haematologica
Journal of the Ferrata Storti Foundation

TP53 mutation analysis in myelodysplastic syndromes – long or short read sequencing?

by Moshe Mittelman and Liran Shlush

Received: March 27, 2026.
Accepted: April 1, 2026.

Moshe Mittelman and Liran Shlush. TP53 mutation analysis in myelodysplastic syndromes – long or short read sequencing?
Haematologica. 2026 Apr 9. doi: 10.3324/haematol.2026.300932 [Epub ahead of print]

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science.
Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication.
E-publishing of this PDF file has been approved by the authors.
After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal.
All legal disclaimers that apply to the journal also pertain to this production process.

TP53 mutation analysis in myelodysplastic syndromes – long or short read sequencing?

Moshe Mittelman[1], Liran Shlush[2]

[1]Department of Hematology, Tel-Aviv Sourasky Medical Center, Tel-Aviv University School of Medicine, Israel

[2]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Correspondence: Moshe Mittelman MD

Tel-Aviv Sourasky Medical Center, Israel

e-mail: moshemt@gmail.com

Contributions: Both authors contributed

Disclosures: Both authors have no relevant COI and nothing to disclose

Diagnosis, prognostication and management of myelodysplastic neoplasms (MDS), like hematology in general, has undergone a paradigm shift over the last few decades. Morphology and blast counting are still relevant, but molecular abnormalities are becoming more relevant [1]. Genetic testing for common myeloid mutations has become an integral part of routine MDS evaluation [1,2]. Practically most labs apply short read sequencing (SRS), as performed by next generation sequencing (NGS), in which multiple short (50–300 base pairs) DNA fragments are generated and simultaneously read [3]. Over the years, we have learnt that it's not only the mutated gene that matters but other features such as, specific hot spots, the variant allele frequency (VAF), the number of mutations, gene-gene interactions can influence prognosis and treatment.

For years, mutations of the *TP53* tumour suppressor gene, the "guardian of the genome", have been known as associated with poor outcomes in MDS [1,4]. The landmark study of Bernard et al. has refined this "all-or-nothing" view [5]. They demonstrated that the adverse prognosis of *TP53* is almost exclusively tied to "multi-hit" (biallelic) inactivation (67% of the *TP53* mutated cases). Patients with a single *TP53* mutation (monoallelic, 33% of MDS patients with *TP53* mutations) often exhibit outcomes similar to those with wild-type *TP53*. In this regard, recent studies have identified mutations in the *TP53* regulator *PPM1D* to be common among patients with therapy-related MDS more than in primary MDS. Furthermore, *PPM1D* carriers had worse prognosis after stem cell transplantation, and they tended to co-occur with *TP53* mutations [6]**.** Monoallelic *TP53* mutations are defined as a single mutation without concurrent deletion or copy neutral loss of heterozygosity (cnLOH). One could argue that biologically a biallelic mutation in *TP53* makes no sense to cells as the mutations have a dominant negative effect. However, the selection pressure for biallelic loss, despite the initial dominant-negative (DN) effect of a single mutation, is a hallmark of clonal evolution. The DN effect, where a mutant *TP53* protein "poisons" the *P53* tetramer, provides an initial advantage by leaving only a small fraction (statistically 1/16 in a heterozygous cell) of functional protein. To achieve total "proliferative freedom," cancer cells benefit from eliminating that last vestige of wild-type activity, which often occurs through Loss of Heterozygosity (LOH) or deletion of the 17p arm, resulting in full biallelic inactivation. The Bernard's discovery prompted the recognition of "MDS with biallelic *TP53* inactivation" as a distinct, high-risk clinical entity in the new 2022 MDS classifications [7,8].

In practice, determining whether two mutations are on the same chromosome (cis), or different chromosomes (trans), a process called phasing, is important for genetic-clinical decision making. The Bernard's paper raised a problem: Short read sequencing (SRS), can identify two or more *TP53* hits, but only when if they are close to each other. If two mutations are far from each other, routine NGS cannot tell if they are on the same allele (*cis*) and mono-allelic or different alleles (trans), biallelic [**Table**]. Thus, accurately defining "biallelic" in the clinic remains a challenge. In practice, when an MDS patient is found to have ≥2 *TP53* mutations, it raises the question: Are these mutations located on the same allele (monoallelic) or on different alleles, indicative of multi-hit *TP53* inactivation ? This has prognostic relevance, dictating the management. Currently, clinicians rely on surrogates and consider as

biallelic any case with multiple mutations, a single mutation with concurrent 17p deletion (via FISH), or a high variant allele frequency (VAF >50%). While these surrogates are highly predictive, they are not direct evidence of phasing, leaving the question of mono or biallelic status open.

