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.[1] 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].[2,3] 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[4]. Conventional karyotyping was performed on bone marrow aspirate material using standard methods as described previously[4]. 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 Nomenclature[5].

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 Rsa1 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.

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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8. Assi R, Gur HD, Loghavi S, et al. P53 protein overexpression in de novo acute myeloid leukemia patients with normal diploid karyotype correlates with FLT3 internal tandem duplication and worse relapse-free survival. *Am J Hematol*. 2018;93(11):1376-1383.

Supplemental Tables and Figures

Table S1. Summary of acute myeloid leukemia frontline therapies.

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

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 (88.89%)			1 (11.11%)	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&ICNnormal	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^{high} (%)**	WHO Diagnosis	Cytogenetic Risk Group
2*	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†	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**		**Low**	**Upper**
Best cut-off value	9.40	-	-	9.40	-	-	7.20	-	-
Sensitivity	89.36%	81.30%	94.78%	89.36%	81.30%	94.78%	91.49%	83.92%	96.25%
Specificity	95.18%	88.12%	98.67%	95.18%	88.12%	98.67%	91.57%	83.39%	96.54%
Positive predictive Value	95.45%	88.75%	97.84%	95.45%	88.75%	97.84%	92.47%	85.03%	96.71%
Negative Predictive Value	88.76%	80.35%	96.74%	88.76%	80.35%	96.74%	90.48%	82.18%	96.07%
Positive Likelihood Ratio	18.54	7.11	48.36	18.54	7.11	48.36	10.85	5.33	22.10
Negative Likelihood Ratio	0.11	0.06	0.20	0.11	0.06	0.20	0.09	0.05	0.18
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**		
	p53 Mutant Expression Pattern	**p53 Wild-Type Expression Pattern**	**Total**
***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.

Table S7. Cox proportional hazards multivariable analysis of factor association with overall survival among frontline acute myeloid leukemia patients with one or more *TP53* mutations and p53^{high} at a cutoff of 20%.*

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	Intermediate/Favorable vs. Adverse	0.45 (0.24, 0.81)	0.0082	0.0082	0.49 (0.29, 0.81)	0.0052	**0.0052**
Allogeneic Stem Cell Transplantation**	Yes vs. No	0.51 (0.29, 0.92)	0.0242	0.0242	0.45 (0.27, 0.75)	0.0025	**0.0025**
TP53 Copy Number	Loss vs. Not loss	0.92 (0.62, 1.37)	0.6886	0.7561		.	.
	NA vs. Not loss	0.85 (0.55, 1.31)	0.4608			.	.
***TP53* Mutation**	>1 vs. =1	2.14 (1.51, 3.04)	<.0001	<.0001	2.16 (1.53, 3.05)	<.0001	**<.0001**
***TP53* VAF**	>40% vs. <=40%	1.83 (1.37, 2.46)	<.0001	<.0001	1.80 (1.35, 2.41)	<.0001	**<.0001**
P53^{high} Immunohistochemistry	**>=20% vs. <20%**	2.35 (1.17, 4.73)	0.0169	0.0544	2.37 (1.19, 4.75)	0.0147	**0.0457**
	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

