SUPPLEMENTARY APPENDIX

Clinical relevance of *IDH1/2* mutant allele burden during follow-up in acute myeloid leukemia. A study by the French ALFA group

Yann Ferret,[1,2,3] Nicolas Boissel,[4,5] Nathalie Helevaut,[1] Jordan Madic,[6] Olivier Nibourel,[1,2,3] Alice Marceau-Renaut,[1,2,3] Maxime Bucci,[1] Sandrine Geffroy,[1] Karine Celli-Lebras,[7] Sylvie Castaigne,[8] Xavier Thomas,[9] Christine Terré,[10] Hervé Dombret,[4,5] Claude Preudhomme[1,2,3] and Aline Renneville[1,2,3]

[1]Hematology Laboratory, Biology and Pathology Center, CHRU of Lille; [2]INSERM, UMR-S 1172, Cancer Research Institute of Lille; [3]University of Lille, F-59000; [4]Hematology Department, Saint-Louis Hospital, AP-HP, Paris; [5]EA3518, Institut Universitaire d'Hématologie (IUH), University 7 Paris Diderot; [6]Circulating Biomarkers Laboratory, Curie Institute, Paris; [7]Acute Leukemia French Association (ALFA) coordination, Saint-Louis Hospital, AP-HP, Paris; [8]Hematology Department, Versailles Hospital, Le Chesnay; [9]Hematology Department, Lyon Sud Hospital, Pierre Benite and [10]Cytogenetic Laboratory, Versailles Hospital, Le Chesnay, France

 doi:10.3324/haematol.2017.183525

Received: October 29, 2017.

Accepted: February 16, 2018.

Pre-published: February 22, 2018.

Correspondence: aline.renneville@gmail.com

Supplemental data

Supplemental data from: Y. Ferret, *et al*. Clinical relevance of *IDH1/2* mutant allele fraction during follow-up in acute myeloid leukemia. A Study by the French ALFA group.

Supplemental data contents:

Supplementary Methods

Cytogenetic analysis

Cytogenetic G-banding analysis was performed according to standard methods. The definition of a cytogenetic clone and descriptions of karyotypes followed the International System for Human Cytogenetic Nomenclature.[1] To establish normal karyotype AML, at least 20 metaphase cells from diagnostic BM had to be evaluated and the karyotype had to be found normal in each metaphase.

Gene mutation analysis and DNA sequencing

FLT3 internal tandem duplication (*FLT3*-ITD),[2] mutations of FLT3 tyrosine kinase domain (*FLT3-TKD*) (*FLT3* D835/I836),[2] *NPM1* (exon 11),[3] *CEBPA,*[4] *IDH1* (exon 4) and *IDH2* (exon 4),[2] were assessed centrally as previously described on genomic DNA extracted from diagnostic BM or PB samples.

Targeted next-generation sequencing (NGS) assays using different gene panels were also performed at AML diagnosis in 100/103 patients to assess the mutational status of *DNMT3A* (exons 2-23), *TET2* (exons 3-11), and for some patients, *IDH1/2* and *NPM1* mutations. Further details regarding these NGS assays are provided below.

Next-generation sequencing analysis

In 100 out of 103 patients included in this study, native genomic DNA was available at AML diagnosis for NGS analysis. For patients included in the ALFA-0702 trial, DNA samples were analyzed with either the Ampliseq 6-gene panel (n=14) or Ampliseq 26-gene panel (n=62) and the Ion PGM sequencing platform. For patients included in the ALFA-0701 trial, DNA samples were analyzed with an Haloplex 42-gene panel and an Illumina sequencing platform (n=24). Mutations of genes shared by the 3 NGS panels and mutated in ≥ 4 patients in our cohort (i.e. *DNMT3A* and *TET2*) were considered for statistical analysis, in addition to *IDH1/2*, *NPM1* and *FLT3*.

