SUPPLEMENTARY MATERIAL

NF1 Mutations are Recurrent in Adult Acute Myeloid Leukemia and Confer Poor Outcome

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Running title: *NF1* mutations in AML

PARTICIPATING INSTITUTIONS

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TREATMENT PROTOCOLS

Patients in this study received intensive cytarabine/daunorubicin-based therapy on one of the following Cancer and Leukemia Group B (CALGB) frontline treatment protocols: 19808 (n=224; ref. 1), 10503 (n=190; ref. 2), 9720 (n=126; ref. 3), 9621 (n=114; ref. 4), 10201 (n=107; ref. 5), 8525 (n=58; ref. 6), 9222 (n=56; ref. 7), 10603 (n=43; ref. 8), 10502 (n=15; ref. 9), 8923 (n=12; ref. 10), 9022 (n=10; ref. 11), 8821 (n=9; ref. 12).

Patients enrolled on CALGB 19808 were randomly assigned to receive induction chemotherapy with cytarabine, daunorubicin, and etoposide with or without PSC-833 (valspodar), a multidrug resistance protein inhibitor (1). On achievement of complete remission (CR), patients were assigned to intensification with high-dose cytarabine and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by autologous peripheral blood stem-cell transplantation (HSCT). Patients enrolled on CALGB 10503 were assigned to receive induction chemotherapy consisting of cytarabine, daunorubicin, and etoposide. Upon achievement of CR, patients received high-dose cytarabine and etoposide for stem-cell mobilization followed by myeloablative treatment with busulfan and etoposide supported by autologous peripheral HSCT. Patients not eligible for HSCT received high-dose cytarabine (HiDAC). After intensification, patients received the DNA methyltransferase inhibitor decitabine for maintenance (2). Patients on CALGB 9720 received induction chemotherapy consisting of cytarabine in combination with or without the multidrug resistance protein modulator PSC-833 (3). Patients enrolled on CALGB 9621 were treated similarly to those on CALGB 19808, as previously reported (4). Patients on CALGB 10201 received induction chemotherapy consisting of cytarabine and daunorubicin, with or without the BCL2 antisense oblimersen sodium. The

consolidation regimen included two cycles of cytarabine (2 g/m^2/d) with or without oblimersen (5). Patients on CALGB 8525 were treated with induction chemotherapy consisting of cytarabine in combination with daunorubicin and were randomly assigned to consolidation with different doses of cytarabine followed by maintenance treatment (6). Patients on protocol CALGB 9222 received induction chemotherapy consisting of cytarabine in combination with daunorubicin followed by consolidation with one cycle of HiDAC. Different doses of mitoxantrone were explored, and the consolidation treatment was randomized to three cycles of monotherapy with HiDAC or consolidation with one cycle of HiDAC, a cycle of cyclophosphamide and etoposide, and one cycle of mitoxantrone and diaziquone (7). In CALGB 10603, cytarabine and daunorubicin followed by consolidation with high-dose cytarabine was applied with or without PKC-412 (8). For the patients on CALGB 10502, bortezomib was added to both induction consisting of cytarabine and daunorubicin and to consolidation with two cycles of intermediate-dose cytarabine (9). The patients enrolled on CALGB 8923 was treated with induction chemotherapy consisting of cytarabine in combination with daunorubicin and was randomly assigned to receive postremission therapy with cytarabine alone or in combination with mitoxantrone (10). Patients enrolled onto CALGB 9022 received induction chemotherapy consisting of cytarabine in combination with daunorubicin followed by consolidation with one cycle of HiDAC, a cycle of cyclophosphamide and etoposide, and one cycle of mitoxantrone and diaziquone (11). After induction consisting of cytarabine in combination with daunorubicin, the patients enrolled on CALGB 8821 received intensive post remission therapy with cytoxan/etoposide and diazaquone/mitoxantrone (12).

DEFINITION OF CLINICAL END POINTS

Complete remission (CR) required an absolute neutrophil count ≥1.5 x 10^9/l, platelet count ≥100 x 10^9/l, no leukemic blasts in the blood, bone marrow (BM) cellularity >20% with maturation of all cell lines, no Auer rods, <5% BM blast cells, and no evidence of extramedullary leukemia, all of which had persisted for at least one month. Relapse was defined by ≥5% BM blasts, circulating leukemic blasts, or the development of extramedullary leukemia. Disease-free survival was measured from the date of CR until the date of relapse or death; patients alive and relapse-free at last follow-up were censored. Overall survival was measured from the date on study until the date of death, and patients alive at last follow-up were censored (13).

MUTATIONAL PROFILING

The mutational status of 80 protein coding genes (*AKT1*, *ARAF*, *ASXL1*, *ATM*, *AXL*, *BCL2*, *BCOR*, *BCORL1*, *BRAF*, *BRD4*, *BRINP3*, *BTK*, *CBL*, *CCND1*, *CCND2*, *CSNK1A1*, *CTNNB1*, *DNMT3A*, *ETV6*, *EZH2*, *FBXW7*, *FLT3* [for *FLT3* tyrosine kinase domain mutations (*FLT3*-TKD) and *FLT3* internal tandem duplications (*FLT3*-ITD)], *GATA1*, *GATA2*, *GSK3B*, *HIST1H1E*, *HNRNPK*, *IDH1*, *IDH2*, *IKZF1*, *IKZF3*, *ILR7*, *JAK1*, *JAK2*, *JAK3*, *KIT*, *KLHL6*, *KMT2A*, *KRAS*, *MAPK1*, *MAPK3*, *MED12*, *MYD88*, *NF1*, *NOTCH1*, *NPM1*, *NRAS*, *PHF6*, *PIK3CD*, *PIK3CG*, *PLCG2*, *PLEKHG5*, *PRKCB*, *PRKD3*, *PTEN*, *PTPN11*, *RAD21*, *RAF1*, *RUNX1*, *SAMHD1*, *SETBP1*, *SF1*, *SF3A1*, *SF3B1*, *SMARCA2*, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *SYK*, *TET2*, *TGM7*, *TP53*, *TYK2*, *U2AF1*, *U2AF2*, *WT1*, *XPO1*, *ZMYM3*, *ZRSR2*) was determined by targeted amplicon sequencing using the MiSeq platform (Illumina, San Diego, CA). DNA library preparations were performed according to the manufacturer's instructions. Briefly, samples were pooled and run on the MiSeq machine using the Illumina MiSeq Reagent Kit v3. Sequenced reads were aligned to the hg19

genome build using the Illumina Isis Banded Smith-Waterman aligner. Single nucleotide variant and indel calling were performed using MuTect and VarScan, respectively (14,15). The MuCor algorithm was used as the baseline for integrative mutation assessment (16). We only considered non-synonymous variants not listed in either the 1000 Genome database or dbSNP142-common variants. All called variants underwent visual inspection of the aligned reads using the Integrative Genomics Viewer (Broad Institute; ref. 17). All variants that occurred with VAFs of <0.10 were considered wild-type; all variants that were sequenced to a depth of <15 reads were excluded from the analysis. In addition, variants were excluded when they occurred only in 1 read direction if sequenced in both directions, if the region contained many variants with low QC scores, or if they occurred in all analyzed samples including run controls. In addition, samples with high background noise were entirely excluded from analysis. Samples were considered non-evaluable for a specific gene if ≥85% of the amplicons covering the target regions within the coding sequence of the gene were sequenced to a depth of <15 reads. Testing for the presence or absence of *FLT3* internal tandem duplication (*FLT3*-ITD) was performed using the Pindel algorithm on the targeted sequencing data. Furthermore, testing for *CEBPA* mutations was performed with Sanger sequencing methods (18), thus adding up to a total of 81 genes analyzed in our study. In accordance with the revision of the World Health Organization classification, only patients with biallelic *CEBPA* mutations were considered as *CEBPA* mutated.

