Prognostic impact of 'multi-hit' *versus* 'single-hit' *TP53* alteration in patients with acute myeloid leukemia: results from the Consortium on Myeloid Malignancies and Neoplastic Diseases

Talha Badar,[1] Ahmad Nanaa,[2] Ehab Atallah,[3] Rory M. Shallis,[4] Emily C. Craver,[5] Zhuo Li,[5] Aaron D. Goldberg,[6] Antoine N. Saliba,[7] Anand Patel,[8] Jan P. Bewersdorf,[6] Adam Duvall,[8] Madelyn Burkart,[9] Danielle Bradshaw,[10] Yasmin Abaza,[9] Maximilian Stahl,[11] Neil Palmisiano,[12] Guru Subramanian Guru Murthy,[3] Amer M. Zeidan,[4] Vamsi Kota,[10] Mrinal M. Patnaik[6] and Mark R. Litzow[6]

[1]Division of Hematology-Oncology and Blood and Marrow Transplantation and Cellular Therapy Program, Mayo Clinic, Jacksonville, FL; [2]John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; [3]Division of Hematology and Medical Oncology, Medical College of Wisconsin, Milwaukee, WI; [4]Section of Hematology, Department of Internal Medicine, Yale School of Medicine, New Haven, CT; [5]Division of Clinical Trials and Biostatistics, Mayo Clinic, Jacksonville, FL; [6]Division of Hematologic Malignancies, Department of Medicine Memorial Sloan Kettering Cancer Center, New York, NY; [7]Division of Hematology, Mayo Clinic, Rochester, MN; [8]Section of Hematology and Oncology, Department of Medicine, University of Chicago, Chicago, IL; [9]Robert H. Lurie Comprehensive Cancer Center, Northwestern Hospital, Chicago, IL; [10]Division of Hematology and Oncology, Georgia Cancer Center, Augusta, GA; [11]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA and [12]Division of Hematology and Oncology, Jefferson University Hospital, Philadelphia, PA, USA

Correspondence: T. Badar
badar.talha@mayo.edu

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Abstract

While there is clear evidence to suggest poorer outcome associated with multi-hit (MH) *TP53* mutation (*TP53*^{MT}) compared to a single-hit (SH) mutation in lower-risk myelodysplastic syndrome (MDS), data are conflicting in both higher-risk MDS and acute myeloid leukemia (AML). We conducted an in-depth analysis utilizing data from ten US academic institutions to study differences in molecular characteristics and outcomes of SH (N=139) *versus* MH (N=243) *TP53*^{MT} AML. Complex cytogenetics were more common in MH than in SH *TP53*^{MT} AML (*P*<0.001); whereas *ASXL1* (*P*<0.001), *RAS* (*P*<0.001), splicing factor (*P*=0.003), *IDH1/2* (*P*=0.001), *FLT3* ITD (*P*<0.001) and *NPM1* (*P*=0.005) mutations clustered significantly with SH *TP53*^{MT} AML. Survival after excluding patients who received best supportive care alone was dismal but not significantly different between patients with SH or MH disease (event-free survival: 3.0 *vs.* 2.20 months, respectively, *P*=0.22; overall survival: 8.50 *vs.* 7.53 months, respectively, *P*=0.13). In multivariable analysis, *IDH1* mutation and allogeneic hematopoietic stem cell transplantation as a time-dependent covariate were associated with superior event-free survival (hazard ratio [HR]=0.44, 95% confidence interval [95% CI]: 0.19-1.01, *P*=0.05 and HR=0.34, 95% CI: 0.18-0.62, *P*<0.001) and overall survival (HR=0.24, 95% CI: 0.08-0.71, *P*=0.01 and HR=0.28, 95% CI: 0.16-0.47, *P*<0.001). Complex cytogenetics (HR=1.56, 95% CI: 1.01-2.40, *P*=0.04) retained an unfavorable significance for overall survival. Our analysis suggests that MH *TP53*^{MT} is less relevant in independently predicting outcomes in patients with AML than in those with MDS.

Introduction

TP53 is the most frequently mutated gene across all malignancies and is associated with a poor prognosis across many cancer types with suboptimal responses to standard-of-care therapies.[1,2] *TP53*-mutated (*TP53*^{MT}) acute myeloid leukemia (AML) is strongly associated with large structural and complex cytogenetic abnormalities, often seen among

recipients of prior cytotoxic therapies.[3-8] Despite the increasing availability of novel therapies, the median overall survival (OS) of patients with *TP53*^{MT} AML remains in the range of 6-9 months, irrespective of therapy intensity.[9-13] Single-hit *TP53*^{MT} is associated with clonal hematopoiesis and may not be directly leukemogenic unless accompanied by subsequent hits that could be secondary to cytotoxic stress.[4,14,15] There are conflicting reports regarding the prognostic impact of allelic state, specifically bi-allelic alteration/"multi-hit" (MH) *TP53*^{MT} *versus* mono-allelic/"single-hit" (SH) *TP53*^{MT} among patients with myeloid neoplasms.[10,16-18] Bernard *et al.* performed extended genetic profiling in a large cohort of patients with myelodysplastic syndrome (MDS) and showed that not all *TP53*^{MT} have equivalent impact on survival.[19] Patients with MDS harboring SH *TP53*^{MT} had similar outcomes to their counterparts with *TP53* wild-type disease. Conversely, multiple hits caused by either multiple mutations of *TP53*/copy-neutral loss of heterozygosity or mono-allelic *TP53*^{MT} with deletion of the other *TP53* allele were associated with inferior clinical outcomes. However, in patients with high-risk MDS with excess blasts or AML, it was recently demonstrated that *TP53* allelic state (SH *vs.* MH *TP53*^{MT}) did not predict differences in clinical outcome.[10] The authors concluded that further risk stratification by *TP53* allelic state may be less relevant among patients with advanced MDS or AML. Here, we present real world data on a large cohort of patients with *TP53*^{MT} AML and report their clinical characteristics, therapy received, and outcome based on *TP53* allelic state.

