

Immunohistochemistry for p53 is a useful tool to identify cases of acute myeloid leukemia with myelodysplasia-related changes that are *TP53* mutated, have complex karyotype, and have poor prognosis

Sebastian Fernandez-Pol, Lisa Ma, Robert S Ohgami and Daniel A Arber

Department of Pathology, Stanford University, Stanford, CA, USA

In this study, we evaluate the expression of p53 in core biopsies with acute myeloid leukemia and correlate the level of expression with acute myeloid leukemia subtype, *TP53* mutation status, karyotype, and survival. Of the 143 cases evaluated, 71 fulfilled the WHO 2016 criteria for acute myeloid leukemia with myelodysplasia-related changes, 40 were acute myeloid leukemia—not otherwise specified, 25 were acute myeloid leukemia with recurrent genetic abnormalities, and 7 were therapy-related acute myeloid leukemia. By immunohistochemistry, 17% showed p53 expression in >5% of the cells. Of the 24 cases with >5% p53-positive cells, 17 were acute myeloid leukemia with myelodysplasia-related changes, 5 were acute myeloid leukemia—not otherwise specified, 1 was acute myeloid leukemia with recurrent genetic abnormalities, and 1 was therapy-related acute myeloid leukemia. In cases for which data was available, expression of >5% p53-positive cells was significantly associated with genotype ($n=67$) and/or karyotype ($n=130$). Among the 115 cases for which clinical follow up was available, the overall survival of cases with p53 expression >15% (Median = 102 days) was significantly shorter compared with cases with p53 expression \leq 15% (Median = 435 days). Within the acute myeloid leukemia with myelodysplasia-related changes group, this association remained significant, with cases with \leq 15% p53-positive cells having a median overall survival of 405 days *versus* 102 days for cases with >15% p53-positive cells. Among acute myeloid leukemia with myelodysplasia-related changes cases with a complex karyotype, the finding of >15% p53-positive cells was significantly associated with worse overall survival. The poor prognosis associated with more than 15% p53-positive cells was independent of age and karyotype. In acute myeloid leukemia with myelodysplasia-related changes, p53 expression may be useful to infer *TP53* mutation status, complex karyotype, and/or poor prognosis in situations where other modalities are not readily available.

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Among the subtypes of acute myeloid leukemia, acute myeloid leukemia with multilineage dysplasia represents a particularly aggressive subtype with a high mortality and poor response to treatment.^{1–3} Although acute myeloid leukemia with myelodysplasia-related changes is defined by particular clinical, morphologic, and cytogenetic features, there remains significant heterogeneity in clinical behavior and response to

therapy among patients with this disease. For this reason, continued efforts to identify prognostic and predictive features are necessary to guide clinical decisions, including decisions regarding intensity of therapy.

The tumor suppressor *TP53* is the most commonly mutated gene in human cancers⁴ and mutations in *TP53* have been demonstrated in several studies to correlate with inferior overall survival among patients with acute myeloid leukemia and myelodysplastic syndrome.^{5–8} In addition, *TP53* mutations are found in ~70% of cases with a complex karyotype, while in cases with normal karyotype, mutations are comparatively rare.⁹ Although genotyping

Correspondence: Dr S Fernandez-Pol, MD, PhD, Department of Pathology, Stanford University, 300 Pasteur Drive, Lane 235, Stanford, CA 94305-5324, USA.

E-mail: sfernand@stanford.edu

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TP53 in clinical specimens has become relatively common, immunohistochemical staining for p53 has been shown to correlate with the presence of missense mutations in *TP53* in several tumor types, and thus has some utility in identifying malignancies with *TP53* mutations which have a worse prognosis.^{10,11} Expression of p53 in acute myeloid leukemia has been examined previously,^{12,13} but limited data are available regarding p53 expression in different World Health Organization acute myeloid leukemia subtypes. In therapy-related myeloid neoplasms, previous work has shown that overexpression of p53 correlates with *TP53* mutation and independently predicts a decreased overall survival.¹⁴ In acute myeloid leukemia arising from myelodysplastic syndrome, p53 was found to be expressed in $\geq 5\%$ of cells in 9 of 39 cases.¹⁵ In this same study, a p53 expression level of $\geq 5\%$ did not stratify higher-risk myelodysplastic syndrome and secondary acute myeloid leukemia into groups with different overall survival. Further work is necessary to evaluate the utility of p53 immunohistochemistry in other acute myeloid leukemia subtypes.

In this study, we set out to correlate expression of p53 with acute myeloid leukemia subtype, karyotype, *TP53* genotype, and survival in a cohort of 143 cases of acute myeloid leukemia. We find that cases of acute myeloid leukemia with a high level of p53 expression are typically acute myeloid leukemia-myelodysplasia-related changes as opposed to acute myeloid leukemia—not otherwise specified or acute myeloid leukemia with recurrent genetic abnormalities. In addition, cases with higher p53 expression frequently have *TP53* mutations, complex karyotypes and show a worse overall survival. Our data suggest that p53 immunohistochemistry may provide useful prognostic information in cases of acute myeloid leukemia with myelodysplasia-related changes.

Materials and methods

Case Selection and Immunohistochemistry

We identified 143 cases of acute myeloid leukemia from the Stanford Department of Pathology (obtained from 12 May 2004 to 25 October 2013) for which the original diagnostic bone marrow core biopsy was available. Cases were classified according to the most recently available 2016 World Health Organization categories.^{1,3} After fixation in Bouin solution and decalcification in Formical-4 (StatLab, McKinney, TX, USA), sections of the paraffin-embedded core biopsy were stained with an antibody to p53 (clone DO-7 mouse, Ventana, 1:400 dilution, Ventana CC1 retrieval solution, pH 6.0). For the entire cohort, core biopsies were considered adequate for evaluation if there were at least ~ 1000 cells present for evaluation and this was the case even for biopsies with hypocellular marrow. This study was approved by Stanford University's institutional review board.

