Abstract # 2687

Development of a New, Highly Sensitive Assay for Circulating Tumor Cell (CTC) Detection Based on the CellSearch® CTC Profile Kit Enrichment and Laser Scanning Cytometry (LSC) Analysis

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Abstract

The presence of rare Circulating Tumor Cells (CTCs) in the peripheral blood, as detected by the FDA-approved CellSearch® CTC kit, is associated with decreased overall and progression free survival in patients with metastatic breast, prostate and colorectal cancers. In addition to CTC enumeration, CellSearch® CTC Kit provides a unique tool for CTC biomarker analysis in cancers of epithelial origin (CD45−, EpCAM+, and cytokeratins (CK) 8,18-positive and/or 19-positive). Considerable cell loss may occur at many of the automated steps of the CellSearch® CTC isolation process, resulting in decreased recovery of CTCs. Herein, we demonstrate an improved, highly sensitive approach for CTC enumeration, which is based on the CellSearch® CTC Profile Kit and quantitative immunofluorescent analysis using Laser Scanning Cytometry (LSC). Profile Kit/LSC-based CTC enumeration consistently detected higher CTC counts in patients with various cancer types, and allowed up to a 470% increase in CTC recovery in prostate cancer patients. Profile Kit/LSC-based CTC enumeration was used in pharmacodynamic studies in a Phase I clinical trial of Bevacizumab and Cediranib. Our preliminary analysis indicated that changes in CTC counts from baseline to C2D26 post-therapy may correlate with changes in tumor size. In conclusion, we developed a new CellSearch® CTC Profile Kit/LSC-based method that offers higher recovery of CTCs and thus provides a broader capability for downstream molecular characterization.

Background and Methods

- CellSearch® CTC kit is designed to enumerate CTCs in 7.5 mL of blood. In order to improve CTC recovery and multiplex downstream biomarker analysis, we integrated CellSearch® Profile kit and Laser Scanning Cytometry (LSC) - based CTC enumeration.
- Laser scanning cytometry is a versatile, robust, quantitative fluorescence cell imaging system that provides high content cytometric information on single cell and cell population levels.
- ***Integrated Method:*** CellSearch® Profile kit captures CTCs using the same anti-EpCAM antibody as CellSerach® CTC enumeration kit. CTCs isolated with Profile Kit are retrieved and immunofluorescent staining is performed using the same clones of anti-CK and anti-CD45 antibodies as in the CellSerach® CTC enumeration kit. CTC enumeration is conducted by LSC using FDA-approved criteria (CK+/CD45-/DAPI+) and can be extensively multiplexed with protein and nucleic acid-based biomarker analysis.

Results

CTC Recovery

Normal donor blood was spiked with 1000 MDA-MB-231 breast cancer EpCAM+ cells (n=2), and recovered by the CellSearch® Profile Kit. Following immunofluorescent staining with anti-CK and anti-CD45 antibody and DAPI, cells were enumerated by LSC.

Sample ID	Number of Cells Spiked	Number of EpCAM+/CK+/ CD45-/DAPI+ Cells Recovered	% Recovery
Donor 1	1139	1128	99%
Donor 2	1232	1694	100%

Table 1. Isolation of tumor cells spiked into whole blood using CellSearch® Profile Kit/LSC-based enumeration yields 99-100% recovery.

CTC Stability

Cancer patient blood was obtained from commercially available source which operates under IRB approval. CTC recovery and enumeration was performed using CellSearch® Profile Kit/LSC enumeration at 24, 72 and 96 hours.

Patient	ApoCell ID	Time Point	CTC Count				
			CTC Count	% Difference from 24h	Mean	SD	CV%
1	D3520107	24hr	8	NA	8	0.82	10.2%
	D3520207	72hr	9	12.5%			
	D3520307	96hr	7	-12.5%			
2	D3520108	24hr	12	NA	12.3	1.25	10.1%
	D3520208	72hr	11	-8.3%			
	D3520308	96hr	14	16.7%			
3	D3520110	24hr	7	NA	6.33	0.47	7.4%
	D3520210	72hr	6	-14.3%			
	D3520310	96hr	6	-14.3%			

Table 2. Percent difference (%RE) in CTC counts ranged from -14 to 17% between the 24 and 72 hour and 24 and 96 hour time points, respectively. CellSearch® Profile Kit/LCS method for CTC isolation meets stability criteria (≤30.0%).

Side-by-Side Comparison between CTC Recovery by Profile Kit/LSC Method and Standard CTC Enumeration Kit

CTC recovery from blood of the same cancer patients was performed using both CellSearch® Profile Kit/LSC and CellSearch® CTC enumeration kit.

