NRAS, WT1, NPM1 and IDH mutation detection for molecular MRD using Flow Cytometry in pediatric AML

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INTRODUCTION

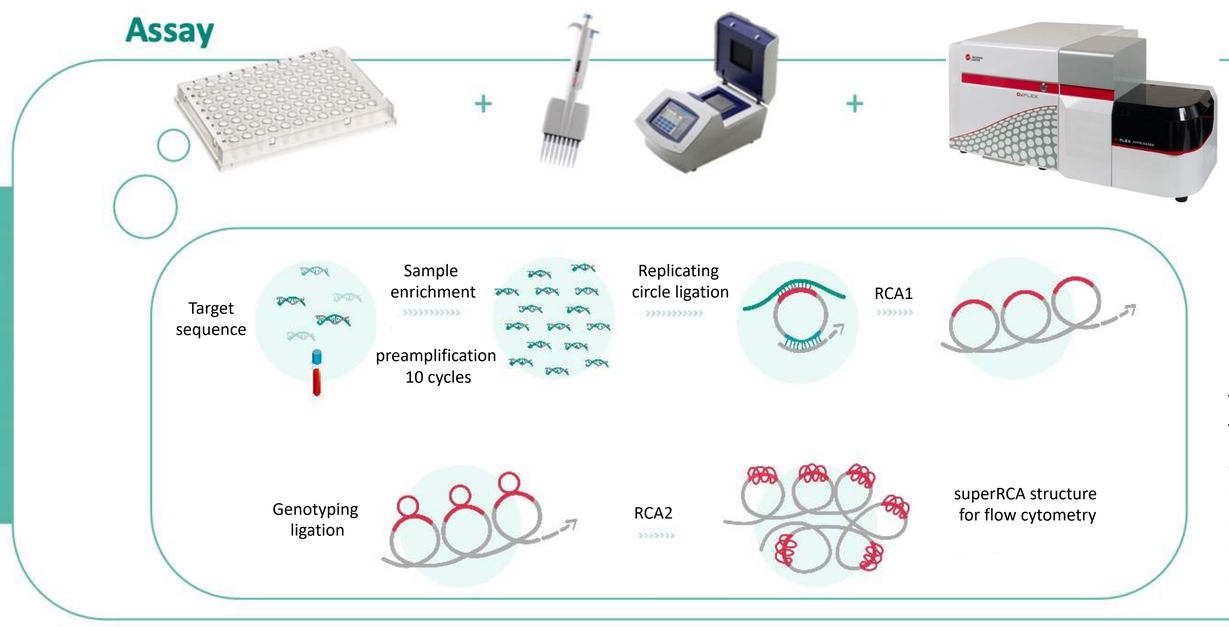
MRD is a key prognostic indicator in pediatric leukemia. Molecular methods complement multiparametric Flow Cytometry (MFC), enhancing sensitivity and early relapse detection. We present data from a novel molecular assay using Flow Cytometry for the multiplex detection of driver mutations, including NRAS, WT1, NPM1, and IDH1/2.

