Posttransplantation MRD monitoring in patients with AML by next-generation sequencing using DTA and non-DTA mutations

Supplemental Data

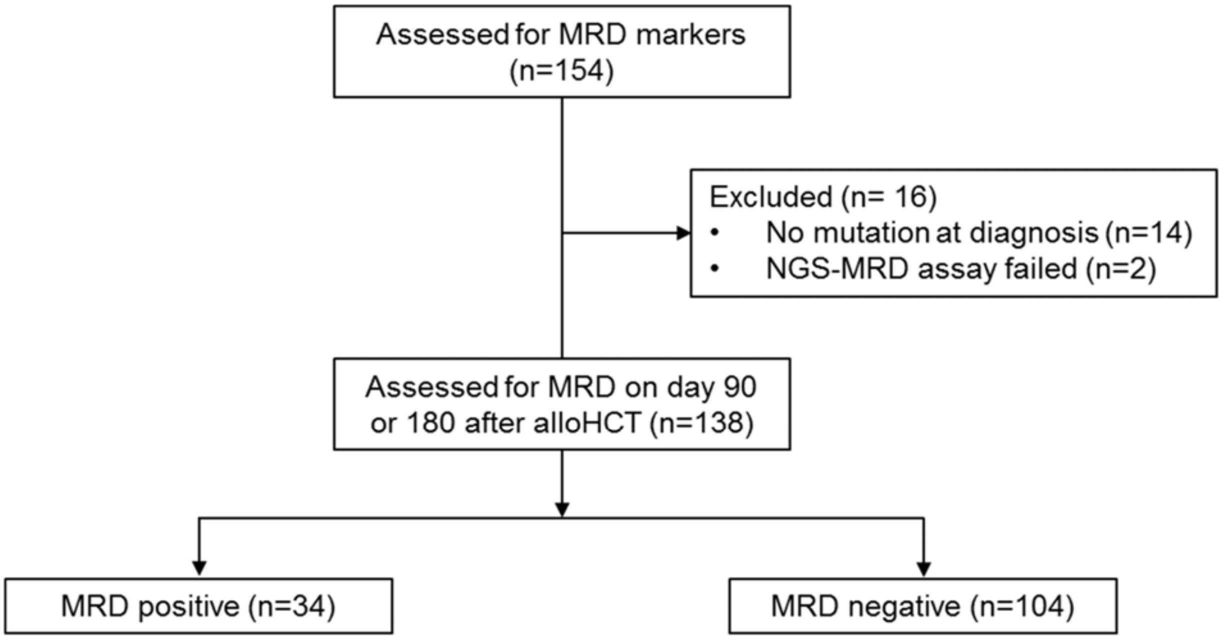
Supplemental Methods

Data sharing statement

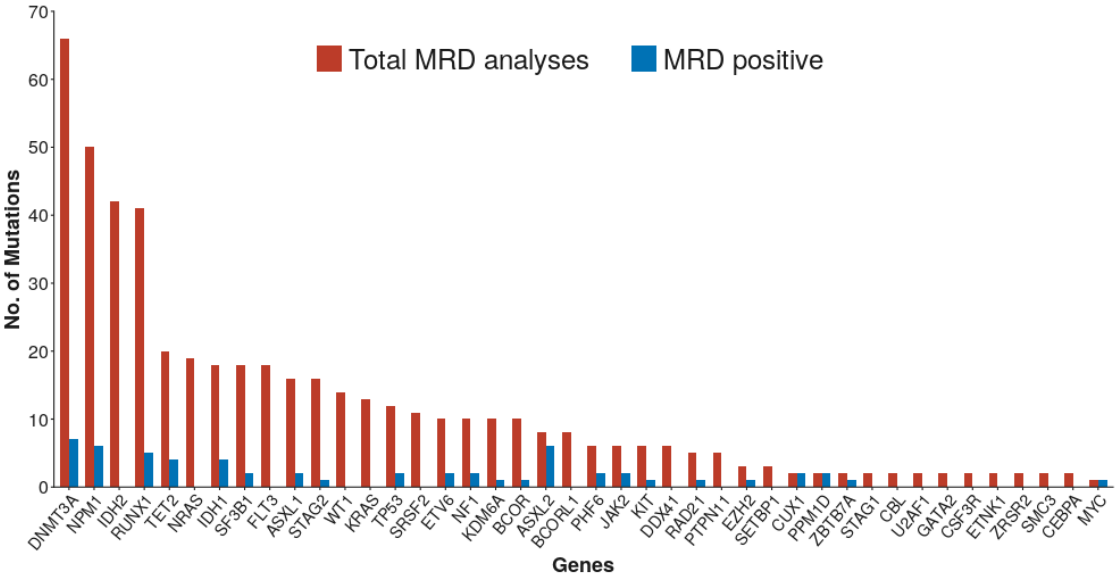
All sequencing results are provided in Supplemental Materials. Flow cytometry standard files from measurable residual disease analyses can be requested from the corresponding authors. The full sequencing data cannot be made available, to comply with the EU General Data Protection Regulation.

Supplemental Figures

Supplemental Figure 1. Consort diagram of studied patients.



Supplemental Figure 2. Frequency of mutations that were used for NGS-MRD analysis in 138 patients and number of mutations that were found MRD positive.

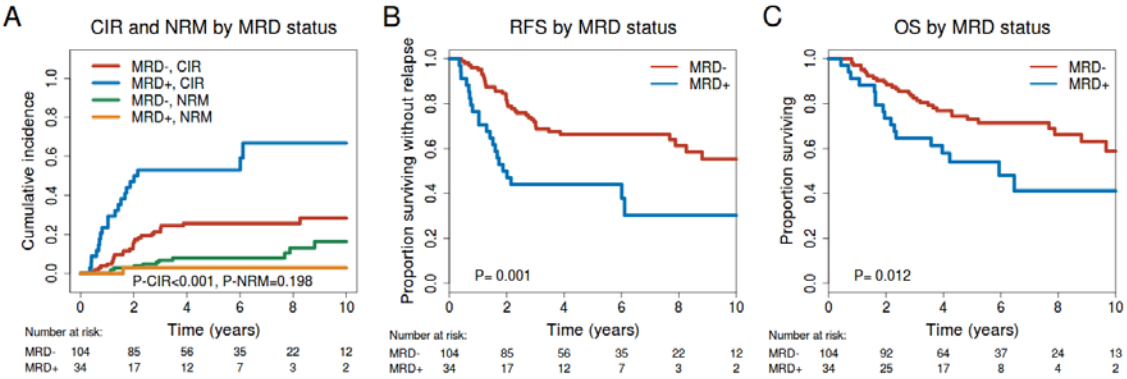


Supplemental Figure 3. CIR, NRM, RFS and OS for patients who were MRD positive on day 90 or 180 compared to MRD negative patients using DTA and non-DTA mutations in 138 patients.

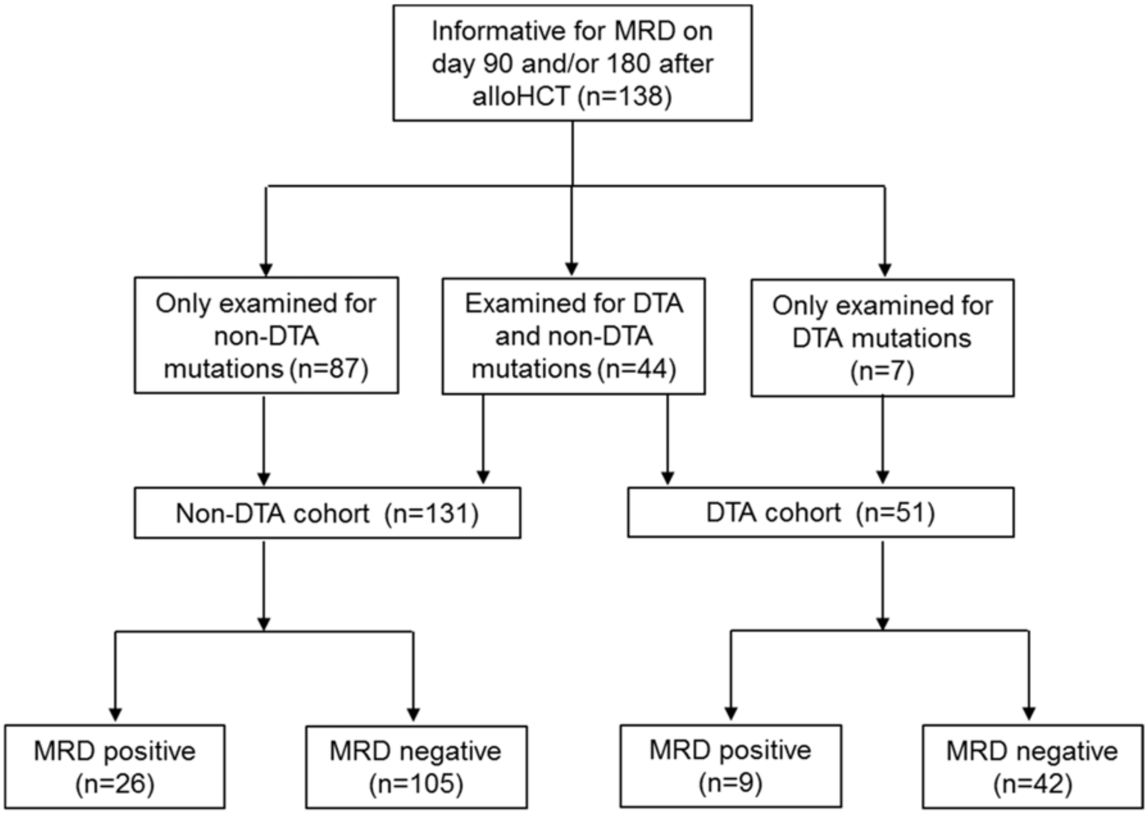
(A) CIR and NRM by competing risk analysis for MRD positive (n=34) and negative (n=104) patients.