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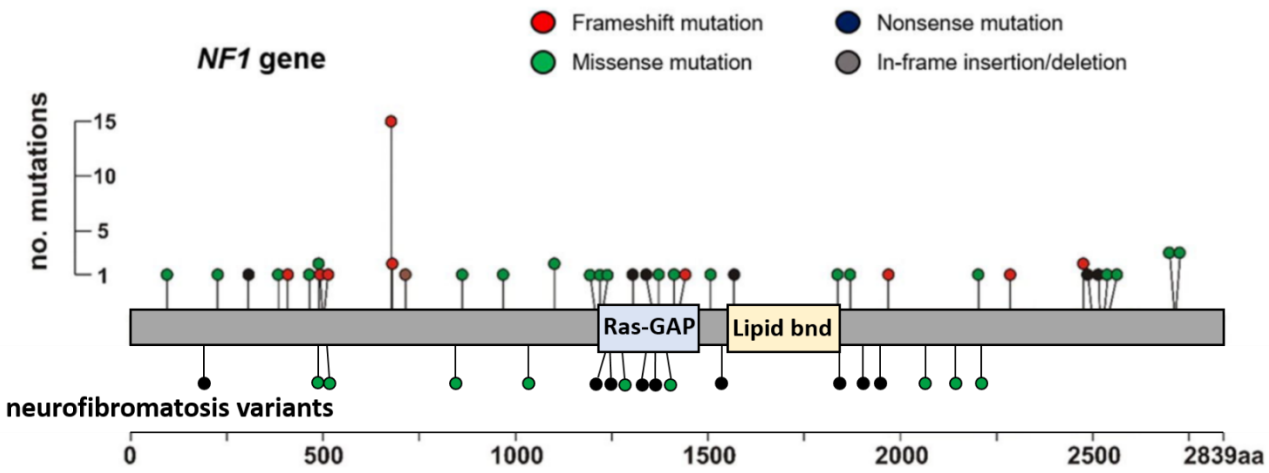
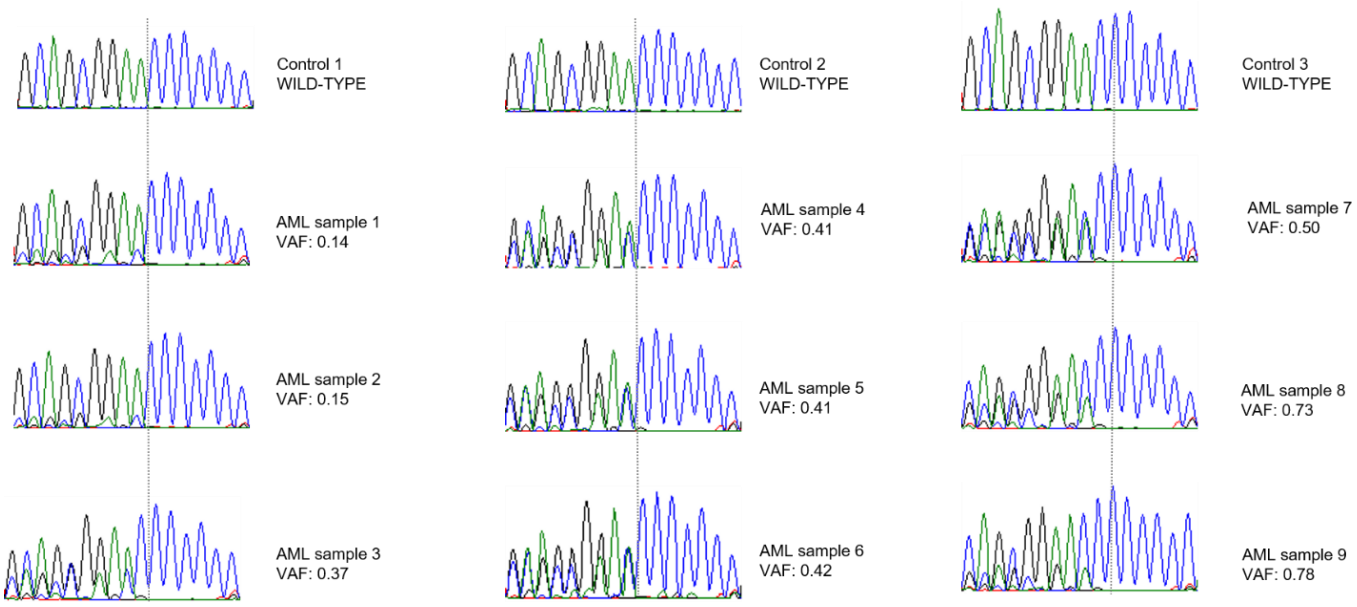
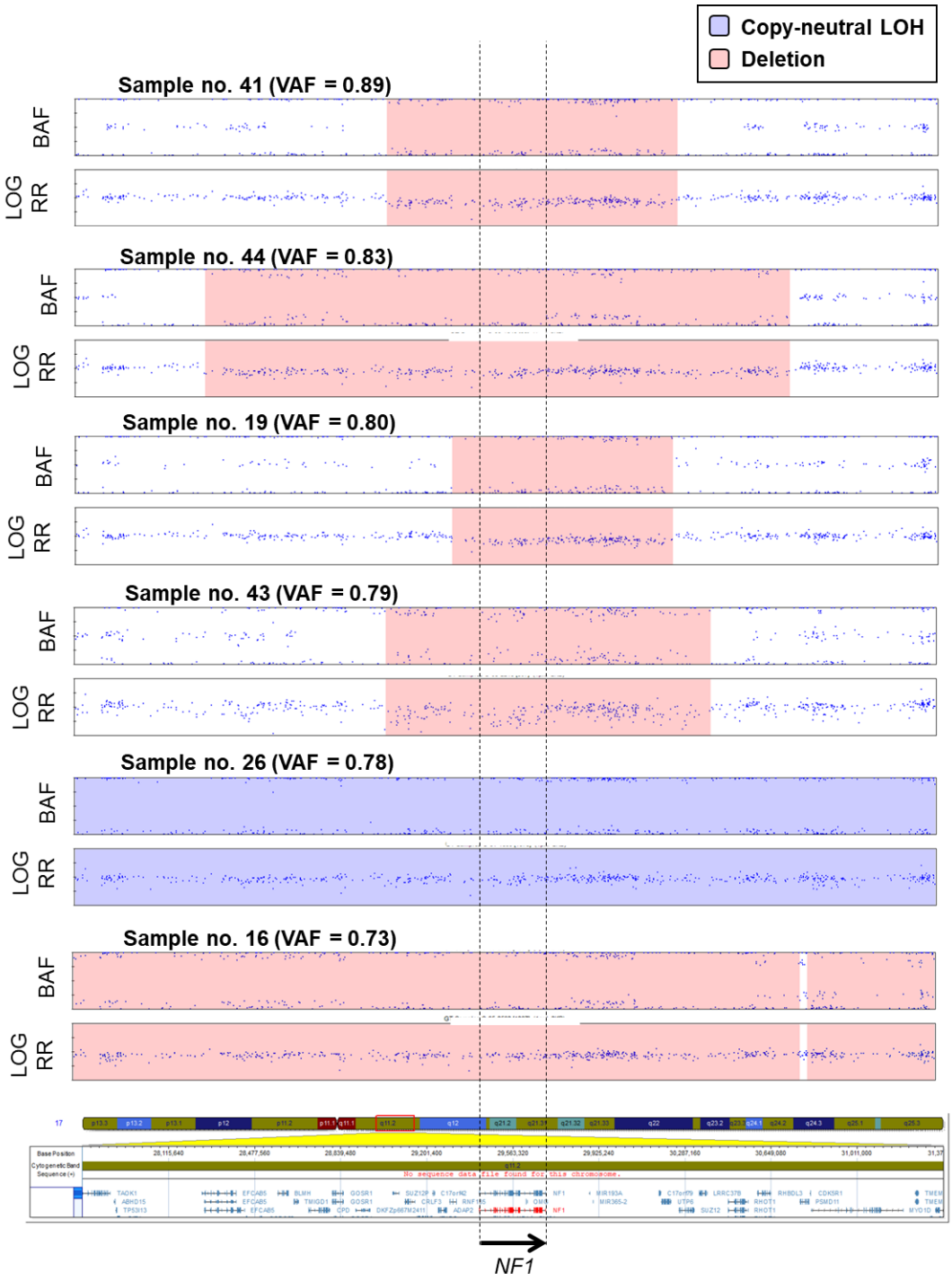
Supplementary Figure S1. Lollipop plot depicting *NF1* mutations detected in 1,021 AML patients (top part), and previously described germline *NF1* mutations in patients with neurofibromatosis type 1 (bottom part).

