Supplemental Table 1.

Patient characteristic and outcomes from overall cohort and derivation cohort and validation cohort

	Overall cohort (N=616)	Derivation cohort (N=308)	Validation cohort (N=308)	*P*
HCT time period	Aug. 1999 - May 2015	Aug. 1999 - April 2009	May 2009 - May 2015	
Survival Follow Up (months)				
median (95% CI)	54.3 (2.0-181.5)	80.8 (72.3-88.6)	36.6 (32.7-41.8)	<.001
range	1.97 - 84.63	11.05- 181.51	1.97 - 84.63	
Outcomes	**2y / LFU**	**2y / LFU**	**2y / LFU**	
Deceased, N (%)	179 (29) / 216 (35)	103 (33) / 132 (43)	76 (25) / 84 (27)	
Events, N (%)	229 (37) / 259 (42)	129 (42) / 152 (49)	100 (32) / 107 (35)	
Relapse incidence, N (%)	157 (25) / 175 (28)	95 (31) / 109 (35)	62 (20) / 66 (21)	
NRM, N(%)	72 (12) / 79 (13)	34 (11) / 38 (12)	38 (12) / 41 (13)	
Sec. Malignancy, N (%)	0 (0) / 5 (1)	0 (0) / 5 (2)	0 (0) / 0 (0)	
	Estimates (%)	**Estimates (%)**	**Estimates (%)**	
2y-OS (95% CI)	70 (66 - 74)	66 (61 - 72)	73 (68 - 79)	.022
4y-OS (95% CI)	63 (59 - 68)	59 (53 - 65)	70 (64 - 75)	.023
2y-EFS (95%CI)	62 (58 - 66)	58 (53 - 64)	66 (60 - 71)	.059
4y-EFS (95%CI)	57 (53 - 61)	53 (47 - 59)	62 (56 - 68)	.027
2y-CIR (95%CI)	27 (23 - 30)	31 (25 - 36)	22 (16 - 27)	.008
4y-CIR (95%CI)	30 (26 - 34)	35 (30 - 40)	24 (19 - 29)	.003
2y-TRM (95% CI)	12 (10 - 15)	11 (8 - 15)	13 (9 - 17)	.518
4y-TRM (95% CI)	14 (11 - 17)	13 (9 - 16)	14 (10 - 18)	.484
aGVHD, N (%)				.864†
Grade 0	225 (37)	109 (35)	116 (38)	
grade 1	138 (22)	78 (25)	60 (19)	
Grade 2	168 (27)	81 (26)	87 (28)	
Grade 3	51 (8)	24 (8)	27 (9)	
Grade 4	25 (4)	12 (4)	13 (4)	
no data	9 (2)	4 (1)	5 (2)	
cGVHD, N(%)				.938†
none	472 (77)	232 (75)	240 (78)	
yes	128 (21)	67 (22)	61 (20)	
extended	33	20	13	
limited	50	29	21	
unspecified state	45	18	27	
no data	16 (3)	9	7	

P values determined using log-rank test.
†P values determined using Fisher exact test.

Supplemental Table 2.

