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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.

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Conflict of interest

TB: Serve in advisory board for Pfizer, Morphosys and Takeda AP: Consulting for Abbvie, research funding from Kronos Bio, Pfizer, Celgene/BMS, Servier, VK: Advisory board for Novartis and Pfizer. Anand Patel: COI: honoraria from AbbVie and BMS, research funding (institutional) from Pfizer and Kronos Bio. Amer Zeidan: Amer Zeidan is a Leukemia and Lymphoma Society Scholar in Clinical Research. Amer M. Zeidan received research funding (institutional) from Celgene/BMS, Abbvie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Cardiff oncology, Incyte, Takeda, Novartis, Shattuck Labs, Geron, and Aprea. AMZ participated in advisory boards, and/or had a consultancy 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. AMZ served on clinical trial committees for Novartis, Abbvie, Gilead, Syros, BioCryst, Abbvie, ALX Oncology, Geron and Celgene/BMS. AMZ received travel support for meetings from Pfizer, Novartis, and Cardiff Oncology.

Data availability

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

Abstract

While there is clear evidence to suggest poorer outcome associated with multi-hit (*MH*) *TP53* mutation compared to single-hit (*SH*) 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 10 US academic institutions to study differences in molecular characteristics and outcomes of *SH* (n= 139) versus *MH* (n= 243) $TP53^{MT}$ AML. Complex cytogenetics (CG) were more common in *MH* compared to *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 significantly clustered with *SH* $TP53^{MT}$ AML. Survival after excluding patients who received best supportive care alone was dismal but not significantly different between *SH* and *MH* (event free survival [EFS]: 3.0 vs 2.20 months, p= 0.22/ overall survival [OS]: 8.50 vs 7.53 months, respectively, p= 0.13). In multivariable analysis, *IDH1* mutation and allogeneic hematopoietic stem cell transplantation (allo-HCT) as a time-dependent covariate were associated with superior EFS (HR; 0.44, 95% CI: 0.19-1.01, p= 0.05/ HR; 0.34, 95% CI: 0.18-0.62, p<0.001) and OS (HR; 0.24, 95% CI: 0.08-0.71, p= 0.01/ HR; 0.28, 95% CI: 0.16-0.47, p<0.001). While complex CG (HR; 1.56, 95% CI: 1.01-2.40, p= 0.04) retained unfavorable significance for OS. Our analysis suggests that unlike in MDS, multi-hit $TP53^{MT}$ is less relevant in independently predicting outcomes in patients with AML.

Introduction

TP53 is the most frequently mutated gene across all malignancies and is associated with a poor prognosis across many cancer types with sub-optimal 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 (CG) abnormalities, often seen among recipients of prior cytotoxic therapies.[3-8] Despite the increasing availability of novel therapies, the median overall survival (OS) amongst 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} vs 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, *MH* 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, the impact of *TP53* allelic state on clinical outcome of high risk MDS with excess blast (MDS-EB) and AML was recently demonstrated to predict no differences in outcome between *SH* vs *MH TP53*^{MT}.[10] The authors concluded that further risk stratification by *TP53* allelic state may be less relevant among patients with advanced MDS or AML.

Here in, we present real world data on a large cohort of patients with *TP53*^{MT} AML and reported clinical characteristics, therapy received, and outcome based on *TP53* allelic state.

Methods

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

Multi-hit *TP53*^{MT} was defined by the presence of (1) 2 or more distinct *TP53*^{MT} regardless of variant allele frequency (VAF) or a single *TP53*^{MT} associated with (i) cytogenetic (CG) abnormalities involving chromosome 17p (e.g. abnormality of 17p or monosomy 17); (ii) a VAF of ≥ 55%, as previously reported by Grob et al.[10] The loss of heterozygosity was not assessed in all patients in this data set.

Acute myeloid leukemia was diagnosed as per 2016 World Health Organization (WHO) classification.[20] Response to treatment was defined according to 2017 European Leukemia Net (ELN) consensus guidelines.[21] Next generation sequencing (NGS) was performed at diagnosis using extracted DNA from bone marrow aspirate specimens with post-sequencing analysis of

tumor-associated mutations. NGS testing was developed, and its performance characteristics determined by the participating institutions in compliance with Clinical Laboratory Improvement Amendments (CLIA) requirements. The NGS panels has a variant sensitivity of ≥ 5% VAF with a minimum depth coverage of 250x.

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

Statistical analysis

Continuous variables were summarized as median (range) while categorical variables were reported as frequency (percentage). Duration of response (CR/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 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 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 (*SH* [n= 139] and *MH* [n= 243]) patients with $TP53^{MT}$ AML were identified. Among 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 CG abnormalities involving chromosome 17p (e.g., abnormality of 17p or monosomy 17). The median age was 67 (range [R], 23-90) and 66.5 (R,18-97) years in the *SH* and *MH TP53*^{MT} AML groups (p= 0.86), respectively (**Table 1**). Thirty-nine (33%) and 70 (29%) patients had secondary (s) AML in the *SH* and *MH* groups, respectively (p= 0.34). Among these 109 secondary AML patients, 11 (10%) patients had *JAK2* mutated myeloproliferative neoplasm in blast phase (MPN-BP), 4 (3%) and 7 (3%) patients in the *SH* and *MH* groups, respectively (p= 0.82). The median *TP53*^{MT} VAF was 22% (R, 5-49%) and 50% (R, 5-98%) in the *SH* and *MH TP53*^{MT} AML groups (p= <0.001), respectively. A higher proportion of patients had complex CG in the *MH* group compared to the *SH* group (93% vs 58%, p= < 0.001). In sub-group analysis, we looked at baseline characteristics of patients with *IDH1* or *IDH2* co-mutated AML. Numerically, patients with secondary AML had a higher proportion of *IDH2*-mutated disease when compared with *IDH1*-mutated disease (46% vs 27%, p= 0.15) and complex CG (54% vs 27%, p= 0.24), but these differences were not statistically significant (Supplementary Table 1).

