The MiSelect R: A novel single-cell retrieval system for CTCs from whole blood with integrated biomarker analysis for targeted and immunotherapy

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Liquid Biopsy 5 Session
4/18, 8:00:00 AM – 12:00 PM
Poster Section 26

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Background

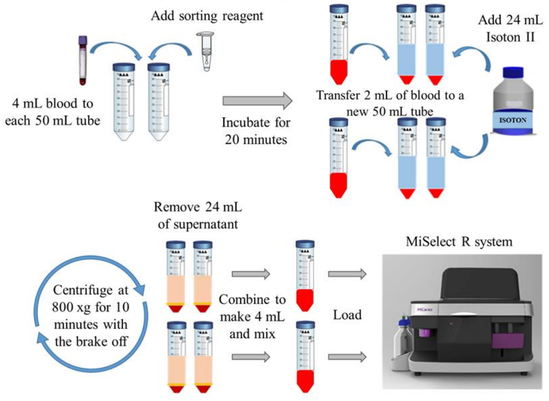
Liquid biopsy tests for circulating tumor cells provide a great source of non-invasive and real-time tumor cells. In addition to cell enumeration, analysis of associated proteins and molecular alterations offer great potential for precision medicine. The MiSelect R was invented for purification and characterization of rare cells, including CTCs. This study evaluates the MiSelect R analytical and clinical performance.

Methods

All blood was collected in K_2EDTA tubes and processed on the MiSelect R using either the SelectChip Dual for automated staining and fluorescence imaging, or the SelectChip Retrieval for Single-Cell analysis.

mBC and NSCLC patient blood samples were drawn at Chang Gung Memorial Hospital and NTUH under IRB approval and patient consent.

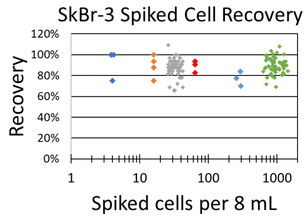
Blood processing on the MiSelect R

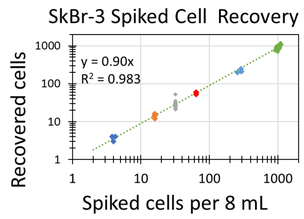


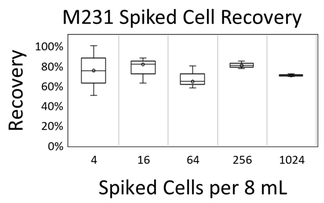
Results

Analytical Validation of the MiSelect R

Cell Recovery, Linearity, Precision and Specificity





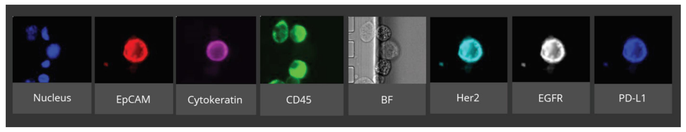


20 Day Precision		
120 total tests	Recovery %	CV %
32 cells in 8 mL	89%	9.2%
1024 cells in 8 mL	90%	7.8%

Healthy Donor Blood Specificity	
Number of Samples	46
Number of Samples with CTCs	0

A total of 139 spiking experiments with SkBr-3 cell numbers of 4, 16, 32, 64, 256, and 1024 added per 8 mL. Using linear regression analysis the average recovery rate is 90% with a R^2 = 0.983, showing a linear measuring range of 0 to 1024 cells per 8 mL. To demonstrate the high sensitivity of the optical detection, low EpCAM expressing MDA-MB-231 cells were added to 8 mL of blood in triplicate. The average recovery rate is 71% with a R^2 = 0.9957.

120 tests were performed over 20 days by three different operators to determine the instrument precision and reproducibility. The within-device coefficient of variation (CV) is 9.2% when 32 cells are added to 8 mL of blood and 7.8% when 1024 cells are added to 8 mL of blood. Blood from 46 healthy donors showed zero CTCs.



