LETTER OPEN

Check for updates

Mutant *TP53* allelic states in human hematopoietic stem and progenitor cells

© The Author(s) 2026, modified publication 2026

Leukemia; https://doi.org/10.1038/s41375-026-03106-x

TO THE EDITOR:

In human hematopoietic stem and progenitor cells (HSPCs), *TP53* mutations characterize clonal hematopoiesis of indeterminate potential (CHIP) and are recognized as a high-risk factor for the development of non-neoplastic and neoplastic disorders, particularly myelodysplastic syndromes (MDS) and acute myeloid leukemia [1, 2]. During leukemic transformation, the remaining *TP53* wild-type (WT) allele is frequently affected, most commonly through loss-of-heterozygosity (LOH), copy-neutral LOH, or a second concurrent mutation. The *TP53* allelic state—reflecting whether one or both alleles are affected—remains a subject of debate regarding its prognostic relevance in patients with MDS [3]. Recently, the biological consequences of monoallelic versus biallelic aberrations were investigated using a Trp53 mouse model [4]. Here, we address this question in human HSPCs by introducing defined *TP53* aberrations via CRISPR/Cas9-mediated genome editing.

Material and methods are described in detail in the *Supplementary information*. Enriched, umbilical cord blood (UCB) derived $CD34^+$ HSPCs were targeted at exon 2 of the *TP53* gene using a CRISPR/Cas9 and recombinant adeno-associated virus serotype 6 mediated knock-in strategy (Fig. 1A and Supplementary Figs. S1A, B and S2) [5]. By targeting exon 2, we were able to generate WT and aberrant *TP53* alleles within the same genomic context while maintaining the endogenous regulatory control. Integration of the donor template at exon 2 disrupts the endogenous *TP53* gene, ensuring that only the donor-encoded cDNA sequences are expressed from the endogenous *TP53* promoter. Mutant *TP53* cDNA—carrying either the variant of interest or a stop codon—or WT cDNA, each linked to a fluorescent reporter (FR) expression cassette, was introduced via homology-directed repair to generate the following monoallelic and biallelic genotypes: $TP53^{R175H/WT}$, $TP53^{R273H/WT}$, $TP53^{KO/WT}$ and $TP53^{R175H/KO}$, $TP53^{R273H/KO}$, $TP53^{KO/KO}$. The p.R175H and p.R273H aberrations are *TP53* hot-spot mutations with reported gain-of-function and dominant-negative/loss-of-function properties, respectively (Supplementary Table S1) [6–8]. FR inserted at the *AAVS1* safe-harbor locus served as $TP53^{WT/WT}$ control. Stable, biallelic integration of *TP53* cDNAs was achieved in 0.5–10.0% of HSPCs (mean, 2.23 ± 1.76 SD) and enriched by flow cytometry (Supplementary Fig. 1C). Site-specific integration was confirmed by PCR and each engineered *TP53* genotype was validated by Sanger sequencing of both genomic DNA and complementary DNA (Fig. 1B and Supplementary Fig. 3A, B), p53 protein expression was confirmed by immunocytochemistry (Supplementary Fig. 3C). Functionally, the introduced *TP53* aberrations resulted in reduced or absent expression of the downstream targets p21 and MDM2 (Supplementary Fig. S4).

We initially investigated whether distinct *TP53* mutant allelic states affect colony growth and self-renewal using methylcellulose-based colony-forming unit (CFU) assays. Reporter-positive, genome-edited HSPCs with biallelic *TP53* aberrations derived from pooled UCB samples generated significantly more colonies than cells with monoallelic aberrations and *AAVS1* controls (Fig. 1C, D). Both CFU-GM and BFU-E colony numbers increased, with erythroid colonies exhibiting a particularly pronounced rise. PCR and Sanger sequencing of colonies showed complete retention of the initially introduced mutations in HSPCs (Supplementary Fig. S5). Serial replating CFU assays demonstrated that HSPCs with WT *TP53* or monoallelic aberrations lost their colony-forming capacity after three passages, whereas cells with biallelic aberrations displayed elevated clonogenic potential (Fig. 1E). Cell cycle analysis revealed a non-significant increase in the proportion of cells with biallelic *TP53* aberrations in the S-phase of the cell cycle (Supplementary Fig. S6).

