Comparison of TP53 mutations in myelodysplasia and acute leukemia suggests divergent roles in initiation and progression

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Key Points

- TP53 mutations in myelodysplastic syndrome and acute myeloid leukemia differ significantly in distribution and functional consequences.
- These differences suggest distinct biological roles for mutated p53 in initiation of myeloid dysplasia and progression to acute leukemia.

TP53 mutation predicts adverse prognosis in many cancers, including myeloid neoplasms, but the mechanisms by which specific mutations affect disease biology, and whether they differ between disease categories, remain unknown. We analyzed TP53 mutations in 4 myeloid neoplasm subtypes (myelodysplastic syndrome [MDS], acute myeloid leukemia [AML], AML with myelodysplasia-related changes [AML-MRC], and therapy-related AML), and identified differences in mutation types, spectrum, and hot spots between disease categories and in comparison to solid tumors. Missense mutations in the DNA-binding domain were most common across all categories, whereas inactivating mutations and mutations outside the DNA binding domain were more common in AML-MRC than in MDS. TP53 mutations in MDS were more likely to retain transcriptional activity, and comutation profiles were distinct between disease categories and mutation types. Our findings suggest that mutated TP53 contributes to initiation and progression of neoplasia via distinct mechanisms, and support the utility of specific identification of TP53 mutations in myeloid malignancies.

Introduction

The transcription factor p53, encoded by the TP53 gene, functions as a tumor suppressor in multiple tissues.1 TP53 is the most frequently mutated gene in human cancers, with mutation incidence exceeding 50% for many cancer types.2 In myeloid neoplasms, TP53 mutations occur in ~10% of cases,3,4 and, as for solid tumors, are strong and independent predictors of adverse prognosis, including failure of induction chemotherapy, relapse after hematopoietic stem cell transplantation, and reduced overall survival.5-8

Myeloid neoplasms have traditionally been divided into myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) by blast enumeration, with >20% blasts in the blood or bone marrow generally required for classification as AML; this division persists in the current World Health Organization (WHO) classification9 and International Consensus Classification (ICC).10 AML arising via progression from MDS, or after cytotoxic chemotherapy or radiation, have worse prognosis than de novo AML and are separately classified; these categories were defined as AML with myelodysplasia-related changes.
(AML-MRC) and therapy-related AML (t-AML) in the revised fourth edition WHO classification, and are maintained in the fifth edition WHO classification and ICC.

The studies of Valk et al11 and Weinberg et al12 demonstrated that the prognostic impact of TP53 mutation cuts across these traditional classifications, with TP53-mutated MDS and AML showing similarly poor prognosis; consequently, the WHO fifth edition and ICC both recognize TP53-mutated myeloid neoplasia as a specific disease category.13,14 Subsequent studies have filled in important details about the relationship between TP53 mutation and clinical outcomes in MDS and AML, including the role of variant allele fraction (VAF) and the impact of multiple “hits” to TP53. It is now well established that increased VAF of the TP53 mutation correlates with worse outcomes.15 The relationship between presence of single vs multiple hits to TP53 and prognosis appears more complex; initial studies reported adverse outcomes only with multiple hits to TP53,16 but subsequent studies demonstrated dismal outcomes even with monoallelic TP53 mutation.12,15 Recent studies have suggested that this distinction may vary by classification (ie, AML vs MDS),16 and based on blast count and genomic landscape.17

