haematologica
Journal of the Ferrata Storti Foundation

Evolution to complex karyotype disease in mono-allelic *TP53*-mutated myelodysplastic syndrome/acute myeloid leukemia: a cohort study

by Priyanka Fernandes, Sergiu Pasca, Tania Jain, Mark J. Levis, Alexander J. Ambinder, Laura Walsh, Ivana Gojo, Douglas B. Smith, Jonathan Webster, Gabriel Ghiaur, W. Brian Dalton, Gabrielle Prince, Richard J. Jones, Amy E. DeZern, Lukasz P. Gondek and Theodoros Karantanos

Received: February 15, 2026.
Accepted: June 3, 2026.

Citation: Priyanka Fernandes, Sergiu Pasca, Tania Jain, Mark J. Levis, Alexander J. Ambinder, Laura Walsh, Ivana Gojo, Douglas B. Smith, Jonathan Webster, Gabriel Ghiaur, W. Brian Dalton, Gabrielle Prince, Richard J. Jones, Amy E. DeZern, Lukasz P. Gondek and Theodoros Karantanos. Evolution to complex karyotype disease in mono-allelic TP53-mutated myelodysplastic syndrome/acute myeloid leukemia: a cohort study.
Haematologica. 2026 June 11. doi: 10.3324/haematol.2026.300734 [Epub ahead of print]

Evolution to complex karyotype disease in mono-allelic *TP53*-mutated myelodysplastic syndrome/acute myeloid leukemia: a cohort study

Priyanka Fernandes[1]*, Sergiu Pasca[1]*, Tania Jain[1], Mark J. Levis[1], Alexander J. Ambinder[1], Laura Walsh[1], Ivana Gojo[1], Douglas B. Smith[1], Jonathan Webster[1], Gabriel Ghiaur[1], W. Brian Dalton[1], Gabrielle Prince[1], Richard J. Jones[1], Amy E. DeZern[1], Lukasz P. Gondek[1]±, Theodoros Karantanos[1]±

* equally contributed first authors

± equally contributed senior authors

1) Division of Hematologic Malignancies and Bone Marrow Transplantation, Department of Medical Oncology, Johns Hopkins University, Baltimore, MD, USA

Corresponding Author: Theodoros Karantanos, Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, 1650 Orleans Street, Baltimore, MD 21287. E-mail: tkarant1@jhmi.edu

Disclosures: The authors declare no relevant conflict of interest

Contributions: PF, SP, LPG and TK conceived the study, performed retrospective data collection and statistical analysis, wrote and revised the manuscript. TJ, MJL, AJA, LW, IG, DBS, JW, GG, WBD, GP, RJJ, AED performed retrospective data collection, edited and revised the manuscript.

Data sharing statement: De-identified data are available upon request to the corresponding author

Mutations in the tumor protein 53 (*TP53*) gene occur in 8-12% of individuals with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), and this percentage increases to 30-40% within those with therapy-related neoplasms[1]. *TP53* mutations or deletions are associated with worse response to both intensive chemotherapy[1] and venetoclax-based therapies[2], as well as shorter relapse-free survival following allogeneic bone marrow transplantation (alloBMT)[3,4]. Numerous studies have highlighted that individuals with multi-hit *TP53* mutations resulting in loss-of-function and complex karyotype (CK) have worse outcomes compared to those with mono-allelic mutations and non-CK[5,6]. Neoplasms with mono-allelic *TP53* mutations can evolve to multi-hit *TP53* mutated disease with CK typically via loss of the wild-type allele which provides survival advantage to mutated clones[5]. However, while the natural history of multi-hit *TP53* mutated MDS/AML with CK is well described there are relatively fewer studies describing the long-term outcomes of patients with mono-allelic *TP53*-mutated MDS/AML[5]. As a result, the incidence of evolution to CK disease among MDS/AML patients with mono-allelic *TP53* mutations and the contributing risk factors are largely unknown. Here, we analyzed a retrospective cohort of 25 consecutive patients with MDS/AML harboring mono-allelic *TP53* mutations treated at a single center and assessed the outcome, rate of complete TP53 loss-of function associated with an acquisition of CK and therapeutic modalities associated with this process. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Johns Hopkins Institutional Review Board (IRB00228638).

For this study, a mono-allelic *TP53*-mutated disease was defined as a single *TP53* mutations with variant allele frequency (VAF)<40% and the absence of CK and alterations in 17/17p. Even though a definitive exclusion of small subclones with multiple mutations or 17p LOH was not possible, this combination minimized the risk of including patient with frank biallelic

mutation or 17p LOH[7,8]. Conversely, multi-hit *TP53*-mutated disease was defined as CK and/or VAF> 40%, as studies suggest that this profile significantly increases the possibility of a completed loss-of-function via biallelic mutations or mutation with 17p LOH [7,8]. Since bulk NGS analysis could not differentiate between biallelic mutations and multiple clones with single mutation, those with two *TP53* mutations were ultimately excluded from the analysis.

