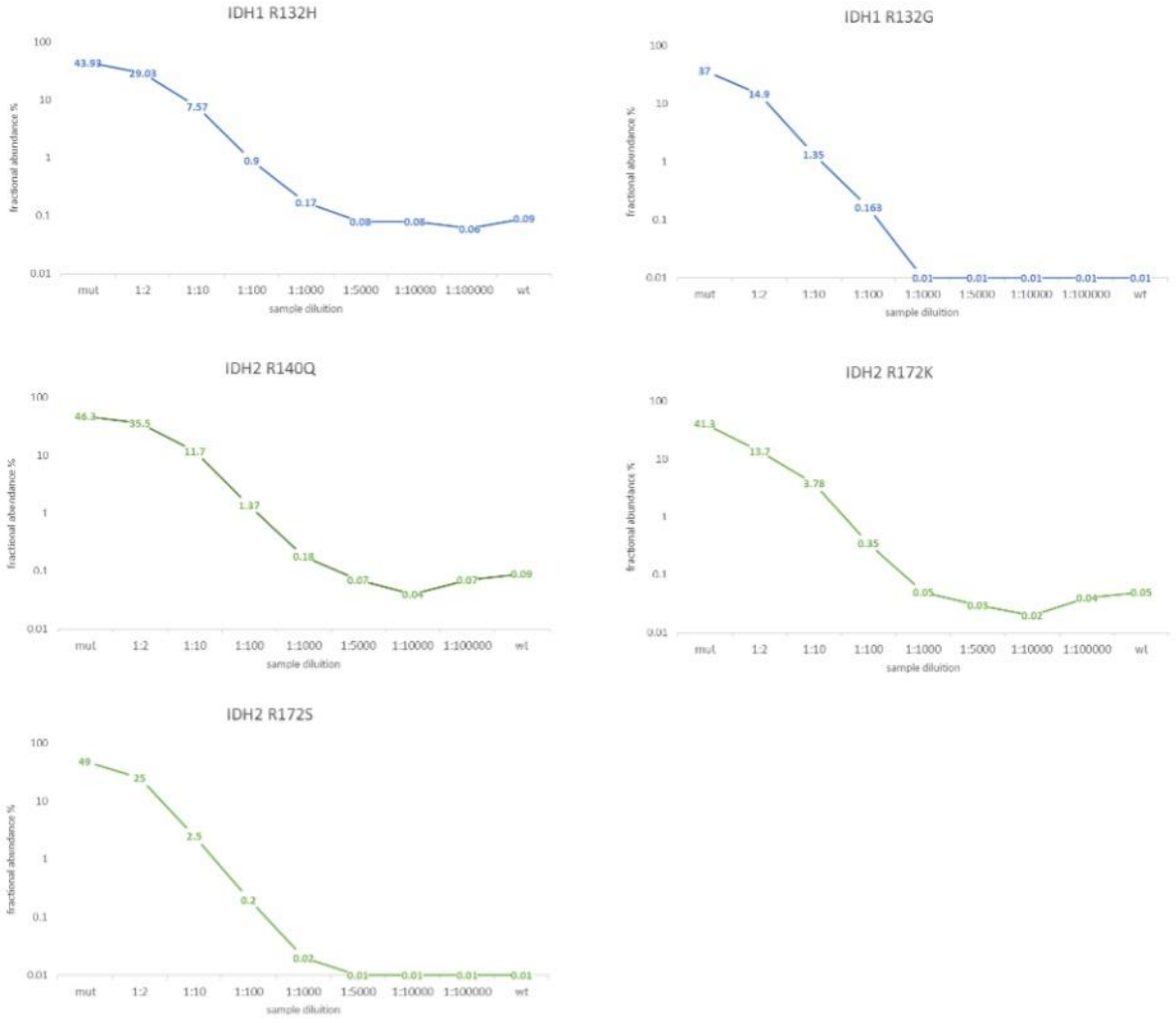
Supplementary

S1: Validation od ddPCR curves and supplementary methods. Charts show IDH1/2 mutation detected in serial dilutions. IDH: isocitrate dehydrogenase; mut: mutated; wt: wild type.