(B) RFS for MRD positive (n=34) and negative (n=104) patients.

(C) OS for MRD positive (n=34) and negative (n=104) patients.



Supplemental Figure 4. Consort diagram of patients with DTA and non-DTA mutations. At diagnosis all patients were examined for DTA and non-DTA mutations by NGS panel sequencing.



Supplemental Figure 5. Mutation status in donors who donated stem cells for patients with positive MRD in DTA genes after alloHCT.

(A) VAF of DTA gene mutations at different time points in patients before and after alloHCT and in donors before stem cell harvest.

(B) CIR and NRM by competing risk analysis for MRD positive (n=7) and negative (n=44) patients.

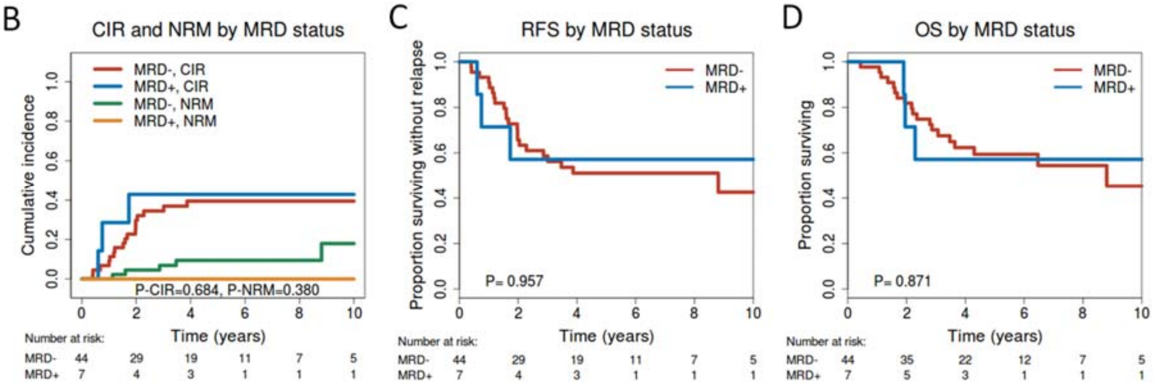
(C) RFS for MRD positive (n=7) and negative (n=44) patients.

(D) OS for MRD positive (n=7) and negative (n=44) patients.

n.d., no data

A

Pub ID	Donor age	Donor sex	Mutation	VAF of patient at diagnosis (%)	VAF of patient at day +90 (%)	VAF of patient at day +180 (%)	VAF of donor pre-donation (%)	Relapse (timepoint post allo-HCT)	Chimerism day +90 (%)	Chimerism day +180 (%)
MRD.078	53	male	DNMT3A, NM_175629.2:c.2645G>A	16.79	0.028	n.d.	0.035	No	100	100
MRD.113	28	male	TET2, NM_001127208.2:c.5260G>C	48.05	0.046	0.031	0	No	100	100
MRD.032	53	female	TET2, NM_001127208.2:c.4532T>A	71.2	0	0.047	0	Yes (day+210)	100	100
MRD.063	59	male	DNMT3A, NM_022552.4:c.2644C>T	45.73	0.015	0.033	0	Yes (day+272)	100	100
MRD.025	47	female	DNMT3A, NM_175629.2:c.2645G>A	38.55	0	0.025	0	No (prophyl. DLI d+344)	99	98
MRD.160	25	female	TET2, NM_001127208.2:c.744C>A	49.22	0.045	0	0	No	100	100
MRD.105	24	female	DNMT3A, NM_175629.2:c.2645G>A	43.62	0.037	0.015	0.075	No	100	100
MRD.164	24	male	ASXL1, NM_015338.5:c.3730A>G	51.16	23.845	0.515	0	Yes (day+691)	54	99
MRD.149	47	female	DNMT3A, NM_175629.2:c.977G>A	27.33	0.009	0	0	No	100	100

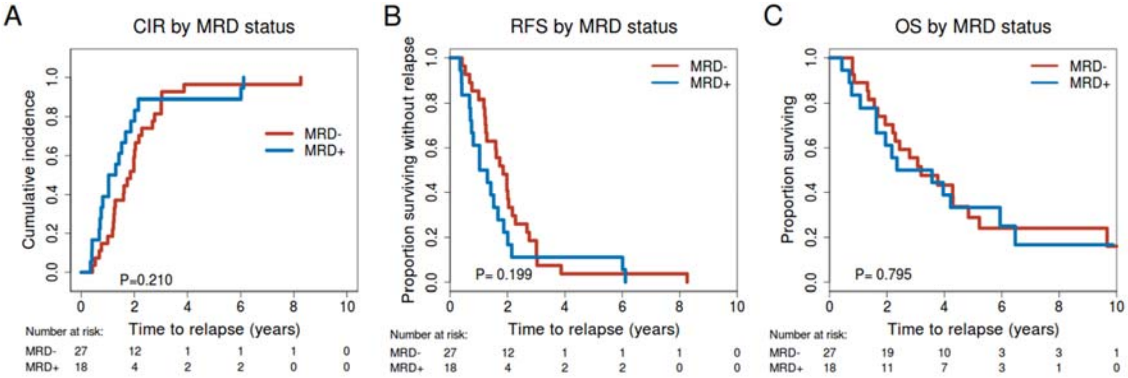


Supplemental Figure 6. Median time to relapse in relapsing patients according to MRD status (restricted to patients with non-DTA mutations).

(A) CIR in relapsing patients based on MRD status. MRD positive vs MRD negative patients: HR 1.49, 95% CI 0.77-2.88, P=0.240, median time to relapse, 1.17 vs 1.85 years. CIR was measured from the date of transplantation.

(B) RFS in relapsing patients based on MRD status. MRD positive vs MRD negative patients: HR 1.49, 95% CI 0.77-2.88, P=0.240, median time to relapse, 1.17 vs 1.85 years. RFS was measured from the date of transplantation.

(C) OS in relapsing patients based on MRD status. MRD positive vs MRD negative patients: HR 1.10, 95% CI 0.55-2.17, P=0.800, median survival time, 2.96 vs 3.07 years.

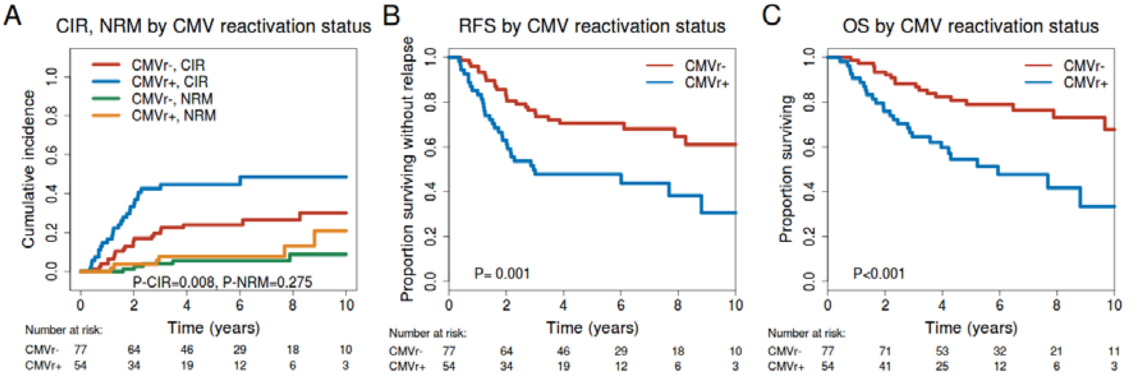


Supplemental Figure 7. Effect of CMV reactivation (CMVr) on CIR, RFS and OS (restricted to patients with non-DTA mutations).

(A) CIR by competing risk analysis in patients with and without CMV reactivation (HR 2.19, 95% CI 1.23-3.92, P=0.008, 5-year CIR 45 vs 24%).

(B) RFS by competing risk analysis in patients with and without CMV reactivation (HR 2.35, 95% CI 1.39-3.95, P=0.001, 5-year RFS 47 vs 71%).

(C) OS by competing risk analysis in patients with and without CMV reactivation (HR 2.92, 95% CI 1.62-5.28, P<0.001, 5-year OS 54 vs 79%).

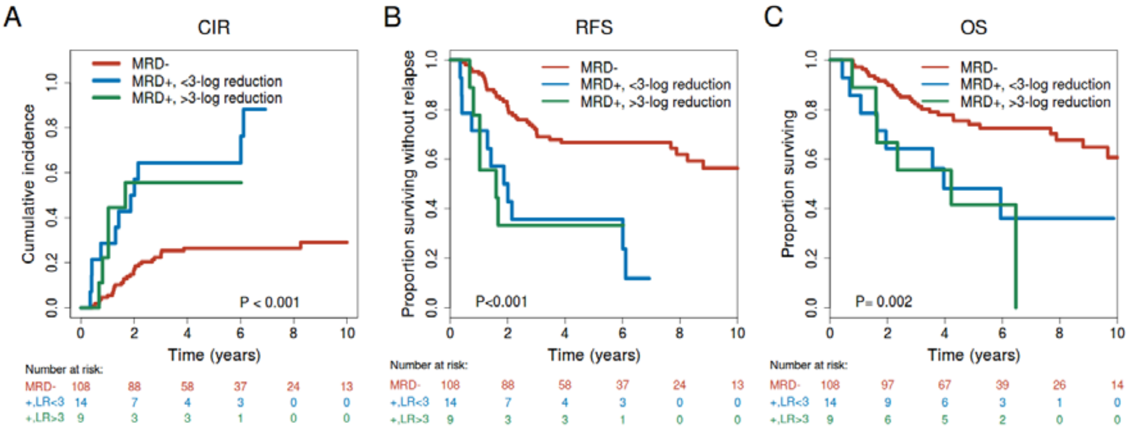


Supplemental Figure 8. Effect of MRD log-reduction (LR) on CIR, RFS and OS for patients who were MRD positive on day 90 or 180 compared to MRD negative patients (restricted to patients with non-DTA mutations). MRD positive patients with less (n=14) or equal/more than (n=9) a 3-log reduction of the VAF of the mutated genes between diagnosis and the post-alloHCT MRD assessment were compared to MRD negative patients (n=108).