1) Library preparation and sequencing

- *Ampliseq 6-gene panel*

Mutations in a selected panel of 6 genes (*ASXL1, EZH2, DNMT3A, RUNX1, TET2, TP53*) (Supplementary Table 1) were screened by a NGS assay using the Ion AmpliSeq Library Kit 2 384 (384 reactions; Thermo FisherScientific, Carlsbad, CA). Multiplex PCR amplifications (196 primer pairs in 3 pools) were performed from 3 x 10 ng of native genomic DNA.

Amplified DNA samples were treated with FuPa reagent to partially digest the primer sequences. Barcodes and adaptors were then added to amplicons by ligation. Products were subjected to 2 successive purifications on AMPure beads (BeckmanCoulter, Brea,CA). The Agilent 2100 Bioanalyzer and the Agilent High sensitivity DNA kit were used to quantify and normalize the libraries, before pooling. Emulsion PCR was performed using the OneTouch 2 instrument (Thermo Fisher Scientific). Enrichment was performed with the ES Ion OneTouch (Ion One Touch 200 Template kit V2) and checked with the Ion Sphere Quality Control Kit. Sequencing was performed with the Ion PGM system (Thermo Fisher Scientific) onto the 316 chip (15 samples per chip).

- *Ampliseq 26-gene panel*

Mutations in a selected panel of 26 genes (*ASXL1, CBL, DNMT3A, ETV6, EZH2, FLT3-TKD, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NRAS, NPM1, PHF6, PTPN11, RIT1, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1, and ZRSR2*) (Supplementary Table 2) were screened by a NGS assay using the Ion AmpliSeq Library Kit 2.0 (384 reactions; Thermo FisherScientific, Carlsbad, CA). Multiplex PCR amplifications (233 primer pairs in 2 pools) were performed from 2 x 10 ng of native genomic DNA. Amplified DNA samples were treated with FuPa reagent to partially digest the primer sequences. Barcodes and adaptors were then added to amplicons by ligation. Products were subjected to 2 successive purifications on AMPure beads (BeckmanCoulter, Brea,CA). The Agilent 2100 Bioanalyzer and the Agilent High sensitivity DNA kit were used to quantify and normalize the libraries, before pooling. Emulsion PCR was performed using the OneTouch 2 instrument (Thermo Fisher Scientific). Enrichment was performed with the ES Ion OneTouch (Ion One Touch 200 Template kit V2) and checked with the Ion Sphere Quality Control Kit. Sequencing was performed with the Ion PGM system (Thermo Fisher Scientific) onto the 318 V2 chip (15 samples per chip).
In addition to those Ampliseq assays, all the samples were also screened for *ASXL1* (including c.1934dupG; p.G646WfsX12) and *SRSF2* mutations by Sanger sequencing.

- *Haloplex 42-gene panel*

Mutations in a selected panel of 37 genes (*ASXL1, BCOR, BCORL1, CALR, CBL,CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, GATA2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NIPBL, NPM1, NRAS, PHF6, PTPN11, RAD21, RIT1, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1,* and *ZRSR2*) (Supplementary Table 3) were screened by a NGS assay using the Haloplex Target Enrichment System (96 reactions; Agilent technology, Santa Clara, CA). Digestion with 8 pairs of restriction enzymes was

performed using 8 x 25 ng of native genomic DNA. After digestion, probes and index were added to the targeted DNA fragments for hybridization (4463 biotinylated probes) and simultaneous circularization. The circularized DNA fragments-probe-index complexes were captured by streptavidin beads. The circular DNA fragments were then closed by ligation, incorporating the index. After elution, the circular DNA fragments were amplified with universal primers containing the sequencing adaptors, and were subjected to a selective purification on AMPure beads (BeckmanCoulter, Brea, CA). Sequencing was performed with the MiSeq (Illumina, San Diego, CA) with the Miseq reagent 300v2 kit (14 samples per flowcell).

