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MYELOID NEOPLASIA

Molecular characterization of mutant *TP53* acute myeloid leukemia and high-risk myelodysplastic syndrome

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KEY POINTS

- **Mutant *TP53* AML and MDS-EB do not differ with respect to molecular characteristics and survival.**
- **Mutant *TP53* AML/MDS-EB should be considered a single molecular disease entity.**

Substantial heterogeneity within mutant *TP53* acute myeloid leukemia (AML) and myelodysplastic syndrome with excess of blast (MDS-EB) precludes the exact assessment of prognostic impact for individual patients. We performed in-depth clinical and molecular analysis of mutant *TP53* AML and MDS-EB to dissect the molecular characteristics in detail and determine its impact on survival. We performed next-generation sequencing on 2200 AML/MDS-EB specimens and assessed the *TP53* mutant allelic status (mono- or bi-allelic), the number of *TP53* mutations, mutant *TP53* clone size, concurrent mutations, cytogenetics, and mutant *TP53* molecular minimal residual disease and studied the associations of these characteristics with overall survival. *TP53* mutations were detected in 230 (10.5%) patients with AML/MDS-EB with a median variant allele frequency of 47%. Bi-allelic mutant *TP53* status was observed in 174 (76%) patients. Multiple *TP53* mutations were found in 49 (21%) patients. Concurrent mutations were detected in 113 (49%) patients. No significant difference in any of the aforementioned molecular characteristics of mutant *TP53* was detected between AML and MDS-EB. Patients with mutant *TP53* have a poor outcome (2-year overall survival, 12.8%); however, no survival difference between AML and MDS-EB was observed. Importantly, none of the molecular characteristics were significantly associated with survival in mutant *TP53* AML/MDS-EB. In most patients, *TP53* mutations remained detectable in complete remission by deep sequencing (73%). Detection of residual mutant *TP53* was not associated with survival. Mutant *TP53* AML and MDS-EB do not differ with respect to molecular characteristics and survival. Therefore, mutant *TP53* AML/MDS-EB should be considered a distinct molecular disease entity.

Introduction

Mutations in *TP53* are present in approximately 10% of patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) and represent a unique subtype with poor outcome.[1-5] *TP53* is located on chromosome 17p13 and is essential for cell cycle control and DNA damage response.[6] Although the exact mechanism of leukemogenesis for mutant *TP53* AML remains unknown, it has been shown that some *TP53* mutations drive a dominant negative effect and typically occur in founding clones that expand after cytotoxic stress.[7,8] Mutant *TP53* is strongly associated with large structural and complex chromosomal aberrations, as illustrated by the co-occurrence of complex karyotypes (CK), which is associated with reduced overall survival in myeloid malignancies.[9-11] In line with the observed poor outcome, mutant *TP53* AML is assigned to the adverse risk category of the 2017 European LeukemiaNet (ELN) risk classification and is recommended to receive intensive consolidation treatments.[11] Although patients with mutant *TP53* AML in complete remission (CR) generally receive allogeneic hematopoietic stem cell transplantation (HSCT), relapse rates remain considerably high.[12]

Recent findings in MDS assign additional prognostic value to the molecular characteristics of mutant *TP53*, including *TP53* mutant allelic status (mono- or bi-allelic) and *TP53* clone size.[4,5,13] However, recent studies exploring the link between these molecular characteristics and outcome in AML were limited and inconclusive.[14,15] Furthermore, it is currently unknown whether mutant *TP53* high risk MDS with excess of blast (MDS-EB) and AML differ in molecular makeup and response to treatment and should be considered as separate entities.

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Here, we present an in-depth characterization of a large cohort of newly diagnosed mutant *TP53* AML and MDS-EB in relation to survival. We performed next-generation sequencing (NGS) to assess the molecular characteristics of mutant *TP53* AML/MDS-EB in detail, including *TP53* mutant allelic status (mono- or bi-allelic), the number of *TP53* mutations, mutant *TP53* clone size, concurrent mutations, cytogenetics, and molecular minimal/measurable residual disease (MRD).

