Letter to *BLOOD*

**Acute myeloid leukemia with TP53 germline mutations**

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**Running title:** TP53 germline mutations in AML

**Keywords:** TP53, Acute Myeloid Leukemia, Germline Mutations, Li-Fraumeni Syndrome, Ionizing Radiation

**Financial Support:** This work was supported by Leukämiehilfe Steiermark (to HS) and the Austrian Science Fund under grant no. P 26619-B19 (to AZ).
To the editor:

Acute myeloid leukemia (AML) is considered a sporadic disease caused by sequential accumulation of somatically acquired mutations in hematopoietic stem or progenitor cells (HSPCs). However, familial clustering of myeloid neoplasms is being increasingly observed and attributed to highly penetrant germline variants in several different genes. The recently published “2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia” incorporated these findings and defined “Myeloid neoplasms with germline predisposition” as a distinct disease entity. In addition to a series of developmental syndromes, this group currently comprises cases with germline mutations in CEBPA, DDX41, RUNX1, ANKRD26, ETV6 and GATA2. Here, we describe familial clustering of AML in a TP53 mutated Li-Fraumeni syndrome (LFS) pedigree. Further screening of 186 primary AML specimens revealed the presence TP53 germline mutations in 1.1% of cases. Finally, we report their frequent occurrence in therapy-related AML (tAML) arising after antecedent ionizing radiation, an observation of relevance for future studies within this area. The study was approved by the ethical committee of the Medical University of Graz, Graz, Austria (votes number 21-065 ex 09/10 and 26-369 ex 13/14, respectively) and performed in accordance with the Declaration of Helsinki.

A 46-year-old Caucasian woman presented with tAML with a complex karyotype arising after previous administration of chemo- and radiotherapy for papillary thyroid carcinoma at the age of 26, colorectal cancer at the age of 30 and bilateral breast cancer at the age of 31 and 41 years, respectively. Despite three lines of intensive AML induction/salvage therapy, the patient never achieved remission and died of progressive disease seven months after diagnosis. Personal and family history indicated a LFS according to Chompret criteria (Figure 1). Interestingly, in addition to the characteristic LFS tumor spectrum, secondary AML following myelodysplasia was observed in the index patient’s father and chronic myeloid leukemia in one of her brothers. Analysis of the TP53 gene was performed using skin
fibroblasts and peripheral blood, respectively, as previously described.\textsuperscript{4,5} A \textit{TP53} c.467G>C germline mutation characterized this LFS pedigree affecting both patients with AML but not the subject suffering from CML (Figure 1, Supplemental Table 2). Although the infrequent occurrence of myeloid neoplasms in individual patients with LFS or Li-Fraumeni-like syndrome is well known,\textsuperscript{4,6-10} these data establish a link between familial clustering of AML and \textit{TP53} germline mutations for the first time. \textit{TP53} c.467G>C, p.R156P is a missense mutation that has been reported as somatic event in different human malignancies previously. Importantly, it confers gain of function of p53 causing malignant transformation of hematopoietic cells \textit{in vitro}. Together with the fact, that loss of the wild-type allele was observed in leukemic specimens, these data suggest, that it is indeed, involved in AML development in this family.\textsuperscript{11} Data of murine leukemia models whereby AML development is significantly aggravated by aberrations in p53 further support this notion.\textsuperscript{12,13}

To determine the frequency of \textit{TP53} germline mutations in AML, a cohort of 186 patients with de novo AML (n=72), secondary AML (sAML; n=66) and tAML (n=48) was investigated (Supplemental Table 1). Exons 2-10 of \textit{TP53} including exon-intron boundaries were analyzed by Sanger and targeted deep sequencing, respectively, using leukemic and constitutional DNA from either buccal swabs or remission material as reported earlier.\textsuperscript{14,15} Mutations were classified according to the “IACR TP53 Database” (http://p53.iarc.fr), “The TP53 Web Site” (http://p53.free.fr) and the “COSMIC database” (http://cancer.sanger.ac.uk/cosmic). Each \textit{TP53} mutation detected was confirmed. In case of a suspected germline variant, DNA from a buccal swab obtained from patients in remission was used for this purpose. \textit{TP53} mutations were found in 35/186 (18.8%) diagnostic leukemia specimens with an equal distribution between different AML subtypes (de novo, 13/72 [18.1%]; sAML 14/66 [21.2%]; t-AML 8/48 [16.7%]; \(P=0.810\) by Pearson’s \(\chi^2\) test). The relatively high number of \textit{TP53} mutated AMLs in this cohort is due to an overrepresentation of high-risk cases and in concordance with published data.\textsuperscript{16} Most importantly, two further \textit{TP53} mutations of germline
origin were detected (2/186; 1.1%; c.673-1G>A and c.733G>A, respectively; Supplemental Table 2) and again, both of them displayed a loss of the wild-type allele in leukemic specimens. Personal and family histories of the patient with the TP53 c.673-1G>A mutation fulfilled Chompret criteria as well (Supplemental Figures 1). In this individual, multiplex testing of 150 genes associated with hereditary cancer has been performed previously revealing an additional CDH1 germline variant. The fact of a cancer associated germline mutation is not only of relevance for genetic counseling of patients as well as family members but has further consequences in the context of myeloid malignancies. AML patients with TP53 aberrations face an exceedingly poor prognosis and are candidates for allogeneic stem cell transplantation. Although no cases of transplantation of p53 mutant/deficient HSPCs have been reported yet, caution is, nevertheless, warranted based on experimental data. Transgenic mice with phosphorylation-site Trp53 mutations experience a depletion of adult stem cells including those of the bone marrow. Furthermore, mice with germline depletion of the Trp53 show - despite an expansion of bone marrow LSK cells - increased mortality rates when compared to Trp53 proficient animals.

