Networking for advanced molecular diagnosis in acute myeloid leukemia patients is possible: the PETHEMA NGS-AML project

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 doi:10.3324/haematol.2020.263806

Received: June 24, 2020.

Accepted: November 3, 2020.

Pre-published: November 12, 2020.

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SUPPLEMENTARY MATERIAL:

Methods

Study design and reference laboratories

The overall population coverage was of 38.5 million habitants[1], with each laboratory receiving samples from institutions located at their assigned geographical areas, ranging from 2.2 to 8.9 million habitants. Bone marrow and peripheral blood samples from acute myeloid leukemia (AML) patients at diagnosis (DX) and at resistance (RS) or first and subsequent relapses (RP) were sent by courier and were isolated according to standardized methods.

Nucleic acid isolation

DNA from white blood cells was obtained in each center following previously established DNA isolation protocols. DNA quantification was assessed with Nanodrop (Thermo Fisher Scientific, Waltham, MA USA) and Qubit fluorometer (Thermo Fisher Scientific). DNA integrity was assessed with Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) or Tape Station 4100 (Agilent Technologies).

Cross-validation

Six of seven reference laboratories had already implemented next-generation sequencing (NGS) assays in a research context before starting the NGS standardization program. The remaining laboratory was included in the second cross-validation round.

Sequencing platforms and panels

The proposed strategy to select the sequencing platform and panel was aimed to allow each laboratory implementing their preferred panel fitting with their available facilities, as well as overlapping with the study of other myeloid neoplasms variants.

NGS was performed either with commercial or custom panels using Ion Torrent or Illumina platforms. Two laboratories implemented the Myeloid Solution panel (SOPHIA Genetics, Lausanne, Switzerland), including 30 genes involved in leukemia and other hematological disorders, such as myelodysplastic and chronic myeloproliferative syndromes (MDS and CMPS). Two laboratories used an extended version of this panel (Pan-Myeloid Panel, SOPHIA Genetics), including 48 genes involved in myeloid neoplasms. Two laboratories performed NGS with the Oncomine Myeloid Research Assay (Thermo Fisher Scientific), including 40 genes involved in diverse myeloid disorders (AML, MDS, and CMPS). One laboratory used a

custom AML panel already implemented in previous research projects that include genes involved in hematological disorders[2].

Table S1. Gene panels employed by central laboratories.

Gene	HULF, HUVR	H12O	HUDN, HURS	UNAV, HUS
	AML Oncomine Research (Ion Torrent)	Custom panel (Ion Torrent)	Myeloid Tumor Solution (Illumina)	Custom PanMyeloid (Illumina)
ASXL1	All exons	All exons	9, 11, 12	12
CEBPA	All exons	All exons	All exons	All exons
FLT3	8, 11 to 16, 20, 23, 24	All exons	13 to 15, 20	14 to 16, 20
IDH1	4	All exons	4	4
IDH2	4	All exons	4	4
NPM1	11	All exons	10, 11	10, 11
RUNX1	All exons	All exons	All exons	All exons
TP53	All exons	All exons	All exons	All exons
ABL1	4 to 9	-	4 to 9	-
BRAF	15	-	15	-
CALR	All exons	All exons	9	9
CBL	8, 9	All exons	8, 9	8, 9
CSF3R	14, 17	All exons	All exons	14 to 17
DNMT3A	11 to 23	All exons	All exons	All exons
ETV6	All exons	All exons	All exons	All exons
EZH2	All exons	All exons	All exons	All exons
GATA2	4, 5	-	-	2 to 6
HRAS	2, 3	-	2, 3	-
JAK2	12 to 15	All exons	All exons	12 to 15
KIT	2, 8 to 11, 13, 17, 18	All exons	2, 8 to 11, 13, 17, 18	2, 8 to 11, 13, 14, 17, 18
KRAS	2 to 6	All exons	2, 3	2 to 4
MPL	3 to 4, 10, 12	All exons	10	3 to 6, 10, 12
NRAS	2 to 4	All exons	2, 3	2 to 4
PTPN11	3, 12, 13	-	3, 7, 13	3, 7, 13
SETBP1	4	All exons	4	4
SF3B1	14 to 21	All exons	10 to 16	11 to 16
SRSF2	1	All exons	1	1
TET2	All exons	All exons	All exons	All exons
U2AF1	2, 6	All exons	2, 6	2, 6
WT1	7, 9	All exons	6 to 10	7, 9

HULF: Hospital Universitario La Fe, HUVR: Hospital Universitario Virgen del Rocío, H12O: Hospital Universitario 12 de Octubre, HUDN: Hospital Universitario de Gran Canaria Dr. Negrín, HURS: Hospital Universitario Reina Sofía, UNAV: CIMA LAB Diagnostics, HUS: Hospital Universitario de Salamanca.

Statistics

Continuous variables were summarized by using median, mean, range and standard deviation (SD). Categorical variables were summarized with relative and absolute frequencies. Association of categorical variables was assessed using the chi-square ($\chi 2$) test. Normal distribution of continuous variables was checked with Shapiro-Wilk test. Association of categorical with continuous variables was performed using Mann-Whitney´s U test (independent groups) or Wilcoxon Test (related groups). Exclusion patterns among genes were analyzed with the Mutually Exclusive Gene Sets (MEGS) analysis, an analytic framework based on a likelihood ratio test and a model selection procedure[3].

