Evaluation of MiSelect R System: A Comparison with Flow Cytometry and/or RT-qPCR Assays for Measurable Residual Disease Monitoring in Acute Myeloid Leukemia

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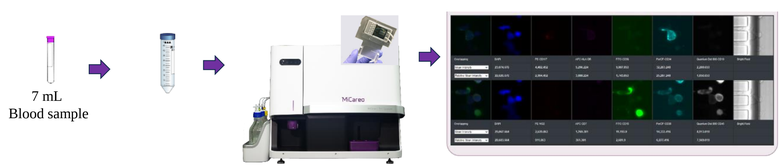
Introduction and Purpose

Two widely used methods for measurable residual disease (MRD) detection in acute myeloid leukemia (AML), multi-parameter flow cytometry (MFC) and real time reverse transcription-quantitative PCR (RT-qPCR), have notable limitations. MFC has suboptimal sensitivity and requires invasive bone marrow (BM) sampling, while RT-qPCR, despite its high sensitivity, is applicable only to patients with specific genetic alterations. MiSelect R, a microfluidic and image-based platform[1,2], isolates rare leukemia-associated cells and employs imaging for accurate enumeration and phenotypic analysis. We aimed to develop a MiSelect R-based MRD method to address the limitations of existing approaches.

Methods

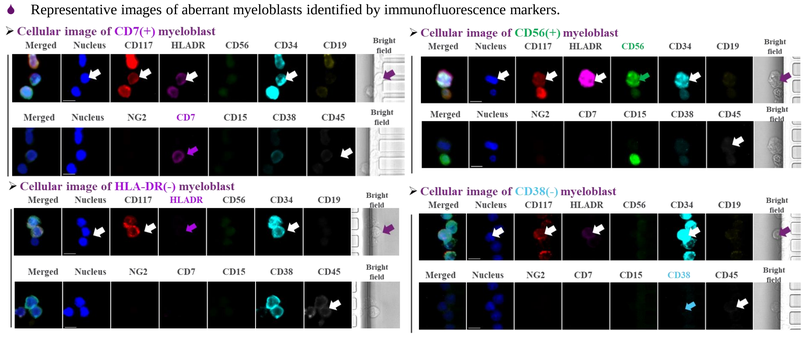
We evaluated MRD in 69 paired bone marrow (BM) and peripheral blood (PB) samples collected after treatment from 43 AML patients. MiSelect R and 8-color MFC were used to detect leukemia-associated immunophenotypes in 58 follow-up samples from 38 patients. RT-qPCR assays were performed in 41 follow-up samples from 26 patients to quantify molecular alterations, including *NPM1* mutations, *RUNX1::RUNX1T1*, *CBFB::MYH11*, *KMT2A*-PTD, and *KMT2A* rearrangements (R). MRD positivity was defined as follows: ≥ 0.01% for MFC, ≥ 5×10^{-7} for MiSelect R, and gene-specific thresholds for RT-qPCR: NCN ratio ≥ 0.01% for *NPM1* mutations; ≤ 3-log reduction for *RUNX1::RUNX1T1* and *CBFB::MYH11* (according to EHA conference 2024); NCN ratio ≥ 0.1% for *KMT2A*-PTD, and ≥ 0.001% for *KMT2A::AF9* and *KMT2A::AFDN* (according to CGMH clinical experience).

Workflow of MiSelect R MRD Assays



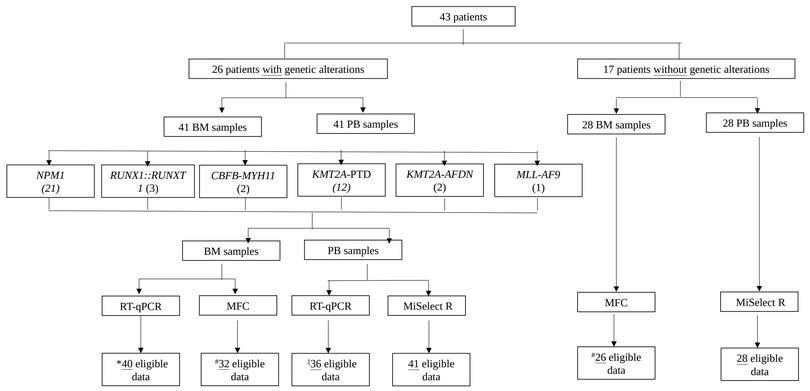
- Whole blood and RBC-lysed samples are labeled with a fluorophore-conjugated anti-CD117 antibody.
- CD117⁺ cells are enriched and stained with an identification antibody panel using the MiSelect R system.
- Myeloblasts are defined as CD117⁺, CD34⁺, HLA-DR⁺, and CD45⁻/dim cells.
- Aberrant marker panels are further analyzed on isolated myeloblasts.
- Differentiation marker panels are evaluated in CD117⁺CD34⁻ and other myeloid populations..

- Representative images of aberrant myeloblasts identified by immunofluorescence markers.



Results

Patient Cohort and MRD Assessment Overview



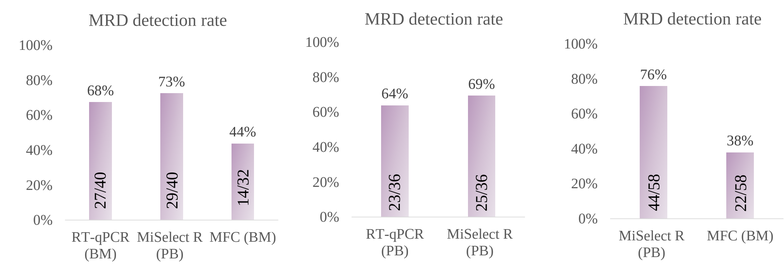
Evelen BM samples with insufficient cells (< 1 x 10^7 cells) for analysis by mpFC to generate eligible data.
[#] One BM samples with insufficient RNA (< 4ug RNA) for analysis by RQ-PCR to generate eligible data
[‡] Five PB samples with insufficient RNA (< 4ug RNA) for analysis by RQ-PCR to generate eligible data.

