



The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS**New Assay Technology and Utility of Peripheral Blood Vs Bone Marrow for Molecular MRD in AML and MDS**Magnus Tobiasson, MD¹, Linus Bosaeus, msc², Lucia Cavelier, PhD³, Lei Chen, PhD^{2,4}¹ Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institutet, Stockholm, SWE² Husargatan 3, Rarity Bioscience AB, Uppsala, Sweden³ Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, SWE⁴ Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden**Background**

Rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy in clinical routine, and improved assay techniques are needed for broad adoption. We describe herein - superRCA assays - which provides 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 this retrospective study have 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), a novel and 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.

For each mutation, sensitivity was assessed through serial dilution and wild type control, with the limit of detection calculated as $Mean(wt) + 3 \cdot SD(wt)$. Samples were analyzed in duplicates or triplicates and sample to sample variation was calculated as $SD/Mean$.

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 demonstrating 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 with the flow cytometer readout. Mean background for superRCA was 0,0016% in wild type controls, and mean limit of detection was 0,0023%. From available serial dilutions, the mean sample to sample variation (CV) at 0,016% was 22,87% and 29,32% at 0,001% dilution.

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.

The low detection limit and high precision of superRCA are consequences of the highly selective genotyping of the repeated target sequences in combination with high input capacity and the large numbers of products that may be conveniently ana-

lyzed by flow cytometry. To further study the utility of using peripheral blood for molecular MRD in MDS and AML patients, more data is needed and studies are ongoing.

Disclosures Bosaeus: *Rarity Bioscience:* Current Employment, Current equity holder in private company, Current holder of stock options in a privately-held company. **Chen:** *Rarity Bioscience:* Current Employment, Current equity holder in publicly-traded company.

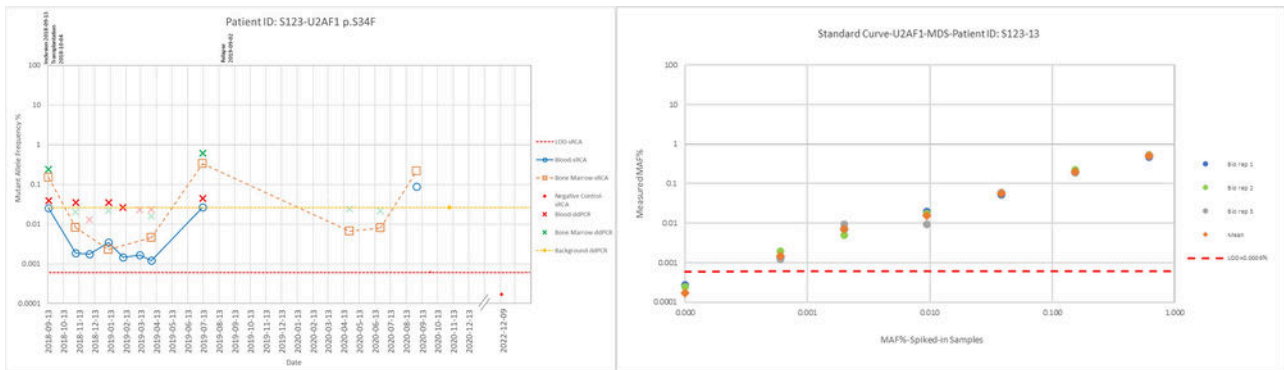


Figure 1

<https://doi.org/10.1182/blood-2023-183013>

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement_1/6039/2/188403/blood-2637-main.pdf by guest on 18 May 2024