Univariate analysis of OS for the overall, derivation and validation cohorts

	Overall cohort N=616		Derivation cohort N=308		Validation cohort N=308	
	2y-OS	*P*	2y-OS	*P*	2y-OS	*P*
HCT time period	Aug. 1999 - May 2015		Aug. 1999 - April 2009		May 2009 - May 2015	
$MRD_{pre\text{-}HCT}$		<.001		<.001		.018
neg	77% (72-81)		74% (68-81)		80% (73-86)	
low	80% (73-88)		81% (68-95)		80% (71-89)	
high	61% (50-72)		57% (43-71)		67% (51-83)	
very high	49% (39-59)		42% (28-55)		58% (43-73)	
Sex		.469		.710		.479
female	69% (63-75)		65% (57-74)		73% (65-81)	
male	72% (67-76)		67% (61-74)		77% (71-83)	
Age groups		.736		.795		.653
< 2 years	77% (61-93)		77% (54-100)		77% (54-100)	
2 to 10 years	71% (66-76)		66% (58-73)		78% (71-84)	
≥ 10 years	70% (65-75)		67% (59-75)		73% (66-80)	
Remission Status		<.001		.001		.095
CR1	75% (69-81)		73% (65-81)		77% (69-84)	
CR2	71% (66-76)		66% (59-73)		76% (70-83)	
>CR2	46% (31-62)		42% (23-61)		53% (28-79)	
Site of relapse		.941		.685		.434
isol. BM	71% (66-77)		65% (57-73)		79% (71-86)	
isol. extramed	71% (58-84)		77% (54-100)		69% (53-85)	
combined	69% (56-81)		62% (43-82)		74% (58-91)	
Time to relapse		<.001		<.001		.015
very early	51% (40-63)		41% (25-58)		61% (45-76)	
early	66% (57-74)		55% (41-68)		75% (65-86)	
late	83% (76-89)		81% (71-90)		85% (77-94)	
Immunophenotype		.558		.075		.241
BCP	70% (66-74)		64% (59-70)		77% (71-82)	
T lineage	73% (65-81)		76% (65-88)		70% (59-81)	
Donor		.007		.264		.010
MSD	79% (73-84)		71% (62-80)		86% (79-93)	
MUD	70% (65-76)		67% (59-75)		74% (66-82)	
MMD	62% (50-75)		58% (42-74)		70% (50-90)	
cord	64% (55-73)		59% (42-76)		65% (55-76)	
Stem-cell source		.021		.110		.072
BM	75% (70-79)		70% (64-77)		79% (73-85)	
PB	64% (55-74)		58% (46-70)		76% (62-90)	
CB	65% (57-74)		61% (46-76)		67% (58-77)	
Conditioning		.011		.051		.179
TBI-based	73% (69-77)		69% (63-75)		77% (72-82)	
Non-TBI-based	60% (50-70)		56% (43-69)		66% (51-81)	

Abbreviations: MRD minimal residual disease, CR complete remission; CR2 second complete remission; BCP B-cell precursor; BM bone marrow; PB peripheral blood; CB cord blood; MSD matched sibling donor; MUD matched unrelated donor; MMD mismatched donor; TBI total body irradiation; qPCR Real-time PCR; MFC multiparameter flow cytometry

P values determined using Log-rank test.

Supplemental Table 3.

Univariate analysis of EFS for the overall, derivation and validation cohorts

	Overall cohort N=616		Derivation cohort N=308		Validation cohort N=308	
	2y-EFS	*P*	2y-EFS	*P*	2y-EFS	*P*
HCT time period	Aug. 1999 - May 2015		Aug. 1999 - April 2009		May 2009 - May 2015	
$MRD_{pre-HCT}$		<.001		<.001		<.001
neg	68% (63-74)		65% (58-72)		72% (65-79)	
low	70% (61-79)		69% (53-85)		71% (60-82)	
high	55% (44-66)		53% (39-67)		58% (40-75)	
very high	34% (25-44)		32% (20-45)		37% (22-52)	
Sex		.671		.657		.253
female	61% (54-67)		60% (51-69)		61% (52-71)	
male	62% (57-67)		57% (50-64)		68% (61-75)	
Age groups		.980		.468		.489
< 2 years	65% (47-84)		77% (54-100)		54% (27-81)	
2 to 10 years	61% (56-67)		56% (48-64)		67% (60-75)	
≥ 10 years	62% (56-67)		58% (50-67)		65% (57-73)	
Remission Status		.001		.002		.331
CR1	66% (60-73)		66% (57-75)		67% (58-76)	
CR2	61% (56-66)		56% (49-64)		66% (58-73)	
>CR2	40% (24-55)		34% (16-53)		51% (25-77)	
Site of relapse		*.754*		.540		.332
isol. BM	62% (55-68)		56% (47-64)		69% (60-78)	
isol. extramed	61% (47-76)		69% (44-94)		58% (40-76)	
combined	57% (43-70)		50% (30-70)		63% (45-81)	
Time to relapse		*<.001*		<.001		.003
very early	40% (28-51)		32% (17-48)		47% (30-64)	
early	55% (46-64)		45% (32-59)		64% (51-76)	
late	75% (68-83)		73% (61-84)		78% (68-88)	
Immunophenotype		.245		.061		.683
BCP	61% (56-65)		56% (49-62)		66% (60-72)	
T lineage	66% (58-75)		69% (57-81)		64% (52-76)	
Donor		.082		.559		.064
MSD	66% (59-73)		60% (50-70)		73% (63-82)	
MUD	62% (56-68)		60% (52-68)		66% (57-74)	
MMD	55% (42-68)		50% (33-66)		65% (43-86)	
cord	56% (46-66)		53% (36-70)		58% (46-69)	
Stem-cell source		.166		.322		.234
BM	64% (59-69)		61% (54-68)		67% (60-74)	
PB	57% (47-67)		51% (38-63)		69% (54-84)	
CB	59% (50-68)		56% (41-71)		60% (49-71)	
Conditioning		.001		.031		.033
TBI-based	64% (60-69)		61% (55-67)		68% (62-73)	
Non-TBI-based	47% (37-57)		45% (32-58)		51% (35-67)	