Methods

We conducted a retrospective study through the Consortium on Myeloid Malignancies and Neoplastic Diseases (COMMAND) consortium (a collaboration of acute leukemia experts from 10 US academic institutions) to analyze the prognostic impact of MH *versus* SH *TP53*^{MT} on outcomes of adult (≥18 years) patients with AML. A total of 382 adults with *TP53*^{MT} (139 SH, 243 MH) AML who were diagnosed between November 2012 and May 2023 were evaluated, and their baseline characteristics, molecular profile, and treatment outcomes were compared based on SH *versus* MH *TP53*^{MT} status. The current cohort of 382 patients was increased from the 291 patients who were included in our previous publication;[9] furthermore, the current cohort has more robust *TP53* gene annotation data and longer follow-up. These features of the cohort reported here enable a more comprehensive evaluation regarding the impact of *TP53* mutation burden on clinical outcome.

MH *TP53*^{MT} was defined by the presence of two or more distinct *TP53*^{MT} regardless of variant allele frequency (VAF) or a single *TP53*^{MT} associated with (i) cytogenetic abnormalities involving chromosome 17p (e.g. abnormality of 17p or monosomy 17) or (ii) a VAF of ≥55%, as previously reported by Grob *et al.*[10] Loss of heterozygosity was not assessed in all patients in this dataset.

AML was diagnosed as per the 2016 World Health Organization classification.[20] Response to treatment was defined according to 2017 European LeukemiaNet consensus guidelines.[21] Next-generation sequencing was performed at diagnosis using DNA extracted from bone marrow aspirate specimens with post-sequencing analysis of tumor-associated mutations. Next-generation sequencing testing was developed, and its performance characteristics determined by the participating institutions in compliance with Clinical Laboratory Improvement Amendments requirements. The next-generation sequencing panel had a sensitivity of ≥5% VAF with a minimum depth coverage of 250x.

The study was conducted after obtaining approval from the Institutional Review Board, adhering to the ethical standards of the Declaration of Helsinki of 1975, as revised in 2000.

Statistical analysis

Continuous variables are summarized as the median (range) while categorical variables are reported as frequency (percentage). Duration of response (complete [CR] or complete with incomplete blood count recovery [CRi]) was defined from the time of onset of response to progression or death due to any reason, whichever occurred earlier. The Kaplan-Meier method was used to estimate event-free survival (EFS), defined as time from diagnosis to relapse or death. The median overall survival (OS) was calculated from time of diagnosis to death or last follow-up. Cox proportional hazards regression models were used to determine the univariate and multivariate predictors of overall mortality and progression. Allogeneic hematopoietic stem cell transplantation (HSCT) was treated as a time-dependent covariate. Multivariable models included all significant univariate predictors. All tests were two-sided with a *P* value <0.05 considered statistically significant.

Results

Baseline characteristics

A total of 382 adult patients with *TP53*^{MT} AML (SH, 139; MH, 243) were identified. Among the 243 patients with MH *TP53*^{MT}, 57 patients had multiple *TP53*^{MT}, 58 patients had *TP53*^{MT} with VAF of ≥55%, and 128 patients had single *TP53*^{MT} associated with cytogenetic abnormalities involving chromosome 17p (e.g., abnormality of 17p or monosomy 17). The median age was 67 (range, 23-90) and 66.5 (range, 18-97) years in the SH and MH *TP53*^{MT} AML groups, respectively (*P*=0.86) (Table 1). Thirty-nine (33%) and 70 (29%) patients had secondary AML in the SH and MH groups, respectively (*P*=0.34). Among these 109 patients with secondary AML, 11 (10%) patients had *JAK2*-mutated myeloproliferative neoplasm in blast phase, of whom four (3%) were in the SH

Table 1. Baseline characteristics, treatment, and outcome in single-hit and multi-hit *TP53*.

Variable	Total N=382	Single-hit *TP53* N=139	Multi-hit *TP53* N=243	*P*
Age in years, median (range)	67 (18-97)	67 (23-90)	66.5 (18-97)	0.86
Age ≥65 years, N (%)	206 (54)	75 (55)	131 (54)	0.83
Gender (male), N (%)	224 (59)	76 (56)	148 (60)	0.38
WBC x 10^9/L, median (range)	2.9 (0.4-460)	2.9 (0.5-460)	3.0 (0.8-288)	0.88
Peripheral blast %, median (range)	10 (0-97)	8 (0-97)	11 (0-97.9)	0.46
Bone marrow blast %, median (range)	35 (2-99)	35 (14-95)	35 (2-99)	0.18
Secondary AML, N (%)	109 (30)	39 (33)	70 (29)	0.34
MPN-blast phase, N (%)	11 (3)	4 (3)	7 (3)	0.82
Therapy-related AML, N (%)	85 (22)	25 (18)	60 (24.5)	0.51
Complex cytogenetics, N (%)	307 (80)	79 (58)	228 (93)	<0.001
TP53 VAF, median (range)	44 (2-98)	22 (2-49)	50 (4-98)	<0.001
Co-mutated, N (%)	239 (63)	91 (67)	148 (60)	0.22
Myeloid co-mutations, N (%)				
TET2	47 (12)	21 (17)	26 (12)	0.18
DNMT3A	41 (11)	15 (10)	26 (11)	0.72
ASXL1	38 (10)	23 (16)	15 (7)	<0.001
RAS	35 (9)	21 (15)	14 (6)	0.001
Splicing factor: *U2AF1, SF3B1, SRSF2*	28 (7)	17 (12)	11 (4)	0.003
JAK2	24 (6)	12 (9)	12 (4)	0.12
RUNX1	25 (7)	12 (9)	13 (6)	0.19
IDH1/2	24 (6)	15 (11)	9 (4)	0.001
FLT3 ITD	19 (5)	15 (11)	4 (2)	<0.001
PTPN11	19 (5)	8 (6)	11 (4)	0.62
GATA2	13 (4)	6 (4)	7 (3)	0.55
NPM1	10 (3)	8 (6)	2 (1)	0.005
BCOR	10 (3)	4 (3)	6 (2)	0.74
CSF3R	10 (3)	6 (4)	4 (2)	0.10
CEBPA	7 (2)	5 (4)	2 (1)	0.10
EZH2	7 (2)	3 (2)	4 (2)	0.69
Type of induction, N (%)				
Intensive chemotherapy	97 (25)	40 (29)	57 (23)	0.22
HMA-based	51 (13)	17 (12.5)	34 (14)	0.75
HMA plus venetoclax	102 (27)	26 (19)	92 (29)	0.01
Other low-intensity chemotherapy*	21 (5.5)	7 (5)	14 (6)	>0.99
Best supportive care	34 (9)	21 (15)	13 (5)	0.001
CR/CRi (N=348 received chemo), N (%)	91 (26)	39 (33)	52 (23)	0.09
Allogeneic HSCT, N (%)	55 (14)	19 (14)	36 (15)	0.53