Results

Platform performance

Table S2. Cross-validation results comparing Ion Torrent and Illumina platforms.

			Illumina					Ion Torrent				
Gene	Coding	Protein	Detected	Included	Error Rate	Mean VAF	SD	Detected	Included	Error Rate	Mean VAF	SD
NPM1 (NM_002520)	c.860_863dupTCTG	p.Trp288Cysfs*12	3	3	0.00%	40.73%	26.61%	2	2	0.00%	43.11%	2.43%
IDH2 (NM_002168.3)	c.419G>A	p.Arg140Gln	3	3	0.00%	42.13%	1.63%	3	3	0.00%	47.32%	2.13%
DNMT3A (NM_022552)	c.2645G>A	p.Arg882His	3	3	0.00%	42.60%	0.78%	3	3	0.00%	44.94%	2.11%
STAG2 (NM_001042749.2)	c.2124del	p.Leu708Phefs*9	1	3	66.67%	NA	NA	0	0	NA	NA	NA
RUNX1 (NM_001754.4)	c.736A>C	p.Thr246Pro	1	3	66.67%	NA	NA	0	3	100.00%	NA	NA
ASXL1 (NM_015338.5)	c.1934dup	p.Gly646Trpfs*12	1	3	66.67%	NA	NA	0	3	100.00%	NA	NA
CEBPA (NM_004364.4)	c.68_78del	p.Pro23Glnfs*81	2	3	33.33%	56.15%	6.72%	2	2	0.00%	46.49%	3.97%
CEBPA (NM_004364.4)	c.895A>G	p.Ser299Gly	3	3	0.00%	43.77%	4.33%	2	2	0.00%	47.64%	2.07%
IDH2 (NM_002168.3)	c.419G>A	p.Arg140Gln	3	3	0.00%	51.67%	7.22%	3	3	0.00%	47.98%	2.38%
NRAS (NM_002524.4)	c.37G>C	p.Gly13Arg	3	3	0.00%	47.27%	2.38%	3	3	0.00%	45.38%	1.35%
EZH2 (NM_004456.4)	c.952del	p.Thr318Glnfs*3	3	3	0.00%	47.54%	2.16%	0	1	100.00%	NA	NA
EZH2 (NM_004456.4)	c.1321G>A	p.Glu441Lys	3	3	0.00%	50.08%	3.44%	1	1	0.00%	NA	NA
DNMT3A (NM_022552)	c.1961G>A	p.Gly654Asp	1	3	66.67%	NA	NA	0	3	100.00%	NA	NA
KMT2A (NM_001197104.1)	c.3253G>A	p.Val1085Met	1	3	66.67%	NA	NA	0	1	100.00%	NA	NA
GATA2 (NM_032638.4)	c.1084C>T	p.Arg362*	1	3	66.67%	NA	NA	0	2	100.00%	NA	NA
ASXL1 (NM_015338.5)	c.1934dup	p.Gly646Trpfs*12	2	3	33.33%	39.82%	4,41%	0	3	100.00%	NA	NA
DNMT3A (NM_022552)	c.2678G>C	p.Trp893Ser	3	3	0.00%	45.00%	3.49%	3	3	0.00%	44.08%	0.78%
TP53 (NM_000546.5)	c.652_670del	p.Val218fs	2	3	33.33%	75.75%	3.18%	3	3	0.00%	62.14%	25.26%
STAG2 (NM_001042749.2)	c.2858G>A	p.Arg953Gln	1	3	66.67%	NA	NA	0	0	NA	NA	NA
CUX1 (NM_181552.4)	c.1588A>C	p.Lys530Gln	1	3	66.67%	NA	NA	0	0	NA	NA	NA
ASXL1 (NM_015338.5)	c.1934dup	p.Gly646Trpfs*12	1	3	66.67%	NA	NA	0	3	100.00%	NA	NA
TP53 (NM_000546.5)	c.392A>T	p.Asn131Ile	3	3	0.00%	47.33%	2.02%	3	3	0.00%	47.33%	2.07%
EZH2 (NM_004456.4)	c.553G>C	p.Asp185His	1	3	66.67%	NA	NA	0	1	100.00%	NA	NA
ASXL1 (NM_015338.5)	c.1934dup	p.Gly646Trpfs*12	1	3	66.67%	NA	NA	0	3	100.00%	NA	NA
NPM1 (NM_002520)	c.863_864insCCTG	p.Trp288Cysfs*12	4	4	0.00%	33.29%	6.08%	3	3	0.00%	37.00%	12.17%
FLT3 (NM_004119.2)	c.1801_1802ins30	p.Asp600_Leu601ins10	4	4	0.00%	28.57%	11.75%	3	3	0.00%	31.03%	6.97%
