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Letter to Blood

TO THE EDITOR:

TP53 mutation in therapy-related myeloid neoplasm defines a distinct molecular subtype

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Recent World Health Organization (WHO)[1] classifications (5th edition), International Consensus Classification (ICC),[2] and the European LeukemiaNet (ELN) guidelines[3] included new categories for *TP53*-mutated (*TP53*^{mut}) myeloid neoplasms (MNs) to acknowledge their uniformly poor outcomes and stimulate clinical research. However, there are critical differences in the details between these classifications, especially regarding single-hit *TP53*^{mut} status, and their significance relating to therapy-related myeloid neoplasm (t-MN), a rare but often fatal malignancy diagnosed following exposure to cytotoxic therapies, remains unclear. Here, we report an international cohort consisting of t-MN with full characterization of *TP53*^{mut} allele status and provide compelling evidence of poor outcome of *TP53*^{mut} t-MN irrespective of the allelic status of *TP53*.

The WHO defines a single category of myelodysplastic syndrome (MDS) with biallelic *TP53* inactivation (MDS-bi*TP53*) irrespective of the blast percentage[1] but excludes single-hit *TP53*^{mut} MDS with bone marrow (BM) blasts <20%. Likewise, in the ICC, single-hit *TP53*^{mut} MDS with blasts <10% is excluded from the definition of *TP53*^{mut} MN.[2] Similarly, the recent International Prognostic Scoring System-Molecular acknowledged the poor outcome of multi-hit *TP53*^{mut} but excluded single-hit *TP53*^{mut}.[4] The ICC and ELN guidelines emphasize *TP53*^{mut} variant allele frequency (VAF) >10% regardless of single- or multi-hit status for MDS/acute myeloid leukemia (AML) and AML.[2,3] These critical differences in the classification of the *TP53*^{mut} MN reveal a lack of consensus among experts that is likely driven by limited evidence or conflicting results.[1-3] For example, in a large cohort consisting predominantly (93%) of de novo MDS, single-hit *TP53*^{mut} had outcomes similar to wild-type *TP53* (*TP53*^{wt}), whereas the association with complex karyotype (CK), high risk of AML transformation, and poor overall survival (OS) were limited to multi-hit *TP53*^{mut}.[5] In contrast, in another study the OS of *TP53*^{mut} AML and MDS with excess blasts were equally poor irrespective of single- or multi-hit *TP53*^{mut}.[6] Notably, MDS <10% blasts were not included in this study. Similarly, survival of MDS and AML with CK was equally poor irrespective of single- or multi-hit *TP53*^{mut} status, and the distinction between MDS and AML by blast percentage did not hold any predictive value.[7] Thus, the majority of the studies driving changes in classification are derived predominantly from de novo MDS and AML with only a small fraction of t-MN[5,7] or were restricted to MDS and AML with CK.[7] Overall, this highlights a lack of data in the clinical context of t-MN to resolve the complex interactions between *TP53*^{mut} single- versus multi-hit status, blast percentage, and VAF.

To address these gaps, we performed a comprehensive analysis of an international cohort of 377 t-MN patients that included 245 t-MDS (65%) and 132 t-AML (35%) (supplemental Methods, available at the *Blood* website). The median age at t-MN diagnosis was 67 years. Somatic mutation analysis identified 185 putative oncogenic mutations in *TP53* at VAF ≥2% in 132 (35%) patients (supplemental Figure 1 and Figure 1A). The majority of the *TP53*^{mut} patients with available information (n = 128; 96.9%) had a VAF >10% (n = 113; 88.2%), and only 15 (11.7%) had VAF ≤10% (supplemental Figure 1). Allelic imbalances overlapping the *TP53* locus were detected in 56 (14.8%) patients, including *TP53*^{mut} VAF >10% (n = 46) and <10% (n = 2) and without *TP53*^{mut} (n = 8, supplemental Figure 1). In summary, 123 t-MN patients had *TP53*^{mut} VAF >10% or LOH or cnLOH involving the *TP53* locus (supplemental Figure 1). Genomic instability including CK, monosomal karyotype, chromosome 5 aberrancies, and marker chromosomes were enriched in *TP53*^{mut} VAF >10% and/or LOH of *TP53* locus compared with *TP53*^{wt} t-MN (supplemental Table 1). Median OS was significantly shorter in the *TP53*^{mut} cases compared with in *TP53*^{wt} cases (8.3 vs 19.4 months; $P < .001$) (Figure 1B). The OS in *TP53*^{mut} t-MN with VAF ≤10% was similar to *TP53*^{wt} (Figure 1B), although the number of cases with *TP53*^{mut} VAF ≤10% were limited and requires further validation.

