HHS Public Access

Author manuscript

Am J Hematol. Author manuscript; available in PMC 2022 November 01.

Published in final edited form as:

Am J Hematol. 2021 November 01; 96(11): 1420–1428. doi:10.1002/ajh.26314.

Development of *TP53* Mutations Over the Course of Therapy for Acute Myeloid Leukemia

Yasmin Alwash[1], **Joseph D. Khoury**[2], **Mehrnoosh Tashakori**[2], **Rashmi Kanagal-Shamanna**[2], **Naval Daver**[1], **Farhad Ravandi**[1], **Tapan M. Kadia**[1], **Marina Konopleva**[1], **Courtney D. Dinardo**[1], **Ghayas C. Issa**[1], **Sanam Loghavi**[2], **Koichi Takahashi**[1], **Elias Jabbour**[1], **Veronica Guerra**[1], **Steven Kornblau**[1], **Hagop Kantarjian**[1], **Nicholas J. Short**[1]

[1]The Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, Texas.

[2]The Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, Texas.

Abstract

TP53 mutations in acute myeloid leukemia (AML) are associated with resistance to standard treatments and dismal outcomes. The incidence and prognostic impact of the emergence of newly detectable *TP53* mutations over the course of AML therapy has not been well described. We retrospectively analyzed 200 patients with newly diagnosed *TP53* wild type AML who relapsed after or were refractory to frontline therapy. Twenty-nine patients (15%) developed a newly detectable *TP53* mutation in the context of relapsed/refractory disease. The median variant allelic frequency (VAF) was 15% (range, 1.1% - 95.6%). *TP53* mutations were more common after intensive therapy versus lower-intensity therapy (23% versus 10%, respectively; P=0.02) and in patients who had undergone hematopoietic stem cell transplant versus those who had not (36% versus 12%, respectively; P=0.005). Lower *TP53* VAF was associated with an increased likelihood of complete remission (CR) or CR with incomplete hematologic recovery (CRi) compared to higher *TP53* VAF (CR/CRi rate of 41% for VAF <20% versus 13% for VAF 20%, respectively). The median overall survival (OS) after acquisition of *TP53* mutation was 4.6 months, with a 1-year OS rate of 19%. *TP53* VAF at relapse was significantly associated with OS; the median OS of patients with *TP53* VAF 20% was 3.5 months versus 6.1 months for those with *TP53* VAF <20% (P<0.05). In summary, new *TP53* mutations may be acquired throughout the course of AML therapy. Sequential monitoring for *TP53* mutations is likely to be increasingly relevant in the era of emerging *TP53*-targeting therapies for AML.

Correspondence: Nicholas J. Short, MD, Assistant Professor, Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 0428, Houston, TX, 77030, Phone: 713-563-4485, nshort@mdanderson.org.

Authorship Contributions:
Conception and design: Nicholas Short, Yasmin Alwash
Editing and supervision: Nicholas Short
Collection and assembly of data: Yasmin Alwash
Validation and investigation: Joseph Khoury, Mehrnoosh Tashakori
Data analysis and interpretation: Yasmin Alwash, Nicholas Short, Veronica Guerra
Final approval of manuscript: All authors reviewed and provided final approval of this manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Institutional review board approval: Obtained

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Keywords

TP53 mutated AML; emergence; treatment; relapsed/refractory

Introduction

Although most patients with newly diagnosed acute myeloid leukemia (AML) achieve complete remission after induction chemotherapy, approximately 50% relapse, after which outcomes are generally poor[1]. These relapses are often driven by expansion of a previously existing mutations or emergence of new ones[2]. Several of these mutations, including *FLT3*, *IDH1* and *IDH2*, are targetable with commercially available small molecular inhibitors, and others may be targetable with new, emerging therapeutic approaches[3]. Accurate detection of these mutations therefore plays an integral role in the current management of AML, and as the treatment landscape of AML expands, the number of potentially actionable mutations is likely to increase.

Mutations in the tumor protein *53 (TP53)* gene are detectable in approximately half of human cancers and in approximately 20% of newly diagnosed AML[4–7]. These mutations often result in p53 protein accumulation are frequently associated with therapy-related AML and complex karyotype[8–10]. *TP53* mutations confer resistance to currently available therapies, and particularly to intensive chemotherapy[11]. Consequently, the outcomes of *TP53*-mutated AML are poor, with high rates of relapse and dismal outcome; it is therefore important to identify these mutations from a prognostic standpoint, as their presence warrants strong consideration of hematopoietic stem cell transplant (HSCT) in first remission[11–14]. New novel therapies currently in clinical trials are poised to become important strategies for *TP53*-mutated AML. These agents include eprenetapopt (APR-246) and magrolimab, both of which have shown promising data in the treatment of *TP53*-mutated myeloid malignancies[15,16]. Alternatively, other emerging agents such as MDM2 inhibitors work through *TP53*-dependent mechanisms and require intact p53 function in order to be effective[4]. In this context, detection of *TP53* mutations is important in order to select alternative strategies.