FLT3 (NM_004119.2)	c.2505T>A	p.Asp835Glu	3	4	25.00%	2.15%	0.39%	2	3	33.33%	2.93%	0.31%
PHF6 (NM_032458.2)	c.548C>T	p.Ser183Phe	1	3	66.67%	NA	NA	3	3	0.00%	51.12%	1.93%
DNMT3A (NM_022552)	c.2264T>C	p.Phe755Ser	4	4	0.00%	42.12%	7.39%	3	3	0.00%	42.09%	4.57%
NRAS (NM_002524.4)	c.34G>A	p.Gly12Cys	3	4	25.00%	1.78%	0.37%	1	3	66.67%	NA	NA
RUNX1 (NM_001754.4)	c.1306dupT	p.Ser436Phefs*164	3	4	25.00%	40.69%	2.76%	3	3	0.00%	45.78%	4.45%
IDH1 (NM_005896.3)	c.394C>T	p.Arg132Cys	4	4	0.00%	16.64%	3.06%	3	3	0.00%	15.73%	1.16%
TET2 (NM_001127208.2)	c.3866G>T	p.Cys1289Phe	4	4	0.00%	40.83%	8.82%	3	3	0.00%	45.95%	0.48%
PHF6 (NM_032458.2)	c.346C>T	p.Arg116*	1	2	50.00%	NA	NA	3	3	0.00%	42.83%	1.85%
EZH2 (NM_004456.4)	c.2255G>C	p.*752Ser	4	4	0.00%	10.98%	1.95%	3	3	0.00%	9.60%	0.53%
SRSF2 (NM_003016.4)	c.161C>T	p.Ser54Phe	3	4	25.00%	5.85%	0.92%	3	3	0.00%	5.60%	0.43%
JAK2 (NM_004972.3)	c.1849G>T	p.Val617Phe	3	4	25.00%	2.35%	0.15%	3	3	0.00%	3.10%	0.80%
FLT3 (NM_004119.2)	c.2028C>G	p.Asn676Lys	2	4	50.00%	25.57%	3.56%	3	3	0.00%	19.91%	0.92%
FLT3 (NM_004119.2)	c.2504A>C	p.Asp835Ala	3	4	25.00%	5.21%	0.28%	3	3	0.00%	5.58%	0.88%
SH2B3 (NM_005475.2)	c.557G>T	p.Ser186Ile	0	0	NA	NA	NA	2	3	33.33%	56.40%	0.57%
PHF6 (NM_032458.2)	c.129_130insGG	p.Lys44Glyfs*38	1	2	50.00%	NA	NA	3	3	0.00%	51.12%	1.09%
EZH2 (NM_004456.4)	c.2212_2231del	p.Ala738Argfs*18	4	4	0.00%	26.68%	7.01%	2	3	33.33%	35.11%	0.47%
NRAS (NM_002524.4)	c.35G>A	p.Gly12Asp	4	4	0.00%	16.73%	2.38%	3	3	0.00%	17.54%	2.68%
EZH2 (NM_004456.4)	c.796G>A	p.Gly266Arg	1	4	75.00%	NA	NA	3	3	0.00%	4.40%	0.13%
ASXL1 (NM_015338.5)	c.1772dup	p.Tyr591*	4	4	0.00%	22.04%	1.58%	3	3	0.00%	21.23%	3.44%
ASXL1 (NM_015338.5)	c.1745_1758del	p.Pro582Argfs*32	3	4	25.00%	14.67%	3.79%	3	3	0.00%	13.26%	2.18%
TP53 (NM_000546.5)	c.916C>T	p.Arg306*	3	4	25.00%	4.31%	0.28%	3	3	0.00%	4.67%	0.16%
SF3B1 (NM_012433.3)	c.1873C>T	p.Arg625Cys	3	4	25.00%	7.99%	0.13%	3	3	0.00%	8.06%	1.67%
RUNX1 (NM_001754.4)	c.593A>G	p.Asp198Gly	4	4	0.00%	76.59%	2.58%	3	3	0.00%	79.85%	1.91%
ASXL1 (NM_015338.5)	c.2463_2478del	p.Asp821Glufs*12	4	4	0.00%	37.12%	10.03%	3	3	0.00%	44.93%	1.81%
ASXL1 (NM_015338.5)	c.2537G>A	p.Ser846Asn	4	4	0.00%	54.17%	6.94%	3	3	0.00%	55.40%	8.12%
SF3B1 (NM_012433.3)	c.2098A>G	p.Lys700Glu	3	4	25.00%	46.40%	1.40%	3	3	0.00%	48.53%	1.80%
CSF3R (NM_156039.3)	c.1853C>T	p.Thr618Ile	3	4	25.00%	46.23%	1.51%	3	3	0.00%	47.88%	1.12%
CSF3R (NM_156039.3)	c.2346dup	p.Ser783Glnfs*6	3	4	25.00%	37.77%	2.92%	0	3	100.00%	NA	NA