A total of 282 individuals with MDS/AML with *TP53* mutations evaluated at Johns Hopkins University Hospital between 1/1/2014 and 2/1/2025 were identified. All mutations were assessed from clinical bulk DNA sequencing. All the *TP53* mutations were reported as somatic based on their presence in COSMIC database and variant allele frequency. Among those, 209 (74.1%) were found to have CK with or without 17/17p alterations at diagnosis. Out of the remaining 73, 48 (65.7%) had *TP53* mutation with VAF ≥40% suggesting deletion copy-neutral loss-of-heterozygosity of 17p , or they had two or more *TP53* mutations[9] (**Supplementary Figure 1A**). As a result, 25 patients with MDS/AML and likely mono-allelic *TP53* mutations (sole mutation with VAF <40% and non-CK) were identified (**Supplementary Figure 1A**). The median (range) age at diagnosis was 65.4 years (23.3 – 88.2 years) and 13 patients (52%) were females. Seventeen patients (68%) had MDS at diagnosis, and 8 (32%) had AML. Importantly, 8 individuals (32%) had history of cytotoxic therapy prior to diagnosis. Most patients had at least one mutation in a non-*TP53* gene (**Figure 1A**). All *TP53* mutations were limited to the DNA-binding domain (**Supplementary Figure 1B)**. The baseline characteristics of the patients and their first- and second-line therapies are shown in **Supplementary Table 1**. Twelve (48%) patients received genotoxic therapy during their treatment including 4 (16%) as front-line therapy, 3 as a salvage genotoxic therapy, 1 (4%) as salvage therapy alone and 7 (25%) as transplant conditioning only.

The median (range) follow up was 19.6 months (5.4 – 118 months). Among the patients with available response data (21 patients), 13 patients (61.9%) achieved complete remission with or without complete count recovery (CR/CRi) or partial remission (PR) and 8 patients (38.1%) had stable or progressive disease (SD/PD). Eighteen patients had longitudinal assessment of their *TP53* mutational burden at diagnosis and following first-line therapy. Eight patients (44.4%) had >50% reduction of *TP53* VAF. Out of the 8 patients who received second line therapy, 6 (75%) achieved a CR/CRi/PR. In total, 10 individuals (40%) underwent alloBMT. Seven (70%) in CR1 and 3 (30%) in CR2 or later. Six patients (24%) had disease relapse following remission (3 patients, 50%) or alloBMT (3 patients, 50%). The overall survival (OS) at 5 years was 32.8% for the entire cohort (**Figure 1B**), 31.2% in AML and 35.7% in MDS patients (**Supplementary Figure 1C**).

In total 5 patients (20%) developed CK. Among them, 3 (60%) had AML and 2 (40%) had MDS. Three of these patients (60%) were found to have CK disease at relapse following alloBMT and the other 2 (40%) were found to have CK disease following first line therapy. Notably, these patients accounted for 83% (5 of 6) of the relapsed individuals. The characteristics of the cohort of patients who developed CK are shown in **Table 1**. Patients who developed CK disease had significantly inferior overall survival (HR=13.6; 95% CI 3.5-51.9, $p < 0.001$) (**Figure 1C**).

The *TP53* VAF at diagnosis and history of genotoxic therapy were evaluated as factors possibly contributing to the evolution of mono-allelic *TP53* mutated MDS/AML to CK disease. *TP53* VAF of 10% was used as a cut-off point based on recent data supporting that *TP53* VAF >10% is associated with significantly worse outcomes among patients with MDS and treatment-related myeloid neoplasms[10,11]. Patients with a *TP53* VAF > 10% at diagnosis had a higher incidence of CK disease, although the difference was not statistically significant ($p = 0.08$) (**Figure 1D**). Four out of 5 individuals (80%) who developed CK received genotoxic therapy following

their initial diagnosis either as part of their induction therapy (7+3, 50%), or conditioning regimen (50%). In total 4/12 (33.3%) patients who received genotoxic therapy developed CK disease compared to 1/13 (7.7%) among those who did not receive genotoxic therapy (**Supplementary Figure 1D**). Time-dependent survival analysis of patients who received treatment other than supportive care (n = 22) showed that recipients of genotoxic therapy had increased incidence of CK disease (HR=9.9; 95% CI 1.0-101.0, p = 0.05) (**Figure 1E**).

The karyotype for each of the 5 patients who developed CK disease had at least 7 abnormalities at the time of evolution, and 3/5 (60%) demonstrated loss of 17/17p. The remaining 2 patients had an increase in their *TP53* mutational burden to >50% VAF indicating a copy neutral loss of heterozygosity (CN-LOH) or microdeletion of the wild-type allele. In most of these patients, the *TP53* mutated clone expanded during progression to CK (**Figure 2**).