Molecular profile and somatic co-mutation pattern

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, respectively (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 compared to the *MH* group, respectively. The somatic co-mutation patterns and frequency of co-mutations in *SH* and *MH* groups, are illustrated in **Figure 2 & Supplementary Figure 1**, respectively. Eleven (46%) patients had *IDH1,* and 13 (54%) patients

had *IDH2* mutations. Two (18%) and 7 (53%) patients with *IDH1* and *IDH2* mutations had *MH TP53*^{MT}, respectively. There were no differences in the co-mutational patterns amongst 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) (Supplementary Table 1).

Treatment and outcome

A significantly higher proportion of patients in the *MH* group received hypomethylating agents (HMA) plus venetoclax (VEN) compared to *SH* group (29% vs. 19%, p= 0.01). However, the proportion of patients who received intensive chemotherapy, HMA 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 patients with CR/CRi (91 [26%]), 28 (31%) patients were measurable residual disease (MRD) negative by flow cytometry after induction. The MRD negative CR rates with intensive vs non-intensive chemotherapy was not significantly different (10% vs 7%, p= 0.78), respectively. Similarly, a comparable proportion of patients received allogeneic stem cell transplantation (allo-HCT) after induction (12% vs 14%; p= 0.53). In sub-group analysis, there was significant difference in response rate amongst patients with *IDH1* co-mutated (54.4%) vs *IDH2* co-mutated (0%) disease, p= 0.003 (**Supplementary Table 1**). The median duration of response was 7.77 vs 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 results are summarized in **Supplementary Table 2**. The co-occurrence of *RAS* (*NRAS* or *KRAS*) (p= 0.02)

and *IDH2* mutations (p= 0.03), negatively impacted response rate. Conversely, the co-occurrence of *IDH1* mutation (p= 0.02) and induction with HMA plus VEN (p= <0.001) were associated with better responses. In this cohort of adverse risk $TP53^{MT}$ AML, age ≥ 65 years (p= >0.99), secondary (p= 0.58) or therapy related (p= >0.99) AML, and complex CG (p= > 0.99) did not have a significant impact in achieving response.

Event Free Survival

Considering the significantly higher proportion of patients receiving supportive care alone in the *SH* 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* group (3.0 vs 2.20, p= 0.22 [**Figure 3B**]), respectively. However, there was a statistically significant difference in EFS between the *SH* and *MH* groups(3.0 vs 2.13, p= 0.02), utilizing *MH* definition as per ICC classification (2 distinct $TP53^{MT}$ with VAF > 10% or single $TP53^{MT}$ with [1] 17p deletion; [2] VAF of >50%; or [3] copy-neutral loss of heterozygosity at the 17p *TP53* locus).[22] In univariate analysis for EFS (**Supplementary Table 3**), complex CG adversely affected outcome (p= 0.04). In contrast, *ASXL1* mutation (p= 0.02), *IDH1* mutation (p= 0.01), HMA plus VEN induction (p= <0.001), and allo-HCT 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), HMA plus VEN induction (HR; 0.53, 95% CI: 0.41-0.70, p= <0.001) and allo-HCT (HR; 0.34, 95% CI: 0.18-0.62, p= <0.001) retained a significant association 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* group (8.50 vs 7.53, p= 0.13 [**Figure 3C**]), respectively. Likewise, we did not observe significant difference in OS between *SH* and *MH* (8.0 vs 8.0, p= 0.32), utilizing *MH* definition as per ICC classification. We looked at impact of complex CG on OS in *SH* and *MH* group, OS was better in *SH* and *MH* sub-group without complex CG (9.97 and 10.07 months) compared with complex CG (6.2 and 7.13 months), respectively, p= 0.008 (**Figure 3D**). We performed landmark analysis from the time of achievement of CR/CRi till last follow up or death; allo-HCT recipients had better OS compared to non-allo-HCT recipient in *SH* (not reached [NR] vs 9.63 months) and *MH* (24.3 vs 9.6 months) group, respectively (p= 0.001 [**Figure 3E**]). In another subset analysis among transplanted patients, those who were transplanted in MRD negative CR (n=12) had numerically higher median OS compared to those with MRD positive disease (n=43) (46.1 vs 25.47 months, p= 0.15) respectively, however it was not statistically significant probably due to a smaller sample size. We performed similar analysis to looked at OS in relation to complex CG and *TP53* allelic state, among patients who achieved CR/CRi and received allo-HCT versus no allo-HCT. In subset analysis, we looked at the impact of co-occurring complex CG on survival outcome of patients in *SH* and *MH* groups. The median OS in months was 23.6, NR, 20.2 and NR in patients having *SH* with complex CG, *SH* without complex, *MH* with complex CG and *MH* without complex CG, 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 median OS of 9.97 vs 5.82 months, respectively (p= 0.04). However, the benefit of intensive chemotherapy in improving OS compared to non-

intensive chemotherapy in *MH TP53*^{MT} was less clear with median OS 8.03 vs 6.7 months (p=0.07), respectively.

In univariate analysis for OS (**Supplementary Table 4**), age as continuous variable (every 10 years) (p= 0.02), complex CG (p= 0.002), and other low intensity chemotherapy (p= 0.01) were associated with inferior outcomes. *RUNX1* 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 allo-HCT as a time dependent co-variate (p= <0.001) were associated with favorable OS in univariate analysis. In multivariable analysis for OS, complex CG (HR; 1.56, 95% CI: 1.01-2.40, p= 0.04) retained unfavorable; *IDH1* mutation (HR; 0.24, 95% CI: 0.08-0.71, p= 0.01) and allo-HCT (HR; 0.28, 95% CI: 0.16-0.47, p= <0.001) retained favorable significance.