Detected: Number of centers which have detected the mutation; Included: Number of centers which include each variant in its NGS assay. Error Rate: Number of centers which failed to detect the variant regarding the total of centers. VAF: Variant allele frequency. SD: Standard deviation of VAF establishment among centers. NA: Not applicable; variants only were detected by one center

Mutation distribution

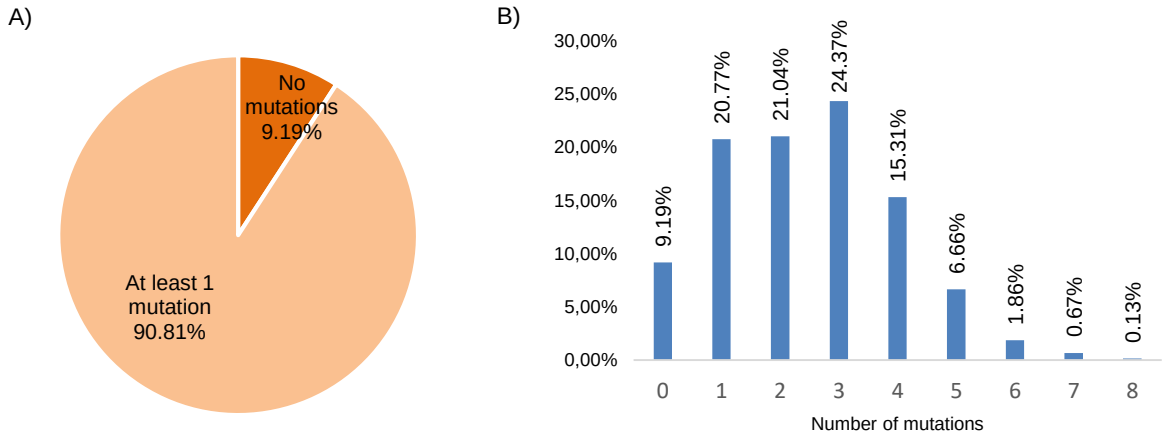

Figure S1. A) Percentage of patients according to the presence/absence of mutations. B) Samples distribution according to mutation number.

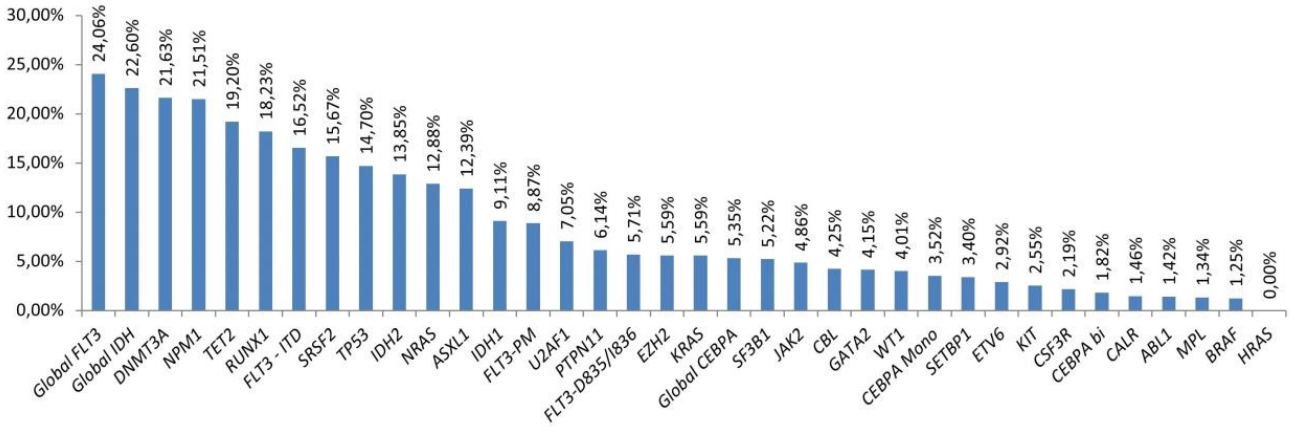

Figure S2. Mutational prevalence in 823 AML samples. Mono: monoallelic variant; bi: biallelic variant; PM: point mutations; ITD: internal tandem duplication. Global IDH: All variants detected in *IDH1* and *IDH2*. Global *FLT3*: All variants detected in *FLT3*.

Exclusion patterns. MEGS analysis

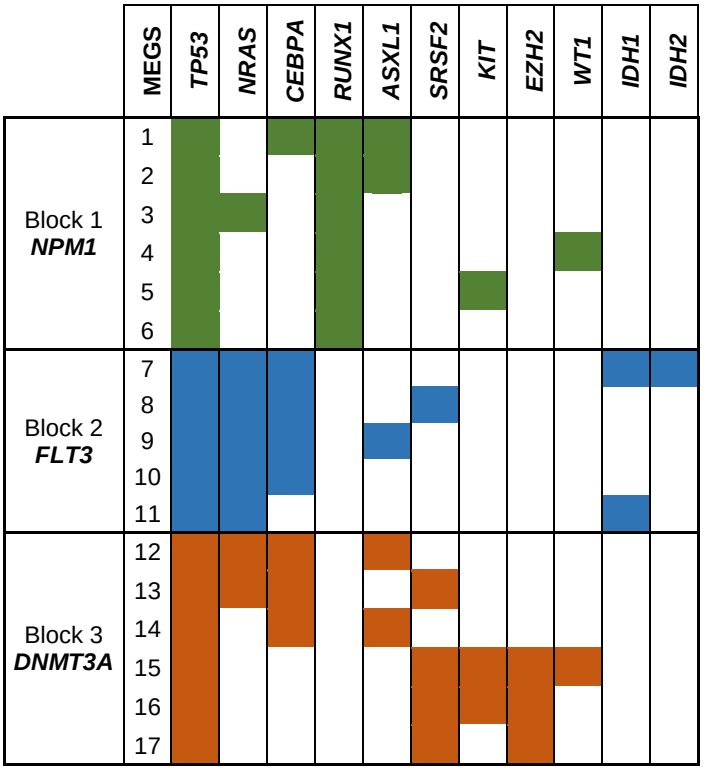

Figure S3. Schematic representation of gene exclusion patterns. 17 mutually exclusive gene sets (MEGS) are grouped in 3 blocks. All genes derived from MEGS analysis are shown in upper line. Colored squares indicate the included genes in each MEGS (line).