In this retrospective analysis of 25 patients with a single *TP53* mutation and no evidence of CK or 17/17p alteration MDS/AML, we observed that clonal evolution to CK after genotoxic therapy is common affecting 20% of patients across the entire cohort, 33% of individuals who received genotoxic therapy and 83% of relapsed patients and is associated with significantly poorer survival. Patients with *TP53* VAF >10% appear to be at a higher risk, particularly when treated with genotoxic chemotherapy (80% of progression to CK) which is consistent with prior studies in patients with MDS[10] and therapy-related myeloid neoplasms[11]. Moreover, we demonstrated that individuals who received genotoxic therapy as part of their treatment are at a significantly higher risk of progressing to CK disease.

Of note, 83% percent of relapsed patients in our cohort developed CK disease with a concurrent 17p LOH. The most plausible explanation is therapy-induced random loss of 17p, which in persons with heterozygous *TP53* mutation leads to a loss of WT allele, and thus the p53 function[12,13]. The

clones without functional p53, avert apoptosis despite numerous structural chromosomal defects resulting in CK[5,12]. Of note, none of the patients who progressed to CK disease acquired new *TP53* mutations suggesting that loss of the wild-type allele under genotoxic stress is the most common mechanism. The fact that the incidence of CK disease was higher among patients who received genotoxic therapy either as first or second line therapy or as part of their conditioning supports the hypothesis that genotoxic therapy may induce evolution of *TP53*-mutated clones via WT allele loss.

Our study has several limitations. First, despite screening 282 patients with *TP53*-mutated MDS/AML, we identified only 25 MDS/AML patients with likely mono-allelic mutations and without evidence of loss-of-*TP53* function based on their chromosomal findings. The small size of our cohort precludes comparative statistical analyses. Moreover, our cohort was heterogeneous with regards to disease biology including patients with various blast percentages. Additionally, since *TP53* mutations were assessed from bulk DNA sequencing data and single-cell sequencing was not performed, we have based the distinction between mono-allelic and multi-hit *TP53* mutated cases solely on VAF and absence of CK or 17/17p deletions. Cases with two mutations were excluded to reduce the risk of including cases with loss-of-function of *TP53*. As a result, confirmation of mono-allelic status of *TP53* mutations at the single cell level is missing from our analysis.

In conclusion, our study demonstrates that patients with single *TP53* mutation remain at considerable risk of progressing to CK disease particularly with VAF >10% when treated with genotoxic agents. Thus, non-genotoxic regimens may be a preferable choice for these individuals. Further investigation in larger prospective cohorts is warranted to better define this risk and the

contributing factors and sequencing at single cell level is required to better understand the underlying biologic mechanisms.

REFERENCES

1. 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.

2. Döhner H, Pratz KW, DiNardo CD, et al. Genetic risk stratification and outcomes among treatment-naive patients with AML treated with venetoclax and azacitidine. Blood. 2024;144(21):2211-2222.

3. Lindsley RC, Saber W, Mar BG, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. N Engl J Med. 2017;376(6):536-547.

4. Sinanidis I, Hochman MJ, Tsai HL, et al. Favorable outcomes in MDS and oligoblastic AML-MR after reduced-intensity conditioning allogeneic bone marrow transplantation with post-transplantation cyclophosphamide. Bone Marrow Transplant. 2024;59(8):1178-1180.

5. 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.

6. Pasca S, Haldar SD, Ambinder A, et al. Outcome heterogeneity of TP53-mutated myeloid neoplasms and the role of allogeneic hematopoietic cell transplantation. Haematologica. 2024;109(3):948-952.

7. Badar T, Nanaa A, Atallah E, et al. 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. Haematologica. 2024;109(11):3533-3542.

8. Sallman D, 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. Short NJ, Montalban-Bravo G, Hwang H, et al. Prognostic and therapeutic impacts of mutant TP53 variant allelic frequency in newly diagnosed acute myeloid leukemia. Blood Adv. 2020;4(22):5681-5689.

10. Montalban-Bravo G, Kanagal-Shamanna R, Benton CB, et al. Genomic context and TP53 allele frequency define clinical outcomes in TP53-mutated myelodysplastic syndromes. Blood Adv. 2020;4(3):482-495.

11. Shah MV, Tran ENH, Shah S, et al. TP53 mutation variant allele frequency of ≥10% is associated with poor prognosis in therapy-related myeloid neoplasms. Blood Cancer J. 2023;13(1):51.

12. Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol. 2001;19(5):1405-1413.

13. Jasek M, Gondek LP, Bejanyan N, et al. TP53 mutations in myeloid malignancies are either homozygous or hemizygous due to copy number-neutral loss of heterozygosity or deletion of 17p. Leukemia. 2010;24(1):216-219.