2) Bioinformatic analysis

- *Ion PGM platform*

The hg19 version of the human genome was used as a reference sequence. Base calls were generated by the Torrent Browser software (5.0.4 version) using the included variant caller with an additional plug-in (Thermo Fisher Scientific). The .bam and.vcf files were used for further analysis. The .vcf files were annotated with the Ion reporter software (Thermo Fisher Scientific) and processed for a second analysis of the indexed files using the Sequence Pilot software (4.2.1 version) (JSI Medical Systems, Ettenheim, Germany). Results were compared with selection of variants that will be further considered. For each variant, depth at the variant position (number of mutated reads and unmutated reads) was considered to calculate variant allele frequency (VAF), which is the proportion of mutated reads among total reads.

- *Illumina platform*

The hg19 version of the human genome was used as a reference sequence. The .fastq files were generated by the Miseq reporter software (2.6 version, Illumina). Base calls and variant annotations were performed with different bioinformatics analysis tools (GATK, Broad Institute; Burrows-Wheeler; Picard-tool, Broad Institute; Trim-galore, Babraham Bioinformatics). A second analysis of the indexed files was processed using the Sequence Pilot software (4.2.1 version) (JSI Medical Systems, Ettenheim, Germany). Results were compared with selection of variants that will be further considered.

Supplementary Tables

Table S1. Limit of detection of the droplet digital PCR assays measured on genomic DNA extracted from *IDH1/2* wild-type pooled blood lymphocytes (PBL), according to the type of *IDH1/2* mutations.

Mutation	No. of wells tested	Mean VAF (%)	SD	LOD (%)
IDH2 p.R140Q	30	0.034	0.018	0.09
IDH2 p.R172K	20	0.019	0.008	0.04
IDH1 p.R132C	10	0.107	0.023	0.18
IDH1 p.R132H	10	0.055	0.012	0.09
IDH1 p.R132G	10	0.012	0.015	0.058

Abbreviations: VAF, variant allele fraction**,** SD, Standard deviation; LOD, limit of detection.

Table S2. List of sequenced regions in the Ampliseq 6-gene panel

Gene	Reference sequence	Exons analyzed
ASXL1	NM_015338	2-12
DNMT3A	NM_022552	2-23
EZH2	NM_004456	2-20
RUNX1	NM_001001890	1-6
TET2	NM_001127208	3-11
TP53	NM_001126112	2-11

Table S3. List of sequenced regions in the Ampliseq 26-gene panel

Gene	Reference sequence	Exons analyzed
ASXL1	NM_015338	11-12
CBL	NM_005188	8-9
DNMT3A	NM_022552	2-23
ETV6	NM_001987	1-8
EZH2	NM_004456	2-20
FLT3	NM_004119	14, 15, 20
IDH1	NM_005896	4
IDH2	NM_002168	4
JAK2	NM_004972	12, 14
KIT	NM_000222	8, 11, 17
KRAS	NM_033360	2-3
MPL	NM_005373	10
NPM1	NM_002520	11
NRAS	NM_002524	2-3
PHF6	NM_001015877	2-10
PTPN11	NM_002834	3
RIT1	NM_006912	5
RUNX1	NM_001001890	1-6
SETBP1	NM_015559.2	4
SF3B1	NM_012433	13-16
SRSF2	NM_003016	1
TET2	NM_001127208	3-11
TP53	NM_001126112	2-11
U2AF1	NM_006758	2-6
WT1	NM_024426	7, 9
ZRSR2	NM_005089	1-11

