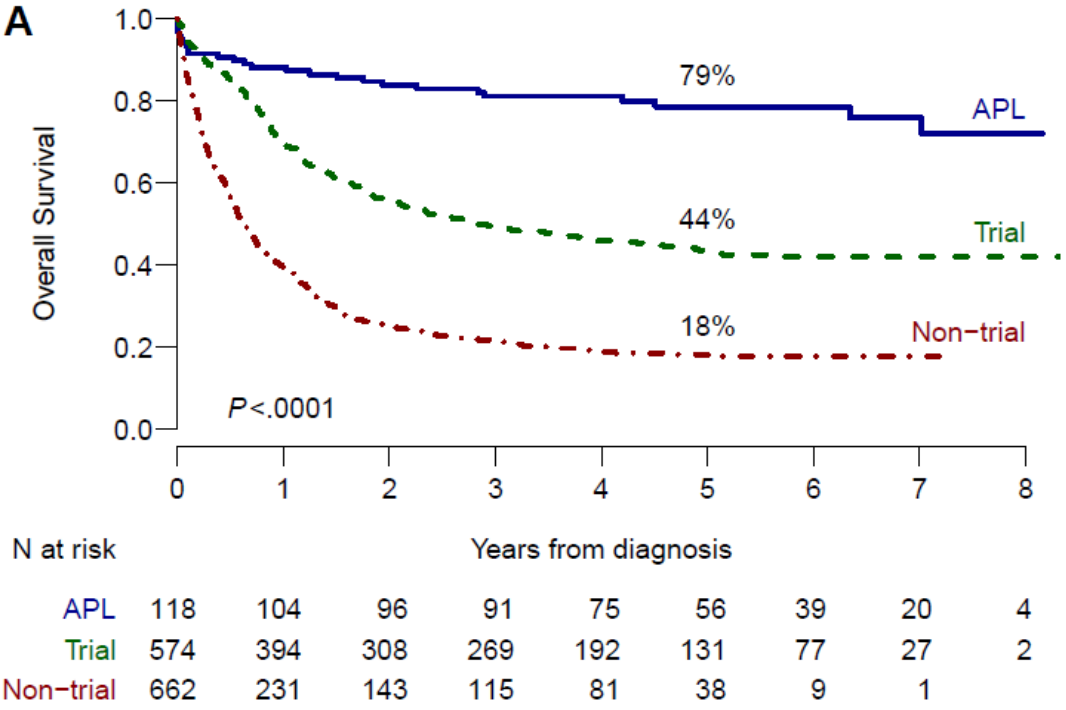


Figure S3.1.2: Kaplan-Meier plots show 5-year overall survival (OS) and disease-free survival (DFS). Subgroups show rates for a different disease category and risk classes. Colored lines indicate survival for groups with different final post-remission therapies; *black*: Hematopoietic stem cell transplantation (HSCT); *red*: chemotherapy (CHT).

S3.2: Amendments and protocol versions (NILG AML 02/06)

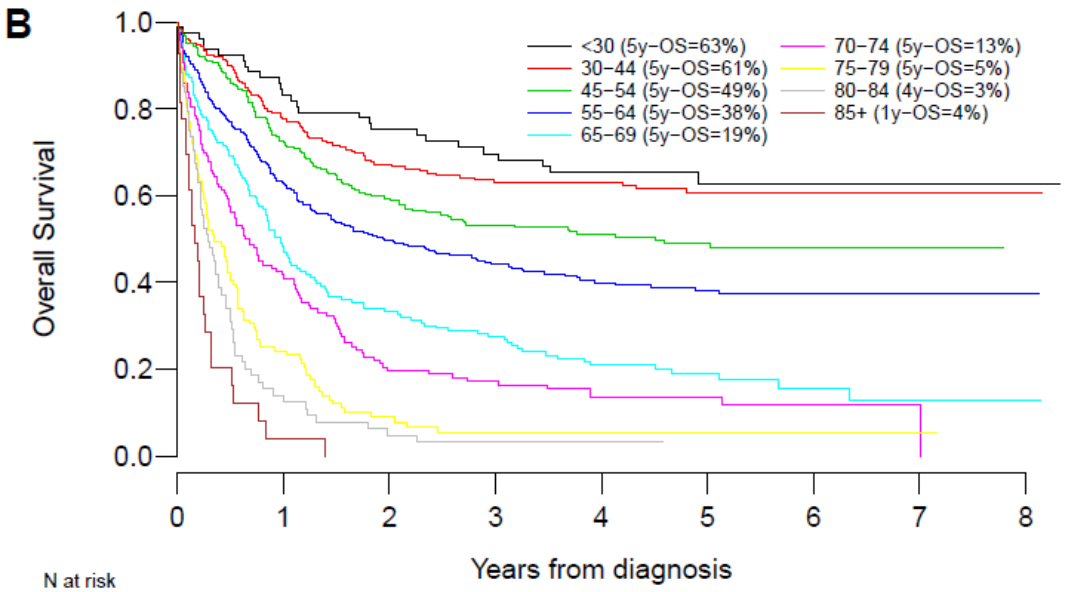
Protocol version	Date issued	Amendment
1	Jul 03, 2006	-
2	May 22, 2007	Lower cytarabine dose in sHD induction regimen (from 2 to 1 g/m^2) for patients deemed unfit to tolerate full dose cytarabine and/or aged >60 years
3	Sep 28, 2009	Upper age limit for enrolment and randomization set at 65 years, as recommended by the Data Monitoring and Safety Board following the interim analysis of Random 1 results in patients older than 65 years (n=35): CR rate 14/17 (82.4%) in arm A patients vs. 9/18 (50%) in arm B patients (*P*=.07). Recomendation based on poor accrual rate, comparable CR rates and higher toxicity of arm B in older patient group.
4	Apr 09, 2010	Ancillary subproject approved: immunophenotypic study of early blast cell clearance in the peripheral blood during induction course 1
5	Jan 16, 2013	Extension of study duration (from 5 to 6 years, including 1 year of follow-up from the date that the last patient was randomized), as recommended by the Data Monitoring and Safety Board following reassessment of low accrual rate to Random 2 and need to implement subset analyses in smaller diagnostic and prognostic groups.