*Other low-intensity therapy includes low-dose cytarabine, an IDH2 inhibitor alone or an investigational agent. WBC: white blood cell count; AML: acute myeloid leukemia; MPN: myeloproliferative neoplasm; VAF: variant allele frequency; ITD: internal tandem duplication; HMA: hypomethylating agent; CR: complete remission; CRi: complete remission with incomplete count recovery; chemo; chemotherapy; HSCT: hematopoietic stem cell transplantation.

group and seven (3%) in the MH group (*P*=0.82). The median $TP53^{MT}$ VAF was 22% (range, 5-49%) and 50% (range, 5-98%) in the SH and MH $TP53^{MT}$ AML groups (*P*<0.001), respectively. The proportion of patients with complex cytogenetics was higher in the MH group than in the SH group (93% *vs.* 58%, *P*<0.001). In subgroup analysis, we looked at baseline characteristics of patients with *IDH1* or *IDH2* co-mutated AML. Patients with secondary AML had a higher proportion of *IDH2*-mutated disease than *IDH1*-mutated disease (46% *vs.* 27%, *P*=0.15) and complex cytogenetics (54% *vs.* 27%, *P*=0.24), but these differences were not statistically significant (*Online Supplementary Table S1*).

Molecular profile and somatic co-mutation pattern

An overview of *TP53* domains, distribution of *TP53* variants and position on the TP53 protein are illustrated in Figure 1. The occurrences of somatic co-mutations were comparable between the SH (67%) and MH (60%) groups (*P*=0.22). *ASXL1* (16% *vs.* 7%, *P*<0.001), *RAS* (15% *vs.* 6%, *P*<0.001), splicing factor (12% *vs.* 4%, *P*=0.003), *IDH1/2* (11% *vs.* 4%, *P*=0.001), *FLT3*-ITD (11% *vs.* 2%, *P*<0.001) and *NPM1* (6% *vs.* 1%, *P*=0.005) mutations were more frequent in the SH group

