CASE REPORT

Blood Science

OPEN

Identification of two novel TP53 mutations in secondary acute myeloid leukemia following multiple myeloma: a case report

Maria Antonia De Francesco[a,b], Rossella Ribolla[c], Alessandra Sottini[a,b], Diego Bertoli[a,b], Carlotta Giupponi[a,b], Ottavia Bonuccelli[a,b], Roberto Bresciani[a,b], Alessandra Tucci[c], Giorgio Biasiotto[a,b,*]

[a]Department of Molecular and Translational Medicine, University of Brescia, Brescia 25123, Italy; [b]Highly Specialized Laboratory, ASST Spedali Civili di Brescia, Brescia 25123, Italy; [c]Department of Hematology, ASST Spedali Civili di Brescia, Brescia, Italy

1. INTRODUCTION

The incidence and mortality of multiple myeloma (MM), the second most widespread hematological malignancy, increased during the last 30 years with approximately 156,000 incident cases, 113,000 deaths and 2.50 million disability-adjusted life years (DALYs) reported globally in 2019.[1] In 2020, the age-standardized incidence rate of MM was 1.78 per 100,000 persons, and the age-standardized mortality rate was 1.14 per 100,000 persons,[2] while 188,000 new cases of MM and 121,000 related deaths have been reported worldwide in 2022.[3]

Secondary myeloid malignancies, such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), may arise as late complications in patients with MM, particularly after prolonged treatment with alkylating agents and immunomodulatory drugs.[4,5]

While the absolute risk of developing therapy-related MDS (t-MDS)/therapy-related AML (t-AML) in MM remains low, its occurrence is clinically significant, particularly in the presence of TP53 mutations and cytogenetically complex profiles.

Here, we report on the case of a patient with an initial diagnosis of MM who developed secondary MDS and a subsequent evolution to AML. The patient exhibited 2 novel TP53 mutations with a significant negative impact both on prognosis and clinical outcome.

2. RESULTS

A 64-year-old Caucasian man was diagnosed with IgAκ monoclonal gammopathy of undetermined significance (MGUS) in December 2016. Hemoglobin levels were within the normal range, and there was no evidence of hypercalcemia, renal insufficiency, or lytic bone lesions. The serum M component was 5 g/L, while total IgA levels were 571 mg/dL (reference range: 85–410 mg/dL). Serum-free light chains κ (sFLCκ) and λ (sFLCλ) were 41.30 mg/L (6.70–22.40 mg/L) and 11.90 mg/L (8.30–27.00 mg/L), respectively, resulting in an abnormal κ/λ ratio of 3.47 (reference: 0.31–1.56). No proteinuria was detected. The patient was referred to the Hematology Unit at Spedali Civili of Brescia for annual follow-up.

In October 2020, the patient developed anemia (hemoglobin 8.7 g/dL) and neutropenia (neutrophils 700/mm^3), accompanied by hypercalcemia (serum calcium 11.25 mg/dL) and mild renal impairment (serum creatinine 1.33 mg/dL). Serum protein electrophoresis revealed an M-protein spike of 38 g/L, and total IgA increased to 3910 mg/dL. Urine immunofixation was positive for κ light chains, with mild 24-hour proteinuria of 500 mg. sFLCκ and sFLCλ were 840.18 and 3.58 mg/L, respectively, yielding a markedly elevated κ/λ ratio of 234.68. Serum β2-microglobulin was 3.7 mg/L, and lactate dehydrogenase (LDH) levels were within the normal range.

Bone marrow aspiration and biopsy were performed, revealing a CD38+ CD138+ plasma cell infiltrate accounting for 60% of marrow cellularity. Interphase fluorescence in situ hybridization (FISH) analysis on enriched bone marrow plasma cells did not reveal any cytogenetic abnormalities. Specifically, the FISH panel was negative for deletion of 17p13.1 (TP53), amplification of 1q21, translocations t(4;14)(p16.3;q32.3) and t(14;16)(q32.3;q23), and deletion of 1p32.3.