Methods

Patients and samples

In total, 2200 patients with AML and MDS-EB (international prognostic scoring system [IPSS] ≥ 1.5 or revised IPSS > 4.5) were assessed for eligibility and treated in the Haemato-Oncology Foundation for Adults in the Netherlands and Swiss Group for Clinical Cancer Research (HOVON-SAKK) clinical trials between 2001 and 2017 (supplemental Figure 1 available on the *Blood* Web site). All patients received standard induction chemotherapy and were consolidated according to the HOVON-SAKK study protocols. Details of treatment protocols were described previously (www.hovon.nl).[16-21] All trial participants provided written informed consent in accordance with the Declaration of Helsinki. DNA was isolated from diagnostic bone marrow samples of 2200 patients with AML/MDS-EB and 537 CR samples (supplementary Methods). In 33 patients with AML/MDS-EB carrying *TP53* variants with a variant allele frequency (VAF) >40%, DNA from saliva was available to verify the germline status.

Cytogenetics and SNP array analyses

Cytogenetic analysis was carried out at the local reference centers using standard protocols. These data, including karyotypes and FISH, were centrally peer-reviewed by clinical genetics laboratory specialists. The clonal structural and numerical chromosomal abnormalities were reported in accordance with the International System for Human Cytogenetic Nomenclature and the ELN 2017 recommendations.[11] CK was defined by 3 or more unrelated chromosome abnormalities in the absence of one of the World Health Organization–designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3), AML with *BCR-ABL1*.[11] Single nucleotide polymorphism (SNP) array was performed according to the manufacturer's instructions using Illumina Infinium GSA+MD-24 version 3.0 BeadChip (Illumina, Inc., San Diego, CA) on 134 of 230 mutant *TP53* AML samples (110 bi-allelic and 24 mono-allelic mutants). The array was scanned with the Illumina iScan Control. Genome studio version 2.1 and Nexus Discovery version 10.0 (Biodiscovery, El Segundo, CA) were used for data analysis.

Targeted NGS and *TP53* deep sequencing

The TruSight Myeloid Sequencing panel (Illumina) was used to detect the presence of driver mutations at diagnosis. Details were described previously.[22] Only pathogenic *TP53* variants were included as defined by occurrence in the COSMIC and IARC TP53 database as well as by analyses in silico with programs such as Polyphen-2, SIFT, FATHMM, MetaSVM, MetaLR, CADD, DANN, and ClinVar. The limit of detection was VAF 1% at diagnosis. To detect *TP53* mutations in CR, we used Illumina-based deep sequencing (supplementary Methods). The limit of detection in the follow-up samples was VAF 0.001% (variable depending on *TP53* mutation type). In the case of multiple *TP53* mutations, the highest VAF was chosen for MRD analysis. Of note, patients with *TP53* germline mutations were excluded from MRD assessment (n = 2).

Allocation of patients based on *TP53* mutant allelic status

Patients with mutant *TP53* AML/MDS-EB were considered bi-allelic when (1) 2 or more *TP53* gene variants were detected, regardless of the VAF; (2) at least 1 *TP53* gene variant co-occurred with a cytogenetic aberration involving chromosome 17p (eg, abnormality of 17p or monosomy 17); or (3) *TP53* mutations were detected with a VAF >55% (supplemental Figure 2). The allocation to the bi-allelic mutant *TP53* group by a VAF threshold of >55% was confirmed in all 15 of 110 patients with bi-allelic mutant *TP53* AML that could be evaluated for copy number alterations by SNP array analyses (ie, either loss of the wild-type allele or segmental uniparental disomy of the mutant *TP53* allele).

Statistical analysis

Associations between variables were tested by the Fisher's exact test for categorical variables and by the Mann-Whitney *U* test for continuous variables. The primary endpoint of the study was overall survival, defined as death from any cause. Survival time was calculated from the start of induction chemotherapy until the event of interest or censoring. Of note, the survival time in the analysis evaluating allogeneic HSCT started at the date of transplant. To compare the survival distributions, we used the log-rank test and the Cox proportional hazards model. The proportional hazards assumption was tested by interaction with time. All *P* values were two sided, and *P* values <.05 were considered statistically significant. Statistical analyses were executed with Stata Statistical Software, Release 16.0 (College Station, TX).

Results

Molecular characteristics of mutant *TP53* AML and MDS-EB

We detected 283 *TP53* mutations in 230 of 2200 (10.5%) patients with AML/MDS-EB by NGS (Table 1; supplemental Figure 1). Of 230 patients with AML/MDS-EB, 44 (19%) were diagnosed with MDS-EB. No significant difference in age, sex, white blood cells, remission rate, and consolidation treatment was present between AML and MDS-EB (Table 1). Deletion 5q was the only cytogenetic aberration significantly more frequently present in MDS-EB (*P* = .025). Of note, in 112 patients with mutant *TP53* AML/MDS-EB, concurrent chromosomal aberrations involving *TP53* (eg, abnormality 17p or loss of chromosome 17) were detected.