Concordance of MRD Assessment Between BM and PB Samples by RT-qPCR

		RT-qPCR (BM)			
		Positive	Negative	Total	Predictive Value (95% CI)
RT-qPCR (PB)	Positive	22	1	23	PPA :95.7% (79 – 99.2%)
	Negative	2	10	12	NPA :83.3% (55.2 - 95.3%)
	Total	24	11	35	
		Sensitivity:91.7%	Specificity:90.9%		Concordance : 91.4% Cohen's Kappa: 0.81

- RT-qPCR demonstrated strong concordance between BM and PB, with a 91.4% agreement observed across 35 paired samples.
- These results support the utility of PB as a reliable surrogate for BM in MRD assessment.

MRD Detection Rates by RT-qPCR, MiSelect R, and MFC in BM and PB Samples



- MiSelect R-MRD demonstrated a detection rate comparable to RT-qPCR–MRD.
- The detection rates of MiSelect R-MRD in PB samples were significantly higher than those of MFC-MRD in BM samples

Conclusion

MiSelect R using PB samples demonstrated higher sensitivity than MFC in BM, and comparable performance to RT-qPCR in both BM and PB. It showed strong concordance with RT-qPCR (BM) in both cross-sectional and longitudinal MRD assessments, achieving high agreement across paired samples. As a novel, non-invasive tool, MiSelect R combines the high sensitivity of RT-qPCR with the broad applicability of MFC, highlighting its potential for reliable MRD monitoring in AML. Future studies may help further support its generalizability across broader clinical contexts.

Concordance Between MiSelect R (PB) and RT-qPCR (BM) in MRD Detection

A. Samples of AML Patients with *NPM1*-mutated and core-binding factor leukemias

		RT-qPCR (BM)			
		Positive	Negative	Total	Predictive Value (95% CI)
MiSelect R (PB)	Positive	17	1	18	PPA : 94.4% (74.2 - 99%)
	Negative	0	8	8	NPA : 100% (67.6 - 100%)
	Total	17	9	26	
		Sensitivity: 100%	Specificity: 88.9%		Concordance: 96.2% Cohen's Kappa: 0.91

B. All patients

		RT-qPCR (BM)			
		Positive	Negative	Total	Predictive Value (95% CI)
MiSelect R (PB)	Positive	26	3	29	PPA : 89.7%(73.6 – 96.4%)
	Negative	2	9	11	NPA : 81.8%(52.3 – 94.9%)
	Total	28	12	40	
		Sensitivity: 92.9%	Specificity: 75%		Concordance: 87.5% Cohen's Kappa: 0.70

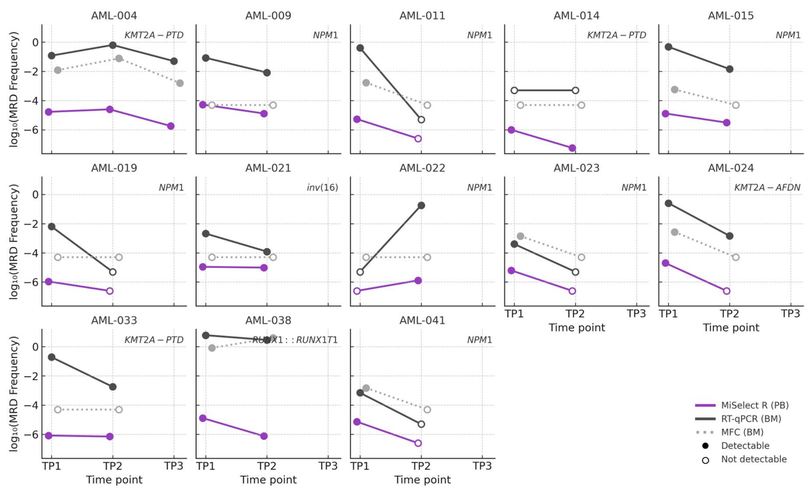
- MRD assessments demonstrated high concordance between RT-qPCR in BM and MiSelect R in PB, with 96.2% agreement observed in patients with NPM1 mutations or core-binding factor leukemias.
- An overall concordance rate of 87.5% was observed across 40 paired samples, highlighting MiSelect R's reliability compared to the current gold standard.

Concordance Between MiSelect R (PB) and MFC (BM) in MRD Detection

		MiSelect R (PB)			
		Positive	Negative	Total	Predictive Value (95% CI)
MFC (BM)	Positive	21	1	22	PPA : 95.5% (78.2 -99.2%)
	Negative	23	13	36	NPA : 36.1% (47.6 -77.5%)
	Total	44	14	58	
		Sensitivity: 47.7%	Specificity: 92.9%		Concordance: 58.6% Cohen's Kappa: 0.26

- MiSelect R detected 23 MRD-positive cases in PB that were missed by MFC in BM, underscoring its superior sensitivity.
- Only one case was observed in which MiSelect R yielded a negative result while MFC was positive; notably, this PB sample exhibited low white blood cell count and lacked detectable myeloblasts.

Longitudinal MRD Tracking in AML Patients by MiSelect R,RT-qPCR, and MFC



- MiSelect R (PB) demonstrated consistent MRD trends over time in AML patients, closely matching RT-qPCR (BM) across 13 cases.
- In contrast, MFC (BM) showed weaker correlation due to its lower sensitivity, with a higher frequency of non-detectable results.

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