(A) CIR by competing risk analysis in MRD positive and MRD negative patients according to VAF log-reduction from diagnosis to post alloHCT MRD assessment (MRD positive <3-log reduction vs MRD negative: HR 3.79, 95% CI 1.97-7.29, P<0.001, 5-year CIR 64% vs 27%; MRD positive >3-log reduction vs MRD negative: HR 3.40, 95% CI 1.25-9.23, P=0.016, 5-year CIR 56% vs 27%).

(B) RFS in MRD positive and MRD negative patients according to VAF log-reduction from diagnosis to post alloHCT MRD assessment (MRD positive <3-log reduction vs MRD negative: HR 3.79, 95% CI 1.97-7.29, P<0.001, 5-year RFS 36% vs 67%; MRD positive >3-log reduction vs MRD negative: HR 3.40, 95% CI 1.25-9.23, P=0.016, 5-year RFS 33% vs 67%).

(C) OS in MRD positive and MRD negative patients according to VAF log-reduction from diagnosis to post alloHCT MRD assessment (MRD positive <3-log reduction vs MRD negative: HR 2.71, 95% CI 1.23-6.01, P=0.014, 5-year OS 48% vs 74%; MRD positive >3-log reduction vs MRD negative: HR 3.48, 95% CI 1.53-7.91, P=0.003, 5-year OS 42% vs 74%).


A
CIR
Cumulative incidence
MRD-
MRD+, <3-log reduction
MRD+, >3-log reduction
P < 0.001
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
Time (years)
Number at risk:
MRD- 108 88 58 37 24 13
+,LR<3 14 7 4 3 0 0
+,LR>3 9 3 3 1 0 0
B
RFS
Proportion surviving without relapse
MRD-
MRD+, <3-log reduction
MRD+, >3-log reduction
P<0.001
Time (years)
Number at risk:
MRD- 108 88 58 37 24 13
+,LR<3 14 7 4 3 0 0
+,LR>3 9 3 3 1 0 0
C
OS
Proportion surviving
MRD-
MRD+, <3-log reduction
MRD+, >3-log reduction
P= 0.002
Time (years)
Number at risk:
MRD- 108 97 67 39 26 14
+,LR<3 14 9 6 3 1 0
+,LR>3 9 6 5 2 0 0

Supplemental Figure 9. Prognostic impact of MRD depending on a 0.1% VAF cutoff in 131 patients based on mutations in non-DTA genes.

(A) CIR in MRD positive and MRD negative patients according to a VAF cutoff of 0.1% based on the marker with the *highest* VAF if multiple markers were measured (MRD 0%<VAF<0.1% vs VAF=0%: HR 2.93, 95% CI 1.20-7.14, P=0.018, 5-year CIR 57% vs 25%; MRD VAF >0.1% vs VAF=0%: HR 4.33, 95% CI 2.19-8.58, P<0.001, 5-year CIR 67% vs 25%).

(B) RFS in MRD positive and MRD negative patients according to a VAF cutoff of 0.1% based on the marker with the *highest* VAF if multiple markers were measured (MRD 0%<VAF<0.1% vs VAF=0%: HR 2.93, 95% CI 1.20-7.14, P=0.018, 5-year RFS 36% vs 67%; MRD VAF >0.1% vs VAF=0%: HR 4.33, 95% CI 2.19-8.58, P<0.001, 5-year RFS 33% vs 67%).

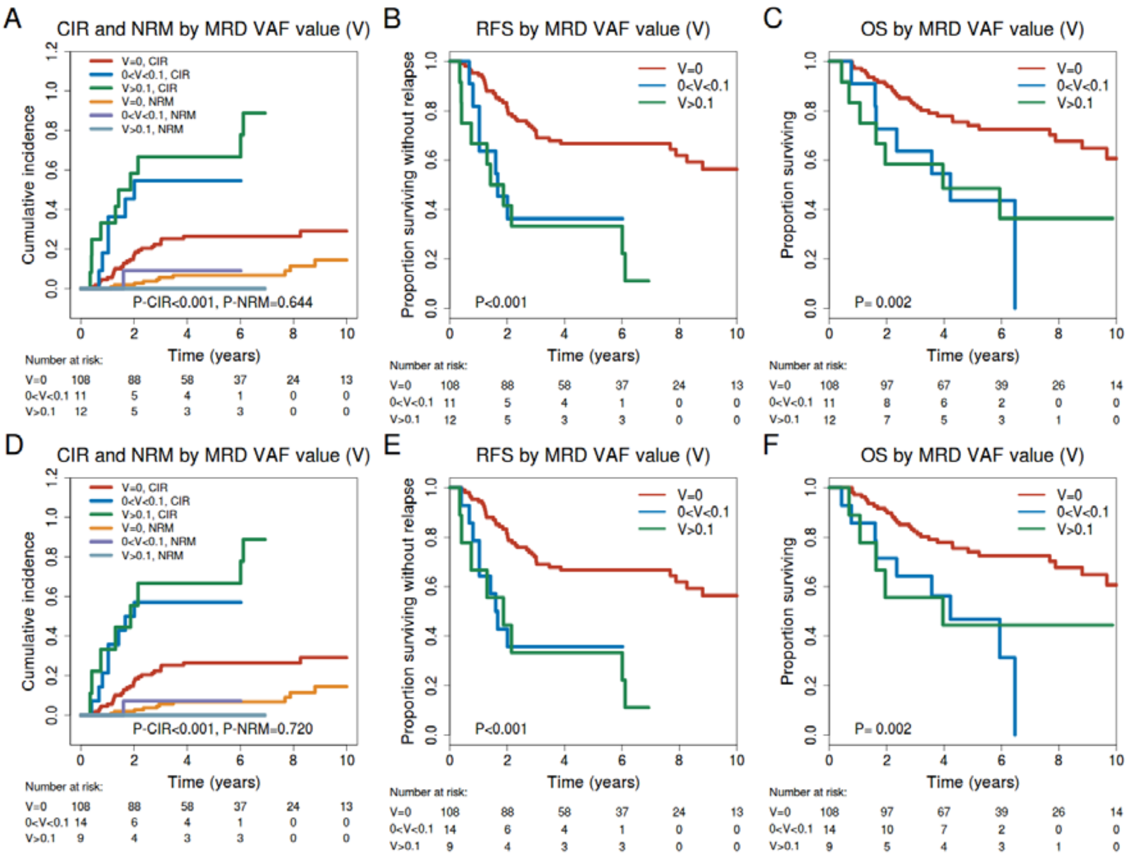
(C) OS in MRD positive and MRD negative patients according to a VAF cutoff of 0.1% based on the marker with the *highest* VAF if multiple markers were measured (MRD 0%<VAF<0.1% vs VAF=0%: HR 3.12, 95% CI 1.45-6.70, P=0.016, 5-year OS 44% vs 74%; MRD VAF >0.1% vs VAF=0%: HR 2.89, 95% CI 1.22-6.88, P=0.033, 5-year OS 49% vs 74%).

(D) CIR in MRD positive and MRD negative patients according to a VAF cutoff of 0.1% based on the marker with the *lowest* VAF if multiple markers were measured (MRD 0%<VAF<0.1% vs VAF=0%: HR 3.15, 95% CI 1.41-7.04, P=0.005, 5-year CIR 59% vs 25%; MRD VAF >0.1% vs VAF=0%: HR 4.28, 95% CI 2.07-8.84, P<0.001, 5-year CIR 67% vs 25%).

(E) RFS in MRD positive and MRD negative patients according to a VAF cutoff of 0.1% based on the marker with the *lowest* VAF if multiple markers were measured (MRD 0%<VAF<0.1% vs VAF=0%: HR 3.15, 95% CI 1.41-7.04, P=0.005, 5-year RFS 36% vs 67%; MRD VAF >0.1% vs VAF=0%: HR 4.28, 95% CI 2.07-8.84, P<0.001, 5-year RFS 33% vs 67%).

(F) OS in MRD positive and MRD negative patients according to a VAF cutoff of 0.1% based on the marker with the *lowest* VAF if multiple markers were measured (MRD 0%<VAF<0.1% vs VAF=0%: HR 3.33, 95% CI 1.68-6.61, P=0.001, 5-year OS 47% vs 74%; MRD VAF >0.1% vs VAF=0%: HR 2.64, 95% CI 0.93-7.51, P=0.069, 5-year OS 44% vs 74%).

Abbreviation: V=VAF



Supplemental Figure 10. Prognostic impact of MRD depending on a 1% VAF cutoff in 131 patients based on mutations in non-DTA genes.

(A) CIR in MRD positive and MRD negative patients according to a VAF cutoff of 1% based on the marker with the *highest* VAF if multiple markers were measured (MRD 0%<VAF<1% vs VAF=0%: HR 3.24, 95% CI 1.62-6.48, P<0.001, 5-year CIR 56% vs 25%; MRD VAF >1% vs VAF=0%: HR 5.20, 95% CI 2.57-10.5, P<0.001, 5-year CIR 80% vs 25%).

(B) RFS in MRD positive and MRD negative patients according to a VAF cutoff of 1% based on the marker with the *highest* VAF if multiple markers were measured (MRD 0%<VAF<1% vs VAF=0%: HR 3.24, 95% CI 1.62-6.48, P<0.001, 5-year RFS 36% vs 68%; MRD VAF >1% vs VAF=0%: HR 5.20, 95% CI 2.57-10.5, P<0.001, 5-year RFS 25% vs 68%).