Discussion

In our real-world, multicenter analysis in 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* vs *MH TP53*^{MT} AML with *IDH1* co-mutations imparting favorable prognostic significance and use of allo-HCT associating with improved OS, irrespective of *SH* vs *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-EB/AML harboring *SH* or *MH TP53*^{MT} had comparable outcomes. Similar to Grob et al.[10], we did not observe significant differences in response rate or survival between *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, *SH TP53*^{MT} had an abundance of somatic co-mutations, while *MH TP53*^{MT} was significantly associated with occurrence of complex CG.[23]

IDH1/2 mutations are observed in approximately 20% of patients with AML (6-16% *IDH1*, 8-19% *IDH2*). [24] *IDH1/2* mutations are more frequently seen in elderly AML, 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, outcomes of *IDH1/2* mutated AML patients have significantly improved, especially those who are ineligible for intensive therapies.[26] Interestingly, we observed significantly improved EFS and OS among patients with *IDH1* co-mutations and favorable significance was retained in multivariate analysis. Moreover, only a small proportion of these patients received VEN plus HMA as first line (2/11 [18%]) or as a salvage therapy (1/11 [9%]) and only 2/11 (18%) patients received allo-HCT in this sub-group. None of the patients received IDH1 inhibitor alone or in combination with chemotherapy upfront. One patient each received HMA/venetoclax plus IDH1 inhibitor and 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.

While allo-HCT is universally considered a potential curative option for patients with adverse risk AML, earlier studies have shown dismal outcomes or patients with *TP53*^{MT} AML receiving allo-HCT.[27] Lack of benefit was attributed to inability to achieve complete response and persistence of *TP53*^{MT} clone pre-alloHCT. In our earlier report utilizing data from 10 US academic centers, we showed that allo-HCT improved survival of patients with *TP53*^{MT} AML.[13] We have now re-confirmed this finding to also be irrespective of *TP53* allelic state. In our study, we also demonstrated in multivariable analysis a significantly better EFS associated HMA plus

VEN induction when compared with other therapies. However, this did not translate into improved OS, suggesting evolution of resistant clones that were not suppressed long-term with VEN plus HMA therapy alone, as previously reported.[28] Secondly, in a subset analysis we observed a better OS with intensive chemotherapy compared to non-intensive chemotherapy induction in *SH* and *MH* sub-groups probably due to the fact that patients eligible for intensive chemotherapy generally have good performance status/less co-morbidities and are more likely candidates for allo-HCT. Furthermore, intensive chemotherapy induction did not retain significance in multivariate analysis for better survival.

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

In conclusion, unlike lower risk MDS, we did not find a significant difference in response rate or survival outcome among patients with *SH* vs *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.

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Table 1. Baseline characteristics, treatment, and outcome: (%), [range]				
Variable	**Total (N= 382)**	**Single-Hit *TP53* (N= 139)**	**Multi-hit *TP53* (N= 243)**	**p value**
Age, years	67 [18-97]	67 [23-90]	66.5 [18-97]	0.86
Age ≥ 65 years	206 (54)	75 (55)	131 (54)	0.83
Gender (Male)	224 (59)	76 (56)	148 (60)	0.38
WBC 10 (9)/L	2.9 [0.4-460]	2.9 [0.5-460]	3.0 [0.8-288]	0.88
Peripheral blast %	10 [0-97]	8 [0-97]	11 [0-97.9]	0.46
Bone marrow blast %	35 [2-99	35 [14-95]	35 [2-99]	0.18
sAML	109 (30)	39 (33)	70 (29)	0.34
MPN-blast phase	11 (29)	4 (3)	7 (3)	0.82
tAML	85 (22)	25 (18)	60 (24.5)	0.51
Complex cytogenetics	307 (80)	79 (58)	228 (93)	**<0.001**
***TP53* VAF**	44 [2-98]	22 [2-49]	50 [4-98]	**<0.001**
Co-mutated	239 (63)	91 (67)	148 (60)	0.22
Myeloid co-mutations				
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				
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)	91 (26)	39 (33)	52 (23)	0.09
Allogeneic HCT	55 (14)	19 (14)	36 (15)	0.53

WBC; white blood cell, sAML; secondary acute myeloid leukemia, MPN; myeloproliferative neoplasm, HMA; hypomethylating agent, CR; complete remission, i; incomplete count recovery, HCT; hematopoietic stem cell transplantation, chemo; chemotherapy

* Other low intensity therapy includes low dose cytarabine, IDH2 inhibitor alone or investigational agent.

Table Legend

Table 1. Baseline characteristics, treatment, and outcome in Single-Hit and Multi-Hit *TP53*

Figure Legend

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.

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

Figure 3. **Kaplan Meier Survival Curves for *SH* vs *MH TP53*** (a) Duration of response (DOR) (b) event free survival (EFS) and (c) overall survival (OS) in single-hit (SH) vs multi-hit (MH) *TP53* mutated acute myeloid leukemia (AML). Subset analysis for OS (d) curves showing impact of complex cytogenetics (CK) on overall survival in single-hit (SH) and multi-hit (MH) TP53 mutated AML. (e) landmark analysis for OS among patients with complete remission receiving allogeneic hematopoietic stem cell transplantation (allo-HCT) in SH vs MH *TP53* AML (f) landmark analysis for OS among patients with complete remission receiving allogeneic hematopoietic stem cell transplantation (allo-HCT) in respect to CK and *TP53* allelic burden.

