## SUPPLEMENTAL DATA

**Supplemental Methods**

**Study group**
All patients underwent full diagnostic bone marrow evaluation at presentation, which included trephine biopsy, clot preparation, aspirate smears, and touch preparations. Wright-Giemsa-stained air-dried BM aspirate smears and/or touch preparations were evaluated, and the percentage of blast or blast-equivalent population was determined by manual differential count of 300-500 cells as part of standard clinical evaluation. Clinical and laboratory data at presentation were obtained by chart review of electronic medical records.

**Mutation analysis**
Mutation analysis was performed using DNA extracted from bone marrow aspirate samples in all patients using clinically validated next-generation sequencing (NGS) mutation panels. While panel design changed over the years (53-, 28-, and 81-gene panels), all provided substantial coverage of the $TP53$ gene [exons (codons): 2 (1-12), 4 (69-112), 5-7 (126-253), 8 (267-306), 10 (332-342; 2-11 (1-394); 1-12 (1-394); and, 2 (1-25), 4 (33-34), 4-11 (80-394), respectively]. In this study, data from the most current (81-gene) NGS mutation panel were used for genomic profiling of AML cases included in this study.

Sequencing libraries were prepared using 250 ng of genomic DNA and respective sequencing libraries were be ran on the Illumina MiSeq (Illumina, Inc., San Diego, CA, USA) sequencer. Integrative Genomics Viewer (IGV, Broad Institute) was used for variant calling. A minimum sequencing coverage of ×250 (bidirectional true paired-end sequencing) was required. The analytic sensitivity of the platforms was established at 1-5% mutant reads in a background of wild-type reads. Publically available knowledgebases (dbSNP, VarSome, ExAC Browser), data from in silico functional prediction tools (SIFT, PolyPhen), as well as variant allele frequency (VAF) were used to infer somatic origin.

The $TP53$ evolutionary action score (EAp53) was obtained from the EAp53 server at http://mammoth.bcm.tmc.edu/EAp53 (accessed in March 2021). For patients with multiple $TP53$ mutations, the variant with highest allelic frequency was used for EAp53 calculation.

**Conventional karyotyping, fluorescence in situ hybridization, and array chromosomal genomic hybridization**
Conventional karyotyping was performed on bone marrow aspirate material using standard methods as described previously. Conventional karyotyping was performed on bone marrow aspirate material using standard methods as described previously. Complex karyotype was defined as the presence of ≥3 independent chromosomal abnormalities in the absence of a WHO-designated AML-associated recurrent cytogenetic abnormality. Fluorescence in situ hybridization (FISH) was performed to confirm CN changes involving the $TP53$ locus. This was accomplished using a probe set specific for the $TP53$ locus at 17p13.1 and the centromeric region of chromosome 17 (CEP17) in cases with structural chromosome 17p alterations on conventional karyotyping. Array comparative genomic hybridization (aCGH) was performed using an oligonucleotide genomic array targeting cancer genes (4 x 180 K format; Agilent Technologies, Santa Clara, CA). The analytical sensitivity (lower limit of detection) in a given sample is approximately 1 in 5 (20%) aberration-containing cells. The average resolution of the assay is
25 kb. Cytogenetic and FISH findings were reported in accordance with the 2017 International System for Human Cytogenetic Nomenclature5.

Array comparative genomic hybridization (aCGH) was performed using an oligonucleotide genomic array targeting cancer genes (4 x 180 K format; Agilent Technologies, Santa Clara, CA). Copy number analysis was performed using a combined aCGH and single nucleotide polymorphism (SNP) platform (SurePrint G3, 4 x 180 K; Agilent Technologies, Santa Clara, CA) containing ~120,000 CGH and 60,000 SNP probes. Following extraction of genomic DNA from bone marrow aspirate samples, both patient and control DNA were subjected to restriction enzyme digestion using Alu1 and Rsal followed by labeling with Cy5-dUTP and Cy3-dUTP respectively (Agilent DNA Labeling Kit Plus) followed by hybridization per manufacturer’s recommendations. Reference human (female) DNA (Promega Corporation, Madison, WI) was used as control. The slides were scanned using a high-resolution microarray scanner (Agilent Technologies, CA) after washing. Data analysis was done using CytoGenomics software and interpretation was performed using standard cutoffs. The analytical sensitivity (lower limit of detection) in a given sample is approximately 1 in 5 (20%) aberration-containing cells.