Table S3. Results from MEGS analysis. Exclusion patterns among genes.

	MEGS	Coverage	LRT	pNominal	pCorrected
1	*NPM1 TP53 RUNX1 CEBPA ASXL1*	0.63380282	74.7289156	2.70E-18	0
2	*NPM1 TP53 RUNX1 ASXL1*	0.60093897	69.5037372	3.81E-17	0
3	*NPM1 TP53 RUNX1*	0.53364632	100.110776	7.21E-24	0
4	Global *FLT3 TP53 NRAS CEBPA ASXL1*	0.61345853	64.7959538	4.15E-16	0
5	Global *FLT3 TP53 NRAS CEBPA*	0.53521127	57.9715794	1.33E-14	0
6	Global *FLT3 TP53 NRAS IDH1*	0.55399061	58.9706952	8.00E-15	0
7	Global *FLT3 TP53 NRAS CEBPA IDH2 IDH1*	0.66979656	82.5232132	5.22E-20	0
8	Global *FLT3 TP53 NRAS CEBPA SRSF2*	0.63380282	68.5592285	6.16E-17	0
9	*NPM1 TP53 RUNX1 KIT*	0.55242567	102.412508	2.25E-24	0
10	*DNMT3A TP53 CEBPA ASXL1*	0.50234742	47.0179694	3.52E-12	0
11	*DNMT3A TP53 SRSF2 EZH2 KIT*	0.55086072	68.2360199	7.25E-17	0
12	*DNMT3A TP53 SRSF2 EZH2 KIT WT1*	0.5743349	75.8696006	1.52E-18	0
13	*NPM1 TP53 RUNX1 WT1*	0.55555556	98.7308566	1.45E-23	0
14	*DNMT3A TP53 NRAS CEBPA ASXL1*	0.58685446	46.4554172	4.69E-12	0
15	*DNMT3A TP53 NRAS CEBPA SRSF2*	0.61032864	53.8452803	1.08E-13	0
16	*DNMT3A TP53 SRSF2 EZH2*	0.53051643	59.9203368	4.94E-15	0
17	*NPM1 TP53 RUNX1 NRAS*	0.61189358	72.6084052	7.91E-18	0

MEGS: mutually exclusive gene sets. Coverage: proportion of samples covered by the MEGS, LRT: likelihood ratio test for examining mutual exclusivity for a subset of genes

Age-related mutations

Table S4. Age-related mutational prevalence and p-values

	Diagnosis			Refractory AML			Relapsed AML		
	<65	≥65	p-value	<65	≥65	p-value	<65	≥65	p-value
ABL1	1.55%	0.97%	0.676	3.45%	3.03%	1.00	0.00%	3.03%	0.429
ASXL1	7.54%	15.95%	0.001	12.12%	15.15%	1.00	3.57%	20.00%	0.011
BRAF	1.04%	1.45%	1.00	0.00%	3.03%	1.00	0.00%	0.00%	NA
CALR	1.64%	1.23%	0.745	0.00%	3.03%	1.00	1.79%	2.22%	1.00
CBL	2.95%	5.83%	0.085	6.06%	3.03%	1.00	1.79%	4.44%	0.584
CEBPA mono	4.3%	3.1%	0.525	0.00%	3.03%	1.00	1.79%	8.90%	0.169
CEBPA bi	1.3%	1.8%	0.753	0.00%	3.03%	1.00	1.79%	4.44%	0.584
CEBPA	5.57%	4.91%	0.724	0.00%	6.06%	0.492	3.57%	13.33%	0.134
CSF3R	2.3%	2.76%	0.803	0.00%	0.00%	NA	3.57%	0.00%	0.501
DNMT3A	22.62%	19.33%	0.328	24.24%	12.12%	0.339	23.21%	26.67%	0.817
ETV6	1.97%	3.99%	0.165	0.00%	3.03%	1.00	1.79%	6.67%	0.321
EZH2	2.62%	7.98%	0.040	6.06%	9.09%	1.00	5.36%	6.67%	1.00
FLT3-PM	10.49%	7.06%	0.158	9.09%	15.15%	0.708	10.71%	6.67%	0.727
FLT3-ITD	19.34%	12.88%	0.030	15.15%	18.18%	1.00	21.43%	15.56%	0.610
GATA2	2.2%	4.74%	0.202	6.67%	3.33%	1.00	6.12%	8.33%	0.695
HRAS	0.0%	0.0%	NA	0.00%	0.00%	NA	0.00%	0.00%	NA
IDH1	7.54%	8.59%	0.663	12.12%	12.12%	1.00	8.93%	22.22%	0.090
IDH2	9.84%	16.34%	0.042	12.12%	15.15%	1.00	16.07%	26.67%	0.223
Global *IDH*	16.7%	23.9%	0.030	24.20%	27.30%	1.00	25.00%	46.70%	0.035
JAK2	2.3%	7.36%	0.003	3.03%	6.06%	1.00	5.36%	6.67%	1.00
KIT	3.28%	1.84%	0.314	3.03%	3.03%	1.00	3.57%	0.00%	0.501
KRAS	4.92%	6.44%	0.493	6.06%	12.12%	0.672	3.57%	0.00%	0.501
MPL	1.64%	1.84%	1.00	0.00%	0.00%	NA	0.00%	0.00%	NA
NPM1	29.18%	17.18%	0.001	9.09%	12.12%	1.00	17.86%	24.44%	0.466
NRAS	14.75%	12.27%	0.414	9.09%	15.15%	0.708	15.50%	8.89%	0.750
PTPN11	7.49%	5.54%	0.386	6.06%	15.15%	0.427	0.00%	2.63%	0.422
RUNX1	13.77%	19.02%	0.086	24.24%	30.30%	0.783	14.29%	24.44%	0.211
SETBP1	1.64%	3.99%	0.095	3.03%	9.09%	0.613	3.57%	6.67%	0.654
SF3B1	1.97%	6.44%	0.006	6.06%	12.12%	0.672	10.71%	4.44%	0.293
SRSF2	7.54%	22.09%	0.000	12.12%	30.30%	0.130	8.93%	22.22%	0.090
TET2	11.8%	27.91%	0.000	6.06%	24.24%	0.082	12.50%	24.44%	0.190
TP53	11.48%	19.33%	0.008	15.15%	9.09%	0.708	10.71%	15.56%	0.556
U2AF1	4.26%	8.9%	0.025	9.09%	12.12%	1.00	5.36%	4.44%	1.00
WT1	3.93%	2.45%	0.365	15.15%	0.00%	0.053	10.71%	4.44%	0.293