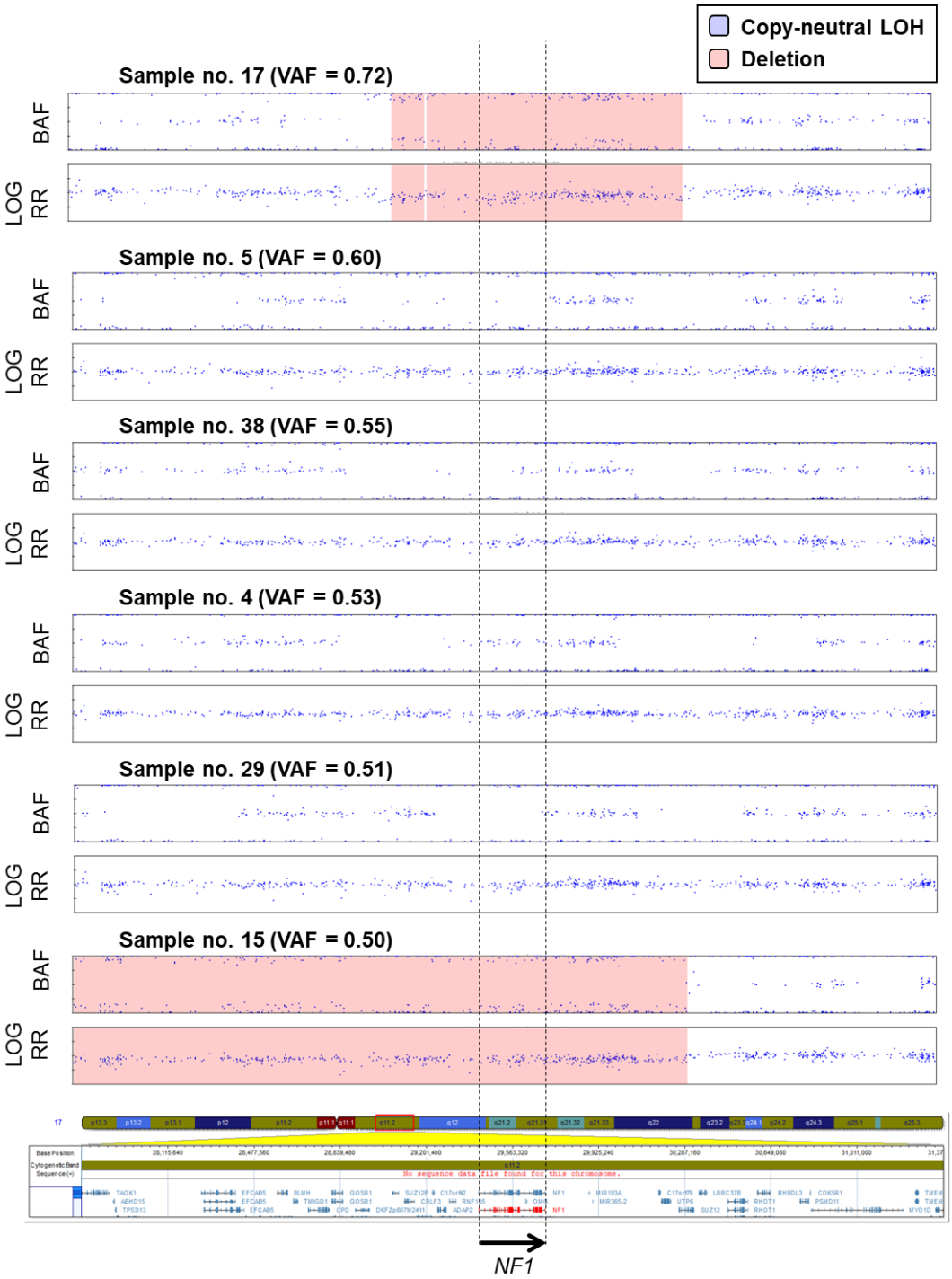


Supplementary Figure S2. Sanger sequencing traces of *NF1* Thr676-mutated samples with available DNA, as well as three *NF1* Thr676 wild-type controls. The variant allele fractions (VAF) observed in the corresponding Miseq sequencing are indicated on the right side of each trace.

