# Detectable mutations precede late myeloid neoplasia in aplastic anemia

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SUPPLEMENTARY DATA

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**Supplemental Table 1**

**A) Methods and samples used for mutation discovery and tracking**

| | At clonal evolution | Prior timepoints |
|---|---|---|
| **UPN 1** | Research NGS (CD117 sorted PBMCs) | ddPCR (*NPM1*) |
| **UPN 2** | Research NGS (CD117 sorted PBMCs) | ddPCR (*RUNX1*) |
| **UPN 3** | Clinical NGS, scDNA-seq | Clinical NGS |
| **UPN 4** | Clinical NGS, Research NGS (CD34 sorted PBMCs) | ddPCR (*RUNX1*) |

DNA-sequencing was performed using targeted myeloid panels of commonly mutated gene regions in myeloid malignancies by either a commercial clinical laboratory ("Clinical NGS") or using an anchored multiplex PCR-based panel incorporating molecular barcode/unique molecular identifiers (ArcherDx, CO) in a NIH research laboratory ("Research NGS").

ddPCR was performed using approximately 1µg of DNA isolated from bone marrow cells (BMCs), using Custom TaqMan SNP Genotyping Assays (cat# 4351379, Thermo Fisher Scientific, Waltham, MA) on the RainDrop platform (RainDance Technologies, Lexington, MA). Data were analyzed using RainDrop Analyst II software.

Single-cell DNA sequencing (scDNA-seq) assay utilizing cell-identifying molecular barcodes, covering 45-genes commonly mutated in myeloid malignancies (Mission Bio, CA), was employed on BMCs from one patient, with paired-end 150-bp sequencing performed utilizing unique dual sample indices on an Illumina HiSeq 2500 (rapid run mode).

FASTQ files are available in the NCBI Small Reads Archive (SRA) (Accession: PRJNA645315).

**B) Clinical NGS Results from UPN 3 (unsorted bone marrow)**

| Gene | Mutation | Baseline (%) | Year 2 (%) | Year 4 (%) | Year 5 (%) | Year 7 (%) |
|---|---|---|---|---|---|---|
| *ASXL1* | c.1900_1922del; p.E635RfsTer15 | 19.3 | 24.6 | 42.0 | 50.7 | 23.3 |
| *RUNX1* | c.319C>T; p.R107C | | 33.3 | 33.3 | 44.4 | 51.6 |
| *SETBP1* | c.2602G>A; D868N | | | 38.8 | 39.4 | 53.1 |
| *PHF6* | c.821G>A; p.R274Q | | | | | 5.7 |