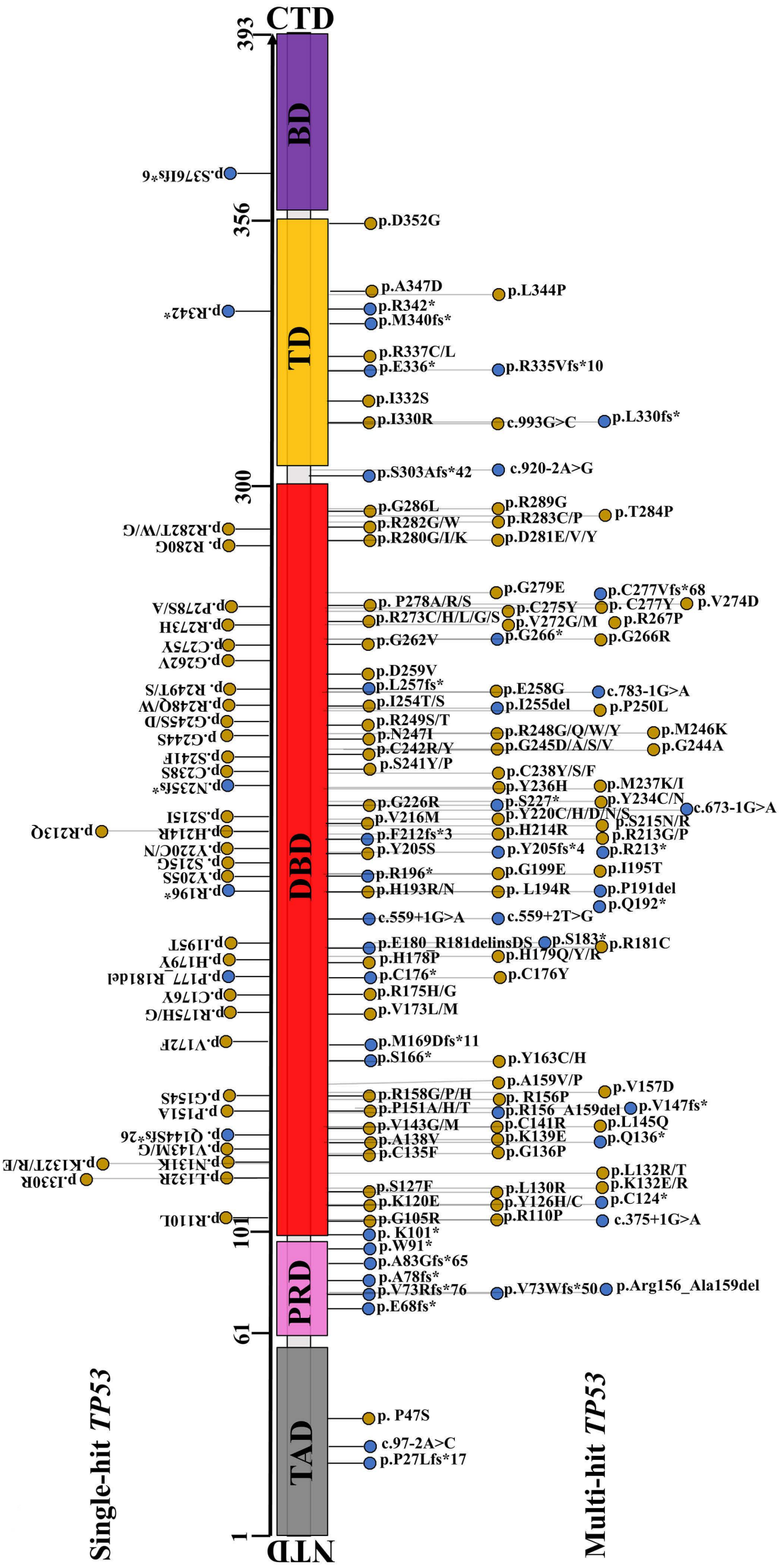

Figure 1. Overview of *TP53* domains, structures, and distribution of *TP53* variants detected, positioned on the TP53 protein. Variants from patients with monoallelic *TP53* are depicted at the top and those from patients with multiple *TP53* hits at the bottom. Missense mutations are shown as gold circles and all other variants, including truncated mutations corresponding to splice site variants, nonsense, nonstop, and frameshift deletions or insertions, are shown as blue circles. NTD: N-terminal domain; TAD: transactivation domain; PRD: proline-rich domain; DBD: DNA-binding domain; TD: tetramerization domain; BD: basic domain; CTD: C-terminal domain.

than in the MH group. The somatic co-mutation patterns and frequency of co-mutations in the SH and MH groups, are illustrated in Figure 2 and *Online Supplementary Figure S1*, respectively. Eleven (46%) patients had *IDH1*, and 13 (54%) patients had *IDH2* mutations. Two (18%) and seven (53%) patients with *IDH1* and *IDH2* mutations, respectively, had MH $TP53^{MT}$. There were no differences in the co-mutational patterns among patients with *IDH1/IDH2* co-mutated disease with the lone exception of *JAK2* mutations, which were more common in the *IDH2* co-mutated group (38.5% *vs.* 0%, *P*=0.04) (*Online Supplementary Table S1*).

Treatment and outcome

A significantly higher proportion of patients in the MH group received hypomethylating agents plus venetoclax compared to the SH group (29% *vs.* 19%, *P*=0.01). However, the proportion of patients who received intensive chemotherapy, hypomethylating agent-based therapy or other low-intensity chemotherapy (low-dose cytarabine, IDH2 inhibitor alone or an investigational agent) were comparable between the two groups (Table 1). The response rates (CR/CRi) were comparable between the SH and MH groups (28% *vs.* 22%, *P*=0.21). Among the 91 (26%) patients with CR/CRi, 28 (31%) were did not have measurable residual disease (MRD), assessed by flow cytometry, after induction. The MRD-negative CR rates with intensive *versus* non-intensive chemotherapy were not significantly different (10% *vs.* 7%, respectively, *P*=0.78). Similarly, a comparable proportion of patients underwent allogeneic HSCT after induction (12% *vs.* 14%, *P*=0.53). In subgroup analysis, there was a significant difference in response rate between patients with *IDH1* co-mutated disease (54.4%) and those with *IDH2* co-mutated disease (0%) (*P*=0.003) (*Online Supplementary Table S1*). The median duration of response was 7.77 *versus* 12.83 months in the SH and MH groups, respectively (*P*=0.73) (Figure 3A).

Predictors of response

Predictors of response (CR/CRi) to induction chemotherapy were evaluated, and the results are summarized in *Online Supplementary Table S2*. The co-occurrence of *RAS* (*NRAS* or *KRAS*) (*P*=0.02) and *IDH2* mutations (*P*=0.03) had a negative impact on response rate. Conversely, the co-occurrence of *IDH1* mutation (*P*=0.02) and induction with a hypomethylating agent plus venetoclax (*P*<0.001) was associated with better responses. In this cohort of adverse-risk $TP53^{MT}$ AML, age ≥65 years (*P*>0.99), secondary AML (*P*=0.58), therapy-related AML (*P*>0.99), and complex cytogenetics (*P*>0.99) did not have significant impacts on achieving response.

Event-free survival

Considering the significantly higher proportion of patients receiving supportive care alone in the SH group compared to the MH group, we excluded these patients from the survival analysis. The median EFS in months was not significantly different between the SH and MH groups (3.0 and. 2.20, respectively, *P*=0.22) (Figure 3B). However, there was a statistically significant difference in EFS between the SH and MH groups (3.0 *vs.* 2.13, *P*=0.02), utilizing the definition of MH as per the International Consensus Classification, i.e., two distinct $TP53^{MT}$ with VAF >10% or a single $TP53^{MT}$ with (i) 17p deletion; (ii) VAF of >50%; or (iii) copy-neutral loss of heterozygosity at the 17p TP53 locus.[22] In univariate analysis for EFS (*Online Supplementary Table S3*), complex cytogenetics adversely affected outcome (*P*=0.04). In contrast, *ASXL1* mutation (*P*=0.02), *IDH1* mutation (*P*=0.01), hypomethylating agent plus venetoclax induction (*P*<0.001), and allogeneic HSCT as a time-dependent covariate (*P*<0.002) were associated with favorable EFS in univariate analysis. In multivariable analysis for EFS, *IDH1* co-mutation (HR=0.44, 95% CI: 0.19-1.01, *P*=0.05), hypomethylating agent plus venetoclax induction (HR=0.53, 95% CI: 0.41-0.70, *P*<0.001) and allogeneic HSCT (HR=0.34, 95% CI: 0.18-0.62, *P*<0.001) retained statistically significant associations with favorable outcomes.