S3.3. Outcome analysis of all 1354 patients registered during NILG AML 02/06 trial (2007 – 2012). Overall survival (OS, 5-year rates) according to diagnosis of APL (acute promyelocytic leukemia) vs. non-APL AML (trial patients vs. non-trial patients) (A); OS according to patient age at diagnosis (B).



N at risk

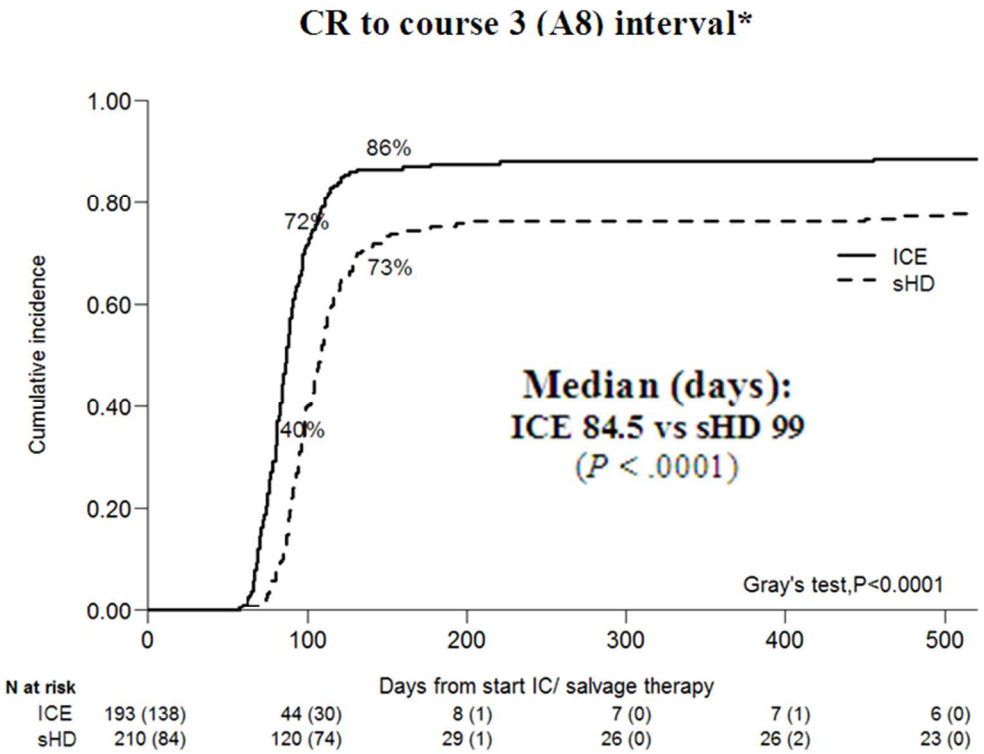
APL	118	104	96	91	75	56	39	20	4
Trial	574	394	308	269	192	131	77	27	2
Non−trial	662	231	143	115	81	38	9	1	



N at risk

<30	80	63	56	50	36	23	16	8	1
30−44	212	160	133	121	96	63	37	13	2
45−54	223	156	125	110	88	59	36	13	
55−64	310	189	146	127	87	53	25	9	2
65−69	159	73	51	40	25	16	7	3	1
70−74	152	53	25	21	13	9	3	1	
75−79	123	26	8	4	2	2	1	1	
80−84	68	8	3	2	1				
85+	27	1							

S3.4. Interval from CR to ID chemotherapy and rates of mobilization of CD34+ blood cells, by randomization arm. Days from CR to ID chemotherapy "A8" course and rates of mobilization of CD34+ blood cells following, by randomization arm.

