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Real world predictors of response and 24-month survival in high-grade *TP53*-mutated myeloid neoplasms

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Current therapies for high-grade *TP53*-mutated myeloid neoplasms (≥10% blasts) do not offer a meaningful survival benefit except allogeneic stem cell transplantation in the minority who achieve a complete response to first line therapy (CR1). To identify reliable pre-therapy predictors of complete response to first-line therapy (CR1) and outcomes, we assembled a cohort of 242 individuals with *TP53*-mutated myeloid neoplasms and ≥10% blasts with well-annotated clinical, molecular and pathology data. Key outcomes examined were CR1 & 24-month survival (OS24). In this elderly cohort (median age 68.2 years) with 74.0% receiving frontline non-intensive regimens (hypomethylating agents +/- venetoclax), the overall cohort CR1 rate was 25.6% (50/195). We additionally identified several pre-therapy factors predictive of inferior CR1 including male gender ($P = 0.026$), ≥2 autosomal monosomies ($P < 0.001$), −17/17p ($P = 0.011$), multi-hit *TP53* allelic state ($P < 0.001$) and *CUX1* co-alterations ($P = 0.010$). In univariable analysis of the entire cohort, inferior OS24 was predicated by ≥2 monosomies ($P = 0.004$), *TP53* VAF > 25% ($P = 0.002$), *TP53* splice junction mutations ($P = 0.007$) and antecedent treated myeloid neoplasm ($P = 0.001$). In addition, mutations/deletions in *CUX1*, *U2AF1*, *EZH2*, *TET2*, *CBL*, or *KRAS* ('*EPI6*' signature) predicted inferior OS24 (HR = 2.0 [1.5–2.8]; $P < 0.0001$). In a subgroup analysis of HMA +/-Ven treated individuals ($N = 144$), *TP53* VAF and monosomies did not impact OS24. A risk score for HMA +/-Ven treated individuals incorporating three pre-therapy predictors including *TP53* splice junction mutations, *EPI6* and antecedent treated myeloid neoplasm stratified 3 prognostic distinct groups: intermediate, intermediate-poor, and poor with significantly different median (12.8, 6.0, 4.3 months) and 24-month (20.9%, 5.7%, 0.5%) survival ($P < 0.0001$). For the first time, in a seemingly monolithic high-risk cohort, our data identifies several baseline factors that predict response and 24-month survival.

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INTRODUCTION

Several studies in the past decade have confirmed the adverse outcome of complex and monosomal karyotype as well as *TP53* alterations in the context of myeloid neoplasms (MN) [1–4]. *TP53*^{MUT} MN are characterized by frequent complex karyotype (CK) and very poor 2-year survival of 12.8%, regardless of blast count [5]. Based on these studies, high-risk myelodysplastic syndrome (MDS) including MDS/AML (MDS/AML) and AML with mutated *TP53* (*TP53*^{MUT}) are now recognized as distinct categories in the World Health Organization 5th edition (WHO5), International Consensus Classification of hematopoietic neoplasms (ICC), and European LeukemiaNet (ELN) risk stratification [6–9].

Beyond assessing complete response to first-line therapy (CR1) and allogeneic hematopoietic stem cell transplantation (allo-SCT) in CR1, there are no well-established pre-therapy prognostic indicators in this cohort of patients [10] who are automatically assigned to the adverse group per ELN2022 risk stratification [9]. Furthermore, there is a significant heterogeneity in response to available therapies (intensive chemotherapy vs. hypomethylating agent (HMA)-based therapy) or agents used in clinical trials (for e.g., APR-246 [11]) with few long-term survivors even in those receiving allo-SCT [12]. Consequently, there is a pressing need to explore better means to identify disease characteristics influencing therapeutic response to optimally select frontline treatments

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Table 1. Baseline characteristics of all 242 patients at diagnosis of *TP53*^{MUT} Myeloid Neoplasm stratified by *TP53* allelic state at diagnosis (see methods section for definition of allelic state).

	TP53^{SH} **N = 69 (28.5%)**	*TP53*^{MH} **N = 173 (71.5%)**	**Test**
Age at diagnosis, Median [IQR]			
Age (years)	68.5 [14−89]	67.9 [23−93]	0.633
Baseline labs, Median [IQR]			
Hemoglobin (g/dL)	8.2 [3−13]	7.9 [4−17]	0.612
Platelet Count (10^9/L)	53.0 [5−344]	40.0 [5−585]	0.572
Abs. Neutrophil Count (10^9/L)	0.5 [0−19]	0.7 [0−100]	0.225
Sex			
Male	33 (47.8%)	103 (59.5%)	0.097
Female	36 (52.2%)	70 (40.5%)	
Era of dx.-no.(%)			
Pre-2018	19 (27.5%)	43 (24.9%)	0.666
2018-current	50 (72.5%)	130 (75.1%)	
Prior Treated Myeloid Neoplasm[a]-no.(%)			
No	55 (79.7%)	140 (80.9%)	0.829
Yes	14 (20.3%)	33 (19.1%)	
MDS/AML-ICC 2022			
MDS/AML (10-19% blasts)	12 (17.6%)	36 (20.8%)	0.580
AML (20%+ blasts)	56 (82.4%)	137 (79.2%)	
Complex karyotype			
Absent	22 (37.9%)	22 (13.8%)	<0.001
Present	36 (62.1%)	137 (86.2%)	
Chromosome 7 loss			
Absent	33 (55.9%)	71 (43.8%)	0.111
Present	26 (44.1%)	91 (56.2%)	
Monosomies-no.(%)			
0–1 Monosomy	34 (58.6%)	49 (30.8%)	<0.001
2+ Monosomies	24 (41.4%)	110 (69.2%)	
Chromosome 12(p) loss			
del(12p) absent	65 (94.2%)	143 (82.7%)	0.020
del(12p) present	4 (5.8%)	30 (17.3%)	
TP53 mutation class[c]-no.(%)			
Missense	50 (72.5%)	109 (63.0%)	0.162
Non-Missense	19 (27.5%)	64 (37.0%)	
Germline alteration.-no.(%)			
Absent	20 (76.9%)	45 (86.5%)	0.283
Present	6 (23.1%)	7 (13.5%)	
Co-alterations-no.(%)			
Absent	31 (44.9%)	62 (35.8%)	0.189
Present	38 (55.1%)	111 (64.2%)	
Therapies-no.(%)			
Low-inten. (HMA ±VEN)	43 (65.2%)	105 (62.5%)	0.795
Intensive (Chemo)	14 (21.2%)	38 (22.6%)	
Best.Supp.Care	7 (10.6%)	15 (8.9%)	
CPX-351/Vyxeos	2 (3.0%)	10 (6.0%)	
Resp. frontline[b]-no.(%)			
No CR/CRi	32 (57.1%)	113 (81.3%)	<0.001
CR/CRi	24 (42.9%)	26 (18.7%)	

Med. Median, *IQR* Interquartile range, *BSC* Best Supportive Care, *CR* Complete Response, *CRi* CR & incomplete hematologic recovery.
[a]These included treated or untreated MDS (Low and High-risk), MDS/MPN and MPN without any *TP53*^{MUT} up until evolution/progression to a *TP53*^{MUT} myeloid neoplasm.
[b]Numbers reported in therapies and response may not add up to cohort total since some patients were either untreated or if treated, response could not evaluated due to various reasons (active second malignancy, early treatment-emergent adverse effects, transferred care elsewhere or early mortality).
[c]In pts with multiple mutations, case was designated *TP53*^{NMIS} if ≥1 *TP53* mutation was a *TP53*^{NMIS} mutation.

and identify patients who are likely to achieve durable benefit from allo-SCT in CR1.