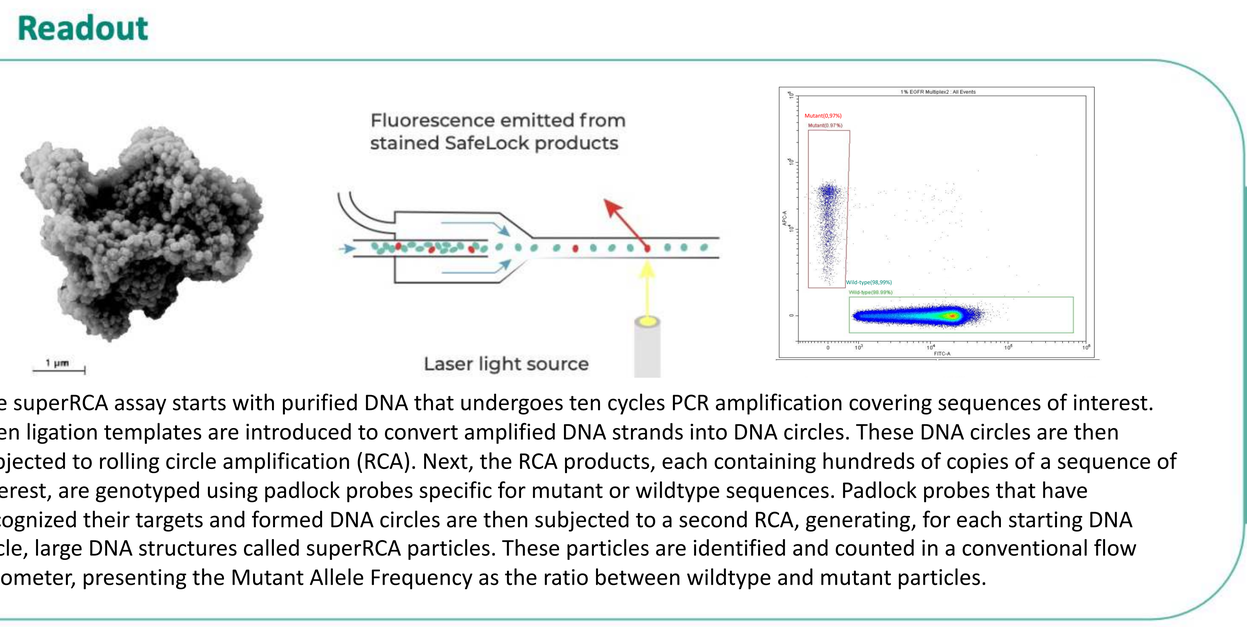
OBJECTIVE

Assessment of new molecular assay technology for MRD using flow cytometry readout.

METHOD

In this retrospective non-interventional study, the initial diagnostic work-up contained mutation screening using targeted Next Generation Sequencing (Trusight Myeloid Panel, Illumina, CA, USA) at the BFM reference laboratory for molecular diagnostics in pediatric acute myeloid leukemia in Essen, Germany. Follow-up samples had previously been analyzed using MFC (DxFLEX, Beckman Coulter, CA, USA) and, when applicable, DNA-based genomic breakpoint specific qPCR following EuroMRD guidelines. For this study, patient samples from diagnosis and follow-up (including relapse timepoints) preserved in liquid nitrogen were analyzed and compared using a new molecular MRD method superRCA (Rarity Bioscience, Uppsala, Sweden) for potential driver gene mutations identified at diagnosis. Analysis was done over two days by two operators, one previously untrained.





The superRCA assay starts with purified DNA that undergoes ten cycles PCR amplification covering sequences of interest. Then ligation templates are introduced to convert amplified DNA strands into DNA circles. These DNA circles are then subjected to rolling circle amplification (RCA). Next, the RCA products, each containing hundreds of copies of a sequence of interest, are genotyped using padlock probes specific for mutant or wildtype sequences. Padlock probes that have recognized their targets and formed DNA circles are then subjected to a second RCA, generating, for each starting DNA circle, large DNA structures called superRCA particles. These particles are identified and counted in a conventional flow cytometer, presenting the Mutant Allele Frequency as the ratio between wildtype and mutant particles.

RESULTS

- 23 bone marrow samples from 5 pediatric AML patients analyzed for potential driver mutations in NRAS (4), WT1 (1), NPM1 (2), IDH1 (1)
- superRCA assays mean Wild-type control= 2,11E-05 (0-8,16E-05)
- superRCA between operators CV= 4.11% (0.33-8.68%)
- Correlation between superRCA and NGS, mean variation of 3,55% across all targets, Pearson correlation of r=0.93, p=0,0009

Table 1. Sample analysis including MFC, PCR, and mutation analysis superRCA (NGS)