Addition of these agents to our therapeutic armamentarium now offers new avenues to treat *TP53*-mutated AML. However, there are limited data regarding the emergence of newly detectable *TP53* mutations over the course of AML therapy and whether repeat testing for this mutation might have clinical implications in the emerging therapeutic landscape of AML. Hence, we sought to evaluate the frequency of *TP53* mutations emerging over the course of therapy in patients with *TP53* wild type (WT) AML who relapsed or were refractory to initial therapy.

Methods

Patients and methods

We retrospectively reviewed the electronic medical records of 1293 patients with newly diagnosed *TP53* WT AML between December 2012 and March 2020 who were treated with

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frontline therapy at our institution. AML was diagnosed per the World Health Organization criteria[17]. Patients with *TP53* mutation at baseline, core binding factor (CBF) AML, and those who did not have mutation data at baseline were excluded (Fig. 1). Among 924 patients with *TP53* WT AML, we limited our analysis to 200 patients who were relapsed or refractory to frontline therapy and had additional mutation profiling performed at relapse or treatment failure. Refractoriness to frontline therapy was defined as lack of response to at least 1 cycle of intensive induction chemotherapy or at least 2 cycles of lower intensity therapy (unless clear evidence of disease progression after 1 cycle). Clinical, cytogenetic, and molecular data were collected through chart review. The study was approved by the institutional review board of The University of Texas MD Anderson Cancer Center.

Karyotyping, target gene sequencing, and immunohistochemistry

Conventional cytogenetic analysis was conducted in the Clinical Cytogenetic Laboratory at MDACC following standard protocols. Cytogenetic results were interpreted and reported according to the International System for Human Cytogenetic nomenclature[18]. To identify *TP53* mutations at baseline or relapse, a targeted next-generation sequencing (NGS) platform covering 28, 53, or 81 genes was performed. The analytical sensitivity of this NGS panel was established at 1–2% mutant reads in a background of WT reads. Mutations were manually reviewed to exclude any artefacts. The European Leukemia Net (ELN) 2017 risk classification was used to categorize patients into their risk group depending on their mutational status and cytogenetic abnormalities[12]. Immunohistochemistry (IHC) was performed on baseline and relapse samples in order to assess p53 protein expression patterns. IHC 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 using the monoclonal anti-p53 antibody clone DO-7 (Dako, Carpinteria, CA, USA) as described previously [19,20]. The extent (percentage) and intensity (0, 1+, 2+, 3+) of staining was evaluated independently by two hematopathologists (J.D.K. and M.T.). Mutant p53 expression pattern (accumulation) was defined as uniform 3+ staining in at least 5% of cells[8,21,22]. Patients with mutant p53 expression patterns were further subdivided into low-burden mutation pattern (10% of cellularity) or high-burden mutation pattern (>10% of cellularity). The other cases were considered to have WT p53 expression pattern.

Response definitions and statistical methods

Complete remission (CR), CR with incomplete hematological recovery (CRi), and morphologic leukemia-free state (MLFS) were defined according to the ELN 2017 criteria[12]. Relapse was defined as recurrence of >5% blasts in the bone marrow. Patient characteristics were summarized using descriptive statistics including median (range) for continuous variables and frequency (%) for categorical variables. The fisher's exact test and Wilcoxon rank-sum test were used to compare categorical and continuous variables, respectively. Overall survival (OS) was defined as the time from the date of first detected *TP53* mutation until death due to any cause or censored at last follow-up. The Kaplan Meier method was performed to estimate OS. All statistical analysis was performed using the IBM SPSS Statistics 23.0 software.

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Results

Patient characteristics

We identified 200 adult patients with non-CBF *TP53* WT AML at diagnosis who relapsed after or were refractory to frontline therapy and are the subject of this analysis (Fig. 1). The median age at diagnosis was 69 years (range, 17–94 years) (Table 1). At diagnosis, 87 patients (44%) had normal karyotype, 41 (21%) had complex karyotype, 15 (8%) had −5/del5q, and 31 (16%) had −7. Eighty-five patients (43%) had ELN adverse risk AML, 94 (47%) were intermediate risk, and 21 (11%) were favorable risk. The most common mutations at baseline were *SRSF2* (24%), *DNMT3A* (23%), *IDH2* mutation (20%), and *NRAS* (18%). Sixty-nine patients (35%) received frontline intensive chemotherapy (defined as cytarabine and anthracycline-based induction) and 131 (66%) received lower-intensity therapy. Among those patients who received lower-intensity therapy, 108 patients (82%) received a hypomethylating agent-based regimen. Twenty-two patients (11%) had undergone prior HSCT in first remission. Eighty-five patients (43%) were refractory to frontline therapy, and 115 (58%) relapsed after initial response to frontline therapy. Among the relapsed patients, the median time to first relapse was 6 months (range 1–53 months).