By contrast, the impact of specific TP53 mutations and mutation types has not been adequately studied. TP53 is unusual among tumor suppressor genes in that mutations are strongly biased toward missense mutations, which potentially retain function, rather than inactivating mutations, and these mutations are enriched in the DNA-binding domain (DBD), with ~30% of mutations occurring at 7 “hot spots,” all within the DBD.18 The vast majority of missense mutations compromise its transcriptional activity, resulting in accumulation of mutant p53 because of its failure to induce MDM2, the E3 ubiquitin ligase that mediates its degradation. However, p53 missense mutations can retain some transcriptional activity: analysis of 2314 p53 mutants in a reporter assay revealed transcriptional activities from none to wild-type (WT) levels, with most DBD mutations reducing activity below 20% of that of WT p53.19 These properties raise the possibility that different TP53 mutation classes (eg, missense vs truncating), or even specific mutations, may drive neoplasia by distinct mechanisms and differentially affect prognosis. In support of this, a recent study demonstrated that the distribution of p53 mutational hot spots varies between AML subtypes, suggesting that there may be distinct and context-dependent roles for different mutations.20 However, there has not been any comprehensive assessment of the distribution of p53 mutations across broader categories of myeloid neoplasia, for example, MDS vs AML, or between hematologic neoplasms and solid tumors; neither WHO fifth edition nor ICC criteria distinguishes between TP53 mutation types. Given accumulating data indicating that TP53 mutational status informs optimal therapy in addition to prognosis,21-25 and development of novel therapies directly targeting mutant p53 protein,26-27 it is critical to understand the nature of p53 mutations in these diseases, and their biological functions in modulating disease initiation or progression.

In this study, we analyzed TP53 mutations in myeloid neoplasms, comparing relative frequencies of mutation types, distributions across the p53 protein, and functional consequences between myeloid neoplasms and solid tumors and across categories of myeloid neoplasia. We identified significant differences between disease categories in types and locations of mutations, transcriptional capacity, and comutation profiles, with the greatest differences occurring between MDS and AML-MRC. These results suggest that TP53 mutations may play distinct pathogenic roles in different categories of myeloid disease, and that different functional categories of TP53 mutation may mediate initiation and progression of myeloid neoplasia.

**Methods**

**Study cohort**

We identified TP53 mutant cases of AML, MDS, AML-MRC, and t-AML in the cBioPortal database, a public-facing repository that incorporates data from multiple studies,28,29 and in the Hematologic Malignancies Data Repository of the Dana-Farber Cancer Institute. From cBioPortal, we incorporated AML data sets from Oregon Health and Science University30 and the Cancer Genome Atlas31; MDS data sets from the University of Tokyo32 and Memorial Sloan-Kettering Cancer Center (MSKCC)33,34; and an MDS/AML data set from Washington University in St. Louis.35 All cases in both data sets were annotated with diagnostic classification and mutation(s) identified by next-generation sequencing, including nucleotide change, amino acid change, and VAF; additional information, including karyotype, treatment history, and clinical outcome, was available for a limited subset of cases. The data sets were not significantly different in terms of the ratios of missense to inactivating TP53 mutations within disease categories. The only exception was the Vanderbilt-Ingram Cancer Center (VICC) data set, which had fewer missense mutations in all disease categories but otherwise reflected the same trends seen in the other data sets. Duplicates within and between databases were manually filtered and removed, and only cases with TP53 mutations annotated as pathogenic or likely pathogenic were included in the analysis (ie, nonpathogenic variants were excluded). After filtering, there were a total of 1117 independent instances of TP53 mutations in myeloid neoplasms, representing 536, 349, 167, and 65 instances in AML, MDS, AML-MRC, and t-AML, respectively.

We classified TP53 mutations as nonsense, frameshift, splice, in-frame indel, or missense, and calculated the frequency of each mutation type in each disease. Information on large deletions was not available. When samples from the same patient were sequenced multiple times, replicates were removed for cataloging p53 mutations but were retained for analyses involving VAF and comutations to capture evolving changes. For survival analysis of individuals with multiple TP53 mutations, the mutation with the highest VAF was considered.

**Peak finding**

Peaks in mutation frequency above background were identified by find_peaks from SciPy’s Signal processing in Python 3.8.

**Comutation analysis**

Data were obtained from cBioPortal using the cBioPortalData Biocompactor package in R version 4.1.3 from the previously identified studies. All comutation plots were created using the CoMut python package.

**Statistical analyses**

Continuous variables were compared by Welch t test. Categorical variables were compared pair-wise by Fisher exact test. Fisher
exact test was also used for comutation studies. In all cases, Bonferroni corrections for multiple hypothesis tests were applied.