With this background, in this study we asked if (1) specific chromosomal alterations within a CK (such as autosomal monosomies), (2) the type of *TP53* mutation (missense [*TP53*^{MIS}] *vs.* non-missense *TP53* mutations [*TP53*^{NMIS}]), and (3) patterns of co-mutations/alterations contribute additional prognostic value beyond *TP53* allelic state in risk-stratifying *TP53*^{MUT} MN.

MATERIALS AND METHODS

Cohort case selection and sample procurement

We identified patients with *TP53*^{MUT} MN carrying ≥1 *TP53* mutation at a VAF ≥ 3% diagnosed between 2014 and 2023 across four US centers with largely similar treatment practices. We excluded individuals with any of the following: a known germline *TP53* mutation, known *TP53*^{MUT} precursor states (CHIP, CCUS), MDS with mutated *TP53* (<10% blasts) [6], and core binding factor-altered AMLs.

Data collection. We collected data on demographics, marrow pathology, molecular and cytogenetic information, and treatment types. Therapies were categorized as: (1) Intensive Chemotherapy (IC) (7 + 3 or high-dose cytarabine), (2) Hypomethylating agent (HMA)-based (without venetoclax), (3) HMA-based with venetoclax, or (4) Best supportive care/palliative regimens. Response was assessed per ELN 2017 guidelines [13] denoting both CR and CR with incomplete hematologic recovery (CRi) as a composite measure of CR1.

Cytogenetic studies

Chromosome analysis was performed following standard cytogenetic laboratory clinical protocol. Fluorescence in situ hybridization (FISH) testing was performed using probe sets targeting most recurring abnormalities in myeloid neoplasms on bone marrow or malignancy-involved peripheral blood. Using previously published criteria, over 90% of the cohort cases met the classification of monosomal karyotype (MK) [1]. Therefore, we applied a revised definition of MK restricting to only unique autosomal monosomies (0−1 vs. 2+) without considering other structural alterations (Supplementary S1).

Next generation sequencing

Somatic next-generation sequencing (NGS) data was available in all cases. To maintain consistency in our analysis, we only included genes that were tested across two or more centers (Supplementary S2). For missense *TP53* mutations, we also examined the evolutionary action score for p53 (Supplementary S3) [14]. Additionally, germline testing data (Supplementary S4) was available in a subset of cases.

Allelic status. *TP53* multi-hit (*TP53*^{MH}) allelic state was designated per ICC 2022 schema [6]. However, single-hit (*TP53*^{SH}) designation used an

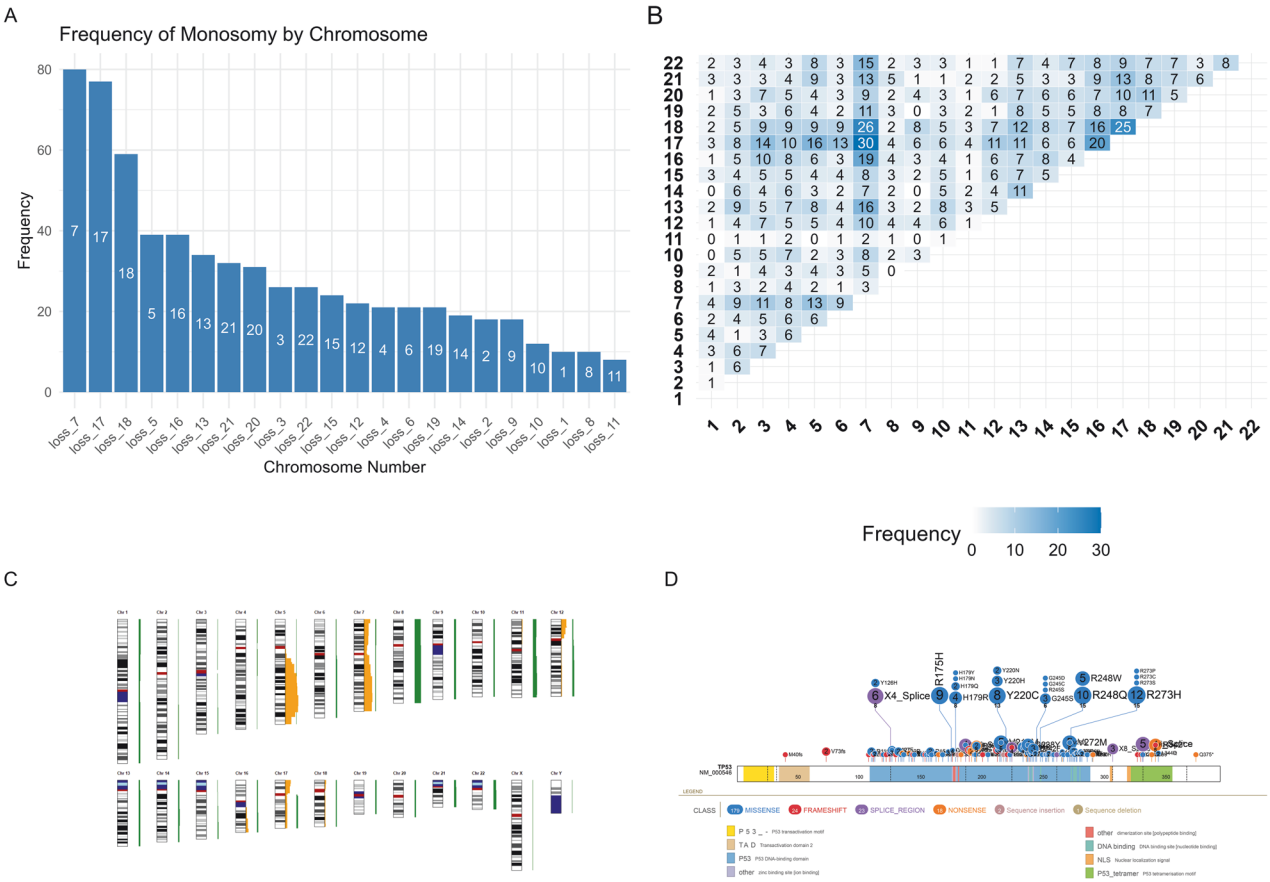

Fig. 1 Summary of cytogenetic alterations and *TP53* mutations. A Frequencies of autosomal monosomies (percentages represent frequencies in patients with available data on karyotype). The most frequent autosomies involved chromosomes 7, 17 and 18. **B** Co-occurrence matrix of autosomal monosomies. Counts indicate frequencies of co-occurrence. The most frequently co-occurring monosomies with loss of 17 were monosomies 7 and 16, followed by monosomies of chromosomes 5, 6 and 12. **C** Ideogram of global losses (orange) and gains (green) across the genome. Recurrent losses are enriched in chromosomes 5, 7 and 17. Furthermore, we observed additional recurrent losses on 12p, besides 16q, 18p, and 18q. Chromosomal gains were prevalent, particularly on chromosomes 8 (trisomy 8), as well as on chromosomes 1, 9, 11, 21, and 22. Clean karyotypes were batched parsed in CytoGPS to create .json files with loss, gain & fusion information. These files were examined using the RCytoGPS package for R, treating them as a binary matrix, to create the ideogram [36]. **D** Summary of *TP53* mutations in the cohort. Somatic variants in *TP53* visualized using lollipop plot generated via the ProteinPaint web-based application [37]. Majority comprised hot-spot DNA-binding domain missense mutations. Chromosomal position coordinates were culled from the IARC database of *TP53* mutations. A few complex mutations seen in a few cases are not depicted and numbers may not match up with that depicted in the results section.

expanded VAF cutoff including cases with VAF between 3 and 10%. Additionally, we modeled any non-missense *TP53* mutations ($TP53^{NMIS}$) as a binary predictor.

Data analysis

The primary endpoint was 24-month overall survival (OS24) from the time of diagnosis of $TP53^{MUT}$ MN to death or last follow-up, censoring patients alive at 24 months. Response to first-line therapy was also evaluated. We employed non-parametric Kaplan-Meier methods, along with Cox proportional hazards (P-H) regression models [15] and, where suitable, flexible parametric models [16] (Supplementary S5).