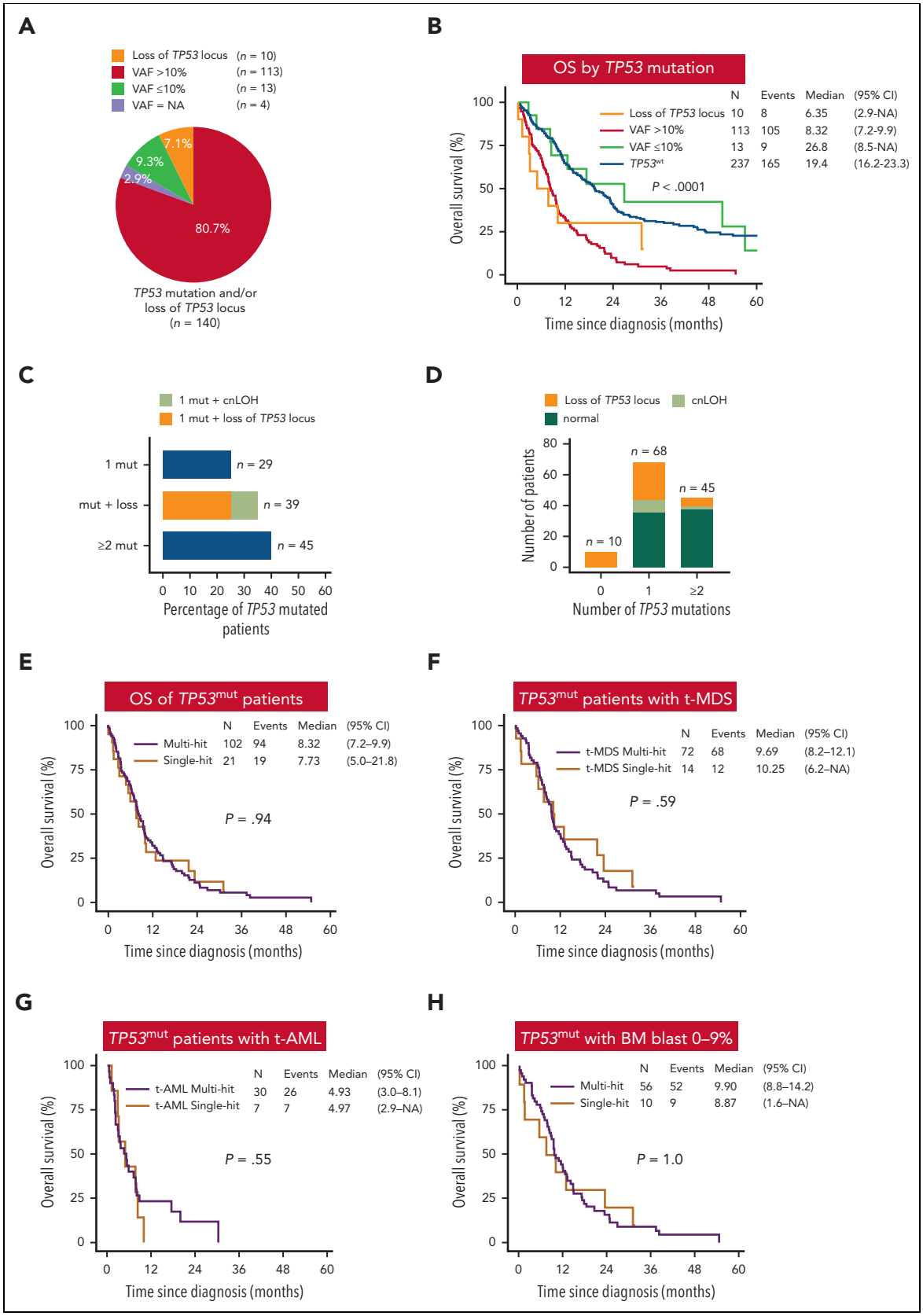

Figure 1. Survival of *TP53*^{mut} t-MN is poor irrespective of the allelic status or bone marrow blast percentage. (A) Proportion of *TP53*^{mut} t-MN patients with *TP53* VAF ≤10% or >10% or loss of *TP53* locus without *TP53*^{mut}. Of the 15 patients with VAF ≤10%, 13 had VAF ≤10% without loss of heterozygosity (LOH). Two patients had LOH across the *TP53* locus and were grouped with "loss of *TP53* locus" (n = 10). (B) OS of *TP53*^{mut} with VAF >10% or loss of *TP53* locus was significantly poor compared with *TP53*^{wt} and *TP53*^{mut} with VAF ≤10% t-MN. (C) The frequency of *TP53*^{mut} subgroup within the *TP53*^{mut} t-MN. *TP53*^{mut} subgroup were defined as cases with single mutation (1 mut),

Table 1. Comparison of genomic instability and other clinical features in single-hit and multi-hit *TP53*-mutated therapy-related myeloid neoplasms

Variables	Multi-hit (n = 102)	Single-hit (n = 21)	*P* value
Age at t-MN diagnosis, y, median (IQR)	67.5 (61.3, 74.0)	67.0 (61.0, 72.0)	.664
Female/Male	46/56	7/14	.346
Hemoglobin, g/L, median (IQR)	91.0 (81.0, 105.0)	87.0 (80.3, 102.0)	.585
WBC $\times 10^9$/L, median (IQR)	2.9 (1.9, 4.2)	2.7 (2.2, 5.3)	.586
ANC $\times 10^9$/L, median (IQR)	0.9 (0.5, 1.9)	1.2 (0.3, 1.8)	.91
Platelets $\times 10^9$/L, median (IQR)	54.5 (30.0, 92.5)	35.0 (25.5, 64.5)	.196
BM blasts %, median (IQR)	6.0 (2.0, 22.0)	4.0 (2.0, 29.0)	.88
t-MN phenotype			
t-MDS	72 (70.6)	14 (66.7)	.795