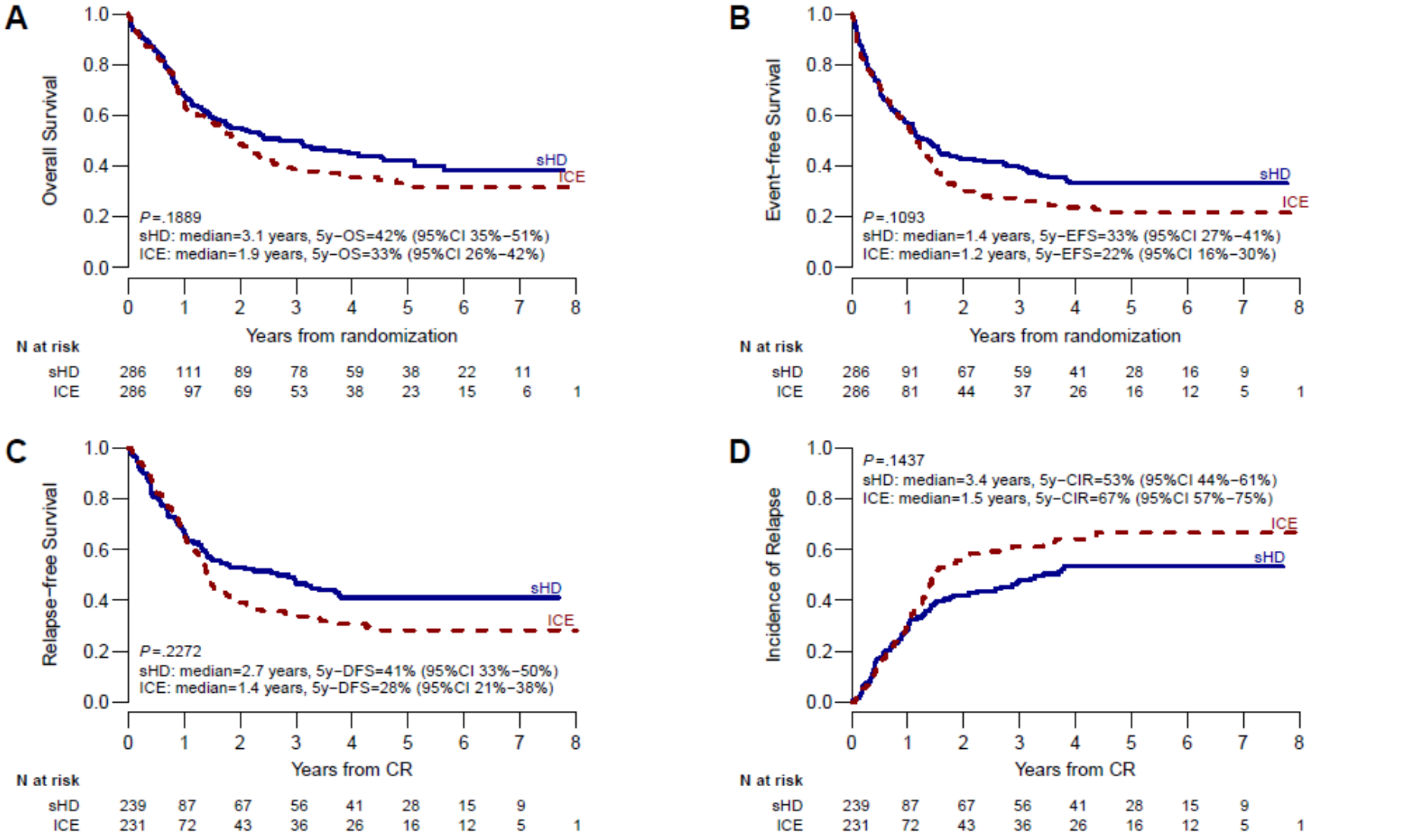


Blood stem cell mobilization (course 3, A8)

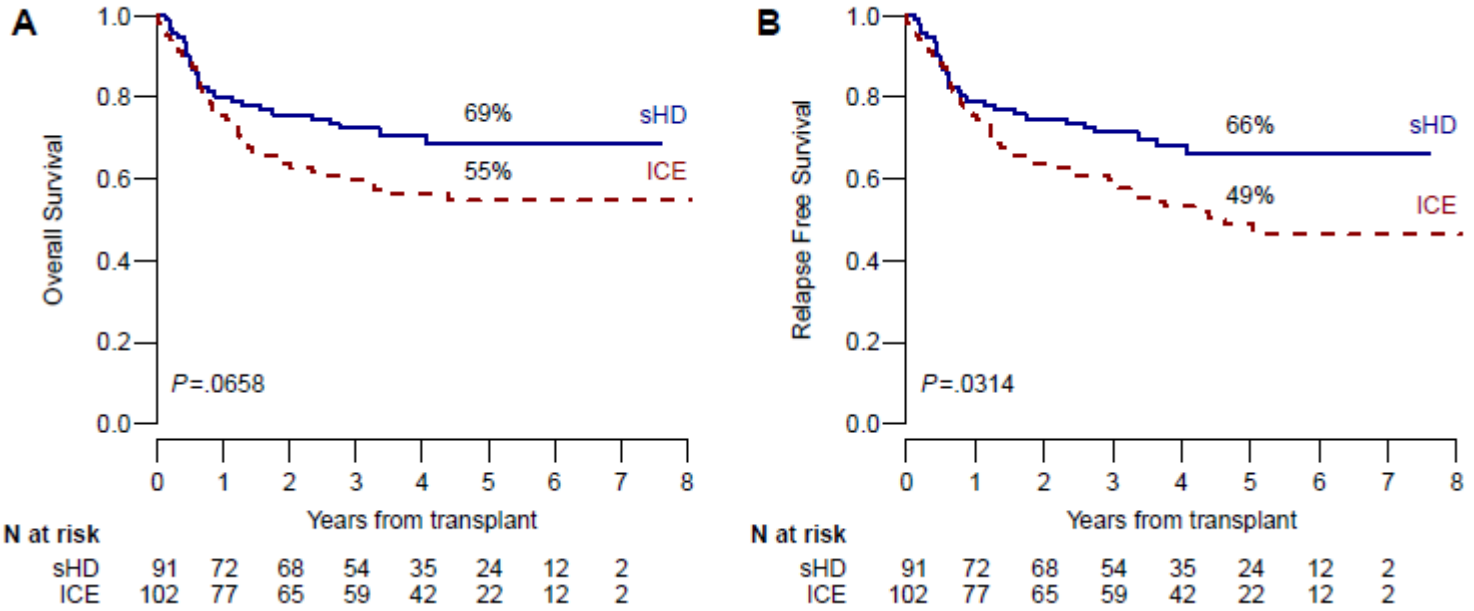
	ICE (n 186)	sHD (n 166)	P
Apheresed, no. (%)	153 (82.2)	123 (74.0)	.06
CD34+ cell harvest*, no. (%)	123 (66.1)	93 (56.0)	.33

