

New assay technology and utility of peripheral blood vs bone marrow for molecular MRD in AML and MDS

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Background

Rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy. However, improved assay techniques are needed for broad adoption in routine clinics. The superRCA assays, which provide for rapid and highly specific detection of DNA sequence variants present at very low frequencies in DNA samples using a standard flow cytometer for readout. We demonstrate and compare the assay against ddPCR for precise, ultra-sensitive detection of single-nucleotide mutant sequences from malignant cells to follow the course of AML and MDS patients following allogeneic stem cell transplantation, both in bone marrow and peripheral blood.

Methods

This study is based on patient material from the Nordic MDS study NMDSG14B (NCT02872662) and AML samples from the U-CAN biobank. The patients in all previously undergone sequencing (TruSight panel, Illumina) upon diagnosis, after which patient specific driver mutations (1-4 per patient) has been chosen and analyzed longitudinally to monitor molecular MRD using ddPCR (Bio-Rad). These longitudinal samples were re-analyzed using superRCA mutation assays (Rarity Bioscience), an ultra-sensitive technique for mutation detection using flow cytometer for readout. Both quarterly bone marrow aspirates and monthly peripheral blood samples post allogeneic stem cell transplantation were included to investigate to what extent frequent blood derived molecular MRD gives equivalent relapse information as bone marrow.

Results

A total of 22 relapse patients and 21 different mutations were analyzed, covering single nucleotide variants, insertions, deletions, as well as mutations with very high GC in ASXL1 and SRSF2.

The analysis demonstrated lower background and limit of detection for the superRCA assays compared to the equivalent ddPCR data. With spike-in dilution series, we demonstrated the superRCA assay could detect single point mutations as low as 1 in 100,000 (mean LOD = 0,0023%) with the flow cytometer readout.

In longitudinal sample analysis of relapse patients, superRCA assay detected remaining mutations after initial treatment and clearly revealed the remaining malignant clone, subsequently leading to a relapse. The superRCA assay also demonstrated the feasibility of detecting corresponding leukemia mutations in the PBMCs when such mutations were present in bone marrow.

Conclusions

The data shows comparable mutant allele frequency results between superRCA and ddPCR upon diagnosis and relapse. The data further demonstrates superRCA mutation assays for the selected driver mutations can reach to very low limit of detection and increasing mutations could be detected

in both blood and bone marrow predicting the relapse of MDS post transplantation in clinical samples.