Table S4. List of sequenced regions in the Haloplex 42-gene panel

Gene	Reference sequence	Exons analyzed
ASXL1	NM_015338	11-12
BCOR	NM_001123385	2-15
BCORL1	NM_021946	1-12
BRAF6	NM_004333	15
CALR	NM_004343.3	9
CBL	NM_005188	8-9
CEBPA	NM_004364	1
CSF3R	NM_172313.2	14
DNMT3A	NM_022552	2-23
ETV6	NM_001987	1-8
EZH2	NM_004456	2-20
FBXW7	NM_033652	9-12
FLT3	NM_004119	14, 15, 20
GATA1	NM_002049	2
GATA2	NM_032638	2-6
IDH1	NM_005896	4
IDH2	NM_002168	4
JAK2	NM_004972	12, 14
JAK3	NM_000215	13
KIT	NM_000222	8-17
KRAS	NM_033360	2-3
MPL	NM_005373	10
NIPBL	NM_133433	2-47
NOTCH1	NM_017617	26-34
NPM1	NM_002520	11
NRAS	NM_002524	2-3
PHF6	NM_001015877	2-10
PTEN	NM_000314	5-7
PTPN11	NM_002834	3-13
RAD21	NM_006265	2-14
RUNX1	NM_001001890	1-6
SETBP1	NM_015559.2	4
SF3B1	NM_012433	13-16
SMC1A	NM_006306	1-25
SMC3	NM_005445	1-29
SRSF2	NM_003016	1
STAG2	NM_001042749	3-35
TET2	NM_001127208	3-11
TP53	NM_001126112	2-11
U2AF1	NM_006758	2-6
WT1	NM_024426	7, 9
ZRSR2	NM_005089	1-11

Table S5. Patients and gene mutations considered for this study.

UPN	Gene	Reference transcript	Exon	Type of mutation	Nucleotide variation	Protein variation	VAF (%)
L1206290	*CEBPA*	NM_004364	1	Frameshift	c.109_123delinsA	p.A37TfsX66	NA
60074	*CEBPA*	NM_004364	1	Frameshift	c.199dupT	p.Y67LfsX41	NA
L1203196	*CEBPA*	NM_004364	1	Frameshift	c.333_334dup	p.P112RfsX49	NA
36979	*CEBPA*	NM_004364	1	Frameshift	c.342delC	p.G116AfsX44	NA
L1206290	*CEBPA*	NM_004364	1	In-frame duplication	c.937_939dup	p.K313dup	NA
53458	*DNMT3A*	NM_022552	8	Missense	c.892G>C	p.G298R	43
L1306600	*DNMT3A*	NM_022552	8	Nonsense	c.958C>T	p.R320X	25
38932	*DNMT3A*	NM_022552	12	Splice site (intron 11)	c.1430-1G>A		40
35066	*DNMT3A*	NM_022552	14	Missense	c.1627G>T	p.G543C	28
60074	*DNMT3A*	NM_022552	14	Missense	c.1644G>A	p.M548I	43
L1201703	*DNMT3A*	NM_022552	15	Nonsense	c.1792C>T	p.R598X	50
44474	*DNMT3A*	NM_022552	15	Nonsense	c.1816C>T	p.Q606X	37
L1201703	*DNMT3A*	NM_022552	16	Missense	c.1904G>C	p.R635P	17
53458	*DNMT3A*	NM_022552	16	Missense	c.1915C>T	p.L639F	37
50609	*DNMT3A*	NM_022552	18	Missense	c.G2159G>A	p.R720H	49
50609	*DNMT3A*	NM_022552	19	Missense	c.2303A>T	p.D768V	48
L1201624	*DNMT3A*	NM_022552	20	Missense	c.2374C>G	p.R792G	22
L1208428	*DNMT3A*	NM_022552	21	Missense	c.2469G>C	p.R823S	48
48483	*DNMT3A*	NM_022552	22	Frameshift	c.2552delT	p.F851SfsX2	43
L1206288	*DNMT3A*	NM_022552	23	Missense	c.2635A>G	p.N879D	43
42190	*DNMT3A*	NM_022552	23	Missense	c.2644C>T	p.R882C	39
38936	*DNMT3A*	NM_022552	23	Missense	c.2644C>T	p.R882C	39
L1200359	*DNMT3A*	NM_022552	23	Missense	c.2644C>T	p.R882C	46
47821	*DNMT3A*	NM_022552	23	Missense	c.2644C>T	p.R882C	57
L1202621	*DNMT3A*	NM_022552	23	Missense	c.2644C>T	p.R882C	43
52584	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	51
43052	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	26
41222	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	45
L1206399	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	48
L1200167	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	43
43506	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	46
54485	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	47
L1200789	*DNMT3A*	NM_022552	23	Missense	c.2645G>A	p.R882H	44
51292	*DNMT3A*	NM_022552	23	Missense	c.2645G>T	p.R882L	44
45278	*DNMT3A*	NM_022552	23	Missense	c.2645G>C	p.R882P	40
57106	*DNMT3A*	NM_022552	23	Missense	c.2645G>C	p.R882P	40
42750	*DNMT3A*	NM_022552	23	Missense	c.2696G>A	p.R899H	23
60074	*DNMT3A*	NM_022552	23	Missense	c.2710C>T	p.P904S	6

Table S5. Patients and gene mutations considered for this study (continued).