Figure 1

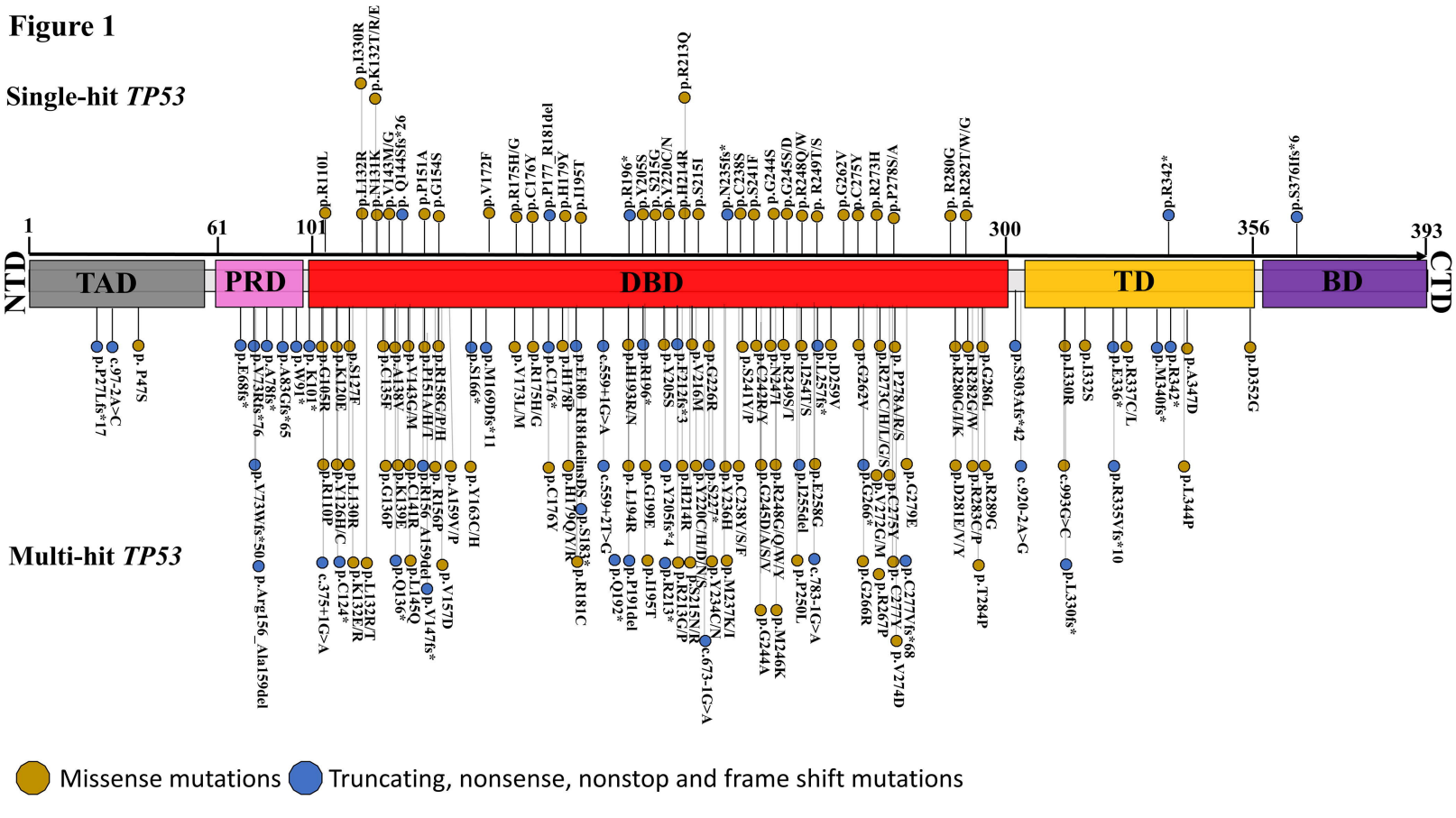