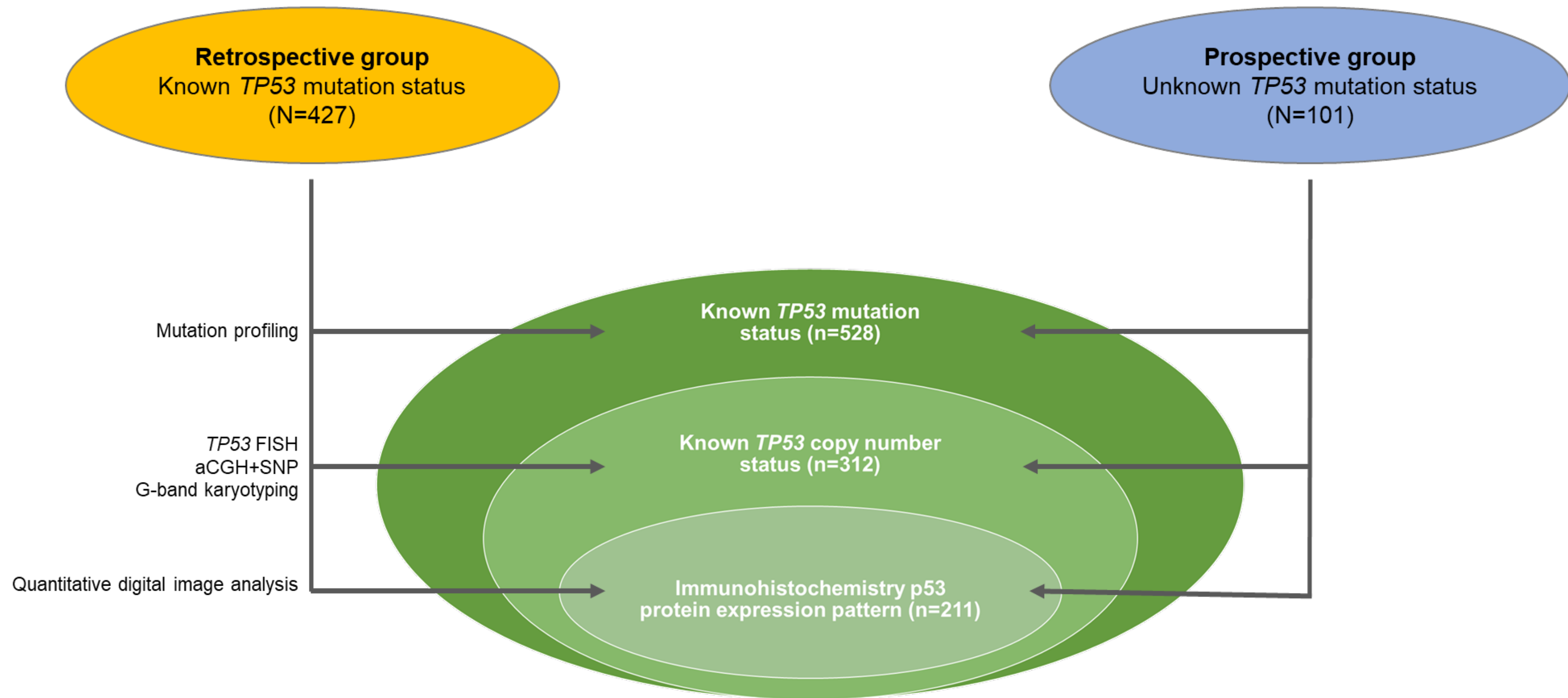

Figure S1. Study design and components. Inclusion criteria into this study included a diagnosis of acute myeloid leukemia and known *TP53* mutation status. Patients in the prospective group were included without *a priori* knowledge of their *TP53* mutation status. Mutation profiling (81-gene panel), *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

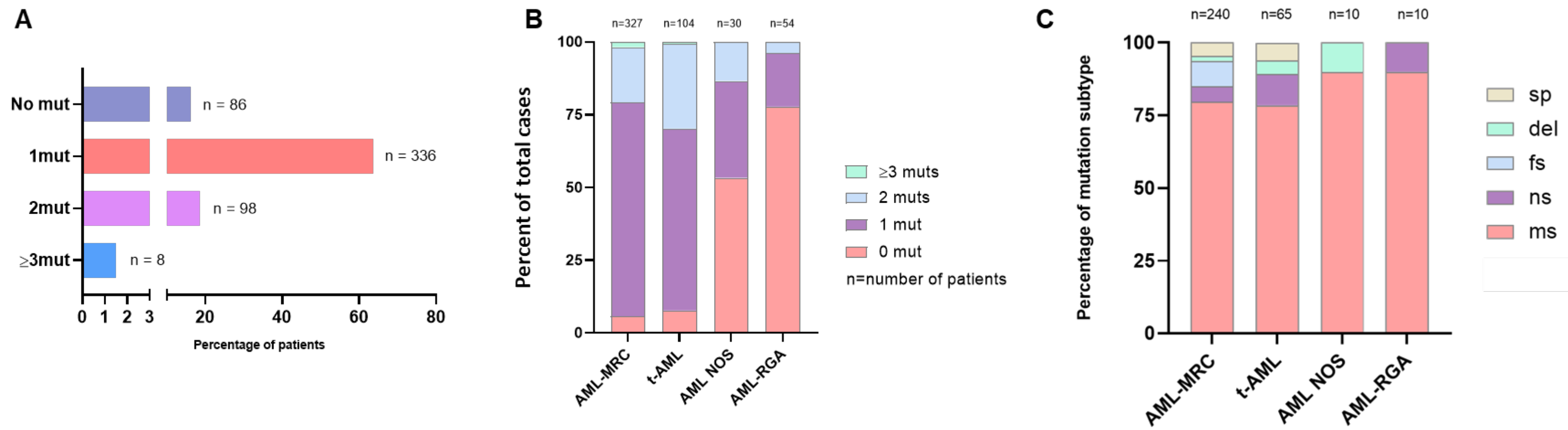

Figure S2. (A) Distribution of study group acute myeloid leukemia (AML) cases by number of *TP53* mutations. (B) Number of *TP53* mutations across World Health Organization diagnostic groups. (C) *TP53* mutation types in cases with a single *TP53* mutation across World Health Organization diagnostic groups.