Method for Quantifying p53 Expression

Examination of the 143 cases revealed that the majority of the core biopsies showed only rare ($< 1\%$) nuclei with dim to moderate expression of p53. These cases also tended to be those that lacked a detectable *TP53* mutation (discussed in detail in the Results section). In contrast, a subset of cases, which tended to have nonsynonymous *TP53* mutations, showed a higher proportion of p53-positive nuclei with intensity ranging from dim to bright. Given that the background level of expression of p53 in *TP53* WT cases was very low in terms of the proportion of positive cells as well as intensity, we reasoned that an increased proportion of even dimly positive cells was significant. For this reason, we chose to count dimly positive cells in all of the counts used in this study. We also think that counting every nucleus that is at least dimly positive may have the added benefit of reducing the interobserver variability compared with choosing a threshold at a moderate level of intensity. The p53-stained core biopsies were evaluated and the percent of all nucleated cells with at least 1+ positive nuclei (defined as above background as compared with negative cases) was determined by manual counting. In core biopsies with variability in p53 staining in different fields, hotspots were counted. After close evaluation, it was decided that precise quantification of p53 staining in cases in the 0–5% ($n=119$) group could not be reliably subcategorized by manual counting without a high degree of interobserver variability. The cases in which p53 expression was greater than 5% were precisely quantified by taking pictures of two optical fields at 200x magnification, printing images of the fields and manually counting the number of p53-positive nuclei. The average total number of cells counted per case was 1790 and the median was 1860 (range 1000–2925). For 15 cases with p53 score $> 5\%$, a second observer counted at least 1000 cells to determine the percent of cells expressing at least dim nuclear p53. A linear regression comparing the two observers resulted in a line with an $R^2=0.97$ (Supplementary Figure 1).

TP53 Sequencing

For 67 of the cases, unstained aspirate smears or frozen bone marrow samples were available to evaluate the *TP53* gene by Sanger sequencing to detect the presence of known frequently occurring mutations. Targeted sequencing of the *TP53* gene was performed as described previously¹⁶ with three primer sets: Set 1 Forward 5'-TCTGTCTCCTTCCTC TTCTAC-3' and Reverse 5'-CTGCTCACCATCGCT ATCTG-3'; Set 2 Forward 5'-CACATGACGGAGGT TGTGAG-3' and Reverse 5'-TAGGGCACCACCACA CTAT-3'; Set 3 Forward 5'-CCTGATTTCTTACT GCCTCTT-3' and Reverse 5'-TCTTGTCTCTGCTTG CTTACC-3'.

Statistical Analysis

Statistical tests used in this study are indicated in the text and in the figure legends and were performed using SPSS software (SPSS, Chicago, IL, USA) and/or R software (<https://cran.r-project.org/>). Cox proportional hazard model was used for univariate and multivariate analyses for overall survival.

Results

Patient Cohort

A summary of cohort characteristics is provided in Table 1. The average age of the study cohort was 55.9 years old (median 60, range 5–80) and consisted of 50% females. Of the 143 cases, 71 (50%) fulfilled the WHO 2008 criteria for acute myeloid leukemia with myelodysplasia-related changes, 40 (28%) were acute myeloid leukemia–not otherwise specified,

25 (18%) were acute myeloid leukemia with recurrent genetic abnormalities, and 7 (5%) were therapy-related acute myeloid leukemia.

p53 Expression in 143 Cases of AML

Of the 143 cases, 24 (17%) contained more than 5% of the cells in the marrow with nuclear p53 expression above background. Among the 119 cases that we categorized as having $\leq 5\%$ p53-positive cells, cases either showed no definite p53-positive cells or at most fewer than 5% nuclei with very dim staining. Within each case with $> 5\%$ p53-positive nuclei, the staining intensity ranged from very dim to very bright. Examples of p53-stained core biopsies, together with relevant clinical information, are provided in Figure 1.

On the basis of the morphologic features, it appeared that the majority of the positive cells were blasts. In 5 cases, p53 expression was seen in

Table 1 Summary of cases evaluated in this study

Case type	Age (mean, median, range)	Males (%)	Complex karyotype ^a	TP53 mutation ^b	Cases with $> 5\%$ p53-positive cells	Cases with $> 15\%$ p53-positive cells
All cases <i>n</i> = 143	55.9 y 60.0 y 5–80	72 (50.3%)	28 of 130 (21.5%)	12 of 67 (17.9%)	24 (17%)	14 (9.8%)
AML-MRC <i>n</i> = 71 49.7%	56.7 y 60 y 11–76	40 (56.3%)	26 of 63 (41.3%)	10 of 33 (30.3%)	17 (24%)	11 (16%)
AML-NOS <i>n</i> = 40 28.0%	59.1 y 61 y 28–80	15 (37.5%)	0 of 36 (0%)	1 of 12 (8.3%)	5 (13%)	2 (5%)
AML-RGA <i>n</i> = 25 17.5%	46.3 y 50 y 5–78	13 (52.0%)	0 of 24 (0%)	0 of 15 (0%)	1 (4%)	0 (0%)
AML-T <i>n</i> = 7 4.9%	63.3 y 61 y 55–76	4 (57.1%)	2 of 7 (28.6%)	1 of 7 (14.3%)	1 (14%)	1 (14%)

