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Comparison of clinical and molecular characteristics of patients with acute myeloid leukemia and either *TP73* or *TP53* mutations

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The transcription factor p53, described as the “guardian of the genome”, becomes activated in response to malignancy-associated stress signals resulting in cell cycle arrest, senescence, differentiation, or apoptosis leading to the inhibition of tumor cell growth.¹ Inactivation of the p53 tumor-suppressor pathway is among the most common escape mechanisms allowing survival of malignant cells subjected to cytotoxic stress.² Inactivating *TP53* mutations have been detected in 50% of cancers.³

Acute myeloid leukemia (AML) is an aggressive bone marrow (BM) malignancy associated with poor long-term survival in most patients. Somatic *TP53* mutations occur in AML less frequently than in solid tumors, in 5–10% of de novo and ~33% of therapy-related or secondary AML cases.^{4–7} They are associated with older age and poor-risk cytogenetic abnormalities [e.g., –5/del(5q), –7/del(7q) and complex karyotype, especially a ‘typical’ complex karyotype].⁸ Patients with *TP53* mutations have very poor outcomes, with complete

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Author contributions

ASM, JK, A-KE, KM, JCB and CDB contributed to the study design; ASM, JK, A-KE, KM, JCB and CDB contributed to the data interpretation, ASM, KM, JK, JCB and CDB wrote the manuscript; ASM, A-KE, SO, DP and DS performed laboratory-based research; JSB performed the data processing; JK and DN performed statistical analysis; RMS, BLP, JEK, KM, JCB and CDB were involved directly or indirectly in the care of patients and/or sample procurement. All authors read and agreed on the final version of the manuscript.

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remission (CR) rates of 20–40% and median overall survival (OS) of 5–9 months.^{5,8,9} Even for patients attaining a CR, allogeneic stem cell transplantation (alloSCT) yields inadequate results with 3-year OS rates of 10–12%.¹⁰

The *TP53* gene, located at 17p13.1, consists of an acidic N-terminus transcription activation domain, a second activation domain, a proline rich domain, a central DNA-binding core domain, a nuclear localization signaling domain, an oligomerization domain, and a C-terminal. A second member of the p53 family is p73, a structural homolog of p53 expressed as multiple protein isoforms that vary in function.¹¹ The *TP73* gene, located at 1p36.32, includes three structural elements similar to *TP53*, including DNA-binding, oligomerization and transactivation domains. The p73 protein induces apoptosis similarly to p53 through activation of its downstream target genes. However, p73 also has functions distinct from p53 with roles in neuronal stem cell maintenance, aging and metabolism, and microRNA regulation.¹² *TP73* is both overexpressed and deleted in cancers but very rarely mutated (<1%).¹³ Despite large sequencing efforts in AML, *TP73* mutations have yet to be described, and it is unknown if they have similar prognostic implications to *TP53* mutations.

Therefore, we analyzed 1029 adults (aged 17 years) with newly diagnosed AML (excluding acute promyelocytic leukemia) using targeted next-generation sequencing (NGS).⁷ The patients were treated on Cancer and Leukemia Group B (CALGB) trials described in the Supplementary Information. Patients dying within 30 days of starting therapy and those who underwent alloSCT in first CR were excluded. Cytogenetic analyses of pretreatment BM and/or blood samples were performed by institutional, CALGB/Alliance for Clinical Trials in Oncology (Alliance)-approved laboratories, and the results confirmed by central karyotype review.¹⁴ Treatment protocols were in accordance with the Declaration of Helsinki and approved by the institutional review boards at each center, and all patients provided written informed consent.

Supplementary Information contains definitions of clinical endpoints [i.e., CR, disease-free survival (DFS), OS] and statistical methods used to compare baseline characteristics, co-occurring mutations and outcomes among three patient groups: those with both *TP53* and *TP73* wild-type, *TP73*-mutated patients with *TP53* wild-type, and *TP53*-mutated patients with *TP73* wild-type. Data collection and statistical analyses were performed by the Alliance Statistics and Data Center using SAS 9.4 and TIBCO Spotfire S+ 8.2 with a dataset locked on January 10, 2019.

The mutational status of 81 protein-coding genes was determined centrally at The Ohio State University by targeted amplicon sequencing using two different gene panels on the MiSeq platform (Illumina, San Diego, CA; details in Supplementary Information). The presence or absence of biallelic *CEBPA* mutations, *FLT3* internal tandem duplications (*FLT3*-ITD), and the *FLT3*-ITD to *FLT3* wild-type allelic ratio were determined as described in Supplementary Information, resulting in 82 genes assessed in our study.