RESULTS

Cohort summary

Table 1 depicts the baseline data of all patients with therapy/response data of all patients receiving definitive therapy stratified by *TP53* allelic state at diagnosis of $TP53^{MUT}$ myeloid neoplasm. Individuals accrued after 2018 were significantly older with 48.3% surpassing 70 years of age as opposed to 30.6% in the pre-2018 period ($P = 0.015$). Additionally, post-2018 individuals frequently received lower-intensity therapies including HMA + /-venetoclax or CPX-351. Furthermore, individuals with AML (blasts ≥20%) were significantly more likely to receive VEN-based regimens compared to patients with MDS/AMLs ($P = 0.003$) at diagnosis.

Most patients (79.7%; 173/217) exhibited complex karyotypes characterized by frequent autosomal monosomies (83.4%; 181/217) along with several recurrent balanced and unbalanced structural alterations in 93.1% of cases (Fig. 1A–C). The two most prevalent single autosomal monosomies affected chromosomes 17 in 31.8% (69/217) and 7 in 31.3% (68/217) (Fig. 1B for co-occurrence plot). There was no association between *TP53* allelic state and prior cytotoxic-therapy ($P = 0.17$). Among germline alterations, *BRCA1*, *BRCA2* and *DDX41* alterations predominated.

$TP53^{NMIS}$ mutations are frequently associated with multi-hit allelic state and autosomal monosomies

Mutational analysis identified 306 pathogenic mutations (200 unique mutations) among the 245 patients comprising mostly hot-spot DNA-binding domain $TP53^{MIS}$ mutations (Fig. 1D). A total of 29.8% (73/245) harbored non-missense *TP53* mutations, either singly or as part of multiple *TP53* mutations. Individuals with single *TP53* mutations were significantly more likely to harbor loss of chromosome 17p either due to monosomy 17 or structural losses of 17p (44.5% vs. 26.8% in multiple *TP53* mutations; $P = 0.019$).

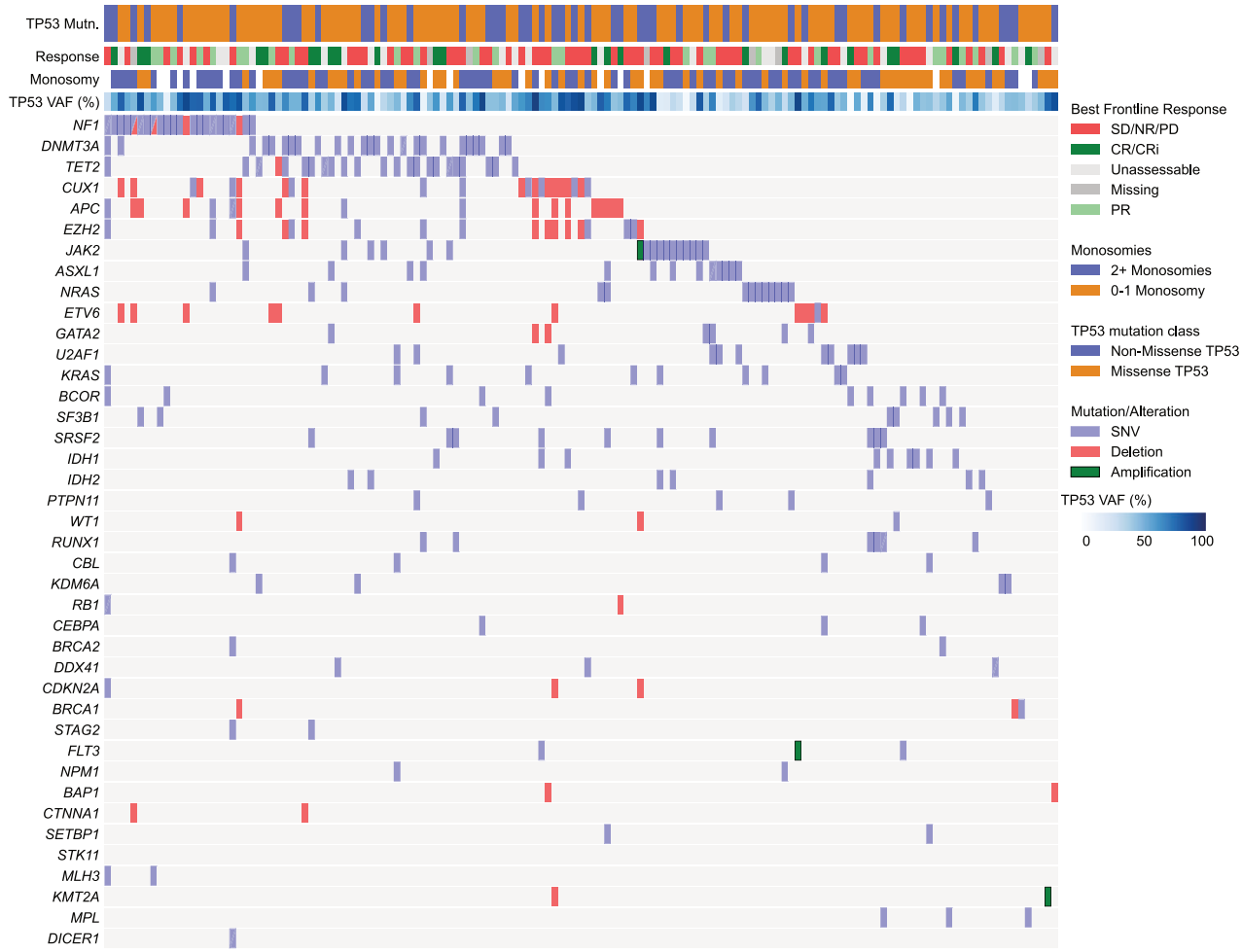

Fig. 2 Co-mutations and structural alterations in patients with any co-mutations. Only variants occurring at least twice within the cohort are represented. Notably, structural alterations/losses were common with certain genes, particularly *CUX1*, *EZH2*, and *APC*.

Among $TP53^{NMIS}$ mutations, splice junction mutations ($TP53^{sjm}$) occurring in 22 (9.0%) were significantly more prevalent in men ($P = 0.038$).

The median *TP53* VAF was 42% (IQR: 23–59%) and did not differ between $TP53^{MIS}$ and $TP53^{NMIS}$ alterations ($P = 0.61$). Sub-clonal *TP53* mutations (VAF < 10%) were observed in 6.5% (16/245) with single mutations and 4.1% (10/245) with 1+ mutations. $TP53^{NMIS}$ were more frequently associated with ≥2 monosomies (74.0% vs. 55.6% in $TP53^{MIS}$; $P = 0.008$), chromosome 7 losses (66.2% vs. 46.3% in $TP53^{MIS}$; $P = 0.005$), multiple *TP53* mutations (41.0% vs. 15.7% in $TP53^{MIS}$; $P < 0.0001$) and germline alterations (33.3% vs. 11.1% with germline in $TP53^{MIS}$; $P = 0.025$). Patients with $TP53^{MIS}$ EAp53 score in upper quartile ($N = 98/135$) frequently had *TP53* VAF > 25% (87.8% *vs.* 72.6%; $P = 0.032$).