Comparison of underlying mutation frequencies across disease categories

Using the data set of missense mutations in AML, MDS, and AML-MRC, letting $d$ denote the disease and $l$ the number of codons, we observed a vector of integers $y_d = (y_{d1}, y_{d2}, ..., y_{dl})$ for each disease. In total, 411 mutations were observed among patients with AML, 286 for those with MDS, and 113 for those with AML-MRC. Considering only codons with at least 1 mutation recorded under any disease, we have that $l = 121$. Assuming $y_d \sim \text{Multinomial} \left( \sum y_d, \theta_d \right)$ and $\theta_d \sim \text{Dirichlet}(\alpha)$, then $\theta_d | y_d \sim \text{Dirichlet}(\alpha + y_d)$. We set $\alpha$ such that $\alpha_l = \sum d y_d$, as an empirical prior. We compared myeloid neoplasms with the Institute for Systems Biology Cancer Genomics Cloud (ISB-CGC) data set using a similar framework.

In total, the analyzed data sets included 414 unique mutations; $\sim$20% of samples contained multiple $\text{TP53}$ mutations, with <3% having a mutant, consistent with previously reported statistics.3 In all disease categories, >80% of DBD mutations were missense, consistent with reports in other cancer types (t-AML was omitted from this and subsequent analyses, because of its small sample size).34,35 By contrast, frameshift and nonsense mutations occurred relatively more frequently in other regions, particularly in the nuclear localization sequence (Figure 1E). The first activation domain did not contain any nonsense mutations in any disease category (Figure 1C). Overall, these results recapitulated the general trend of enrichment of missense mutations in the $\text{TP53}$ DBD,44,45 and reveal differences in mutation type and location between myeloid neoplasms, including relative enrichment of null and splice-site mutations in AML-MRC compared with MDS.

Table 1. Percentage of cases harboring the indicated number of distinct $\text{TP53}$ mutations

<table>
<thead>
<tr>
<th>Disease</th>
<th>1, % (n)</th>
<th>2, % (n)</th>
<th>$\geq$3, % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>78.6 (338)</td>
<td>19.1 (82)</td>
<td>2.3 (10)</td>
</tr>
<tr>
<td>MDS</td>
<td>77.3 (214)</td>
<td>20.6 (57)</td>
<td>2.2 (6)</td>
</tr>
<tr>
<td>AML-MRC</td>
<td>79.5 (109)</td>
<td>19.0 (26)</td>
<td>1.5 (2)</td>
</tr>
<tr>
<td>t-AML</td>
<td>81.8 (45)</td>
<td>18.2 (10)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Cases were stratified by disease type, and the percentage of cases with 1, 2, and $\geq$3 mutations was determined. For patients of whom multiple samples were sequenced, the largest number of distinct $\text{TP53}$ mutations identified in a single sample was considered.

Results

$\text{TP53}$ mutation types and locations vary between myeloid neoplasms

In the analyzed data sets, 6.9% to 23.5% myeloid neoplasia cases were $\text{TP53}$ mutant, consistent with previously reported statistics.9 In total, the analyzed data sets included 414 unique mutations; $\sim$20% of samples contained multiple $\text{TP53}$ mutations, with <3% containing $\geq3$ mutations (Table 1). The distribution of VAFs was similar between disease categories except for MDS, which had significantly lower VAFs, consistent with expected lower blast counts (supplemental Figure 1A; supplemental Table 1). Patterns of single-nucleotide substitutions in $\text{TP53}$ were similar between diseases; C→T mutations, which showed a coding strand bias, comprised approximately half of all single-nucleotide changes, consistent with mutations arising from DNA methylation (supplemental Figure 1B). In samples with multiple mutations, VAFs were generally within a twofold range, indicating that the mutations arose at similar points in disease progression (supplemental Figure 1C, region between gray lines).