TP53 aberrations are closely linked to structural chromosomal abnormalities (CAs). Using single-cell template strand sequencing of genome-edited HSPCs derived from single UCB donors, we analyzed CAs induced by *TP53* aberrations [9]. Cells were treated with either doxorubicin, a potent cellular stressor, or DMSO as control (Supplementary Figs. S7, S8). Upon doxorubicin treatment, cells with *TP53* monoallelic aberrations already exhibited a significantly higher frequency of CAs compared to *AAVS1*-targeted controls. In addition, a spontaneous and significant increase in CAs was observed in HSPCs carrying the $TP53^{R175H}$ mutation (Fig. 2A, Supplementary Fig. S9). In HSPCs harboring the *TP53* R175H and R273H mutations, chromosomes 3, 5, 7 and 17 were among the most frequently affected. Aberrations of chromosomes 5 and 7 are also common in patients with *TP53* mutated myeloid neoplasms. Consistent with the clinical situation, we observed terminal losses (64% of all CAs affecting chromosomes 5 and 7), terminal gains (14%), arm gains (7%), whole chromosome gains (7%) and complex aberrations (8%) in our experimental setting (Fig. 2B, Supplementary Fig. S10).

To delineate the transcriptional consequences of monoallelic and biallelic *TP53* aberrations, we performed RNA sequencing on reporter-positive, genome-edited cells from pooled UCB samples. Compared with *AAVS1* controls, the majority of differentially expressed genes (DEGs) were shared between both *TP53* aberrant groups (Fig. 2C, D). Among uniquely expressed genes, monoallelic *TP53* aberrant HSPCs—in particular those with the R175H and R273H genotypes—showed upregulation of PD-L1 (CD274) which was confirmed by flow cytometry (Supplementary Fig. S11). Biallelic *TP53* aberrations were associated with overexpression of HOXA10 (Supplementary Tables S2 and S3). Gene Set Enrichment Analysis of shared DEGs highlighted upregulation of oxidative phosphorylation, heme metabolism and inflammatory signaling including TNFα/NF-κB, IL6-JAK-STAT3 and IL2-STAT5 (Supplementary Fig. S12), whereas downregulated genes were enriched for mitotic spindle assembly and G_2M checkpoint regulation (Fig. 2E). Furthermore, we observed negative enrichment of a set of 135

Received: 16 February 2026 Revised: 11 July 2026 Accepted: 31 July 2026
Published online: 18 August 2026