A 6-gene co-alteration signature including *CUX1* deletion is associated with higher *TP53* VAF

Co-alterations were present in 61.6% ($N = 149$) with a median of 1 co-alteration (range: 0–16 co-alteration). Co-alterations in epigenetic pathways (*DNMT3A* and *TET2*) genes predominated (Fig. 2), in line with prior observations [5]. Males had a higher frequency of mutations in genes of the spliceosome complex (*SF3B1*, *SRSF2*, *U2AF1* or *ZRSR2*) (19.1% vs. 5.7% in females; $P = 0.002$) and the nine myelodysplasia-related genes [6] (31.6% vs. 17.9% in females; $P = 0.015$). Among structural alterations detected by NGS, losses at the *CUX1* (Cut Like Homeobox 1) locus occurring in 9.7% (22/227) of individuals were the most frequent structural alteration (after losses at the *TP53* locus). Patients with any co-alteration had a significantly higher median *TP53* VAF (45.2% vs. 42%; $P = 0.013$). Likewise, individuals carrying *CUX1* alterations had almost double the *TP53* VAF compared to those without *CUX1* alterations (78.5% vs. 43%; $P < 0.0001$).

We constructed an '*EPI6*' signature based on alterations co-occurring in ≥4 individuals and defined by the presence of mutations or deletions in at least one of six key genes associated with methylation (*TET2*, *EZH2*), mitogen-activated protein kinase (*KRAS*), signaling (*CBL*), splicing (*U2AF1*) pathways or *CUX1*. We selected these genes based on their frequency of occurrence and impact in initial exploratory univariable analysis. This *EPI6* signature was present in 24.8% (60/242) of individuals with a significantly higher proportion harboring *TP53* VAF > 25% (88.3% vs. 74.7% with VAF > 25% in individuals lacking *EPI6*; $P = 0.027$). We used this *EPI6* signature going forward in our analyses of treatment outcomes and long-term survival.

Response data: *CUX1* alterations and ≥2 autosomal monosomies predict inferior CR1

First-line treatment and response information. Among the 234 patients with available treatment information, low-intensity regimens (mostly HMA-based) were used in 148 (63.2%), intensive chemotherapy in 22.2% ($N = 52$), CPX-351 in 12 (5.1%) while 22 (9.4%) received only best supportive care. Treatment information was not available in 8 since these patients were either lost to follow up or experienced early mortality before commencing

treatment. Among HMA-based therapies ($N = 144$), HMA (with or without magrolimab) was used in 60 (41.7%) while HMA + VEN was used in 58.3% ($N = 84$). A total of 14.5% (35/242) went on to receive allogeneic stem cell transplantation with 69.7% receiving myeloablative conditioning regimens. First-line response was evaluable in 195 patients with 25.6% achieving CR, 24.1% with partial response, and 50.3% with non-response/stable-progressive disease. A small subset of patients could not be evaluated for treatment response because of treatment discontinuation due to treatment-emergent adverse effects, developed active second non-hematopoietic malignancies, or were lost to follow-up shortly after diagnosis. These patients were excluded from all response analyses but were included in the analysis of 24-month overall survival (OS24).

Univariable predictors of inferior CR1. There was no difference in CR rates between lower-intensity (28.7%; $N = 35/122$) and intensive regimens (25.0%; $N = 12/48$, $P = 0.63$). although surprisingly, CR rates were higher in females (33.7% vs. 19.6% CR1 in Male; $P = 0.026$).

Looking at the entire cohort (intensively and non-intensively treated patients), the biological predictors of inferior CR1 were ≥2 monosomies (15.9% vs. 40.3% CR1 in 0–1 Monosomy; $P < 0.001$), losses on chromosome 17 (15.5% vs. 32.4%; $P = 0.011$), $TP53^{MH}$ allelic state (18.7% vs. 42.9%; $P < 0.001$), myelodysplasia-related gene mutations (13.3% vs. 31.1%; $P = 0.009$) as well as *EPI6* (14.3% vs. 29.5%; $P = 0.035$). Remarkably, none of the individuals with *CUX1* alterations achieved a CR (0.0%; 0/19) vs. 43/162 (26.5%) CR1 without *CUX1* alterations. Chromosome 5 ($P = 0.67$) and chromosome 7 losses ($P = 0.39$) did not impact frontline response. Among the intensively treated subgroup, hot-spot *TP53* mutations were associated significantly inferior frontline response (17.9% vs. 57.1% CR1 in Non-hotspot; $P = 0.025$).

Among individuals treated with HMA-based regimens, HMA + VEN did not result in significantly higher CR rates (31.9%) vs. 24.0% in HMA only ($P = 0.35$). These data are in line with several recent reports [17–20]. However, among patients with blast counts over 20%, HMA+Ven resulted in marginally higher CR rates (32.1% vs. 16.7% in HMA only; $P = 0.12$).

Multivariable response prediction models in entire cohort and HMA subgroup. In a multi-variable logistic regression model on the entire cohort ($N = 168$) including gender and the aforementioned biologic predictors (monosomies, *TP53* allelic state, gender and *EPI6*), ≥2 monosomies (OR = 0.29 [95% CI: 0.13–0.63]; $P = 0.002$), and *TP53* allelic state (OR = 0.43 [95% CI: 0.19–0.95]; $P = 0.036$) predicted significantly inferior response with a marginal effect for *EPI6* ($P = 0.049$) (Supplementary Fig. 1, ROC curve). Among the subgroup treated only with HMA-based therapies ($N = 114$), the logistic regression model identified ≥2 monosomies (OR = 0.22 [95% CI: 0.09–0.55]; $P = 0.001$), and *TP53* allelic state (OR = 0.36 [95% CI: 0.14–0.91]; $P = 0.031$) while *EPI6* ($P = 0.32$) was not relevant.

Baseline outcome data

The median duration of follow up from diagnosis of $TP53^{MUT}$ MN to study exit was 6.1 months (range: 0.2–72.8 months) with 202 deaths in 242 patients and a death rate of 87.0 per 1000 patient-years with a 24-month survival of 16% (95% CI = 11.3–21.5%).

Uni-variable analyses of OS24

Age at diagnosis >70 years (HR = 1.5 [1.1–2.0]; $P = 0.005$), antecedent treated myeloid neoplasm (HR = 1.8 [1.2–2.5]; $P = 0.002$; Fig. 3A) and complex karyotype (HR = 1.8 [1.2–2.7]; $P = 0.009$) all predicted worse OS24. Blast count at diagnosis and therapy-related myeloid neoplasm did not influence outcomes. Among chromosomal alterations, del(7q) (HR = 1.5 [1.1–2.1]; $P = 0.009$) and monosomy of chromosomes 17 (HR = 1.4 [1.0–2.0]; $P = 0.032$) predicted inferior outcomes. Neither del(5q), del(17p), nor monosomies of 5 or 7 impacted survival. Comparing 0−1 vs. ≥ 2 monosomies, the latter group experienced significantly higher hazard of mortality (HR = 1.7 [1.2–2.3]; $P = 0.002$) (Fig. 3B).

$TP53^{NMIS}$ *but not multi-hit allelic state is adverse*. We next assessed different measures of *TP53* mutations and their associations with outcome. The number of *TP53* mutations (1 vs. 1 +) did not impact OS24. In analysis agnostic to the number of hits in TP55, $TP53^{NMIS}$ did not impact OS24. However, among individuals with single *TP53* mutations, $TP53^{NMIS}$ predicted significantly inferior median survival (4.0 vs. 9.2 mos.; $P_{Log\text{-}rank} = 0.037$) with early mortality. Among $TP53^{NMIS}$, splice junction mutations were associated with particularly poor outcomes (HR = 1.9 [1.2–3.1]; $P = 0.007$) (Fig. 3C). Neither underlying germline alterations ($P = 0.38$) nor $TP53^{MH}$ allelic state ($P = 0.09$) conferred worse outcomes.

While all *TP53* VAF cutoffs (>10%, >25%, >40%, and >50%) predicted inferior OS24, we selected VAF > 25% (HR = 1.8 [1.2–2.6]; $P = 0.002$) for all subsequent analysis based on the balance of cases across both groups for an appropriately powered analysis while also assessing the Youden index that reflected maximizing separation (Fig. 3D). Intriguingly, the beneficial impact of a lower VAF within each of these binary cut-point was restricted only to male gender when examined separately by gender (Supplementary Fig. 2) although the analysis was slightly underpowered.

