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

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

Criteria
- 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 .fastq file .csv file
- chromosome position
- genomic variant

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

Lower disease-free survival	HR 3.4, p=0.005
Lower overall survival	HR 4.2, p<0.001
Risk of death	HR 4.54, p =0.005*
Risk of relapse	HR 3.76, p =0.012*

***Multivariate analysis**

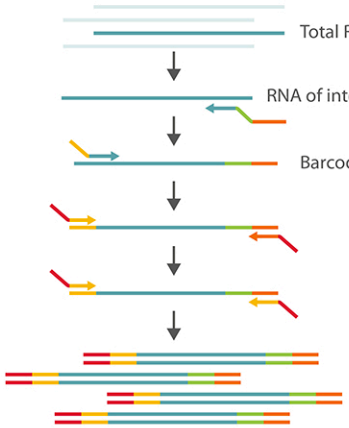
Onecha *et al.*, Haematologica, 2019

Serial dilution:

Peripheral blood from healty adult donor

Cell lines or AML patient cells

Digital targeted RNA-sequencing-based-approach



Targeted reverse transcription and molecular barcoding

Targeted PCR of barcoded cDNA

Library construction and sample indexing

- 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

Dillon *et al.*, Haematologica, 2019