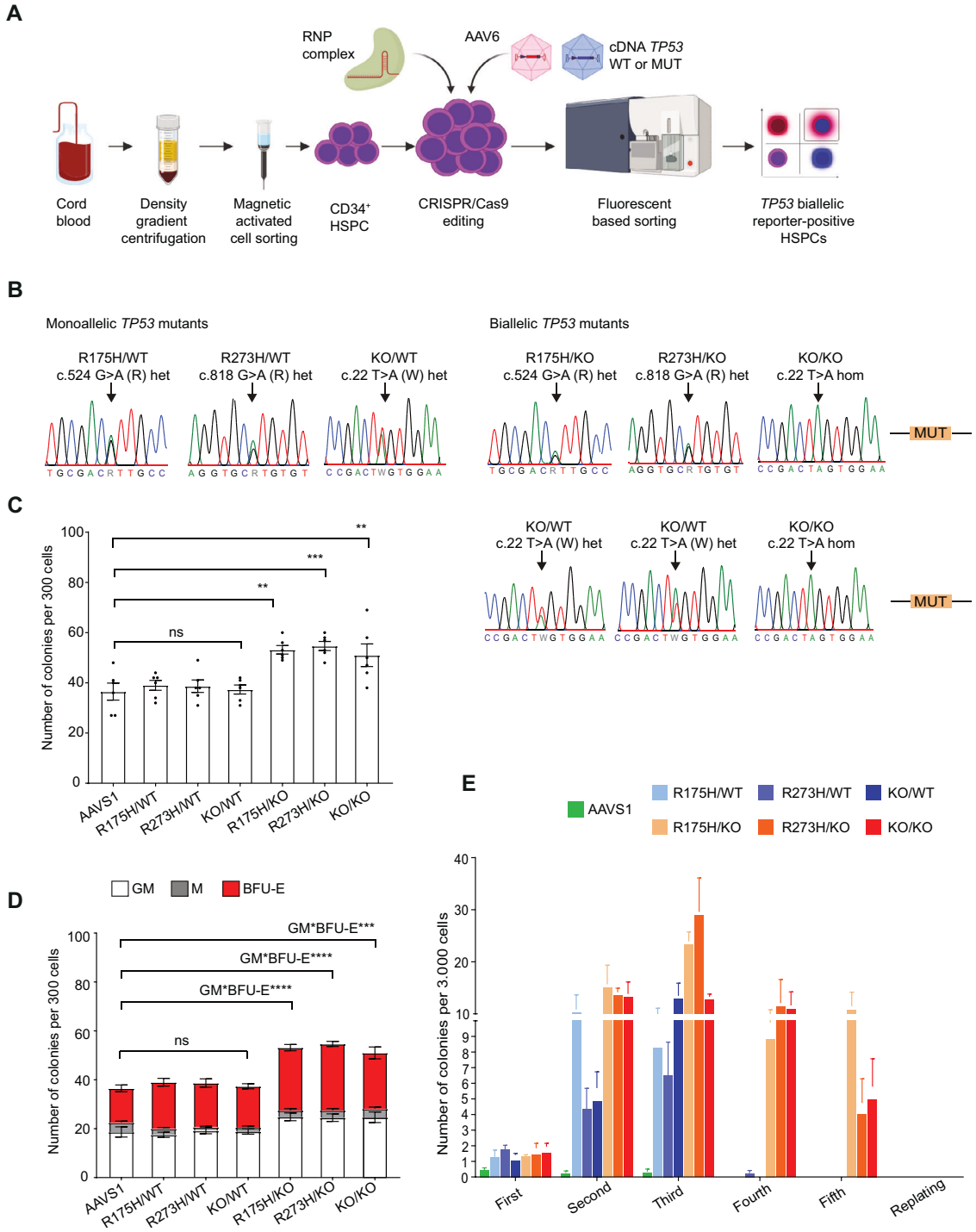


Fig. 1 The *TP53* allelic state differentially modulates human HSPC growth and self-renewal. A CRISPR-mediated knock-in of wild-type (WT) or mutant (MUT) *TP53* cDNA into cord blood-derived CD34⁺ HSPCs. **B** Representative genomic DNA chromatograms displaying monoallelic (R175H/WT, R273H/WT, KO/WT) and biallelic (R175H/KO, R273H/KO, KO/KO) *TP53* genotypes. **C** CFUs from different genotypes 14 days after seeding into methylcellulose. **D** Morphological classification of colonies as BFU-E (red), monocyte (M, gray) or granulocyte/macrophage (GM, white) from primary plating after 14 days. **E** CFUs from different genotypes upon replating. **C**–**E** Data are presented as mean ± s.e.m. of two pooled samples plated in triplicates. Statistical comparisons to *AAVS1* control by one-way (**C**) or two-way (**D**) ANOVA. ns not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. HSPCs hematopoietic stem and progenitor cells, CFUs colony-forming units, BFU-E burst-forming unit erythroid.

myeloid-specific genes in HSPCs carrying either monoallelic or biallelic *TP53* aberrations, while 100 erythroid-associated genes were positively enriched in both groups compared to *TP53* WT HSPCs. The ratio of CEBPA to erythroid-specific transcripts such as GATA1 and PLIN2 was reduced in both monoallelic and biallelic mutant HSPCs, consistent with a shift in lineage commitment as we have observed in the CFU assays (Supplementary Fig. S13).