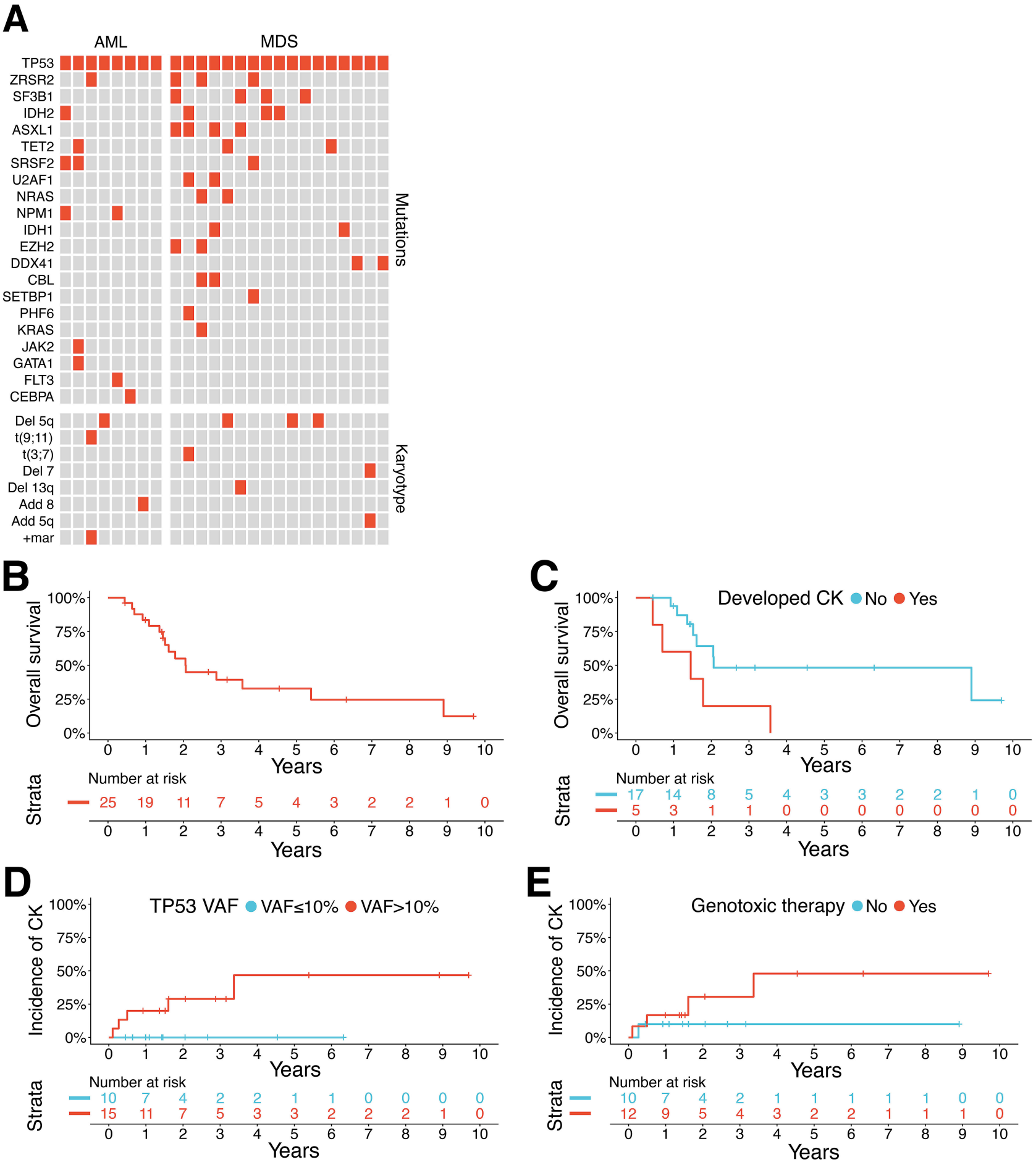
Table 1. Baseline characteristics of patients who progressed to CK *TP53*-mutated AML/MDS

