Utility of Blood vs Bone marrow for molecular MRD in AML and MDS

- superRCA, an ultra-sensitive mutation detection method using flow cytometer readout

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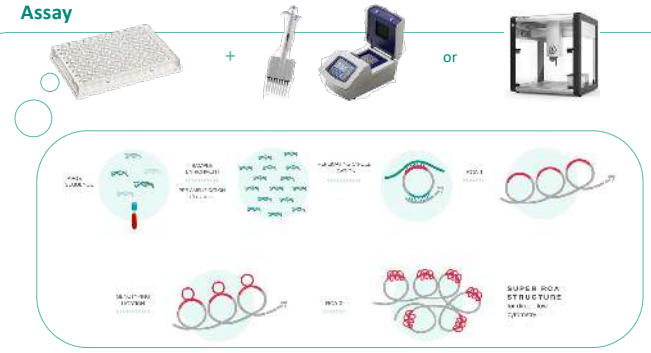
Introduction: This study aims at investigating the utility of peripheral blood for molecular MRD in patients diagnosed with MDS or AML. This is an ongoing study and a subset of data from the initial pilot cohort is presented in this poster.

Method: Patients in this retrospective study have all undergone sequencing (TruSight panel, Illumina) upon diagnosis, after which patient specific driver mutations (1-5 per patient) has been chosen to monitor molecular MRD using ddPCR mutation assays (Bio-Rad). These longitudinal samples has now been re-analysed using superRCA mutation assays (Rarity Bioscience), a novel and ultra-sensitive technique for mutation detection using flow cytometer for readout. Both bone marrow aspirates and peripheral blood are included to investigate to what extent blood derived molecular MRD gives equivalent relapse information as bone marrow.

Assay



Readout

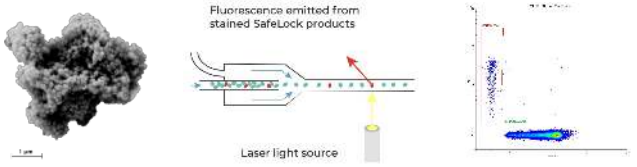


Figure1: Using superRCA assay, sequences of interest are developed into giant DNA particles with existing lab equipment. During such giant DNA particle generation process, a majority voting-based sequence genotyping step is embedded, and the fluorescent signal of each DNA particle reflects the genotyping result. It is possible to detect up to 50 different mutations present in the same DNA sample at a sensitivity of 1 in 100,000 readout by standard flow cytometer.

Preliminary Data - Long Longitudinal monitoring of point mutations in both Blood DNA samples and Bone Marrow DNA samples

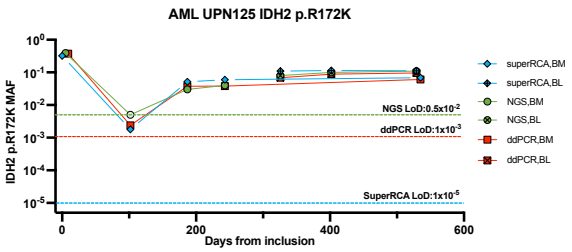


Figure 2: AML Patient UPN125 was included in the study from Day 0. The level of IDH2 p.R172K positive clone in blood and bone marrow indicates residual disease levels during treatment. NGS, ddPCR and superRCA were analyzed side by side on the same patient samples for comparison.

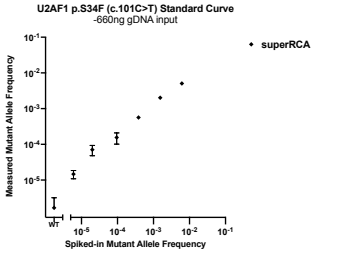


Figure 3: 4-fold dilution curve of superRCA U2AF1 p.34F mutation assay using U2AF1 p.S34F positive genomic DNA samples spiked into wild-type genomic DNA, total 660ng assay input. Mean and SD from 3 replicates*.

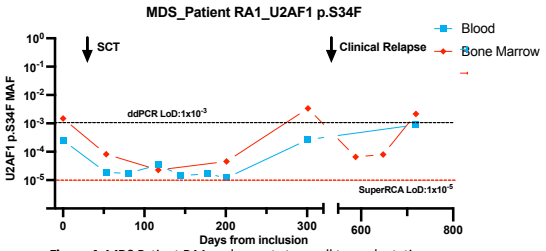


Figure 4: MDS Patient RA1 underwent stem cell transplantation (SCT) in 20 days after inclusion into the study. The levels of U2AF1 p.S34F in blood and bone marrow indicates residual disease and molecular relapse before confirmed clinically relapse after 330 days from inclusion*.

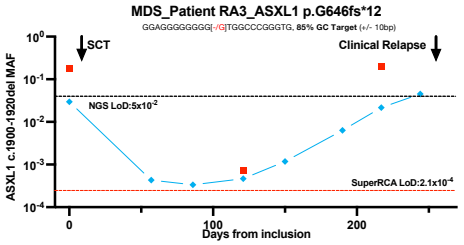


Figure 5: MDS Patient underwent stem cell transplantation (SCT) 25 days after the inclusion. The levels of ASXL1 p.G646fs*12 positive clone in blood and bone arrow indicates residual disease and molecular relapse before it was confirmed clinically in 245 days after the inclusion*.

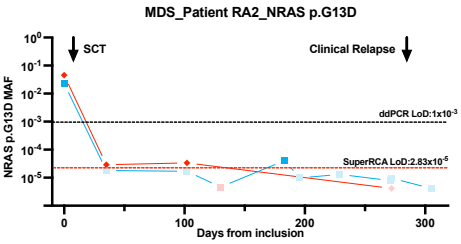


Figure 6: MDS Patient RA 2 underwent stem cell transplantation (SCT) 8 days after the inclusion of the study. The levels of NRAS p.G13D positive clone disappeared both in the blood and bone marrow sample while the patient still had a clinical relapse confirmed at Day 306 from the inclusion of the study*.

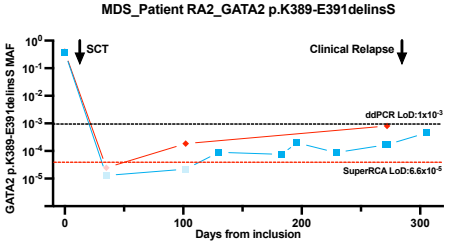


Figure 7: MDS Patient RA2 underwent stem cell transplantation (SCT) 8 days after the inclusion of the study. The levels of GATA2 p.K389_E391delinsS positive clone in blood and bone marrow indicates residual disease and molecular relapse before it was confirmed clinically at Day 306 from the inclusion of the study *.

Conclusion: Preliminary data demonstrates superRCA mutation assays for the selected driver mutations can reach to very low limit of detection (10^{-5} level) and mutations could be detected in both blood and bone marrow upon relapse.

**Unpublished data, public access is restricted.*