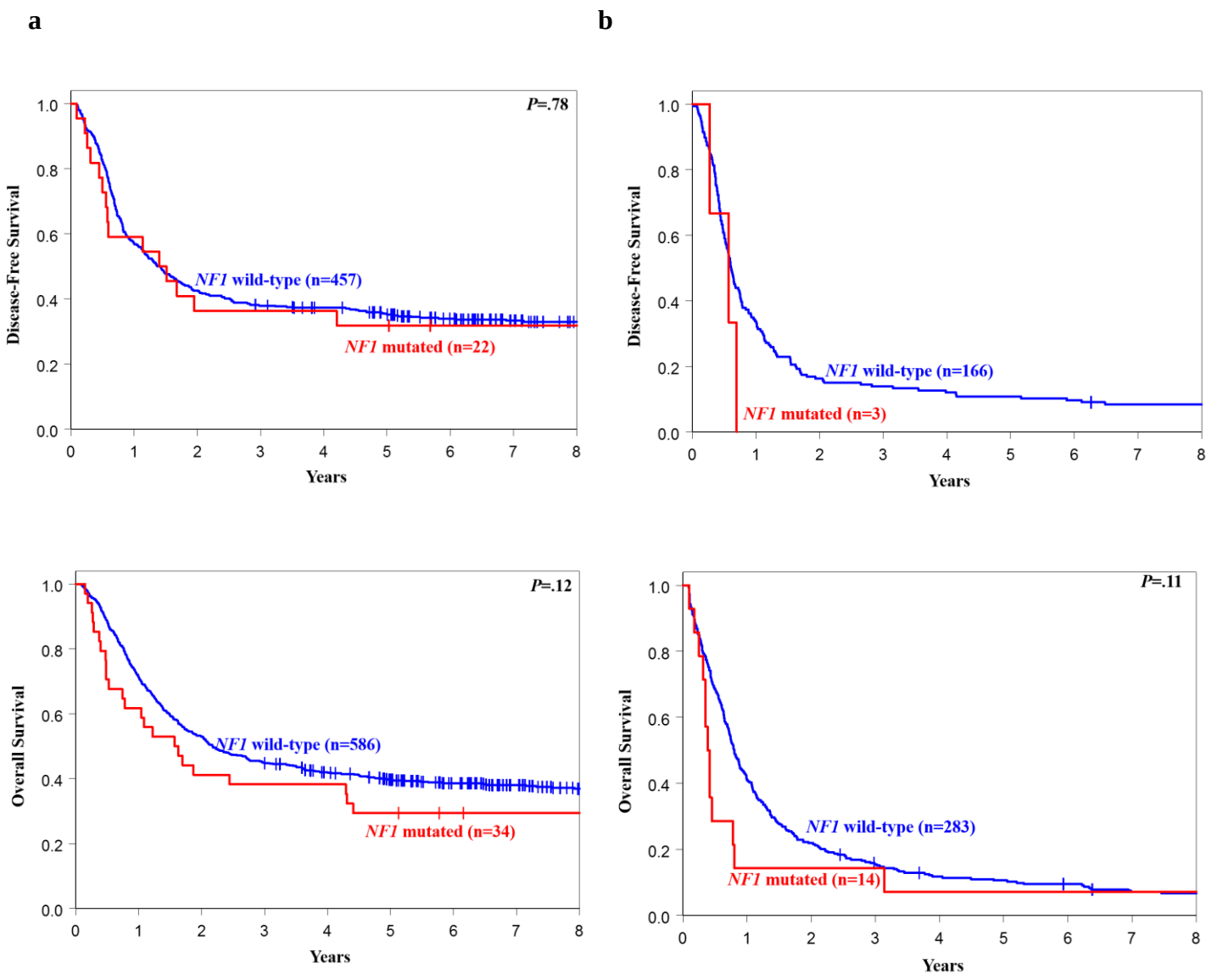


Supplementary Figure S3. Copy number variations (CNVs) in *NF1*-mutated samples. Shown are the B-allele frequency (BAF) and LogR ratio (LOG RR) plots from SNP-array genotyping. Copy number loss is shown in red and copy neutral loss of heterozygosity (homozygous stretches > 1Mb) is shown in purple.




Copy-neutral LOH
Deletion
Sample no. 17 (VAF = 0.72)
BAF
LOG RR
Sample no. 5 (VAF = 0.60)
BAF
LOG RR
Sample no. 38 (VAF = 0.55)
BAF
LOG RR
Sample no. 4 (VAF = 0.53)
BAF
LOG RR
Sample no. 29 (VAF = 0.51)
BAF
LOG RR
Sample no. 15 (VAF = 0.50)
BAF
LOG RR
NF1

Supplementary Figure S4. a, Kaplan-Meyer curves depicting disease-free (upper panel) and overall survival (lower panel) of patients <60 years of age with (red) and without *NF1* mutations (blue). b, Kaplan-Meyer curves depicting disease-free (upper panel, no *P*-values are provided due to the small sample size in the *NF1* mutated group) and overall survival (lower panel) of patients ≥60 years of age with (red) and without *NF1* mutations (blue).



Supplementary Table S1. Characteristics of patients with acute myeloid leukemia harboring *NF1* mutations

Sample ID	*NF1* amino acid change[a,b]	Mutation type	VAF	Read depth	Co-mutated genes (VAF)	Karyotype
1	L94P T2204I S404Sfs	Missense Missense Frameshift	0.39 0.45 0.36	57 475 33	*DNMT3A*-R882 (0.38), *EZH2* (0.74), *TET2* (0.42)	45,XX,-7[6]/46,XX,-7,+21[11]
2	N226T	Missense	0.36	100	*BRD4* (0.52), *FLT3*-TKD (0.38), *NPM1* (0.31)	46,XX[20]
3	R304*	Nonsense	0.27	624	*KRAS* (0.15)	46,XY,inv(16)(p13.1q22)[1]/47,idem,+8[19]
4	H389R	Missense	0.53	197	*ASXL1* (0.40), *NRAS* (0.25), *SRSF2* (0.46), *TET2* (0.43)	47,XX,+8[9]/46,XX[11]
5	S465R D919E T676Tfs I2548M A1224V	Missense Missense Frameshift Missense Missense	0.22 0.10 0.18 0.20 0.60	251 739 123 227 88	*KIT* (0.18), *NPM1* (.43), *PTPN11* (0.25), *SMARCA2* (0.20)	46,XY[19][c]

6	Y489C	Missense	0.31	207	*NPM1* (.21), *NRAS* (0.21), *TYK2* (0.43)	46,XX[20]
7	K498Kfs	Frameshift	0.24	286	*TET2* (0.39), *TP53* (0.55)	44,XX,-5,trp(8)(q24.3q13),del(16)(q22),add(17)(p11.2), -21[12]/44,idem,add(11)(q23)[9]/46,XX[1]
8	P504Pfs	Frameshift	0.41	310	*RUNX1* (0.35), *SETBP1* (0.42), *SF3B1* (0.88)	46,XY[20]
9	T676Tfs	Frameshift	0.42	272	*RAF1* (0.47), *TP53* (0.80)	46,XY,add(1)(p11.2),-5,der(7)t(1;7)(p11.2;q22),i(8)(q10), add(17)(p11.2),-21,+mar1,+mar2[3]/46,idem,add(18)(q21), -mar2,+mar3[10]/46,idem,+8,-i(8)(q10),i(11)(q10)[2]/ 46,XY[4][c]
10	T676Tfs	Frameshift	0.41	345	*FLT3*-ITD, *NPM1* (0.43), *WT1* (0.42)	46,XY[20]
11	T676Tfs	Frameshift	0.78	409	*ASXL1* (0.31), *EZH2* (0.94), *STAG2* (0.75), *TET2* (0.49)	47,XY,+8[5]/46,XY,del(16)(q13q24)[14]/46,XY[2]
12	T676Tfs	Frameshift	0.14	516	*NPM1* (0.44), *SMC1A* (0.17), *XPO1* (0.15)	46,XX[20]