Abbreviations: MRD minimal residual disease, CR complete remission; CR2 second complete remission; BCP B-cell precursor; BM bone marrow; PB peripheral blood; CB cord blood; MSD matched sibling donor; MUD matched unrelated donor; MMD mismatched donor; TBI total body irradiation; qPCR Real-time PCR; MFC multiparameter flow cytometry

P values determined using Log-rank test.

Supplemental Table 4.

Univariate analysis of CIR for the overall, derivation and validation cohorts

	Overall cohort N=616		Derivation cohort N=308		Validation cohort N=308	
	2y-CIR	*P*	2y-CIR	*P*	2y-CIR	*P*
HCT time period	Aug. 1999 - May 2015		Aug. 1999 - April 2009		May 2009 - May 2015	
$MRD_{pre-HCT}$		<.001		.002		.001
neg	20% (16-24)		24% (18-30)		15% (10-21)	
low	19% (11-26)		25% (10-40)		16% (8-24)	
high	35% (25-46)		41% (27-55)		27% (12-42)	
very high	44% (34-54)		47% (34-61)		40% (25-54)	
Sex		.913		.270		.128
female	26% (20-31)		27% (19-35)		24% (17-32)	
male	25% (21-30)		33% (27-40)		17% (12-23)	
Age groups		.625		.141		.579
< 2 years	19% (4-34)		8% (0-22)		31% (6-56)	
2 to 10 years	27% (22-32)		34% (27-42)		20% (13-26)	
≥ 10 years	24% (19-29)		29% (21-37)		20% (13-26)	
Remission Status		.042		.122		.444
CR1	20% (15-25)		24% (16-32)		17% (10-24)	
CR2	28% (23-33)		34% (27-41)		22% (16-28)	
>CR2	34% (20-49)		38% (20-57)		27% (4-49)	
Time to relapse		<.001		<.001		.042
very early	46% (34-57)		62% (45-78)		32% (17-46)	
early	32% (24-41)		42% (28-55)		25% (14-35)	
late	16% (10-23)		19% (10-29)		13% (5-22)	
Site of relapse		.532		.696		.680
isol. BM	29% (23-34)		36% (27-44)		20% (13-28)	
isol. extramed	20% (8-32)		23% (0-46)		19% (5-32)	
combined	31% (19-44)		33% (14-52)		30% (12-47)	
Immunophenotype		.251		.332		.034
BCP	27% (23-30)		32% (26-38)		21% (16-26)	
T lineage	21% (14-29)		25% (14-37)		18% (9-27)	
Donor		.707		.799		.959
MSD	28% (21-34)		35% (25-44)		20% (12-29)	
MUD	25% (20-31)		30% (22-37)		21% (13-28)	
MMD	29% (17-40)		31% (16-46)		25% (6-44)	
cord	20% (13-27)		25% (10-40)		18% (9-26)	
Stem-cellsource		.132		.240		.726
BM	26% (22-31)		30% (24-37)		22% (16-28)	
PB	31% (22-40)		37% (26-49)		19% (6-32)	
CB	18% (11-25)		22% (9-35)		16% (8-24)	
Conditioning		.005		.081		.050
TBI-based	23% (20-27)		29% (23-34)		19% (14-23)	
Non-TBI-based	37% (27-47)		40% (28-53)		32% (17-46)	

Abbreviations: MRD minimal residual disease, CR complete remission; CR2 second complete remission; BCP B-cell precursor; BM bone marrow; PB peripheral blood; CB cord blood; MSD matched sibling donor; MUD matched unrelated donor; MMD mismatched donor; TBI total body irradiation; qPCR Real-time PCR; MFC multiparameter flow cytometry

P values determined using Gray's test.