Sample collection and bio-banking

After informed consent was signed, DNA was extracted from bone marrow of patients with AML , prospectively and longitudinally collected since 2010 in accordance with GCP and as approved by the local ethical review board (Ethical Committee approval code: 012/2009/U/Tess,

01/2011/U/Tess, 10/2011/U/Tess and 253/2013/O/Tess). Samples were maintained at -20° C with continuous temperature monitoring.

Patient/sample selection

Molecular data analyzed for standard clinical practice were stored in a custom database; patients with known IDH1 or IDH2 mutation were selected out of 883 unique patients (patients that are not followed from the diagnosis or that were not tested for IDH1/2 were excluded). Clinical data were collected retrospectively from clinical records. Cytogenetic-molecular risk, response and survival are defined according to 2022 ELN recommendations.

Cytogenetic and mutation testing

Review of cytogenetic status included karyotype based on a minimum of 20 metaphase cells from the bone marrow. Karyotypes were analyzed after G banding and defined according to International System for Human Cytogenetic Nomenclature.

Rapid detection of FLT3 and NPM1 mutation were performed as previously described [33]. Direct sequencing was performed using ABI PRISM 3700 DNA sequencing (Applied Biosystems©). Fifty ng of precipitated DNA was amplified, tested with agarose gel purified with EXOSAP, and was sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems©). To amplify NPM1-mutated transcripts, we prepared complementary DNA from total RNA. We detected MRD on reverse-transcriptase quantitative polymerase chain reaction using mutation-specific primers and probes. Assays were run in duplicate with commercial kit (Qiagen) on the ABI 7900 platform (Applied Biosystems), and mutated transcript levels were compared to expression of the ABL1 reference gene with the use of plasmid standards (Qiagen). Assays were performed under PCR conditions proposed by the Europe Against Cancer program, as in previous works on NPM1 (ref. 34,35). A panel with 30 genes were used for next generation sequencing

(Sophia© Myeloid Solution). Next generation sequencing was performed according to manufacturing instruction. Sequencing was performed on Illumina MiSeq© and results were analyzed using the Sophia© DDM platform.