Missense mutations were most prevalent across all disease categories, as expected.20,24,25 Nevertheless, the spectrum of mutation types varied between neoplasms, with MDS showing significantly more missense mutations and fewer splice-site mutations than AML-MRC (Figure 1A; supplemental Figure 1D). This trend was maintained among cases with multiple $\text{TP53}$ mutations (Figure 1B) or with VAF of $\geq0.55$, a proxy for loss of heterozygosity (Figure 1C). We did not observe significant differences in mutation spectrum between cases with and without complex karyotype; however, only a subset of cases in the data set contained full karyotype information, limiting the statistical power of our analysis. Because the data sets pooled multiple studies, resulting in heterogeneous patient populations, we repeated this analysis on 2 individual studies, the MSKCC (2020) cohort and the VICC cohort. In both cases, results were similar to those observed with the parent (pooled) data set, with missense mutations enriched and splice mutations depleted in MDS compared with AML-MRC (supplemental Figure 1E-F). Notably, these results narrowly failed to achieve significance in the Memorial Sloan-Kettering data set, demonstrating the importance of analyzing the larger, pooled data sets to obtain sufficient sample sizes to achieve statistical significance while retaining the overall trends of individual studies.

While mutations were strongly enriched in the DBD for all disease categories, as reported,25 DBD enrichment was significantly higher in MDS than in AML or AML-MRC (Figure 1D). A nuclear localization sequence—containing region adjacent to the DBD was significantly enriched for mutations in AML-MRC (Figure 1D). In all disease categories, >80% of DBD mutations were missense, consistent with reports in other cancer types (t-AML was omitted from this and subsequent analyses, because of its small sample size).34,35 By contrast, frameshift and nonsense mutations occurred relatively more frequently in other regions, particularly in the nuclear localization sequence (Figure 1E). The first activation domain did not contain any nonsense mutations in any disease category (Figure 1C). Overall, these results recapitulated the general trend of enrichment of missense mutations in the $\text{TP53}$ DBD,44,45 and reveal differences in mutation type and location between myeloid neoplasms, including relative enrichment of null and splice-site mutations in AML-MRC compared with MDS.

Analysis of distribution of $\text{TP53}$ mutations reveals myeloid-specific and disease-specific hot spots

To obtain a more granular view of the types and locations of $\text{TP53}$ mutations, we constructed lollipop plots depicting the frequency of missense, nonsense, and frameshift mutations at each codon in each disease (Figure 2A). Of 393 amino acids, 195 were mutated at least once; 128 mutated positions harbored missense mutations, and 27 positions showed examples of both missense and null mutations, with a general bias toward missense mutations. To determine whether the overall pattern of mutations (their locations and frequencies) differed between neoplasms, we plotted the cumulative fraction of all mutations along the length of the protein (Figure 2B). The greatest differences in the cumulative curves were seen between MDS and AML-MRC, with AML-MRC being biased toward C-terminal mutations (Figure 2B, lower). To identify
individual hot spot mutations in a quantitative and unbiased manner, we applied an algorithm that detects peaks in mutation frequency above local background levels. This analysis was conducted only for missense mutations because of the relative paucity of other mutation types. Of the 7 most common TP53 mutation sites in human cancers (subsequently referred to as “pan-cancer hot spots”), 6 were identified as peaks in AML (R175, Y220, G245, R248, R273, and R282); R249 was the only hot spot in the pan-cancer set not identified as such in myeloid neoplasms (Figure 2C). MDS and AML-MRC additionally lacked hot spots at G245 and R282. Contact mutations (R248Q, R273H, and R282W) were more common in AML-MRC.
Figure 2.
(16.8%) than in either AML or MDS (10.9% and 12.9%, respectively), whereas conformational mutations (R175H, Y220C, G245S, and R249S) occurred with nearly equal prevalence in all categories (11.4%-12.4%). In addition to the 7 pan-cancer hot spots, we identified several hot spots in 2 of 3 diseases, which we termed “myeloid specific” (irrespective of their mutation rates in individual solid tumors). “Disease-specific” hot spots were defined as being present in only 1 disease. These results highlight the overall similarities in mutation patterns between myeloid neoplasms while also revealing differences in mutation distributions (frequency and location).