TP53 mutation acquisition and association with baseline features

Overall, 29 patients (15%) developed a newly detectable *TP53* mutation by NGS at any point over the course of therapy in the context of relapsed/refractory disease. Nineteen of these pts (66%) acquired a detectable mutation after the first line of therapy, 6 patients (21%) after two lines of therapy, and 4 patients (14%) after three lines of therapy. Sixteen patients (55%) with newly detectable *TP53* mutation had received frontline intensive chemotherapy, and 13 patients (45%) had received a frontline lower-intensity regimen; only 2 of these patients (13%) received venetoclax plus a hypomethylating agent. Twenty-four patients (83%) acquired one *TP53* mutation, among these patients; 21 patients acquired a missense mutation, and 3 patients acquired a frameshift mutation. Five patients (17%) acquired 2 *TP53* mutations; 3 of these patients acquired 2 missense mutations, 1 patient acquired 2 frameshift mutations, and 1 patient acquired 1 missense and 1 nonsense mutation. The median variant allelic frequency (VAF) of the *TP53* mutation at the time of first detection was 15% (range 1.1–95.6%). Five patients (17%) had a VAF >40%, 5 (17%) had a VAF of 20%–40%, 6 (21%) had a VAF of 10%–20%, and 13 (45%) had a VAF of <10%. Overall, 11 patients (38%) had a VAF <5%. The median time from diagnosis to first detection of *TP53* mutation was 10 months (range 1–23 months).

We identified factors at baseline associated with increased likelihood of developing a newly detectable *TP53* mutations. New *TP53* mutations were more common in patients with a baseline chromosome 5 abnormality versus those without a chromosome 5 abnormality (23% versus 10%, respectively; P=0.02) and in those with a baseline *IDH2* mutations versus no *IDH2* mutation (28% versus 12%, respectively; P=0.02). Newly detected *TP53* mutations were also more common after intensive therapy versus lower-intensity therapy (23% versus 10%, respectively; P=0.02) and in patients who had undergone HSCT in first remission versus those who had not (36% versus 12%, respectively; P=0.005). No other baseline features were associated with the development of a newly detectable *TP53* mutation,

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including age or other cytogenetic/molecular features. Interestingly, there was no association with the development of *TP53* mutation in patients with complex karyotype versus non-complex karyotype at baseline (15% versus 17%, respectively; P=0.95). Additionally, even though therapy-related AML is commonly associated with *TP53* mutations, among the 26 patients with therapy-related *TP53* WT AML with relapsed/refractory disease, only 1 patient (4%) developed a detectable *TP53* mutation over the course of therapy.

At the time of emergence of *TP53* mutation, 13 of the newly detectable *TP53* mutations (45%) occurred in the context of complex cytogenetics. In 7 of the 13 cases, both the cytogenetic complexity and *TP53* mutation emerged concomitantly and were not present at baseline. Among the 13 total cases where *TP53* mutations occurred with complex cytogenetics, the median VAF was 20.9% (range, 1.4% - 95.6%). Eight patients (28%) had diploid cytogenetics at time of detection of *TP53* mutation, with a median VAF of 3.2% (range, 1.2% - 36.5%). Among patients who developed a newly detectable *TP53* mutation, the most common co-mutations at the time of *TP53* detection were *DDX41*, *DNMT3A, IDH2* and *NRAS,* each of which was mutated in 30%, 22%, 22%, and 18% of *TP53*-mutated relapses, respectively. In most cases, the co-mutations had also been present at diagnosis. However, at time of detection of *TP53* mutation, 3 patients acquired a newly detectable *NRAS* mutation.

p53 protein accumulation by IHC and relationship with NGS

We performed IHC for p53 protein accumulation in baseline and relapse samples as an orthogonal method to assess *TP53* mutation status. The relationship between *TP53* VAF by NGS and p53 protein expression by IHC at baseline and relapse is shown in (Table 2). Among the 29 patients who relapsed with a newly detectable *TP53* mutation by NGS, 17 patients (59%) had available bone marrow samples at baseline or at relapse for assessment of p53 protein expression by IHC, and 16 patients (55%) had paired baseline and relapse samples. Evaluation of p53 accumulation by IHC at baseline demonstrated a WT p53 expression pattern at baseline in 12 patients (71%) and a mutant p53 staining pattern in 5 patients (29%); 4 of these patients had IHC findings consistent with a low-frequency mutant *TP53* clones (10% of marrow cellularity) and 1 patient had a mutant high-frequency clone estimated to comprise 20% of marrow cellularity. Conversely, at time of *TP53* development by NGS, mutant p53 expression by IHC was identified in 14 patients (82%), 6 patients with a low-frequency clone and 8 patients with a high-frequency clone. Of note, 3 patients (18%) out of the 17 patients with a detectable *TP53* mutation by NGS had WT p53 expression pattern by IHC; the *TP53* VAFs in these patients were 2.7%, 5.5% and 36.5%. At the time of *TP53* mutation detection by NGS, the VAF of *TP53* mutations in patients with WT p53 protein expression or mutant-low p53 protein expression by IHC was numerically lower than in patients with mutant-high p53 protein expression (5.5% versus 20.4%, respectively; P=0.3). At this same time point, patients with mutant-high p53 protein expression by IHC were more likely to have complex cytogenetics than patients with WT or mutant-low p53 protein expression by IHC (75% versus 22%, respectively; P=0.05). These data suggest that p53 expression could support conclusions regarding p53 pathway altering the pathogenic impact of *TP53* mutations when detected.