t-AML	30 (29.4)	7 (33.3)	
Cytogenetic changes			
Any cytogenetic aberrancies	101 (99.0)	20 (95.2)	.313
Complex karyotype, n (%)	91 (89.2)	18 (85.7)	.706
Monosomal karyotype, n (%)	85 (83.3)	16 (76.2)	.531
Marker chromosome, n (%)	59 (57.8)	9 (42.9)	.235
Ring chromosome, n (%)	21 (20.6)	4 (19.0)	1
Abnormal Chrom 17, n (%)	31 (30.4)	10 (47.6)	.136
Abnormal Chrom 5, n (%)	83 (81.4)	14 (66.7)	.148
Abnormal Chrom 7, n (%)	61 (59.8)	14 (66.7)	.63
Abnormal Chrom 3, n (%)	33 (32.4)	4 (19.0)	.3
Trisomy 8, n (%)	20 (19.6)	5 (23.8)	.766
Abnormal Chrom 9, n (%)	21 (20.6)	4 (19.0)	1
Abnormal Chrom 11, n (%)	27 (26.5)	5 (23.8)	1
Abnormal Chrom 12, n (%)	37 (36.3)	6 (28.6)	.619
Abnormal Chrom 13, n (%)	36 (35.3)	6 (28.6)	.622
Abnormal Chrom 16, n (%)	26 (25.5)	3 (14.3)	.399
Abnormal Chrom 18, n (%)	34 (33.3)	8 (38.1)	.801
Abnormal Chrom 19, n (%)	27 (26.5)	7 (33.3)	.594
Abnormal Chrom 20, n (%)	24 (23.5)	5 (23.8)	1
Abnormal Chrom 21, n (%)	34 (33.3)	5 (23.8)	.451
Somatic mutations on NGS			
TP53^{mut} VAF, median (IQR)	42.0 (31.6, 69.0)	37.70 (20.0, 43.0)	**.03**
Co-mutations (excluding *TP53*^{mut})			
≥ 2 mutations, n (%)	18 (17.6)	7 (33.3)	.06
1 mutation, n (%)	26 (25.5)	8 (38.1)	
No mutations, n (%)	58 (56.9)	6 (28.6)	
ASXL1, n (%)	7 (6.9)	3 (14.3)	.372

ANC, absolute neutrophil count; Chrom, chromosome; DMT, disease modifying therapy; HMA, hypomethylating agents; IQR, interquartile range; NGS, next-generation sequencing; SCT, stem cell transplant; WBC, white blood count.