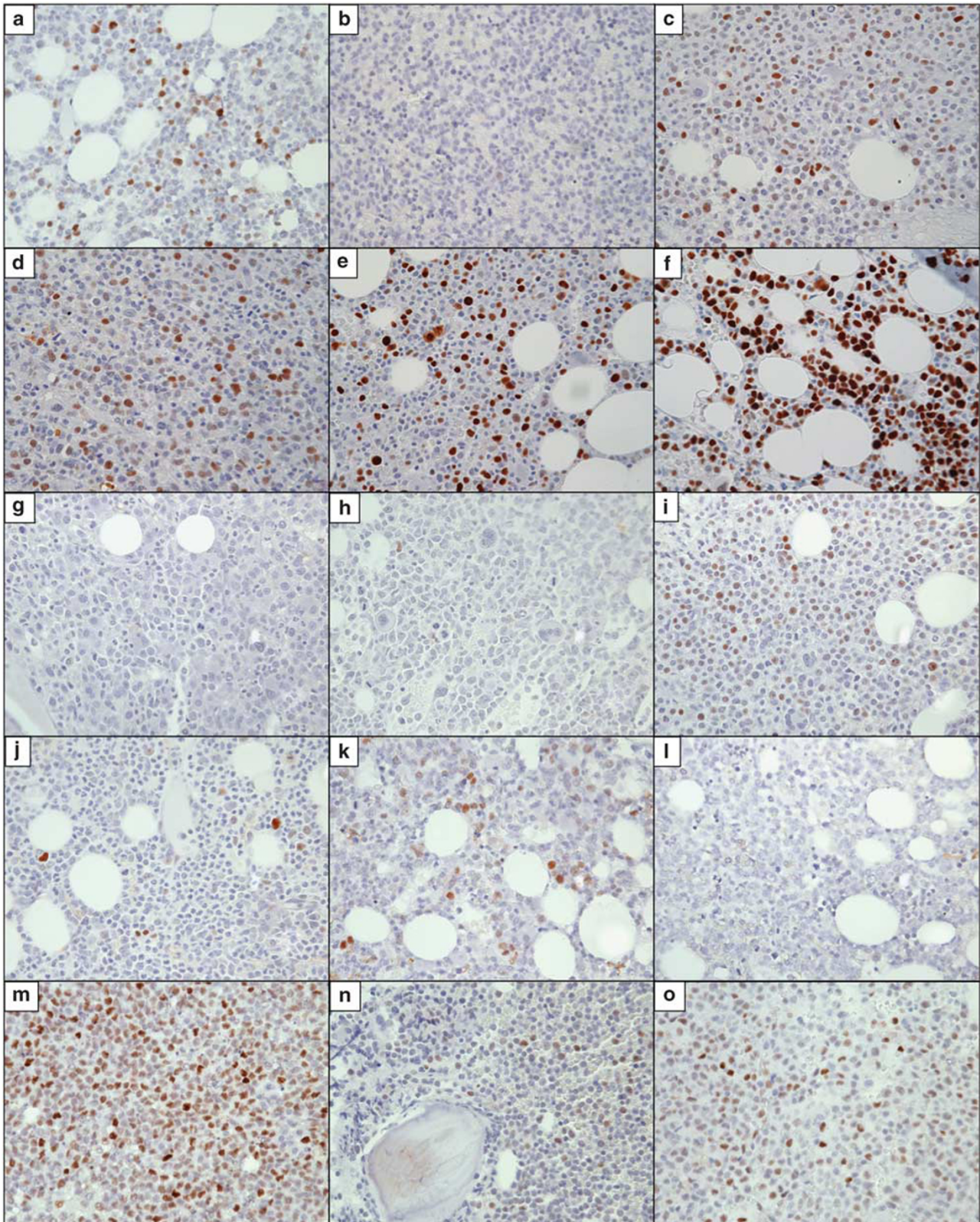
Abbreviations: AML, acute myeloid leukemia; MRC, myelodysplasia-related changes; RGA, recurrent genetic abnormalities; T, therapy related.

^aKaryotype data were available for 130 of the cases.

Figure 1 Representative core biopsies stained with p53 antibody. (a) Acute myeloid leukemia–not otherwise specified, *TP53* not sequenced, 12.5% p53-positive cells by manual count. Patient alive at 1771 days. (b) Acute myeloid leukemia–not otherwise specified with normal karyotype, *TP53* not sequenced, patient alive at 319 days, 4% p53-positive cells by manual count. (c) Acute myeloid leukemia with myelodysplasia-related changes with complex karyotype, *TP53* not sequenced, 16% positive cells, patient deceased at 10 days. (d) Acute myeloid leukemia with myelodysplasia-related changes with complex karyotype, *TP53* not sequenced, 19% p53-positive cells, patient deceased at 102 days. (e) Acute myeloid leukemia with myelodysplasia-related changes with complex karyotype, *TP53* not sequenced, 26% p53-positive cells, patient deceased at 247 days. (f) Acute myeloid leukemia with myelodysplasia-related changes with complex karyotype with *TP53* mutation (C176F) detected, 79% p53-positive cells, patient alive at 286 days. (g) Acute myeloid leukemia with myelodysplasia-related changes with complex karyotype, frameshift *TP53* mutation, clinical follow up not available. (h) Acute myeloid leukemia with myelodysplasia-related changes (biphenotypic) with complex karyotype, only rare positive cells, no *TP53* mutation detected, patient alive at 234 days. (i) Acute myeloid leukemia with myelodysplasia-related changes with complex karyotype, R273H *TP53* mutation detected, 14% p53-positive cells, clinical follow up not available. (j) Therapy-related acute myeloid leukemia with normal karyotype with rare strongly positive cells, no *TP53* mutation detected, patient deceased at 189 days. (k) Acute myeloid leukemia with myelodysplasia-related changes normal karyotype with 11% p53-positive cells, no *TP53* mutation detected, clinical follow up not available. (l) Acute myeloid leukemia with myelodysplasia-related changes complex karyotype with rare dim positive cells, R196* *TP53*, patient deceased at 594 days. (m) Acute myeloid leukemia–not otherwise specified with WT *TP53*, alive at 423 days of follow up with 46% p53-positive cells. (n) Acute myeloid leukemia–not otherwise specified with *TP53* V274F mutation and 7% p53-positive cells. (o) Acute myeloid leukemia with recurrent genetic abnormalities with mutated NPM1, wild-type *TP53*, 15% p53-positive cells.