CUX1 *deletions and* EPI6 *signature including* CUX1 *are both adverse*. The presence of any co-alteration occurring in 149/242 (61.6%) did not impact survival ($P = 0.50$). *CUX1* alterations (mostly losses detected by NGS) were associated with particular very poor survival (1.2% vs. 15.8%; $P_{fpm} < 0.001$) without any survivor beyond 12 months of diagnosis (Supplementary Fig. 3). Looking at combinations of co-altered genes, co-alterations in myelodysplasia-related genes ($P = 0.54$) or spliceosome complex genes did not impact survival ($P = 0.87$). In an age-adjusted model, *EPI6* predicted significantly inferior OS24 (3.4% vs. 18.7% for no *EPI6*; $P_{fpm} < 0.0001$; HR = 2.0 [1.5–2.8]; $P < 0.0001$ Fig. 4) with a differential impact of each of *EPI6* genes when stratified by gender (Fig. 4A).

Venetoclax-based regimens do not offer significant survival benefit. Using low-intensity therapies as a referent group, patients receiving intensive regimens (7 + 3 or HiDAC-based) experienced slightly better outcomes in an age-adjusted analysis (HR = 0.7 [0.5–1.1]; $P = 0.10$; $N = 200$) but significantly better in the unadjusted OS24 (27.5% vs. 13.1% in low-intensity; $P_{fpm} = 0.021$). See Supplementary Fig. 4 for outcomes by major therapy classes. Among patients treated with intensive chemotherapy (excluding patients treated with CPX-351), VAF > 25% (HR = 3.3 [1.3–8.5]; $P = 0.016$; $N = 52$) and EAp53 score (analyzing only those who had a $TP53^{MIS}$ mutation) in the highest quartile (HR = 2.1 [1.0–4.6]; $P = 0.055$; $N = 39$) were both adverse. In a subgroup analysis by VAF, the beneficial effect of intensive regimens over non-intensive regimens was restricted only to individuals with VAF ≤ 25%.

Among HMA-treated individuals, HMA+venetoclax was marginally adverse in patients older than 70 years (HR = 1.4 [0.8–2.5]; $P = 0.24$; $N = 76$) with no OS benefit compared to HMA alone, in line with recent data [21]. Within this subgroup ($N = 144$), only antecedent treated myeloid neoplasm (HR = 1.8 [1.1–2.8]; $P = 0.016$; $N = 144$), $TP53^{sjm}$ (HR = 2.0 [1.0–3.9]; $P = 0.044$; $N = 144$) and *EPI6* predicted poor outcomes (HR = 2.0 [1.0–3.9]; $P = 0.044$; $N = 144$). *TP53* allelic status, *TP53* VAF, monosomies and EAp53 score (Supplementary Fig. 5) were not prognostic in this subgroup (Supplementary Fig. 6). Achieving CR1 to first-line therapy significantly improved outcomes (OS24 40.6% vs. 8.0% for

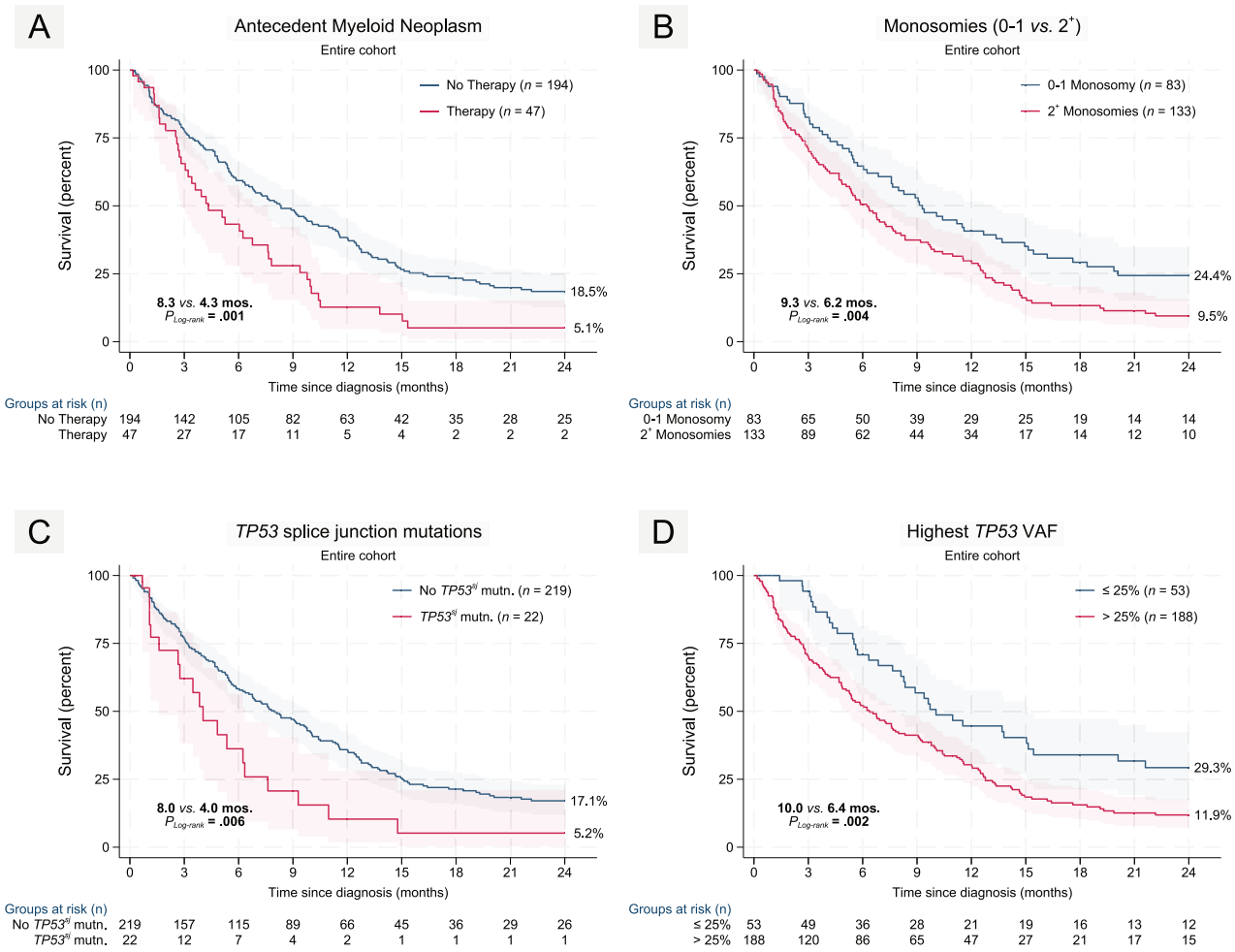

Fig. 3 Univariable K-M plots of key pre-therapy adverse predictors. A Antecedent myeloid neoplasm, **B** Autosomal monosomies and **D**. Highest *TP53* VAF ≥ 25%, all adversely impact overall survival (OS24). **C** *TP53* splice junction mutations were significantly associated with male gender and were associated with worse survival compared to all other classes of *TP53* mutations. Patients with antecedent treated myeloid neoplasm included individuals receiving HMA as well as supportive care (transfusions).

no CR1; $P < 0.0001$) overall and within treatment subgroups of intensively treated as well as HMA treated individuals (OS24 33.2% vs. 4.2% for HMA + /-Ven subgroup; $P < 0.0001$).