Therefore, he was diagnosed with IgAκ MM, stage IIIA Durie-Salmon[6] and stage II disease according to the revised International Staging system (R-ISS).[7]

In November 2020, the patient started induction therapy with bortezomib (1.3 mg/m^2 twice weekly on a 21-day cycle), thalidomide (50 mg/d—dose reduced due to newly diagnosed atrial fibrillation during an infectious complication), and dexamethasone (40 mg weekly) for a total of 4 cycles. He subsequently underwent mobilization chemotherapy with cyclophosphamide, followed by collection of autologous hematopoietic stem cells. In April 2021, the patient received autologous stem cell transplantation after conditioning with high-dose melphalan (200 mg/m^2).

At the end of first-line treatment, the patient achieved a very good partial response (VGPR) according to the International Myeloma Working Group (IMWG) response criteria,[8] with minimal residual disease (MRD) still detectable in the bone marrow. In September 2021, he initiated lenalidomide maintenance therapy at a dose of 10 mg daily for 21 days of each 28-day cycle.

The patient continued maintenance therapy without significant complications until July 2024, when reappearance of a detectable monoclonal component in the serum prompted a

*Address correspondence: Prof. Giorgio Biasiotto, Department of Molecular and Translational Medicine, University of Brescia, Brescia 25123, Italy. E-mail address: giorgio.biasiotto@unibs.it (G. Biasiotto).

Conflict of interest: The authors declare that they have no conflict of interest.

Blood Science (2026) 8, 1–4:e00282.

Received August 11, 2025; Accepted January 29, 2026.

http://dx.doi.org/10.1097/BS9.0000000000000282

bone marrow re-evaluation. Bone marrow examination revealed a plasma cell infiltration of less than 10%, but marked dysplasia across all 3 hematopoietic lineages. A diagnosis of MDS with multilineage dysplasia (MDS-MLD) was established according to the World Health Organization (WHO) 5th edition classification.[9] Given the diagnosis of myelodysplasia and biochemical relapse of MM, lenalidomide treatment was permanently discontinued and clinical and laboratory follow-up was initiated.

In October of the same year, due to a sudden worsening of peripheral blood counts, a repeat bone marrow aspiration was performed, revealing progression of myelodysplasia with 25% blast cells and relapsed MM with an increase in plasma cell infiltration to 15%. The complex karyotype (59~60,XY,+1,+2,+4,+6,+8,+9,+add(11)(p14),+13,+14,-18,-19,+20,-21,+22,+6~7mar[cp9]/44,XY,-5,psu dic(7;12)(q11;p12)[8]/43,XY,-5,psu dic(7;12)(q11;p12),der(15;21)(q10;q10)[3]), the somatic molecular characterization and WT1 overexpression led to a diagnosis of AML, in the setting of concomitant relapsed MM. Given the presence of dual hematologic malignancies and the lack of therapeutic options, the patient was transitioned to supportive and palliative care.

Low-dose corticosteroids were introduced for cenesthetic relief, and the patient received periodic transfusions with red blood cell concentrates and pooled platelets to manage anemia and hemorrhagic diathesis at the hematology day hospital.

Due to increasing transfusion requirements and a decline in general condition that made home management difficult, the patient was transferred to a palliative care hospice in December 2024. He subsequently passed away on January 30, 2025, due to the natural progression of his acute leukemia.

3. DISCUSSION

Next generation sequencing (NGS) analysis of bone marrow blood was performed by SOPHIA DDM™ for Genomics software in order to identify somatic mutations including p53 variants and respective variant allele frequency (VAF).[10,11]

By using a 51 gene panel (CMYS panel_R_v1 Sophia Genetics), we found that our patient harbored only 2 mutations of TP53 (NM_000546) and no other mutations. The first mutation was the deletion c.576_582delGCATCTT p.His193Serfs*52 with VAF 33%. This mutation was never identified before, to our knowledge. The deletion of 7 bases was in the coding region for the DNA Binding Domain, where it generated a change in the amino acid sequence starting from the Histidine 193 to Serine, therefore resulting in a new random sequence of amino acids that ended after 52 residues (**Fig. 1**). In biophysical and structural studies, the DNA binding domain was reported as inherently unstable and very sensitive to any mutation. Therefore, the frameshift mutation in DBD has a dominant-negative effect by disrupting DNA binding and preventing the organization of the active normal homotetramers responsible for tumor progression.[12] Furthermore, this mutation was located in the L2 region (from 163 to 195 amino acids) that has been previously associated with anthracyclines or mitomycin resistance in breast cancer and other tumors.[13,14]