We detected 13 *TP73* mutations in 12 patients, with two different *TP73* mutations found in one patient. Another patient (no. 12 in Supplementary Table S1) had concurrent *TP73* and *TP53* mutations. All *TP73* mutations were missense and occurred throughout the gene

without any hotspot regions (Figure 1A). In contrast, most of 98 *TP53* mutations found in 88 patients were in the DNA-binding domain within hotspot codons (R175, Y220, G245, and R248)³ (Figure 1B). Eight patients had 2 and one patient had 3 *TP53* mutations. Among 98 *TP53* mutations, 72 were missense, 18 frameshift, 6 nonsense, and 2 deletions. Variant allele fractions (VAFs) were >0.20 in 92% of patients with *TP73* mutations and in all *TP53*-mutated patients. Nine-hundred-thirty patients had wild-type *TP53* and *TP73*.

We found no significant differences in pretreatment characteristics between *TP73*-mutated and *TP53*-mutated patients, except for *TP73*-mutated patients being classified half as often in the adverse-risk category of the 2017 European LeukemiaNet (ELN) classification¹⁵ ($P<0.001$; Supplementary Table S2). The only difference between *TP73*-mutated and wild-type patients was lower presenting white blood cell (WBC) counts in the former ($P=0.03$). Compared with wild-type patients, *TP53*-mutated patients were older ($P<0.001$), had lower platelet ($P=0.02$) and WBC counts ($P<0.001$), lower percentages of blood ($P<0.001$) and BM blasts ($P=0.01$), and were more often classified in the 2017 ELN adverse-risk category ($P<0.001$).

Cytogenetically, *TP73*-mutated patients had normal karyotypes most often [six (50%) patients], followed by complex karyotypes, detected in two (16%) patients (Supplementary Table S1). Cytogenetic findings in *TP53*-mutated patients are summarized in Supplementary Table S3. Most patients (70%) had ‘typical’ and 5% ‘atypical’ complex karyotypes.⁸ The remaining patients had other cytogenetic abnormalities in a non-complex karyotype (19%) or a normal karyotype (6%). All patients with microscopically detectable loss of 17p had ‘typical’ complex karyotypes.

Regarding co-occurring mutations, *TP73*-mutated patients harbored more mutations in the cohesin complex (36% vs 8%, $P=0.02$), *NPM1* (36% vs 5%, $P=0.006$) and methylation-related (64% vs 28%, $P=0.03$) genes than *TP53*-mutated patients. *TP73*-mutated patients also had more mutations in the cohesin complex genes than the wild-type patients (36% vs 12%, $P=0.04$; Supplementary Table S4). Compared with wild-type patients, *TP53*-mutated patients had less frequent mutations in chromatin remodeling (7% vs 18%, $P=0.007$), kinases (14% vs 42%, $P<0.001$), methylation-related (28% vs 50%, $P<0.001$), *NPM1* (5% vs 42%, $P<0.001$) and the RAS pathway (14% vs 26%, $P=0.01$) genes. Frequencies of single gene mutations in each group are listed in Supplementary Table S5. Mutations co-occurring with *TP73* and *TP53* mutations with frequencies of ≥2% are depicted in Supplementary Figures S1 and S2, respectively.

Concerning treatment, 83 of 87 (96%) *TP53*-mutated patients and 10 of 11 (91%) *TP73*-mutated patients received intensive induction treatment. The remaining 5 patients were treated on CALGB 11002 with decitabine+/-bortezomib. We found no significant difference in CR rates between *TP73*-mutated and *TP53*-mutated patients (64% vs 40%, $P=0.20$). However, *TP73*-mutated patients had a longer DFS (median, 2.0 vs 0.4 years, $P=0.005$) and OS (median, 3.9 vs 0.4 years; $P<0.001$) than *TP53*-mutated patients (Figure 2 and Table 1). Conversely, there were no significant differences between the *TP73*-mutated and wild-type patients in CR rates (64% vs 71%; $P=0.52$), DFS (median, 2.0 vs 1.1 years; $P=0.59$) or OS

(median, 3.9 vs 1.5 years; $P=0.28$). Median survival of all patients in the study was 8.6 years.