Patient		Diag.	IND1	IND2	CON1	Relapse	Rel_IND1	Rel_IND2
Vie 1	MFC	38%	0	-	-	-	-	-
	NPM1 Mut A	28,28% (28,84%)	0,06%	-	-	-	-	-
	WT1 c.1078_1091dup	21,00% (24,22%)	0,03%	-	-	-	-	-
Vie 2	MFC	34%	0%	-	-	-	-	-
	NPM1 Mut B	47,54% (37,7%)	0,10%	-	-	-	-	-
Vie 3	MFC	61%	-	-	-	6,30%	20,98%	33,60%
	NRAS p.G12S	35,88% (38,2%)	-	-	-	0.207%	2,63%	0,143%
Vie 4	MFC	95%	-	0%	0%	-	-	-
	IDH1 p.R132C	43,96% (45,5%)	-	0,09%	0,39%	-	-	-
	NRAS p.G12D	30,39% (34,8%)		0,02%	0,08%			
	NRAS p.Q61K	8,35% (8,2%)		0,00%	0,01%			
Vie 5	MFC	84%	0%	0%	0%	-	-	-
	PCR - MLL-MLLT3	-	Neg	Neg	Neg	-	-	-
	NRAS p.G12A	40,5% (46,85%)	0,00%	0,00%	0,00%	-	-	-