Two or more *TP53* mutations were found in 49 AML/MDS-EB cases (Table 2; supplemental Figure 2). In total, 206 missense, 16 nonsense, 38 insertion/deletion, and 23 splice-site mutations were detected (supplemental Figure 3). Nearly all missense mutations occurred in the TP53 DNA binding domain (supplemental Figure 3). In total, 56 of the 230 patients with *TP53* mutant AML/MDS-EB (24.3%) were considered mono-allelic and 174 (75.7%) were bi-allelic (Table 2; supplemental Figure 2). The

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Table 1. Patient characteristics of AML/MDS-EB with mutated *TP53* (n = 230)

	AML (n = 186)	MDS-EB (n = 44)	AML/MDS-EB (n = 230)	*P*
Age, y				.820
Median	62	63	62	
Range	18-80	35-73	18-80	
Sex, no. (%)				.736
M	111 (60)	25 (57)	136 (59)	
F	75 (40)	19 (43)	94 (41)	
White blood cells at diagnosis, no. (%)*				
≤100	183 (99)	44 (100)	227 (99)	1.000
>100	2 (1)	0 (0)	2 (1)	
Last treatment before first CR, no. (%)				.106
Refractory	70 (38)	10 (23)	80 (35)	
Cycle I	90 (48)	29 (66)	119 (52)	
Cycle II	26 (14)	5 (11)	31 (13)	
Consolidation therapy, no. (%)				1.000
No allogeneic HSCT	137 (74)	33 (75)	170 (74)	
Allogeneic HSCT	49 (26)	11 (25)	60 (26)	
Cytogenetics, no. (%)†				
Monosomy 5	51 (28)	11 (27)	62 (28)	1.000
Deletion 5q	78 (44)	26 (63)	104 (47)	.025
Monosomy 7	58 (32)	14 (34)	72 (33)	.855
Monosomy 17	71 (40)	10 (24)	81 (37)	.075
Abnormality 17p	33 (18)	6 (15)	39 (18)	.656
Complex karyotype	148 (83)	37 (90)	185 (84)	.343
Monosomal karyotype	139 (78)	35 (85)	174 (79)	.394

*Numbers may not sum to 230 because of missing values.

†Cytogenetics failed in 10 patients.

mutant *TP53* clone size was normally distributed with a median VAF of 47% (supplemental Figure 4A). Concurrent mutations were detected in only 113 (49%) patients with mutant *TP53* AML/MDS-EB (Figure 1). The most frequent concurrent mutations were detected in *DNMT3A*, *TET2*, *ASXL1*, *RUNX1*, and *SRSF2* (Figure 1; Table 2). The *TP53* mutant allelic status, number of *TP53* mutations, *TP53* clone size, and concurrent mutations at diagnosis did not significantly differ between mutant *TP53* AML and MDS-EB (Table 2).

Of note, most (84%) patients with mutant *TP53* AML/MDS-EB have CK, and many associations between CK and the different molecular characteristics were observed (Table 1; supplemental Table 1). CK was detected in most patients with bi-allelic mutant *TP53* (97%), in patients with multiple *TP53* mutations (94%), and in patients with larger *TP53* clones (94% in VAF >40%) (supplemental Table 1; supplemental Figure 4B). Concurrent mutations were enriched in AML/MDS-EB marked by non-CK, yet the most prevailing mutated genes (*DNMT3A* and *TET2*) were not significantly associated with CK (supplemental Table 1).

Association of mutant *TP53* characteristics and outcome in AML and MDS-EB

We next compared outcome of patients with mutant *TP53* AML/MDS-EB in relation to the established ELN 2017 prognostic subgroups. Mutant *TP53* strongly associated with reduced survival in the context of the ELN 2017 adverse risk category (2-year overall survival, 12.8% *TP53* mutant vs 42.5% *TP53* wild-type; $P < .001$) (Figure 2A). Because of the molecular homogeneity of mutant *TP53* AML and MDS-EB, we investigated whether the AML or MDS-EB status associated with survival. No difference in outcome was observed between the AML and MDS-EB mutant *TP53* subgroups ($P = .549$) (Figure 2B). All our findings indicate that mutant *TP53* AML/MDS-EB represents a homogeneous group and is therefore considered a singular entity in the following analysis.