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Treatment response and overall survival of *TP53*-mutated patients

After *TP53* mutation acquisition, 23 patients (79%) received low intensity chemotherapy, 2 patients (7%) received high intensity chemotherapy and 4 patients (14%) did not receive any treatment. Among the 25 patients who received salvage therapy, 6 patients (24%) received a venetoclax-based salvage regimen. Seventeen patients (68%) responded to salvage therapy. The CR/CRi/MLFS rate to salvage therapy was similar regardless of *TP53* VAF (71% for VAF <20% and 63% for VAF 20%). However, there was a trend towards a higher rate of CR/CRi for those with lower *TP53* VAF (41% for VAF <20% and 13% for VAF 20%; P=0.15). Seven of these responders underwent subsequent HSCT. However, all transplanted patients relapsed post-transplant. With a median follow-up of 35 months, the median overall survival (OS) after acquisition of *TP53* mutation was 4.6 months, with a 1-year OS rate of 19% (Fig. 2A). *TP53* VAF was significantly associated with OS; the median OS of patients with *TP53* VAF 20% was 3.5 months versus 6.1 months for those with *TP5*3 VAF <20% (P<0.05) (Fig. 2B). At the time of *TP53* detection by NGS, evaluation of p53 protein staining by IHC was correlated with OS. Patients with mutant-high p53 protein expression showed strong trend towards worse outcomes than those with mutant-low or WT p53 protein expression (3.4 months versus 7.4 months, respectively; P=0.09) (Fig. 2C).

TP53 acquisition during complete remission

We also identified a newly detectable *TP53* mutation while in CR in 5 patients (1%) out of the 555 patients who responded to frontline therapy and were not included in the primary analysis (Fig. 1). The median VAF at the time of *TP53* mutation detection was 2.5% (range 1.0% - 3.3%). Three of these patients had a detectable *TP53* mutation after HSCT. A fourth patient had a *TP53* mutation detected while receiving consolidation therapy; this patient subsequently underwent HSCT, after which the mutation was no longer detectable. A fifth patient developed a newly detectable *TP53* mutation in the setting of flow cytometric measurable residual disease (MRD)-positive disease. This patient received nivolumab maintenance and subsequently achieved flow MRD negativity, although the *TP53* mutation persisted.

Four patients had available bone marrow samples for IHC assessment at baseline and at time of *TP53* mutation detection by NGS (Table 2). All of these patients demonstrated a WT p53 protein expression pattern by IHC at baseline. At the time of *TP53* detection by NGS, two patients had a mutant-low p53 protein expression pattern and 2 patients had a WT p53 protein expression pattern. All 4 patients had diploid karyotype at time of *TP53* detection. With a median follow-up of 27 months since detection of a *TP53* mutation by NGS, none of these patients have developed hematologic relapse, 4 patients are still alive, and one patient died from GVHD complication 4 months after HSCT.

Discussion

In this single-center, retrospective study, we showed that (15%) of patients with WT *TP53* AML acquired a newly detectable *TP53* mutation over the course of therapy. These mutations tended to be subclonal with a median VAF of 15%. Acquisition of a newly detectable *TP53* mutation was associated with receiving frontline intensive chemotherapy or

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HSCT, suggesting that these therapies may select for development of *TP53* mutations and/or *TP53* clonal expansion. Interestingly, despite the strong association of *TP53* mutations with complex karyotype,[10] the rate of *TP53* mutations was the same between patients with complex or non-complex cytogenetics at baseline. Conversely, at relapse, 45% of cases of newly detectable *TP53* mutations were associated with complex karyotype, with both the *TP53* mutation and cytogenetic complexity arising concurrently in most of these cases. Taken together, these findings support the concept that *TP53* mutations generally precede cytogenetic complexity rather than vice versa. We also observed significantly higher *TP53* VAF in patients with complex karyotype compared to those with diploid karyotype, further supporting the association between cytogenetic complexity and TP53 mutations, which has been described predominantly in the context of newly diagnosed AML[10].

