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Myelodysplastic Syndromes

DEVELOPING A SCALABLE MODEL FOR GENETIC ANALYSIS OF EXTRA-MYELOID TISSUES TO RESOLVE SOMATIC-GERMLINE AMBIGUITY IN MYELOID DISORDERS

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Genomic characterization using next-generation sequencing (NGS) has become a widely adopted tool in myeloid neoplasms (MN) and bone marrow failure (BMF) syndromes. It now supports diagnostic classification, prognostic stratification, and the identification of predictive and therapeutic biomarkers. However, the widespread NGS use increasingly reveals variants with interpretation challenges, highlighting the need appropriate tissue selection to distinguish somatic vs. germline variants.

Within the GEMMA Project, a hematology–genetics outpatient program at Rome Policlinico Tor Vergata focused on identifying germline predisposition to BMF/MN, we analyzed samples from 81 individuals (03/2023-10/2025). These included 45 BMF/MN patients and 36 family members at risk. Control DNA was extracted from non-myeloid tissues in probands using CD3+ lymphocytes (n=31), fibroblasts (n=1), and nails (n=16). Peripheral blood mononuclear cell's DNA was analyzed for family members.

We confirmed the germline nature of variants in 13/45 patients. In 5 cases, germline origin was suspected based on variants with ~50% variant allele frequency (VAF) in myeloid panels for *SH2B3* (n=3) and *RUNX1* (n=2) variants, and was confirmed on DNA from nails. Germline confirmation derived from clinical exome sequencing in 8 cases, involving variants in *GATA2*, *DDX41*, *TERC*, *SAMD9*, and *FANCA*. Analysis of CD3+ T cells revealed a revertant mosaicism in a compound heterozygous *FANCA*-mutated patient. The recurrent identification of *GATA2* and *DDX41* variants prompted us to adopt a custom myeloid NGS panel from 30 to 51 genes as standard approach.

Conversely, analysis of non-myeloid tissues excluded the germline nature of the variants in 7 acute myeloid leukemia cases (5 *CEBPA*-mutated, 2 *TP53*). This was further confirmed by therapy-dependent VAF fluctuations and was consistent with the recurrence of variants in these genes as somatic.

The incidental finding of a *MYD88* variant at 52% VAF in a primary myelofibrosis case, prompted nail DNA testing, which excluded the somatic origin and prevented misclassification of clonality.

Accurate tissue selection is crucial for precision diagnostics in MN/BMF, not only to detect germline variants underlying disease predisposition, but also to exclude somatic mutations that could misdirect clinical management. While fibroblasts are the gold standard, nails (and CD3+ cells if chosen wisely) offer a rapid and minimally invasive alternative to distinguish somatic from germline variants. The traditional model of genetic testing management, where germline (hereditary) and somatic (cancer) results are handled through separate pathways and by different specialists, is fragmented, inefficient, and confusing for patients (10.1038/s41591-025-03686-8). We believe our experience supports a "patient-centered" scalable model **(Fig 1)** in which the two streams of information are integrated from the outset and presented to the patient in a unified manner.

MYELODYSPLASTIC SYNDROMES

Fig 1. Scalable model for genetic analysis of extra-myeloid tissues