UPN	Gene	Reference transcript	Exon	Type of mutation	Nucleotide variation	Protein variation	VAF (%)
48483	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	45
39771	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	46
48379	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	42
35992	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	50
L1210793	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	26
L1206288	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	35
53891	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	15
L1204485	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	42
L1303599	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	16
L1304166	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	41
L1201624	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	42
L1203356	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	34
L1200789	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	36
L1209496	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	41
L1201999	*IDH1*	NM_005896	4	Missense	c.394C>T	p.R132C	18
42750	*IDH1*	NM_005896	4	Missense	c.394C>G	p.R132G	23
35140	*IDH1*	NM_005896	4	Missense	c.394C>G	p.R132G	36
57712	*IDH1*	NM_005896	4	Missense	c.394C>G	p.R132G	37
L1208428	*IDH1*	NM_005896	4	Missense	c.394C>G	p.R132G	43
L1205789	*IDH1*	NM_005896	4	Missense	c.394C>G	p.R132G	46
40311	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	46
35066	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	52
38932	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	42
44761	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	NA
L1301870	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	38
54485	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	NA
L1211313	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	40
L1302995	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	45
L1302547	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	41
55766	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	NA
L1305787	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	42
L1306701	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	46
L1206290	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	41
50609	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	NA
51380	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	39
L1202459	*IDH1*	NM_005896	4	Missense	c.395G>A	p.R132H	NA

Table S5. Patients and gene mutations considered for this study (continued).

UPN	Gene	Reference transcript	Exon	Type of mutation	Nucleotide variation	Protein variation	VAF (%)
44434	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	44
48483	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	42
41029	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	51
39550	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	34
L1209948	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	53
42190	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
L1201703	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	50
L1210436	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	30
34795	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	29
38745	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	43
38936	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	25
36979	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	47
37408	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	23
43564	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
43459	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
L1200359	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	51
L1209695	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	50
L1301864	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	26
L1211151	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	47
L1300811	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	44
47821	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	50
L1301608	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	49
52584	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
L1302925	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	35
45278	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	44
L1202031	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
57150	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
42840	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
57106	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	27
L1306600	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	23
L1203196	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	49
L1306882	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	38
43052	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	29
L1202621	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	43
53312	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	46
L1200370	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
L1304314	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
41222	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	49
49624	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	53
L1201626	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	43
50179	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
L1204294	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	40
51292	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	NA
L1201420	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	38
L1206399	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	52
32487	*IDH2*	NM_002168	4	Missense	c.419G>A	p.R140Q	33

Table S5. Patients and gene mutations considered for this study (continued).

UPN	Gene	Reference transcript	Exon	Type of mutation	Nucleotide variation	Protein variation	VAF (%)
32888	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	22
44474	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	39
43515	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	27
34187	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	39
42458	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
37365	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	32
L1302236	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	22
57081	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	24
60074	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	31
L1206397	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
L1202978	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	48
53458	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	34
49614	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
L1200167	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	32
38512	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
L1206032	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
53395	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	45
48978	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
L1300683	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	NA
L1204429	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	6
43506	*IDH2*	NM_002168	4	Missense	c.525G>A	p.R172K	43

Table S5. Patients and gene mutations considered for this study (continued).