13	T676Tfs	Frameshift	0.42	676	*FLT3*-TKD (0.17), *NPM1* (0.29), *RAD21* (0.35), *WT1* (0.17)	46,XX[20]
14	T676Tfs	Frameshift	0.25	293	*PRKCB* (0.18), *TP53* (0.68)	47,X,-Y,dic(5;17)(q11;p13),+11,add(15)(p13),-16,+21, +add(22)(p11.2),+r[20]
15	T676Tfs	Frameshift	0.5	135	*JAK3* (0.49)	48,XY,del(5)(q31),inv(7)(q22q36),der(11)add(11)(p13)add(11)(q13),-17,der(18)del(18)(p11.2)add(18)(q21),+mar1,+mar2, +mar3[19]/46,XY[1]
16	T676Tfs	Frameshift	0.73	458	*TP53* (0.81)	45,XY,del(5)(q15q34),-7,+11,der(16)t(16;17)(q13;q11.2),-17, idic(22)(p11.2)[20]
17	T676Tfs	Frameshift	0.72	410	*BCOR* (0.85), *BCORL1* (0.76), *DNMT3A*-R882 (0.41), *IDH2* (0.44)	46,XY[20]
18	T676Tfs	Frameshift	0.38	325	*DNMT3A*-non-R882 (0.38), *IKZF1* (0.51), *SF1* (0.41), *TP53* (0.51)	43,XX,-3,add(5)(q11.2),der dic(7;11)(11pter→11q11: :7p11.1→7q21::7q31→7q31::?::11q14→11qter),-17[13]/ 86,idemx2[8]
19	T676Tfs	Frameshift	0.80	222	*HNRNPK* (0.50), *IL7R* (0.45), *ZRSR2* (0.36)	46,XX,del(9)(q22q32),del(12)(p12p13),add(14)(q11.2), dup(17)(q21q25)[12]/46,XX[10][c]

20	T676Tfs	Frameshift	0.41	282	*IKZF1* (0.41), *PTPN11* (0.38), *SF3B1* (0.34)	46,XY,inv(3)(q21q26.2)[5]/46,idem,del(7)(p13p15)[13][c]
21	T676Tfs	Frameshift	0.15	374	*PTPN11* (0.47)	48,XY,+X,+11[19]/46,XY[1]
22	T676Tfs	Frameshift	0.11	1152	*DNMT3A*-R882 (0.42), *FLT3*-ITD	46,XX[30]
23	C680Cfs	Frameshift	0.21	467	*BCORL1* (0.49)	46,XX,der(16)inv(16)(p13.1q22)del(16)(q22)[16]/ 47,idem,+22[10]/46,XX[4]
24	F710FL	In-frame Indel[D]	0.14	534	*PIK3CD* (0.64), *TP53* (0.51)	46,XX,inv(16)(p13q22)[19]/46,XX[1]
25	T862S	Missense	0.44	340	*EZH2* (0.88), *FLT3*-ITD, *GATA1* (0.49), *PTPN11* (0.15), *RUNX1* (0.49)	46,XX,del(7)(q22q34)[12]/46,XX,del(7)(q32q34)[8]
26	M968K	Missense	0.78	37	*DNMT3A*-non-R882 (0.45), *HIST1H1E* (0.77), *TP53* (0.96)	46,XX,del(12)(p12p13)[2]/65,XX,+3,+3,+8,+8,+9,+11,+12, del(12)(p12p13),+13,+14,+15,+18,+18,+19,+20,+22,+22, +3mar[3]/65,XX,+3,+3,+8,+8,+9,i(9)(p10),+11,+12, del(12)(p12p13),+13,+14,+15,+18,+18,+19,+20,+22,+22, +3mar[4]/46,XX[2][c]

27	L1102F	Missense	0.32	242	*DNMT3A*-non-R882 (0.25), FLT3-*ITD*, *IDH2* (0.21), *WT1* (0.16)	47,XX,+8[20]/46,XX[1]
28	L1102F	Missense	0.19	169	*ASXL1* (0.11), *DNMT3A*-non-R882 (0.26), *FLT3*-ITD, *IDH2* (0.28), *WT1* (0.11)	46,XX,?del(16)(q12.1)[18]/46,XX[3][c]
29	L1208W	Missense	0.51	867	*FLT3*-TKD (0.33), *NPM1* (0.51), *WT1* (0.75)	46,XX[30]
30	Y489C G1219R	Missense Missense	0.72 0.34	393 542	*ASXL1* (0.31), *IKZF1* (0.46)	46,XX,t(2;3)(p21;q27)[28]
31	R1306*	Nonsense	0.82	169	*ASXL1* (0.32), *PHF6* (0.89), *RUNX1* (0.40), *WT1* (0.43)	46,XY[20]
32	R1362*	Nonsense	0.26	139	*IDH2* (0.29), *NPM1* (0.34)	46,XY[20]
33	H1374Y	Missense	0.47	486	*CEBPA*	46,XY[18][c]
34	P1400Q	Missense	0.42	627	*ASXL1* (0.43), *RAD21* (0.43), *TET2* (0.32), *ZRSR2* (1.00)	45,X,-Y,t(8;21)(q22;q22)[20]

35	A1403fs	Frameshift	0.16	429	*NPM1* (0.40), *SMC1A* (0.35), *TET2* (0.53)	47,XY,+8[2]/46,XY[37]
36	L1486S	Missense	0.39	23	*DNMT3A*-R882 (0.37), *HNRNPK* (0.46), *IDH2* (.45) *KRAS* (0.31), *NPM1* (.41) *SMARCA2* (0.52)	47,XY,+21[43]/46,XY[7]
37	S1567*	Nonsense	0.40	364	*FLT3*-ITD, *IKZF1* (0.30), *SF3A1* (0.44), *SF3B1* (0.47)	46,XY,i(6)(p10)[13]/46,XY[5]
38	S1817C	Missense	0.55	750	*GATA2* (0.54), *NRAS* (0.37)	46,XX,add(5)(q11.2)[3]
39	R1870Q	Missense	0.40	645	*NPM1* (0.37), *NRAS* (0.14), *TET2* (0.49)	46,XY,del(7)(q22q34),dup(12)(q15q24.1)[19]/46,XY[1]
40	A1966	Missense	0.34	35	*BRD4* (0.27), *TP53* (0.29)	45,XX,add(5)(q11.2),+8,qdp(11)(q21q25),del(12)(p12p12), del(16)(p11.2p13.3),add(17)(p13),-18,-20[19]/46,XX[3]
41	Y2264*fs	Nonsense	0.89	347	*BCOR* (0.81), *CBL* (0.81), *GATA2* (0.41), *SF3B1* (0.44)	46,XY,t(3;3)(q21;q26)[1]/45,idem,-7[18][c]

42	T2454*	Missense	0.91	144	*NPM1* (0.49)	46,XY[20]
43	T2454*	Nonsense	0.79	194	*NPM1* (0.53)	46,XY[20]
44	W2473*	Nonsense	0.83	94	*SAMHD1* (0.47), *SF3B1* (0.44)	45,XY,inv(3)(q21q26),-7[13]/46,XY[4]
45	R2496*	Nonsense	0.34	200	*FLT3*-ITD, *NPM1* (0.13), *SAMHD1* (0.20), *SF3B1* (0.19), *SMARCA2* (0.31), *TP53* (0.45)	47-49,XY,t(1;11)(q25;q21),+der(1)t(1;11)(q25;q21), del(5)(q31q35),-17,-18,+19,+20,del(20)(q11.2),+2mar[cp6][c]
46	T2513A	Missense	0.54	506	*DNMT3A*-R882 (0.50), *FLT3*-ITD, *NPM1* (0.51)	47,XY,+8[2]/46,XY[18]
47	W2712R	Missense	0.13	32	*FLT3*-TKD (0.40), *IDH2* (0.47), *NPM1* (0.45), *SF3B1* (0.35), *SYK* (0.46)	46,XY[31]