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Table 2. Molecular characteristics of mutant *TP53* AML/MDS-EB (n = 230)

	AML (n = 186)	MDS-EB (n = 44)	AML/MDS-EB (n = 230)	*P*
***TP53* mutant allelic status, no. (%)**				.241
Mono-allelic	42 (23)	14 (32)	56 (24)	
Bi-allelic	144 (77)	30 (68)	174 (76)	
Number of *TP53* mutations, no. (%)				.153
Single	150 (81)	31 (70)	181 (79)	
Multiple	36 (19)	13 (30)	49 (21)	
Mutant *TP53* clone size, VAF (%)				.409
Median	48	41	47	
Range	1-97	3-91	1-97	
Mutation at diagnosis, no. (%)				
Any concurrent	95 (51)	18 (41)	113 (49)	.244
DNMT3A	25 (13)	6 (14)	31 (13)	1.000
TET2	17 (9)	3 (7)	20 (9)	.773
ASXL1	10 (5)	2 (5)	12 (5)	1.000
RUNX1	10 (5)	1 (2)	11 (5)	.695
SRSF2	11 (6)	1 (2)	12 (5)	.471

We performed survival analysis to evaluate the relationship of molecular characteristics and cytogenetic aberrations to outcome in mutant *TP53* AML/MDS-EB. Mono-allelic mutant *TP53* AML/MDS-EB had a similar dismal survival compared with its bi-allelic counterpart ($P = .327$) (Figure 3A; supplemental Figure 5). Neither the number of *TP53* mutations (Figure 3B) nor aberrations involving chromosome 17 (supplemental Figure 6) associated with altered outcome in patients