The second mutation carried by the patient was c.1049T>C p.Leu350Pro with VAF 30%. This mutation was reported in ClinVar database as a variant with uncertain significance and was never found in MDS/AML to the best of our knowledge. It was positioned in the oligomerization domain present in the second alpha-helix before the unstructured final part of the protein (**Fig. 2**). Because it is known that the cyclic amino acid proline is a destroyer of the secondary structure alpha-helix, the identified mutation p.Leu350Pro might have a role in blocking the assembly of the tetramer, therefore interfering with the normal function of the p53 protein. In support of this hypothesis, it was found that the very similar mutation p.Leu344Pro located in the same domain, near Leucine 350, had the effect of forming predominantly monomers

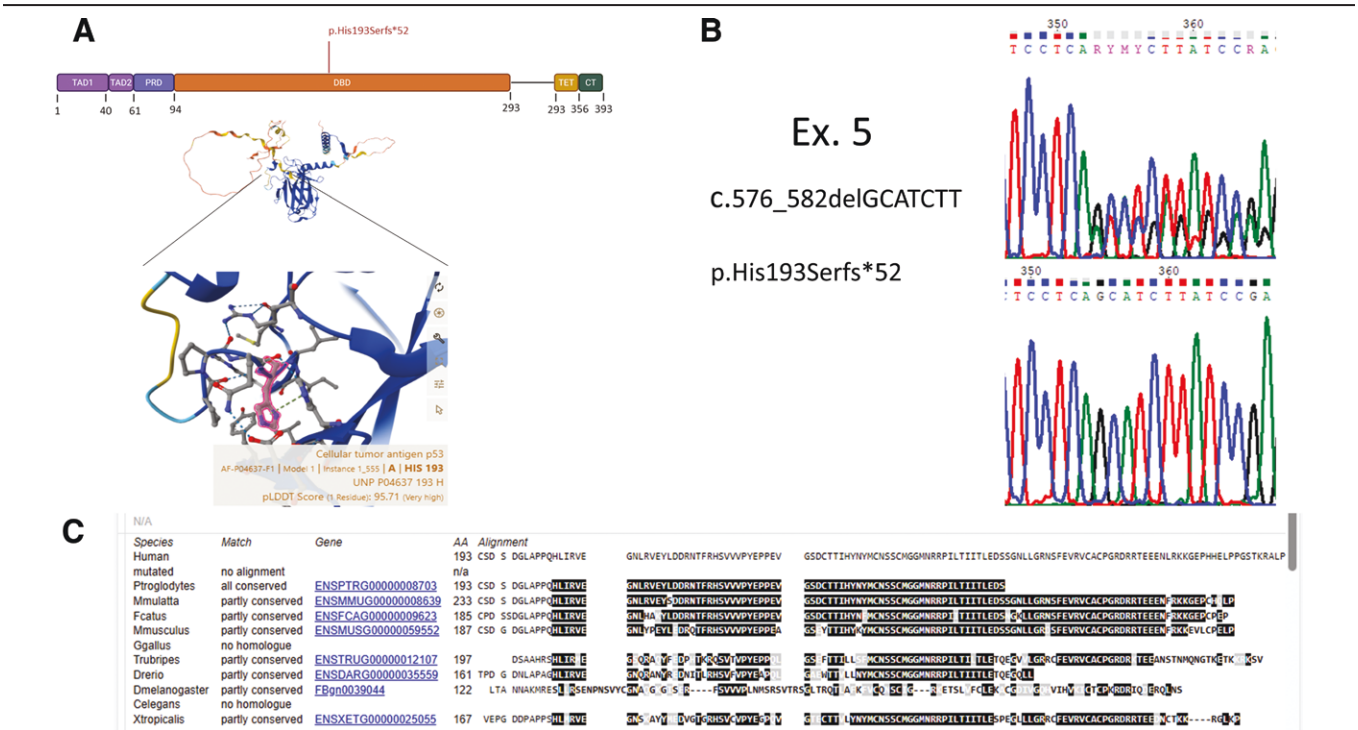

Figure 1. Effects of the p.His193Serfs*52 mutation. (A) This mutation is located in the DBD of the protein between a brief α-helix and a β-sheet domain and stabilizes the tertiary structure interacting with His 214 (www.alphafold.ebi.ac.uk). (B) Sanger sequencing electropherograms of the mutations are reported. (C) Phylogenesis shows that His193 is an amino acid conserved in the phylogenesis until amphibians (Xenopus tropicalis) and fishes (Danio rerio). The 50 amino acids after His193, which were changed in the frameshift, are well conserved in mammals and confirm the importance of this part for the protein functionality (www.mutationtaster.org). A part of the figure has been created by biorender.com. DBD = DNA-binding domain.