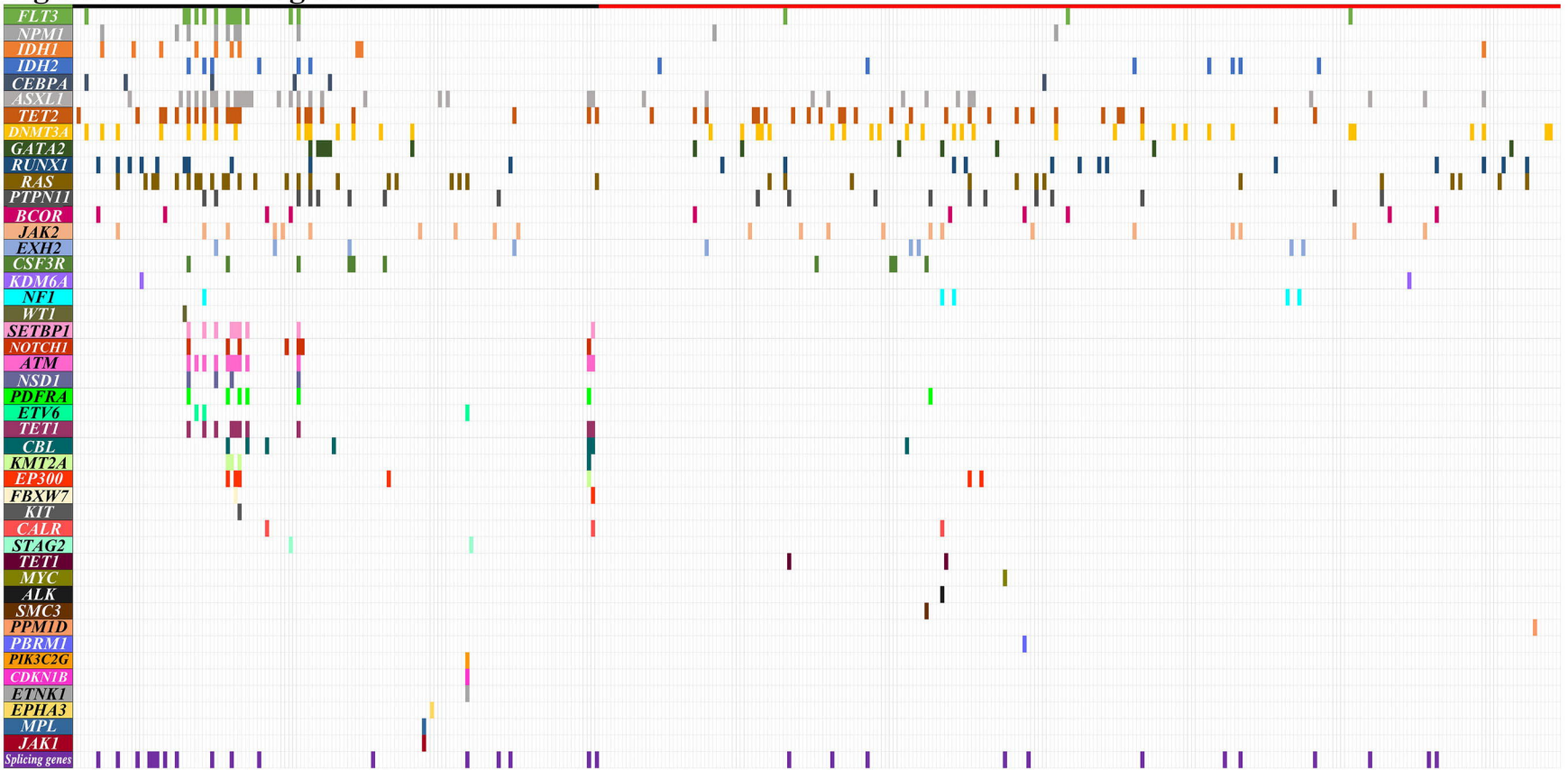
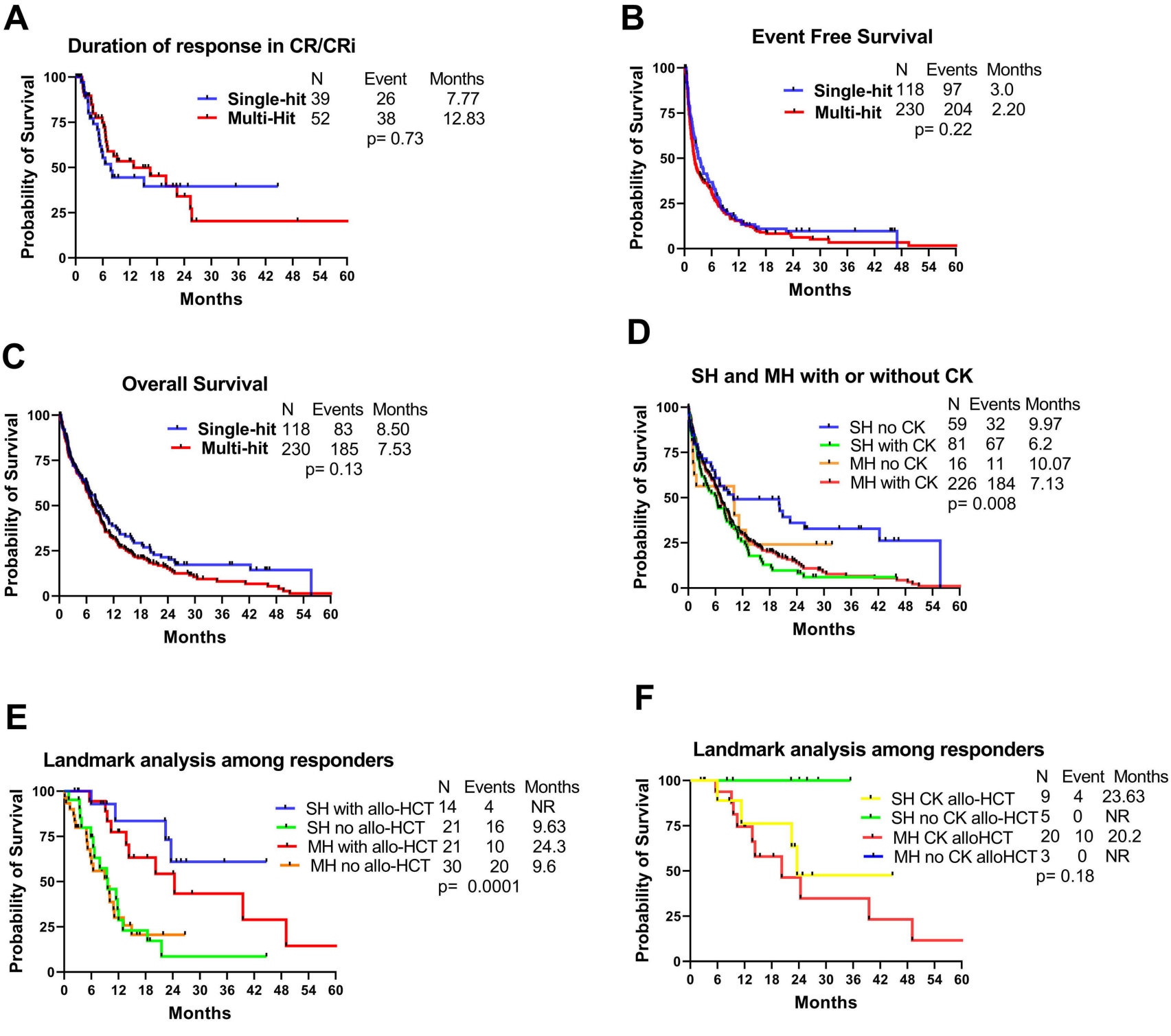