**Immunohistochemistry and digital image analysis**

Immunohistochemistry for p53 detection was performed on automated Leica Bond stainers (Leica Biosystems, Buffalo Gove, Illinois) using 3-4 μm sections from formalin-fixed paraffin-embedded bone marrow tissue samples.6 The assay was validated on decalcified and non-decalcified tissue samples.6 The assay was validated on decalcified and non-decalcified tissue samples. The whole-slide digital scans of p53 immunohistochemistry slides were acquired using the Aperio ScanScope (Aperio Technologies, Vista, CA, USA) system and analyzed digitally using the nuclear algorithm of the Aperio ImageScope software (Aperio Technologies) as described previously.7 Digital image analysis parameters included staining intensity (0-3 scale; 0: no staining; 1+: weak; 2+: moderate; 3+: strong) and percentage of positive cells as a fraction of total bone marrow nucleated cells.8 Control bone marrow samples were used to optimize readout calibrations. Samples were considered adequate for evaluation if at least 1000 intact cells could be analyzed. All digital analysis data were confirmed by manual review. Image analysis was performed on a carefully selected subset of cases representative of various mutation classes and p53 protein domains within the retrospective cohort. All p53 immunohistochemistry stains performed on the prospective cohort were evaluated with digital image analysis regardless of their TP53 mutation status.

**References**

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**Supplemental Tables and Figures**

**Table S1.** Summary of acute myeloid leukemia frontline therapies.

|                        | Intensive Chemotherapy (N=84)                      | Lower-Intensity Chemotherapy (N=221)                  |
|------------------------|----------------------------------------------------|------------------------------------------------------|
|                        | Chemotherapy Type                                   | Chemotherapy Type                                    | N (%)                                      |
| No Venetoclax          | HiDAC-based Induction (CLIA, FLAG-IDA, etc.)        |                                                      | 55 (65)                                   |
|                        | CPX-351                                            |                                                      | 4 (5)                                     |
| Venetoclax-based       | CLIA + Venetoclax                                   |                                                      | 19 (23)                                   |
|                        | FLAG-Ida + Venetoclax                              |                                                      | 6 (7)                                     |
|                        | HMA-based Chemotherapy (N=100)                      |                                                      |                                          |
|                        | HMA-Combinations (non-Ven)                         |                                                      | 47 (21)                                   |
|                        | HMA Alone                                          |                                                      | 53 (24)                                   |
|                        | LDAC Based                                          |                                                      | 29 (13)                                   |
|                        | Other                                              |                                                      | 2 (1)                                     |
|                        | HMA + Venetoclax alone                             |                                                      | 51 (23)                                   |
|                        | HMA + Ven + 3rd Drug                                 |                                                      | 21 (10)                                   |
|                        | Cladribine + LDAC + Venetoclax                      |                                                      | 18 (8)                                     |

Abbreviations: HiDAC: High-dose araC; CLIA: cladribine, idarubicin, araC; FLAG-Ida: Fludarabine, araC, Idarubicin, GCSF; HMA: Hypomethylating agent; LDAC: Low-dose araC; Ven: venetoclax; 3rd Drug in HMA+Ven Combos included: Ivosidenib, Gilteritinib, Quizartinib, Ponatinib, Pevonidostat, APR-246, or gemtuzumab ozogamicin
Table S2. Comparison of *TP53* mutation types across AML-relevant parameters.