Supplemental Table 5.

Univariate analysis of NRM for the overall, derivation and validation cohorts

	Overall cohort N=616		Derivation cohort N=308		Validation cohort N=308	
	2y-NRM	*P*	2y-NRM	*P*	2y-NRM	*P*
HCT time period	Aug. 1999 - May 2015		Aug. 1999 - April 2009		May 2009 - May 2015	
$MRD_{pre-HCT}$		.056		.070		.587
neg	11% (7-14)		10% (6-15)		11% (6-16)	
low	10% (5-16)		6% (0-15)		12% (5-19)	
high	9% (2-15)		6% (0-13)		12% (1-23)	
very high	20% (12-28)		21% (10-32)		19% (7-30)	
Sex		.646		.387		.850
female	12% (8-17)		13% (7-19)		12% (6-18)	
male	11% (8-14)		10% (6-14)		13% (8-17)	
Age groups		.564		.599		.894
< 2 years	15% (2-29)		15% (0-35)		15% (0-35)	
2 to 10 years	10% (7-14)		9% (5-14)		11% (6-17)	
≥ 10 years	13% (9-17)		13% (7-18)		13% (8-18)	
Remission Status		.024		.023		.477
CR1	12% (8-16)		10% (4-15)		14% (8-20)	
CR2	10% (7-13)		9% (5-14)		11% (6-15)	
>CR2	24% (11-38)		27% (10-44)		20% (0-40)	
Time to relapse		.478		*.435*		*.205*
very early	12% (5-20)		6% (0-14)		18% (6-31)	
early	11% (6-17)		13% (4-22)		10% (2-17)	
late	8% (3-12)		8% (1-15)		7% (1-14)	
Site of relapse		.138		.402		.077
isol. BM	8% (5-12)		8% (4-13)		8% (3-14)	
isol. extramed	18% (7-29)		8% (0-22)		22% (8-36)	
combined	12% (3-21)		17% (2-32)		7% (0-17)	
Immunophenotype		.900		.138		.266
BCP	12% (9-15)		12% (8-16)		11% (7-15)	
T lineage	11% (6-17)		5% (0-11)		16% (8-25)	
Donor		<.001		.019		.005
MSD	5% (2-8)		5% (1-9)		4% (0-8)	
MUD	12% (8-15)		11% (6-16)		13% (7-19)	
MMD	16% (6-26)		19% (7-32)		10% (0-23)	
cord	22% (14-30)		22% (8-36)		22% (13-31)	
Stem-cellsource		<.001		.042		.012
BM	9% (6-11)		8% (5-12)		9% (5-13)	
PB	12% (5-18)		12% (4-20)		11% (1-21)	
CB	21% (14-28)		22% (9-35)		21% (12-30)	
Conditioning		0.351		.438		.534
TBI-based	11% (8-14)		10% (7-14)		12% (8-16)	
Non-TBI-based	15% (8-22)		14% (5-23)		16% (4-27)	

Abbreviations: MRD minimal residual disease, CR complete remission; CR2 second complete remission; BCP B-cell precursor; BM bone marrow; PB peripheral blood; CB cord blood; MSD matched sibling donor; MUD matched unrelated donor; MMD mismatched donor; TBI total body irradiation; qPCR Real-time PCR; MFC multiparameter flow cytometry

P values determined using Gray's test.

Supplemental Table 6.

Comparison of the ability of qPCR and MFC methods for detecting $MRD_{pre\text{-}HCT}$ and $MRD_{post\text{-}HCT}$ Assessments on day +30, +60, +90, +180, +365 as defined in the land mark analysis.