Several studies has shown the inferior outcome of *TP5*3-mutated AML[11,13,23–25]. Interestingly, in our study, the outcomes of patients who relapsed with *TP53* mutations were better than expected, with a CR/CRi/MLFS rate of 68%. However, only 32% of patients achieved CR/CRi, and these responses were very short lived, with a median OS of only 4.6 months. The relatively high marrow response rate in these patients may reflect the subclonal nature of these mutations, as the median VAF was only 15% and patients with lower *TP53* VAF had a higher rate of CR/CRi to salvage therapy. It is possible that these very small *TP53*-mutated clones therefore may not impart the same degree of chemotherapy resistance as larger clones. This relationship between *TP53* VAF and clinical outcomes was further supported by our analysis which showed that patients with *TP53* VAF 20% had significantly worse OS than those with VAF <20% (median OS: 3.5 months versus 6.1 months; $P<0.05$). This is consistent with other studies which demonstrated that patients with lower mutant *TP53* burden are more likely to respond to frontline therapy and have better outcomes, particularly with conventional cytarabine-based regimens.[11,26–28] Interestingly, the median OS of 6.1 months for patients with *TP53* mutation with VAF <20% is approximately what would be expected in an unselected relapsed/refractory AML population, and therefore the clinical significance of these very low level subclonal mutations at relapse remains unclear. Our analysis is consistent with another study in the frontline setting that suggested that *TP53* VAF may impact prognosis, and it therefore raises further questions about whether *TP53* VAF should be considered in consensus risk stratification guidelines.[11]

We detected *TP53* mutations in 5 patients who had no morphological disease and who did not subsequently relapse. Overall, this constituted approximately 1% of evaluable patients, suggesting this is a relatively uncommon phenomenon. These mutations were of very low VAFs (median 2.5%). Together, the indolent nature of these mutations and their subclonal nature suggest that these may represent clonal hematopoiesis of indeterminate potential (CHIP).[29] With longer follow-up, it remains uncertain whether these persistent low-level *TP53* mutations could contribute to later relapse or the development of a secondary therapy-related neoplasm.

Interestingly, when we performed IHC on the baseline samples that were *TP53* WT by NGS, we found 5 patients with evidence of mutant p53 expression by IHC (4 with evidence of low-level mutant p53 protein expression and 1 with high-level mutant p53 protein

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expression). In contrast, at time of relapse and *TP53* mutation detection by NGS, we identified 3 patients with WT p53 protein expression by IHC. While these two methods of mutant p53 detection were largely concordant, the few cases of discordant findings suggest these two methods highlight the potentially complementary roles of NGS and IHC in detecting mutant *TP53*, particularly in the era of new *TP53*-specific therapies. More comprehensive studies are needed to further define the relative roles of NGS and IHC in the detection of mutated *TP53* and p53, respectively.

Novel therapies are needed to improve the duration of response and outcome in this group of patients with *TP53*-mutated AML[3]. Two such promising drugs in the treatment of *TP53*-mutated myeloid malignancies are eprenetapopt (APR-246) and magrolimab. Eprenetapopt has been proposed to work through restoration of transcriptional transactivation function of mutant p53, although its precise mechanism is still not fully established[30]. Similarly, the anti-CD47 monoclonal antibody magrolimab has been studied in combination with azacitidine in newly diagnosed AML, including a substantial subgroup of patients with *TP53* mutations[16]. In the 29 patients with *TP53*-mutated AML, the median OS was 12.9 months in patients with *TP53*-mutated AML, which compares very favorably to the median OS of <8 months achieved with a hypomethylating agent plus venetoclax[31,32]. Given the 15% rate of newly detectable *TP53* mutations that we observed in this study, one could consider evaluating these agents in the maintenance setting for patients with high-risk *TP53* WT AML. Such an approach might be especially important for patients treated with chemotherapy and/or HSCT, since these are the main factors that determined *TP53*-mutated relapse in our study. Conversely, the use MDM2 inhibitors should be avoided in patients with *TP53*-mutated disease, as these agents require the presence of functional p53 proteins to be effective[3]. The development of these new agents that may have *TP53*-specific or *TP53*-preferential activity highlights the importance of repeat molecular profiling at time of relapse in order to select optional salvage therapies.

As a retrospective study, this analysis has certain limitations. In our study, we excluded patients with CBF AML because these patients have a distinctly superior OS even in the relapsed/refractory setting, which may have skewed the findings of our post-relapse analysis; a future analysis evaluating these patients and their rates of *TP53* mutation acquisition may be informative. Our analysis specifically focused on the detection of *TP53* mutations by NGS over the course of therapy, and therefore we did not assess the development of new *TP53* deletions, which might also have prognostic or therapeutic importance. Repeated molecular testing over the course of the disease also was not performed regularly and was conducted only in a subset of patients. This could have caused an underestimation of the frequency of newly emergent *TP53* mutations and may have limited our assessment of the exact time of the development of a new mutation. The sensitivity of our *TP53* NGS assay was 1–2%; however most newly detectable *TP53* mutations were of very low VAF (for example, 38% had VAF <5%). Therefore, we cannot definitively determine whether these mutations were truly newly developed mutations or expansion of an already existing subclone at relapse. Higher sensitivity panels or single-cell sequencing could provide more accurate and comprehensive information in a subsequent analysis.

