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Prognostic relevance of *RUNX1* mutations at acute myeloid leukemia diagnosis and as measurable residual disease marker before and after allogeneic stem cell transplantation

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To The Editor,

An allogeneic hematopoietic stem cell transplantation (HSCT) remains the treatment with the highest chance of cure in patients with acute myeloid leukemia (AML) harboring high risk disease. Current risk stratification in patients receiving intensive treatment is usually evaluated according to the European LeukemiaNet (ELN) 2022 classification.[1] *RUNX1* is among the most frequently mutated genes in AML,[2] one of the genes defining AML with myelodysplasia-related gene mutations (AML-MR) according to the International Consensus Classification (ICC) and, subsequently, adverse ELN 2022 risk.[1,3] Thus, the presence of *RUNX1* mutations at AML diagnosis usually identifies patients to whom a consolidating HSCT will be offered. Prior to as well as during follow up after an HSCT, the presence of measurable residual disease (MRD) is a prognostic factor identifying patients with a higher cumulative incidence of relapse (CIR) and shorter overall survival (OS).[4–6] However, molecular MRD has merely been well established for *NPM1* mutations and several AML fusion genes, while most other molecular aberrations were only evaluated as part of larger next generation sequencing (NGS) panels.[7] The prognostic relevance of molecular MRD assessment depends on the measured gene as well as the clinical context. While DTA (*i.e. ASXL1*, *DNMT3A*, and *TET2*) mutations are not suitable MRD markers after chemotherapy due to their presence in pre-leukemic clones,[8–10] they seem to allow valid relapse prediction when measured after an HSCT, since the recipients' hematopoiesis has been replaced.[11] The same may be true for other gene mutations defining AML-MR,[9,10,12] but data on *RUNX1* mutations remains scarce. Here, we opted to describe the prognostic impact of *RUNX1* mutations at diagnosis and evaluate the potential of *RUNX1* mutations as MRD markers in AML before and after an HSCT.

Applying NGS, we analyzed the *RUNX1* mutation status at diagnosis in 308 AML patients, who received an HSCT during further disease course at our institution. In *RUNX1*-mutated patients with suitable follow-up samples in composite complete remission (CRc) available, the *RUNX1* mutation based MRD before (23 patients) and after HSCT (107 samples in 21 patients) was evaluated. Patient-specific digital droplet polymerase chain reaction (ddPCR) assays were developed using a competitive probe approach. A detectable *RUNX1* mutation variant allele frequency (VAF) ≥ 0.05% was regarded as MRD positive. *RUNX1* MRD results were not available to the treating physician and did not prompt

treatment intervention. For details regarding the patient cohort, MRD assays see Table 1, and Supplementary Tables S1-S2. Clinical endpoints were CIR and OS, all statistical analyses were performed using the R statistical software platform (version 4.0.2). Median follow up after HSCT was 1.9 years. Written informed consent was obtained in accordance with the Declaration of Helsinki. Data analyses were approved by the Institutional Review Board of the University Hospital Leipzig (363/16-ek).

At diagnosis, 48 patients (16%) had at least one *RUNX1* mutation (43 patients had one mutation, 5 patients had two mutations), the median VAF was 36.5% (range 3.8% - 95.1%). In line with published data, *RUNX1* mutations at AML diagnosis were more often seen in patients with a secondary or treatment related AML (*P*=.05, Table 1).[13] In contrast, we did not observe the previously described association with male sex or older age, likely due to the bias of selecting patients scheduled for HSCT. Not surprisingly, compared to *RUNX1*-wild type patients, *RUNX1*-mutated patients more often had ELN 2022 adverse genetic risk (*P*<.001), but less often a complex karyotype (*P*=.03). In line with previous reports, *RUNX1*-mutated patients also less frequently showed mutations in *NPM1* (*P*=.004), *FLT3*-TKD (*P*=.006) and *TP53* (*P*=.001), and more often mutations in *ASXL1* (*P*=.001), and *SRSF2* (*P*=.03).[13,14] Previous data suggested that *RUNX1*-mutated patients might benefit from a consolidating HSCT.[13,14] Accordantly, the presence of a *RUNX1* mutation did not associate with a higher CIR (*P*=.33) or shorter OS (*P*=.30, Figure 1A and B) in our cohort of patients who received an HSCT. One large analysis by the European Society for Blood and Marrow Transplantation (EBMT) also showed similar outcomes in patients with or without a *RUNX1* mutation transplanted in first CR, further strengthening the assumption that this procedure may overcome the adverse prognostic effect of mutated *RUNX1*.[14]

The *RUNX1* mutation VAF decreased in all patients who achieved a CRc prior to HSCT compared to the VAF at diagnosis (Figure 1). Still, *RUNX1* mutations persisted in 52% of patients (n=12) at a median VAF of 1.5% (range 0.06% - 10.9%). *RUNX1* MRD-positivity prior to HSCT associated with a significantly higher CIR (*P*=.008) and shorter OS (*P*=.04, Figure 1C,D). Of note, female patients significantly more often achieved a *RUNX1* MRD clearance prior to HSCT (*P*=.001, Supplementary Table S3), while *ASXL1*

co-mutated patients (*P*=.07) and *FLT3*-TKD wild type patients (*P*=.07) tended to more often remain *RUNX1* MRD-positive at HSCT.