	MRD categories				
	neg	low	high	very high	total
Pre-HCT					
MFC	197 (75.8)	6 (2.3)	19 (7.3)	38 (14.6)	260 (100)
qPCR	134 (37.6)	101 (28.4)	63 (17.7)	58 (16.3)	356 (100)
Day +30					
MFC	196 (94.2)	2 (1.0)	5 (2.4)	5 (2.4)	208 (100)
qPCR	147 (57.0)	78 (30.2)	26 (10.1)	7 (2.7)	258 (100)
Day +60					
MFC	55 (93.2)	2 (3.4)	1 (1.7)	1 (1.7)	59 (100)
qPCR	93 (82.3)	14 (12.4)	6 (5.3)	0 (0)	113 (100)
Day +90					
MFC	170 (96.6)	2 (1.1)	3 (1.7)	1 (1.0)	176 (100)
qPCR	162(63.5)	73 (28.6)	15 (5.9)	5 (2.0)	255 (100)
Day +180					
MFC	46 (100)	0 (0)	0 (0)	0 (0)	46 (100)
qPCR	131 (80.8)	19 (11.7)	5 (3.1)	8 (4.9)	162 (100)
Day +365					
MFC	82 (97.6)	1 (1.2)	1 (1.2)	0 (0)	84 (100)
qPCR	114 (84.4)	16 (11.8)	2 (1.5)	3 (2.2)	135 (100)
Day >+365					
MFC	17 (94.4)	1 (5.6)	0 (0)	0 (0)	18 (100)
qPCR	106 (89.1)	5 (4.2)	5 (4.2)	3 (2.5)	119 (100)

Abbreviations: MRD minimal residual disease; neg negative, HCT hematopoietic stem cell transplantation; MFC multiparameter Flow Cytometry; qPCR Real-time PCR.

Supplemental Table 7.

Effect of aGVHD on outcome of patients who were MRD+ and MRD- at day+30, day +90 and day +180 post-HCT. Missing values of aGVHD were not included.