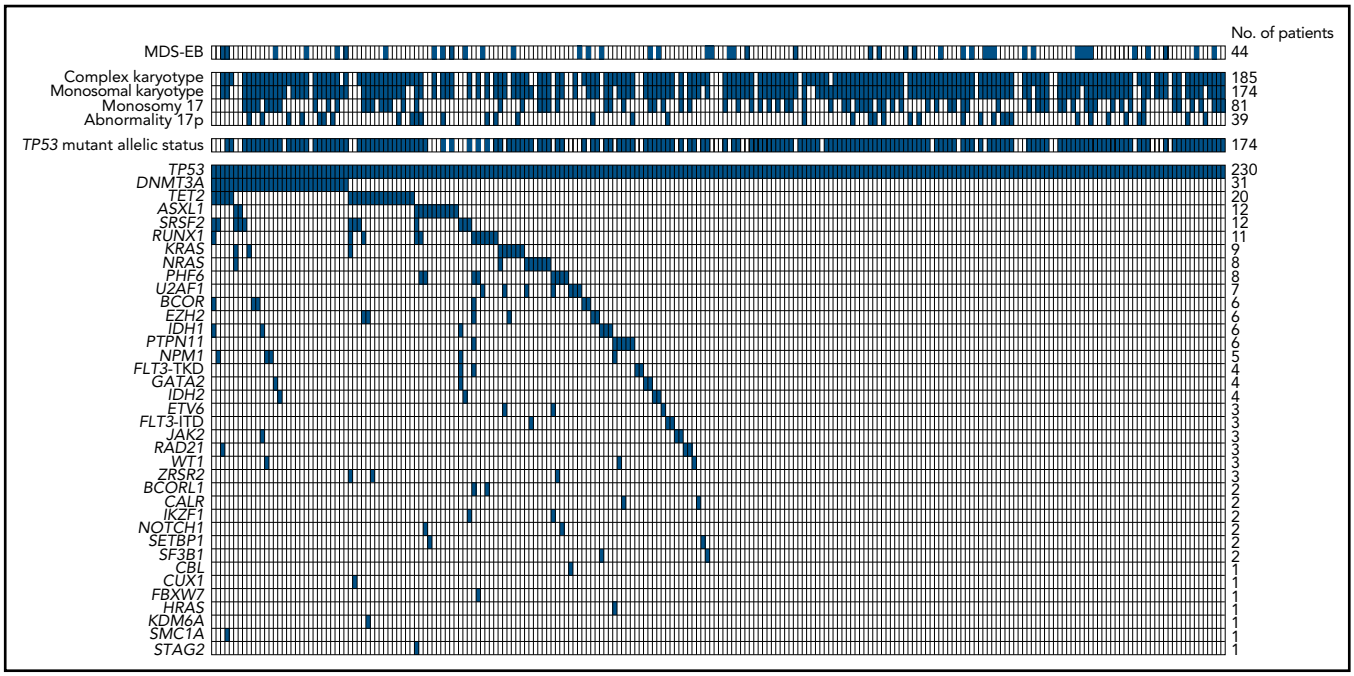

Figure 1. Overview of cytogenetic aberrations and concurrent mutations in mutant *TP53* AML/MDS-EB (n = 230). Each column represents an individual patient, and the presence of the aberration is indicated in blue. The upper panel shows the cytogenetic aberrations, and the lower panel shows the concurrent mutations. Patients with MDS-EB or bi-allelic *TP53* mutant status are also indicated in blue. In case of failed cytogenetics, the cytogenetic aberrations were considered negative.

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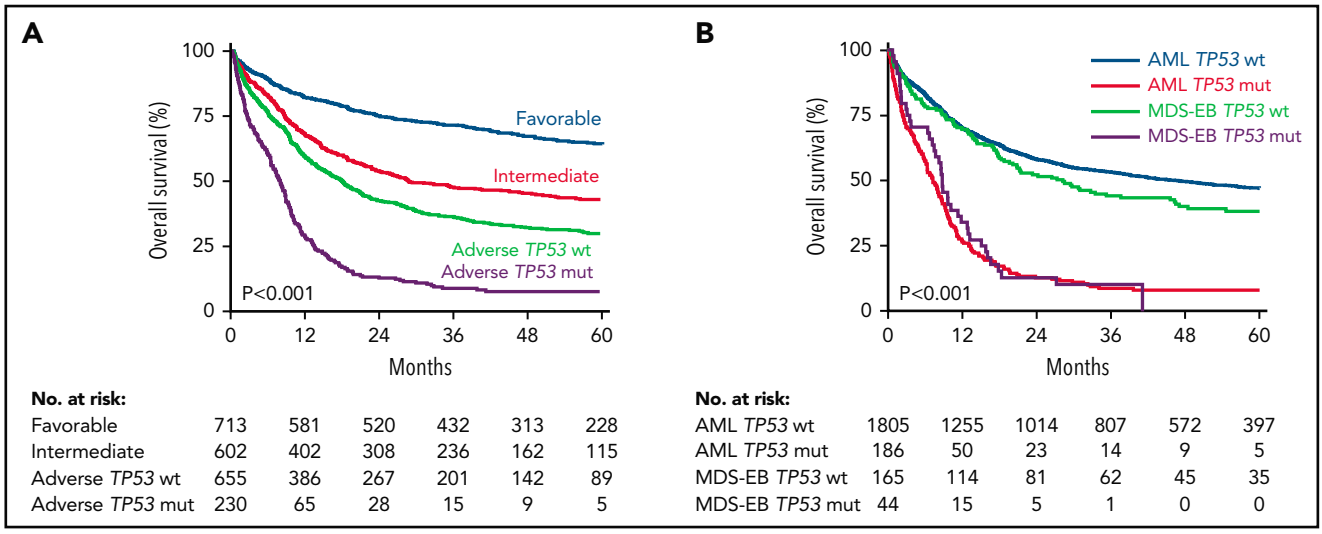

Figure 2. Overall survival of patients with AML and MDS-EB (n = 2200). (A) Overall survival of AML/MDS-EB patients by the ELN 2017 risk classification. Patients in the adverse risk category are segregated by *TP53* wild-type and *TP53* mutant. (B) Overall survival of AML and MDS-EB disease classification at diagnosis stratified according to patients with *TP53* wild-type and *TP53* mutant.

with mutant *TP53* AML/MDS-EB. Concurrent mutations conferred limited but detectable survival benefit (Figure 3C), whereas the presence of specific concurrent mutations provided no further survival advantage (supplemental Figure 7). Clone size, realized by taking decreasing *TP53* mutation VAF thresholds and continuous modeling per 10% VAF, was investigated for impact on outcome. None of the mutant *TP53* VAF thresholds significantly associated with survival: VAF 50% (P = .990); VAF 40% (P = .257); VAF 30% (P = .064); VAF 20% (P = .189); VAF 10% (P = .161); and VAF 5% (P = .226) (supplemental Figure 8A-F) (hazard ratio per 10% VAF, 1.04; 95% CI, 0.99-1.09; P = .141). Hence, the molecular characteristics of mutant *TP53* AML/MDS-EB did not evidently relate to treatment outcome.