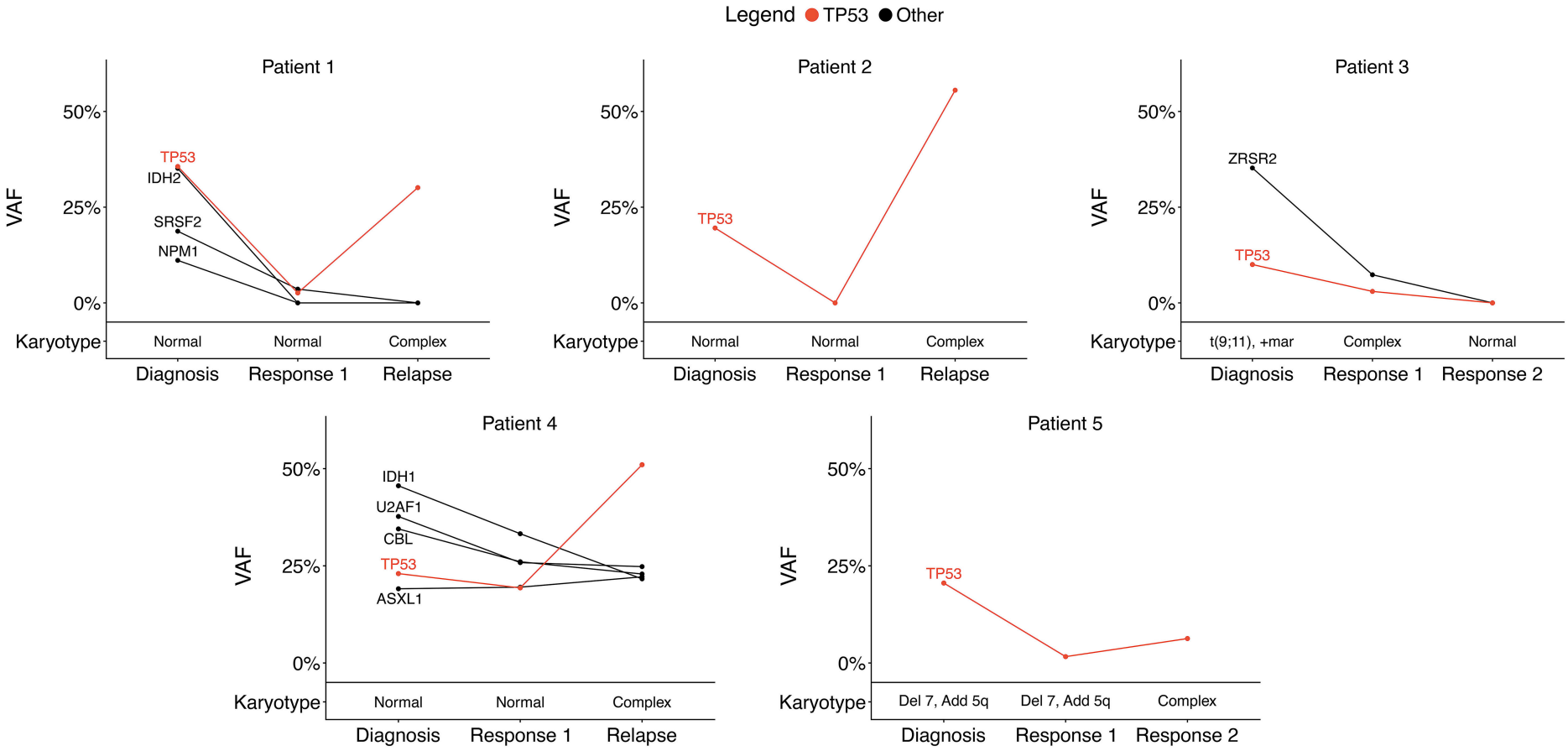
Characteristics	**N = 5 (%)**
Sex	
Females	2 (40)
Males	3 (60)
Disease	
AML	3 (60)
MDS	2 (40)
Karyotype at diagnosis	
Normal	3 (60)
Two chromosomal abnormalities	2 (40)
TP53 mutation VAF at diagnosis median, range	20.6, 10.02 – 35.62
Transformation time	
Post-induction and consolidation	2 (40)
Post-AlloBMT	3 (60)
TP53 mutation VAF above 50% at CK	2 (40)
17/17p deletion at complex karyotype	3 (60)

Figure Legends

Figure 1. Evolution of mono-allelic *TP53*-mutated myeloid neoplasms (single mutation, VAF<40%, no CK) to CK disease is associated with higher VAF at diagnosis and genotoxic therapy and negatively impacts overall survival. **A.** Mutational landscape and cytogenetic abnormalities of the cohort, stratified AML and MDS. **B.** Cohort overall survival (5yr OS: 32.8%). **C.** Comparison of the overall survival between patients who developed CK disease and those who did not. **D.** Incidence of Complex Karyotype, stratified by baseline *TP53* Variant Allele Frequency. High *TP53* Variant Allele Frequency was defined as VAF > 10%. **E.** Incidence of Complex Karyotype stratified by treatment with genotoxic therapy following MDS/AML diagnosis.

Figure 2. Karyotype Evolution and *TP53* VAF %Variant Allele Frequency (VAF) Progression in patients who developed CK disease (N=5).


A
AML
MDS
TP53
ZRSR2
SF3B1
IDH2
ASXL1
TET2
SRSF2
U2AF1
NRAS
NPM1
IDH1
EZH2
DDX41
CBL
SETBP1
PHF6
KRAS
JAK2
GATA1
FLT3
CEBPA
Mutations
Del 5q
t(9;11)
t(3;7)
Del 7
Del 13q
Add 8
Add 5q
+mar
Karyotype
B
Overall survival
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8 9 10
Years
Strata
Number at risk
25 19 11 7 5 4 3 2 2 1 0
0 1 2 3 4 5 6 7 8 9 10
Years
C
Developed CK No Yes
Overall survival
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8 9 10
Years
Strata
Number at risk
17 14 8 5 4 3 3 2 2 1 0
5 3 1 1 0 0 0 0 0 0 0
0 1 2 3 4 5 6 7 8 9 10
Years
D
TP53 VAF VAF≤10% VAF>10%
Incidence of CK
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8 9 10
Years
Strata
Number at risk
10 7 4 2 2 1 1 0 0 0 0
15 11 7 5 3 3 2 2 2 1 0
0 1 2 3 4 5 6 7 8 9 10
Years
E
Genotoxic therapy No Yes
Incidence of CK
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8 9 10
Years
Strata
Number at risk
10 7 4 2 1 1 1 1 1 0 0
12 9 5 4 3 2 2 1 1 1 0
0 1 2 3 4 5 6 7 8 9 10
Years