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In conclusion, this study demonstrated that 15% of patients with *TP53* WT AML can acquire one or more newly detectable *TP53* mutation(s) over the course of their treatment, particularly after intensive chemotherapy and/or HSCT. These newly detectable *TP53* mutations were associated with a complex karyotype in 45% of cases, with both the *TP53* mutation and cytogenetic complexity arising concurrently in a majority of these cases. Post-relapse OS was very poor if the *TP53* mutation was present at a VAF >20%, although the outcomes of patients with lower *TP53* VAF appear similar to previous reports of unselected patients in the relapsed/refractory setting, and thus the impact of these small subclonal mutations remains uncertain. Overall, our findings suggest that sequential monitoring for new, emergent *TP53* mutations over the course of AML therapy might have clinical utility. Such monitoring may be particularly relevant in the era of novels therapies with the potential to target *TP53*-mutated myeloid malignancies.

Funding Statement:

Supported by an MD Anderson Cancer Center Support Grant (CA016672) and SPORE. N.J.S. is supported by the K12 Paul Calabresi Clinical Oncology Scholar Award and the American Society of Hematology Junior Faculty Scholar Award in Clinical Research.

Data Availability Statement:

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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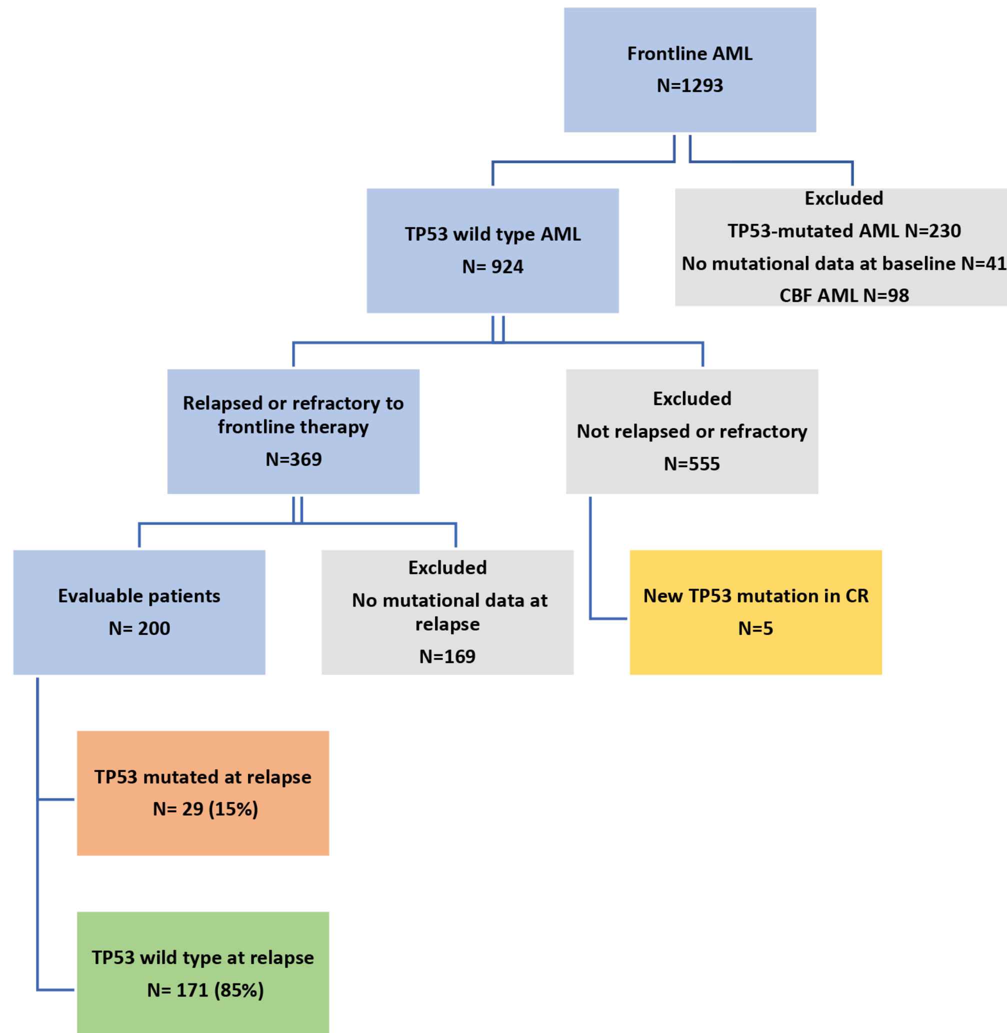