*$\geq 2 \times 10^6$/kg

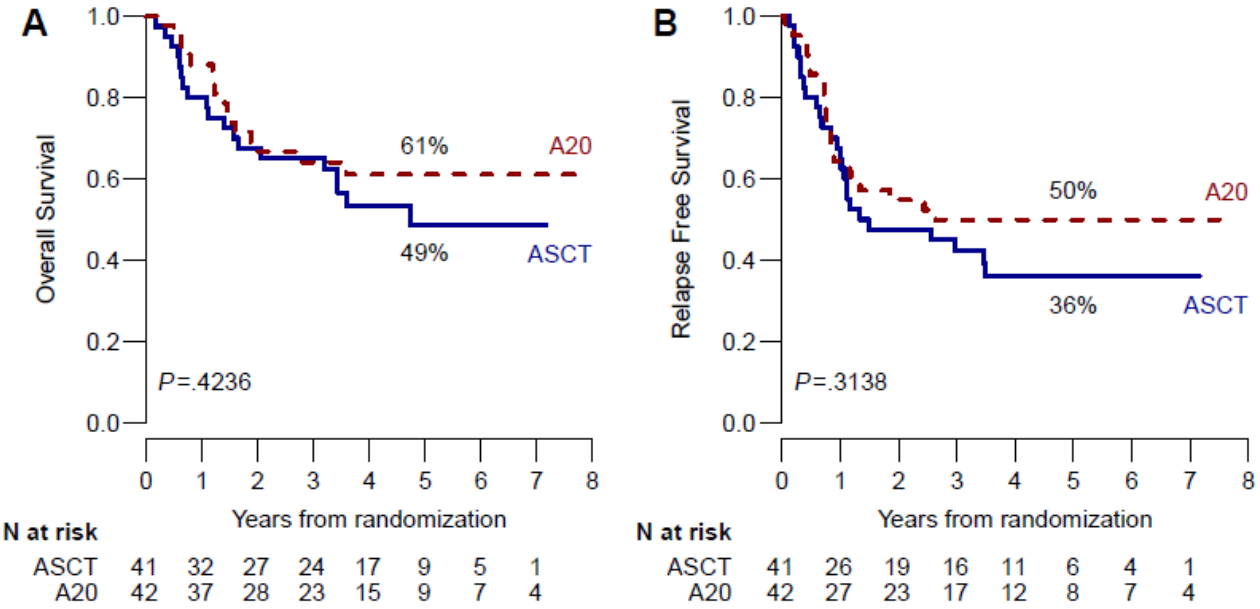
S3.5. Kaplan-Meier survival analyses, according to the randomization arm, after censoring at time of allogeneic hematopoietic cell transplantation. (A) Overall survival (OS); (B) event-free survival (EFS); (C) relapse-free survival (RFS); and (D) cumulative incidence of relapse (CIR)**.**



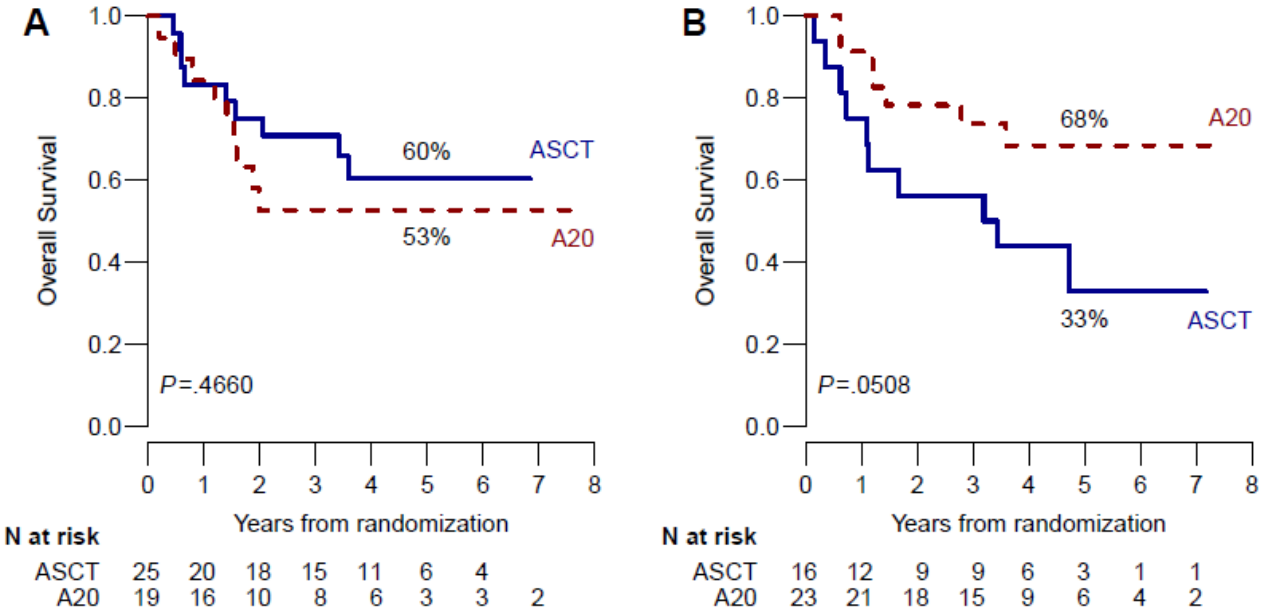
S3.6. Kaplan-Meier survival analyses, according to the randomization arm, in patients receiving an allogeneic hematopoietic cell transplantation in first CR. (A) overall survival (OS); (B) relapse-free survival (RFS).



S3.7.1 Kaplan-Meier survival analyses according to random 2 arm (BU-CY autotransplantation [ASCT] or repetitive blood stem cell-supported HD courses [A20]). (A) overall survival; (B) relapse-free survival.



S3.7.2 Kaplan-Meier survival analyses according to random 1 arm (ICE or sHD induction course 1) and random 2 arm (BU-CY autotransplantation [ASCT] or repetitive blood stem cell-supported HD courses [A20]). (A) overall survival according to random 2 arm for patients receiving ICE chemotherapy at random 1; (B) overall survival according to random 2 arm for patients receiving sHD chemotherapy at random 1.