In line with previous work, we confirmed that CK associates with reduced survival in *TP53* mutant AML/MDS-EB (2-year overall survival, 9% CK vs 34% non-CK; P = .002), regardless of type of consolidation therapy (Figure 4).[9,10] However, the overall survival of patients with non-CK *TP53* mutant AML/MDS remains poor. Because of strong association of CK with all mutant *TP53* molecular characteristics, no further stratification was feasible among patients with AML/MDS-EB with CK (supplemental Table 1). Of note, CK AML/MDS-EB with wild-type *TP53* appeared to have a significantly improved outcome in our cohort of 2200 AML/MDS-EB cases as compared with CK in the context of mutant *TP53* (Figure 4C), indicating that the presence of mutant *TP53* at diagnosis defines a separate CK entity.

Sensitivity analysis, performed to identify potential treatment modification within trial protocols, yielded no significant interactions. Similar results were obtained when elderly patients with AML were excluded (data not shown).

Molecular minimal residual disease in mutant *TP53* AML

Detection of molecular MRD is an important prognostic marker in AML.[22-24] We performed deep targeted sequencing on

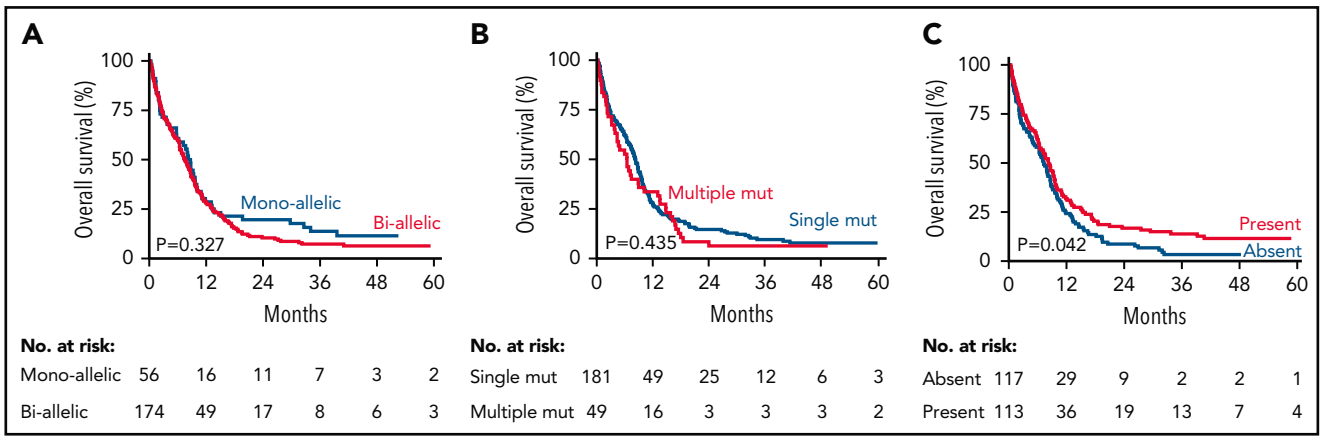

Figure 3. Overall survival of molecular characteristics in mutant *TP53* AML/MDS-EB (n = 230). Overall survival of *TP53* mutant allelic status (mono-allelic versus bi-allelic) (A), the number of *TP53* mutations (B), and the presence or absence of concurrent mutations (C).