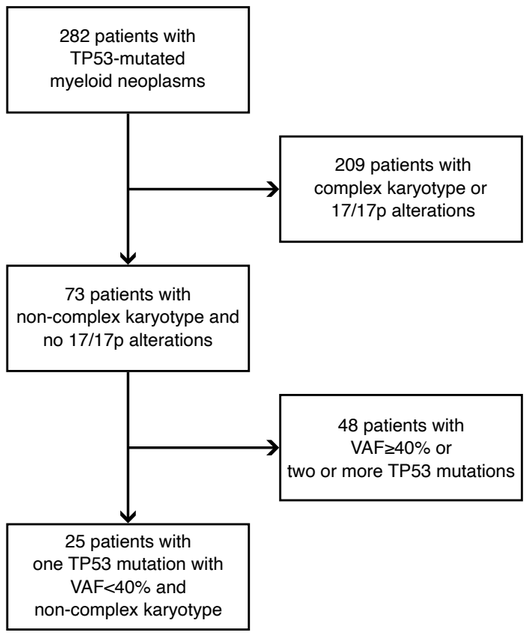

Legend TP53 Other
Patient 1
50%
25%
0%
VAF
TP53
IDH2
SRSF2
NPM1
Karyotype
Normal
Normal
Complex
Diagnosis
Response 1
Relapse
Patient 2
50%
25%
0%
VAF
TP53
Karyotype
Normal
Normal
Complex
Diagnosis
Response 1
Relapse
Patient 3
50%
25%
0%
VAF
ZRSR2
TP53
Karyotype
t(9;11), +mar
Complex
Normal
Diagnosis
Response 1
Response 2
Patient 4
50%
25%
0%
VAF
IDH1
U2AF1
CBL
TP53
ASXL1
Karyotype
Normal
Normal
Complex
Diagnosis
Response 1
Relapse
Patient 5
50%
25%
0%
VAF
TP53
Karyotype
Del 7, Add 5q
Del 7, Add 5q
Complex
Diagnosis
Response 1
Response 2

Supplementary Table 1: Clinical and Demographic Characteristics of Patients with Mono-allelic *TP53*-Mutated MDS/AML with Non-Complex Karyotypes

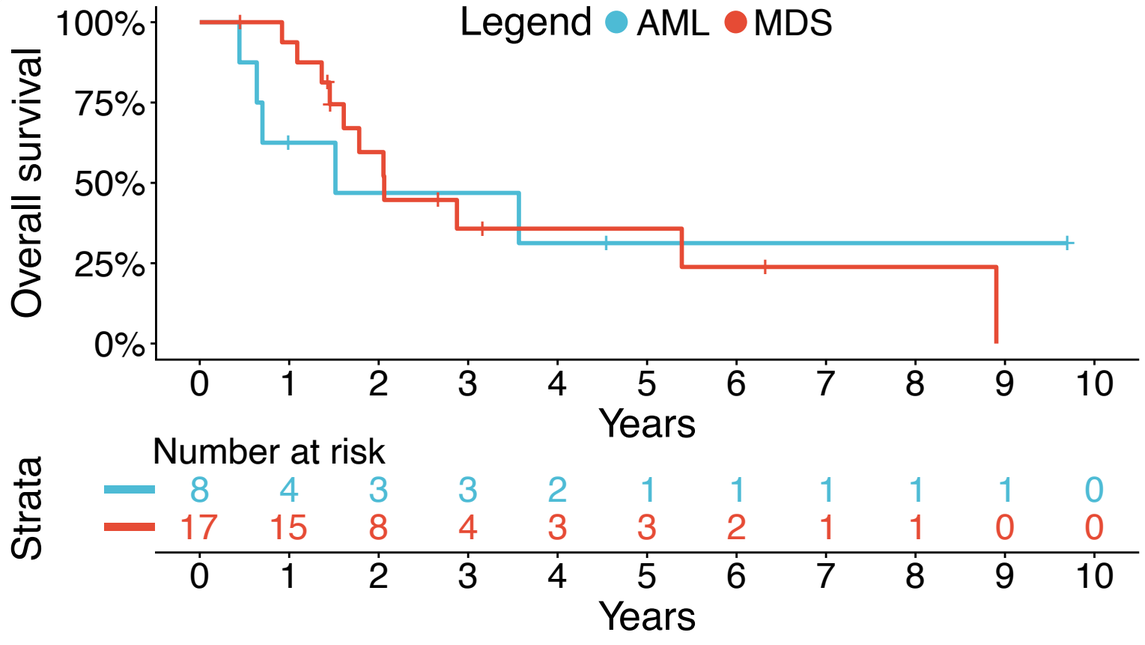
Characteristic	**N = 25 (%)**
Sex	
Female	13 (52)
Male	12 (48)
Age at diagnosis	65.4 years (23.3 – 88.2 years)
Diagnosis	
AML	8 (32)
MDS	17 (68)
Treatment-related	8 (32)
Blasts%, range	7.5 (0-90)
CNS disease	3 (12)
Karyotype	
Good	20 (80)
Intermediate	4 (16)
Poor	1 (4)
TP53 variant allele frequency %, range	15 (2.54 – 38.8)
Prognostic score	
ELN 2024 for AML	
Intermediate	2 (25)
Adverse	6 (75)
R-IPSS-M for MDS	-0.3 (-1.48 – 1.36)
First line therapy	
Intensive chemotherapy	4 (16)
HMA-based therapy	15 (60)
Lenalidomide	1 (4)
IDH inhibitor alone	1 (4)
Growth factors	1 (4)
Luspatercept	1 (4)
Supportive care	2 (8)
Second line therapy (N=8)	
Chemotherapy	4 (50)
HMA-based therapy	4 (50)