megakaryocyte nuclei (Supplementary Table 4). Four of these cases were acute myeloid leukemia with myelodysplasia-related changes and one was therapy-related acute myeloid leukemia. All of these

cases had a complex karyotype and the proportion of p53-positive cells in the 5 cases ranged from 16 to 53%. *TP53* sequence data was available for the therapy-related acute myeloid leukemia case,



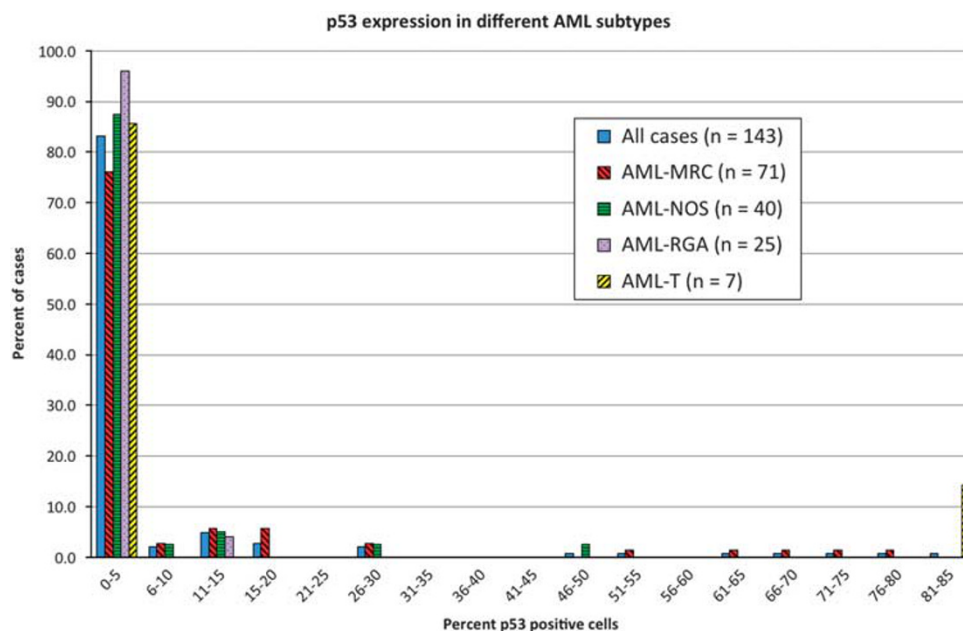


Figure 2 Percent of cases in each category with the indicated level of p53-positive cells. AML-MRC, acute myeloid leukemia with myelodysplasia-related changes, AML-NOS, acute myeloid leukemia—not otherwise specified, AML-RGA, acute myeloid leukemia with recurrent genetic abnormalities, AML-T, therapy-related acute myeloid leukemia.

which showed a mutation leading to an R175H substitution.

On the basis of our evaluation of the stained biopsies, it appeared that distinction of a true p53 ‘null’ phenotype, characterized by a complete absence of detectable p53 expression, was difficult to discern from cases with a very low level of p53 expression. In addition, our cohort contained only two cases in which the *TP53* genotype might predicted to yield a p53 null phenotype due to loss of a significant portion of the WT protein. Both of these cases were acute myeloid leukemia with myelodysplasia-related changes and both showed very few p53-positive cells and included 1 case with a frameshift mutation (shown in Figure 1g) and 1 case with a nonsense mutation (shown in Figure 1l). Our conclusion was that it was difficult or impossible to reliably distinguish these cases from the numerous other cases with less than 5% p53-positive cells that were *TP53* WT.

Because of the difficulty in accurately quantifying p53 expression at low levels, we grouped cases with less than or equal to 5% p53-positive cells into one bin, which contained 119 of the cases. We grouped cases with >5% expression into bins with intervals of 6–10%, 11–15%, 15–20%, 21–25%, etc. Of the 24 cases with >5% p53-positive cells, 17 were acute myeloid leukemia with myelodysplasia-related changes (71%), 5 were acute myeloid leukemia—not otherwise specified (21%), 1 was therapy-related acute myeloid leukemia (4%), and 1 (4%) was acute myeloid leukemia—with recurrent genetic abnormalities. The distribution of p53 expression for all of the cases, as well as for each

acute myeloid leukemia subcategory, is shown in Figure 2.