UPN	Gene	Reference transcript	Exon	Type of mutation	Nucleotide variation	Protein variation	VAF (%)
34795	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	17
35066	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	31
35140	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	21
35992	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	32
37408	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	10
38932	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	27
38936	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	23
39550	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	13
39771	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	28
40311	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	28
42190	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
42750	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	12
42840	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
43564	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
44434	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	27
44761	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
48483	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	26
50179	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
50609	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
51292	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
51380	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	14
52584	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
54485	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
55766	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
L1201420	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	31
L1201626	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	31
L1203356	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	30
L1204294	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	34
L1204485	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	42
L1205789	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	36
L1209496	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	36
L1210436	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	27
L1300811	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	40
L1302547	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	37
L1302995	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	35
L1304166	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	39
L1304314	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	NA
L1305787	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	35
L1306600	*NPM1*	NM_002520	11	Frameshift	c.860_863dupTCTG	p.W288CfsX12	20
43459	*NPM1*	NM_002520	11	Frameshift	c.863_864insCATG	p.W288CfsX12	NA
57712	*NPM1*	NM_002520	11	Frameshift	c.863_864insCATG	p.W288CfsX12	31
L1202031	*NPM1*	NM_002520	11	Frameshift	c.863_864insCATG	p.W288CfsX12	NA
L1301870	*NPM1*	NM_002520	11	Frameshift	c.863_864insCATG	p.W288CfsX12	35
L1202459	*NPM1*	NM_002520	11	Frameshift	c.863_864insCGTG	p.W288CfsX12	NA
L1306701	*NPM1*	NM_002520	11	Frameshift	c.863_864insCCAG	p.W288CfsX12	30
38745	*NPM1*	NM_002520	11	Frameshift	c.863_864insCCTG	p.W288CfsX12	29
41029	*NPM1*	NM_002520	11	Frameshift	c.863_864insCCTG	p.W288CfsX12	29
57150	*NPM1*	NM_002520	11	Frameshift	c.863_864insCCTG	p.W288CfsX12	NA
L1200370	*NPM1*	NM_002520	11	Frameshift	c.863_864insCCTG	p.W288CfsX12	NA
L1306882	*NPM1*	NM_002520	11	Frameshift	c.863_864insTATG	p.W288CfsX12	36
48483	*TET2*	NM_001127208	3	Frameshift	c.2348_2367del	p.E783AfsX12	71
41029	*TET2*	NM_001127208	3	Frameshift	c.2565delA	p.A855AfsX18	NA
42190	*TET2*	NM_001127208	6	Missense	c.G3637G>A	p.V1213M	16
L1201624	*TET2*	NM_001127208	11	In-frame deletion	c.4615_4635del	p.Q1541_Q1547del	48

Abbreviations: UPN: unique patient number; VAF, variant allele frequency; NA: not available.

Supplementary Figures

Figure S1. Representative examples of 2-D plots of droplet fluorescence for *IDH1/2* wild-type controls and *IDH1/2* mutated samples, according to the type of *IDH1/2* mutations.