|                     | MS  | DEL  | NS   | FS   | SP  | Overall |
|---------------------|-----|------|------|------|-----|---------|
| **Total**           | N=333 (87.63%) | 8 (2.11%) | 24 (6.32%) | 15 (3.95%) | 380 (100%) |
| **AML WHO Category** |     |      |      |      |     |         |
| AML MRC             | 236 (88.39%) | 4 (1.50%) | 14 (5.24%) | 13 (4.87%) | 267 (70.26%) | 0.350 |
| AML NOS             | 10 (90.91%)  | 1 (9.09%) |           |      | 11 (2.89%) |
| AML post MPN        | 8 (88.99%)   |           |           |      | 9 (2.37%) |
| AML RGA             | 9 (90.00%)   | 1 (10.00%) |           |      | 10 (2.63%) |
| t-AML               | 70 (84.34%)  | 3 (3.61%) | 9 (10.84%) | 1 (1.20%) | 83 (21.84%) |
| **Number of TP53 mutations** |     |      |      |      |     |         |
| 1                   | 259 (88.40%) | 8 (2.73%) | 15 (5.12%) | 11 (3.75%) | 293 (77.11%) | 0.140 |
| >1                  | 74 (85.06%)  | 9 (10.34%) | 4 (4.60%) |      | 87 (22.89%) |
| **TP53 mutation & allelic state** |     |      |      |      |     |         |
| 1mut&CNloss         | 139 (84.76%) | 6 (3.66%) | 10 (6.10%) | 9 (5.49%) | 164 (61.65%) | 0.758 |
| 1mut&CNnormal       | 45 (91.84%)  | 1 (2.04%) | 3 (6.12%) |      | 49 (18.42%) |
| >1mut&CNloss        | 21 (84.00%)  | 2 (8.00%) | 2 (8.00%) |      | 25 (9.40%) |
| >1mut&CNnormal      | 25 (89.29%)  | 2 (7.14%) | 1 (3.57%) |      | 28 (10.53%) |
| **Complex cytogenetics** |     |      |      |      |     |         |
| No                  | 43 (91.49%)  | 1 (2.13%) | 2 (4.26%) | 1 (2.13%) | 47 (12.57%) | 0.809 |
| Yes                 | 284 (86.85%) | 7 (2.14%) | 22 (6.73%) | 14 (4.28%) | 327 (87.43%) |
Table S3. Clinical and pathologic characteristics of acute myeloid leukemia cases with TP53 copy neutral loss of heterozygosity.

| Number of TP53 mutations | TP53 VAF (%) | HGVS Nomenclature | p53 Domain | Mutation Type | Coverage | P53\textsuperscript{high} (%)\textsuperscript{**} | WHO Diagnosis | Cytogenetic Risk Group |
|--------------------------|--------------|--------------------|------------|---------------|----------|---------------------|--------------|----------------------|
| 2\textsuperscript{*}     | 88.37        | NM_000546.5(TP53):c.524G>A p.R175H | DBD        | Missense      | 6338     | n/a                 | AML-MRC      | Adverse              |
| 1                        | 95.42        | NM_000546.5(TP53):c.1024C>T p.R342* | TETRAMER   | Nonsense      | 3581     | 3.61                | AML-MRC      | Adverse              |
| 1                        | 33.37        | NM_000546.5(TP53):c.578A>T p.H193L | DBD        | Missense      | 859      | 40.2                | AML post MPN | Adverse              |
| 1                        | 88.67        | NM_000546.5(TP53):c.286del p.S96fs | OTHER      | Frameshift    | 2982     | n/a                 | AML-MRC      | Adverse              |
| 1                        | 93.00        | NM_000546.5(TP53):c.659A>G p.Y220C | DBD        | Missense      | n/a      | 31.1\textsuperscript{†} | AML-MRC      | Adverse              |

\*Values in table are for the dominant mutation; second mutation VAF: 6.12%

\**Percentage of nuclei with 3+ staining intensity by immunohistochemistry.

\†See Figure 3G.