Five Cases of Acute Myeloid Leukemia–Not Otherwise Specified and 1 Case of Acute Myeloid Leukemia With Recurrent Genetic Abnormalities With Elevated p53 Expression

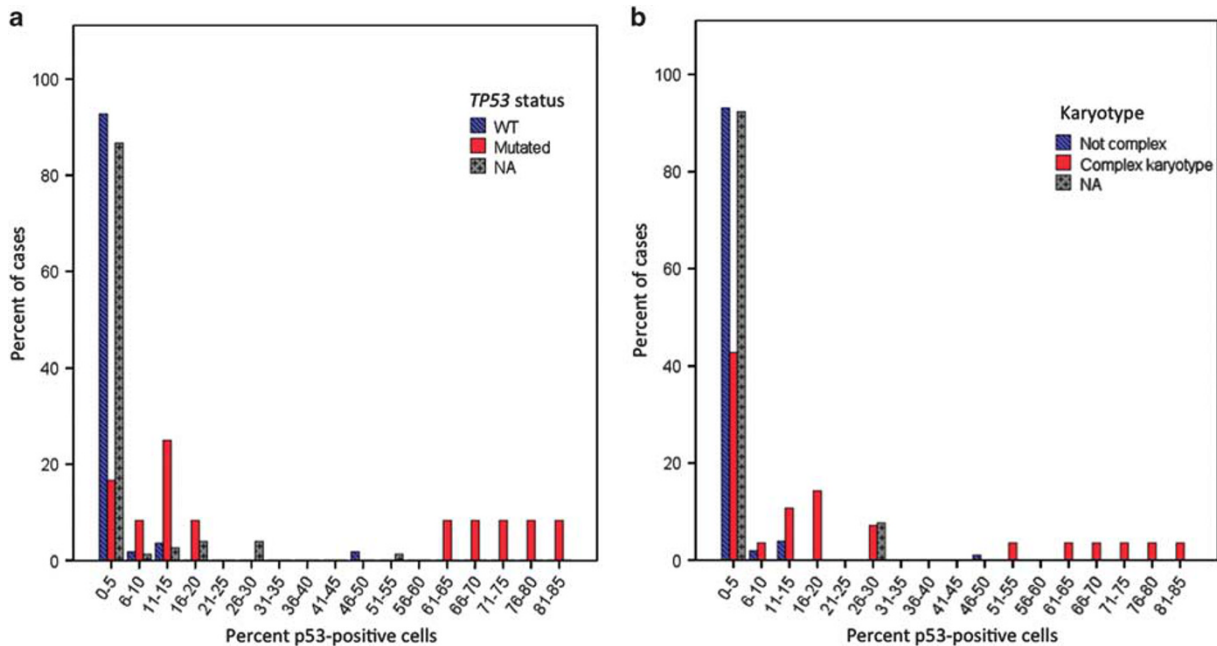
While cases with an elevated proportion of p53-positive cells were more frequently seen in the acute myeloid leukemia with myelodysplasia-related changes subcategory, 5 cases of acute myeloid leukemia—not otherwise specified and 1 case of acute myeloid leukemia with recurrent genetic abnormalities showed >5% p53-positive nuclei. Details regarding these cases are provided in Table 2 and representative images of a subset of these cases are provided in Figure 1 (images a, n, and o). Thus, while an elevated proportion of p53-positive cells is found more frequently in acute myeloid leukemia with myelodysplasia-related changes (Table 1), our data does not support the notion that positive p53 immunohistochemistry can be used as a surrogate for acute myeloid leukemia with myelodysplasia-related changes.

High p53 Expression Correlates With *TP53* Mutation

Evaluation of the 67 cases for which *TP53* genotype data was available showed that cases with higher p53 expression appeared to be enriched for cases with non-synonymous mutations in *TP53* (Figure 3a). There were two cases with *TP53* mutations in which the proportion of p53-positive

Table 2 Five cases of acute myeloid leukemia—not otherwise specified (AML-NOS) and one case of acute myeloid leukemia with recurrent genetic abnormalities (AML-RGA) with elevated p53 expression

AML subtype	p53-positive cells	Follow up	TP53 status
AML-NOS Figure 1a	12.5%	Alive at 1771 days	Not available
AML-NOS Figure 1n	7.1%	Not available	V274F
AML-NOS	11%	Died at 31 days	Not available
AML-NOS	27%	Died at 27 days	Not available
AML-NOS	46%	Alive at 423 days	WT
AML-RGA (NPM1 mutated) Figure 1o	15%	Not available	WT

**Figure 3** Distribution of p53 expression among cases for which TP53 was genotyped (**a**, $n = 67$) and for which karyotype data was available (**b**, $n = 130$). In (**a**) and (**b**), the * highlights one case of acute myeloid leukemia—not otherwise specified with normal karyotype from a patient who was alive at 423 days of follow up. WT, wild type. NA indicates cases for which TP53 was not sequenced (**a**) and cases for which karyotype was not available (**b**).

cells was $< 5\%$. One case had a frameshift mutation and another case had an R196* nonsense mutation (representative image of the latter case provided in Figure 1l). In examining these two cases, we could not identify a staining pattern that made these cases clearly discernible from the numerous wild-type TP53 cases with $< 5\%$ p53-positive cells.

Using the Fisher's exact test, a cutoff of $> 5\%$ was significantly associated with TP53 mutation ($P < 0.0001$). In order to evaluate the performance characteristics of utilizing p53 expression as a predictor for TP53 mutation, we constructed a receiver operating characteristic curve using different p53 expression levels and the results are shown in Table 3. The area under the curve when using p53 expression to detect TP53 mutations was 0.873 (95% CI = 0.745–1.000). Using a cutoff of $> 5\%$ p53-positive cells resulted in a sensitivity of 83% and a specificity of 89% for detecting a TP53 mutation.

Using a cutoff of $> 10\%$ reduced the sensitivity to 0.75 and yielded an increase in specificity to 91%.