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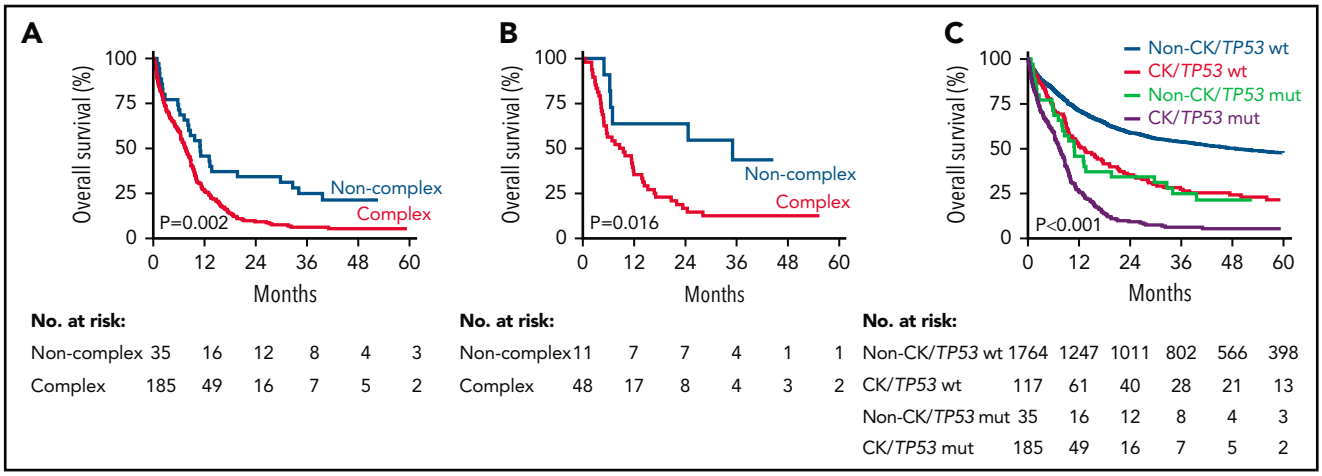

Figure 4. Overall survival of patients with AML/MDS-EB by CK. Overall survival of patients with mutant *TP53* AML/MDS-EB by CK (n = 220) (A) and for mutant *TP53* AML/MDS-EB patients who received allogeneic HSCT (n = 59) (B). Of note, the survival time starts at the date of transplant. (C) Overall survival of patients with AML/MDS-EB by CK and mutant *TP53* status (n = 2101).

complete morphological remission bone marrow samples from 62 patients with mutant *TP53* AML to assess molecular MRD. Mutant *TP53* is often the only suitable marker for molecular MRD detection because the prevalence of concurrent mutations at diagnosis is relatively low and most concurrently mutated genes may associate with antecedent clonal hematopoiesis (*DNMT3A*, *TET2*, and *ASXL1*) rather than residual leukemia (Figure 1). In total, 45 of 62 patients with AML/MDS-EB had detectable *TP53* mutations in CR, for which the status did not associate with overall survival ($P = .653$) (Figure 5).

Discussion

Substantial heterogeneity within the mutant *TP53* AML/MDS-EB subgroup on a clinical and molecular level precludes the exact assessment on prognostic impact for individual patients with AML/MDS-EB. Here, we report the detailed molecular characterization of mutant *TP53* in a large cohort of patients with AML and MDS-EB. No significant differences in the distribution of *TP53* molecular characteristics and outcome between patients with AML and MDS-EB were observed. In fact, the 5-year overall survival of patients with mutant *TP53* AML and MDS-EB in our study is similar to others.[13] Mutant *TP53* AML/MDS-EB represents a molecular homogeneous group with distinct clinicopathologic characteristics and outcomes. Therefore, we propose that mutant *TP53* MDS-EB and AML should be considered a single entity, regardless of the requisite blast percentage at diagnosis.

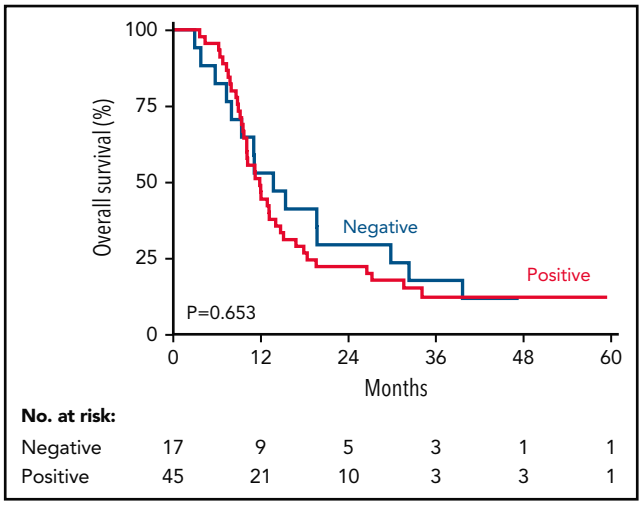

Figure 5. Overall survival of patients with AML/MDS-EB by *TP53* mutations detected in CR (n = 62).