Next, we performed a Bayesian analysis to determine to what degree codons with missense mutations differed in their underlying mutation frequency between diseases. This analysis was performed for the pooled data set of myeloid neoplasms compared with the Institute for Systems Biology Cancer Genomics Cloud (ISB-CGC) data set of mutations derived from all cancer types to assess differences between hematologic malignancies and solid tumors. It was separately conducted pair-wise for the different categories of myeloid neoplasms (Figure 2D). Sampling from the distributions of inferred mutation frequencies and then calculating the difference between them yielded a simulated expected difference with a 95% Bayesian credible interval for each codon. The largest expected difference between myeloid neoplasms and the pan-cancer data set was observed for mutations at codon Y220, which were enriched in myeloid neoplasms; mutations at R175, R248, and R273 were also over-represented in myeloid neoplasms, whereas mutations at R245, R249, and R282 were under-represented. (Figure 2D, top panel). The estimated differences between the categories of myeloid neoplasms were smaller than those between myeloid neoplasms and solid tumors, with no individual codons at which the 95% Bayesian credible interval of the difference in mutation frequency excluded 0 occurring in any of the pair-wise comparisons (Figure 2D, bottom).

We then cataloged the amino acid changes resulting from missense mutations in each disease. Overall, 77 codons showed at least 2 different substitutions (Figure 3; supplemental Figure 2). C176 had the greatest diversity of substitutions, with mutations to F, G, R, S, W, and Y detected across diseases. The frequencies of specific substitutions varied between diseases at some positions. R273H was more prevalent in AML-MRC than in AML (9.5% vs 5.8%), with MDS showing an intermediate prevalence (7.3%; Figure 3). R248 was biased toward substitution to Q over W in MDS, whereas AML-MRC showed the opposite trend, and AML showed almost no bias. These patterns suggest both flexibility and specificity in pathogenic TP53 mutations.

Overall, our findings demonstrate a distinct distribution of p53 mutations in myeloid neoplasms compared with solid tumors, and suggest distinct mutational patterns between individual myeloid neoplasms. The differences in amino acid substitutions between diseases at particular codons suggest distinct functional roles for specific p53 mutations in disease initiation and progression.

Transcriptional activity and comutation profiles of TP53 mutations differs between categories of myeloid neoplasia

To assess whether the biological consequences of mutated p53 might vary between types of myeloid neoplasia, we first calculated the distribution of transcriptional activity scores for missense mutations in each neoplasm (see “Methods”), and found that it was significantly higher in MDS than in AML-MRC, with AML falling between these (Figure 4A). This result held true when the analysis was performed only on the subset of cases with VAF of ≥0.55, indicating that it was not an artifact of the status of the second allele (Figure 4B).

To gain further insight into the interactions of mutant TP53 with other oncogenic pathways, we investigated patterns of comutation between TP53 and known leukemic driver genes. DNMT3A, TET2, and NRAS were commonly comutated with TP53, whereas FLT3 and NPM1 comutations were significantly less common than in cases with WT TP53, consistent with previous studies. FLT3 and NPM1 mutations were under-represented in TP53-mutant AMLs compared with TP53-WT AML (supplemental Figure 3; supplemental Table 3). When parsed by TP53 mutation type, comutations in 26 driver genes were under-represented in TP53 nonsense and splice mutations (Figure 4C; supplemental Table 4): across samples with both single and multiple TP53 mutations, the average number of comutations was 0.36 and 0.59 per TP53 nonsense or splice mutation, respectively, compared with a comutation rate of 0.88 for TP53 missense mutations. In AML-MRC, nonsense TP53 mutations were significantly associated with comutation of DNMT3A (Figure 4D), with 2 of 6 DNMT3A mutations occurring with nonsense TP53 (for comparison, none of the 19 DNMT3A mutations in MDS cooccurred with nonsense TP53). These findings provide further evidence that TP53 null and splice-site mutations may mediate pathogenesis of myeloid neoplasms through distinct mechanisms from those of the more common missense mutations.