In this study, we established a model of mutant *TP53* allelic states in human HSPC using CRISPR/Cas9 genome engineering. By

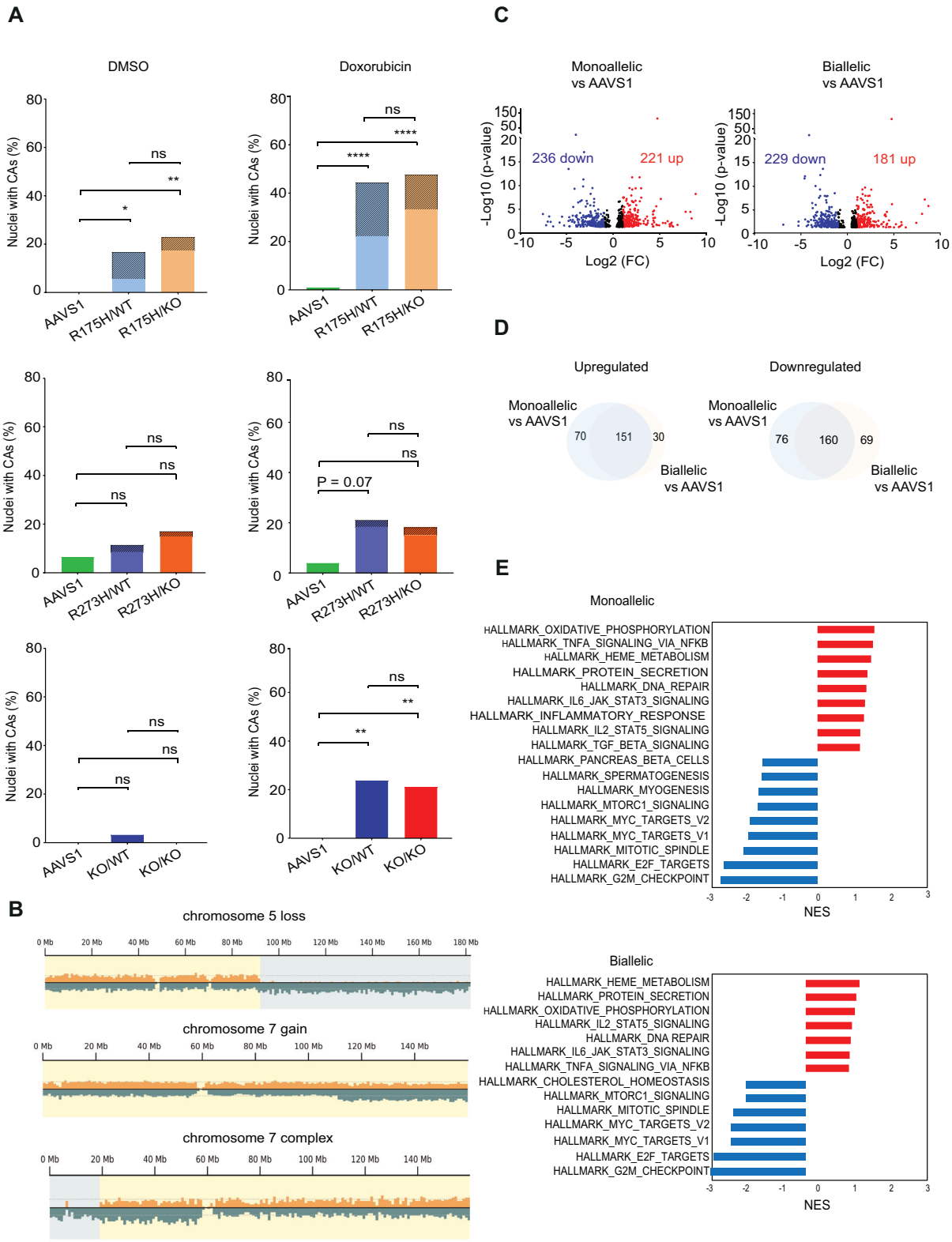


Fig. 2 *TP53* mutations in human HSPCs drive chromosomal aberrations (CAs) and activate inflammatory transcriptional programs. **A** Frequency of CAs in *TP53* mutant HSPCs treated with DMSO or doxorubicin; shaded areas of the bars indicate multiple CAs per single nucleus. Statistical comparisons to *AAVS1* controls were performed using two-sided Fisher's exact test. Data were generated from one biological donor per genotype. **B** Representative CA plots for chromosome 5 loss, chromosome 7 gain and complex aberrations on chromosome 7 with sequencing reads aligned to the positive (Watson, orange) or negative (Crick, green) strands. **C** Volcano plots depicting differentially expressed genes (DEGs) in monoallelic and biallelic *TP53* mutants versus *AAVS1* controls. **D** DeepVenn diagrams showing DEG overlaps. (E) Gene set enrichment analysis (MSigDB hallmark gene sets) highlighting relevant enriched pathways (FDR < 0.25); positive normalized enrichment scores (NES) in red, negative NES in blue. **C**–**E** Data were generated from three independent biological donor-derived HSPC pools.

employing UCB-derived cells, we were able to investigate the functional consequences of particular *TP53* aberrations without confounding effects of an aging cellular microenvironment. We found that genomic aberrations can be triggered by a single *TP53* aberration, consistent with dysregulation of mitotic spindle and G_2M checkpoints, as revealed by transcriptome analysis. The spectrum of CAs mirrors that observed in *TP53*-mutated myeloid malignancies indicating that these lesions constitute