(C) OS in MRD positive and MRD negative patients according to a VAF cutoff of 1% based on the marker with the *highest* VAF if multiple markers were measured (MRD 0%<VAF<1% vs VAF=0%: HR 2.95, 95% CI 1.48-5.86, P=0.002, 5-year OS 48% vs 74%; MRD VAF >1% vs VAF=0%: HR 3.14, 95% CI 0.95-10.4, P=0.062, 5-year OS 40% vs 74%).

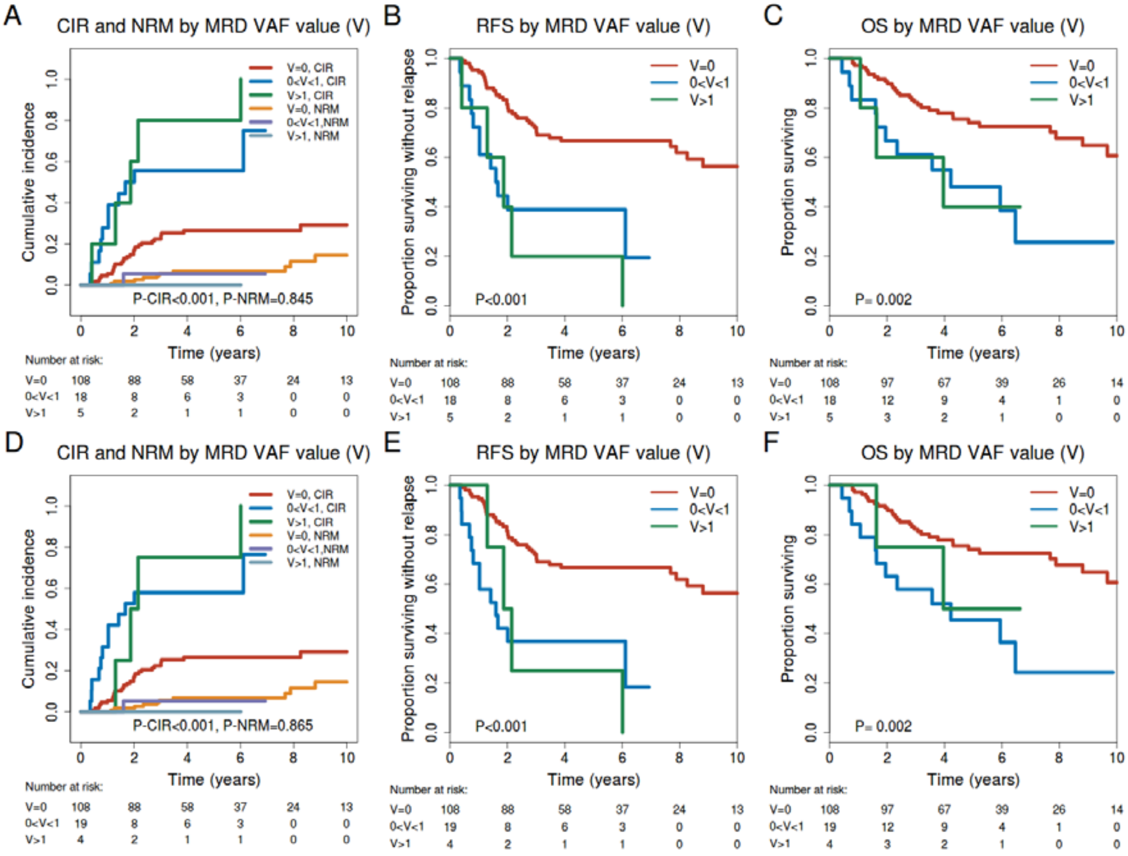
(D) CIR in MRD positive and MRD negative patients according to a VAF cutoff of 1% based on the marker with the *lowest* VAF if multiple markers were measured (MRD 0%<VAF<1% vs VAF=0%: HR 3.47, 95% CI 1.76-6.85, P<0.001, 5-year CIR 58% vs 25%; MRD VAF >1% vs VAF=0%: HR 4.21, 95% CI 2.34-7.58, P<0.001, 5-year CIR 75% vs 25%).

(E) RFS in MRD positive and MRD negative patients according to a VAF cutoff of 1% based on the marker with the *lowest* VAF if multiple markers were measured (MRD 0%<VAF<1% vs VAF=0%: HR 3.47, 95% CI 1.76-6.85, P<0.001, 5-year RFS 36% vs

68%; MRD VAF >1% vs VAF=0%: HR 4.21, 95% CI 2.34-7.58, P<0.001, 5-year RFS 25% vs 68%).

(F) OS in MRD positive and MRD negative patients according to a VAF cutoff of 1% based on the marker with the *lowest* VAF if multiple markers were measured (MRD 0%<VAF<1% vs VAF=0%: HR 3.17, 95% CI 1.62-6.21, P=0.001, 5-year OS 46% vs 74%; MRD VAF >1% vs VAF=0%: HR 2.17, 95% CI 0.58-8.18, P=0.250, 5-year OS 50% vs 74%).

Abbreviation: V=VAF

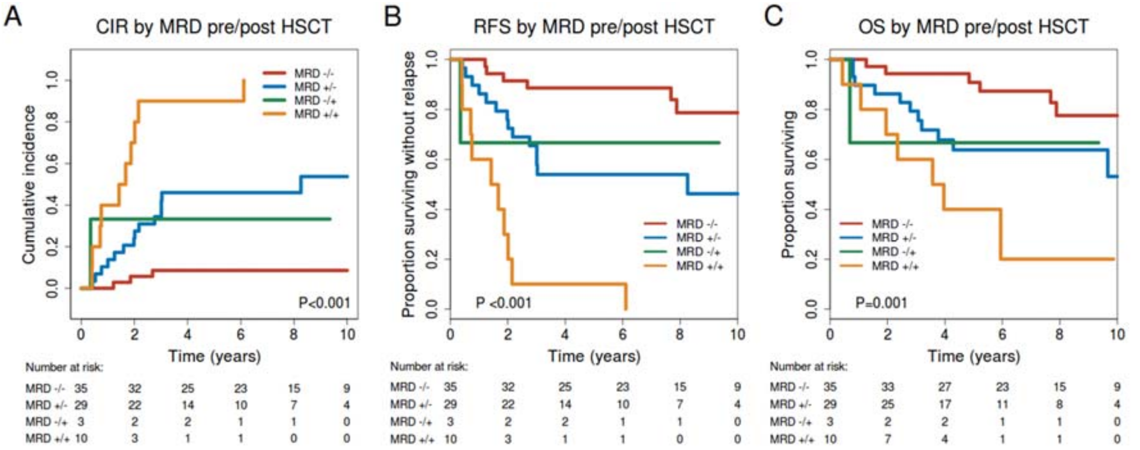


Supplemental Figure 11. Prognostic impact of MRD depending on pre-alloHCT MRD status in CR patients based on mutations in non-DTA genes.

(A) CIR in post alloHCT MRD positive and MRD negative patients who had been either MRD+ or MRD- before alloHCT.

(B) RFS in post alloHCT MRD positive and MRD negative patients who had been either MRD+ or MRD- before alloHCT.

(C) OS in post alloHCT MRD positive and MRD negative patients who had been either MRD+ or MRD- before alloHCT.

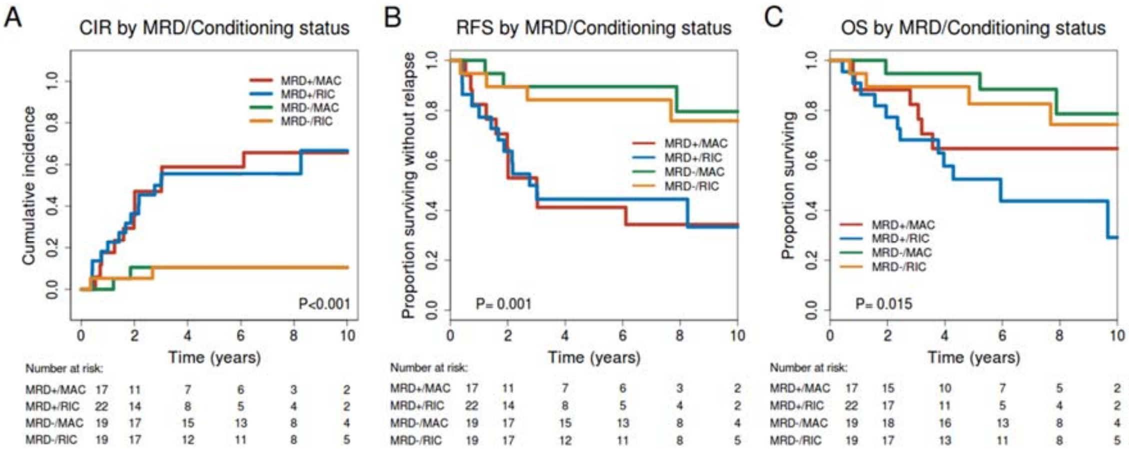


Supplemental Figure 12. Prognostic impact of conditioning intensity on MRD positive and MRD negative patients measured in CR before alloHCT and based on mutations in non-DTA genes (n=77 patients).