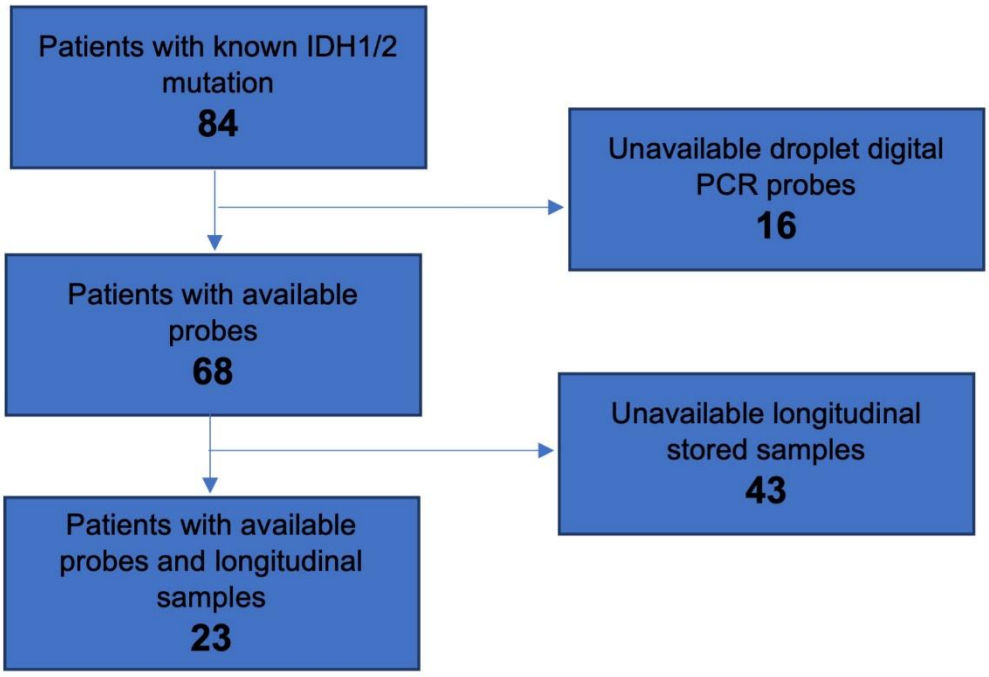
Droplet digital PCR

DdPCR was performed with Bio-Rad's Qx200 DdPCR System© according manufacture instructions. DdPCR for all the samples were performed in duplicate. 105 ng of DNA in 3 µl were diluted with 10 µl of Mastemix (Bio-Rad©), 1 µl of mutation-specific mutated probe, 1 µl of mutation-specific wild-type probe, and 5 µl of nuclease-free water. After preparation, droplet generator microfluidic generated droplets with oil (Bio-Rad©), then samples underwent 40 PCR cycles. Droplet reader Qx200 (Bio-Rad©) was used for droplet reading. Quanta Studio© software was used for data analysis. Serial dilutions were used to identify sensitivity limit of the method for each mutation included in the study. All the assays were performed in duplicate.

Statistics

Analyses were performed on the entire study population, if not differently specified. Rates and medians are reported descriptively with interquartile range (IQR). Receiver operating characteristic (ROC) curves were used to test sensitivity and specificity of sanger sequencing and ddPCR. Gold standard is defined for each test, as specified below. K-means were used to classify samples according to IDH1/2 fractional abundance when appropriate. Box plot representation includes median and 95% confidence interval. Survival was reported as a median and Kaplan Meier representation was provided. Subgroup analyses were conducted whenever appropriate. Differences in survival between sub-groups were assessed with 2-sided log-rank test. SPSS Statistic 25 was used for the analysis.

S2: Consort diagram of patients selected in this study from historical records and biobanking facility. IDH: isocitrate dehydrogenase



58 ***S3: Samples collected and analyzed at various timepoints considering patients from 1st sample***
59 ***in the study; Blue squares represent available and analyzed samples; grey squares represent***
60 ***unavailable samples; TP: time point; PT : patient.***

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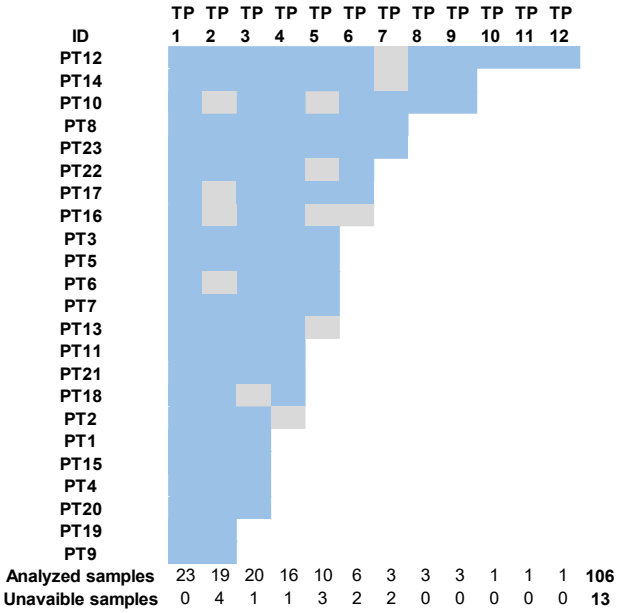

Timepoint	*Number of samples analyzed*	*Percent*
Diagnosis	*15*	*14%*
CR	*54*	*51%*
SD/relapse	*37*	*35%*