Recent studies revealed important associations of *TP53* mutant allelic status and mutant *TP53* clone size with a more favorable outcome for patients with MDS and AML.[5,13,15] These studies established significant associations and interactions of *TP53* mutant allelic status and mutant *TP53* clone size with CK. Remarkably, in our study based on a substantial number of patients with mutant *TP53* AML and MDS-EB undergoing standard induction chemotherapy, we did not reveal an association between any of the *TP53* molecular characteristics and survival. Although the distribution of molecular characteristics and outcome of mutant *TP53* AML and MDS-EB in HOVON-SAKK clinical trials is comparable to other clinical trials, our analysis did not include low-risk MDS patients who often associate with non-CK.[5,13] It is thought that the presence of wild-type *TP53* is critical for maintaining chromosomal stability. During progression from MDS to high-risk MDS-EB or AML, mutant *TP53* clones often become bi-allelically mutated and genomically unstable, which is reflected by the strong association between bi-allelic *TP53* mutants and CK in our study. However, some patients with mono-allelic mutant *TP53* AML/MDS-EB also had CK. In 8 (2 non-CK and 6 CK) of 24 patients with mono-allelic mutant *TP53* AML/MDS-EB in whom high-quality DNA was available, we indeed confirmed, by SNP array analyses, the presence of uniparental disomy or focal 17p deletions that had been missed with conventional cytogenetics. Those patients can easily be misclassified as having mono-allelic *TP53* mutation. Reallocation of these 8 patients with mono-allelic mutant *TP53* AML/MDS-EB to the mutant *TP53* bi-allelic group did not affect our results (data not shown). Additional studies are required to investigate whether other (epigenetic) mechanisms are affecting the wild-type *TP53* allele in mono-allelic cases without copy number

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alterations. Altogether, our results indicate that further stratification by the molecular characteristics of mutant *TP53* appears to be less relevant when patients have progressed to MDS-EB or AML.

Although molecular MRD has prognostic value for predicting impending relapse in AML/MDS-EB,[22-24] we did not observe such association in mutant *TP53* AML/MDS-EB. Despite using deep sequencing, which revealed MRD in most cases, molecular MRD detection in mutant *TP53* AML/MDS-EB did not yield prognostic value. It is conceivable that all patients with mutant *TP53* AML/MDS-EB achieving CR have MRD, sometimes at levels undetectable with current NGS approaches. In fact, the high relapse rates in patients with AML/MDS-EB without detectable mutant *TP53* MRD in CR illustrates the critical role of mutant *TP53* in chemotherapeutic response and implies that small refractory clones are present below our NGS detection limit.[7] Although concurrent mutations are present in mutant *TP53* AML/MDS-EB, mutant *TP53* itself appeared to be exclusive in half of the patients. Of note, most concurrent mutations are known contributors of age-related clonal hematopoiesis, in which we and others previously showed lack of prognostic significance.[25,26] Although the applicability of molecular MRD detection in patients with mutant *TP53* AML/MDS-EB in our study is limited, future clinical trials with new drugs and other quantified MRD endpoints may benefit from molecular MRD detection based on mutant *TP53*.

Although very poor, better overall survival is observed in a minority of patients with AML/MDS-EB with non-CK mutant *TP53*. Possible explanations for the improved outcome in selected cases may be the enrichment of single *TP53* mutations with low VAFs as well as higher frequencies of concurrent mutations in this group, indicating that mutant *TP53* in these cases may represent clonal hematopoiesis rather than subclonal disease. Previous work in therapy-related AML indicates that mutant *TP53* will eventually be the founding clone[7]; however, additional studies, including those involving relapse of patients with non-CK mutant *TP53*, are needed to demonstrate that mutant *TP53* may be responsible for early relapse. Nevertheless, whether patients with mono-allelic non-CK mutant TP53 AML/MDS-EB have better outcome requires additional investigations on larger numbers of patients.

In conclusion, from a clinical and molecular perspective, we propose to consider mutant *TP53* AML/MDS-EB a distinct disease entity.

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Authorship

Contribution: T.G., A.S.A.A.H., B.L., M.J.-L., and P.J.M.V. designed the study and wrote the manuscript with input from the remaining authors; and all authors vouch for the completeness and accuracy of the data and analysis.

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Footnotes

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