Supplementary Figure 1. A. Patient selection flowchart and inclusion criteria for the final cohort of 25 patients with MDS/AML harboring monoallelic *TP53* mutations (VAF <40% and non-CK/17/17p alterations). **B.** Distribution of *TP53* mutations within the cohort and their location within the DNA Binding Domain. **C.** Kaplan Meier Overall Survival Curve, stratified by disease subtype of AML (5yr OS: 31.3%) and MDS (5yr OS: 35.7%).

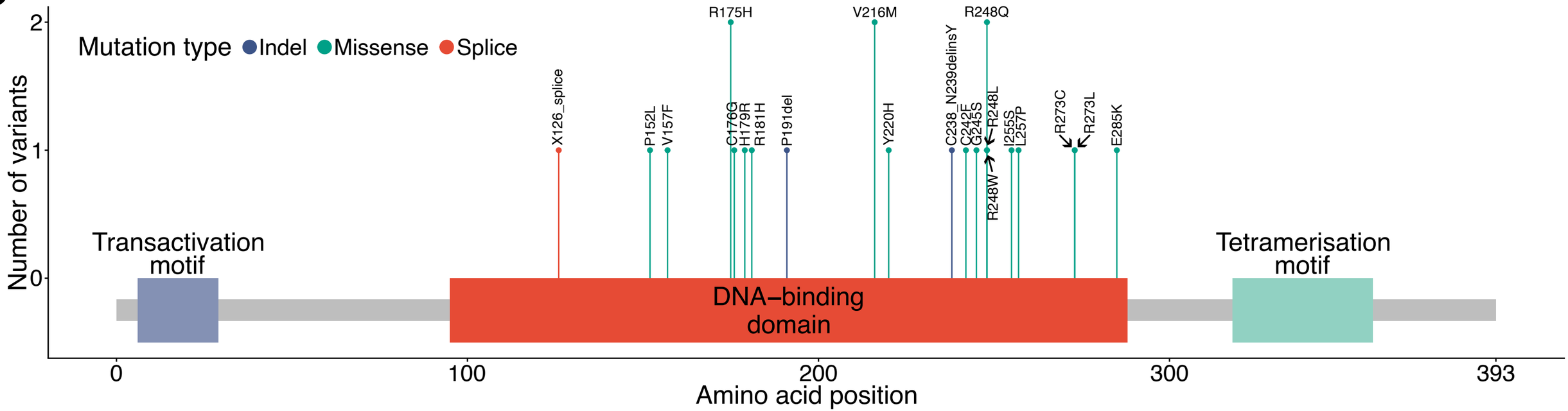
A


282 patients with TP53-mutated myeloid neoplasms
209 patients with complex karyotype or 17/17p alterations
73 patients with non-complex karyotype and no 17/17p alterations
48 patients with VAF≥40% or two or more TP53 mutations
25 patients with one TP53 mutation with VAF<40% and non-complex karyotype