Analysis of impact of TP53 mutation class on outcomes in AML and AML-MRC

To investigate whether TP53 mutation types affect patient outcomes, we first grouped TP53 mutations as missense or null, with
the latter category comprised of frameshift, nonsense, and splice mutations. This grouping was chosen because of the distinct effects of the mutation type on p53 protein: null type mutations do not express detectable protein, whereas missense mutants tend to overexpress it. We observed a modest trend toward longer survival for p53-null cases in both AML and AML-MRC (Figure 4E-F), although survival for both null and missense mutations was shorter than that reported for WT p53.11,12 These differences did not achieve statistical significance, and the small sample sizes involved and potential for confounding variables (eg, differences in treatment among patients at different institutions) precludes drawing definitive conclusions. However, these results suggest that TP53 missense mutations may exert oncogenic effects in AML and AML-MRC beyond simply inactivating its tumor-suppressive function.

Discussion

In this study, we identified differences in TP53 mutations between myeloid neoplasms and solid tumors, as well as between subtypes of myeloid neoplasms, particularly between MDS and AML-MRC. These findings suggest discrete biological mechanisms for mutant p53 in hematologic malignancies, particularly in driving initiation and progression of myeloid neoplasia, and indicate that molecular and functional characterization of TP53 mutations, as opposed to p53 immunohistochemistry alone, may be required for optimal assessment of TP53-mutant myeloid neoplasms in clinical practice. Notably, our analyses took advantage of large consortia that pool data from multiple independent studies and institutions (eg, cBioPortal) to uncover associations between TP53 mutation types, functions, and disease categories. This approach has been
Figure 4. p53 transcriptional activity and comutations vary between classes of neoplasia and TP53 mutation types. (A) Box and whisker plots of transcriptional activity scores of missense mutants in the indicated disease categories; AML, n = 408; MDS, n = 282; and AML-MRC, n = 113; *P < .05 by Welch t test with Bonferroni correction. (B) Box and whisker plots of transcriptional scores of missense mutants with VAF of ≥0.55 in the indicated neoplasms; AML, n = 79; MDS, n = 32; and AML-MRC, n = 30. In panels A-B, horizontal line indicates median, “X” marks the mean, and box top and bottom represent the 75th and 25th percentiles, respectively. Median (lower) and mean (upper) values are given for each bar. Error bars represent the 95% confidence interval. (C) Comutations in TP53 with the indicated leukemic driver genes, segregated by TP53 mutation type. (D) Comutations of DNMT3A with the indicated classes of TP53 mutations in AML, MDS, or AML-MRC. Color indicates the percent of DNMT3A mutations cooccurring with the indicated class of TP53 mutation. *P < .05 by Fisher exact test comparing the association of DNMT3A mutation with the indicated TP53 mutation type vs all other TP53 mutation types. (E) Survival curves of AML (n = 10 null, and n = 15 missense TP53) or (F) AML-MRC (n = 13 null, and n = 22 missense TP53) patients with the indicated TP53 mutation types. For panels E-F, P values calculated by Gehan-Breslow-Wilcoxon test.
fruitful in yielding both novel mechanistic insights and clinically useful correlations in solid tumors.40-42 However, data derived from such sources are, by nature, heterogeneous in their analytical methods and clinical treatment protocols. Future studies using cohorts derived from single institutions will be critical to ensure that the conclusions are not influenced by hidden confounding variables that can occur in both overly homogeneous or heterogeneous data sets; furthermore, these studies may uncover additional associations or determine impacts of other variables (eg, blast count or monoallelic vs biallelic mutation) on the correlation between individual mutations/mutation classes and clinical outcomes.

We identified significant differences in TP53 mutations between myeloid neoplasms and solid tumors. Most notably, we observed marked enrichment of mutations at codon Y220, which are relatively uncommon in solid tumors but were among the most frequent mutations in myeloid neoplasms in our data set. This finding is of particular interest because the properties of this mutant protein render it uniquely susceptible to reactivation by small-molecule stabilizers, particularly carbazole-based agents.43-45 The p53-stabilizing agent enrenetapost has demonstrated efficacy in TP53-mutant myeloid neoplasms when used in combination with azacitidine and venetoclax.44-46 Although this specific agent appears to have limited activity against Y220 mutants,46 carbazoles are being evaluated in clinical trials for solid tumors.47,48 Our findings thus argue for prioritizing evaluation of these agents in myeloid neoplasms. Furthermore, given that several p53-targeting agents currently in clinical trials have differential activity against specific mutations,48 our findings illustrate the potential benefit of systematically identifying TP53 mutations in myeloid neoplasms to identify patients who might benefit from this and other mutation-specific therapies.