Abbreviations: VAF: variant allelic frequency; HGVS: Human Genome Variation Society; DBD: DNA-binding domain; n/a: not applicable/available; IHC: immunohistochemistry; WHO: World Health Organization; AML-MRC: acute myeloid leukemia with myelodysplasia-related changes; MPN: myeloproliferative neoplasm.
Table S4. Optimal p53 immunohistochemistry cutoff point (% nuclei with 3+ staining intensity) in patients without truncating pattern.

| Method                        | Youden | ROC01     | MAXSpSe     |
|-------------------------------|--------|-----------|-------------|
|                               |        | 95%CI     | 95%CI       | 95%CI       |
|                               |        | Low       | Upper       | Low         | Upper       |
| Best cut-off value            | 9.40   | -         | -           | 9.40        | -           |
| Sensitivity                   | 89.36% | 81.30%    | 94.78%      | 89.36%      | 81.30%      |
|                               |        | 94.78%    |             | 94.78%      |             |
| Specificity                   | 95.18% | 88.12%    | 98.67%      | 95.18%      | 88.12%      |
|                               |        | 98.67%    |             | 98.67%      |             |
| Positive predictive Value     | 95.45% | 88.75%    | 97.84%      | 95.45%      | 88.75%      |
|                               |        | 97.84%    |             | 97.84%      |             |
| Negative Predictive Value     | 88.76% | 80.35%    | 96.74%      | 88.76%      | 80.35%      |
|                               |        | 96.74%    |             | 96.74%      |             |
| Positive Likelihood Ratio     | 18.54  | 7.11      | 48.36       | 18.54       | 7.11        |
|                               |        | 48.36     |             | 48.36       |             |
| Negative Likelihood Ratio     | 0.11   | 0.06      | 0.20        | 0.11        | 0.06        |
|                               |        | 0.20      |             | 0.20        |             |
| False Positive                | 4      | 4         | 7           |             |             |
| False Negative                | 10     | 10        | 8           |             |             |
| Optimal criterion             | 0.85   | 0.01      | 0.91        |             |             |
| Accuracy                      | 0.921  | 0.921     | 0.915       |             |             |
| AUC (p<0.001)                 | 0.965  | 0.939     | 0.991       |             |             |
**Table S5.** Concordance table and corresponding analytic performance metrics of digital image analysis-assisted p53 immunohistochemistry (7.2% cutoff, including truncating pattern) in predicting TP53 mutation status in acute myeloid leukemia.

| Molecular | Immunohistochemistry | Total |
|-----------|----------------------|-------|
|           | p53 Mutant Expression Pattern | p53 Wild-Type Expression Pattern |       |
| TP53 mutant | 120                  | 8     | 128    |
| TP53 wild-type | 7                    | 76    | 83     |
| Total    | 127                  | 84    | 211    |

| Statistic                  | Value       | 95% CI                   |
|----------------------------|-------------|--------------------------|
| Sensitivity                | 94.49       | 88.97% to 97.76%         |
| Specificity                | 90.48%      | 82.09% to 95.08%         |
| Positive Likelihood Ratio  | 9.92        | 5.13 to 19.20            |
| Negative Likelihood Ratio  | 0.06        | 0.03 to 0.13             |
| Positive Predictive Value  | 93.75%      | 88.57% to 96.67%         |
| Negative Predictive Value  | 91.57%      | 84.04% to 95.72%         |
| Accuracy                   | 92.89%      | 88.55% to 95.97%         |
Table S6. Cox proportional hazards multivariable analysis of factor association with leukemia-free survival among frontline acute myeloid leukemia patients with one or more TP53 mutations.

| Parameter                        | Full Model (N=123 with 115 events) | Reduced Model |
|----------------------------------|------------------------------------|---------------|
|                                  | HR (95%CI) | Individual P value | Overall P value | HR (95%CI) | Individual P value | Overall P value |
| Age, continuous                  | 1.00 (0.98, 1.02) | 0.8680 | 0.8680 | . | . |
| Cytogenetic Risk Group           | Intermediate/Favorable vs. Adverse | 0.41 (0.20, 0.84) | 0.0144 | **0.0144** | 0.57 (0.34, 0.97) | 0.0369 | **0.0369** |
| Allogeneic Stem Cell Transplantation* | Yes vs. No | 0.57 (0.29, 1.12) | 0.1018 | 0.1018 | . | . |
| TP53 Copy Number                 | Loss vs. Not loss | 0.64 (0.36, 1.14) | 0.1306 | 0.2695 | . | . |
| TP53 Mutation                    | >1 vs. =1 | 1.58 (0.95, 2.62) | 0.0781 | 0.0781 | . | . |
| TP53 VAF                         | >40% vs. <=40% | 1.96 (1.32, 2.92) | 0.0009 | **0.0009** | 1.89 (1.28, 2.77) | 0.0012 | **0.0012** |