Breast Cancer Samples on the MiSelect R

Study Population

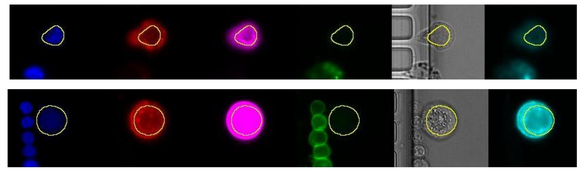
Baseline characteristics	
Median Age (Range)	57 (42-73)
Disease state	Metastatic breast cancer
Patients with prior treatment	21

New line Therapy			Subject number	CTC detected subject number
1st line	Naïve		8	8
	Adjuvant	Hormone	4	2
		Chemo	2	0
		Targeted	1	0
2nd line or subsequent			14	4
Total			29	14

CTCs were detected in 100% (8/8) of treatment naïve mBC patients.

HER2 Expression in mBC CTCs

HER2 status analysis in primary tumors and CTCs in 9 mBC patients

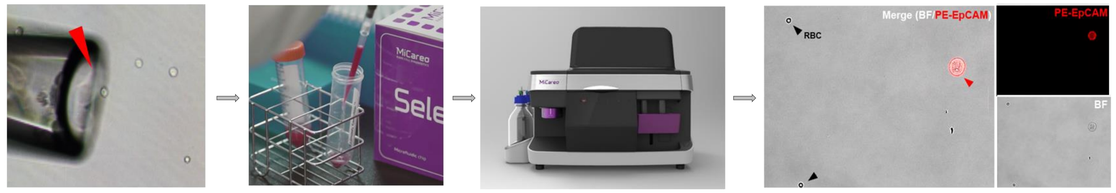


HER2 testing on CTCs \ HER2 testing by IHC	HER2(+)	HER2(-)
HER2(+)	1	0
HER2(-)	0	8

The Her2 score found by the MiSelect R agreed with the IHC analysis of the primary tumor.

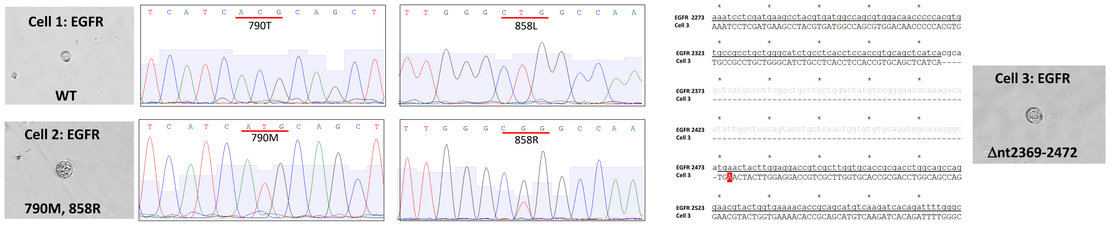
Single CTC Retrieval from Whole Blood with the SelectChip Retrieval

Seven experiments are conducted where 16 cultured cells are added to 4 mL of whole blood and labeled with a fluorescent antibody. Labeled cells are sorted out of the whole blood by the MiSelect R System using the SelectChip Retrieval and output into a microcentrifuge tube. All cells are imaged and 61% of the spiked cells were recovered with a cell purity of 53%.



Single tumor cell retrieval and RT-PCR analysis of EGFR mutations from the blood of a NSCLC patient

3 individual tumor cells are collected using the SelectChip Retrieval from the blood of a NSCLC patient. Each cell was examined for EGFR mutations by RT-PCR and sequencing analysis. 1 cell had 858R and 790M mutations, 1 cell was WT EGFR, and 1 cell showed in-frame deletion (Δnt2369-2472)



Conclusion

The MiSelect R System demonstrated outstanding analytical performance and clinical sensitivity for CTC detection, as well as versatile capability for multiple-biomarker characterizations. It also showed excellent capability for single cell retrieval of rare cells from whole blood and pleural effusion. It provides consistent and automated cellular and molecular information at the single cell level.