Overall survival

After excluding patients who received supportive care alone, we calculated the median OS. The median OS in months was not significantly different between the SH and MH groups (8.50 *vs.* 7.53, respectively, *P*=0.13) (Figure 3C). Likewise, we did not observe a significant difference in OS between the SH and MH groups (8.0 *vs.* 8.0, respectively, *P*=0.32), utilizing the MH definition as per the International Consensus Classification. We looked at the impact of complex cytogenetics on OS in the SH and MH groups and found that OS was better in the SH and MH subgroups without complex cytogenetics (9.97 and 10.07 months, respectively) than in those with complex cytogenetics (6.2 and 7.13 months, respectively) (*P*=0.008) (Figure 3D). We performed landmark analysis from the time of achievement of CR/CRi until last follow-up or death; allogeneic HSCT recipients had better OS compared to non-allogeneic HSCT recipients in both the SH group (not reached *vs.* 9.63 months, respectively) and the MH group (24.3 *vs.* 9.6 months, respectively) (*P*=0.001) (Figure 3E). In another subset analysis among transplanted patients, those who were transplanted in MRD-negative CR (N=12) had a longer median OS compared to those with MRD-positive disease (N=43) (46.1 *vs.* 25.47 months, respectively, *P*=0.15) although the difference was not statistically significant probably due to a smaller sample size. We performed a similar analysis to look at OS in relation to complex cytogenetics and *TP53* allelic state among patients who achieved CR/CRi and did or did not receive allogeneic HSCT. In subset analysis, we looked at the impact of co-occurring complex cytogenetics on survival outcome of patients in the SH and MH groups. The median OS was 23.6 months, not reached, 20.2 months and not reached in patients with SH and complex

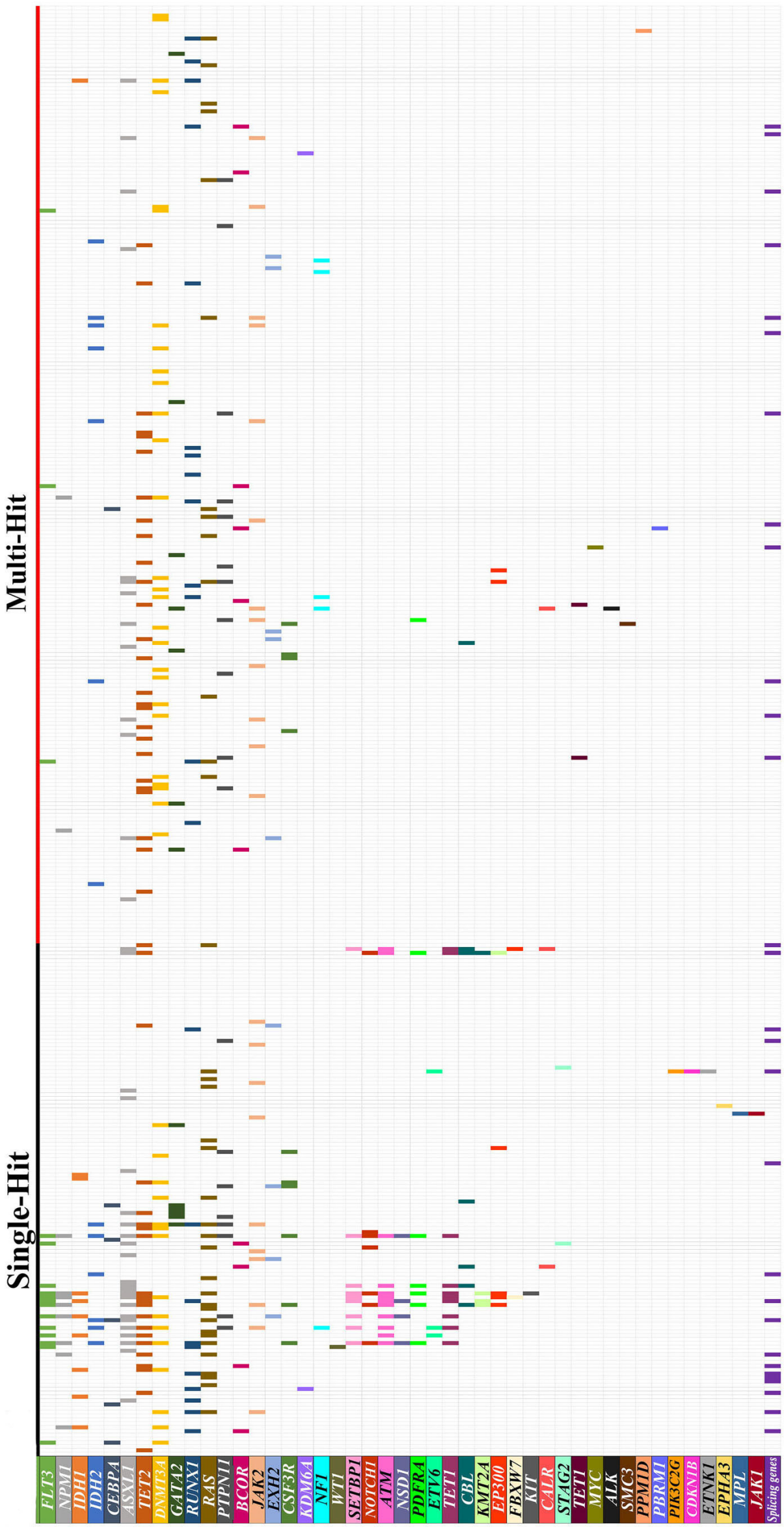

Figure 2. Patterns of the co-mutations identified in the *TP53* cohort. Patients with single-hit *TP53* are depicted at the left (black) and patients with multi-hit *TP53* at the right (red).