*First line of therapy only.

†Six patients with muti-hit did not receive chemotherapy and/or radiotherapy. They had only immunosuppression.

Figure 1 (continued) ≥2 mutations without the loss of chromosome 17p13 across *TP53* locus (≥2 mut), mutation(s) plus copy neutral LOH (cnLOH) and/or loss of the *TP53* locus (mut + loss). (D) Number of patients with 0, 1, or ≥2 *TP53*^{mut}. Colors represent the status of chromosome 17 at the *TP53* locus, to include cnLOH, loss of *TP53* locus, and no detected aberration (normal). Unbalanced translocations leading to loss of *TP53* locus are encoded as "loss." OS of single- vs multi-hit *TP53*^{mut} in the whole cohort (E), t-MDS (F), t-AML (G), and bone marrow blast 0% to 9% (H).

Table 1 (continued)

Variables	Multi-hit (n = 102)	Single-hit (n = 21)	*P* value
RAS, n (%)	2 (2.0)	2 (9.5)	.135
RUNX1, n (%)	5 (4.9)	1 (4.8)	1
SF3B1, n (%)	2 (2.0)	0 (0.0)	1
SRSF2, n (%)	4 (3.9)	2 (9.5)	.272
TET2, n (%)	7 (6.9)	2 (9.5)	.65
DNMT3A, n (%)	11 (10.8)	5 (23.8)	.148
FLT3, n (%)	0 (0.0)	1 (4.8)	.171
IDH2, n (%)	1 (1.0)	0 (0.0)	1
Disease-modifying therapy for t-MN*			
No DMT, n (%)	20 (19.6)	6 (28.6)	.17
Intensive chemotherapy, n (%)	14 (13.7)	4 (19.0)	
HMA-based chemotherapy, n (%)	43 (42.2)	9 (42.9)	
Venetoclax-based therapy, n (%)	24 (23.5)	1 (4.8)	
Unknown, n (%)	1 (1)	1 (4.8)	
Allogeneic SCT, n (%)	16 (15.7)	3 (14.3)	1
Months between primary to t-MN, median (IQR)	103.4 (48.2, 165.5)	79.4 (43.9, 151.9)	.685
Clinical features at primary disease			
Age at primary disease, y, median (IQR)	57.0 (49.5, 65.0)	55.5 (48.3, 60.8)	.464
Hematologic malignancy, n (%)	61 (59.8)	14 (66.7)	.777
Solid cancer, n (%)	34 (33.3)	7 (33.3)	
Other, n (%)	7 (6.9)	0 (0.0)	
Treatment for primary cancer/disease†			
Chemotherapy alone for primary cancer/disease, n (%)	53 (52%)	9 (42.8%)	.603
Chemotherapy plus radiotherapy for primary cancer, n (%)	35 (34.3%)	9 (42.8%)	.621
Radiation only for primary cancer, n (%)	8 (7.8%)	3 (14.4%)	.602
Immunosuppression, n (%)	12 (11.8)	0 (0.0)	.217
Auto SCT for primary cancer, n (%)	26 (25.5)	6 (28.6)	.788

ANC, absolute neutrophil count; Chrom, chromosome; DMT, disease modifying therapy; HMA, hypomethylating agents; IQR, interquartile range; NGS, next-generation sequencing; SCT, stem cell transplant; WBC, white blood count.

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Among patients with *TP53*^{mut} VAF >10% (n = 113), 75% had single *TP53*^{mut} plus loss of *TP53* locus or cnLOH (n = 39; 34.5%) or ≥2 *TP53*^{mut} (n = 45; 39.8%), whereas 25% (n = 29) had single *TP53*^{mut} (Figure 1C). Of the 29 patients with single *TP53*^{mut}, 18 (62%) and 11 (37.9%) patients had VAF >50% and 10% to 50%, respectively (supplemental Figure 1). Additionally, 10 patients had loss of the *TP53* locus without evidence of *TP53*^{mut} (n = 8) or *TP53*^{mut} VAF ≤10% (n = 2) (Figure 1D and supplemental Figure 1). Loss or cnLOH of *TP53* locus was more prevalent in cases with single *TP53*^{mut} compared with ≥2 *TP53*^{mut} (57.4% vs 15.5%, $P < .0001$) (Figure 1D).