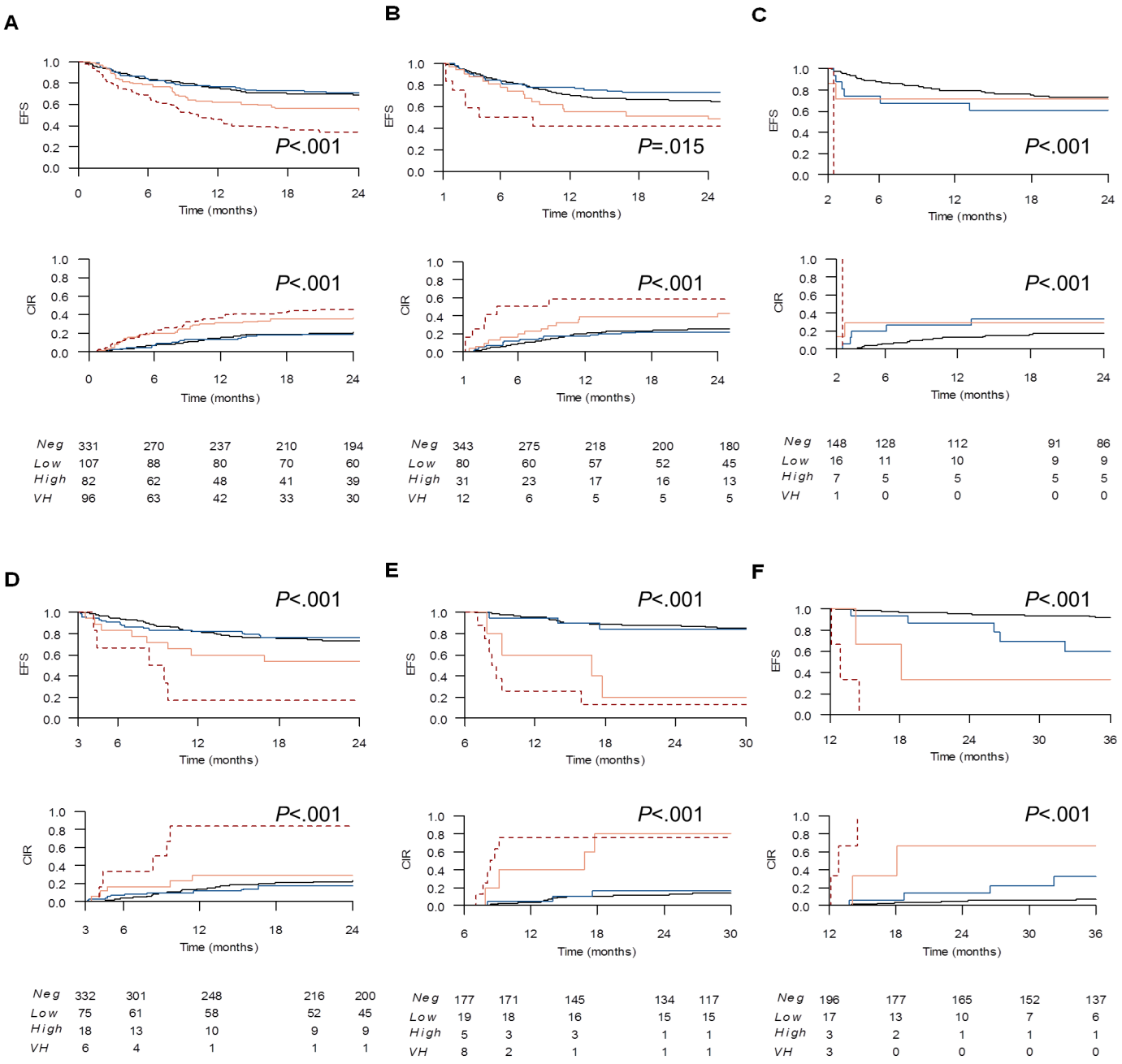
	N	(%)	2y-EFS (%) (95% CI)	*P*	HR (95% CI)	*P*	2y-CIR (%) (95% CI)	*P*	HR (95% CI)	*P*
MRD positive										
Day +30				.001		.012		.008		<.001
MRD positive - no aGVHD	33	27	51 (36 - 71)		1		49 (32 - 67)		1	
MRD positive - aGVHD	90	73	68 (59 - 79)		0.35 (0.16 - 0.80)		24 (15 - 33)		0.29 (0.16 - 0.55)	
Day +90				.040		.045		<.001		<.001
MRD positive - no aGVHD	23	23	55 (37 - 80)		1		46 (25 - 66)		1	
MRD positive - aGVHD	75	75	70 (61 - 82)		0.49 (0.25 - 0.98)		17 (8 - 25)		0.26 (0.12 - 0.57)	
Day +180				.094		.145		.301		.190
MRD positive - no aGVHD	12	38	42 (21 - 81)		1		50 (22 - 78)		1	
MRD positive - aGVHD	20	62	64 (47 - 90)		0.51 (0.21 - 1.26)		35 (14 - 56)		0.56 (0.23 - 1.35)	
MRD negative										
Day +30				.017		.021		<.001		<.001
MRD negative - no aGVHD	116	34	53 (45 - 64)		1		39 (30 - 48)		1	
MRD negative - aGVHD	221	66	71 (65 - 77)		0.66 (0.47 - 0.94)		18 (13 - 23)		0.45 (0.30 - 0.69)	
Day +90				.016		.107		.002		<.001
MRD negative - no aGVHD	113	35	63 (54 - 73)		1		35 (26 - 44)		1	
MRD negative - aGVHD	214	65	77 (72 - 83)		0.62 (0.42 - 0.92)		17 (12 - 22)		.44 (0.29 - 0.69)	
Day +180				.002		.003		.001		.003
MRD negative - no aGVHD	60	34	80 (70 - 91)		1		20 (9 - 31)		1	
MRD negative - aGVHD	115	66	89 (83 - 95)		0.34 (0.17 - 0.70)		9 (4 - 15)		0.31 (0.15 - 0.67)	

Abbreviations: aGVHD acute graft versus host disease; MRD minimal residual disease; HCT hematopoietic cell transplantation; EFS Event-free survival; CIR cumulative incidence of relapse; CI confidence interval; HR hazard ratio.

Supplemental Table 8.