Single-hit TP53
Multi-hit TP53
NTD
CTD
TAD
PRD
DBD
TD
BD
1
61
101
300
356
393
Missense mutations
Truncating, nonsense, nonstop and frame shift mutations

Figure 2


Single-Hit
Multi-Hit
FLT3
NPM1
IDH1
IDH2
CEBPA
ASXL1
TET2
DNMT3A
GATA2
RUNX1
RAS
PTPN11
BCOR
JAK2
EXH2
CSF3R
KDM6A
NF1
WT1
SETBP1
NOTCH1
ATM
NSD1
PDFRA
ETV6
TET1
CBL
KMT2A
EP300
FBXW7
KIT
CALR
STAG2
TET1
MYC
ALK
SMC3
PPM1D
PBRM1
PIK3C2G
CDKN1B
ETNK1
EPHA3
MPL
JAK1
Splicing genes


A
Duration of response in CR/CRi
Probability of Survival
Months
N Event Months
Single-hit 39 26 7.77
Multi-Hit 52 38 12.83
p= 0.73
B
Event Free Survival
Probability of Survival
Months
N Events Months
Single-hit 118 97 3.0
Multi-hit 230 204 2.20
p= 0.22
C
Overall Survival
Probability of Survival
Months
N Events Months
Single-hit 118 83 8.50
Multi-hit 230 185 7.53
p= 0.13
D
SH and MH with or without CK
Probability of Survival
Months
N Events Months
SH no CK 59 32 9.97
SH with CK 81 67 6.2
MH no CK 16 11 10.07
MH with CK 226 184 7.13
p= 0.008
E
Landmark analysis among responders
Probability of Survival
Months
N Events Months
SH with allo-HCT 14 4 NR
SH no allo-HCT 21 16 9.63
MH with allo-HCT 21 10 24.3
MH no allo-HCT 30 20 9.6
p= 0.0001
F
Landmark analysis among responders
Probability of Survival
Months
N Event Months
SH CK allo-HCT 9 4 23.63
SH no CK allo-HCT 5 0 NR
MH CK alloHCT 20 10 20.2
MH no CK alloHCT 3 0 NR
p= 0.18

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.

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Figures: 3
Tables: 1
Supplementary material: 5

Author contribution

TB: Conceptualization, data curation, writing original draft, and submission. AN: helped in data collection and making figures. EA, RMS, AP, ANS, MS, JPB: contributed patients, review and edit manuscript. MB, MS, GCC, GM, YA, AD, DB, VK, SD, ADG, NP, AAK, AZ, MP: contributed patients and review manuscript. MRL: contributed patients, supervise, review, and edit the manuscript.

Conflict of interest

TB: Serve in advisory board for Pfizer, Morphosys and Takeda AP: Consulting for Abbvie, research funding from Kronos Bio, Pfizer, Celgene/BMS, Servier, VK: Advisory board for Novartis and Pfizer. Anand Patel: COI: honoraria from AbbVie and BMS, research funding (institutional) from Pfizer and Kronos Bio. Amer Zeidan: Amer Zeidan is a Leukemia and Lymphoma Society Scholar in Clinical Research. Amer M. Zeidan received research funding (institutional) from Celgene/BMS, Abbvie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Cardiff oncology, Incyte, Takeda, Novartis, Shattuck Labs, Geron, and Aprea. AMZ participated in advisory boards, and/or had a consultancy 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. AMZ served on clinical trial committees for Novartis, Abbvie, Gilead, Syros, BioCryst, Abbvie, ALX Oncology, Geron and Celgene/BMS. AMZ received travel support for meetings from Pfizer, Novartis, and Cardiff Oncology.