As most of the cases that express p53 at $> 5\%$ in our study were acute myeloid leukemia with myelodysplasia-related changes, we performed a receiver operating characteristic curve analysis to evaluate the utility of using p53 expression for predicting a TP53 mutation in this subset of acute myeloid leukemias. As shown in Table 3, the area under the curve (0.844 95% CI = 0.689–0.999) was similar to that seen when including all acute myeloid leukemia subtypes. The sensitivities and specificities using > 5 , > 10 , and $> 15\%$ cutoffs were also similar to those calculated when including all cases for which TP53 was genotyped. We conclude that p53 expression as determined by immunohistochemistry accurately predicts TP53 mutation status in acute myeloid leukemia when more than 5% of cells are positive.

Table 3 Results of receiver operating characteristic curve for p53 expression and *TP53* mutation

	<i>TP53</i> status	Number of cases	AUC 95% CI	Sensitivity			Specificity		
				> 5%	> 10%	> 15%	> 5%	> 10%	> 15%
All cases	WT	55	0.873	0.83	0.75	0.5	0.89	0.91	0.94
	Mutated	12	0.745–1.000						
AML-MRC	WT	23	0.844	0.8	0.8	0.5	0.85	0.89	0.90
	Mutated	10	0.689–0.999						

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplasia-related changes, AUC, area under the curve.

Association Between p53 Expression and Complex Karyotype

In our cohort, information regarding karyotype was available for 130 cases, of which 102 (78%) had a normal karyotype and 28 (22%) had a complex karyotype. Of these 130 cases, 63 (48%) were acute myeloid leukemia with myelodysplasia-related changes, 36 (28%) were acute myeloid leukemia—not otherwise specified, 24 (18%) were acute myeloid leukemia with recurrent genetic abnormalities, and 7 (5%) were therapy-related acute myeloid leukemia. To evaluate the association between p53 expression and karyotype, we examined the distribution of p53 expression and observed that cases with higher levels of p53 expression appeared to consist preferentially of cases with complex karyotype (Figure 3b). A cutoff of >5% p53-positive cells was significantly associated with a complex karyotype ($P < 0.0001$, Fisher's exact test). To evaluate the sensitivity and specificity of p53 immunohistochemistry for detecting cases with complex karyotype, we created a receiver operating characteristic curve using the two variables. Using all subtypes of acute myeloid leukemia, the calculated area under the curve was 0.761 (95% CI = 0.642–0.880) and cutoffs of >5, >10, and >15% resulted in specificities of >90% but low sensitivities for detecting cases with complex karyotype (Table 4). Restricting the analysis to cases of acute myeloid leukemia with myelodysplasia-related changes, and using the same p53 expression cutoffs, similarly showed specificities of >90% but low sensitivities. We conclude that expression p53 in >5% of cells in a bone marrow core biopsy involved by acute myeloid leukemia is not particularly sensitive but is highly specific for a complex karyotype.

p53 Expression in Cases With Abnormalities of Chromosome 17

As the *TP53* gene resides on the short arm of chromosome 17 (17p13),¹⁷ we decided to evaluate whether p53 expression was related to cytogenetic abnormalities that included this region. Eleven of the 130 that were evaluated for cytogenetic abnormalities had -17 on karyotype. Of these 11 cases, 10 cases were acute myeloid leukemia with

myelodysplasia-related changes and 1 case was therapy-related acute myeloid leukemia and 5 of the 11 cases had >5% p53-positive cells. *TP53* was sequenced in 7 of the 11 cases, and 5 of these had a mutation in the gene, consistent with previous observations in other malignancies in which loss of one *TP53* gene is often associated with mutation in the remain *TP53* allele.¹⁸ The 2 cases that had a -17 and were *TP53* WT were both acute myeloid leukemia with myelodysplasia-related changes with ≤5% p53-positive cells.

p53 Expression is an Independent Poor Prognostic Factor

In order to determine whether p53 expression correlates with prognosis, we obtained progression-free survival and overall survival for 115 cases for which follow-up data were available. Evaluation of different cutoffs of p53 expression showed that there was no effect on progression free survival but that overall survival was significantly shorter in cases with >5% p53 expression when examining all subcategories together as well as when restricting the analysis to cases of acute myeloid leukemia with myelodysplasia-related changes or simply acute myeloid leukemia with myelodysplasia-related changes cases with complex karyotype (Figure 4). We tested the effect of different cutoffs of p53 expression on overall survival and found that cutoffs between >5% and >20% stratified all acute myeloid leukemia cases into prognostically significant groups (Supplementary Table 1). These same cutoffs remained significantly associated with overall survival when limiting the survival analysis to the acute myeloid leukemia with myelodysplasia-related changes group (Supplementary Table 2). Within the group of acute myeloid leukemia with myelodysplasia-related changes with complex karyotype, cutoffs of >5 to >15% stratified these cases into prognostically significant groups (Supplementary Table 3).

To determine if the prognostic impact of p53 expression was independent of the factors of age, karyotype, and *TP53* genotype, we initially performed univariate regression analysis (Table 5). This analysis showed that age, karyotype, and p53 score (using a cutoff of >15%) were significantly

Table 4 Results of receiver operating characteristic curve for p53 expression and karyotype

Karyotype	Number of cases	AUC 95% CI	Sensitivity			Specificity		
			> 5%	> 10%	> 15%	> 5%	> 10%	> 15%
<i>All cases</i>								
Normal karyotype	102	0.761	0.57	0.54	0.43	0.93	0.95	0.98
Complex karyotype	28	0.642–0.880						
<i>AML-MRC</i>								
Normal karyotype	39	0.776	0.58	0.54	0.42	0.96	0.98	1
Complex karyotype	26	0.650–0.903						

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplasia-related changes, AUC, area under the curve.