Mono: monoallelic variant; bi: biallelic variant; PM: point mutations; ITD: internal tandem duplication. Global IDH: All variants detected in *IDH1* and *IDH2*. NA: Not applicable. Shaded results are statistically significant.

Mutational stability in paired samples

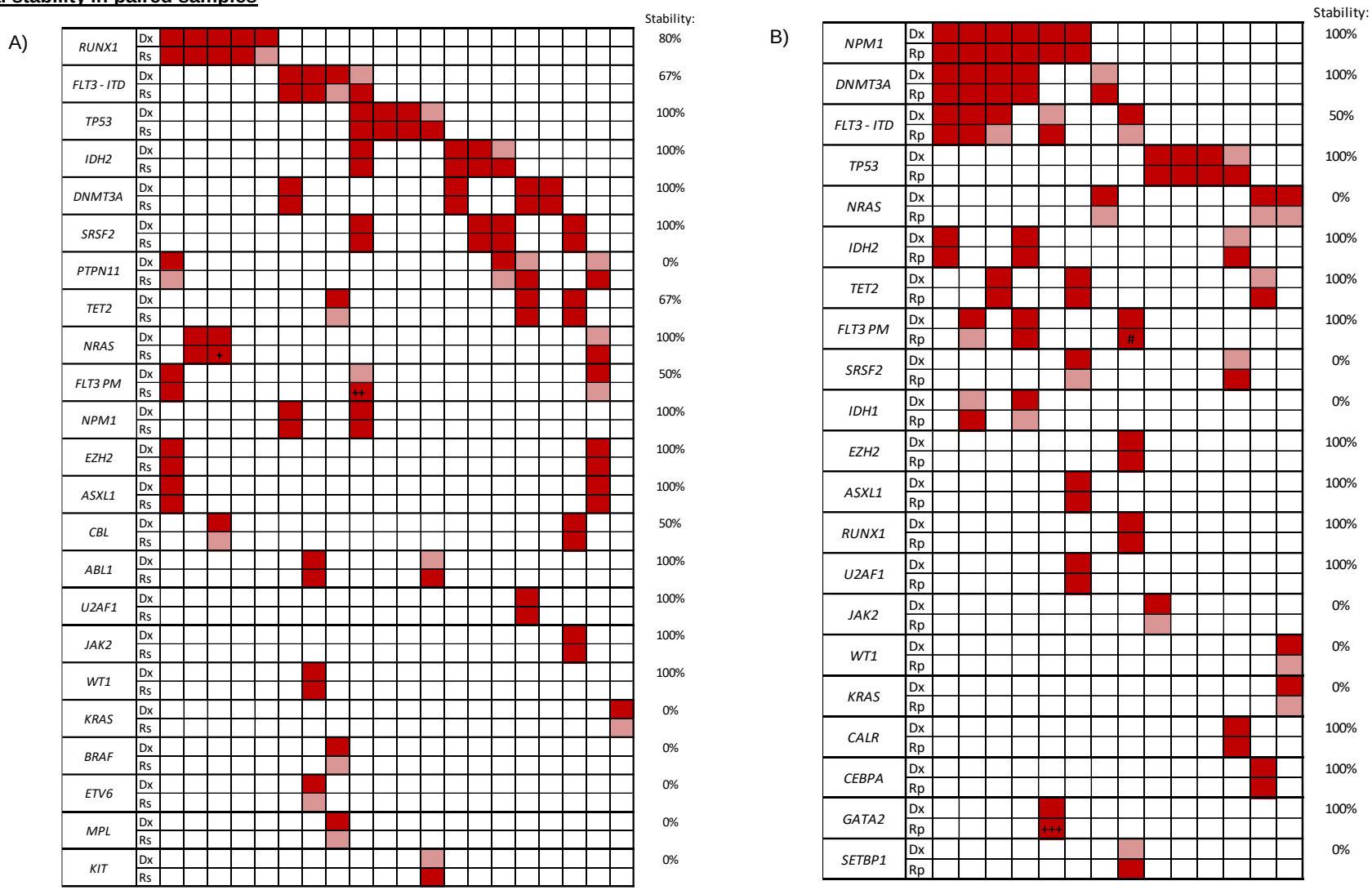

Figure S4. Mutation stability in diagnosis (DX) /refractory (RS) AML (panel A) and DX/relapse (RP) (Panel B). Dark red: mutated gene; Light red: lack of mutation detected in other moment disease; White: wild-type. #: one diagnosis mutation is lost at relapse. +: A second mutation is acquired. ++: Two new mutations are acquired. +++: keep the diagnosis mutation and acquires two new. ITD: Internal tandem duplication. PM: Point mutations.

Clinically relevant mutations

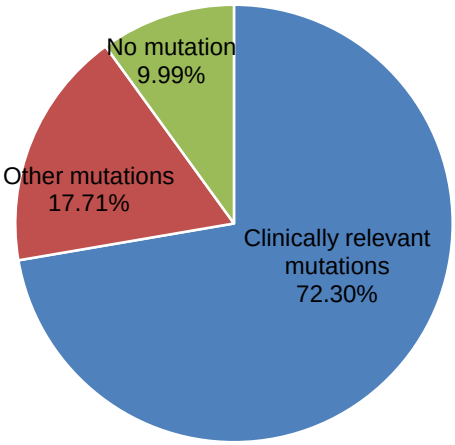

Figure S5. Percentage of patients with clinically relevant mutations.

***NPM1* mutations**

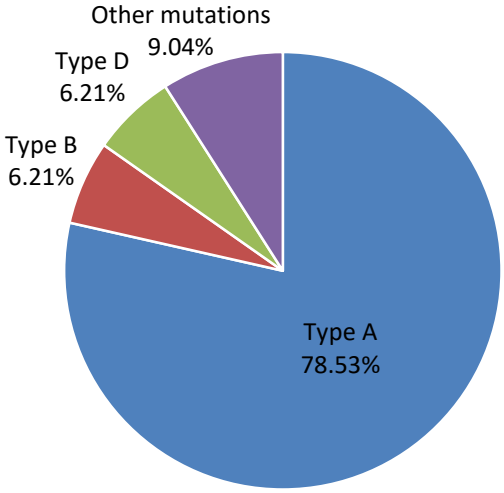