Data availability

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

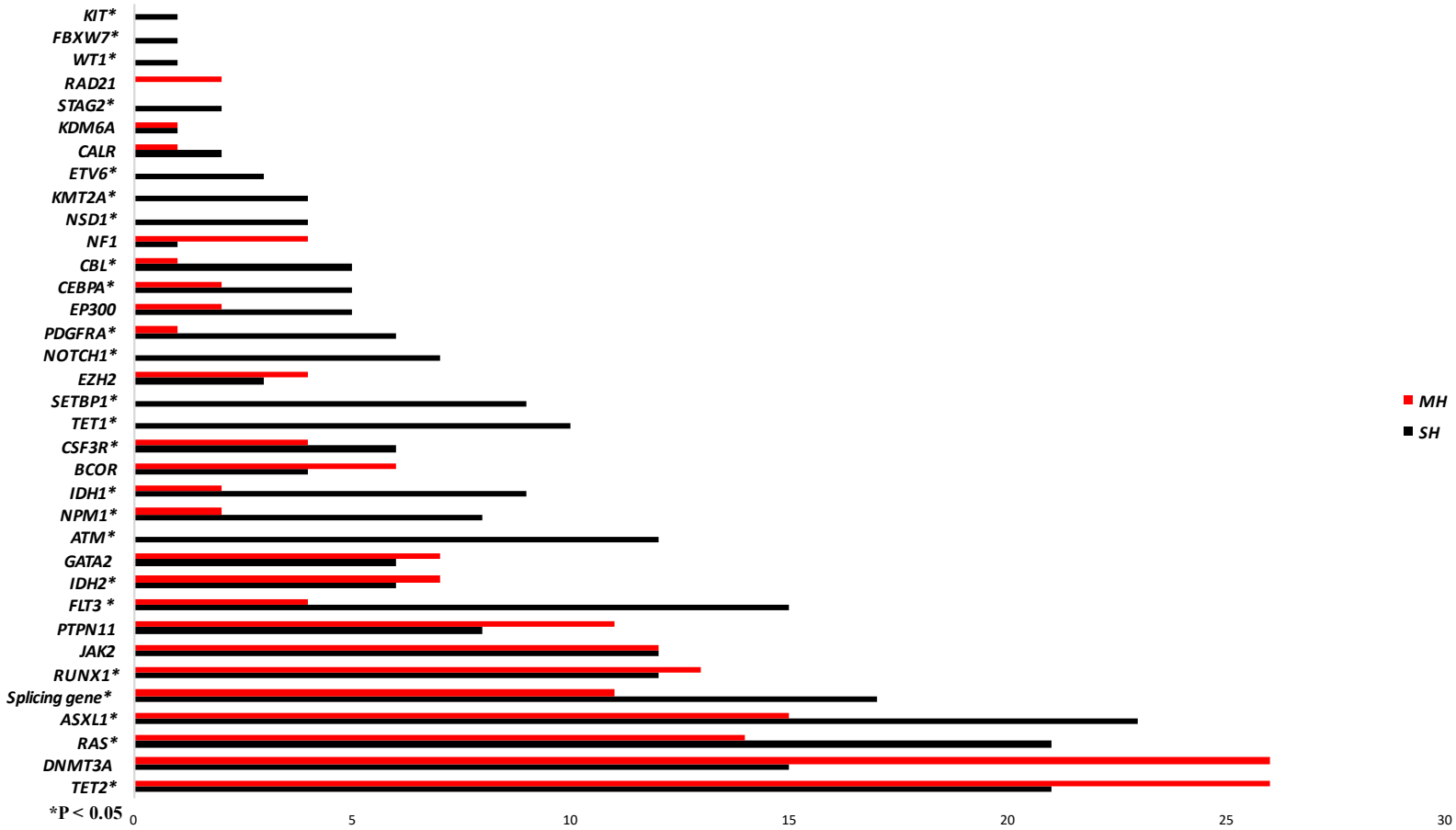
Supplementary Table 1. Baseline characteristics and outcome with *IDH1* (N=11) and *IDH2* (13) mutations			
Variables	IDH1	IDH2	P Value
Age	66 [24-73]	70 [36-85]	>0.99
sAML	3 (27)	6 (46)	0.15
Complex CG	3 (27)	7 (54)	0.24
TP53 VAF (%)	24 [5-32]	68 [7-89]	0.52
Multi-hit *TP53*	2 (18)	7 (53)	0.10
Co-mutations other than *IDH1/2*			
ASXL1	5 (45)	5 (38)	>0.99
TET2	5 (45)	4 (31)	0.41
FLT3 ITD	4 (36)	3 (23)	0.65
DNMT3A	4 (36)	6 (46)	0.69
NPM1	3 (27)	2 (15)	0.63
RAS	2 (18)	5 (38)	0.40
RUNX1	2 (18)	2 (15)	>0.99
Splicing factor (U2AF1, SF3B1, SRSF2)	1 (9)	4 (31)	0.33
GATA2	1 (9)	1 (7)	>0.99
EZH2	1 (9)	0	0.45
JAK2	0	5 (38.5)	0.04
PTPN11	0	3 (23)	0.59
CSF3R1	0	2 (15)	0.48
CEBPA	0	1 (7)	>0.99
BCOR	0	0	-
Venetoclax plus HMA (1st line)	2 (18)	1 (7)	0.21
Venetoclax, HMA, IDH1 inhibitor (Salvage)	1 (9)	-	-
HMA plus IDH2 inhibitor (Salvage)	-	1 (7)	-
IDH1 inhibitor alone (Salvage)	1 (9)	-	-
IDH2 inhibitor alone (Salvage)	-	1 (7)	-
CR/CRi rate	6 (54.5)	0	0.003
Allogeneic-HCT	2 (18)	1 (8)	0.57
sAML; secondary acute myeloid leukemia, HMA; hypomethylating agent, CR; complete remission, i; incomplete count recovery, HCT; hematopoietic stem cell transplantation			

Supplementary Table 2. Predictors of complete remission (N= 91/382; 24%)				
Variable	Total (N= 382)	CR/CRi	No CR/CRi	p value
Age ≥ 65 years	206 (54)	49 (24)	156 (76)	>0.99
Gender (Male)	224 (59)	40 (18)	118 (82)	0.62
sAML	109 (30)	28 (26)	81 (74)	0.58
tAML	85 (22)	20 (23.5)	65 (76.5)	>0.99
Complex cytogenetics	307 (80)	73 (24)	234 (76)	>0.99
Single hit *TP53*	137 (36)	38 (28)	99 (72)	0.211
Multi hit *TP53*	245 (64)	53 (22)	192 (78)	0.211
Co-mutated	239 (63)	55 (23)	183 (77)	0.71
Myeloid co-mutations				
TET2	47 (12)	7 (15)	40 (85)	0.19
DNMT3A	41 (11)	9 (22)	32 (78)	>0.99
ASXL1	38 (10)	11 (29)	27 (71)	0.41
RAS	35 (9)	3 (9)	32 (91)	0.02
Splicing factor *(U2AF1, SF3B1, SRSF2)*	28 (7)	5 (18)	23 (82)	0.64
JAK2	24 (6)	5 (21)	19 (79)	>0.99
RUNX1	25 (7)	6 (24)	19 (76)	>0.99
IDH1	11 (3)	6 (55)	5 (45)	0.02
IDH2	13 (3)	0	13 (100)	0.03
FLT3 ITD	19 (5)	8 (42)	11 (68)	0.09
PTPN11	19 (5)	3 (16)	16 (84)	0.58
GATA2	13 (4)	2 (15)	11 (85)	0.74
NPM1	10 (3)	3 (30)	7 (70)	0.70
BCOR	10 (3)	5 (50)	5 (50)	0.06
CSF3R	10 (3)	2 (20)	8 (80)	>0.99
CEBPA	7 (2)	3 (43)	4 (57)	0.36
EZH2	7 (2)	2 (28.5)	5 (71.5)	>0.99
Type of Induction				
Intensive induction	97 (25)	25 (26)	72 (74)	0.67
HMA based	50 (13)	9 (18)	41 (82)	0.37
HMA plus venetoclax	102 (27)	37 (36)	65 (64)	<0.001
Other low intensity chemotherapy	21 (5)	2 (10)	19 (90)	0.12
WBC; white blood cell, sAML; secondary acute myeloid leukemia, HMA; hypomethylating agent, CR; complete remission, i; incomplete count recovery, HCT; hematopoietic stem cell transplantation				