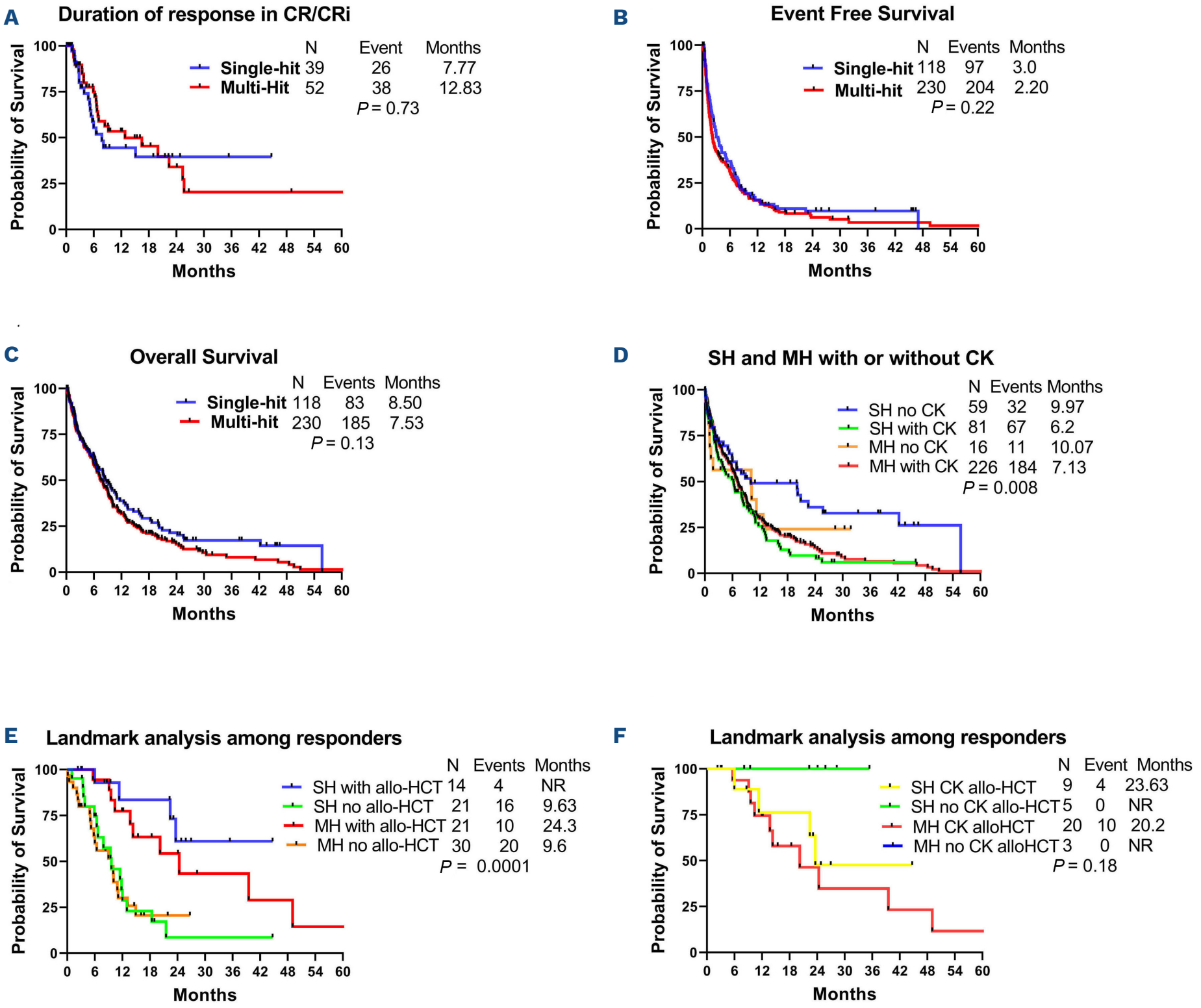

Figure 3. Kaplan-Meier survival curves for patients with single-hit and multi-hit *TP53*-mutated acute myeloid leukemia. (A) Duration of response, (B) event-free survival and (C) overall survival (OS) in single-hit (SH) *versus* multi-hit (MH) *TP53*-mutated acute myeloid leukemia (AML). (D) Subset analysis showing the impact of complex cytogenetics on OS in SH and MH *TP53*-mutated AML. (E) Landmark analysis for OS among patients with complete remission receiving allogeneic hematopoietic stem cell transplantation in SH *versus* MH *TP53* AML. (F) Landmark analysis for OS among patients with complete remission undergoing allogeneic hematopoietic stem cell transplantation with respect to the presence or absence of complex cytogenetics and *TP53* allelic burden. CR: complete remission; CRi: complete remission with incomplete blood count recovery; CK: complex cytogenetics; allo-HCT: allogeneic stem cell transplantation; NR: not reached.

cytogenetics, SH without complex cytogenetics, MH with complex cytogenetics and MH without complex cytogenetics, respectively (*P*=0.18) (Figure 3F). Between patients with SH $TP53^{MT}$, those who received intensive chemotherapy induction had significantly better outcome compared to those who received non-intensive chemotherapy with a median OS of 9.97 *versus* 5.82 months, respectively (*P*=0.04). However, the benefit of intensive chemotherapy compared to non-intensive chemotherapy in improving OS in MH $TP53^{MT}$ was less clear with a median OS of 8.03 *versus* 6.7 months, respectively (*P*=0.07).