Figure S3

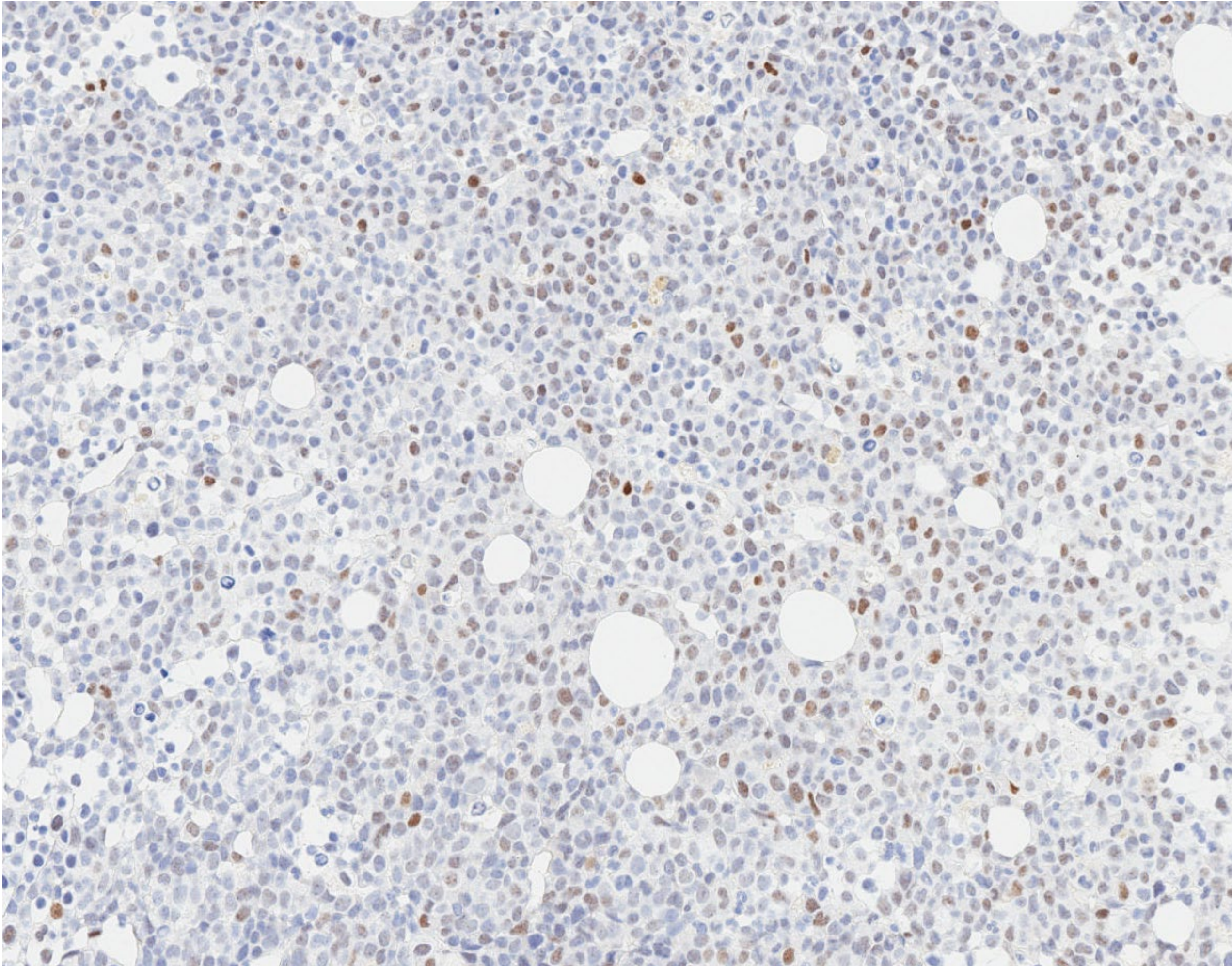

Figure S3. Representative wild-type p53 expression pattern by immunohistochemistry in a bone marrow trephine biopsy with acute myeloid leukemia wild-type *TP53*. [20x; hematoxylin counterstain]