Allogeneic stem cell transplant, ongoing CR at day 100 post-alloSCT and chronic GVHD are favorable. A total of 35 (14.5%) patients underwent alloSCT including nearly 50% of those who had achieved CR1 to prior first-line therapy. Patients with monosomy 7, *TP53* VAF > 25% and $TP53^{MH}$ at diagnosis were significantly less likely to undergo alloSCT ($P = 0.036$, $P = 0.018$, $P = 0.042$ respectively). Transplanted individuals enjoyed significantly superior median survival (Not reached vs. 5.6 mos. for no alloSCT; $P_{Log\text{-}rank} < 0.0001$) and OS24 (59.8% vs. 5.5% for no alloSCT; $P_{fpm} < .0001$). Likewise, among transplanted individuals, ongoing CR at day +100 post-alloSCT was significantly associated with better OS24 (63.5% *vs.* 39.6% for no CR; $P_{fpm} = .09$), in congruence with results from prior work demonstrating significant beneficial impact of alloSCT in $TP53^{MUT}$ MNs [10]. Early relapse by day+100 was significantly more likely in patients harboring *EPI6* signature ($P = .012$), *CUX1* alterations ($P = .045$) or high EAp53 score ($P = .08$) at diagnosis.

Among 31 patients evaluated for GVHD, 51.6% (16/31) experienced acute GVHD comprising 3/15, 11/15, 1/15 with grades 1, 2 and 3 respectively with a marginal survival benefit with any acute GVHD. A total of 50.0% (17/34) developed chronic GVHD comprising 12/17 and 2/17 with moderate and severe cGVHD respectively. Moderate-severe cGVHD was associated with significant OS benefit (see Supplementary Fig. 7 for 100-day post allo-SCT landmark analysis of OS).

TP53 Risk Score (*TP53*RS) for $TP53^{MUT}$ myeloid neoplasms for patients treated with HMA-based therapies

Multivariable model for entire cohort. Based on the predictors relevant in univariable analysis, a multivariable model for the entire cohort was constructed using 5 predictors including antecedent treated myeloid neoplasm, *TP53* VAF, $TP53^{sjm}$, monosomies and *EPI6* signature. The models include regular Cox, 45-day landmark Cox, and a competing risk model modeling only leukemia-specific deaths in the competing risk model (See Table 2 and Fig. 5A for details).

Multivariable model and Risk score for HMA-treated subgroup. Since HMA-based therapies are the most predominant frontline choice in this cohort, we developed a separate risk score for this subgroup excluding *TP53* VAF and monosomies (which were relevant only in intensively-treated subgroups as noted earlier) (See Table 3 for details).

This *TP53* risk score (*TP53*RS) for HMA-treated individuals delineated three risk groups comprising intermediate (0 factors, *TP53*RS0), intermediate-poor (1 factor, *TP53*RS1), and poor (2+ factors, *TP53*RS2) risk groups with significantly different median survival (12.8 vs. 6.0 vs. 4.3 mos.; $P_{Log\text{-}rank} < 0.001$) as well as 24-

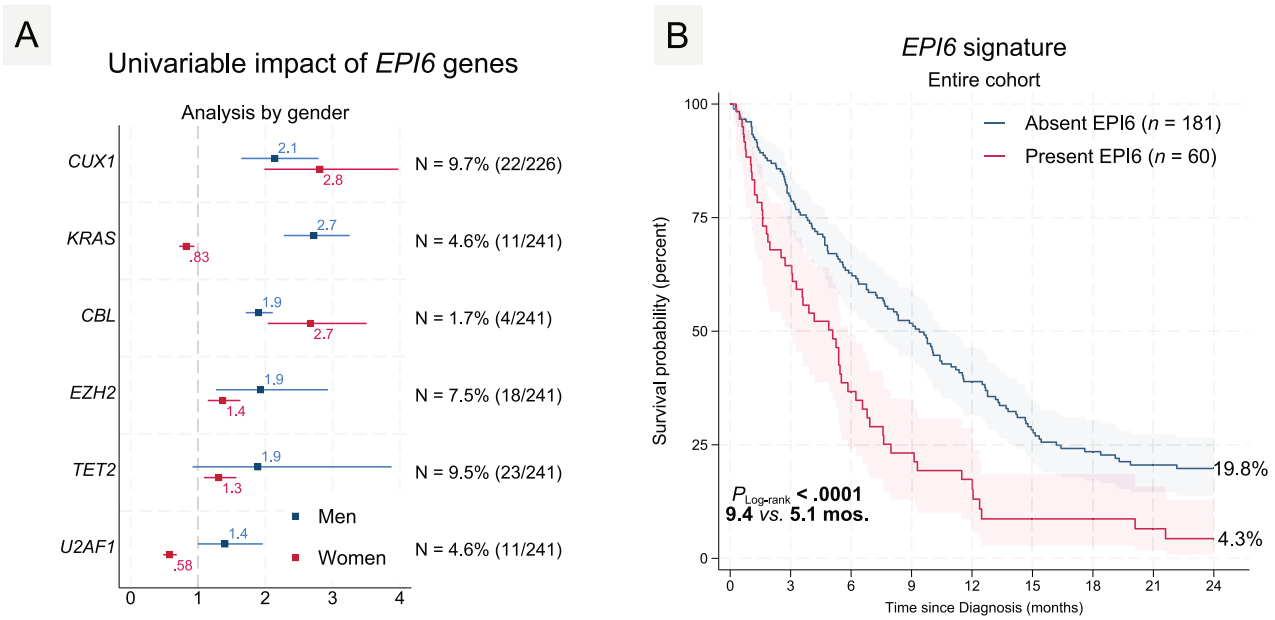

Fig. 4 Univariable adverse impact of *EPI6* genes and outcome. A Forest plot of the hazard ratios of each of the six "*EPI6*" genes in the OS24 analysis with numbers and frequencies of occurrence depicted on the right. There were gender specific differences in some of the genes and hence these genes were included in the *EPI6* signature despite borderline adverse significance in the combined analysis. **B** Patients carrying co-mutations/alterations in any of the 6 "*EPI6*" genes including *CBL*, *CUX1*, *EZH2*, *TET2*, *KRAS*, or *U2AF1* experienced shorter median and overall survival. *CUX1* was the most influential gene in the *EPI6* signature with overall survival of *CUX1* altered individuals approaching 0% by 12 months after diagnosis.

Table 2. Multivariable models fitted incorporating the four relevant pretherapy predictors adjusted for age at diagnosis and a clustered sandwich estimator for standard errors accounting for clustering across contributing centers.

	Cox		45-day landmark		Compet. Risk	
	HR	**95% CI**	**HR**	**95% CI**	**SHR**	**95% CI**
Age at Dx.						
≤70 yrs	1.00		1.00		1.00	
>70 yrs	1.34**	[1.05, 1.70]	1.44***	[1.16, 1.79]	1.22	[0.95, 1.57]
Anteced. Treated MN						
No	1.00		1.00		1.00	
Yes	1.72*	[0.92, 3.21]	1.95*	[0.88, 4.28]	1.48	[0.80, 2.73]
TP53 Splice Mutn.						
Absent	1.00		1.00		1.00	
Present	1.73	[0.90, 3.33]	1.70	[0.88, 3.30]	1.79	[0.81, 3.97]
Highest *TP53* VAF						
≤25%	1.00		1.00		1.00	
>25%	1.70***	[1.33, 2.16]	1.55***	[1.29, 1.86]	1.33**	[1.05, 1.68]
Monosomies						
0–1 monosomy	1.00		1.00		1.00	
2+ monosomies	1.62***	[1.23, 2.15]	1.61***	[1.19, 2.19]	1.31	[0.85, 2.02]
EPI6 signature						
Absent *EPI6*	1.00		1.00		1.00	
Present *EPI6*	1.96***	[1.45, 2.64]	1.90***	[1.54, 2.34]	1.48***	[1.20, 1.82]

Only TP53 VAF and EPI6 remained relevant in all three models with monosomies retaining relevance in the regular and 45-day landmark models while TP53sjm and antecedent treated myeloid neoplasm were less relevant although adverse in multivariable analysis too despite not being statistically significant. Importantly EPI6 had independent prognostic value beyond all predictors in the multivariable model.
Hazard ratio (HR) for competing risk model denotes Sub-Hazard Ratio.
***$p < 0.01$, **$p < 0.05$, *$p < 0.1$.

