Use of the new superRCA mutation assay for molecular MRD monitoring using flow cytometry in pediatric AML

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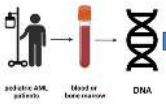
Introduction: Acute myeloid leukemia (AML) is the second most acute leukemia in children. Thanks to the use of study-validated combination chemotherapies in conjunction with sensitive diagnostic detection methods, more than 70% of patients can currently be cured. The superRCA method, which can quantitatively detect biomarkers in leukemic AML blasts as a combination of molecular and flow cytometric methods with high sensitivity, could usefully supplement the diagnostic methods available to date. Based on the detection of mutations in the Wilms-Tumor gene (WT1; HGNC 12796), which is mutated in 6% of all pediatric AML patients, superRCA assays were established for a patient with two WT1 mutations as part of an ongoing validation study by analyzing samples at diagnosis and after treatment (peripheral blood (PB) and bone marrow(BM)) were analyzed. For comparison and assessment, the results were evaluated using an amplification-refractory mutation assay (ARMS). The aim was to clarify whether the superRCA assay can be used for MRD diagnostics.

Aim: This project is designed to test the suitability of the superRCA method for the supplementary diagnosis of pediatric AML.

Material & Methods: DNA was extracted from PB and BM samples of a patient with AML (AML with maturation; WHO 2022) using an automated process (Maxwell RSC Cultured Cells DNA Kit Promega). Mutation screening was performed with a custom myeloid panel from Sophia Genetics (Sophia Genetics, Rolle, Switzerland). Next generation sequencing (NGS) was performed on an NextSeq 2000 sequencer (Illumina, San Diago; USA).



For data analysis, SOPHiA DDM™ Software was used. Padlock probes for the identified WT1-mutations were designed and synthesized as described by Chen et al 2022. Rolling circle amplification of DNA was performed as described by the manufacturer (Rarity Bioscience, Uppsala, Sweden). Measurement and analysis of mutant allel frequency (MAF) of the products was done using a DxFLEX Flow Cytometer (Beckman Coulter, CA, USA). For comparison an DNA based quantitative amplification-refractory mutation system (ARMS) was established for one mutation.

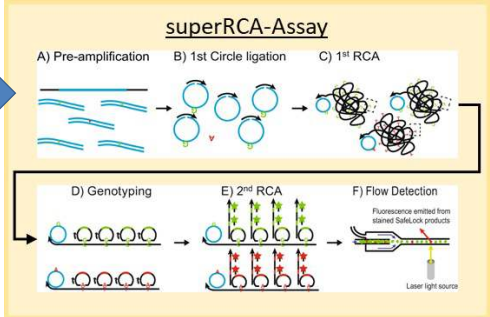


Figure 1: superRCA-Assay (adapted from Chen et al. 2022[1])

Results:

Two mutations in the WT1 gene (c.1078_1091dup; c.1086dup) were detected using NGS. Padlock probes were successfully developed for both mutations. During follow-up, PB and BM samples were analyzed in parallel for six time points. The superRCA showed positive results for both WT1 mutations in all six BM and all three PB samples. Comparing the results of both mutations in the BM, only minor deviations (mean MAF: 8.6%) were shown.

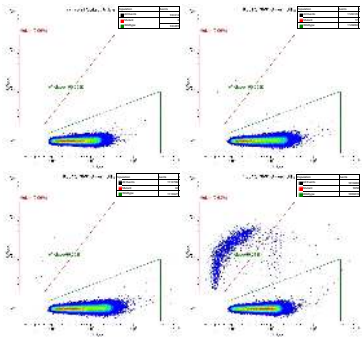


Figure 2: WT1 *c.1078_1091dup* assay. Example data: Top left: Negative control. Top right: Positive control 0.001% MAF. Bottom left: Positive control 0.004% MAF. Positive control 1.024% MAF. (Courtesy from Rarity Bioscience).

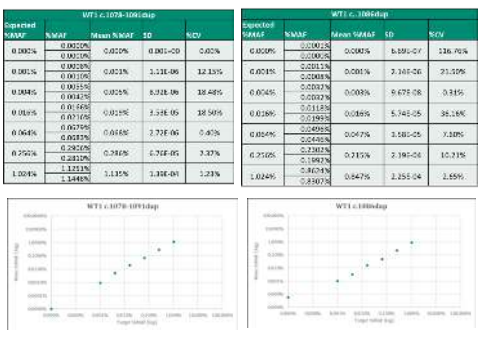


Figure 3: Results of spike-in experiments for *WT1*:c.1078-1091dup and *WT1*:c.1086dup for the calculation and quality control of Mutant Allel Frequencies (%MAF).

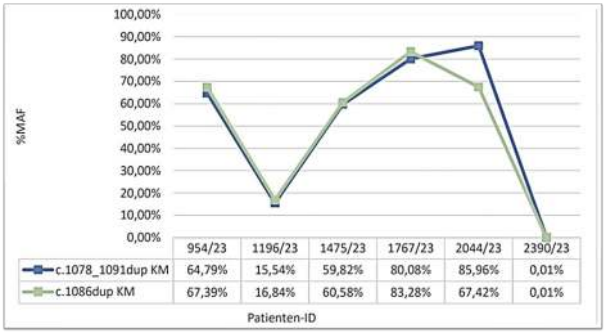


Figure 4: SuperRCA MRD diagnostic using two *WT1-Mutations* (***c.1078_1091dup; c.1086dup***) *as biomarker.* **Timepoints**: **954/23**: start of therapy; **1196/23**: after Induction1; **1475/23:** after induction 2; **1767/23**: after FLA+GO; **2044/23**: before stem cell transplantation (SCT); **2390/23**:after SCT.

Conclusion: The superRCA assay's detection of molecular biomarkers shows promise for MRD monitoring in pediatric AML. As it uses absolute quantification between wild-type and mutant, no reference to an initial sample is needed for MRD load calculation. The method's robustness and specificity is shown by the high concordance of results when detecting two mutations in the same gene.

References: 1. Chen et al. 2022. Ultra-sensitive monitoring of leukemia patients using superRCA mutation detection assays; Nature Communications

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