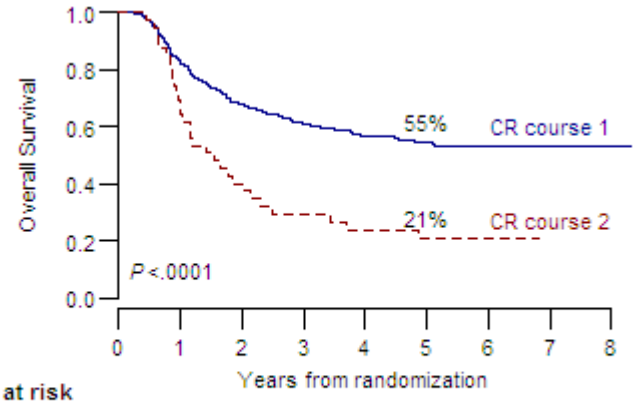


S3.8. Comprative toxicity analysis in random 2 patients (BU-CY autotransplantation [ASCT] or repetitive blood stem cell-supported HD courses [A20]; incidence data > 3% are reported).

Toxicity type	**N (%)**	**ASCT (N=41)**	**A20 (N=42)**	***P***
Infections (etiology)				
Any	60 (72.3)	27 (65.9)	33 (78.6)	.22
Bacterial	31 (37.3)	9 (22)	22 (52.4)	.01
Gram+	18 (21.7)	7 (17.1)	11 (26.2)	.007
Gram-	19 (22.9)	4 (9.8)	15 (35.7)	.008
Fungal	1 (1.2)	0 (0)	1 (2.4)	.27
Viral	6 (7.2)	2 (4.9)	4 (9.5)	.37
Unknown	26 (31.3)	12 (29.3)	14 (33.3)	.42
Infections (clinical picture)				
Fever	59 (71.1)	27 (65.9)	32 (76.2)	.27
Bacteremia	20 (24.1)	6 (14.6)	14 (33.3)	.11
Sepsis	16 (19.3)	3 (7.3)	13 (31)	.02
Pneumonia	10 (12)	2 (4.9)	8 (19)	.10
Other involved site	9 (10.8)	4 (9.8)	5 (11.9)	.44
Gastrointestinal system	3 (3.6)	3 (7.3)	0 (0)	.06
Skin	4 (4.8)	1 (2.4)	3 (7.1)	.75
Urinary system	2 (2.4)	0 (0)	2 (4.8)	.54
Other toxicity	51 (61.4)	30 (73.2)	21 (50)	.04
Hemorrhage	6 (7.2)	3 (7.3)	3 (7.1)	.08
Grade >2	1 (1.2)	1 (2.4)	0	
Metabolism	14 (16.9)	5 (12.2)	9 (21.4)	.01
Grade >2	3 (3.6)	1 (2.4)	2 (4.8)	
Gastrointestinal system	40 (48.2)	25 (61)	15 (35.7)	.05
Grade >2	10 (12.0)	9 (22)	1 (2.4)	
Pulmonary	3 (3.6)	1 (2.4)	2 (4.8)	.04
Grade >2	1 (1.2)	1 (2.4)	0	
Cardiovascular system	7 (8.4)	5 (12.2)	2 (4.8)	.07
Grade >2	0	0	0	
Liver	23 (27.7)	11 (26.8)	12 (28.6)	.03
Grade >2	7 (8.4)	5 (12.2)	2 (4.8)	
Central/peripheral nervous system	2 (2.4)	0 (0)	2 (4.8)	.01
Grade >2	2 (2.4)	0	2 (4.8)	
Skin	8 (9.6)	3 (7.3)	5 (11.9)	.03
Grade >2	1 (1.2)	0	1 (2.4)	
Allergy	5 (6)	3 (7.3)	2 (4.8)	.10
Grade >2	0	0	0	

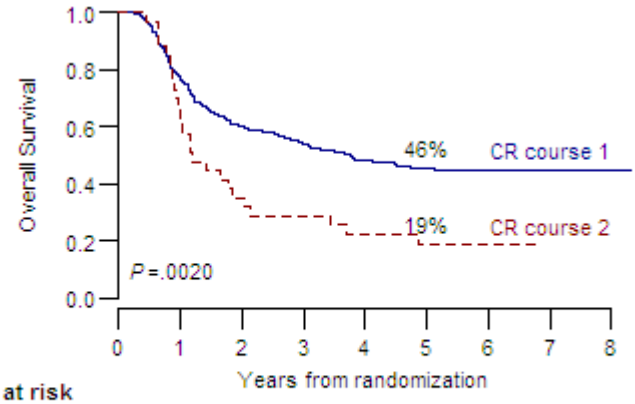
S3.9. Landmark analysis of OS in patients that achieved early or late CR (following course 1 or course 2, respectively). Data are cumulative and landmark time was fixed at 90 days, that is the median time from randomization to late CR evaluation: (A) all patients, (B) patients at high risk, and (C) patients at standard risk, according to study definitions.