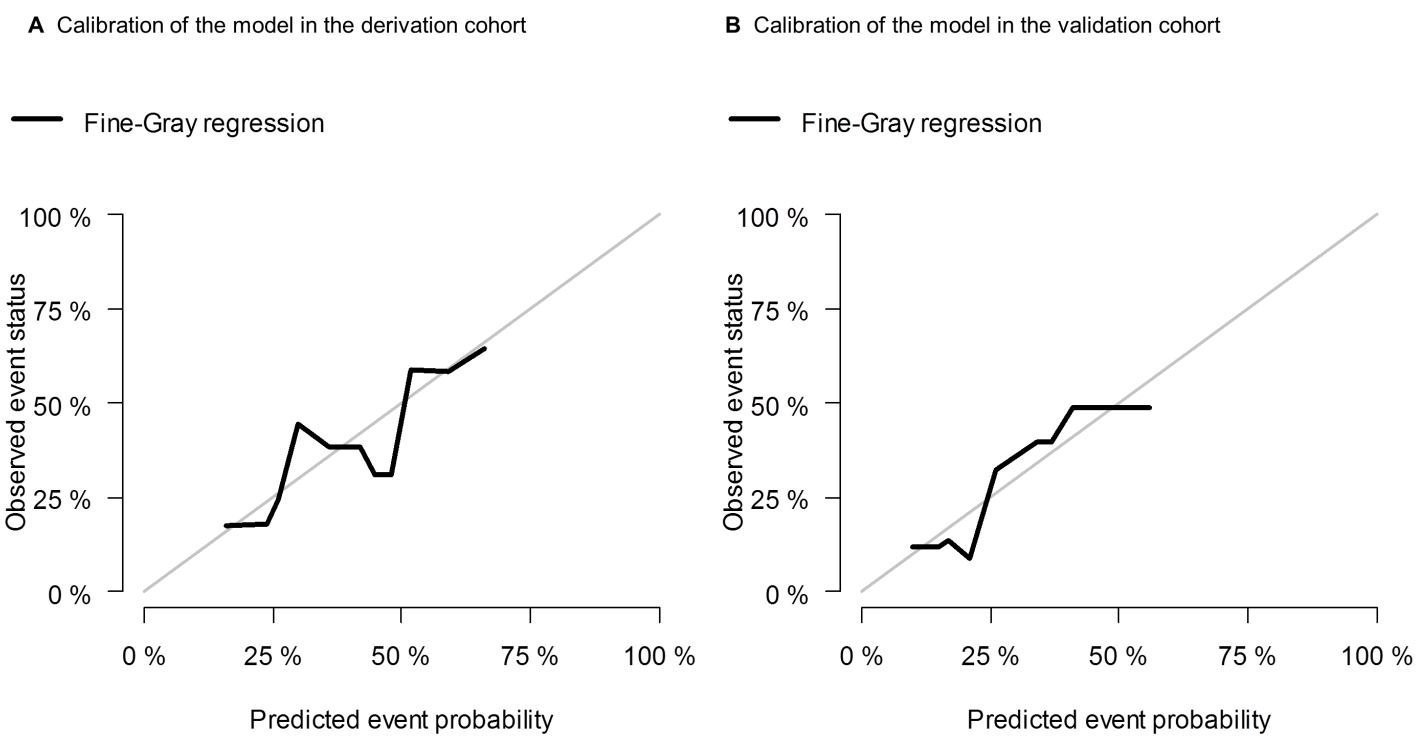
Testing for Interaction between MRD and aGVHD on relapse. Coefficients and hazard ratios are from time dependent Cox regression model.

	Coef	se (coef)	z	*P*	HR (95% CI)
MRD					
negative	reference				
positive	1.2441	3.4698	0.2256	<.001	3.47 (2.23 - 5.40)
AGVHD					
negative	reference				
Grade I-II	-1.1071	0.2374	-4.663	<.001	0.33 (0.21 - 0.53)
Grade III-IV	-0.7495	0.4026	-1.861	.0628	0.47 (0.21 - 1.04)
Interaction					
MRD pos: aGVHD grade I-II	0.2570	0.3378	0.761	.4466	1.29 (0.67 - 2.51)
MRD pos: aGVHD grade III-IV	-1.0852	0.663	-1.2629	.1034	0.34 (0.09 - 1.25)



Supplemental Figure 1.

Event-free survival (EFS) and cumulative incidence of relapse (CIR) in children with ALL who were MRD negative (solid blue), MRD low positive (solid gold), MRD high positive (solid grey) or MRD very high positive (dashed red) at different time points (A) prior to HCT (B) day +30; (C) day +60; (D) day +90; (E) day +180 and (F) day +365. P-values were determined using the log-rank test for EFS and the Gray test for CIR.



Supplemental Figure 2.
Calibration Plots for the integrated risk scores of the prognostic model based on pre-HCT MRD and baseline patient characteristics.