48	W2712R	Missense	0.10	39	*AXL* (0.49), *BCOR* (0.52), *CBL* (0.42), *IDH1* (0.45), *NPM1* (0.46), *SMC3* (0.24)	46,XX[30]
49	W2712R	Missense	0.12	34	*FLT3*-ITD, *NPM1* (0.40), *RAD21* (0.46)	46,XY[20]
50	R2713W	Missense	0.10	48	*NRAS* (0.43)	46,XY,t(6;9)(p23;q34)[20]
51	R2713W	Missense	0.15	34	*ASXL1* (0.53) *DNMT3A*-non-R882 (0.47) *ETV6* (0.52) *IDH2* (0.44) *SMC3* (0.48)	46,XX[20]
52	R2713W	Missense	0.17	35	*KIT* (0.42)	46,XY,inv(16)(p13q22)[17]/47,idem,+Y[3]

Abbreviation: VAF, variant allele fraction.

[a] * denotes nonsense mutation; fs, frame shift mutation.

[b] Patients #1,5 and 30 harbored three, five and two different *NF1* mutations, respectively.

[c] A non-clonal cell or cells were also detected.

[d] In-frame insertion/deletion, grouped together with missense mutations.

Supplementary Table S2. Variant allele fractions of *NF1* mutations compared with the largest variant allele fractions of co-occurring mutations in other genes in patients with no evidence of copy number variations (CNVs) in the *NF1* gene

Sample ID	*NF1* mutation VAF	The largest VAF of the co-occurring mutation	Difference between VAF of *NF1* mutation and VAF of co-occurring mutation
1	**0.45**	**0.42**	**0.03**
2	0.36	0.52	-0.16
4[b]	**0.53**	**0.46**	**0.07**
5[a]	**0.60**	**0.43**	**0.17**
6	0.31	0.43	-0.12
7	0.24	0.55	-0.21
8	**0.41**	**0.42**	**-0.01**
9	**0.42**	**0.47**	**-0.05**
10	**0.41**	**0.43**	**-0.02**
12	0.14	0.44	-0.30
13	**0.42**	**0.35**	**0.07**
14	**0.25**	**0.18**	**0.07**
15	**0.50**	**0.49**	**0.01**
18	0.38	0.51	-0.13
21	0.15	0.47	-0.32
22	0.11	0.42	-0.31
25	**0.44**	**0.49**	**-0.05**
27	**0.32**	**0.25**	**0.07**
28	0.19	0.26	-0.07
29	**0.51**	**0.51**	**0**
32	0.26	0.34	-0.08
35	0.16	0.53	-0.37
36	0.39	0.52	-0.13
37	0.40	0.47	-0.07
38	**0.55**	**0.54**	**0.01**
39	0.40	0.49	-0.09
40	**0.34**	**0.29**	**0.05**
45	0.34	0.45	-0.11
46	**0.54**	**0.51**	**0.03**
47	0.13	0.47	-0.34
48	0.10	0.52	-0.42
49	0.12	0.46	-0.34
51	0.15	0.53	-0.38

Abbreviation: VAF, variant allele fraction.
Bold type and gray shading indicates patients in whom the *NF1* mutation was presumably found in the main clone. Patients with loss-of-heterozygosity encompassing *NF1* locus, *NF1*-mutated samples with VAF >0.60, or co-mutated genes with VAFs > 0.60 have not been considered.
[a] indicates sample with multiple *NF1* mutations, for which the largest *NF1* mutation VAF is listed.
[b] indicates hemizygous mutation.

Supplementary Table S3. Comparison of pretreatment clinical characteristics of acute myeloid leukemia patients with and without *NF1* mutations analyzed by targeted next-generation sequencing

Characteristic	***NF1* mutated (*n*=52)**	***NF1* wild-type (*n*=969)**	***P*[a]**
Age, yr			0.49
Median	50	52	
Range	19-82	17-84	
Sex, *n* (%) of females	22 (42)	425 (44)	0.89
Race, *n* (%)			0.50
White	44 (92)	833 (88)	
Nonwhite	4 (8)	119 (13)	
Hemoglobin, g/dL			
Median	9.0	9.2	0.17
Range	3.0-13.0	2.3-25.1	
Platelet count, x 10^9/L			
Median	51	55	0.38
Range	7-317	4-850	
White blood cell count, x 10^9/L			
Median	19.4	26.6	0.18
Range	1.3-156	0.4-475	
Bone marrow blasts, %			<0.001
Median	52	67	
Range	10-86	0-99	
Blood blasts, %			0.18
Median	45	54	
Range	0-95	0-99	
Extramedullary involvement, *n* (%)	11 (22)	235 (26)	0.74
ELN classification, *n* (%)			0.02
Favorable	22 (42)	425 (44)	
Intermediate	8 (15)	223 (23)	
Adverse	22 (42)	251 (26)	
Unclassifiable	0 (0)	70 (7)	

Characteristic	***NF1* mutated (*n*=52)**	***NF1* wild-type (*n*=969)**	***P*[a]**
Cytogenetic groups, *n* (%)			0.002
Normal karyotype	19 (37)	526 (54)	
inv(16)/t(16;16)	4 (8)	73 (8)	
t(8;21)	1 (2)	40 (4)	
inv(3)/t(3;3)	3 (6)	9 (1)	
t(6;9)	1 (2)	5 (1)	
Sole +8	5 (10)[b]	38 (4)[c]	
Complex karyotype	10 (19)	84 (9)	
Other abnormalities	9 (17)	194 (20)	

Abbreviations: ELN, European LeukemiaNet; *n*, number.

[a] *P*-values for categorical variables are from Fisher's exact test, *P*-values for continuous variables are from the Wilcoxon rank sum test.

[b] One patient (no. 11 in Supplemental Table S1) had a clone with sole del(16)(q13q24) in addition to a clone with sole +8.

[c] Five patients had another abnormal clone(s) in addition to a clone with sole +8.

Supplementary Table S4. Frequencies of functional groups comprising mutations detected in *NF1* mutated versus *NF1* wild-type patients

Functional group[a]	***NF1* mutated (*n*=52)**	***NF1* wild-type (*n*=969)**	***P***[b]
Chromatin remodeling, *n* (%)			0.06
Mutated	14 (27)	158 (16)	
Wild type	38 (73)	811 (84)	
Cohesin complex, *n* (%)			0.38
Mutated	8 (15)	113 (12)	
Wild type	44 (85)	856 (88)	
Kinases, *n* (%)			0.77
Mutated	17 (33)	341 (37)	
Wild type	35 (67)	588 (63)	
Methylation-related, *n* (%)			0.32
Mutated	20 (38)	449 (46)	
Wild type	32 (62)	520 (54)	
NPM1, *n* (%)			0.77
Mutated	18 (35)	362 (38)	
Wild type	34 (65)	603 (62)	
RAS pathway, *n* (%)			1.00
Mutated	13 (25)	237 (24)	
Wild type	39 (75)	732 (76)	
Spliceosome, *n* (%)			0.86
Mutated	10 (19)	180 (19)	
Wild type	42 (81)	784 (81)	
Transcription factors, *n* (%)			1.00
Mutated	10 (20)	181 (20)	
Wild type	40 (80)	703 (80)	
Tumor suppressors, *n* (%)			0.02
Mutated	15 (29)	152 (16)	
Wild type	37 (71)	817 (84)	

Abbreviations: *n*, number.