During follow-up after HSCT, 23 of 48 (48%) of *RUNX1*-mutated patients relapsed. Of those, 8 had material for *RUNX1* mutation analysis available at relapse, and all samples were positive for the *RUNX1* mutation present at diagnosis. Still, we cannot rule out the possibility of lost *RUNX1* mutations at relapse, as not all patients had relapse material available. Of all relapsing patients, 8 had at least one follow up sample in CRc available prior to relapse. In 7/8 of those patients (88%), impending relapse was preceded by a *RUNX1* MRD-positive sample with a median VAF of 2.6% (range 0.08% - 10.0%) at a median of 95 days (range 28-177) prior to relapse. Only one patient remained *RUNX1* MRD-negative prior to relapse with the last negative sample taken 83 days prior to morphologic relapse. All patients with post-HSCT material available that remained in morphologic remission until last follow up (n=11) had no detectable *RUNX1* mutation at any timepoint after HSCT.

Irrespective of sampling timepoints after HSCT, *RUNX1* MRD had a high specificity as all patients tested *RUNX1* MRD-positive relapsed eventually, while *RUNX1* MRD sensitivity increased with longer time after HSCT (day +28 75%, day +100 80%, and day +365 100%, respectively, Figure 2A). This correlation matches previous analyses showing the highest risk of relapse despite a negative MRD test result very early after HSCT.[11]

By using receiver operating characteristic (ROC) curves the feasibility of *RUNX1* MRD to predict relapse during follow-up after HSCT was further evaluated. Here, detectable *RUNX1* MRD - as continuous values above the limit of detection - was an excellent marker for relapse prediction within 28 days (AUC_{RUNX1} = 0.99), 56 days (AUC_{RUNX1} = 0.97), 84 days (AUC_{RUNX1} = 0.85) and 112 days after sampling (AUC_{RUNX1} = 0.84, Figure 2B,C). *RUNX1* MRD also was superior to the bone marrow chimerism in predicting relapse within 28 days (AUC_{Chim} = 0.83, ROC comparison *P*<.01) and 56 days after sampling (AUC_{CD34} = 0.82, ROC comparison *P*<.01). With longer latency between sampling and relapse, *RUNX1* and the bone marrow chimerism allowed for comparable relapse prediction, *i.e.*, within 84 days (AUC_{Chim} = 0.82, ROC comparison *P*=.87), and 112 days (AUC_{CD34} = 0.81, ROC comparison *P*=.67, Figure 2B,C).

RUNX1 is usually part of MRD panels showing the feasibility of NGS-based MRD detection, but only one previous report analysed *RUNX1* individually, and suggested it as a suitable marker to track AML during and after treatment.[15] In a total of six patients - four treated with intensive chemotherapy followed by HSCT, one with hypomethylating (HMA) agents alone, and one with HMA and venetoclax – Nachmias *et al.* showed that *RUNX1* mutation levels decline when a CR is reached and rise again at relapse of the disease. Together with our data, these results suggest that *RUNX1* mutations can be used as suitable MRD markers, both prior to and after an HSCT.

We cannot rule out the possibility of the presence of germline *RUNX1* mutations in our analysis (which would hamper pre-HSCT MRD analysis), although the low VAF in remission prior to HSCT and the absence of such patient history make it less likely. While our study is also limited by the relatively low number of patients included in MRD analyses, and further validation is needed, we are the first to provide evidence that *RUNX1* allows for a feasible MRD evaluation before and after HSCT. Notably, this distinguishes *RUNX1* from other genes described in the context of AML-MR, *i.e. SRSF2*, and *SF3B1*,[10–12] which – like DTA mutations - often persist in remission and are not suitable MRD markers when patients are treated without an HSCT. Since *RUNX1* mutations are not regularly co-occurring with genetic aberrations currently recommended for MRD analyses by the ELN - *i.e. NPM1* mutations or core binding factor fusion genes - they may increase the number of patients to whom molecular MRD monitoring can be offered.[7,13,14]

In conclusion, in contrast to patients consolidated by chemotherapy, the presence of a *RUNX1* mutation at diagnosis did not associate with adverse outcomes after HSCT, which supports previous assumptions that *RUNX1*-mutated patients may benefit from this consolidation.[13,14]

RUNX1 MRD detection during follow up by patient-specific ddPCR assays allows for a reliable MRD analysis with a short turn-around time, high sensitivity and low cost, resulting in clinically meaningful MRD detection before and after HSCT. The long median latency (95 days) between the first MRD-positive sample and morphologic relapse suggests that, for most patients, sufficient time would remain to plan potential *RUNX1* MRD-guided treatment interventions, such as inclusion into clinical trials, faster tapering of immunosuppression, or planning of donor lymphocyte infusions.

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Table 1. Patient' characteristics* according to the *RUNX1* mutation status at diagnosis.

	***RUNX1* wild type n=260**	***RUNX1* mutated n=48**	***P***
Age at HSCT, years			.50
median (range)	60 (21-76)	63 (20-73)	
Sex, n (%)			.99
male	137 (53)	25 (52)	
female	123 (47)	23 (48)	
Disease origin, n (%)			.05
secondary/treatment related	94 (36)	25 (52)	
de novo	166 (64)	23 (48)	
ELN 2022 risk, n (%)			<.001
favorable	41 (16)	1 (2)	
intermediate	64 (26)	0 (0)	
adverse	146 (58)	46 (98)	
***ASXL1* mutation, n (%)**			.002
wild type	230 (89)	34 (71)	
mutated	27 (11)	14 (29)	
***FLT3*-ITD mutation, n (%)**			.36
wild type	223 (86)	44 (92)	
mutated	37 (14)	4 (8)	
***FLT3*-TKD mutation, n (%)**			.006
wild type	237 (92)	35 (78)	
mutated	20 (8)	10 (22)	
***NPM1* mutation, n (%)**			.006
wild type	192 (81)	41 (98)	
mutated	45 (19)	1 (2)	
***SRSF2* mutation, n (%)**			.03
wild type	202 (88)	33 (75)	
mutated	28 (12)	11 (25)	
***TP53* mutation, n (%)**			.001
wild type	204 (79)	46 (98)	
mutated	54 (21)	1 (2)	
Remission status at HSCT, n (%)			>.99
CRc1	163 (63)	30 (63)	
CRc2	28 (11)	5 (10)	
PR/relapsed/refractory	69 (27)	13 (27)	
Donor type, n (%)			.63
matched related	32 (12)	7 (15)	
unrelated, HLA matched	172 (66)	35 (73)	
HLA mismatched	46 (18)	5 (10)	
haploidentical	10 (4)	1 (2)	
Conditioning regimen, n (%)			.55
nma	114 (44)	21 (44)	
ric	97 (37)	21 (44)	
mac	49 (19)	6 (12)	
HCT-CI Score, n (%)			.76
0	91 (40)	19 (44)	
1/2	75 (33)	15 (35)	
≥ 3	61 (27)	9 (21)	