(A) Overall Survival according to early or late CR (course 1 vs. course 2)



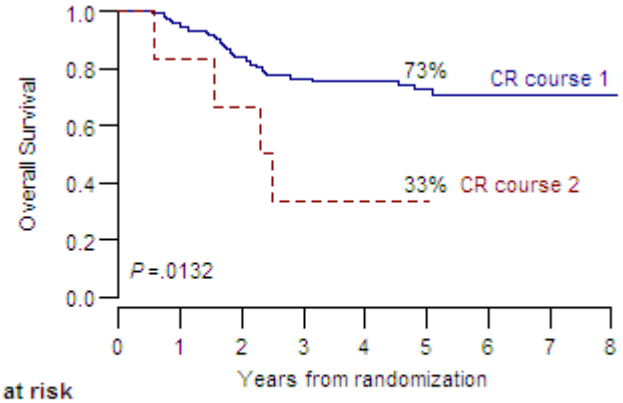
N at risk									
CR course 1	424	346	280	247	175	119	74	26	2
CR course 2	39	26	15	11	9	6	1		

(B) Overall Survival according to early or late CR (course 1 vs. course 2) in patients at high risk



N at risk									
CR course 1	286	217	168	148	99	63	40	11	1
CR course 2	33	21	11	9	7	5	1		

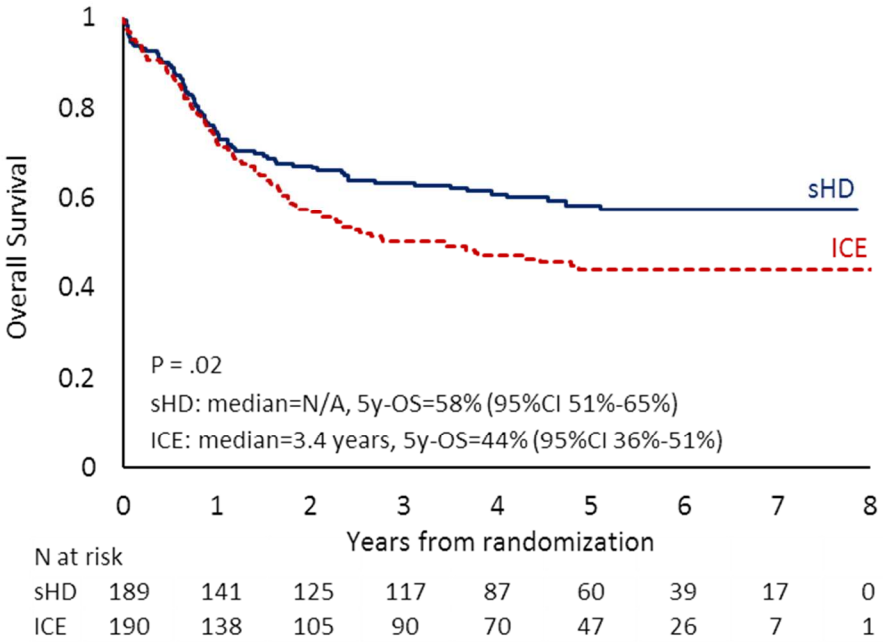
(C) Overall Survival according to early or late CR (course 1 vs. course 2) in patients at standard risk

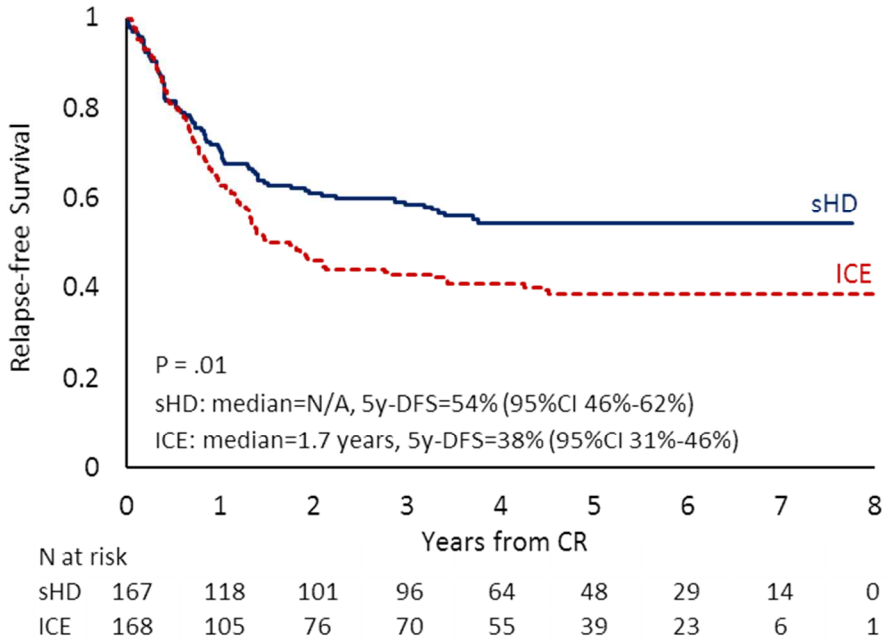


N at risk									
CR course 1	138	129	112	99	76	56	34	15	1
CR course 2	6	5	4	2	2	1			

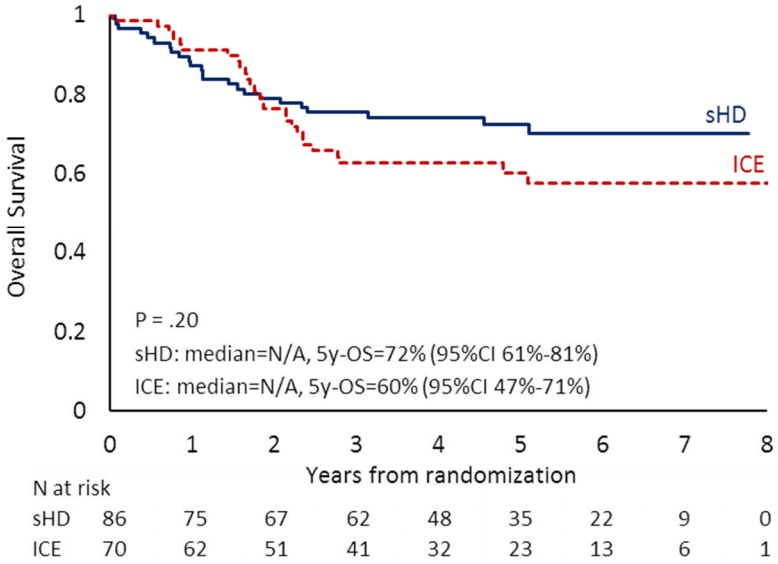
S3.10. **Kaplan-Meier estimates of OS and RFS, according to randomization arm, in patients aged ≤60 years with *de novo* AML, patients with SR or HR AML and patients with favorable genetic risk AML, according to the ELN 2010 stratification.** (A) Patients aged ≤60 years with *de novo* AML, (B) unselected patients with (*left*) standard-risk or (*right*) high-risk AML, (C) patients aged ≤60 years with *de novo* AML and with (*left*) standard-risk or (*right*) high-risk AML, and (D) patients with genetic risk subsets according to the ELN 2010 stratification.