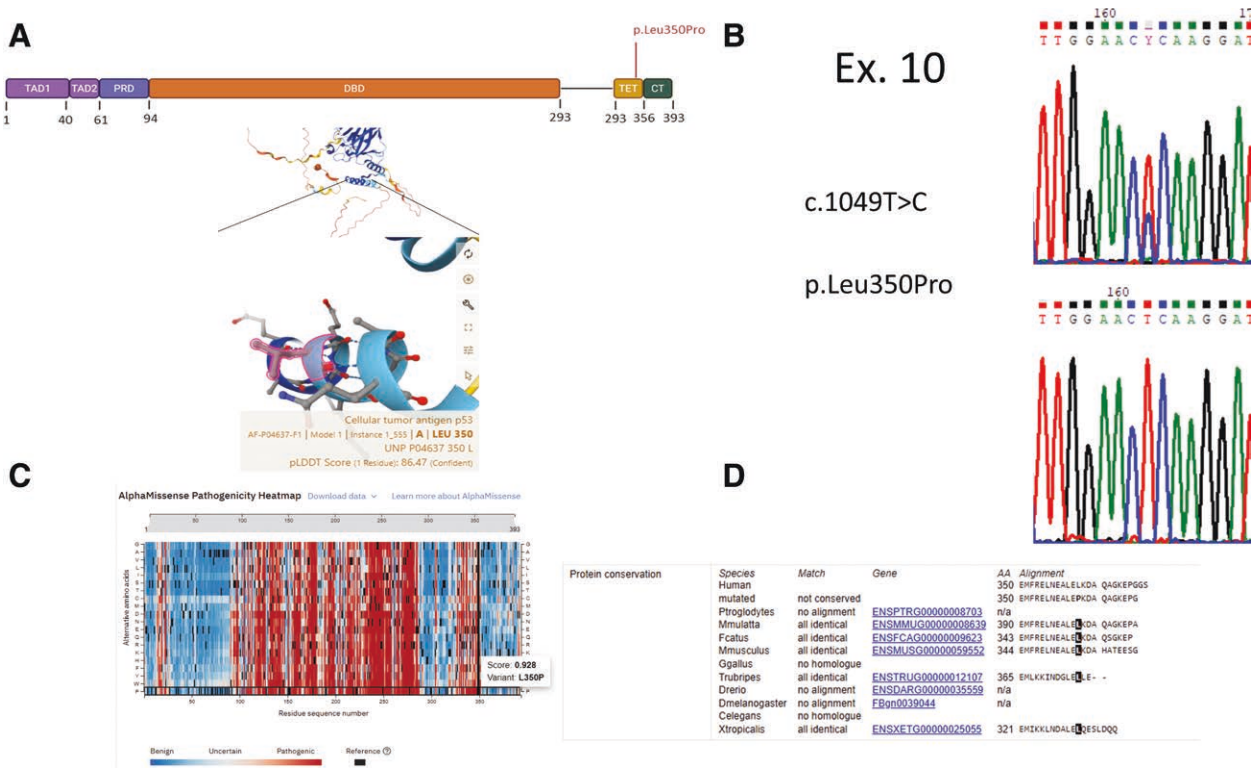

Figure 2. Effects of p.Leu350Pro mutation. (A) This amino acid variation changes the sequence of the last α-helix of the oligomerization domain of the protein (www.alphafold.ebi.ac.uk). (B) Sanger sequencing electropherogram shows the base variation. (C) The AlphaMissense Pathogenicity Heatmap is reported, evidencing the pathogenic prediction of the p.Leu350Pro mutation (www.alphafold.ebi.ac.uk). (D) The protein conservation in phylogenesis evidences that the mutated Leu is maintained unchanged until amphibians (Xenopus tropicalis) and fishes (Takifugu rubripes), underlining its crucial function (www.mutationtaster.org). A part of the figure has been created by biorender.com.