**For each assessed parameter, only patients with data available are shown.*

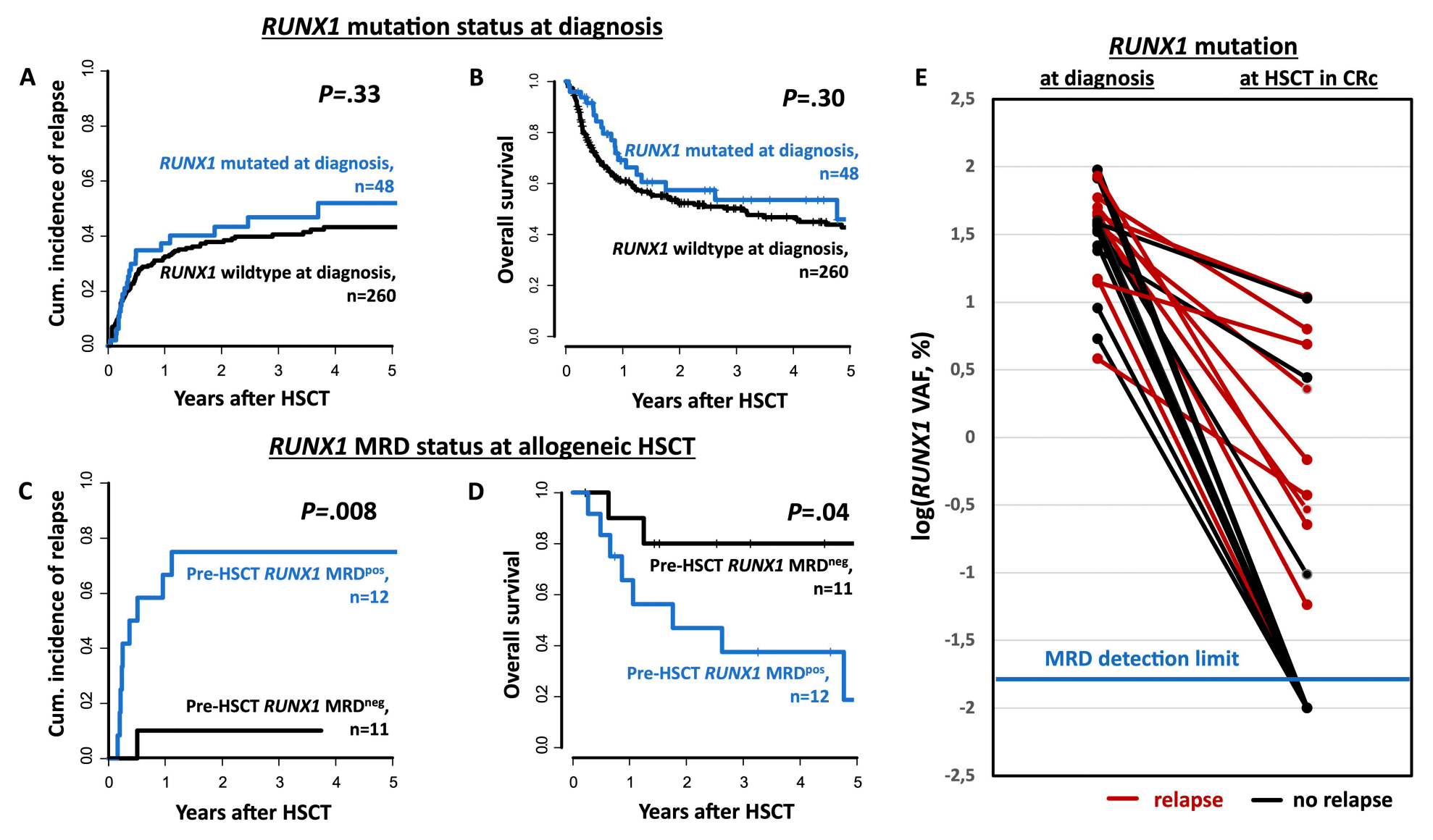
Abbreviations: CR, complete remission; CRi, complete remission with incomplete peripheral recovery; ELN, European Leukemia Net; HCT-CI, hematopoietic cell transplantation comorbidity index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; mac, myeloablative conditioning (2x60 mg/kg body weight Cyclophosphamide and 12 Gy total body irradiation or 5x30 mg/m2 Fludarabine and 8 Gy total body irradiation); nma, non-myeloablative conditioning (3x30 mg/m2 Fludarabine and 2 Gy total body irradiation);