In univariate analysis for OS (*Online Supplementary Table S4*), age as a continuous variable (every 10 years) (*P*=0.02), complex cytogenetics (*P*=0.002), and other low-intensity chemotherapy (*P*=0.01) were associated with inferior outcomes. *RUNX*1 mutation (*P*=0.01), *IDH1* mutation (*P*<0.001), *FLT3* ITD mutation (*P*=0.003), *NPM1* mutation (*P*=0.02), intensive induction (*P*=0.007) and allogeneic HSCT as a time-dependent co-variate (*P*<0.001) were associated with favorable OS in univariate analysis. In multivariable analysis

for OS, complex cytogenetics (HR=1.56, 95% CI: 1.01-2.40, *P*=0.04) retained an unfavorable significance, whereas *IDH1* mutation (HR=0.24, 95% CI: 0.08-0.71, *P*=0.01) and allogeneic HSCT (HR=0.28, 95% CI: 0.16-0.47, *P*<0.001) retained favorable significance.

Discussion

In our real-world, multicenter analysis of a large cohort of patients with *TP53*^{MT} AML, we did not observe significant differences in remission rates or survival based on *TP53* allelic state. We found that distinct myeloid co-mutation patterns exist between patients with SH and those with MH *TP53*^{MT} AML, with *IDH1* co-mutations imparting a favorable prognostic significance, and that the use of allogeneic HSCT associating with improved OS, irrespective of SH or MH *TP53*^{MT} status.

Recent studies have explored the clinical significance of *TP53*^{MT} allelic status in patients with MDS and AML.[4,10,23] While patients with MDS harboring SH *TP53*^{MT} tend to have similar outcomes compared to their *TP53* wild-type counterparts and better outcomes than those with MH *TP53*^{MT}, patients with MDS with excess blasts/AML harboring SH or MH *TP53*^{MT} had comparable outcomes. Similarly to Grob *et al.*,[10] we did not observe significant differences in response rate or survival between patients with SH or MH *TP53*^{MT} AML. These data suggest that *TP53* allelic state in advanced MDS or AML is less relevant in predicting clinical outcome. Similar to what has been observed in MDS studies, patients with SH *TP53*^{MT} AML had an abundance of somatic co-mutations, while MH *TP53*^{MT} AML was significantly associated with occurrence of complex cytogenetics.[23]

IDH1/2 mutations are observed in approximately 20% of patients with AML (*IDH1*, 6-16%; *IDH2*, 8-19%).[24] *IDH1/2* mutations are more frequently seen in elderly AML patients, especially those with diploid or intermediate-risk cytogenetics, and frequently co-occur with *FLT3* ITD and *NPM1* mutations.[25] With the development of venetoclax and *IDH1/2* inhibitors, the outcomes of *IDH1/2*-mutated AML patients have improved significantly, especially those who are ineligible for intensive therapies.[26] Interestingly, we observed significantly improved EFS and OS among patients with *IDH1* co-mutations and the favorable significance was retained in multivariate analysis. Moreover, only a small proportion of these patients received venetoclax plus a hypomethylating agent as first-line treatment (2/11 [18%]) or as a salvage therapy (1/11 [9%]) and only 2/11 (18%) patients in this subgroup underwent allogeneic HSCT. None of the patients received an IDH1 inhibitor alone or in combination with chemotherapy upfront. One patient each received a hypomethylating agent/venetoclax plus IDH1 inhibitor and an IDH1 inhibitor alone as a salvage therapy with no response. While these findings are intriguing, they need to be validated in a larger group of patients.

Although allogeneic HSCT is universally considered a potentially curative option for patients with adverse-risk AML, earlier studies showed dismal outcomes for patients with *TP53*^{MT} AML undergoing allogeneic HSCT.[27] Lack of benefit was attributed to inability to achieve complete response and persistence of the pre-transplant *TP53*^{MT} clone. In our earlier report utilizing data from ten US academic centers, we showed that allogeneic HSCT improved survival of patients with *TP53*^{MT} AML.[13] We have now re-confirmed this finding and shown that it holds true irrespective of *TP53* allelic state. The multivariable analysis in this study also demonstrated a significantly better EFS associated with induction with a hypomethylating agent plus venetoclax when compared with other therapies. However, this did not translate into improved OS, suggesting evolution of resistant clones that were not suppressed in the long-term by venetoclax plus hypomethylating agent therapy alone, as previously reported.[28] Secondly, in a subset analysis we observed better OS with intensive chemotherapy compared to non-intensive chemotherapy induction in the SH and MH subgroups, probably due to the fact that patients eligible for intensive chemotherapy generally have good performance status/fewer co-morbidities and are more likely candidates for allogeneic HSCT. Furthermore, intensive chemotherapy induction did not retain significance for better survival in multivariate analysis.

We acknowledge some limitations of our analysis including a selection bias inherent to a retrospective analysis and some overlap with our prior work.[9] However, our current analysis includes 382 patients followed longitudinally, significantly strengthening our previous cohort of 291 patients, with more robust *TP53* gene annotation data, and these patients have longer follow-up. This strengthened cohort enabled a more comprehensive evaluation of the impact of *TP53* mutation burden on clinical outcome. Second, cases with apparent mono-allelic *TP53*^{MT} may have hidden clones with bi-allelic *TP53* inactivation which were not detected by widely used sequencing methods. Furthermore, although loss of heterozygosity to determine *TP53*^{MT} allelic state was not assessed in all patients in this dataset, we defined SH and MH *TP53*^{MT} based on earlier observations by Grob *et al.*[10] Moreover, we did not observe significant differences in survival outcomes using the definition of MH *TP53* as per the International Consensus Classification or by Grob *et al.*[10]

In conclusion, unlike in lower-risk MDS, we did not find a significant difference in response rate or survival outcomes between patients with SH or MH *TP53*^{MT} AML, which is consistent with recent reports.[10,18] Prospective studies are needed to better understand the effect of *TP53* allelic state on the outcomes of patients with *TP53*^{MT} AML.