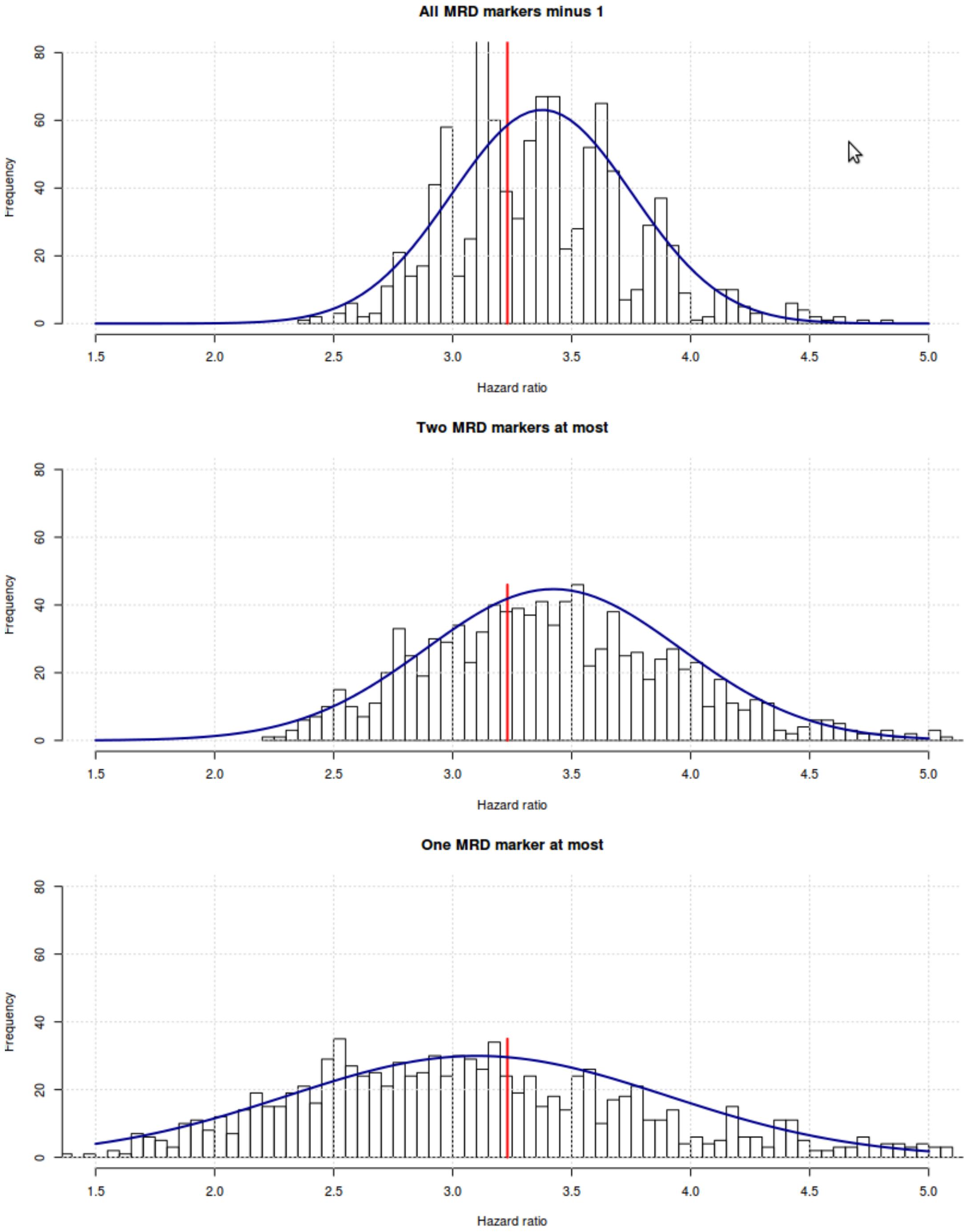
(A) Cumulative incidence of relapse in MRD positive and MRD negative patients according to conditioning intensity (MRD+/RIC vs MRD+/MAC: HR 1.01, 95% CI 0.48-2.13, P=0.97, 5-year CIR 57% vs 59%; MRD-/RIC vs MRD-/MAC: HR 1.05, 95% CI 0.16-7.12, P=0.960, 5-year CIR 11% vs 10%).

(B) Relapse free survival in MRD positive and MRD negative patients according to conditioning intensity (MRD+/RIC vs MRD+/MAC: HR 1.09, 95% CI 0.52-2.29, P=0.810, 5-year RFS 38% vs 41%; MRD-/RIC vs MRD-/MAC: HR 1.43, CI 0.34-6.09, P=0.630, 5-year RFS 84% vs 90%).

(C) Overall survival in MRD positive and MRD negative patients according to conditioning intensity (MRD+/RIC vs MRD+/MAC: HR 2.05, 95% CI 0.79-5.31, P=0.140, 5-year OS 45% vs 65%; MRD-/RIC vs MRD-/MAC: HR 1.52, 95% CI 0.36-6.49, P=0.570, 5-year OS 83% MRD-/RIC vs 95% MRD-/MAC).



Supplemental Figure 13. Estimated hazard ratios for CIR from 1000 permutations using 1, 2, or all minus 1 of the available MRD measurements per patient for MRD monitoring (including mutations in DTA and non-DTA genes). The red line indicates the hazard ratio for MRD positive patients for CIR calculated from all available MRD markers (HR 3.23, see **Supplemental Table 7**).



Supplemental Tables

Supplemental Table 1. Genes included in our custom myeloid panel (based on GRCh37/hg19).

Gene	**Exons**	**Gene**	**Exons**	**Gene**	**Exons**
ASXL1	12	*GATA2*	2-6	*RUNX1*	complete
ASXL2	11+12	*IDH1*	4	*SETBP1*	4
BCOR	complete	*IDH2*	4	*SF3B1*	13-16
BCORL1	complete	*JAK2*	12, 14	*SMC1A*	2, 11, 16, 17
BRAF	Exon15	*KDM6A*	complete	*SMC3*	10, 13, 19, 23, 25, 28
CALR	9	*KIT*	2, 8-11, 13, 17	*SRSF2*	1
CBL	8, 9	*KRAS*	2-5	*STAG1*	complete
CEBPA	complete	*MPL*	10	*STAG2*	complete
CSF3R	14-17	*MYC*	2	*TET2*	3-11
CSNK1A1	3, 4	*NF1*	complete	*TP53*	2-11
DDX41	complete	*NPM1*	11	*U2AF1*	2, 6
DNMT3A	complete	*NRAS*	2-5	*WT1*	7, 9
ETNK1	3	*PHF6*	complete	*ZBTB7A*	2, 3
ETV6	complete	*PPM1D*	1-6	*ZRSR2*	complete
EZH2	complete	*PTPN11*	3, 13		
FLT3	14-16, 20	*RAD21*	complete		

Supplemental Table 2. Comparison of clinical and transplantation-associated characteristics between MRD positive and MRD negative patients (full cohort of 138 patients, mutations in DTA and non-DTA genes were used as MRD markers).

Characteristic	**All Patients n=138**	**MRD positive n=34**	**MRD negative n=104**	**P**
Age				0.617
Median (years)	52.7	51.5	53	
Range (years)	19-73.8	21.5-68.8	19-73.8	
Patient sex				0.700
Male - no. (%)	77 (56)	18 (53)	59 (57)	
Female - no. (%)	61 (44)	16 (47)	45 (43)	
ECOG performance status before				0.807
ECOG 0-1 - no. (%)	133 (96)	33 (97)	100 (96)	
ECOG ≥2 - no. (%)	5 (4)	1 (3)	4 (4)	
FAB-subtype				0.622
M0 - no. (%)	14 (10)	4 (12)	10 (10)	
M1 - no. (%)	21 (15)	2 (6)	19 (18)	
M2 - no. (%)	20 (14)	6 (18)	14 (13)	
M4 - no. (%)	27 (20)	6 (18)	21 (20)	
M5 - no. (%)	13 (9)	4 (12)	9 (9)	
M6 - no. (%)	4 (3)	1 (3)	3 (3)	
M7 - no. (%)	1 (1)	0 (0)	1 (1)	
Missing – no. (%)	38 (28)	11 (31)	27 (26)	
AML type				0.548
De novo - no. (%)	100 (72)	26 (76)	74 (71)	
Secondary* - no. (%)	38 (28)	8 (24)	30 (29)	
2017 ELN risk group				0.738
Favorable – no. (%)	31 (22)	8 (24)	23 (22)	
Intermediate – no. (%)	42 (30)	10 (29)	32 (31)	
Adverse – no. (%)	62 (46)	14 (41)	48 (46)	
Missing – no. (%)	3 (2)	2 (6)	1 (1)	
Cytogenetic risk group				0.657
Favorable – no. (%)	6 (4)	1 (3)	5 (5)	
Intermediate – no. (%)	106 (77)	28 (82)	78 (75)	
Adverse – no. (%)	26 (19)	5 (15)	21 (20)	
Complex karyotype				0.466
Absent – no. (%)	126 (91)	30 (88)	96 (92)	
Present – no. (%)	12 (9)	4 (12)	8 (8)	
WBC count				0.512
Median - (x10^9/l)	14.6	16.1	8.3	
Range - (x10^9/l)	0.7-283.5	1.2-141.2	0.7-283.5	
Hemoglobin				0.851
Median – g/dL	9.8	9.8	9.7	
Range – g/dL	4-13.8	5.5-13.8	4-13.2	
Platelet count				0.691
Median - (x10^9/l)	63	69	60	