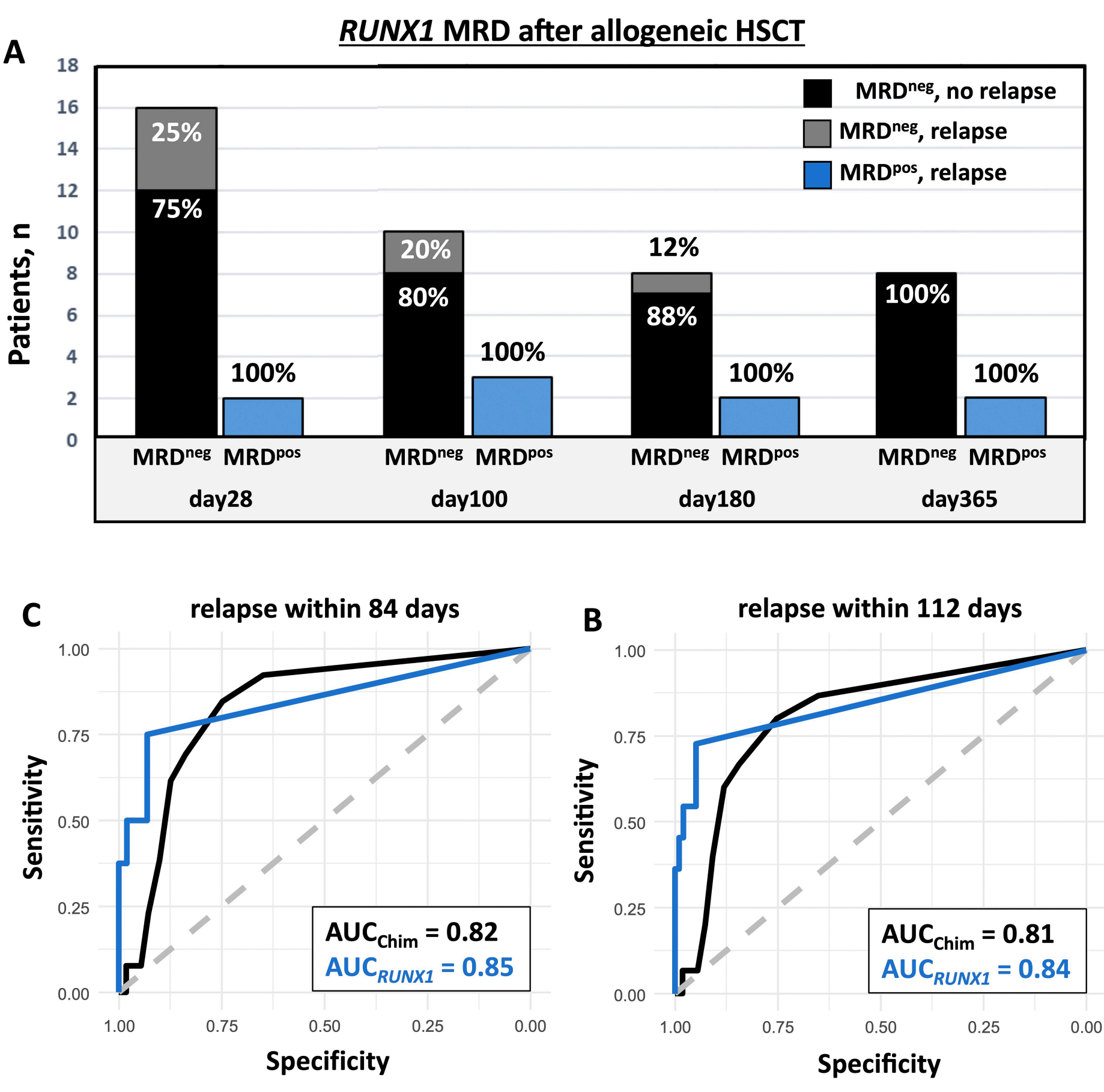
NPM1, nucleophosmin 1; PR, partial remission; ric, reduced intensity conditioning (busulfan 8 mg/kg orally or 6.4 mg/kg intravenously or Treosulfan 3x10 g/m2 and 5x30 mg/m2 Fludarabine).

Figure Legends

Figure 1: Outcomes according to the *RUNX1* mutation status. (A) Cumulative incidence of relapse and (B) Overall survival according to the presence or absence of a *RUNX1* mutation at diagnosis. (C) Cumulative incidence of relapse and (D) Overall survival in *RUNX1* mutated patients according to the *RUNX1* MRD status in CRc prior to allogeneic HSCT. (E) Comparison of *RUNX1* mutation burden at diagnosis and in CRc at HSCT in *RUNX1* mutated patients (black lines: no relapse after HSCT, red lines: relapse after HSCT).

Figure 2: *RUNX1* mutation based MRD after allogeneic HSCT. (A) Percentage of patients with a positive or negative *RUNX1* MRD status at days +28, +100, +180, and +365 after allogeneic HSCT. (B,C) ROC curve analysis for relapse prediction according to *RUNX1* MRD levels as well as the CD34-selected bone marrow chimerism measured after allogeneic HSCT. (B) Relapse prediction within 84 days after sampling and (C) Relapse prediction within 112 days after sampling.