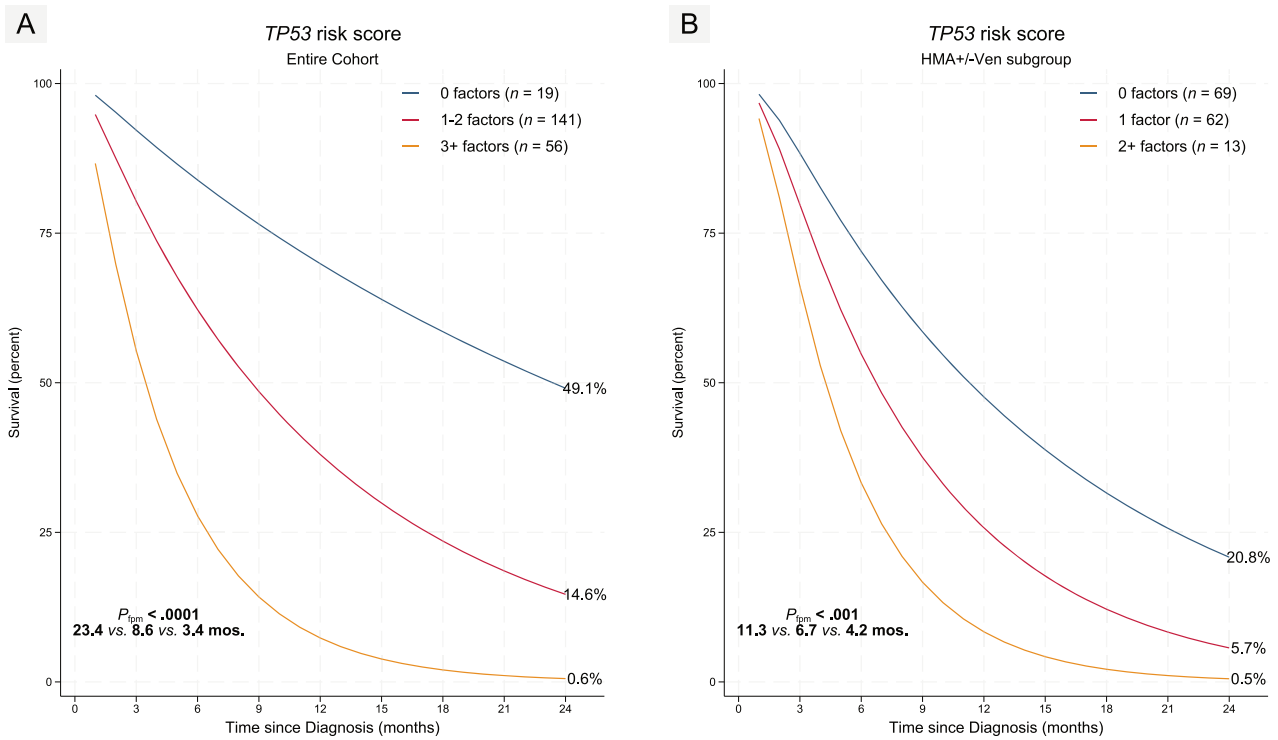

Fig. 5 Impact of *TP53* risk score with relevant predictors on OS24 by flexible parametric analysis in entire cohort and HMA subgroup. **A** Entire cohort analysis utilizing *TP53* VAF and monosomies in addition to antecedent treated myeloid neoplasm, $TP53^{sjm}$ and *EPI6* separates three prognostic groups corresponding to risk score cutoffs of 0 factors, 1–2 factors, and 3+ factors. **B** *TP53* risk score for HMA-treated patients using parsimonious 3-parameter model in sensitivity analysis agnostic to monosomies and VAF information.

Table 3. Multivariable (Cox, 45-day landmark Cox and competing risk) subgroup analysis in HMA+/-Ven treated individuals including only three relevant baseline predictors adjusted for age at diagnosis.

	Cox		45-day landmark		Compet. Risk	
	HR	**95% CI**	**HR**	**95% CI**	**SHR**	**95% CI**
Age at Dx.						
≤70 yrs	1.00		1.00		1.00	
>70 yrs	1.35***	[1.15, 1.59]	1.43***	[1.14, 1.78]	1.26**	[1.00, 1.57]
Anteced. Treated MN						
No	1.00		1.00		1.00	
Yes	1.74**	[1.14, 2.65]	2.10**	[1.16, 3.82]	1.55*	[0.94, 2.56]
TP53 Splice Mutn.						
Absent	1.00		1.00		1.00	
Present	1.97**	[1.06, 3.68]	2.07**	[1.02, 4.19]	1.94	[0.81, 4.65]
EPI6 signature						
Absent *EPI6*	1.00		1.00		1.00	
Present *EPI6*	1.83***	[1.47, 2.29]	1.80***	[1.52, 2.13]	1.50***	[1.24, 1.81]

These three (antecedent treated myeloid neoplasm, TP53sjm and EPI6) were used in development of the TP53RS for this group of individuals.
Hazard ratio (HR) for competing risk model denotes Sub-Hazard Ratio.
***$p < 0.01$, **$p < 0.05$, *$p < 0.1$.

month survival (20.9% vs. 5.7% vs. 0.5%; $P_{fpm} < 0.0001$) respectively (Fig. 5B). Patients in the poor risk group (2+ factors) were marginally older than 70 years of age (76.9% vs. 50.4%; $P = 0.07$) with none of these patients receiving an alloSCT.

Sensitivity analysis excluding low TP53 *VAF patients*. Since inclusion of cases with *TP53* VAF < 10% could potentially inflate the impact of the proposed score, we performed an additional sensitivity analysis excluding 17 (7.0%) such individuals. *TP53*RS retained its prognostic value in this last analysis as well (HR = 1.8 [1.3–2.5]; $P < .001$; $N = 133$).

DISCUSSION

While most prior studies focused on comparing the impact of pretherapy predictors (viz. any *TP53* mutations or *TP53* VAF) in

myeloid neoplasms across all risk strata, our cohort is remarkable for being the first to look within just the ELN 2022 high-risk subgroup. We identified several novel factors that reliably predict poor response to first-line therapy (monosomal karyotype, multi-hit *TP53* allelic state, *CUX1* deletions) and inferior survival including a novel *EPI6* co-alteration signature including *CUX1*. We specifically focused on developing a model/risk score solely with pre-therapy predictors rather than well-known therapy-related factors (such as CR1 and transplant).

While the proportion of patients treated with chemotherapy was still small in our cohort, it is remarkable that *TP53* allelic state, *TP53* VAF and monosomies were all predictive for survival only in the intensively treated subgroup of patients. While the 25% *TP53* VAF cutoff in our study was admittedly data-driven, it was nevertheless in line with the 23% cutoff proposed by Bahaj and coworkers [22] as well as the 25% cutoff used by Grob and coworkers [5] although this latter study was an exclusively chemotherapy-treated, and slightly younger cohort. In comparing the impact of VAF by treatment subgroups, we note as well that *TP53* VAF is relevant only within the intensive chemotherapy treated subgroups, in line with prior studies [17, 23]. We were however surprised by the higher CR rates in females possibly attributable to less frequent $TP53^{MH}$ in females. Whether additional fitness-related factors or better compliance in females play a role remains to be seen.