rather than tetramers.[15] Another mutation in the same domain p.Ala347Asp also interfered with the tetramerization forming predominantly dimers.[15]

In t-MDS and t-AML, the most frequent observed mutations are localized in *TP53* gene and are detected in approximatively 30% to 40% of patients; on the contrary, only 5% to 10% of patients with de novo MDS and AML exhibit TP53 mutations.[16]

However, to validate the hypothesis that these 2 TP53 variants are pathogenic and contribute to a more aggressive phenotype, future studies are needed based on functional assays performed in appropriate cell model such as the MOLM cell line, which is FLT3-ITD–positive and harbors wild-type TP53—a genetic context that simulates aggressive AML biology. They will include the transfection of expression vectors carrying the mutant cDNAs, both individually and in combination, to mimic their interaction with endogenous WT p53 in forming protein complexes and the study of the variation in the expression of apoptosis, ferroptosis, and autophagy markers to verify the effects in cell viability.

Recent genomic evidence suggests that selective pressure induced by treatment makes already pre-existing subclonal TP53 mutations become dominant.[17–19] This hypothesis supports a model of parallel clonal evolution alongside myeloid hematopoiesis.[20,21] Unfortunately, in this case, we were not able to discriminate whether the observed TP53 mutations were due to pre-existing clones or new ones acquired during lenalinomide therapy because the Italian guidelines of the Italian Society of Hematology (2021) do not require the research of the somatic mutation for MM patients, and so molecular characterization was not performed at that time. However, despite the use of unsorted bone marrow samples, the observed TP53 variant allele frequencies were compatible with the proportion of myeloid blast, although lineage-specific attribution cannot be formally proven.

Missense mutations, producing single amino acid changes, constitute the majority of mutations occurring in *TP53* gene and were mainly localized in DBD (95.3%).[22] Furthermore, both the frameshift mutations and those which insert a new stop codon, leading to a truncated protein, are considered strongly disruptive of p53 functions.[23] Biallelic mutations of TP53 are leukemogenic and are often defined as part of multihit mutations.[17,24] In an MDS and AML cohort of mutated TP53 patients, it was shown that 36% of the patients were carriers of single hits, while the remaining 64% had double or multihit mutations. This last category of patients, carrying missense and/or nonsense/frameshift mutations, had worse overall survival if compared with the single-mutated patients.[25] Finally, the co-occurrence of multihit TP53 alterations and a highly complex karyotype, as found in our patient, is consistent with data showing that TP53-mutant myeloid neoplasms frequently harbor extensive structural chromosomal abnormalities, reflecting a profound genomic instability that underlies aggressive disease progression and poor clinical outcome.[26] Multi-hit TP53 mutations in therapy-related myeloid neoplasms (t-MDS/t-AML), especially with complex karyotype, define a uniformly aggressive disease subtype. These patients typically show very poor responses to standard therapies, high chemoresistance, and median overall survival often under 10 months. In this setting, even blast count or history (MDS vs AML) loses prognostic significance while multihit TP53 status remains the strongest independent predictor of dismal outcome.[16,26]

4. CONCLUSIONS

Therefore, our study describes 2 novel mutations of TP53 in secondary AML that led to a poor clinical outcome of the patient. However, further studies are needed to definitively address the

role played by these identified mutations in the already complex molecular diversity of AML, although because of the lack of TP53 analysis at MM diagnosis, we cannot rule out the possibility that these mutations were already present before AML.

Nevertheless, this case underlines the complexity of hematological disorders such as MM and MDS, which can change their morphological features and evolve. It highlights the importance of flexible and adaptive management of patients, opening new questions about therapeutic strategies. In fact, the use of lenalidomide exhibits a double face, being useful in both plasma cells and myeloid disorders but raising queries about whether protracted lenalidomide exposure could favor the development of acquired new mutation, especially in TP53, leading to secondary malignancies with poor outcome.

AUTHOR CONTRIBUTIONS

M.A.D.F. and G.B. designed the study and wrote the manuscript. R.R. and A.T. cared for the patient and contributed to the writing of the first draft of the manuscript. A.S. performed molecular analysis. D.B., A.S., C.G., O.B., and R.B. performed results analysis. All authors revised the manuscript critically. All authors read the manuscript and approved the final version for submission.