early events in leukemogenesis. Notably, the R175H mutation led to a spontaneous rise in CAs, supporting its previously described gain-of-function activity in acute leukemia models [6]. The early onset of copy-number alterations has also been reported in patients with Li-Fraumeni syndrome carrying *TP53* germline mutations [10]. Monoallelic *TP53* aberrations also induced inflammatory pathways characteristic of preleukemic states. Similar pathway upregulation has been described in heterozygous *TP53* mutant HSPCs from patients with myeloproliferative syndromes and was shown to confer a fitness advantage of *Trp53* mutant clones in experimental in vivo models [11]. *TP53* mutations have been reported to confer an immunosuppressive phenotype. We observed upregulation of the immune checkpoint ligand PD-L1 in edited HSPCs harboring monoallelic *TP53* aberrations, particularly with the R175H and R273H genotypes. PD-L1 expression has also been described in HSPCs of patients with *TP53* mutant MDS and AML where it correlates with poor survival outcomes [12]. However, disruption of a single *TP53* allele is insufficient to drive the outgrowth of UCB-derived human HSPCs. Following inactivation of the second allele, *TP53* aberrant HSPCs acquired a clonogenic growth and self-renewal advantage. The homeobox transcription factor HOXA10 was identified among upregulated genes that potentially contribute to this transition. In transgenic mice, HOXA10 has been reported to promote both proliferation and lineage commitment of hematopoietic stem cells in a concentration-dependent manner [13]. Enhanced self-renewal of biallelic mutations, considered a prerequisite for leukemic transformation, has also been described recently in a novel *Trp53* mouse model [4].