RUNX1 mutation status at diagnosis
A
P=.33
RUNX1 mutated at diagnosis, n=48
RUNX1 wildtype at diagnosis, n=260
Cum. incidence of relapse
Years after HSCT
B
P=.30
RUNX1 mutated at diagnosis, n=48
RUNX1 wildtype at diagnosis, n=260
Overall survival
Years after HSCT
RUNX1 MRD status at allogeneic HSCT
C
P=.008
Pre-HSCT RUNX1 MRDpos, n=12
Pre-HSCT RUNX1 MRDneg, n=11
Cum. incidence of relapse
Years after HSCT
D
P=.04
Pre-HSCT RUNX1 MRDneg, n=11
Pre-HSCT RUNX1 MRDpos, n=12
Overall survival
Years after HSCT
E
RUNX1 mutation
at diagnosis
at HSCT in CRc
log(RUNX1 VAF, %)
MRD detection limit
relapse
no relapse


A
RUNX1 MRD after allogeneic HSCT
Patients, n
18
16
14
12
10
8
6
4
2
0
MRDneg, no relapse
MRDneg, relapse
MRDpos, relapse
25%
75%
100%
20%
80%
100%
12%
88%
100%
100%
100%
100%
MRDneg MRDpos
day28
MRDneg MRDpos
day100
MRDneg MRDpos
day180
MRDneg MRDpos
day365
C
relapse within 84 days
Sensitivity
1.00
0.75
0.50
0.25
0.00
AUCChim = 0.82
AUCRUNX1 = 0.85
1.00 0.75 0.50 0.25 0.00
Specificity
B
relapse within 112 days
Sensitivity
1.00
0.75
0.50
0.25
0.00
AUCChim = 0.81
AUCRUNX1 = 0.84
1.00 0.75 0.50 0.25 0.00
Specificity

Supplementary Information to

Prognostic relevance of *RUNX1* mutations at acute myeloid leukemia diagnosis and as measurable residual disease marker before and after allogeneic stem cell transplantation

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Supplementary Table S1. Primer and probe sequences and PCR conditions for *RUNX1* MRD evaluation.

To measure *RUNX1*-based MRD, mutation-specific digital droplet (dd)PCR assays were developed using a competitive probe-approach for all *RUNX1* mutated patients with DNA or RNA of peripheral blood or bone marrow follow-up samples available. For every gene's (mutation and wild type) copy number determination, the droplets were generated using the Automated Droplet Generator (BioRad). PCR was performed using the Thermocycler T100 (BioRad). The PCR consists of an initial denaturation (95°C 10 minutes) followed by 40 cycles (denaturation 94°C 30 seconds; annealing/extension 1 minute, please refer to the table for the used duration and temperature) with a ramp rate set to 2°C/min and a final extension (98°C 10 min). *RUNX1* MRD positivity was defined as VAF ≥ 0.05% for all *RUNX1* mutations.

mutation	**probe sequence**	**primer forward sequence**	**primer reverse sequence**	**Annealing/extension Temperature**
D93Rfs*45 c.276dup	**wildtype** 5' GGTGCGCACCGACAG 3' **mutation** 5′ GGTGCGCACCCGACAG 3′	**cDNA and gDNA** 5′ CATGGTGGAGGTGCTGGC 3′	**cDNA and gDNA** 5′ GTCTTGTTGCAGCGCCAG 3′	54.6°C
P103fs c.306delG	**wildtype** 5' GTGCTGCCTACGCACT 3' **mutation** 5′ CGTGCTCCTACGCACT 3′	**cDNA and gDNA** 5′ GACAGCCCCAACTTCCTCT 3′	**cDNA and gDNA** 5′ GCCAGTACCTTGAAAGCGAT 3′	54.6°C
H105Vfs*36 c.312_313insGTGC ACAACG	**wildtype** 5' CTACGCACTGGCGCT 3' **mutation** 5′ CTACGGTGCACAACGCAC 3′	**cDNA and gDNA** 5′ GACAGCCCCAACTTCCTCT 3′	**cDNA and gDNA** 5′ GCCAGTACCTTGAAAGCGAT 3′	50.9°C
W106* c.317G>A	**wildtype** 5' CTACGCACTGGCGCT 3' **mutation** 5′ CTACGCACTAGCGCT 3′	**cDNA and gDNA** 5′ GACAGCCCCAACTTCCTCT 3′	**cDNA and gDNA** 5′ GCCAGTACCTTGAAAGCGAT 3′	54.6°C
W106* c.318G>A	**wildtype** 5' CTACGCACTGGCGCT 3' **mutation** 5′ CTACGCACTGACGCT 3′	**cDNA and gDNA** 5′ GACAGCCCCAACTTCCTCT 3′	**cDNA and gDNA** 5′ GCCAGTACCTTGAAAGCGAT 3′	54.6°C
C108Wfs*30 c.323_324insG	**wildtype** 5' CTACGCACTGGCGCT 3' **mutation** 5′ GCACTGGCGCTGGCAACAA 3′	**cDNA and gDNA** 5′ GACAGCCCCAACTTCCTCT 3′	**cDNA and gDNA** 5′ GCCAGTACCTTGAAAGCGAT 3′	54.6°C
I114Afs*25	**wildtype**	**cDNA and gDNA**	**cDNA and gDNA**	50°C