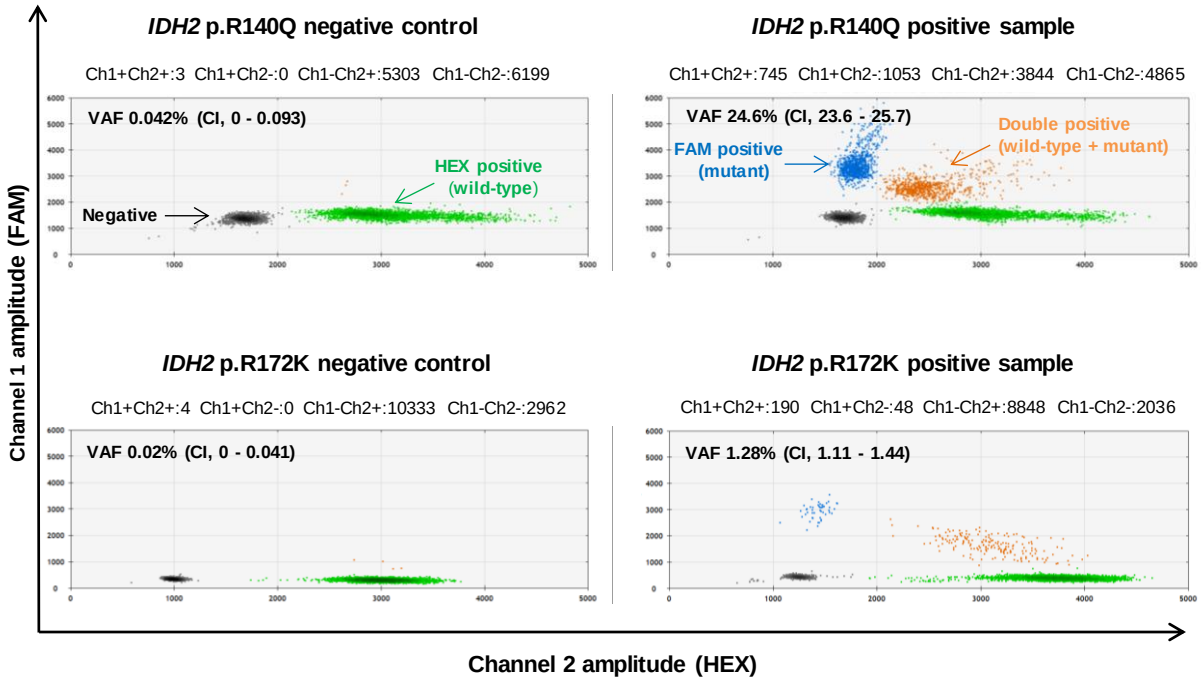
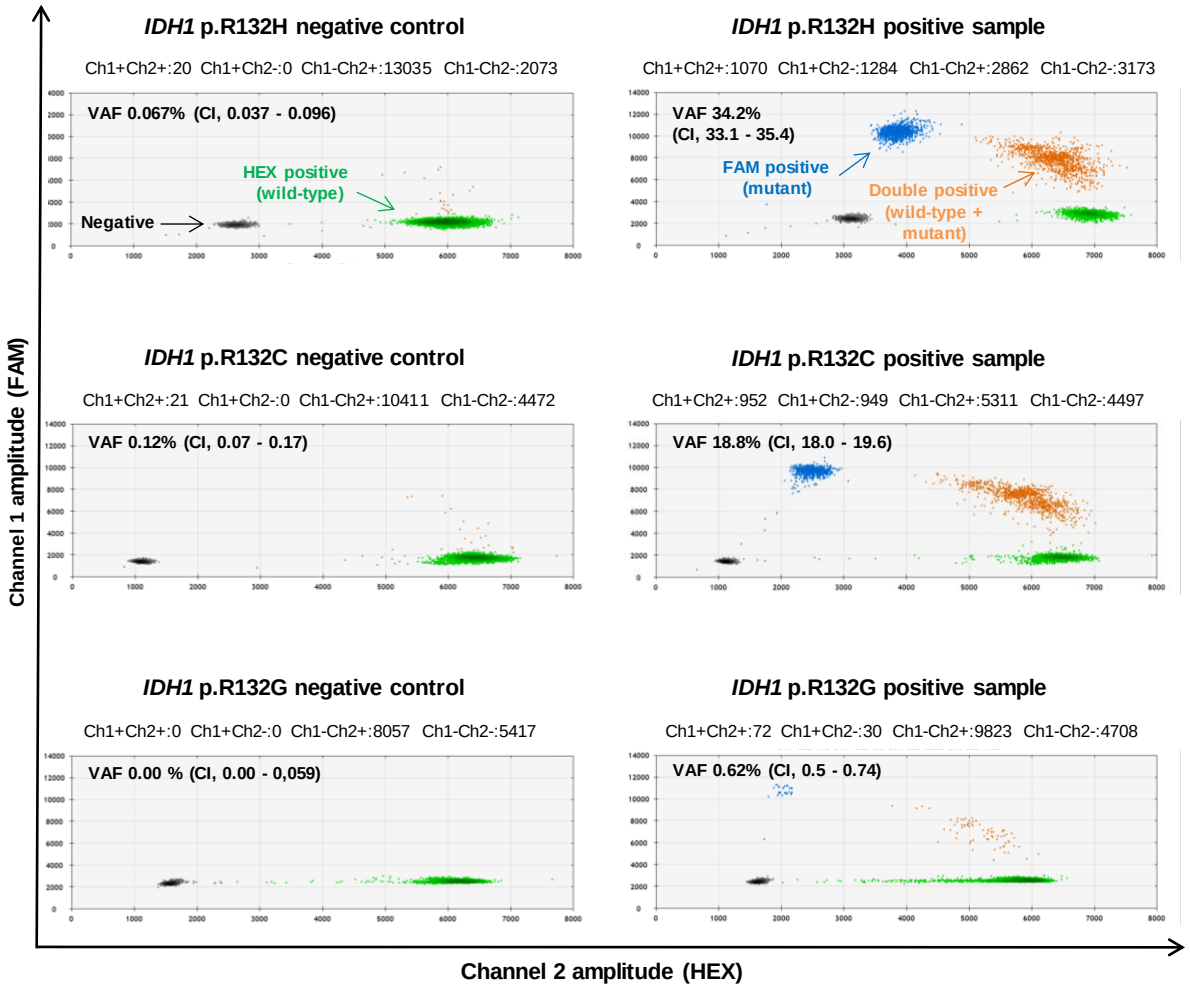