*Time-dependent covariate.
| Parameter                                      | Full Model (N=223 with 205 events) | Reduced Model |                    |                    |                    |
|------------------------------------------------|---------------------------------|---------------|--------------------|--------------------|--------------------|
|                                                | HR (95%CI)                       | Individual P value | Overall P value | HR (95%CI) | Individual P value | Overall P value |
| Age, continuous                                | 1.01 (0.99, 1.02)                | 0.2890         | 0.2890             | .            | .                  |
| Cytogenetic Risk Group                         |                                 |                |                    | 0.49 (0.29, 0.81)| 0.0052             |
| Intermediate/Favorable vs. Adverse             | 0.45 (0.24, 0.81)                | 0.0082         | 0.0082             | 0.49 (0.29, 0.81)| 0.0052             |
| Allogeneic Stem Cell Transplantation**         |                                 |                |                    | 0.45 (0.27, 0.75)| 0.0025             |
| Yes vs. No                                     | 0.51 (0.29, 0.92)                | 0.0242         | 0.0242             | 0.45 (0.27, 0.75)| 0.0025             |
| TP53 Copy Number                               |                                 |                |                    | 0.45 (0.29, 0.81)| 0.0025             |
| Loss vs. Not loss                              | 0.92 (0.62, 1.37)                | 0.6886         | 0.7561             | 0.45 (0.29, 0.81)| 0.0025             |
| NA vs. Not loss                                | 0.85 (0.55, 1.31)                | 0.4608         |                    | 0.45 (0.29, 0.81)| 0.0025             |
| TP53 Mutation                                  |                                 |                |                    | 2.14 (1.51, 3.04)| <.0001             |
| >1 vs. =1                                      | 2.14 (1.51, 3.04)                | <.0001         | <.0001             | 2.16 (1.53, 3.05)| <.0001             |
| TP53 VAF                                       |                                 |                |                    | 2.16 (1.53, 3.05)| <.0001             |
| >40% vs. <=40%                                 | 1.83 (1.37, 2.46)                | <.0001         | <.0001             | 1.80 (1.35, 2.41)| <.0001             |
| P53^{high} Immunohistochemistry                |                                 |                |                    | 1.80 (1.35, 2.41)| <.0001             |
| >=20% vs. <20%                                 | 2.35 (1.17, 4.73)                | 0.0169         | 0.0544             | 2.37 (1.19, 4.75)| 0.0147             |
| NA vs. <20%                                    | 1.73 (0.94, 3.19)                | 0.0779         |                    | 1.71 (0.93, 3.13)| 0.0833             |

*Excluding p53^{truncated}

**Time-dependent covariate.
Figure S1. Study design and components. Inclusion criteria into this study included a diagnosis of acute myeloid leukemia and known TP53 mutation status. Patients in the prospective group were included without a priori knowledge of their TP53 mutation status. Mutation profiling (81-gene panel), TP53 copy number status, and p53 immunohistochemistry data were available on all patients in the prospective group and on 72, 101, and 110 patients in the retrospective group, respectively. Criteria for p53 immunohistochemistry evaluation on the retrospective group included availability of adequate bone marrow trephine biopsy material, known copy number status, all non-missense mutations, and representative missense mutations up to 3 representative cases per mutation where applicable. Outcome analysis was performed on 360 patients treated at our institution and with available outcome data. Abbreviations: FISH: fluorescence in situ hybridization; aCGH: array comparative genomic hybridization; SNP: single-nucleotide polymorphism analysis.
Figure S2. (A) Distribution of study group acute myeloid leukemia (AML) cases by number of TP53 mutations. (B) Number of TP53 mutations across World Health Organization diagnostic groups. (C) TP53 mutation types in cases with a single TP53 mutation across World Health Organization diagnostic groups.
Figure S3. Representative wild-type p53 expression pattern by immunohistochemistry in a bone marrow trephine biopsy with acute myeloid leukemia wild-type TP53. [20x; hematoxylin counterstain]