Range - (x10^9/l)	10-475	11-475	10-427	
Number of chemotherapy cycles				0.501
One cycle – no. (%)	11 (8)	1 (3)	10 (10)	
Two cycles – no. (%)	47 (34)	11 (32)	36 (35)	
≥ Three cycles – no. (%)	26 (19)	6 (18)	20 (19)	
Missing – no. (%)	54 (39)	16 (47)	38 (36)	
Remission status				0.366
First CR – no. (%)	90 (65)	20 (58)	70 (67)	
CRi – no. (%)	4 (3)	1 (3)	3 (3)	
Second CR – no. (%)	17 (12)	5 (15)	12 (12)	
No CR – no. (%)	27 (20)	8 (24)	19 (18)	
HCT-CI before transplantation				0.838
0-2 – no. (%)	112 (81)	28 (82)	84 (81)	
>2 – no. (%)	26 (19)	6 (18)	20 (19)	
Donor match				0.872
MRDonor – no. (%)	35 (25)	7 (21)	28 (27)	
MUDonor – no. (%)	74 (54)	21 (61)	53 (51)	
MMR/MMUDonor – no. (%)	29 (21)	6 (18)	23 (22)	
Conditioning therapy				0.512
Myeloablative – no. (%)	47 (34)	10 (29)	37 (36)	
Reduced intensity – no. (%)	91 (66)	24 (71)	67 (64)	
Stem cell source				0.466
Peripheral blood stem cells -- no.	12 (9)	4 (12)	8 (8)	
Bone marrow – no. (%)	126 (91)	30 (88)	96 (92)	
Donor sex				0.013
Male - no. (%)	93 (67)	17 (50)	76 (73)	
Female - no. (%)	45 (33)	17 (50)	28 (27)	
CMV status				0.365
Donor neg/patient neg – no. (%)	41 (30)	8 (24)	33 (32)	
Any other constellation – no. (%)	97 (70)	26 (76)	71 (68)	
CMV reactivation				0.375
No – no. (%)	82 (59)	18 (53)	64 (62)	
Yes – no. (%)	56 (41)	16 (47)	40 (38)	
aGvHD				0.238
No aGvHD – no. (%)	60 (43)	19 (56)	41 (39)	
Grade 1/2 – no. (%)	64 (46)	12 (35)	52 (50)	
Grade 3/4 – no. (%)	14 (10)	3 (9)	11 (11)	
cGvHD				0.052
No cGvHD – no. (%)	73 (53)	23 (68)	50 (48)	
Limited – no. (%)	46 (33)	10 (29)	36 (35)	
Extensive – no. (%)	19 (14)	1 (3)	18 (17)	
Type of CR sample for MRD day 90				0.504
Bone marrow – no. (%)	12 (9)	2 (6)	10 (10)	
Peripheral blood – no. (%)	126 (91)	32 (94)	94 (90)	
MRD status before alloHCT				0.155
MRD negative CR	39 (28)	6 (18)	33 (32)	
MRD positive CR	42 (30)	11 (32)	31 (30)	
No CR	27 (20)	8 (24)	19 (18)	
Missing – no. (%)	30 (22)	9 (26)	21 (20)	

* AML secondary to myelodysplastic syndromes (MDS) or therapy related AML

Abbreviations: aGvHD, acute graft versus host disease; alloHCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; cGvHD, chronic graft versus host disease; CMV, cytomegalovirus; CR, complete remission; CRi, CR with incomplete hematologic recovery; ECOG, eastern cooperative oncology group; ELN, European LeukemiaNet; FAB, French American British Classification; HCT-CI: hematopoietic cell transplantation comorbidity index; MRD, measurable residual disease; MRDonor, matched related donor; MUDonor, matched unrelated donor; MMRDonor, mismatched related donor; MMUD, mismatched unrelated donor; P, P value; WBC, white blood cell count;

Supplemental Table 3. Results of NGS-MRD analysis in 138 patients using mutations in 42 different genes as MRD markers. See excel file “Supplemental Table 3”.

Supplemental Table 4. Frequency of genes used for NGS-MRD analysis and limit of detection by gene.

Gene	**Number of MRD-measurements (n)**	**Percentage of MRD-measurements (%)**	**Number of patients (n)**	**Percentage of patients (%)**	**Median limit of detection (VAF %)**
ASXL1	16	3.2	7	5.1	0.0106
ASXL2	8	1.6	4	2.9	0.0187
BCOR	10	2.0	5	3.6	0.0105
BCORL1	8	1.6	4	2.9	0.0140
CBL	2	0.4	1	0.7	0.0125
CEBPA	2	0.4	1	0.7	0.0232
CSF3R	2	0.4	1	0.7	0.0073

CUX1	2	0.4	1	0.7	0.3294
DDX41	6	1.2	3	2.2	0.0139
DNMT3A	66	13.3	38	27.5	0.0076
ETNK1	2	0.4	1	0.7	0.0033
ETV6	10	2.0	5	3.6	0.0072
EZH2	3	0.6	2	1.4	0.0570
FLT3[1]	18	3.6	10	7.2	0.0150
GATA2	2	0.4	1	0.7	0.0051
IDH1	18	3.6	10	7.2	0.0059
IDH2	42	8.5	21	15.2	0.0110
JAK2	6	1.2	3	2.2	0.0066
KDM6A	10	2.0	5	3.6	0.0187
KIT	6	1.2	3	2.2	0.0153
KRAS	13	2.6	8	5.8	0.0072
MYC	1	0.2	1	0.7	0.0034
NF1	10	2.0	5	3.6	0.0060
NPM1	50	10.1	27	19.6	No LOD[2]
NRAS	19	3.8	12	8.7	0.0084
PHF6	6	1.2	3	2.2	0.0079
PPM1D	2	0.4	1	0.7	0.0653
PTPN11	5	1.0	3	2.2	0.0068
RAD21	5	1.0	3	2.2	0.0068
RUNX1	41	8.3	22	15.9	0.0125
SETBP1	3	0.6	2	1.4	0.0112
SF3B1	18	3.6	10	7.2	0.0127
SMC3	2	0.4	1	0.7	0.0247
SRSF2	11	2.2	7	5.1	0.0279
STAG1	2	0.4	1	0.7	0.0347
STAG2	16	3.2	11	8.0	0.0189

TET2	20	4.0	10	7.2	0.0155
TP53	12	2.4	7	5.1	0.0148
U2AF1	2	0.4	1	0.7	0.0032
WT1	14	2.8	7	5.1	0.0518
ZBTB7A	2	0.4	1	0.7	0.0253
ZRSR2	2	0.4	1	0.7	0.007
Total	**495**	**100**	**270**	**Median 2 genes per patient**	**Median 0.0112**

[1] FLT3-ITD was used 3 times for 2 FLT3-ITD mutated patients and FLT3-TKD was used 15 times for 8 FLT3-TKD mutated patients.

[2] Limit of detection is defined as 10 supporting reads.

Supplemental Table 5. MRD and remission status before alloHCT and on day 90 and day 180 after alloHCT (the reason for non-assessment was lack of sample availability).

Remission status pre-Tx	n=138	Remission status day 90	n=138	Remission status day 180	n=138
CR MRD- (n)	39	CR MRD-	32	CR MRD-	27
				CR MRD+	2
				MRD not assessed	3
		CR MRD+	4	CR MRD-	1
				CR MRD+	1
				MRD not assessed	2
		MRD not assessed	3	CR MRD-	3
				CR MRD+	0
CR MRD+ (n)	42	CR MRD-	31	CR MRD-	24
				CR MRD+	2
				MRD not assessed	5
		CR MRD+	9	CR MRD-	1
				CR MRD+	6
				MRD not assessed	2
		MRD not	2	CR MRD-	2

		assessed		CR MRD+	0
Non-CR (n)	27	CR MRD-	19	CR MRD-	19
				CR MRD+	0
		CR MRD+	8	CR MRD-	4
				CR MRD+	3
				MRD not assessed	1
MRD not assessed (n)	30	CR MRD-	22	CR MRD-	21
				CR MRD+	1
		CR MRD+	8	CR MRD-	5
				CR MRD+	3
Total MRD- (n)			104		107
Total MRD+ (n)			29		18

Supplemental Table 6. Univariate and multivariate analysis for CIR, NRM, RFS and OS in 138 AML patients (full cohort, mutations in DTA and non-DTA genes were used as MRD markers).

Endpoint	**Variables in the model**	**No. of Patients**	**Events**	**5-year outcome**	**Univariate analysis**			**Multivariate analysis**		
		first/ second category	**first/ second category**	**% first/ second category**	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
CIR	Complex karyotype positive vs negative	12/126	10/37	75/28	6.34	3.01-13.36	<0.001	3.65	1.42-9.38	0.007
	CMV status all other combinations vs D+P negative	97/41	41/6	41/12	3.48	1.48-8.17	0.004	2.81	1.17-6.75	0.020
	MRD positive vs negative	34/104	20/27	53/26	3.23	1.80-5.78	<0.001	2.57	1.32-5.02	0.006
NRM	AML type secondary vs de novo	38/100	7/5	16/3	4.45	1.48-13.42	0.008	4.45	1.48-13.42	0.008
RFS	Complex karyotype positive vs negative	12/126	10/49	25/64	5.12	2.42-10.85	<0.001	3.18	1.28-7.90	0.013
	CMV status all other combination vs D+P negative	97/41	50/9	51/83	3.01	1.50-6.04	0.002	2.58	1.25-5.31	0.010
	MRD positive vs negative	34/104	21/38	44/66	2.48	1.43-4.32	0.001	2.04	1.09-3.83	0.026
OS	Complex karyotype positive vs negative	12/126	9/39	22/71	6.24	2.66-14.67	<0.001	4.85	1.89-12.44	0.001
	CMV status all other combination vs D+P negative	97/41	41/7	59/85	3.09	1.40-6.86	0.005	2.50	1.12-5.57	0.025
	K/NRAS mutated vs wildtype	29/109	14/34	52/71	1.97	1.08-3.59	0.027	2.25	1.26-4.02	0.006

	MRD positive vs negative	34/104	17/31	54/71	2.09	1.16-3.79	0.015	1.80	0.95-3.41	0.070

Abbreviations: CI, confidence interval; CIR, cumulative incidence of relapse; CMV, cytomegalovirus; D+P, donor and patient; HR, hazard ratio; MRD, measurable residual disease; non-DTA, mutation in genes other than *DNMT3A, TET2*, and *ASXL1*; NRM, non-relapse mortality; OS, overall survival; P, P value; RFS, relapse-free survival;

NOTE. Hazard ratios greater than or less than 1 indicate an increased or decreased risk, respectively, of an event for the first category listed

Supplemental Table 7. Univariate analysis of all considered variables for CIR, NRM, RFS and OS using mutations in DTA and non-DTA genes in 138 patients. See excel file “Supplemental Table 7”.