Patient ID	Primary Diagnosis	CTC Count per 7.5mL blood	
		CellSearch CTC Kit	CellSearch Profile Kit + LSC
1	Hepatocellular carcinoma	0	29
2	Hepatocellular carcinoma	0	88
3	Hepatocellular carcinoma	0	29
4	Prostate cancer	56	84
5	Prostate cancer	38	180
6	Prostate cancer	0	39

Table 3. In a side-by side comparison using same patient blood, CellSearch® Profile Kit/LCS method recovered more CTCs as compared with CellSearch® CTC kit.

Overall Comparison between CTC Recovery by Profile Kit/LSC Method and Standard CTC Enumeration Kit

CTC recovery in 90 cancer patients by CellSearch® CTC kit was compared with CTC recovery in 52 cancer patients by Profile Kit/LSC method. Cancer types: breast, colorectal cancer, head and neck, renal cell, basal cell, prostate, non-small cell lung, small cell lung, alveolar soft tissue sarcoma, ovarian, endometrial, adenocarcinoma of duodenum, sebaceous gland, leiomyosarcoma, mesothelioma and others.

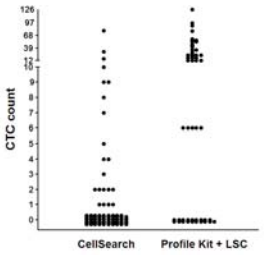


Figure 1. CellSearch® Profile Kit/LCS method recovered a significantly larger number of CTCs compared with CellSearch® CTC kit (Figure 2, p<0.01). Eighty eight % of patients (79 out of 90) were CTC-negative based on CellSearch® CTC kit, while only 37% of patients (18 out of 52) were CTC-negative based on Profile Kit/LSC method.

CTC enumeration in a Phase I Trial of a Combination of Bevacizumab and Cediranib (AZD2171)

CTCs were enumerated using Profile Kit/LSC in a Phase I trial of a Combination of the VEGFR Kinase Inhibitor Cediranib (AZD2171) and Bevacizumab in advanced malignancies (NCI Protocol #7534). RECIST was assessed at the end of Cycle 2 on 9 patients.

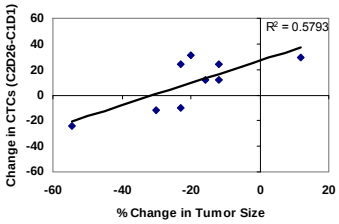


Figure 2. Change in CTC counts between C1D1 (baseline) and C2D26-30 is plotted as a function of % Change in Tumor Size after 2 Cycles of therapy (9 patients). Greater than 25% change in tumor size correlated with the decrease in CTC counts in the peripheral blood.

Increased CTC recovery allows multiplex molecular profiling

Integration of CTC Detection and Protein Biomarker Analysis

Examination of phospho-protein expression in enriched CTCs from cancer patients.

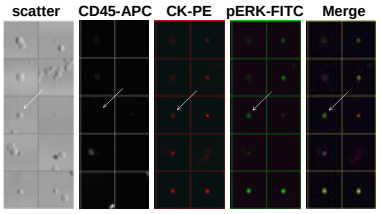
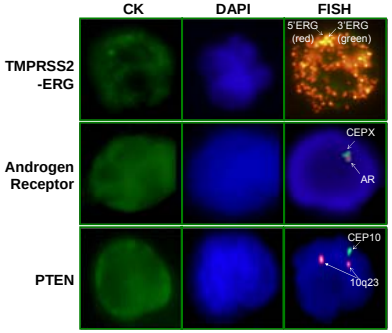


Figure 3. LSC-based CTC enumeration using the CK and CD45 markers and scatter (cells vs debris) allowed detection of ERK phosphorylation in CTCs. This type of analysis can be utilized in real time during Pharmacodynamic studies of investigational drugs.

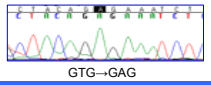
FISH-Based Examination of Genetic Alterations in Enriched CTCs

Figure 4. CTCs were isolated from cancer patients and detected using Profile Kit/LSC. Using Metafer® relocation software, CTCs were re-examined by FISH.



Gene Mutations in CTCs

Figure 5. EpCAM+ cells were isolated from cancer patient blood using CellSearch® Profile Kit. DNA was isolated and analyzed by Allele-Specific PCR for the presence of *BRAFV600E* hot spot mutation. Sequencing analysis confirmed presence of GTG→GAG *BRAFV600E* mutation in CTCs.



Conclusions

- We have developed a new sensitive method for CTC analysis based on CellSearch® Profile Kit isolation and LCS detection. This method yields higher recovery than CellSearch® CTC enumeration kit and allows multiplex analysis of protein and nucleic acid-based biomarkers in CTCs.
- Interim analysis of Phase I study of a combination of Bevacizumab and Cediranib demonstrated that CTC counts, as detected by the new method, may correlate with clinical response to antiangiogenic therapy.

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