c.335_338dup	5’ CAAGACCCTGCCCATCGCT 3‘ **mutation** 5´ CAAGACCCTGCCTGCCCAT 3´	5´ GACAGCCCCAACTTCCTCT 3´	5´ TCTCCGGGCCAGTACCTT 3´	
N136I c.407A>T	**Wildtype** 5’ GCTGGCAATGATGAAAACTACT 3‘ **mutation** 5´ GCTGGCATTGATGAAAACTACT 3´	**gDNA** 5´ AGATGGCACTCTGGTCACTG 3´	**gDNA** 5´ GGTTCTTCATGGCTGCGG 3´	50.9°C
S141* c.422C>A	**wildtype** 5‘ GAAAACTACTCGGCTGAGCT 3‘ **mutation** 5´ GAAAACTACTAGGCTGAGCT 3´	**cDNA and gDNA** 5’ CTCTGGTCACTGTGATGGCT 3‘	**cDNA and gDNA** 5´ GTTCTTCATGGCTGCGGTAG 3´	50.9°C
L144P c.431T>C	**wildtype** 5’ CTACTCGGCTGAGCTGAGAAAT 3‘ **mutation** 5´ CTACTCGGCTGAGCCGAGAAAT 3´	**cDNA and gDNA** 5’ CTCTGGTCACTGTGATGGCT 3‘	**cDNA and gDNA** 5´ GTTCTTCATGGCTGCGGTAG 3´	50.9°C
R166Q c.497G>A	**wildtype** 5‘CGGTCGAAGTGGAAGAGG 3‘ **mutation** 5´ CTTCCACTT[CAA]CCGACA 3´	**gDNA** 5´ CCATGAAGAACCAGGTTGCA 3´ **cDNA** 5‘ CTACCGCAGCCATGAAGAAC 3‘	**gDNA** 5´ TGTGGGTTTGTTGCCATGAA 3´ **cDNA** 5’ TGATGGCTCTGTGGTAGGTG 3‘	54.6°C
R166* c.496C>T	**wildtype** 5‘CGGTCGAAGTGGAAGAGG 3‘ **mutation** 5´ GTTTGTCGGTTGAAGTGGA 3´	**gDNA** 5´ CCATGAAGAACCAGGTTGCA 3´ **cDNA** 5‘ CTACCGCAGCCATGAAGAAC 3‘	**gDNA** 5´ TGTGGGTTTGTTGCCATGAA 3´ **cDNA** 5’ TGATGGCTCTGTGGTAGGTG 3‘	54.6°C
S167N c.500G>A	**wildtype** 5‘CGGTCGAAGTGGAAGAGG 3‘ **mutation** 5´ CGGTCGAAATGGAAGAGG 3´	**gDNA** 5´ CCATGAAGAACCAGGTTGCA 3´ **cDNA** 5‘ CTACCGCAGCCATGAAGAAC 3‘	**gDNA** 5´ TGTGGGTTTGTTGCCATGAA 3´ **cDNA** 5’ TGATGGCTCTGTGGTAGGTG 3‘	54.6°C
S167fs c.497dup	**wildtype** 5‘CGGTCGAAGTGGAAGAGG 3‘ **mutation** 5´ CGGTCGGAAGTGGAAGAG 3´	**gDNA** 5´ CCATGAAGAACCAGGTTGCA 3´ **cDNA** 5‘ CTACCGCAGCCATGAAGAAC 3‘	**gDNA** 5´ TGTGGGTTTGTTGCCATGAA 3´ **cDNA** 5’ TGATGGCTCTGTGGTAGGTG 3‘	54.6°C
K171* c.511A>T	**wild type** 5´ GGAAGAGGGAAAAGCTTCA 3’	**gDNA** 5´ GCCACCAACCTCATTCTGTT 3´	**gDNA** 5´ CTTGCGGTGGGTTTGTGAAG 3´	50.9°C

	mutation 5´ GGAAGAGGGTAAAGCTTCA 3´	**cDNA** 5‘CTACCGCAGCCATGAAGAAC 3‘	**cDNA** 5’ CTTGCGGTGGGTTTGTGAAG 3‘	
I177N c.530T>A	**wild type** 5´ CTCTGACC[ATC]ACTGTCTTCA 3’ **mutation** 5´ CTCTGACC[AAC]ACTGTCTTCA 3´	**gDNA** 5´ GCCACCAACCTCATTCTGTT 3´	**gDNA** 5‘ TGATGGCTCTGTGGTAGGTG 3´	54.6°C
R201Q c.602G>A	**wild type** 5‘ GCCCCGAGAACCTCGA 3’ **mutation** 5´ GCCCCAAGAACCTCGA 3´	**gDNA** 5´ CTTCACAAACCCACCGCAAG 3´ **cDNA** 5‘CACCTACCACAGAGCCATCA 3‘	**gDNA** 5´ TGGATGCACTTACTTCGAGGT 3´ **cDNA** 5’ GCTCGGAAAAGGACAAGCTC 3‘	50.9°C
R201* c.601C>T	**wild type** 5‘ GCCCCGAGAACCTCGA 3’ **mutation** 5´ GCCCTGAGAACCTCGA 3´	**gDNA** 5´ CTTCACAAACCCACCGCAAG 3´ **cDNA** 5‘CACCTACCACAGAGCCATCA 3‘	**gDNA** 5´ TGGATGCACTTACTTCGAGGT 3´ **cDNA** 5’ GCTCGGAAAAGGACAAGCTC 3‘	50.9°C
R204Q c.611G>A	**wild type** 5‘GAGAACCTCGAAGACATCGG 3’ **mutation** 5´ GAGAACCTCAAAGACATCGG 3´	**gDNA** 5´ CTTCACAAACCCACCGCAAG 3´ **cDNA** 5‘CACCTACCACAGAGCCATCA 3‘	**gDNA** 5´ TGGATGCACTTACTTCGAGGT 3´ **cDNA** 5’ GCTCGGAAAAGGACAAGCTC 3‘	50.9°C
Q213Rfs24 c.637del	**wild type** 5’ CTAGATGATCAGACCAAGCCC 3‘ **mutation** 5´ CTAGATGATAGACCAAGCCCG 3´	**gDNA** 5´ CTTCACAAACCCACCGCAAG 3´ **cDNA** 5‘CACCTACCACAGAGCCATCA 3‘	**gDNA** 5´ TGGATGCACTTACTTCGAGGT 3´ **cDNA** 5’ GCTCGGAAAAGGACAAGCTC 3‘	50.9°C
R232W c.694C>T	**wild type** 5´ CAGCTGCGGCGCACA 3´ **mutation** 5´ CAGCTGTGGCGCACA 3’	**cDNA and gDNA** 5´ GAGCTTGTCCTTTTCCGAGC 3´	**cDNA and gDNA** 5´ CTGGGTGGTGTGGGCTGA 3´	50.9°C
T270Nfs*330 c.808dup	**wild type** 5´ [+G][+A]TACAAGGCAGATCCAAC 3´ **mutation** 5´ [+G][+A]TAACAAGGCAGATCCAAC 3’	**gDNA** 5´ AAATCCCACCCCACTTTACAT 3´ **cDNA** 5´ CACTGCCTTTAACCCTCAGC 3´	**cDNA and gDNA** 5´ AGGACTGATCGTAGGACCAC 3´	50.9°C
P278Tfs*322 c.830dup	**wild type** 5‘ATCCCCACCGTGGTCC 3’	**gDNA** 5´ CTTCAGATACAAGGCAGATCCA 3´	**cDNA and gDNA** 5´ GCACAGAAGGAGAGGCAATG 3´	50.9°C