Fig. 1 - Patient selection flow chart

Abbreviations: AML, acute myeloid leukemia; CBF, core-binding factor

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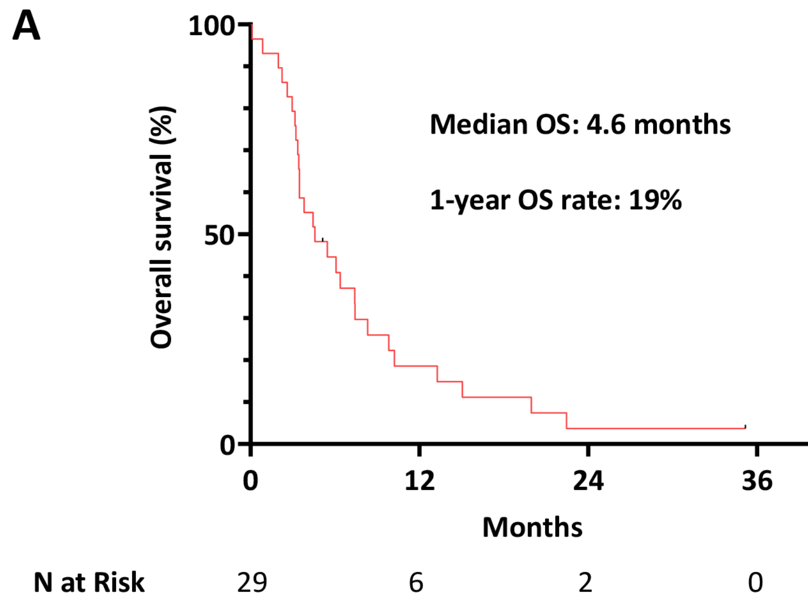

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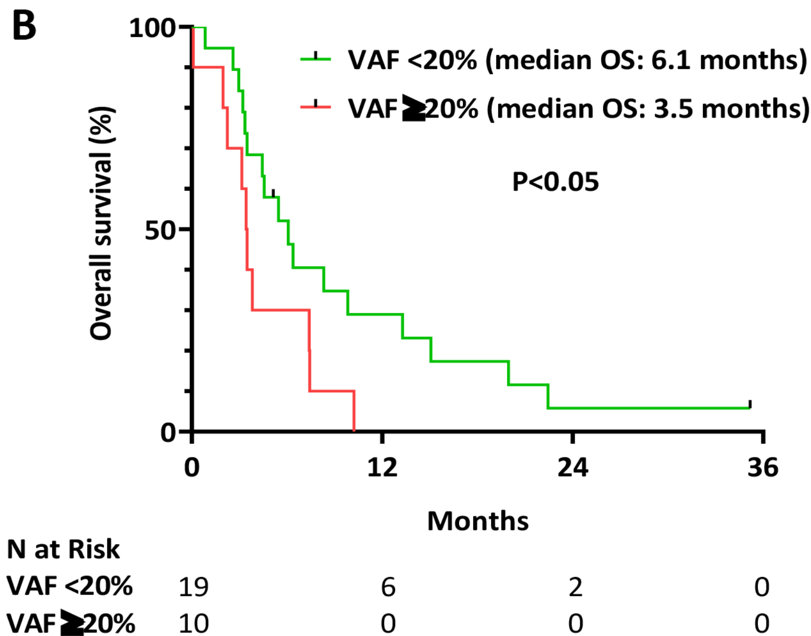

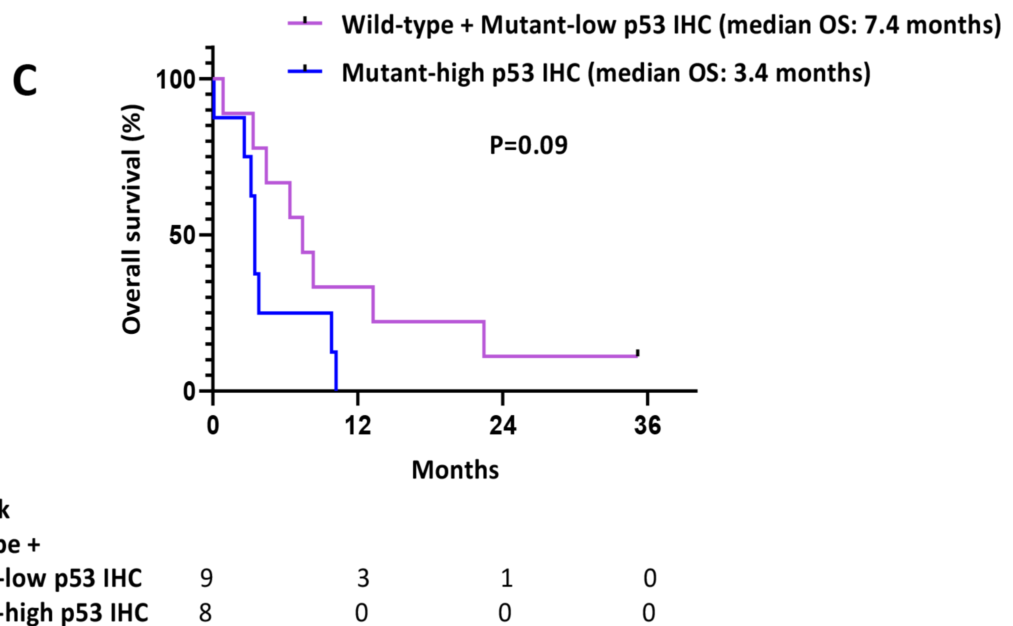

Fig. 2. Overall survival from the time of *TP53* mutation.