These findings provide mechanistic insights into early *TP53*-driven transformation of human HSPCs and highlight potential clinical implications. In particular, *TP53* mutant CHIP clones expand during cytotoxic therapy, ultimately giving rise to therapy-related myeloid neoplasms that are aggressive disorders associated with a dismal outcome [14]. In a recent report, patients with solid cancers and *TP53* mutant clonal hematopoiesis undergoing chemotherapy were additionally treated with the CDK4/6 inhibitors trilaciclib or palbociclib. Compared with placebo, these agents substantially mitigated the expansion of the mutant clone without causing severe side effects [15]. Given that even monoallelic *TP53* aberrations induce profound functional changes, our data support the application of such strategies targeting mutant *TP53* as early as possible—at the monoallelic CHIP stage.

DATA AVAILABILITY

Single-cell template strand sequencing data are deposited at the European Genome-phenome Archive (EGA) under accession number EGAS50000001965. RNA-seq data are publicly available through the European Nucleotide Archive (ENA) under accession number PRJEB101389 (BioStudy: S-BSST2246).

Karin Lind[1,13], Sayantanee Dutta[1,2,13]✉, Marco Raffaele Cosenza[3], Thomas Eder[4], Johannes Foßelteder[1], Gabriel Pabst[1], Wolfgang Schöll[5], Andreas Prokesch[6], Holger W. Auner[7], Albert Wölfler[1], Armin Zebisch[1,8], Andreas Reinisch[1,9], Florian Grebien[4,10,11], Jan O. Korbel[3,12] and Heinz Sill[1]✉

[1]*Division of Hematology, Medical University of Graz, Graz, Austria.* [2]*Division of Oncology, Medical University of Graz, Graz, Austria.* [3]*Genome Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany.* [4]*Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria.* [5]*Department of Obstetrics & Gynecology, Medical University of Graz, Graz, Austria.* [6]*Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Division of Cell Biology, Histology and Embryology, Medical University of Graz, Graz, Austria.* [7]*Division of Haematology and Central Haematology Laboratory, Lausanne University Hospital, Lausanne, Switzerland.* [8]*Otto Loewi Research Center for Vascular Biology, Immunology and Inflammation, Division of Pharmacology, Medical University of Graz, Graz, Austria.* [9]*Department of Blood Group Serology and Transfusion Medicine, Medical University of Graz, Graz, Austria.* [10]*St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria.* [11]*CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.* [12]*Bridging Research Division on Mechanisms of Genomic Variation and Data Science, German Cancer Research Center (DKFZ), Heidelberg, Germany.* [13]*These authors contributed equally: Karin Lind, Sayantanee Dutta.*✉*email: sayantanee.dutta@medunigraz.at; heinz.sill@medunigraz.at*