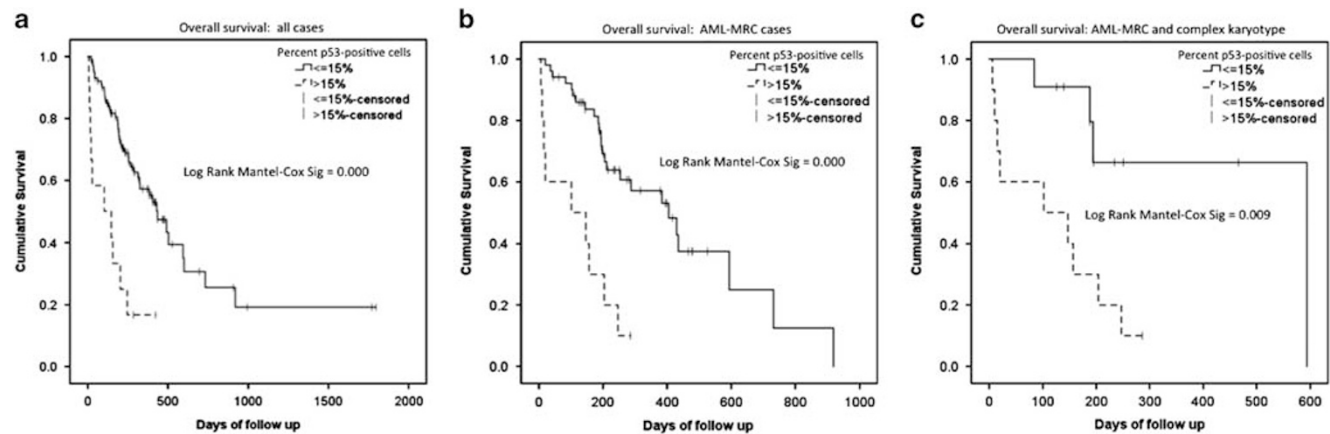


Figure 4 Overall survival in cases with $\leq 15\%$ p53 expression compared with $> 15\%$ p53 expression in all cases of acute myeloid leukemia (a, $n = 115$), cases of acute myeloid leukemia with myelodysplasia-related changes (b, $n = 61$), and acute myeloid leukemia-myelodysplasia-related changes cases with complex karyotype (c, $n = 21$). Kaplan–Meier curves and statistical tests were performed using IBM SPSS software. AML-MRC, acute myeloid leukemia with myelodysplasia-related changes.

Table 5 Univariate analyses for overall survival in all cases with clinical follow up

All cases	Hazard ratio for death	95% confidence interval	P-value ^a
Age, $n = 115$	1.03	1.01–1.05	0.002
TP53 mutation, $n = 47$	2.43	0.80–7.4	0.12
Complex karyotype, $n = 105$	2.51	1.33–4.74	0.005
p53 expression $> 15\%$, $n = 87$	4.13	2.0–8.3	7.9×10^{-5}

^aWald test.

associated with survival. The lack of an association with survival with *TP53* mutational status in our analysis is likely because only 47 of the cases that were genotyped for *TP53* had data regarding overall survival. Subsequent multivariate analysis using age, karyotype, and p53 expression cutoff of $> 15\%$ showed that age and p53 expression were independently significantly associated with survival (Table 6).

A similar analysis using the variables of age, karyotype and p53 expression performed with only acute myeloid leukemia with myelodysplasia-related changes cases showed that age and p53 expression

were associated with hazard of death in univariate analysis (Table 7). In a multivariate analysis using age and p53 expression, a p53 expression level of $> 15\%$ was associated with a hazard ratio (HR) for death of 4.7 (95% CI 2.08–10.6; $P = 0.0002$; Table 8). Age was also independently associated with death (HR = 1.03, 95% CI 1.003–1.06, $P = 0.033$). As seen in the analysis performed using all cases, in the acute myeloid leukemia with myelodysplasia-related changes subgroup, the presence of *TP53* mutation was not independently associated with an increased risk of death. The discrepancy between the association between *TP53* mutation and p53 expression

may be due to the fewer number of cases for which *TP53* mutation and survival data were available ($n=46$, with 20 events) compared with the number of cases for which data regarding p53 expression and survival data were available ($n=105$, with 48 events).

Discussion

Our findings largely agree with previous work showing that p53 is overexpressed in myeloid neoplasms with nonsynonymous *TP53* mutations.^{19,20} Lepelley *et al*¹² reported that 2 of 19 cases of acute myeloid leukemia demonstrated detectable p53 expression by immunohistochemistry and these cases also had missense *TP53* mutations. In this study two other cases with no p53-positive cells had a nonsense mutation or a frameshift mutation in *TP53*. However, this study preceded subclassification of acute myeloid leukemia into more modern prognostically relevant subtypes.¹² A later study by Zolota and colleagues evaluated p53 expression in 42 bone marrow biopsies classified according to FAB classification.¹³ In contrast to our findings, in their study, they found a higher proportion of cases with an elevated fraction of p53-positive nuclei. Specifically, 81% of the cases in their cohort showed $\geq 10\%$ p53-positive nuclei. We speculate that this difference is due to the higher titer of DO-7 antibody used in their study (1:150) compared with the titer used in our study (1:400).