Figure S6. *NPM1* mutation type distribution.

Table S5. *NPM1* mutations. Coding sequence, mutation type and percentage are shown.

Coding	Protein	Type	N	%
c.860_863dupTCTG	p.Trp288Cysfs*12	A	139	78.53%
c.863_864insCATG	p.Trp288Cysfs*12	B	11	6.21%
c.863_864insCCTG	p.Trp288Cysfs*12	D	11	6.21%
c.863_864insTATG	p.Trp288Cysfs*12	R	2	1.13%
c.863_864insCAGA	p.Trp288Cysfs*12	ZM	2	1.13%
c.863_864insTCGC	p.Trp288Cysfs*12	YJ	2	1.13%
c.863_864insCCAG	p.Trp288Cysfs*12	K	1	0.56%
c.863_864insCAGG	p.Trp288Cysfs*12	G	1	0.56%
c.863_864insCCGG	p.Trp288Cysfs*12	J	1	0.56%
c.863_864insCTTG	p.Trp288Cysfs*12	I	1	0.56%
c.863_864insTAGG	p.Trp288Cysfs*12	ZA	1	0.56%
c.863_864insCGCG	p.Trp288Cysfs*12	-	1	0.56%
c.863_864insCTGC	p.Trp288Cysfs*12	-	1	0.56%
c.863_864insTGTA	p.Trp288Cysfs*12	-	1	0.56%
c.499G>A	p.Asp167Asn	-	1	0.56%
c.868_869delinsCGGTTC	p.Trp290Argfs*10	-	1	0.56%

N: number of positive samples; %: percentage of each mutation type.