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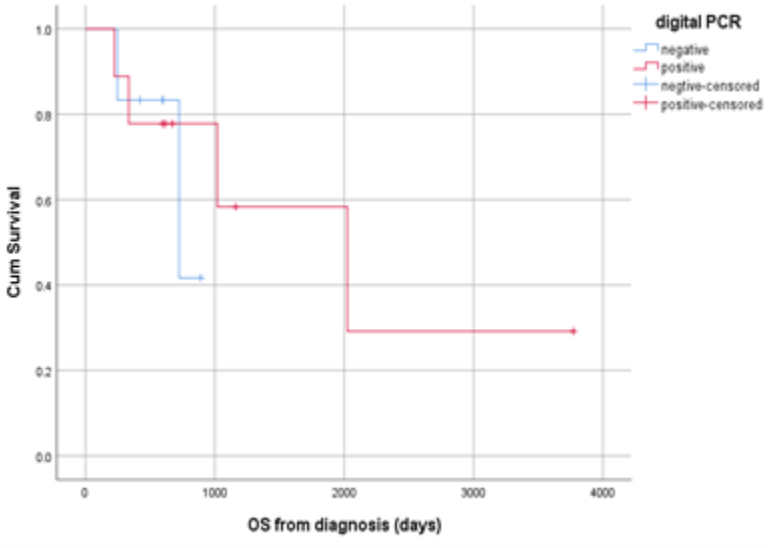
S4: Impact of DDPCR positivity on overall survival



S5: Cut-off for fractional abundance interpretation in different scenarios; orange zone represent values of c for which IDH1/2/ mutation is likely to be related to clonal hematopoiesis rather than AML; green zone represent values of c for which IDH1/2/ mutation is likely to be sub clonal; c= cut-off value; IDH: isocitrate dehydrogenase.

c	**IDH1/2 fractional abundance %**										
Bone marrow blasts %	**50.0**	**40.0**	**30.0**	**20.0**	**10.0**	**5.00**	**4.00**	**3.00**	**2.00**	**1.00**	**0.50**
0.50	0.01	0.01	0.02	0.03	0.05	0.10	0.13	0.17	0.25	0.50	1.00
1.00	0.02	0.03	0.03	0.05	0.10	0.20	0.25	0.33	0.50	1.00	2.00
2.00	0.04	0.05	0.07	0.10	0.20	0.40	0.50	0.67	1.00	2.00	4.00
5.00	0.10	0.13	0.17	0.25	0.50	1.00	1.25	1.67	2.50	5.00	10.0
6.00	0.12	0.15	0.20	0.30	0.60	1.20	1.50	2.00	3.00	6.00	12.0
7.00	0.14	0.18	0.23	0.35	0.70	1.40	1.75	2.33	3.50	7.00	14.0
8.00	0.16	0.20	0.27	0.40	0.80	1.60	2.00	2.67	4.00	8.00	16.0
9.00	0.18	0.23	0.30	0.45	0.90	1.80	2.25	3.00	4.50	9.00	18.0
10.0	0.20	0.25	0.33	0.50	1.00	2.00	2.50	3.33	5.00	10.0	20.0
15.0	0.30	0.38	0.50	0.75	1.50	3.00	3.75	5.00	7.50	15.0	30.0
20.0	0.40	0.50	0.67	1.00	2.00	4.00	5.00	6.67	10.0	20.0	40.0
30.0	0.60	0.75	1.00	1.50	3.00	6.00	7.50	10.0	15.0	30.0	60.0
40.0	0.80	1.00	1.33	2.00	4.00	8.00	10.0	13.3	20.0	40.0	80.0
50.0	1.00	1.25	1.67	2.50	5.00	10.0	12.5	16.7	25.0	50.0	100
60.0	1.20	1.50	2.00	3.00	6.00	12.0	15.0	20.0	30.0	60.0	120
100	2.00	2.50	3.33	5.00	10.0	20.0	25.0	33.3	50.0	100	200

S6: IDH1/2 fractional abundance kinetics in patients who obtained complete remission after intensive chemotherapy, azacytidine+venetoclax or IDH1 or IDH2 inhibitor.