Supplemental Table 8. Comparison of clinical and transplantation-associated characteristics between MRD positive and MRD negative patients (51 patients, restricted to mutations in DTA genes).

Characteristic	**All patients n=51**	**MRD pos n=9**	**MRD neg n=42**	**P**
Age				0.976
Median (years)	52.6	54.9	52.2	
Range (years)	26.4-73.8	28.7-65.3	26.4-73.8	
Patient sex				0.025
Male - no. (%)	23 (45)	1 (11)	22 (52)	
Female - no. (%)	28 (55)	8 (89)	20 (48)	
ECOG performance status before alloHCT				0.643
ECOG 0-1 - no. (%)	50 (98)	9 (100)	41 (98)	
ECOG ≥2 - no. (%)	1 (2)	0 (0)	1 (2)	
FAB-subtype				0.75
M0 - no. (%)	5 (10)	0 (0)	5 (12)	
M1 - no. (%)	5 (10)	0 (0)	5 (12)	
M2 - no. (%)	9 (18)	3 (33)	6 (14)	
M4 - no. (%)	10 (20)	2 (22)	8 (19)	
M5 - no. (%)	9 (18)	1 (11)	8 (19)	
M6 - no. (%)	1 (2)	0 (0)	1 (2)	
M7 - no. (%)	0 (0)	0 (0)	0 (0)	
Missing – no. (%)	12 (24)	3 (33)	9 (21)	
AML type				0.68
De novo - no. (%)	43 (84)	8 (89)	35 (83)	
Secondary* - no. (%)	8 (16)	1 (11)	7 (17)	
2017 ELN risk group				0.255
Favorable – no. (%)	16 (31)	1 (11)	15 (36)	
Intermediate – no. (%)	15 (29)	3 (33)	12 (29)	
Adverse – no. (%)	19 (37)	4 (44)	15 (36)	
Missing – no. (%)	1 (2)	1 (11)	0 (0)	
Cytogenetic risk group				0.097
Favorable – no. (%)	2 (4)	1 (11)	1 (2)	
Intermediate – no. (%)	42 (82)	8 (89)	34 (81)	
Adverse – no. (%)	7 (14)	0 (0)	7 (17)	
Complex karyotype				0.281
Absent – no. (%)	46 (90)	9 (100)	37 (88)	
Present – no. (%)	5 (10)	0 (0)	5 (12)	
WBC count				0.82

Median - (x10^9/l)	15.6	8.7	17.7	
Range - (x10^9/l)	0.9-223.5	1.2-53.5	0.9-223.5	
Hemoglobin				0.82
Median – g/dL	9.8	9.8	9.8	
Range – g/dL	5.5-13.8	6.5-12.1	5.5-13.8	
Platelet count				0.975
Median - (x10^9/l)	72	96	67.5	
Range - (x10^9/l)	11-402	23-333	11-402	
Number of chemotherapy cycles before				0.599
One cycle – no. (%)	2 (4)	0 (0)	2 (5)	
Two cycles – no. (%)	11 (22)	2 (22)	9 (21)	
≥ Three cycles – no. (%)	14 (27)	3 (33)	11 (26)	
Missing – no. (%)	24 (47)	4 (44)	20 (48)	
Remission status				0.847
First CR – no. (%)	29 (57)	5 (56)	24 (57)	
CRi – no. (%)	2 (4)	0 (0)	2 (5)	
Second CR – no. (%)	10 (20)	2 (22)	8 (19)	
No CR – no. (%)	10 (20)	2 (22)	8 (19)	
HCT-CI before transplantation				0.178
0-2 – no. (%)	42 (82)	6 (67)	36 (86)	
>2 – no. (%)	9 (18)	3 (33)	6 (14)	
Donor match				0.729
MRDonor – no. (%)	12 (24)	2 (22)	10 (24)	
MUDonor – no. (%)	29 (57)	6 (67)	23 (55)	
MMR/MMUDonor – no. (%)	10 (20)	1 (11)	9 (21)	
Conditioning therapy				0.06
Myeloablative – no. (%)	20 (39)	1 (11)	19 (45)	
Reduced intensity – no. (%)	31 (61)	8 (89)	23 (55)	
Stem cell source				0.413
Peripheral blood stem cells -- no. (%)	3 (6)	0 (0)	3 (7)	
Bone marrow – no. (%)	48 (94)	9 (100)	39 (93)	
Donor sex				0.273
Male - no. (%)	31 (61)	4 (44)	27 (64)	
Female - no. (%)	20 (39)	5 (56)	15 (36)	
CMV status				0.806
Donor neg/patient neg – no. (%)	13 (25)	2 (22)	11 (26)	
Any other constellation – no. (%)	38 (75)	7 (78)	31 (74)	
CMV reactivation				0.930
No – no. (%)	29 (57)	5 (56)	24 (57)	
Yes – no. (%)	22 (43)	4 (44)	18 (43)	
aGvHD				0.188
No aGvHD – no. (%)	21 (41)	6 (67)	15 (36)	
Grade 1/2 – no. (%)	25 (49)	3 (33)	22 (52)	
Grade 3/4 – no. (%)	5 (10)	0 (0)	5 (12)	
cGvHD				0.322
No cGvHD – no. (%)	21 (41)	5 (56)	16 (38)	
Limited – no. (%)	22 (43)	4 (44)	18 (43)	
Extensive – no. (%)	8 (16)	0 (0)	8 (19)	
Type of CR sample for MRD day 90				0.281

Bone marrow – no. (%)	5 (10)	0 (0)	5 (12)	
Peripheral blood – no. (%)	46 (90)	9 (100)	37 (88)	
MRD status before alloHCT				0.815
MRD negative CR	13 (25)	3 (33)	10 (24)	
MRD positive CR	13 (25)	2 (22)	11 (26)	
No CR	10 (20)	2 (22)	8 (19)	
Missing – no. (%)	15 (29)	2 (22)	13 (31)	

Abbreviations: aGvHD, acute graft versus host disease; alloHCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; cGvHD, chronic graft versus host disease; CMV, cytomegalovirus; CR, complete remission; CRi, CR with incomplete hematologic recovery; ECOG, eastern cooperative oncology group; ELN, European LeukemiaNet; FAB, French American British Classification; HCT-CI: hematopoietic cell transplantation comorbidity index; MRD, measurable residual disease; MRDonor, matched related donor; MUDonor, matched unrelated donor; MMRDonor, mismatched related donor; MMUD, mismatched unrelated donor; P, P value; WBC, white blood cell count;

Supplemental Table 9. Comparison of molecular characteristics between MRD positive and MRD negative AML patients (131 patients, restricted to mutations in non-DTA genes).

Characteristic	All Patients n=131	MRDpos n=26	MRDneg n=105	P
ASXL1				0.118
mutated - no. (%)	24 (18)	2 (8)	22 (21)	
wildtype - no. (%)	107 (82)	24 (92)	83 (79)	
ASXL2				0.058
mutated - no. (%)	6 (5)	3 (12)	3 (3)	
wildtype - no. (%)	125 (95)	23 (88)	102 (97)	
BCOR				0.906
mutated - no. (%)	16 (12)	3 (12)	13 (12)	
wildtype - no. (%)	115 (88)	23 (88)	92 (88)	
BCORL1				0.591
mutated - no. (%)	8 (6)	1 (4)	7 (7)	
wildtype - no. (%)	123 (94)	25 (96)	98 (93)	
BRAF				NA
mutated - no. (%)	0 (0)	0 (0)	0 (0)	
wildtype - no. (%)	131 (100)	26 (100)	105 (100)	
CALR				0.617
mutated - no. (%)	1 (1)	0 (0)	1 (1)	
wildtype - no. (%)	130 (99)	26 (100)	104 (99)	
CBL				0.040
mutated - no. (%)	3 (2)	2 (8)	1 (1)	
wildtype - no. (%)	128 (98)	24 (92)	104 (99)	
CEBPAsm				0.591
mutated - no. (%)	8 (6)	1 (4)	7 (7)	
wildtype - no. (%)	123 (94)	25 (96)	98 (93)	
CEBPAdm				0.842
mutated - no. (%)	6 (5)	1 (4)	5 (5)	
wildtype - no. (%)	125 (95)	25 (96)	100 (95)	
CSF3R				0.383
mutated - no. (%)	3 (2)	0 (0)	3 (3)	
wildtype - no. (%)	128 (98)	26 (100)	102 (97)	
DDX41				0.257
mutated - no. (%)	5 (4)	0 (0)	5 (5)	
wildtype - no. (%)	126 (96)	26 (100)	100 (95)	
DNMT3A				0.544
mutated - no. (%)	47 (36)	8 (31)	39 (37)	
wildtype - no. (%)	84 (64)	18 (69)	66 (63)	
ETNK1				0.478
mutated - no. (%)	2 (2)	0 (0)	2 (2)	
wildtype - no. (%)	129 (98)	26 (100)	103 (98)	