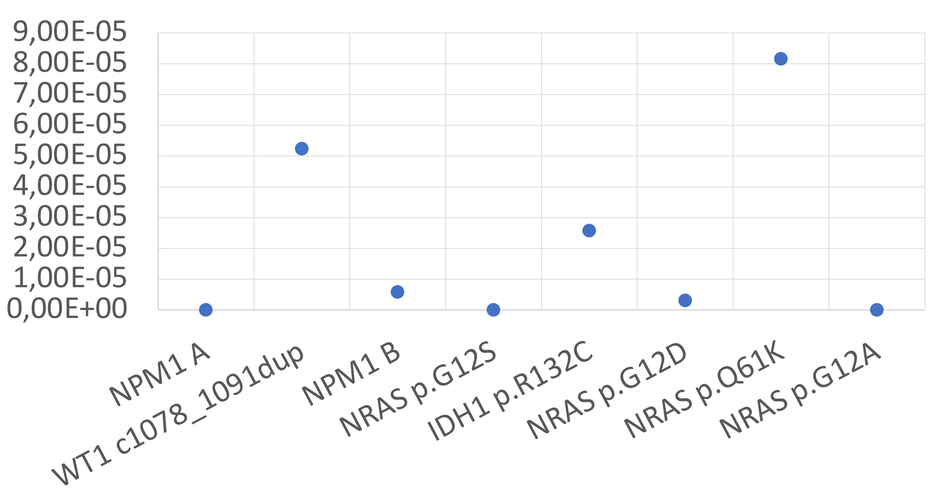


Figure 1. Mean Wild type background, control samples in replicates

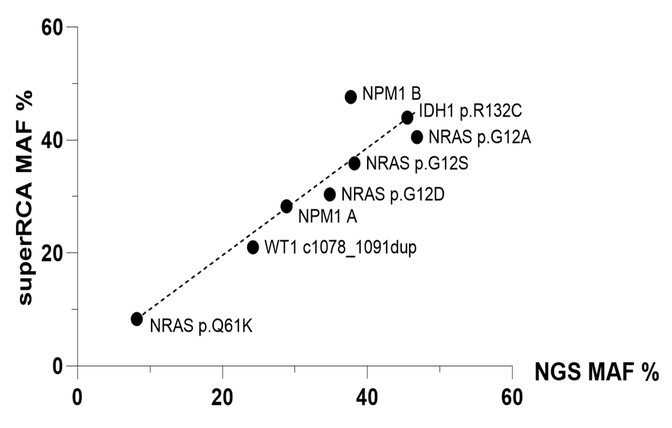


Figure 2. Correlation between NGS and superRCA MAF all targets

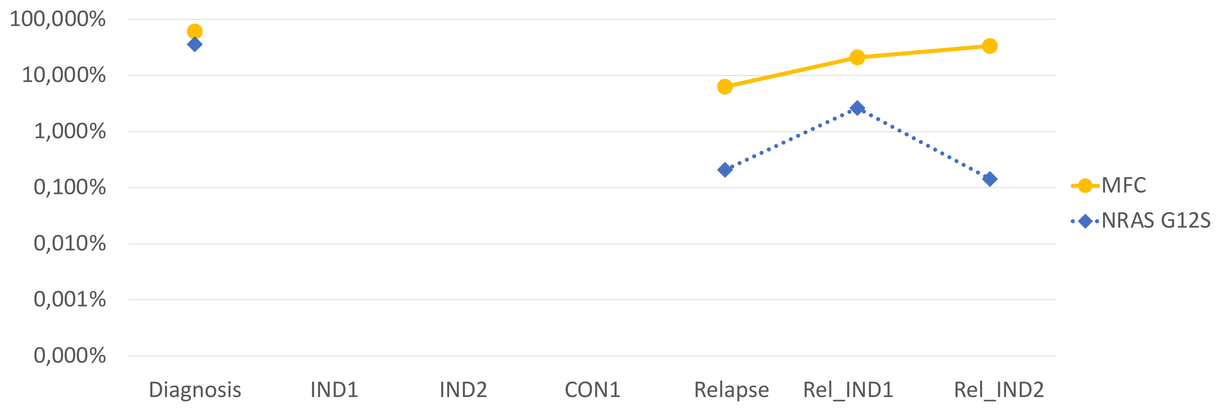


Figure 3: Correlation between MFC and superRCA at diagnosis, relapse, and during follow-up in a patient (Vie3) who developed treatment resistance after relapse. Divergence between MFC and superRCA suggests the presence of subclonal mutations. Abbreviations: IND1, post-Induction 1; IND2, post-Induction 2; CON1, post-Consolidation 1; Rel, Relapse

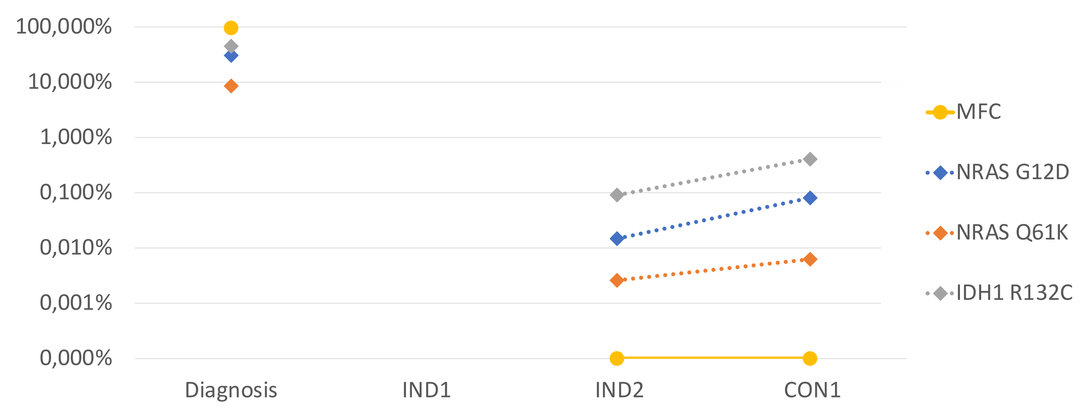


Figure 4. **Correlation between MFC and superRCA** at diagnosis and two follow-up time points of Patient Vie4**.** Instances where MFC is negative but superRCA remains positive may indicate either that blast counts are below MFC's detection threshold or that mutations exist in mature cells, which are not captured when monitoring MRD via MFC. Abbreviations: IND1, post-Induction 1; IND2, post-Induction 2; CON1, post-Consolidation 1.

CONCLUSION

Analysis of molecular MRD using flow cytometry is viable approach and may particularly add value to pediatric leukemia patients with identified driver mutations. The superRCA assay shows strong correlation to the NGS data at diagnosis and demonstrated a very low limit of detection for molecular MRD analysis at follow up. This warrants further evaluation in prospective studies.