	mutation 5′ ATCCCCCACCGTGGTC 3′	**cDNA** 5′ AGCCTCAGAGTCAGATGCAG 3′		
S303* c.908C>A	**wild type** 5′ GCCCATTTCACCTGGAC 3’ **mutation** 5′ GCCCATTTAACCTGGAC 3′	**cDNA and gDNA** 5′ CATTGCCTCTCCTTCTGTGC 3′	**cDNA and gDNA** 5′ TTCTGCAGAGAGGGTTGTCA 3′	54.6°C
Glu316* c.946G>T	**wild type** 5′ TCTCTGCAGAACTTTCCAGTC 3’ **mutation** 5′ TCTCTGCATAACTTTCCAGTC 3′	**gDNA** 5′ CAACGCCCATTTCACCTGG 3′ **cDNA** 5′ CGTCGGGGAGTAGGTGAAG 3′	**cDNA and gDNA** 5′ GCTCAGCTGCAAAGAATGTG 3′	50.9°C
Q397Kfs*197 c.1189del	**wild type** 5′ GCCCGTTCCAAGCCAGCT 3’ **mutation** 5′ GCCCGTTCAAGCCAGCT 3′	**cDNA and gDNA** 5′ TACCACACCTACCTGCCG 3′	**cDNA and gDNA** 5′ GGAGAACTGGTAGGAGCCG 3′	55°C

Abbreviations: cDNA, complementary DNA; gDNA, genomic DNA.

Supplementary Table S2. Additional patient' characteristics* according to the *RUNX1* mutation status at diagnosis.

	***RUNX1* wild type n=260**	***RUNX1* mutated n=48**	***P***
Hemoglobin at diagnosis, g/dL			.91
median (range)	8.5 (4.3-14.7)	8.7 (6-14.88)	
Platelet count at diagnosis, x 10^9/L			.25
median (range)	57 (2-728)	64 (3-270)	
WBC at diagnosis, x 10^9/L			.38
median (range)	4.4 (0.7-385)	6 (0.7-238)	
Blood blasts at diagnosis, %			.81
median (range)	20 (0-97)	22 (2-85)	
Bone marrow blasts at diagnosis, %			.25
median (range)	48 (3-95)	55 (6-95)	
CD34+/CD38- cell burden at diagnosis, %			.15
median (range)	0.5 (0-64)	1.6 (0-28)	
LDH at diagnosis, ukat/l			.43
median (range)	5.8 (1.45-37.2)	5.4 (3.14-30)	
Normal karyotype, n (%)			.19
absent	168 (65)	26 (54)	
present	90 (35)	22 (46)	
Complex karyotype (ELN 2022), n (%)			.03
absent	183 (72)	42 (87.5)	
present	72 (28)	6 (12.5)	
***CEBPA* mutation, n (%)**			.80
wild type	228 (89)	43 (92)	
mutated	27 (11)	4 (8)	
***IDH1* mutation, n (%)**			.99
wild type	227 (89)	43 (90)	
mutated	29 (11)	5 (10)	
***IDH2* mutation, n (%)**			.13
wild type	222 (87)	43 (96)	
mutated	33 (13)	2 (4)	
***SF3B1* mutation, n (%)**			.17
wild type	213 (94)	36 (88)	
mutated	13 (6)	5 (12)	
***U2AF1* mutation, n (%)**			.39
wild type	216 (96)	36 (92)	
mutated	9 (4)	3 (8)	
Donor sex, n (%)			.99
female into male	34 (13)	6 (12.5)	
all others	224 (87)	42 (87.5)	
CMV status, n (%)			.17
recipient + / donor –	74 (29)	19 (40)	
all others	183 (71)	29 (60)	
Acute GvHD ≥ grade 2, n (%)			.70
absent	172 (76)	36 (80)	
present	55 (24)	9 (20)	
Chronic GvHD, n (%)			.87
absent	85 (52)	21 (55)	
limited	28 (17)	7 (18)	
extended	52 (32)	10 (26)	

**For each assessed parameter, only patients with data available are shown.*

Abbreviations: CMV, cytomegalovirus; GvHD, graft-versus-host disease; LDH, lactatedehydrogenase.

Supplementary Table S3. Clinical and genetic characteristics* for *RUNX1* mutated patients according to the presence or absence of *RUNX1* MRD in CRc prior to allogeneic HSCT.