While *TP53* allelic state did not impact overall survival, it nevertheless remains useful in assessing response to front-line therapy (more so in patients receiving non-intensive therapies). Looking at the different *TP53* mutation classes, patients with any $TP53^{NMIS}$ mutation face significant early mortality. Two prior studies [24, 25] evaluating MDS demonstrated worse OS with $TP53^{NMIS}$ mutations compared to $TP53^{MIS}$ with the latter looking at post-transplant survival. However, an AML study from the German-Austrian AMLSG did not find any impact on OS based on mutation class (but with a trend towards worse event-free survival with $TP53^{NMIS}$) in a cohort treated primarily with intensive chemotherapy [26]. In our cohort, *TP53* splice junction mutations were associated with the worst outcomes compared to all other mutations. While they occurred much more frequently in men without any impact on CR1 rates, their adverse impact on survival was much more pronounced in women. These findings are congruent with recent demonstration of gender specific differences in the frequencies of AML-associated genetic alterations and less frequent complex karyotype and mutation in genes related to the spliceosome machinery in women (including *U2AF1* and *SRSF2*) [27].

On the other hand, the predictive relevance of autosomal monosomies is quite remarkable and admittedly somewhat surprising in our high-risk cohort with complex karyotype. While it has long been known that monosomal karyotype is adverse and often enriched in complex karyotype AMLs [4, 28] with frequent del(17p) [29], these cohorts comprised a heterogeneous mix of AML patients of all risk groups without specifically focusing on *TP53*-mutated individuals. Considering the high frequency of complex karyotype in our cohort, we restricted the analysis to monosomies only while ignoring structural alterations which were near-universal. Importantly, monosomy 7 was more relevant than monosomy involving chromosomes 5 or 17. We observed that the predictive value of 2+ monosomies for inferior CR1 was restricted only to the subgroup receiving non-intensive therapies underscoring its relevance in an increasingly HMA-treated elderly cohort. However, neither monosomies, nor *TP53* VAF nor allelic state impacted overall survival in the HMA-treated subgroup.

In the context of the *EPI6* signature, *CUX1* emerged as the most pivotal gene. While previous studies [30, 31] have highlighted the detrimental effects of *CUX1* deletions and mutations in AML, their significance in $TP53^{MUT}$ MN has not been evaluated. Notably, in our cohort, *CUX1* losses (which were more prevalent than mutations) were observed in 10% of cases. These losses identified a subgroup with an abysmal response rate (0%) to frontline treatment with significantly worse 2-year survival (Fig. 4A) particularly among females. It is important to emphasize that while *CUX1* alterations frequently co-occurred with losses of chromosome 7/7q [32], isolated losses at this genomic locus were still associated with adverse clinical outcomes, underscoring their independent relevance beyond karyotypically detectable -7/7q. Our observations of *RAS* pathway co-alterations (*CBL* and *KRAS*) in the *EPI6* signature aligns with the documented activation of this pathway in *CUX1*-altered MNs, as recently demonstrated in the context of 7q alterations in myeloid neoplasms [33]. Consistent with a previous study by Badar and coworkers which identified a significant association between *TP53* mutations and the Q157 variant in *U2AF1*-mutated MNs, our cohort also exhibited a marked predominance of the *U2AF1* Q157 variant compared to the S34F variant [34].

We note as well that blast counts at diagnosis are irrelevant once there are more than 9% blasts, affirming that MDS/AML and AML with $TP53^{MUT}$ are indeed a single biologic entity. That said, establishing a diagnosis of morphologic CR or morphologic leukemia-free state (MLFS) based on blast counts after therapy however is particularly problematic in $TP53^{MUT}$ MN where significant erythroid-predominant leukemic hematopoiesis (often CD34-/strong p53 +) is frequently observed. Post-therapy marrow biopsies frequently show less than 5% CD34-expressing blasts with a significant component of strong p53-expressing erythroid component (frequently corresponding to an underlying *TP53* mutation) highlighting that neither blast count nor CD34 are good surrogates for pathologic CR or MLFS in $TP53^{MUT}$ MNs despite significant cytoreduction. To this end, the fast turnaround time of IHC compared to all the other tests (NGS and karyotype) makes it an attractive surrogate for most if not all non-truncating *TP53* mutations [35], particularly in elderly and therapy-related AMLs at diagnosis and after therapy.

Two major limitations of our study include 1] lack of molecular MRD in response assessment and 2] lack of an external validation cohort. To the second point, most published AML and MDS cohort lack public data on alterations (especially losses) at the *CUX1* locus hampering their use as a validation cohort for the *EPI6* signature. Furthermore, prior studies included higher proportions of intensively treated patients limiting their use as a representative validation cohort even if there was available NGS data. Lastly, we were unable to ascertain if some of the observed *TP53* mutations in patients with low VAF merely corresponded to age-related clonal hematopoiesis-associated mutations unrelated to the main clone (see Supplementary Fig. 8 for analysis of the missense *TP53* substitutions and *TP53* domain relevance in the University of Chicago cohort). Importantly, our work underscores that not all classes of *TP53* mutations are equal given the adverse outcomes with splice junction mutations and early mortality with any $TP53^{NMIS}$ mutations.

In the real-world setting, all candidate components of the proposed risk score (NGS, FISH, karyotype) are typically available within two weeks of diagnosis. Furthermore, most candidate genes of *EPI6* are common genes included in standard NGS panels, except perhaps *CUX1*, for which we recommend an algorithm in the NGS pipeline for calling *CUX1* loss detection. Among HMA-treated individuals, patients in intermediate/*TP53*RS0 risk group with 0−1 monosomies enjoy a 63% CR1 (vs. 16% CR1 with 2+ monosomies; $P < 0.001$) and are most likely to respond significantly to frontline therapies and have the best chance at alloSCT with durable post-transplant survival. However, most other patients in intermediate-poor/*TP53*RS1 and poor/*TP53*RS2 groups still face early mortality due to comorbidities, active second malignancies or treatment-emergent adverse effects including neutropenic sepsis. As a result, these latter groups derive little to no benefit with existing therapies, highlighting the need for the developing novel agents with better safety and efficacy profiles for these subgroups.

In conclusion, the several novel proposed pre-therapy biologic predictors (*CUX1* deletions, allelic state, *TP53* splice junction mutations, *TP53* VAF, autosomal monosomies, and specific co-mutation patterns) as well as antecedent treatment (HMA exposure) for a myeloid neoplasm have differential prognostic utility depending on treatment subgroups (intensive vs. HMA-based). These data will better inform frontline response and aid in identifying the best candidates for alloSCT in this high-risk, elderly cohort.

DATA AVAILABILITY

Please contact corresponding author Girish Venkataraman for with any data requests. Upon reasonable requests appropriate de-identified data frames can be shared.

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AUTHOR CONTRIBUTIONS

AK and AER performed chart review, curated mutations and edited manuscript. PMS and KK performed chart review. ES performed chart review and collated scripts for

Figures and manuscript. HVS. Visiting pathologist curated data from IARC and EAP53 server. SYY collated all *TP53* mutations for lollipop plot generation, BA, MS, JLP, ABP and MPM contributed cases, did chart review and mutation curation of contributed cases. MT, JS and PW curated mutation data and examined pipeline for NGS data. AL and CF reviewed cytogenetic data. AP, MWD, CS and JEC, TK, provided patient care, accrued patients on clinical trials (HMA/Magrolimab, Gilead), designed some analyses, and edited the manuscript. MEM reviewed the data related to *CUX1* biology and edited those portions of the manuscript. LW, EH, DA, JXC, SG, SBG, and GV reviewed pathology data and edited final version of manuscript. OL and PK analyzed the *TP53* missense mutations in the University of Chicago subset reported in the supplementary data. PW and PW analyzed the CNA data. GV initiated, designed, supervised study, conducted all statistical analysis, & wrote the manuscript. All authors edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Waiver of consent for retrospective chart review per University of Chicago Institutional Review Board (IRB20-1250)

ADDITIONAL INFORMATION

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