Integrating data from next generation sequencing, copy number, cytogenetic banding, fluorescence in situ hybridization, and single nucleotide polymorphism microarray analyses, *TP53*^{mut} were classified as multi- or single-hit following ICC[2] (supplemental Methods). In total, 21 (17.1%) of the 123 patients with *TP53*^{mut} and/or loss of *TP53* locus were considered single-hit and 102 (82.9%) were considered multi-hit (supplemental Figure 1). The clinical parameters and OS of t-MN with single-hit *TP53*^{mut} and 17p loss across the *TP53* locus were comparable (8.3 vs 6.3 months, $P = .58$) (supplemental Figure 2A and supplemental Table 2) and were combined for subsequent analyses.

We next compared the clinical features, profiles of genome stability, and patterns of co-mutation for each *TP53* state. In contrast to the findings for predominantly de novo MDS,[5] no difference in the frequency of structural chromosomal aberrancies including CK, monosomal karyotype, chromosome 5 aberrancy, or comutation pattern between single- and multi-hit *TP53*^{mut} t-MN were observed (Table 1). Similarly, no significant differences were observed in age, latency, blood counts, BM blast percentage, and cytogenetic aberrancies. Critically, OS was not significantly different between the single- and multi-hit *TP53*^{mut} in the whole t-MN cohort and for the t-MDS subgroup or the t-AML subgroup or when stratified by blast

percent categories (Figure 1E-H and supplemental Figure 2B). There was also no difference in the incidence of progression to AML between single- vs multi-hit *TP53*^{mut} t-MDS (supplemental Figure 2C). The striking enrichment of CK in single-hit *TP53*^{mut} t-MN (85.7%) compared with reported frequency of 13% in de novo MDS[5] and VAF cut-off >10% vs 2% could partly explain the difference between the 2 studies. Single nucleotide polymorphism microarray analyses and copy number analysis were unavailable for 7 cases with single-hit *TP53*^{mut} with VAF 10% to 50%, and thus few cases of multi-hit *TP53*^{mut} could have been missed. However, the OS between single- and multi-hit *TP53*^{mut} was still not significantly different after excluding these cases (supplemental Figure 2D).

These findings are highly relevant considering the changes proposed in the recent classifications. The ICC[2] and the ELN[3] removed the subcategory of "therapy-related," substituting it with diagnostic qualifiers instead. The WHO[1] has grouped t-MN with secondary MN and renamed it as "myeloid neoplasm post-cytotoxic therapy," with the assertion that the majority of MDS and AML occurring post–cytotoxic therapy have *TP53*^{mut} and that multi-hit *TP53*^{mut} have poor outcome compared with single-hit.[1] The underlying assumption of these changes is that *TP53*^{mut} MNs, regardless of the underlying etiology, have similar genomic characteristics and outcomes.

Our results provide compelling evidence that neither the allelic status nor the BM blast percentage of t-MDS provides meaningful prognostic information and that the *TP53* VAF of 10% is a clinically useful threshold to identify patients with poor survival. Underestimation of the poor prognosis of single-hit *TP53*^{mut} t-MDS, by exclusion from *TP53*^{mut} MN, has significant implications on patient management such as consideration for allogeneic transplantation and exclusion from clinical trials. Our findings, therefore, warrant reconsideration of the allelic status in *TP53*^{mut} t-MN.

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Authorship

Contribution: D.H. designed the study, contributed the patient data, analyzed the data, and wrote the manuscript; E.N.H.T. and R.C. collated the data, analyzed the data, and edited the manuscript; C.H.K. performed statistical analysis and edited the manuscript; C.H., E.N.H.T., A. Brown, and H.S. contributed to variant annotation; D.L. contributed to cytogenetic analysis; P.W. contributed to variant annotation and CNA analysis; S.K. contributed DDR expertise and edited the manuscript; N.S. reviewed the manuscript; A. Brown collected the data and contributed patients; A.A., H.A., T.B., M.R.L., A.M., W.J.H., N.G., M.P., K.B., A. Beligaswatte, R.H., P.G., M.K., D.M.R., D.S., N.S., P.B., D.Y., P.G., C.L., A.Y., N.H., R.G., I.S.W., A.W., and A.T. contributed patients and edited manuscript; D.T. edited the manuscript; M.V.S. designed the study, contributed the patient data, and edited the manuscript; and all authors agreed to the final version of the manuscript.

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Footnotes

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Data are available on request from the corresponding authors.

The online version of this article contains a data supplement.

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