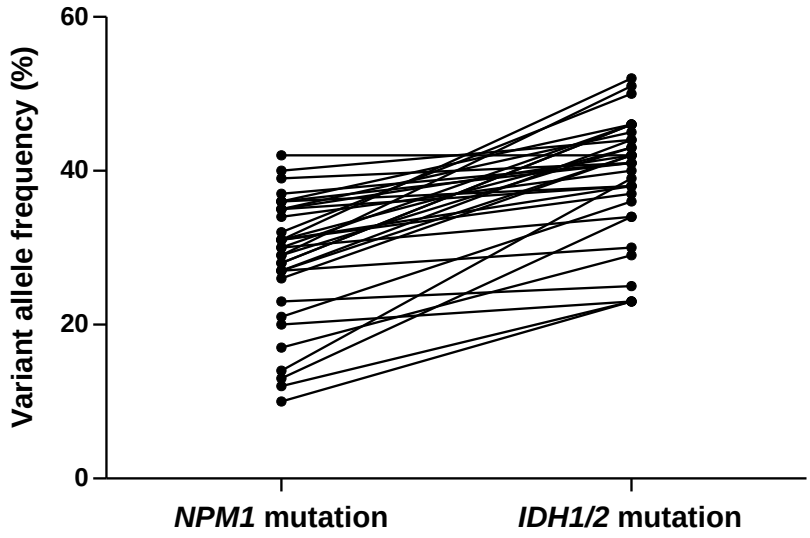
Figure S1. Representative examples of 2-D plots of droplet fluorescence for *IDH1/2* wild-type controls and *IDH1/2* mutated samples, according to the type of *IDH1/2* mutations (continued).



Channel 1 fluorescence (FAM) is plotted against channel 2 fluorescence (HEX) for each droplet. The number of droplets considered per well is indicated above each 2-D plot.

Abbreviations: VAF, variant allele fraction; CI, confidence interval; Ch, channel.

Figure S2. Comparison of the *NPM1* and *IDH1/2* variant allele frequencies in diagnostic samples from 34 patients carrying both mutations.



Supplementary References

1 ISCN. In: Mitelman F, ed. ISCN 1995, Guidelines for Cancer Cytogenetics, Supplement to: An International System for Human Cytogenetic Nomenclature. S. Karger; *Basel*, 1995; 1-110.

2 Renneville A, Boissel N, Nibourel O, et al. Prognostic significance of DNA methyltransferase 3A mutations in cytogenetically normal acute myeloid leukemia: a study by the Acute Leukemia French Association. Leukemia 2012;26(6):1247–1254.

3 Boissel N, Renneville A, Biggio V, et al. Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. Blood 2005;106(10):3618–3620.

4 Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood 2002;100(8):2717–2723.