Development of highly reproducible and sensitive sequencing-based methods for the detection of measurable (minimal) residual disease (MRD) in acute myeloid leukemia

Patients with *de novo* or secondary non-M3 AML

- Presence of NPM1 type A mutation or single nucleotide variants (SNVs) in FLT3, IDH1 and/or IDH2 at diagnosis
- Availability of one follow-up genomic (g)-DNA sample

**Deep targeted NSG-based method with digital PCR**

**Experimental workflow**
- DNA amplification
- Library preparation
  - Sequencing 1,000,000 of reads
  - .fastq file

**Bioinformatic workflow**
- Data input: FASTA
- Data output: .csv file
- Ratio: mutated sequences/wt sequences

- Sensitivity of $10^4$ for SNVs and $10^5$ for NPM1 insertions/deletions
- MRD positive status by NSG sequencing

**Serial dilution:**
- Peripheral blood from healthy adult donor
- Cell lines or AML patient cells

**Digital targeted RNA-sequencing-based approach**

- Total RNA
- Targeted reverse transcription and molecular barcoding
- RNA of interest
- Targeted PCR of barcoded cDNA
- Barcoded cDNA
- Library construction and sample indexing
- Targeted library

- Leukemic cells could be detected down to as low as 1 per 100,000 healthy donor cells
- Detection sensitivity between 1:10,000 to 1:100,000 for the fusion and mutated NPM1 transcripts
- 1M reads are needed for the detection of fusion transcripts and 3M reads for the detection of NPM1 insertion mutations

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*Multivariate analysis

Onecha *et al.*, Haematologica, 2019

Dillon *et al.*, Haematologica, 2019