[a] A given functional group is considered mutated if at least one gene belonging to this group is mutated. Functional groups comprise specific genes as follows: chromatin remodeling: *ASXL1*, *BCOR*, *BCORL1*, *EZH2* and *SMARCA2*; cohesin: *RAD21*, *SMC1A*, *SMC3 and STAG2; kinases: AXL, FLT3-ITD, FLT3-TKD and KIT, TYK2; methylation-related: DNMT3A, IDH1, IDH2 and TET2; NPM1: NPM1; RAS pathway: CBL, KRAS, NRAS and PTPN11; spliceosome: SF3B1, SRSF2, U2AF1 and ZRSR2; transcription factors: CEBPA, ETV6, GATA2, IKZF1, NOTCH1 and RUNX1; and tumor suppressor: PHF6, TP53 and WT1*.

[b] *P*-values are from Fisher's exact test.

Supplementary Table S5. Frequencies of co-existing single gene mutations detected in patients with *NF1* mutations compared to *NF1* wild-type patients

Gene[a]	*NF1* mutated (*n*=52)	*NF1* wild-type (*n*=969)	*P*[b]
ASXL1, *n* (%)			0.39
Mutated	5 (10)	63 (7)	
Wild type	47 (90)	906 (93)	
BCOR, *n* (%)			0.74
Mutated	3 (6)	47 (5)	
Wild type	49 (94)	922 (95)	
BCORL1, *n* (%)			0.33
Mutated	2 (4)	21 (2)	
Wild type	50 (96)	948 (98)	
BRD4, *n* (%)			0.16
Mutated	2 (4)	12 (1)	
Wild type	50 (96)	957 (99)	
CBL, *n* (%)			0.29
Mutated	2 (4)	19 (2)	
Wild-type	50 (96)	950 (98)	
DNMT3A, *n* (%)			0.51
Mutated	10 (19)	236 (24)	
R882	5	165	
Non-R882	5	72	
Wild type	42 (81)	733 (76)	
EZH2, *n* (%)			0.18
Mutated	3 (6)	26 (3)	
Wild type	49 (94)	943 (97)	
FLT3-ITD, *n* (%)			0.40
Mutated	9 (17)	221 (23)	
Wild type	43 (83)	738 (77)	
FLT3-TKD, *n* (%)			0.79
Mutated	4 (8)	70 (7)	
Wild type	48 (92)	890 (93)	
GATA2, *n* (%)			1.00
Mutated	2 (4)	44 (5)	
Wild type	50 (96)	925 (95)	
HNRNPK, *n* (%)			0.05
Mutated	2 (4)	5 (1)	
Wild type	50 (96)	964 (99)	
IDH1, *n* (%)			0.62
Mutated	3 (6)	87 (9)	
Wild type	49 (94)	882 (91)	
IDH2, *n* (%)			0.82
Mutated	6 (12)	107 (11)	
Wild type	46 (88)	862 (89)	

Gene[a]	*NF1* mutated (*n*=52)	*NF1* wild-type (*n*=969)	*P*[b]
IKZF1, *n* (%)			0.005
Mutated	4 (8)	11 (1)	
Wild type	48 (92)	958 (99)	
KIT, *n* (%)			1.00
Mutated	2 (4)	43 (4)	
Wild type	50 (96)	926 (96)	
KRAS, *n* (%)			1.00
Mutated	2 (4)	36 (4)	
Wild type	50 (96)	932 (96)	
NPM1, *n* (%)			0.77
Mutated	18 (35)	362 (38)	
Wild type	34 (65)	603 (62)	
NRAS, *n* (%)			0.53
Mutated	5 (10)	130 (13)	
Wild type	47 (90)	839 (87)	
PTPN11, *n* (%)			0.55
Mutated	4 (8)	58 (6)	
Wild type	48 (92)	911 (94)	
RAD21, *n* (%)			0.10
Mutated	3 (6)	19 (2)	
Wild type	49 (94)	950 (98)	
RUNX1, *n* (%)			0.47
Mutated	3 (6)	100 (10)	
Wild type	49 (94)	869 (90)	
SAMHD1, *n* (%)			0.09
Mutated	2 (4)	8 (1)	
Wild type	50 (96)	961 (99)	
SF3B1, *n* (%)			0.002
Mutated	7 (13)	29 (3)	
Wild type	45 (87)	940 (97)	
SMARCA2, *n* (%)			0.14
Mutated	3 (6)	23 (2)	
Wild type	49 (94)	946 (98)	
SMC1A, *n* (%)			0.68
Mutated	2 (4)	30 (3)	
Wild type	50 (96)	939 (97)	
SMC3, *n* (%)			0.68
Mutated	2 (4)	30 (3)	
Wild type	50 (96)	939 (97)	
TET2, *n* (%)			1.00
Mutated	7 (13)	137 (14)	
Wild type	45 (87)	832 (86)	
TP53, *n* (%)			0.007
Mutated	9 (17)	62 (6)	
Wild type	43 (83)	907 (94)	

Gene[a]	***NF1* mutated (*n*=52)**	***NF1* wild-type (*n*=969)**	***P***[b]
WT1, *n* (%)			0.26
Mutated	6 (12)	66 (7)	
Wild type	46 (88)	903 (93)	
ZRSR2, *n* (%)			1.00
Mutated	2 (4)	48 (5)	
Wild type	50 (96)	921 (95)	

Abbreviation: *n*, number.

[a] Listed are only those genes that were found mutated in at least two *NF1*-mutated patients.

[b] *P*-values relate to the comparison of *NF1*-mutated versus *NF1* wild-type patients. *P*-values are from Fisher's exact test.

Supplementary Table S6. Comparison of the pre-treatment clinical characteristics of patients with *NF1* Thr676, other *NF1* frameshift and nonsense mutations, and patients harboring *NF1* missense mutations

Characteristic	***NF1* Thr676 mutations[a] (*n*=14)**	***NF1* nonsense and frameshift mutations other than Thr676[a] (*n*=12)**	***NF1* missense Mutations[a] (*n*=26)**	***P*[b]**
Age, yr				0.78
Median	51	49	50	
Range	19-70	23-73	22-82	
Sex, *n* (%) of females	5 (36)	3 (25)	14 (54)	0.23
Race, *n* (%)				0.65
White	11 (85)	0 (0)	23 (92)	
Nonwhite	2 (15)	10 (100)	2 (8)	
Hemoglobin, g/dL				0.05
Median	8.1	9.1	9.2	
Range	6.4-10.5	3.0-13.0	4.9-12.1	
Platelet count, x 10^9/L				0.95
Median	54	51	50	
Range	14-191	7-317	15-277	
White blood cell count, x 10^9/L				0.02
Median	7.9	14.8	27.2	
Range	1.8-99.9	3.3-115.2	1.3-155.9	
Blood blasts, %				0.72
Median	36	53	45	
Range	14-91	12-82	0-95	
Bone marrow blasts, %				0.33
Median	41	51	62	
Range	17-82	12-80	10-86	
Extramedullary involvement, *n* (%)	0 (0)	1 (9)	10 (40)[c]	0.007

Characteristic	***NF1* Thr676 mutations[a] (*n*=14)**	***NF1* nonsense and frameshift mutations other than Thr676[a] (*n*=12)**	***NF1* missense Mutations[a] (*n*=26)**	***P*[b]**
ELN classification, *n* (%)				0.07
Favorable	2 (14)	5(42)	15 (58)	
Intermediate	3 (21)	1 (8)	4 (15)	
Adverse	9 (64)	6 (50)	7 (27)	

Abbreviation: *n*, number.