In this issue of ***Haematologica***, Zeuthen and colleagues address this question [9]. They utilized the Oxford Nanopore platform, and long-read sequencing (LRS) of the *TP53* coding regions (exons 2-11) in 29 MDS patients with ≥2 *TP53* mutations, 62 mutations totally. They obtained perfect concordance between SRS and LRS for mutation detection (62/62 mutations). Moreover, they could determine the allelic status, and in 28 of 29 patients (96.6%), multiple mutations indeed represented a multi-hit configuration. Only a single patient (3.4%) was found to have two mutations in *cis* (multi-hit but monoallelic status). Their findings provide a reassuring "reality check" for current diagnostic practice: Once SRS demonstrates ≥2 *TP53* mutations, they are very likely multi-hit, and you don't need fancy expensive tools to count or locate those hits. Thus, in a way, for ≥2 *TP53* mutations in NGS, “we have come back to square one”, or in other words, indeed, we are dealing with poor prognostic disease.

LRS also enables detection mutations that cannot be found by SRS and might shed light on the disease biology [10]. However, the main contribution of this paper is determining the allele status of *TP53* multiple mutations, and delivering the message that continuing with SRS in routine practice is enough in most cases.

The study has some limitations. The relatively small sample size (n=29), the lack of cytogenetic data for some patients (e.g., del(17p) or copy-neutral loss of heterozygosity) remains a hurdle. Without chromosomal data, a "monoallelic" sequencing result cannot definitively rule out biallelic inactivation occurring at the structural level. Also, one unresolved nuance discussed by the authors is "sub-clonal mosaicism". There are some technical VAF discrepancies, and lack of clinical correlation. Nevertheless, the pragmatic conclusion of the authors remains: While LRS is technically superior for direct phasing, the high prevalence of true biallelic status in patients with multiple mutations suggests that routine SRS, combined with VAF analysis and cytogenetics, remains sufficient and cost-effective for most MDS patients with multiple *TP53* mutations. LRS may be reserved for "selected patients", those where the VAF is ambiguous or where treatment decisions (transplant ? intensive chemotherapy ?) hinge on the precise definition of the *TP53* state.

References

1. Cazzola M, Malcovati L. Genome sequencing in the management of myelodysplastic syndromes and related disorders. Haematologica. 2025;110(2):312-329.

2. Oster HS, Van de Loosdrecht AA, Mittelman M. Diagnosis of myelodysplastic syndromes: the classic and the novel. Haematologica. 2025;110(2):300-311.

3. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241-247.

4. Bejar R. Implications of molecular genetic diversity in myelodysplastic syndromes. Curr Opin Hematol. 2017;24(2):73-78.

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. 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.

7. 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.

8. Arber DA, Orazi A, Hasserjian R, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.

9. Zeuthen M, Christoffersen LS, Hildonen M, et al. Long-read sequencing resolves TP53 allelic status in myeloid neoplasms. Haematologica. xxx

10. Abel HJ, Mahgoub M, Davarapalli N, et al. Evaluation of long-read genome sequencing for genomic profiling of myeloid cancers. J Mol Diagn. 2025;27(12):1242-1254.

Table 1. Allelic state assessment: standard short-reading sequencing (SRS) vs. long-reading sequencing (LRS).

Feature	Short-Read Sequencing (SRS)	Long-Read Sequencing (LRS)
Technology examples	Illumina (MiSeq/NextSeq), Ion Torrent	Oxford Nanopore Technologies (ONT), PacBio
Typical read length	150 – 300 base pairs	10,000+ base pairs (can span the full gene)
Phasing capability	Indirect / Inferential: Limited to variants within the same 300bp fragment.	Direct / Definitive: Can phase variants across the entire *TP53* locus (Exons 2–11).
Biallelic detection	Based on surrogates (VAF >50%, multiple mutations, or concurrent del(17p) via FISH).	Direct observation of whether mutations are on the same (*cis*) or different (*trans*) alleles.
Resolution of distant variants	Cannot determine if mutations in different exons (e.g., Exon 4 and Exon 8) are on the same molecule.	Easily resolves distance variants due to long contiguous reads.
Clinical classification	Generally sufficient for ICC/WHO 2022 biallelic definitions in most cases.	Highly precise; eliminates rare false positives for "multi-hit" status (approx. 3.4% in Zeuthen et al.).
Logistics	Standard of care, high throughput, widely available.	Emerging; offers rapid turnaround but requires specialized bioinformatic pipelines.