Supplementary Table 3. Cox regression models predicting for event free survival				
Variable	Univariate analysis for EFS		Multivariate analysis for EFS	
	EFS (mo)	P value	HR (95% CI)	P value
Age (every 10 years)	-	0.27		
Gender (Male vs Female)	1.80 vs 2.27	0.99		
sAML	1.73 vs 2.13	0.20		
tAML	2.27 vs 1.93	0.17		
Complex cytogenetics	1.97 vs 2.27	0.04	1.42 (0.98, 2.07)	0.06
Single hit *vs* Multi hit *TP53*	2.27 vs 1.90	0.40		
Co-mutated	2.13 vs 1.93	0.39		
Myeloid co-mutations				
TET2	1.97 vs 2.13	0.69		
DNMT3A	1.87 vs 2.13	0.72		
ASXL1	3.00 vs 2.03	0.02	0.73 (0.44, 1.21)	0.22
RAS	1.97 vs 2.13	0.98		
Splicing factor *(U2AF1, SF3B1, SRSF2)*	1.90 vs 2.13	0.51		
JAK2	1.30 vs 2.13	0.68		
RUNX1	1.17 vs 2.13	0.17		
IDH1	8.23 vs 2.07	0.01	0.44 (0.19, 1.01)	0.05
IDH2	1.17 vs 2.13	0.17		
FLT3 ITD	2.13 vs 2.0	0.04	0.98 (0.48, 2.01)	0.96
PTPN11	1.97 vs 2.13	0.87		
GATA2	1.20 vs 2.13	0.35		
NPM1	2.83 vs 2.03	0.06		
BCOR	1.47 vs 2.07	0.20		
CSF3R	2.13 vs 2.03	0.91		
CEBPA	3.67 vs 2.13	0.25		
EZH2	1.50 vs 2.10	0.52		
Type of Induction				
Intensive induction	1.33 vs 2.20	0.20		
HMA based	5.10 vs 1.80	0.09		
HMA plus venetoclax	3.73 vs 1.63	<0.001	0.53 (0.41, 0.70)	<0.001
Other low intensity chemotherapy	1.43 vs 2.13	0.11		
Allogeneic-HCT[1]	-	0.002	0.34 (0.18, 0.62)	<0.001
mo; months, HR; hazard ratio, CI; confidence interval, WBC; white blood cell, sAML; secondary acute myeloid leukemia, HMA; hypomethylating agent, HCT; hematopoietic stem cell transplantation [1]Allogeneic- HCT was treated as a time-dependent covariate.				

Supplementary Table 4. Cox regression models predicting for overall survival				
Variable	Univariate analysis for OS		Multivariate analysis for OS	
	OS (mo)	P value	HR (95% CI)	P value
Age (every 10 years)	-	0.002	1.07 (0.95, 1.21)	0.27
Gender (Male vs Female)	6.57 vs 8.07	0.59		
sAML	5.90 vs 7.53	0.06		
tAML	8.20 vs 6.60	0.86		
Complex cytogenetics	6.67 vs 10.07	0.002	1.56 (1.01, 2.40)	0.044
Single hit vs Multi hit TP53	6.87 vs 7.13	0.35		
Co-mutated	6.70 vs 8.0	0.60		
Myeloid co-mutations				
TET2	6.57 vs 6.63	0.35		
DNMT3A	5.90 vs 7.10	0.89		
ASXL1	11.33 vs 6.60	0.18		
RAS	4.03 vs 6.90	0.78		
Splicing factor (*U2AF1, SF3B1, SRSF2*)	6.57 vs 6.67	0.34		
JAK2	5.87 vs 7.10	0.58		
RUNX1	9.93 vs 6.57	0.01	0.73 (0.42, 1.25)	0.25
IDH1	55.73 vs 7.10	<0.001	0.24 (0.08, 0.71)	0.010
IDH2	23.07 vs 7.03	0.52		
FLT3 ITD	22.53 vs 6.90	0.003	0.90 (0.39, 2.12)	0.82
PTPN11	3.37 vs 6.70	0.45		
GATA2	4.40 vs 6.70	0.07		
NPM1	NR vs 7.10	0.02	0.31 (0.07, 1.37)	0.12
BCOR	6.57 vs 6.90	0.20		
CSF3R	6.70 vs 7.03	0.97		
CEBPA	8.83 vs 6.90	0.35		
EZH2	2.73 vs 7.03	0.29		
Type of Induction				
Intensive induction	9.13 vs 6.47	0.007	0.86 (0.62, 1.18)	0.34
HMA based	9.17 vs 6.67	0.85		
HMA plus venetoclax	7.53 vs 6.87	0.28		
Other low intensity chemotherapy	1.93 vs 7.30	0.01	3.66 (2.09, 6.39)	<0.001
Allogeneic-HCT[1]	-	<0.001	0.28 (0.16, 0.47)	<0.001

mo; months, HR; hazard ratio, CI; confidence interval, WBC; white blood cell, sAML; secondary acute myeloid leukemia, HMA; hypomethylating agent, HCT; hematopoietic stem cell transplantation, NR; not reached.
[1]Allogeneic- HCT was treated as a time-dependent covariate.



Supplementary Figure 1. *Patterns and frequency of co-mutations identified in the TP53 cohort by allelic state. Single-hit TP53 are depicted in black and those co-mutated with multi-hit TP53 in red. *P < 0.05, Chi Square approximation & two-sided Fisher's exact test.*