Disclosures

TB has served on advisory boards for Pfizer, Morphosys and Takeda. AP has provided consultancy services for AbbVie; has

received research funding from Kronos Bio, Pfizer, Celgene/BMS, and Servier; has received honoraria from AbbVie and BMS and has received institutional research funding from Pfizer and Kronos Bio. VK has served on advisory boards for Novartis and Pfizer. AMZ is a Leukemia and Lymphoma Society Scholar in Clinical Research; has received institutional research funding from Celgene/BMS, AbbVie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Cardiff Oncology, Incyte, Takeda, Novartis, Shattuck Labs, Geron, and Aprea; has participated in advisory boards, and/or had a consultancy role with and received honoraria from AbbVie, Pfizer, Celgene/BMS, Jazz, Incyte, Agios, Servier, Boehringer-Ingelheim, Novartis, Astellas, Daiichi Sankyo, Geron, Taiho, Seattle Genetics, BeyondSpring, Takeda, Ionis, Amgen, Janssen, Genentech, Epizyme, Syndax, Gilead, Kura, Chiesi, ALX Oncology, BioCryst, Notable, Orum, Mendus, Foran, Syros, and Tyme; has served on clinical trial committees for Novartis, AbbVie, Gilead, Syros, BioCryst, Abbvie, ALX Oncology, Geron and Celgene/BMS; and has received travel support for meetings from Pfizer, Novartis, and Cardiff Oncology. The other authors have no conflicts of interest to disclose.

Contributions

TB conceived the study, curated the data, wrote the original draft, and was responsible for the submission. AN helped in data collection and making figures. ECC and ZL helped with the statistical analysis. EA, RMS, AP, ANS, MS, and JPB contributed patients, and reviewed and edited the manuscript. MB, MS, GSGM, YA, AD, DB, VK, ADG, NP, AMZ, and MMP contributed patients and reviewed the manuscript. MRL contributed patients, and supervised, reviewed, and edited the manuscript.

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Daver NG, Maiti A, Kadia TM, et al. TP53-mutated myelodysplastic syndrome and acute myeloid leukemia: biology, current therapy, and future directions. Cancer Discov. 2022;12(11):2516-2529.
2. Sabapathy K, Lane DP. Therapeutic targeting of p53: all mutants are equal, but some mutants are more equal than others. Nat Rev Clin Oncol. 2018;15(1):13-30.
3. Badar T, Szabo A, Sallman D, et al. Interrogation of molecular profiles can help in differentiating between MDS and AML with MDS-related changes. Leuk Lymphoma. 2020;61(6):1418-1427.
4. Hiwase D, Hahn C, Tran ENH, et al. TP53 mutation in therapy-related myeloid neoplasm defines a distinct molecular subtype. Blood. 2023;141(9):1087-1091.
5. Haase D, Stevenson KE, Neuberg D, et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. Leukemia. 2019;33(7):1747-1758.
6. Kandoth C, McLellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502(7471):333-339.
7. Weinberg OK, Siddon A, Madanat YF, et al. TP53 mutation defines a unique subgroup within complex karyotype de novo and therapy-related MDS/AML. Blood Adv. 2022;6(9):2847-2853.
8. Sallman DA, Komrokji R, Vaupel C, et al. Impact of TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. Leukemia. 2016;30(3):666-673.
9. Badar T, Atallah E, Shallis RM, et al. Outcomes of TP53-mutated AML with evolving frontline therapies: impact of allogeneic stem cell transplantation on survival. Am J Hematol. 2022;97(7):E232-E235.
10. Grob T, Al Hinai ASA, Sanders MA, et al. Molecular characterization of mutant TP53 acute myeloid leukemia and high-risk myelodysplastic syndrome. Blood. 2022;139(15):2347-2354.
11. Rücker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood. 2012;119(9):2114-2121.
12. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209-2221.
13. Badar T, Atallah E, Shallis R, et al. Survival of TP53-mutated acute myeloid leukemia patients receiving allogeneic stem cell transplantation after first induction or salvage therapy: results from the Consortium on Myeloid Malignancies and Neoplastic Diseases (COMMAND). Leukemia. 2023;37(4):799-806.
14. Boettcher S, Miller PG, Sharma R, et al. A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies. Science. 2019;365(6453):599-604.
15. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015;518(7540):552-555.
16. Zeidan AM, Bewersdorf JP, Hasle V, et al. Prognostic implications of mono-hit and multi-hit TP53 alterations in patients with acute myeloid leukemia and higher risk myelodysplastic syndromes treated with azacitidine-based therapy. Leukemia. 2023;37(1):240-243.
17. Bahaj W, Kewan T, Gurnari C, et al. Novel scheme for defining the clinical implications of TP53 mutations in myeloid neoplasia. Res Sq. 2023 Mar 9:rs.3.rs-2656206. doi: 10.21203/rs.3.rs-2656206/v1. [preprint not peer-reviewed].
18. Stengel A, Meggendorfer M, Walter W, et al. Interplay of TP53 allelic state, blast count, and complex karyotype on survival of patients with AML and MDS. Blood Adv. 2023;7(18):5540-5548.
19. Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med. 2020;26(10):1549-1556.
20. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
21. Döhner H, Estey E, Grimwade D, et al. Diagnosis and

management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.
22. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.
23. Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. Nat Med. 2020;26(10):1549-1556.
24. DiNardo CD, Ravandi F, Agresta S, et al. Characteristics, clinical outcome, and prognostic significance of IDH mutations in AML. Am J Hematol. 2015;90(8):732-736.
25. Paschka P, Schlenk RF, Gaidzik VI, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. J Clin Oncol. 2010;28(22):3636-3643.
26. Pollyea DA, DiNardo CD, Arellano ML, et al. Impact of venetoclax and azacitidine in treatment-naïve patients with acute myeloid leukemia and IDH1/2 mutations. Clin Cancer Res. 2022;28(13):2753-2761.
27. Mohr B, Schetelig J, Schäfer-Eckart K, et al. Impact of allogeneic haematopoietic stem cell transplantation in patients with abnl(17p) acute myeloid leukaemia. Br J Haematol. 2013;161(2):237-244.
28. DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. Blood. 2020;135(11):791-803.