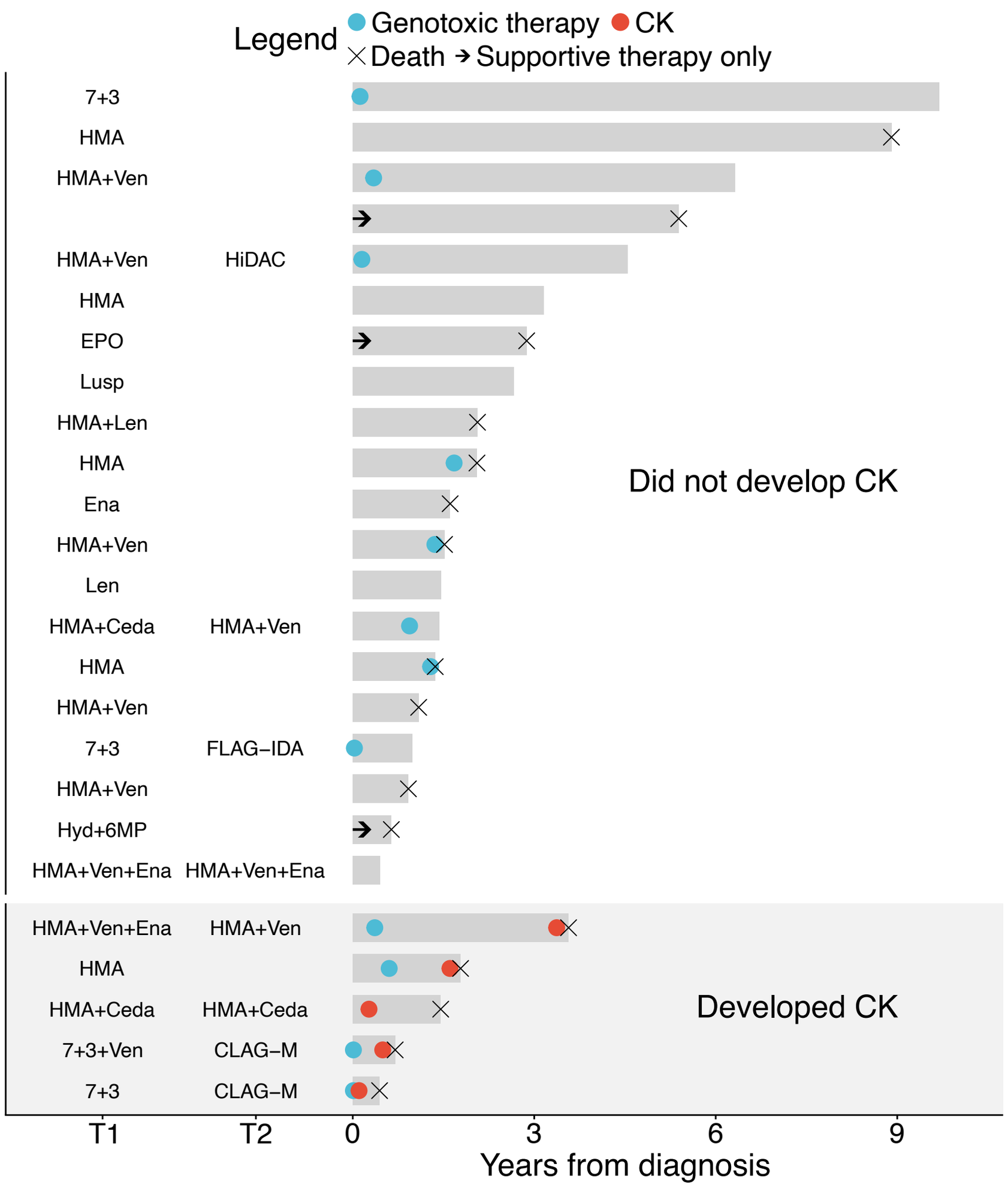
C


Legend AML MDS
Overall survival
100%
75%
50%
25%
0%
0 1 2 3 4 5 6 7 8 9 10
Years
Number at risk
Strata
8 4 3 3 2 1 1 1 1 1 0
17 15 8 4 3 3 2 1 1 0 0
0 1 2 3 4 5 6 7 8 9 10
Years

B


Mutation type Indel Missense Splice
Number of variants
2
1
0
X126_splice
P152L
V157F
R175H
C176G
H179R
R181H
P191del
V216M
Y220H
C238_N239delinsY
C242F
G245S
R248Q
R248L
R248W
I255S
L257P
R273C
R273L
E285K
Transactivation motif
DNA–binding domain
Tetramerisation motif
0
100
200
300
393
Amino acid position

Supplementary Figure 2. Swimmers plot showing the first use of cytotoxic therapy, development of CK, and death in patients receiving treatment other than supportive care. Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CK, complex karyotype; HMA, hypomethylating agent; Ven, venetoclax; EPO, erythropoietin; Lusp, luspatercept; Len, lenalidomide; Ceda, cedazuridine; Hyd, hydroxyurea; 6MP, 6 mercaptopurine; HiDAC, high-dose ara-C; T1, first line of therapy; T2, second line of therapy.


Legend
Genotoxic therapy
CK
Death
Supportive therapy only
7+3
HMA
HMA+Ven
HMA+Ven
HiDAC
HMA
EPO
Lusp
HMA+Len
HMA
Ena
HMA+Ven
Len
HMA+Ceda
HMA+Ven
HMA
HMA+Ven
7+3
FLAG–IDA
HMA+Ven
Hyd+6MP
HMA+Ven+Ena
HMA+Ven+Ena
Did not develop CK
HMA+Ven+Ena
HMA+Ven
HMA
HMA+Ceda
HMA+Ceda
7+3+Ven
CLAG–M
7+3
CLAG–M
Developed CK
T1
T2
0
3
6
9
Years from diagnosis