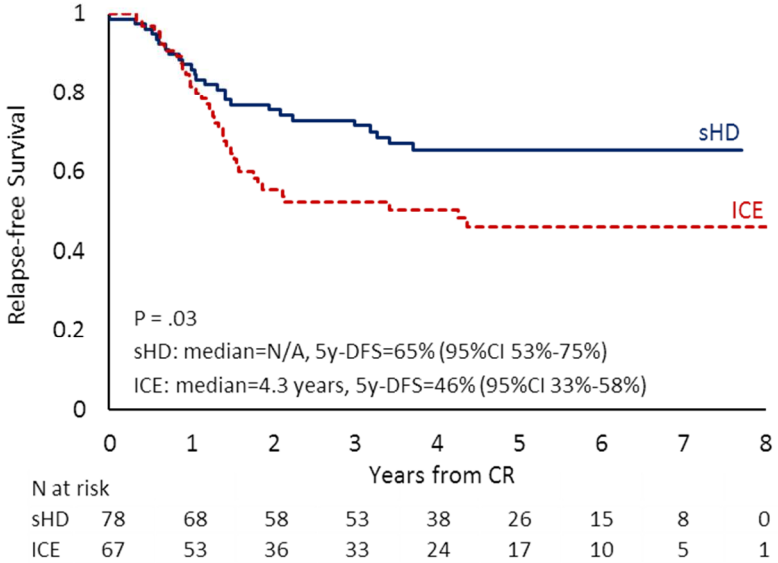
(A) Patients subset: age ≤60 years with *de novo* AML