A.) Overall survival for the whole population, B.) Overall survival stratified by *TP53* variant allelic frequency <20% and 20%, C.) Overall survival stratified by p53 protein expression by immunohistochemistry

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Table 1.

Baseline patient characteristics of patients with *TP53*-wild type AML who were refractory to or relapsed after frontline therapy

Characteristic (N=200)	Median [range] / N (%)
Age, years	69 [17–94]
Therapy-related AML	26 (13)
Frontline therapy	
Intensive chemotherapy	69 (35)
Low-intensity therapy	131 (66)
Response to frontline therapy	
Relapsed	115 (58)
Refractory	85 (43)
European LeukemiaNet 2017 risk	
Favorable	21 (11)
Intermediate	94 (47)
Adverse	85 (43)
Cytogenetics	
Diploid	87 (44)
11q23 rearrangement	7 (4)
−7	31 (16)
−5/−5q	15 (8)
Complex	41 (21)
Other abnormalities	19 (10)
Mutations *	
SRSF2	1¼6 (24)
DNMT3A	46 (23)
IDH2	39 (20)
NRAS	36 (18)
ASXL1	35 (18)
RUNX1	33 (17)
FLT3-ITD	32 (16)
TET2	30 (15)
NPM1	29 (15)
DDX41	3/23 (13)
IDH1	22 (11)
CEBPA	22 (11)
SF3B1	3/31 (10)
PTPN11	14 (7)
U2AF1	¾6 (7)
ETV6	3/51 (6)
FLT3-D835	10 (5)
KRAS	10 (5)

*Only mutations present in 5% of cases are included

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Table 2.

Paired assessment of patients with newly detectable *TP53* mutation at time of relapse or refractory disease and of patients in remission at time of new *TP53* mutation detection

Paired assessment of patients with newly detectable *TP53* mutation at baseline and at time of relapsed or refractory disease					
Patient [a]	**p53 protein expression by IHC at baseline**	**Cytogenetics at baseline**	**p53 protein expression by IHC at relapse**	**Cytogenetics at relapse**	***TP53* VAF (%)**
1	Wild-type pattern	Complex	Wild-type pattern	Complex	5.5
2	Mutant-high pattern	Complex	Mutant-high pattern	Complex	95.6
3	Mutant-low pattern	Other	Mutant-low pattern	Other	16.7
4	Mutant-low pattern	Other	Wild-type pattern	Diploid	36.5
5	Mutant-low pattern	Diploid	Mutant-high pattern	Complex	43.3
6	Wild-type pattern	Other	Mutant-low pattern	Other	15.0
7	Wild-type pattern	Diploid	Wild-type pattern	Diploid	2.7
8	n/a	Diploid	Mutant-high pattern	Complex	19.1
9	Wild-type pattern	Diploid	Mutant-high pattern	Complex	19.9
10	Wild-type pattern	Other	Mutant-low pattern	Diploid	3.2
11	Wild-type pattern	Other	Mutant-high pattern	Other	1.1
12	Wild-type pattern	Other	Mutant-high pattern	Other	2.7
13	Wild-type pattern	Other	Mutant-low pattern	Other	9.3
14	Wild-type pattern	Other	Mutant-low pattern	Diploid	1.5
15	Wild-type pattern	Diploid	Mutant-high pattern	Complex	20.9
16	Wild-type pattern	Other	n/a	Other	1.1
17	Wild-type pattern	Diploid	Mutant-low pattern	Complex	1.4
18	Mutant-low pattern	Complex	Mutant-high pattern	Complex	95.2
Paired assessment of patients at baseline and at time of new *TP53* mutation detection while in remission					
Patient [b]	**p53 protein expression by IHC at baseline**	**Cytogenetics at baseline**	**p53 protein expression by IHC at time of new *TP53* mutation**	**Cytogenetics at time of new *TP53* mutation**	***TP53* VAF (%)**
1	Wild-type pattern	Diploid	Wild-type pattern	Diploid	1.6
2	Wild-type pattern	Diploid	Mutant-low pattern	Diploid	5.7
3	Wild-type pattern	Other	Wild-type pattern	Diploid	1.0
4	Wild-type pattern	Diploid	Mutant-low pattern	Diploid	1.6

Abbreviations: IHC, immunhistochemistry; VAF, variant allelic frequency

[a] 11 patients did not have samples available for IHC at baseline or relapse/refractory disease

[b] 1 patient did not have samples available for IHC at baseline or at time of new *TP53* mutation detection in remission