ETV6				0.705
mutated - no. (%)	7 (5)	1 (4)	6 (6)	
wildtype - no. (%)	124 (95)	25 (96)	99 (94)	
EZH2				0.058
mutated - no. (%)	6 (5)	3 (12)	3 (3)	
wildtype - no. (%)	125 (95)	23 (88)	102 (97)	
FLT3-ITD				0.482
mutated - no. (%)	15 (11)	4 (15)	11 (10)	
wildtype - no. (%)	116 (89)	22 (85)	94 (90)	
FLT3-TKD				0.772
mutated - no. (%)	12 (9)	2 (8)	10 (10)	
wildtype - no. (%)	119 (91)	24 (92)	95 (90)	
FLT3 other				0.281
mutated - no. (%)	2 (2)	1 (4)	1 (1)	
wildtype - no. (%)	129 (98)	25 (96)	104 (99)	
GATA2				0.478
mutated - no. (%)	2 (2)	0 (0)	2 (2)	
wildtype - no. (%)	129 (98)	26 (100)	103 (98)	
IDH1				0.298
mutated - no. (%)	13 (10)	4 (15)	9 (9)	
wildtype - no. (%)	118 (90)	22 (85)	96 (91)	
IDH2				0.196
mutated - no. (%)	21 (16)	2 (8)	19 (18)	
wildtype - no. (%)	110 (84)	24 (92)	86 (82)	
JAK2				0.005
mutated - no. (%)	4 (3)	3 (12)	1 (1)	
wildtype - no. (%)	127 (97)	23 (88)	104 (99)	
KDM6A				0.519
mutated - no. (%)	11 (8)	3 (12)	8 (8)	
wildtype - no. (%)	120 (92)	23 (88)	97 (92)	
KIT				0.793
mutated - no. (%)	4 (3)	1 (4)	3 (3)	
wildtype - no. (%)	127 (97)	25 (96)	102 (97)	
KRAS				0.772
mutated - no. (%)	12 (9)	2 (8)	10 (10)	
wildtype - no. (%)	119 (91)	24 (92)	95 (90)	
MPL				
mutated - no. (%)	0 (0)	0 (0)	0 (0)	
wildtype - no. (%)	131 (100)	26 (100)	105 (100)	
MYC				0.554
mutated - no. (%)	3 (2)	1 (4)	2 (2)	
wildtype - no. (%)	128 (98)	25 (96)	103 (98)	
NF1				0.885
mutated - no. (%)	11 (8)	2 (8)	9 (9)	
wildtype - no. (%)	120 (92)	24 (92)	96 (91)	

NOTCH1				0.281
mutated - no. (%)	2 (2)	1 (4)	1 (1)	
wildtype - no. (%)	129 (98)	25 (96)	104 (99)	
NPM1				0.820
mutated - no. (%)	33 (25)	7 (27)	26 (25)	
wildtype - no. (%)	98 (75)	19 (73)	79 (75)	
NRAS				0.619
mutated - no. (%)	21 (16)	5 (19)	16 (15)	
wildtype - no. (%)	110 (84)	21 (81)	89 (85)	
PHF6				0.552
mutated - no. (%)	7 (5)	2 (8)	5 (5)	
wildtype - no. (%)	124 (95)	24 (92)	100 (95)	
PPM1D				0.044
mutated - no. (%)	1 (1)	1 (4)	0 (0)	
wildtype - no. (%)	130 (99)	25 (96)	105 (100)	
PTPN11				0.519
mutated - no. (%)	11 (8)	3 (12)	8 (8)	
wildtype - no. (%)	120 (92)	23 (88)	97 (92)	
RAD21				0.706
mutated - no. (%)	8 (6)	2 (8)	6 (6)	
wildtype - no. (%)	123 (94)	24 (92)	99 (94)	
RUNX1				0.813
mutated - no. (%)	28 (21)	6 (23)	22 (21)	
wildtype - no. (%)	103 (79)	20 (77)	83 (79)	
SETBP1				0.257
mutated - no. (%)	5 (4)	0 (0)	5 (5)	
wildtype - no. (%)	126 (96)	26 (100)	100 (95)	
SF3B1				0.115
mutated - no. (%)	14 (11)	5 (19)	9 (9)	
wildtype - no. (%)	117 (89)	21 (81)	96 (91)	
SMC1A				0.281
mutated - no. (%)	2 (2)	1 (4)	1 (1)	
wildtype - no. (%)	129 (98)	25 (96)	104 (99)	
SMC3				0.212
mutated - no. (%)	6 (5)	0 (0)	6 (6)	
wildtype - no. (%)	125 (95)	26 (100)	99 (94)	
SRSF2				0.247
mutated - no. (%)	13 (10)	1 (4)	12 (11)	
wildtype - no. (%)	118 (90)	25 (96)	93 (89)	
STAG1				0.617
mutated - no. (%)	1 (1)	0 (0)	1 (1)	
wildtype - no. (%)	130 (99)	26 (100)	104 (99)	
STAG2				0.486
mutated - no. (%)	21 (16)	3 (12)	18 (17)	
wildtype - no. (%)	110 (84)	23 (88)	87 (83)	

TET2				0.085
mutated - no. (%)	11 (8)	0 (0)	11 (10)	
wildtype - no. (%)	120 (92)	26 (100)	94 (90)	
TP53				0.853
mutated - no. (%)	9 (7)	2 (8)	7 (7)	
wildtype - no. (%)	122 (93)	24 (92)	98 (93)	
U2AF1				0.249
mutated - no. (%)	5 (4)	2 (8)	3 (3)	
wildtype - no. (%)	126 (96)	24 (92)	102 (97)	
WT1				0.706
mutated - no. (%)	8 (6)	2 (8)	6 (6)	
wildtype - no. (%)	123 (94)	24 (92)	99 (94)	
ZBTB7A				0.005
mutated - no. (%)	4 (3)	3 (12)	1 (1)	
wildtype - no. (%)	127 (97)	23 (88)	104 (99)	
ZRSR2				0.706
mutated - no. (%)	8 (6)	2 (8)	6 (6)	
wildtype - no. (%)	123 (94)	24 (92)	99 (94)	
Epigenetic modifiers*				0.975
mutated - no. (%)	86 (66)	17 (65)	69 (66)	
wildtype - no. (%)	45 (34)	9 (35)	36 (34)	
Nucleophosmin				0.820
mutated - no. (%)	33 (25)	7 (27)	26 (25)	
wildtype - no. (%)	98 (75)	19 (73)	79 (75)	
Cohesin complex*				0.308
mutated - no. (%)	30 (23)	4 (15)	26 (25)	
wildtype - no. (%)	101 (77)	22 (85)	79 (75)	
Signal transduction*				0.233
mutated - no. (%)	72 (55)	17 (65)	55 (52)	
wildtype - no. (%)	59 (45)	9 (35)	50 (48)	
Spliceosome*				0.948
mutated - no. (%)	41 (31)	8 (31)	33 (31)	
wildtype - no. (%)	90 (69)	18 (69)	72 (69)	
Myeloid TFs*				0.456
mutated - no. (%)	64 (49)	11 (42)	53 (50)	
wildtype - no. (%)	67 (51)	15 (58)	52 (50)	
Tumor suppressors*				0.364
mutated - no. (%)	18 (14)	5 (19)	13 (12)	
wildtype - no. (%)	113 (86)	21 (81)	92 (88)	

Abbreviations: ITD, internal tandem duplication; MRD, measurable residual disease; NA, not applicable; neg, negative; P, P value; pos, positive; TFs, transcription factors; TKD, tyrosine kinase domain.

* Genes included in these categories are: epigenetic modifiers: *ASXL1, ASXL2, DNMT3A, EZH2, IDH1, IDH2, KDM6A, KMT2A, TET2*; cohesin complex: *RAD21, SMC1A, SMC3, STAG1, STAG2*; signal transduction: *CBL, ETNK1, FLT3, JAK2, KIT, KRAS, NF1, NRAS, PTPN11*; spliceosome: *DDX41, SF3B1, SRSF2, U2AF1, ZBTB7A, ZRSR2*; myeloid transcription factors: *BCOR, BCORL1, CEBPA, CUX1, ETV6, GATA2, MYC, NOTCH1, PHF6, RUNX1, SETBP1*; tumor suppressors: *PPM1D, TP53, WT1*.

Supplemental Table 10. Univariate analysis of all considered variables for CIR, NRM, RFS and OS using mutations only in non-DTA genes in 131 patients. See excel file “Supplemental Table 10”.

Supplemental Table 11. Sensitivity, specificity and accuracy of NGS-MRD and conventional chimerism analysis for relapse prediction in 131 patients in whom non-DTA genes were quantified by NGS-MRD.

(A) MRD measured on day 90 and/or day 180 after alloHCT.

	Relapse	**No relapse**
MRD positive	18	8
MRD negative	27	78
Sensitivity	0.40	
Specificity	0.91	
Accuracy	0.73	

(B) Donor chimerism measured on day 90 and/or day 180 after alloHCT.

	Relapse	**No relapse**
Chimerism < 100%	16	9
Chimerism = 100%	28	75
Sensitivity	0.36	
Specificity	0.89	
Accuracy	0.71	

(C) MRD and donor chimerism measured on day 90 and/or day 180 after alloHCT.

	Relapse	**No relapse**
MRD positive and/or chimerism < 100%	23	15
MRD negative and chimerism = 100%	22	71
Sensitivity	0.51	
Specificity	0.83	
Accuracy	0.72	

Supplemental Table 12. MRD is found in a wide range of genes in relapsing patients. The genes that were used for MRD assessment in eighteen patients who were MRD positive and who relapsed together with the number of patients tested and the number of patients found MRD positive are listed.

Gene	Tested (number of patients)	MRD positive (number of patients)
ASXL2	8	6
BCOR	4	1
BCORL1	4	0
CUX1	2	2
ETV6	4	2
EZH2	3	1
FLT3	7	0
IDH1	4	3
IDH2	10	0
JAK2	4	2
KDM6A	6	1
KIT	2	0
KRAS	3	0
NF1	4	2
NPM1	11	3
NRAS	8	0
PHF6	6	2
PPM1D	2	2
PTPN11	3	0
RAD21	1	1
RUNX1	10	4
SF3B1	11	1
SMC3	2	0
SRSF2	2	0
STAG2	4	1
TP53	6	1
U2AF1	2	0
WT1	4	0