***FLT3* mutations**

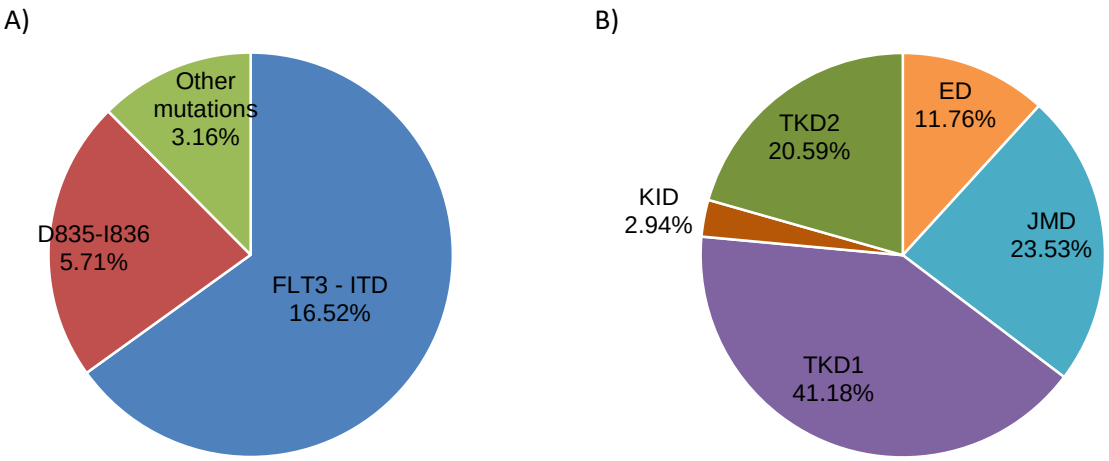

Figure S7. A) Percentage of samples with *FLT3*-internal tandem duplications (ITD), D835/I836 and other *FLT3* mutations. B) Location of other *FLT3* mutations (*FLT3*-ITD and FLT3-D835/I836 excluded). ED: extracellular domain, JMD: juxtamembrane domain, TKD1: tyrosine kinase 1 domain, TKD2: tyrosine kinase 2 domain, KID: kinase insert domain

***CEBPA* mutations**

Table S6. Biallelic mutations in *CEBPA*.

ID	Coding	Protein	VAF (%)
1	c.934_936dup	p.Gln312dup	37.57
	c.139_155delinsAGGC	p.Ala47Argfs*109	42.10
2	c.224del	p.Asp75Alafs*85	19.30
	c.92dup	p.Gly32Argfs*76	14.30
3	c.296_302del	p.Gly99Alafs*59	25.90
	c.542_543insTT	p.Gln182Serfs*137	26.30
4	c.112_116del	p.Gly38Argfs*68	41.67
	c.916_917delins21	p.Arg306delins8	45.02
5	c.341_350del	p.Gly114Alafs*43	37.80
	c.949_950insGTC	p.Glu316_Leu317insArg	41.40
6	c.949_951dup	p.Leu317dup	42.76
	c.120_121del	p.Gln41Alafs*66	39.17
7	c.476dup	p.Ile160Aspfs*10	45.81
	c.68_78del	p.Pro23Glnfs*81	44.98
8	c.45_56delinsCC	p.Met15Ilefs*142	19.31
	c.971T>C	p.Leu324Pro	16.41
9	c.917_918insTTG	p.Arg306_Asn307insCys	49.12
	c.247del	p.Gln83Serfs*77	47.18
10	c.997_1003del	p.Arg333Trpfs*87	30.48
	c.198_201dup	p.Ile68Leufs*41	32.03
11	c.1070_1071insTTGGGG	p.Cys357_Ala358insTrpGly	20.67
	c.4G>T	p.Glu2*	17.56
12	c.78_87dup	p.Ala30Glnfs*81	10.49
	c.74_84dup	p.Ala29Argfs*135	9.27
13	c.955_971del	p.Ser319Alafs*78	50.69
	c.971T>C	p.Leu324Pro	46.07
14	c.245_249dup	p.His84Serfs*78	43.65
	c.815dup	p.Lys273Glnfs*48	42.55
15	c.247del	p.Gln83Serfs*77	50.22
	c.917_918insTTG	p.Arg306_Asn307insCys	44.49

VAF: Variant allele frequency

***IDH1* and *IDH2* mutations**

Table S7. Protein and coding change for mutations in A) *IDH1* and *IDH2* and B) patients with mutations in both genes.

	Gene	Coding	Protein	N	%
A)	*IDH1*	c.394C>T	p.Arg132Cys	39	52.00%
		c.395G>A	p.Arg132His	23	30.67%
		c.394C>G	p.Arg132Gly	8	10.67%
		c.394C>A	p.Arg132Ser	3	4.00%
		c.395G>T	p.Arg132Leu	2	2.67%
	IDH2	c.419G>A	p.Arg140Gln	91	79.82%
		c.515G>A	p.Arg172Lys	18	15.79%
		c.418C>T	p.Arg140Trp	3	2.63%
		c.419G>T	p.Arg140Leu	2	1.75%

	Gene	Coding	Protein	VAF (%)	ID
B)	*IDH1*	c.395G>A	p.Arg132His	26.77	1
	IDH2	c.419G>A	p.Arg140Gln	8.15	
	IDH1	c.395G>A	p.Arg132His	7.22	2
	IDH2	c.419G>A	p.Arg140Gln	22.10	
	IDH1	c.395G>A	p.Arg132His	40.50	3
	IDH2	c.418C>T	p.Arg140Trp	2.00	

N: number of positive samples; %: percentage of each protein change; VAF: Variant allele frequency

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Appendix

Institutions and clinicians participating in the PETHEMA epidemiologic registry of acute myeloid leukemia and acute promyelocytic leukemia:

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