REFERENCES

[1] Lv JT, Jiao YT, Han XL, et al. Integrating p53-associated genes and infiltrating immune cell characterization as a prognostic biomarker in multiple myeloma. *Heliyon* 2024;10(8):e30123.

[2] Huang J, Chan SC, Lok V, et al. The epidemiological landscape of multiple myeloma: a global cancer registry estimate of disease burden, risk factors, and temporal trends. *Lancet Haematol* 2022;9(9):e670–e677.

[3] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024;74(3):229–263.

[4] Reddi DM, Lu CM, Fedoriw G, et al. Myeloid neoplasms secondary to plasma cell myeloma: an intrinsic predisposition or therapy-related phenomenon? A clinicopathologic study of 41 cases and correlation of cytogenetic features with treatment regimens. *Am J Clin Pathol* 2012;138(6):855–866.

[5] Gramegna D, Bertoli D, Cattaneo C, et al. The role of clonal hematopoiesis as driver of therapy-related myeloid neoplasms after autologous stem cell transplantation. *Ann Hematol* 2022;101(6):1227–1237.

[6] Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer* 1975;36(3):842–854.

[7] Palumbo A, Avet-Loiseau H, Oliva S, et al. Revised international staging system for multiple myeloma: a report from International Myeloma Working Group. *J Clin Oncol* 2015;33(26):2863–2869.

[8] Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. *Leukemia* 2006;20(9):1467–1473.

[9] Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia* 2022;36(7):1703–1719.

[10] GLASS. station2.arrest.tools/glass/. Accessed March 24, 2023.

[11] Pal K, Bystry V, Reigl T, et al. GLASS: assisted and standardized assessment of gene variations from Sanger sequence trace data. *Bioinformatics* 2017;33(23):3802–3804.

[12] Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. *Annu Rev Biochem* 2008;77:557–582.

[13] Berge EO, Huun J, Lillehaug JR, Lønning PE, Knappskog S. Functional characterisation of p53 mutants identified in breast cancers with suboptimal responses to anthracyclines or mitomycin. *Biochim Biophys Acta* 2013;1830(3):2790–2797.

[14] Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med* 2007;357(25):2552–2561.

[15] Annor GK, Elshabassy N, Lundine D, et al. Oligomerization of Mutant p53 R273H is not required for gain-of-function chromatin associated activities. *Front Cell Dev Biol* 2021;9:772315.

[16] Santini V, Stahl M, Sallman DA. Mutations in acute leukemias and myelodysplastic syndromes: insights and treatment updates. *Am Soc Clin Oncol Educ Book* 2024;44(3):e432650.

[17] Grob T, Al Hinai ASA, Sanders MA, et al. Molecular characterization of mutant TP53 acute myeloid leukemia and high-risk myelodysplastic syndrome. *Blood* 2022;139(15):2347–2354.

[18] 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.

[19] Welch JS, Petti AA, Miller CA, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. *N Engl J Med* 2016;375(21):2023–2036.

[20] Bolton KL, Ptashkin RN, Gao T, et al. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nat Genet* 2020;52(11):1219–1226.

[21] Sperling AS, Guerra VA, Kennedy JA, et al. Lenalidomide promotes the development of TP53-mutated therapy-related myeloid neoplasms. *Blood* 2022;140(16):1753–1763.

[22] Tashakori M, Kadia T, Loghavi S, et al. TP53 copy number and protein expression inform mutation status across risk categories in acute myeloid leukemia. *Blood* 2022;140(1):58–72.

[23] Kotler E, Shani O, Goldfeld G, et al. A systematic p53 mutation library links differential functional impact to cancer mutation pattern and evolutionary conservation. *Mol Cell* 2018;71(5):873.

[24] Donehower LA, Soussi T, Korkut A, et al. Integrated analysis of TP53 gene and pathway alterations in the Cancer Genome Atlas. *Cell Rep* 2019;28(11):3010.

[25] Bahaj W, Kewan T, Gurnari C, et al. Novel scheme for defining the clinical implications of TP53 mutations in myeloid neoplasia. *J Hematol Oncol* 2023;16(1):91.

[26] Weinberg OK, Siddon A, Madanat YF, et al. TP53 mutation defines a unique subgroup within complex karyotype de novo and therapy-related MDS/AML. *Blood Adv* 2022;6(9):2847–2853.