REFERENCES

1. Usui Y, Endo M, Iwasaki Y, Iijima H, Nakagawa H, Matsuda K, et al. Clinical significance of TP53-mutant clonal hematopoiesis across diseases. Blood Cancer Discov. 2025;6:298–306.
2. Weeks LD, Niroula A, Neuberg D, Wong W, Lindsley RC, Luskin M, et al. Prediction of risk for myeloid malignancy in clonal hematopoiesis. NEJM Evid. 2023;2. https://doi.org/10.1056/evidoa2200310.
3. Shah MV, Hung K, Baranwal A, Kutyna MM, Al-Kali A, Toop C, et al. Evidence-based risk stratification of myeloid neoplasms harboring TP53 mutations. Blood Adv. 2025;9:3370–80.
4. Fullin J, Topcu E, Zielinska KA, Schimmer RR, Klemm N, Koch C, et al. The pathogenesis of therapy-related myeloid neoplasms from TP53-mutant clonal hematopoiesis. Leukemia. 2026;40:279–92.
5. Fosselteder J, Pabst G, Sconocchia T, Schlacher A, Auinger L, Kashofer K, et al. Human gene-engineered calreticulin mutant stem cells recapitulate MPN hallmarks and identify targetable vulnerabilities. Leukemia. 2023;37:843–53.
6. Loizou E, Banito A, Livshits G, Ho YJ, Koche RP, Sánchez-Rivera FJ, et al. A gain-of-function p53-mutant oncogene promotes cell fate plasticity and myeloid leukemia through the pluripotency factor FOXH1. Cancer Discov. 2019;9:962–79.
7. Boettcher S, Miller PG, Sharma R, McConkey M, Leventhal M, Krivtsov AV, et al. A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies. Science. 2019;365:599–604.
8. Wang Z, Burigotto M, Ghetti S, Vaillant F, Tan T, Capaldo BD, et al. Loss-of-function but not gain-of-function properties of mutant TP53 are critical for the proliferation, survival, and metastasis of a broad range of cancer cells. Cancer Discov. 2024;14:362–79.
9. Cosenza MR, Gaiatto A, Erarslan Uysal B, Andrades A, Sautter NL, Simunovic M, et al. Origins of chromosome instability unveiled by coupled imaging and genomics. Nature. 2025;648:383–93.
10. Light N, Layeghifard M, Attery A, Subasri V, Zatzman M, Anderson ND, et al. Germline TP53 mutations undergo copy number gain years prior to tumor diagnosis. Nat Commun. 2023;14:77.
11. Rodriguez-Meira A, Norfo R, Wen S, Chedeville AL, Rahman H, O'Sullivan J, et al. Single-cell multi-omics identifies chronic inflammation as a driver of TP53-mutant leukemic evolution. Nat Genet. 2023;55:1531–41.
12. Sallman, McLemore DA, Aldrich AL AF, Komrokji RS, McGraw KL, Dhawan A, et al. TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. Blood. 2020;136:2812–23.
13. Magnusson M, Brun AC, Miyake N, Larsson J, Ehinger M, Bjornsson JM, et al. HOXA10 is a critical regulator for hematopoietic stem cells and erythroid/megakaryocyte development. Blood. 2007;109:3687–96.
14. Hiwase D, Hahn C, Tran ENH, Chhetri R, Baranwal A, Al-Kali A, et al. TP53 mutation in therapy-related myeloid neoplasm defines a distinct molecular subtype. Blood. 2023;141:1087–91.
15. Chan ICC, Zhang P, Pan X, Castro C, Fox N, Lewis AM, et al. CDK4/6 inhibition mitigates chemotherapy-induced expansion of TP53-mutant clonal hematopoiesis. Nat Genet. 2026;58:582–92.

ACKNOWLEDGEMENTS
We thank all mothers and their newborns for donating cord blood-derived hematopoietic stem and progenitor cells. ChatGPT (OpenAI) was used to improve the clarity of the manuscript.

AUTHOR CONTRIBUTIONS
KL and SD contributed equally. HS, JF, GP, and AR conceived and designed the experiments. KL, SD, MRC, JF, and GP performed the experiments. HS, KL, SD, MRC, TE, JF, GP, AP, HWA, AW, AZ, AR, FG, and JOK analyzed and interpreted data. WS, AR, and JOK contributed reagents, materials and analysis tools. HS, KL, and SD wrote the manuscript. All authors read and approved the manuscript.

FUNDING
This work was supported by the Austrian Science Fund FWF (grant number P31430-B26 to HS; grant-doi 10.55776/P3667, 10.55776/PAT1753824 and 10.55776/I6101 to AZ; grant-doi 10.55776/P32783 and 10.55776/I5021 to AR), Leukämiehilfe Steiermark (to HS), Austrian Cancer Aid-Styria (to SD) and the European Research Council (ERC Advanced Grant, number 101098056 to JOK).

COMPETING INTERESTS
JOK has previously disclosed a patent application (no. EP19169090) that is relevant to the use of single-cell template strand-sequencing for somatic structural variation analysis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE STATEMENT
The study has been approved by the ethics committee of Medical University of Graz, Graz, Austria (vote number 33-534 ex 20/21) and informed consent was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41375-026-03106-x.

Correspondence and requests for materials should be addressed to Sayantanee Dutta or Heinz Sill.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2026, modified publication 2026