In contrast to the prior studies, our cohort is subclassified according to the 2016 WHO subgroups. Our study also included a relatively high percentage of acute myeloid leukemia with myelodysplasia-related changes, possibly due to the predominance of older adults in the study, which is a particularly aggressive subtype of acute myeloid leukemia. To our knowledge, our study is the largest cohort of acute myeloid leukemia with myelodysplasia-related changes to be analyzed for p53 expression and correlated with *TP53* mutation and survival. Another study evaluated p53 expression in acute myeloid leukemia evolving from a preceding myelodysplastic syndrome.¹⁵ In this report, 9 of 39 cases (23%) of secondary acute myeloid leukemia showed strong nuclear staining (score 3+) in at least 5% of the cells in the entire bone marrow. Their scoring strategy differed from ours in that they only counted strongly positive cells. The authors sequenced *TP53* in 37 randomly selected bone marrow samples from their cohort of higher-risk myelodysplastic syndrome and secondary acute myeloid leukemia and found substantial agreement with p53 (Cohens Kappa = 0.645). In lower risk myelodysplastic syndrome with del(5q)¹⁹ and in therapy-related myeloid neoplasms,²⁰ a p53 cutoff of $\geq 1\%$ was found to independently predict outcomes. Overall, there is likely inter-laboratory variability in terms of p53 expression and thus any published cutoff for interpretation of

Table 6 Multivariate Cox proportional hazard model for overall survival in all cases with clinical follow up

All cases n = 115	Hazard ratio for death	95% confidence interval	P-value ^a
Age	1.04	1.01–1.06	0.0016
Karyotype	1.50	0.64–3.51	0.35
p53 expression > 15%	3.30	1.22–8.93	0.019

^aWald test.

Table 7 Univariate analyses for overall survival in acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) subgroup

AML-MRC n = 61	Hazard ratio for death	95% confidence interval	P-value ^a
Age, n = 51	1.03	1.003–1.06	0.033
Complex karyotype, n = 39	1.78	0.85–3.74	0.13
p53 expression > 15%, n = 51	4.70	2.08–10.6	0.0002

^aWald test.

Table 8 Multivariate analyses for overall survival in acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) subgroup

AML-MRC n = 61	Hazard ratio for death	95% confidence interval	P-value ^a
Age, n = 51	1.03	1.003–1.07	0.032
p53 expression > 15%, n = 51	4.75	2.09–10.8	0.0002

^aWald test.

immunohistochemistry expression should be validated with cases known to have abnormal *TP53*. Furthermore, it is probably prudent to evaluate *TP53* genotype in cases with elevated p53 expression.

Potential limitations of our study include our use of the proportion of nucleated cells expressing p53 in the marrow rather than the proportion of blasts expressing p53. However, it is likely that at least a subset of the non-blast cells in marrows with leukemia are derived from the neoplastic clone, have *TP53* mutations, and consequently exhibit a p53-mutant immunophenotype. This proposal is supported by the cases in our cohort that had megakaryocytes with strong nuclear p53 staining and we are not the first group to report strong p53 expression in non-blast cells in cases of myelodysplastic syndrome with *TP53* mutations.^{19,20} In addition, accurate quantification of the fraction of blasts expressing p53 would be challenging and would likely require staining with additional markers to

accurately quantify blasts in the marrow. Thus, we do not see assessment of p53 expression as a proportion of all nucleated cells as a limitation. In any case, our study demonstrates that determining p53 expression as a fraction of all nucleated cells in the marrow is prognostically useful, predicts *TP53* mutation, and is likely easily incorporated into clinical practice. It is also important to note, that, because our cohort contains relatively few cases with a high level of p53 expression and because we did not test cutoffs with a validation set, the cutoffs used here should be used as general guideline rather than as definitive values.

An additional limitation of our study is the approach used to sequence the *TP53* gene. First, we did not sequence the entire *TP53* gene, and thus there are possibly cases in which *TP53* is mutated that were called *TP53* WT in our study. As our sequencing targets known hotspots, the number of cases with mutations that were called WT is likely small, if indeed there are any. Despite these limitations, which would potentially result in an underestimate of the sensitivity and specificity of p53 immunohistochemistry to predict mutation status, our study suggests that p53 immunohistochemistry has a reasonable sensitivity and specificity for detecting *TP53* mutations. In addition, the sensitivity and specificity of p53 immunohistochemistry for identifying *TP53*-mutated cases determined in our study are similar that seen in lung and ovarian tumors,^{17,21} suggesting that at least some of the limitations facing the use of immunohistochemistry in predicting *TP53* abnormalities are shared across diverse neoplasms.

As sequencing of *TP53* becomes routinely incorporated into sequencing panels used in the diagnostic workup of acute myeloid leukemia, it is reasonable to ask whether p53 immunohistochemistry would be redundant and unnecessary in most cases. Although our data suggest that p53 immunohistochemistry may be a useful ancillary test in cases of acute myeloid leukemia with myelodysplasia-related changes to identify patients with a particularly poor prognosis, it is probably unnecessary to perform immunohistochemistry for p53 in all cases of acute myeloid leukemia. Because the turnaround time for immunohistochemistry is typically much shorter than molecular assays to assess *TP53* genotype, it may be useful to perform p53 immunohistochemistry when a bone marrow biopsy shows features that are suspicious for dysplasia or in the case of patients with acute myeloid leukemia evolving from a preceding myelodysplastic syndrome. In addition, there are situations in which cytogenetic data is unavailable (eg, due to technical limitations, insufficient material, absent analyzable metaphases, etc) and in these cases, p53 immunohistochemistry may be useful to suggest whether a case of acute myeloid leukemia will be particularly aggressive.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Modern Pathology website (<http://www.nature.com/modpathol>)