[a] Patients harboring multiple *NF1* mutations were classified in the following way: Patient #1 (harboring 2 missense mutations and 1 frameshift mutation) was classified as frameshift mutation, patient #5 (harboring 4 missense mutations and 1 Thr676 mutation) was classified as Thr676 mutation, and patient #30 (harboring 2 missense mutations) was classified as missense mutation). Patient #24 (harboring an in-frame insertion/deletion) was classified as missense mutation.

[b] P-values for categorical variables are from Fisher's exact test, P-values for continuous variables are from Wilcoxon rank sum test.

[c] Five patients had lymphadenopathy (including one patient with skin infiltrates and another with hepatosplenomegaly), four patients had splenomegaly (including the aforementioned patient with hepatomegaly and lymphadenopathy, and another with hepatomegaly), and one patient had gum hypertrophy.

Supplementary Table S7. Comparison of the frequencies of gene mutations in functional groups among patients with *NF1* Thr676, other *NF1* frameshift and nonsense mutations, and patients harboring *NF1* missense mutations

Functional group[a]	***NF1* Thr676 mutations**[B] **(*n*=14)**	***NF1* nonsense and frameshift mutations other than Thr676**[b] **(*n*=12)**	***NF1* missense Mutations**[b] **(*n*=26)**	***P***[c]
Chromatin remodeling, *n* (%)				0.11
Mutated	2 (14)	6 (50)	6 (23)	
Wild type	12 (86)	6 (50)	20 (77)	
Cohesin complex, *n* (%)				0.28
Mutated	3 (21)	0 (0)	5 (19)	
Wild type	11 (79)	12 (100)	21 (81)	
Kinases, *n* (%)				0.40
Mutated	3 (21)	3 (25)	11 (42)	
Wild type	11 (79)	9 (75)	15 (58)	
Methylation-related, *n* (%)				0.53
Mutated	4 (29)	4 (33)	12 (46)	
Wild type	10 (71)	8 (67)	14 (54)	
NPM1, *n* (%)				0.26
Mutated	3 (21)	3 (25)	12 (46)	
Wild type	11 (79)	9 (75)	14 (54)	
RAS Pathway, *n* (%)				0.59
Mutated	2 (14)	3 (25)	8 (31)	
Wild type	12 (86)	9 (75)	18 (69)	
Spliceosome, *n* (%)				0.12
Mutated	2 (14)	5 (42)	3 (12)	
Wild type	12 (86)	7 (58)	23 (88)	
Transcription factors, *n* (%)				0.38
Mutated	2 (14)	4 (36)	4 (16)	
Wild type	12 (86)	7 (64)	21 (84)	

Functional group[a]	***NF1* Thr676 mutations**[B] **(*n*=14)**	***NF1* nonsense and frameshift mutations other than Thr676**[b] **(*n*=12)**	***NF1* missense Mutations**[b] **(*n*=26)**	***P***[c]
Tumor suppressors, *n* (%)				0.44
Mutated	6 (43)	3 (25)	6 (23)	
Wild type	8 (57)	9 (75)	20 (77)	

Abbreviation: *n*, number.

[a] A given functional group is considered mutated if at least one gene belonging to this group is mutated. Functional groups comprise specific genes as follows: chromatin remodeling: *ASXL1*, *BCOR*, *BCORL1*, *EZH2* and *SMARCA2*; cohesin: *RAD21*, *SMC1A*, *SMC3 and STAG2; kinases: AXL, FLT3-ITD, FLT3-TKD and KIT, TYK2; methylation-related: DNMT3A, IDH1, IDH2 and TET2; NPM1: NPM1; RAS pathway: CBL, KRAS, NRAS and PTPN11; spliceosome: SF3B1, SRSF2, U2AF1 and ZRSR2; transcription factors: CEBPA, ETV6, GATA2, IKZF1, NOTCH1 and RUNX1; and tumor suppressor: PHF6, TP53 and WT1*.

[c] *P*-values are from Fisher's exact test.

Supplementary Table S8. Outcomes of younger AML patients with *NF1* mutations according to variant allele fractions.

Outcome	***NF1* mutated VAF≥0.50 n=13**	***NF1* mutated VAF <0.50 n=21**	***P*[a]**
Complete remission, *n* (%)	6 (46)	17 (81)	0.06
Relapse rate, *n* (%)	5 (100)[b]	6 (35)	0.04
Disease-free survival			0.09
Median, years	0.6	1.9	
% Disease-free at 1 year (95% CI)	40 (5-75)	65 (38-82)	
% Disease-free at 3 years (95% CI)	0	47 (23-68)	
Overall survival			0.03
Median, years	0.8	2.4	
% Alive at 1 year (95% CI)	46 (19-70)	71 (47-86)	
% Alive at 3 years (95% CI)	23 (6-47)	48 (26-67)	

Abbreviation: VAF, variant allele fraction.

[a] *P*-values for categorical variables are from Fisher's exact test, *P*-values for the time to event variables are from the log-rank test. The median follow-up for those alive is 9.0 years, range: 5.1-16.4 years (n=10). The median follow-up for those who have not had an event is 9.7 years, range: 5.1-16.4 years (n=7).

[b] Follow-up data not available for one patient who achieved a CR.

Supplementary Table S9. Outcomes of younger (aged <60 years) AML patients with and without *NF1* Thr676 mutations.

Endpoint	***NF1* Thr676 (n=10)[a]**	***NF1* wild-type (n=586)[a]**	***P*[b]**
Complete remission, *n* (%)	5 (50)	463 (79)	.04
Disease-free survival[c]			--
Median, years	1.4	1.4	
% Disease-free at 1 year (95% CI)	60 (13-88)	57 (52-61)	
% Disease-free at 3 years (95% CI)	0	38 (34-42)	
Overall survival[c]			.01
Median, years	0.8	2.2	
% Alive at 1 year (95% CI)	50 (18-75)	71 (67-75)	
% Alive at 3 years (95% CI)	20 (3-47)	45 (41-49)	

Abbreviations: CI, confidence interval; *n*, number.
[a] Among patients who achieved a CR, only those who received at least one cycle of post-remission chemotherapy according to protocol were included in the outcome analysis.
[b] *P*-values for complete remission are from Fisher's exact test, *P*-values for disease-free and overall survival are from the log-rank test.
[c] Patients who received allogeneic hematopoietic stem cell transplantation in first CR were excluded from disease-free survival and overall survival analyses.