Among myeloid neoplasms, MDS and AML-MRC showed the greatest differences in terms of their types, distributions, and predicted transcriptional activities of TP53 mutations. These findings suggest that distinct types of TP53 mutations mediate initiation of MDS and progression of MDS to AML. We found that R175H, G245S, and R282W were underrepresented in MDS and AML-MRC compared with AML; the R175H result is consistent with the study of Tashakori et al.20 Conversely, R273H mutations were more common in MDS and AML-MRC than in AML. We found that missense mutations, particularly those retaining higher levels of transcriptional activity, are overrepresented in MDS, whereas mutations resulting in complete loss of expression or transcriptional activity of p53 protein are relatively overrepresented in AML-MRC. This result suggests that p53 missense mutations may play a more active role in initiation of MDS, possibly by acquiring gain-of-function properties, whereas p53 inactivation is sufficient for progression to AML-MRC. The specific association of TP53 nonsense mutations with DNM3A mutations in AML-MRC supports this hypothesis, because DNM3A mutations (and, more broadly, mutations in genes that regulate chromatin accessibility and/or epigenetic control of gene expression) are common founder mutations in both MDS and clonal hematopoiesis of indeterminate potential.50 Notably, WT p53 protein can assume a “pseudomutant” conformation in hematopoietic stem cells, frequently in the presence of DNM3A mutations, leading to mutant-like activation of downstream transcriptional pathways.50 Thus, in this setting, TP53 missense mutation may not provide additional selective advantage to preleukemic clones, whereas truncating mutations may be capable of promoting further leukemogenesis and driving progression to AML-MRC. Overall, these findings raise the intriguing possibility that specific identification of TP53 mutations in MDS may be useful in predicting which patients are likely to progress to AML. Tracking the evolution of TP53 mutations in individual patients during progression from MDS to AML-MRC will be instrumental in further evaluation of this question. It would also be of interest to analyze the distribution of TP53 mutations as a function of blast percentage in MDS, particularly in light of recent data indicating that the clinical impact of TP53 mutation is different in MDS with and without excess blasts.17 Although such analysis would require larger and more comprehensively annotated data sets than those used for this study, it might identify whether differences in the nature and distribution of mutations manifest at an even earlier stage than the transition from MDS to AML.

We observed that in both AML and AML-MRC, inactivating mutations tended to show longer survival than missense mutations, although these differences were not statistically significant. Prior studies demonstrating no significant survival difference between TP53 mutation types did not distinguish between categories of myeloid neoplasia.26 Our data suggest that more detailed multivariate analyses accounting for both neoplasm type and TP53 mutation type will be required to conclusively determine the effects of TP53 mutation class on patient survival. Notably, any differences in survival could arise from differences in TP53 mutations themselves or from the spectrum of comutations, which varied according to neoplasm subtype as well as TP53 mutation types. Specifically, DNM3A mutations were associated with AML-MRC containing nonsense TP53 mutations, but the consequences of this association remain unknown.

Clinical assessment of TP53 mutation has historically operated under the guiding assumption that loss of functional p53 protein activity mediates its role in driving neoplasia, and, therefore, that specific identification and characterization of mutations are not clinically useful, and that p53 immunohistochemistry (a proxy for loss of protein function) is sufficient for diagnostic workup. This assumption is embedded in recent updates to classification schema for hematologic malignancies; both the fifth edition WHO classification and the ICC of myeloid neoplasms incorporate TP53 mutation status into classification of myeloid malignancies,9,10 but neither scheme incorporates mutation type or functional consequence. Our findings suggest that such assessment may be incomplete, and that assessment of TP53 mutation type and consequences may be necessary to fully account for its impact on disease biology and prognosis.

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Authorship

Contribution: A.J. and S.B.L. designed the concept, developed the study, and wrote the original draft of the manuscript; E.E.A. and B.A.A. performed computational and statistical analysis of data; G.L. advised the study and provided essential resources; and all authors contributed to the final version of the manuscript.

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