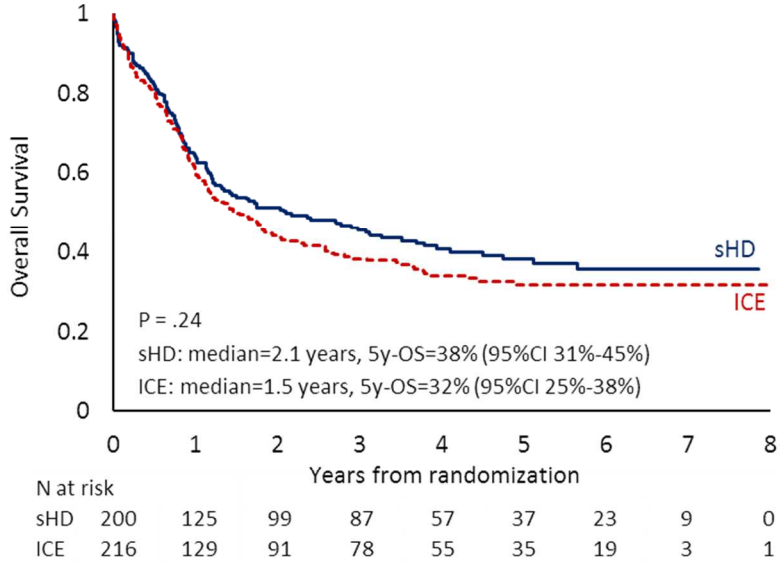


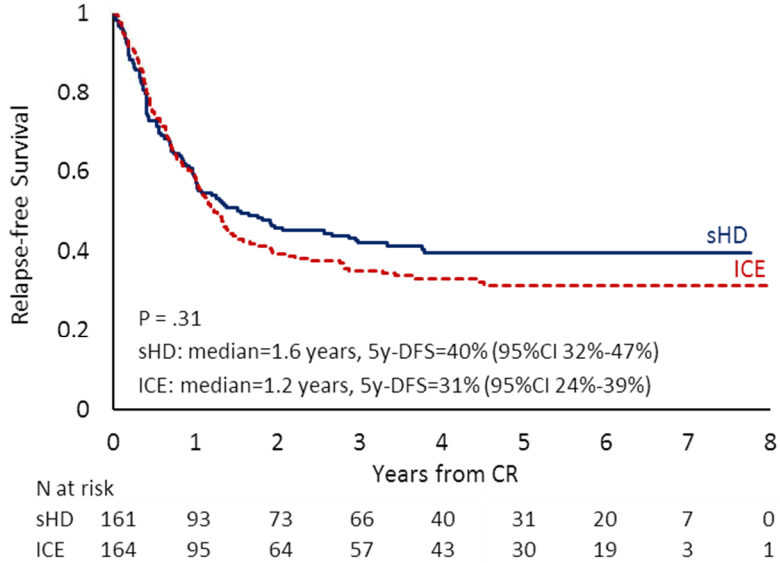
(B) Patients subset: standard risk



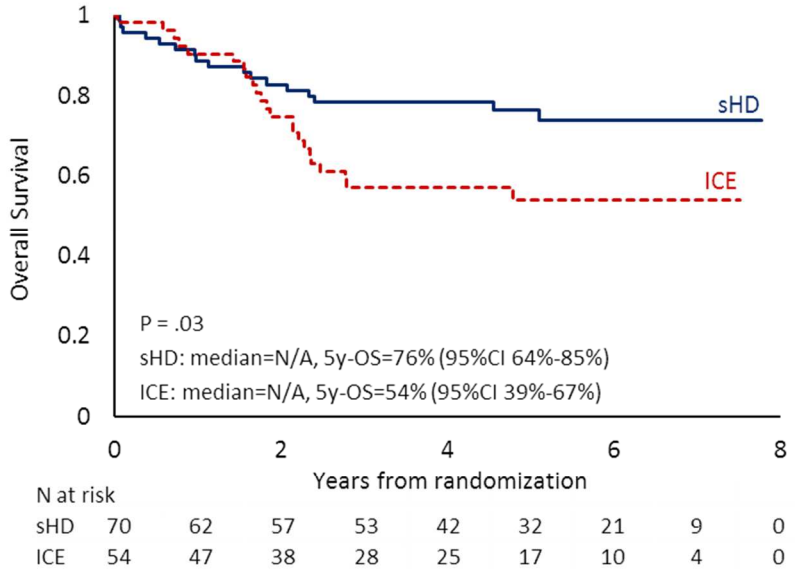


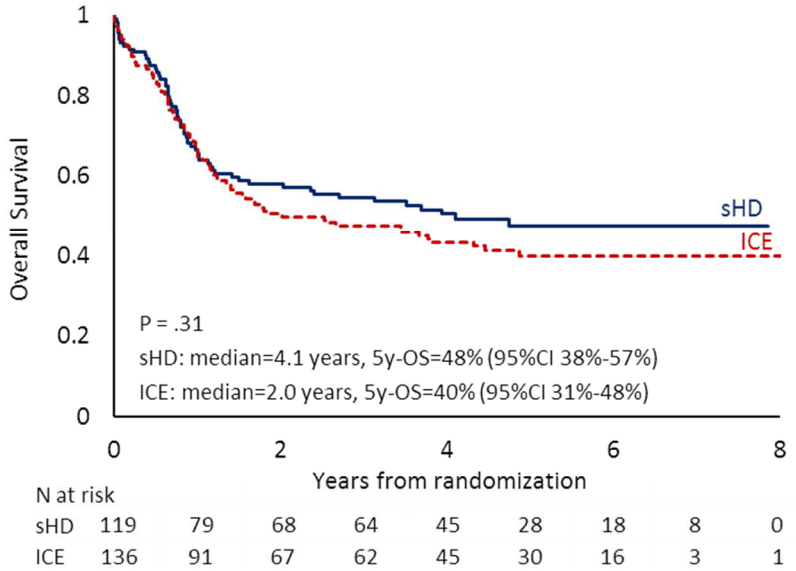
Patient subset: high risk

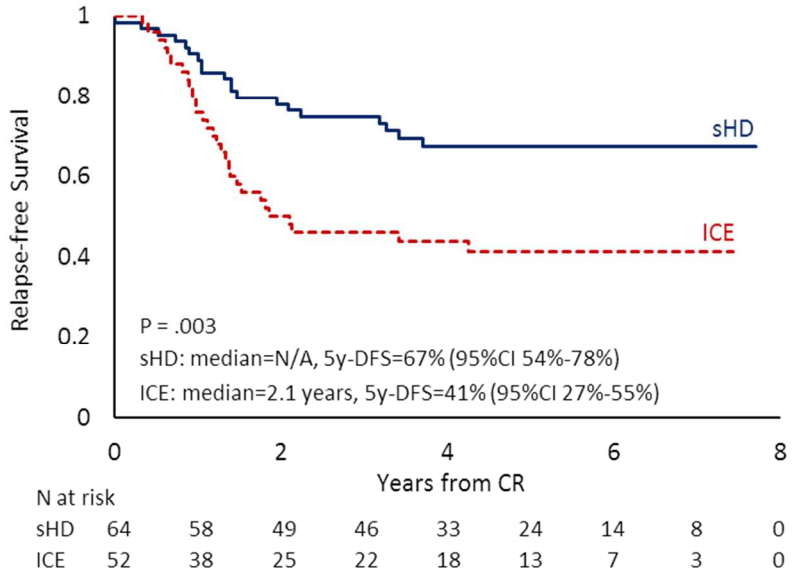


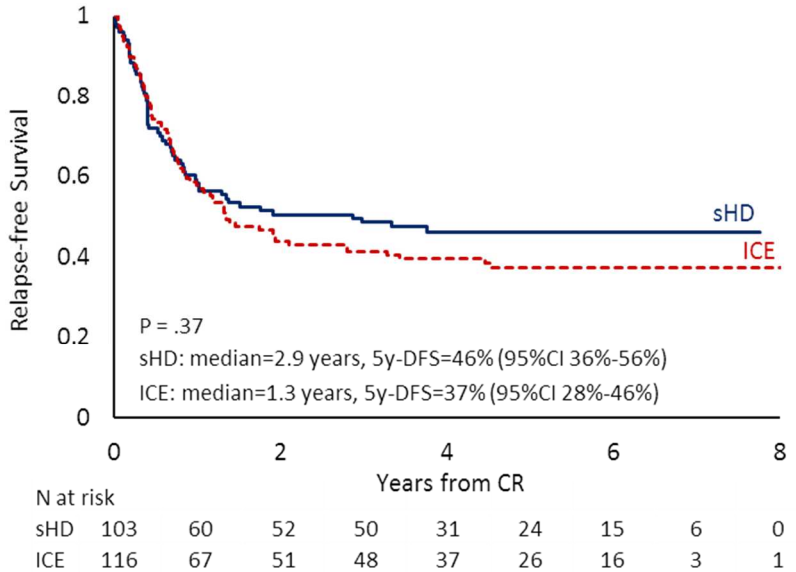


(C) Patients subset: age ≤60 years with *de novo* AML, standard risk (*left*) and high risk (*right*)









(D) Patient subset: ELN 2010 genetic risk groups (all ages)