	***RUNX1* MRD^{neg} n=11**	***RUNX1* MRD^{pos} n=12**	***P***
Parameters at diagnosis			
Sex, n (%)			.001
male	1 (9)	10 (83)	
female	10 (91)	2 (17)	
Disease origin, n (%)			.40
secondary/treatment related	3 (27)	6 (50)	
de novo	8 (73)	6 (50)	
Hemoglobin at diagnosis, g/dL			.10
median (range)	7.6 (6-14.88)	9 (7.5-12.8)	
Platelet count at diagnosis, x 10^9/L			.76
median (range)	58 (15-100)	52 (3-224)	
WBC at diagnosis, x 10^9/L			.20
median (range)	19.2 (1.1-238)	4.7 (1.2-34.9)	
Blood blasts at diagnosis, %			.13
median (range)	49 (4-70)	23 (4-72)	
Bone marrow blasts at diagnosis, %			.21
median (range)	70 (34-95)	43 (26-92)	
CD34+/CD38- cell burden at diagnosis, %			.88
median (range)	2.5 (1-4)	2.6 (0-20)	
LDH at diagnosis, ukat/l			.17
median (range)	7.39 (3.46-30)	5.265 (3.82-14.8)	
Normal karyotype, n (%)			.41
absent	4 (36)	7 (58)	
present	7 (64)	5 (42)	
Complex karyotype (ELN22), n (%)			>.99
absent	11 (100)	11 (92)	
present	0 (0)	1 (8)	
ASXL1 mutation, n (%)			.07
wild type	10 (91)	6 (50)	
mutated	1 (9)	6 (50)	
CEBPA mutation, n (%)			.32
wild type	8 (73)	11 (92)	
mutated	3 (27)	1 (9)	
FLT3-ITD mutation, n (%)			.22
wild type	9 (82)	12 (100)	
mutated	2 (18)	0 (0)	
FLT3-TKD mutation, n (%)			.07
wild type	6 (55)	11 (92)	
mutated	5 (45)	1 (8)	
IDH1 mutation, n (%)			.54
wild type	8 (100)	10 (100)	
mutated	0 (0)	0 (0)	
IDH2 mutation, n (%)			.44
wild type	7 (87.5=	10 (100)	
mutated	1 (12.5)	0 (0)	
NPM1 mutation, n (%)			.45
wild type	8 (89)	11 (100)	
mutated	1 (11)	0 (0)	
SF3B1 mutation, n (%)			.54
wild type	6 (75)	11 (92)	
mutated	2 (25)	1 (8)	
SRSF2 mutation, n (%)			.99

wild type	7 (78)	9 (75)	
mutated	2 (22)	3 (25)	
TP53 mutation, n (%)			>.99
wild type	11 (100)	12 (100)	
mutated	0 (0)	0 (0)	
U2AF1 mutation, n (%)			>.99
wild type	7 (87.5)	10 (91)	
mutated	1 (12.5)	1 (9)	
Parameters assessed prior to allogeneic HSCT			
Age at HSCT, years			.67
median (range)	64 (42-74)	61 (46-73)	
Remission status at HSCT, n (%)			>.99
CRc 1	10 (91)	9 (72)	
CRc 2	1 (9)	2 (18)	
ASXL1 mutation VAF at HSCT, n (%)[1]			NA
median (range)	NA	0.81 (<0.05-14.8)	
number of patients with data available	0	5	
DNMT3A mutation VAF at HSCT, n (%)[1]			NA
median (range)	36.4 (NA-NA)	12.1 (11.7-12.4)	
number of patients with data available	1	2	
TET2 mutation VAF at HSCT, n (%)[1]			NA
median (range)	NA	9.7 (5.2-14.3)	
number of patients with data available	0	2	
SRSF2 mutation VAF at HSCT, n (%)[2]			NA
median (range)	<0.05 (NA-NA)	13.7 (10.3-17.1)	
number of patients with data available	1	2	
Conditioning regimen, n (%)			.45
nma	6 (55)	9 (82)	
ric	4 (36)	1 (9)	
mac	1 (9)	1 (9)	
HCT-CI Score, n (%)			>.99
0	5 (50)	5 (50)	
1/2	3 (30)	2 (20)	
≥ 3	2 (20)	3 (30)	
Donor type, n (%)			.82
matched related	2 (18)	2 (18)	
unrelated, HLA matched	6 (55)	8 (73)	
HLA mismatched	3 (27)	1 (9)	
haploidentical	0 (0)	0 (0)	
Donor sex, n (%)			.22
female into male	0 (0)	3 (25)	
all others	11 (100)	9 (75)	
CMV status, n (%)			.10
recipient + / donor –	3 (27)	8 (67)	
all others	8 (73)	4 (33)	
Outcomes after HSCT			
Acute GvHD ≥ grade 2, n (%)			.32
absent	8 (73)	11 (92)	
present	3 (27)	1 (8)	
Chronic GvHD, n (%)			.46
absent	5 (56)	3 (33)	
limited	2 (22)	1 (11)	
extended	2 (22)	5 (56)	

**For each assessed parameter, only patients with data available are shown.*

[1] Persistence of DNMT3A, TET2, and ASXL1 was evaluated using ddPCR in patients with material available and a known mutation at diagnosis as published previously (Jentzsch et al. Bone Marrow Transplant 2021 doi: 10.1038/s41409-021-01407-6)

[2] Persistence of SRSF2 was evaluated using ddPCR in patients with material available and a known mutation at diagnosis as published previously (Grimm et al. Am J Hematol 2021 doi: 10.1002/ajh.26298)

Abbreviations: CMV, cytomegalovirus; CRc, composite complete remission; ELN, European Leukemia Net; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; HCT-CI, hematopoietic cell transplantation comorbidity index; HSCT, hematopoietic stem cell transplantation; IDH, isocitrate dehydrogenase gene; ITD, internal tandem duplication; LDH, lactatedehydrogenase; MRD, measurable residual disease; nma, non-myeloablative conditioning; PR, partial remission; ric, reduced intensity conditioning; VAF, variant allele frequency.