Interestingly, two of the three TP53 germline mutated patients described above suffered from tAML. As we already observed the occurrence of TP53 germline mutations in 4/58 tAML patients previously, we extended our analyses within this subgroup by pooling these data with those of our index patient and the 48 tAML patients analyzed within the present study, thereby generating a cohort of 107 tAML patients with information available on the TP53 germline mutation status as well as clinical parameters. In total, six out of 107 patients (5.6%) harbored a TP53 germline mutation, which further highlights the importance of this genetic event in tAML (Supplemental Tables 2 and 3). In light of the fact that HSPCs with TP53 aberrations have been shown to expand preferentially after cytotoxic treatment, these data suggest that TP53 germline mutations could indeed predispose to the development of tAML. Interestingly, treatment of the primary malignancy included ionizing radiation in...
five out of six patients (83%) in contrast to 38/101 (38%) TP53 wild-type cases only (Table 1; \( P=0.038 \) in Fisher’s exact test). In light of the fact that susceptibility to radiation induced carcinogenesis has been shown in p53 deficient mouse models previously,\(^{23,24}\) one might speculate that human HSPCs carrying a TP53 germline mutation might also respond inadequately to genotoxic stress induced by ionizing radiation. Despite this tempting assumption, it should be noted that the cohort of tAML patients studied is too small to draw a final conclusion, particularly as \( P \)-values did not reach significance any longer when corrected for multiple hypothesis testing (Table 1). Therefore, analysis of larger tAML cohorts specifically addressing this issue will be needed to further corroborate such a correlation.

Taken together, these data demonstrate that TP53 germline mutations occur in a small fraction of AML patients and are particularly frequent in tAML. Furthermore, they suggest a potential association of these aberrations with tAML occurring after ionizing radiation therapy which will be of relevance for the design of future studies.

**Acknowledgments**

This work was supported by Leukämiehilfe Steiermark (to HS) and the Austrian Science Fund under grant no. P 26619-B19 (to AZ).

**Authorship contributions**

Contribution: HS designed and conceived the study; AZ, RL, MM, KL, KK, MG, DF, JG, AW and HS acquired data; AZ, RL, MM, KL, KK, MG, DF, JG, AW and HS analyzed and interpreted data; AZ and HS wrote and reviewed the manuscript; all authors reviewed and approved the manuscript.

**Disclosure of conflicts of interest**

The authors declare no conflict of interest.
<table>
<thead>
<tr>
<th><strong>Number of patients (n)</strong></th>
<th><strong>TP53 germline status</strong></th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Females / males</td>
<td>Wild-type</td>
<td>Mutated</td>
</tr>
<tr>
<td>101</td>
<td>54 (53%) / 47 (47%)</td>
<td>5 (83%) / 1 (17%)</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
<td>65 (19-85)</td>
<td>53 (8-80)</td>
</tr>
<tr>
<td><strong>LDH (U/L)</strong></td>
<td>268 (121-2489)</td>
<td>413 (127-543)</td>
</tr>
<tr>
<td><strong>WBC at diagnosis (10⁹/L)</strong></td>
<td>4.14 (0.81-305.7)</td>
<td>2.62 (1.47-16.15)</td>
</tr>
<tr>
<td><strong>Antecedent cytotoxic therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>38/101 (38%)</td>
<td>5/6 (83%)</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>48/101 (48%)</td>
<td>2/5 (40%)*</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>47/101 (47%)</td>
<td>1/5 (20%)*</td>
</tr>
<tr>
<td>Topoisomerase inhibitors</td>
<td>18/101 (18%)</td>
<td>2/5 (40%)*</td>
</tr>
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Table 1. Clinical and laboratory characteristics of tAML patients according to the *TP53* germline status. Cytotoxic therapies with leukemogenic potential were classified according to Sill et al.²⁵ *, one patient, whose chemotherapeutic agents administered for the primary malignancies were unknown, was excluded from statistical calculations; *P, P*-value; WBC, white blood cell count; LDH, lactate dehydrogenase.
**Figure legends**

**Figure 1.** A Li-Fraumeni syndrome pedigree showing for the first time familial clustering of AML. The index patient developed therapy-related AML (tAML) (II:4) following cytotoxic treatment of diverse antecedent malignancies, the index patient’s father (I:2) secondary AML (sAML) following myelodysplasia. Filled symbols, subjects with malignancies; asterisk denotes a $TP53$ c.467G>C, p.R156P germline mutation carrier; the “minus” a wild-type $TP53$ germline status. Numbers in brackets indicate age at diagnosis in years. CML, chronic myeloid leukemia; TC, thyroid carcinoma; CRC, colorectal cancer; BC, breast cancer; PNET, primitive neuroectodermal tumor.
References

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