As expected, *TP53*-mutated patients had inferior CR rates (40% vs 71%; $P<0.001$), and shorter DFS (median, 0.4 vs 1.1 years; $P<0.001$) and OS (median, 0.4 vs 1.5 years; $P<0.001$; Figure 2 and Table 1) than wild-type patients. There were only six *TP53*-mutated long-term survivors (OS >2 years). Two patients underwent alloHCT with refractory disease after initial therapy [one patient treated on 10503 with one induction with cytarabine, daunorubicin and etoposide and another (with a concurrent *TP73* mutation) treated on 19808 with 2 inductions of cytarabine, daunorubicin and etoposide+/-valsopodar] with OS of 6.7 and 10.2 years, respectively. Three patients received induction on 10503 and underwent autologous transplantation in first CR per protocol with OS ranging from 3.6 to 6.7 years. The remaining long-term survivor was treated on 9720 with cytarabine, daunorubicin, and etoposide+/-valsopodar and had an OS of 2.2 years.

Determining pretreatment clinical and molecular features and treatment outcomes associated with *TP73* mutations in AML is of interest because p73 is part of the p53 family. *TP73* mutations differ from *TP53* mutations with regard to rate of occurrence, 1% versus 9%, and associations with other mutations. *TP73*-mutated patients more often harbored mutations in cohesin complex, *NPM1* and methylation-related genes. Pretreatment clinical characteristics did not differ significantly between *TP73*-mutated and *TP53*-mutated patients except for the assignment into the 2017 ELN risk groups. Importantly, our study has revealed that the key difference between *TP73*- and *TP53*-mutated patients is that *TP73*-mutated patients have DFS and OS superior to those of *TP53*-mutated patients. However, since the number of *TP73*-mutated patients is relatively small, our findings should be validated in an independent patient cohort.

Most *TP53*-mutated patients in our study responded poorly to intensive cytotoxic approaches, with a 40% CR rate and a 1-year DFS rate of 11% and OS rate of 15%. Conversely, our findings suggest that cytotoxic chemotherapy is more appropriate for both *TP73*-mutated (CR rate, 64%; 1-year DFS and OS rates, 100%) and *TP73* and *TP53* wild-type patients (CR rate, 71%; 1-year DFS rate, 52%; 1-year OS rate, 63%). Identification of the mechanisms underlying chemo-responsiveness of *TP73*-mutated patients and chemoresistance in *TP53*-mutated patients could provide valuable insights for designing therapies to improve the poor outcomes in *TP53*-mutated AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A

B

Figure 1.

A) Lollipop plot depicting 13 *TP73* mutations found in 12 patients with newly diagnosed acute myeloid leukemia. Eleven patients each had a single *TP73* mutation and one patient had two different *TP73* mutations. The patient with dual mutations in both *TP73* and *TP53* is included. The structural domains of the gene are represented by color coding as outlined in the figure. Missense mutations are depicted by the blue circles. **B)** Lollipop plot depicting 98 mutations in the *TP53* gene found in 88 patients with newly diagnosed acute myeloid leukemia. The patient with dual mutations in both *TP73* and *TP53* is included. The structural domains of the gene are represented by color coding as outlined in the figure. Frameshift mutations are depicted by the red circles, nonsense mutations are depicted by the orange circles, missense mutations are depicted by the blue circles, and indel mutations are depicted by the gray circles. The numbers within each circle denote the numbers of mutations at certain sites within the gene.

A

B

Figure 2.

Kaplan-Meier curves depicting the **a** disease-free survival and **b** overall survival of de novo acute myeloid leukemia patients with *TP73* mutations (blue) compared with those of patients with *TP53* mutations (red) and of patients with wild-type (wt) *TP73* and *TP53* genes (black).

Outcomes of acute myeloid leukemia patients with *TP73* mutations, patients with *TP53* mutations, and patients without *TP53* or *TP73* mutation (wild-type)

Table 1

Endpoint	<i>TP73m</i> n=11	<i>TP53m</i> n=87	Wild-type n=930	<i>P</i> ^a <i>TP73m</i> vs <i>TP53m</i>	<i>P</i> ^a <i>TP73m</i> vs wt	<i>P</i> ^a <i>TP53m</i> vs wt
Complete remission, %	64	40	71	0.20	0.52	<0.001
Disease-free survival				0.005	0.59	<0.001
Median, years	2.0	0.4	1.1			
% Disease-free at 1 year (95% CI)	100	11 (4–24)	52 (48–56)			
% Disease-free at 3 years (95% CI)	29 (4–61)	9 (2–21)	33 (30–37)			
Overall survival				<0.001	0.28	<0.001
Median, years	3.9	0.4	1.5			
% Alive at 1 year (95% CI)	100	15 (8–23)	63 (60–66)			
% Alive at 3 years (95% CI)	55 (23–78)	5 (1–10)	36 (33–39)			

Abbreviations: *CI*, confidence interval; *m*, mutated; *n*, number; *wt*, wild-type.

^a *P*-values for categorical variables are from Fisher's exact test, *P*-values for time to event variables are from the log-rank test.