

Molecular Characterization of Circulating Tumor Cells Using A Highly Sensitive Method of Enrichment Based on the CellSearch CTC Profile Kit

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Background

The concept of "liquid biopsy" refers to analysis of rare cells in the blood such as Circulating Tumor Cells (CTC) and represents a powerful tool for molecular characterization of tumors for which the biopsies are not available. Flores et al. (Br. J. Cancer, 2009) has recently demonstrated that more patients can be identified as carrying HER2-amplification when analyzed using CTCs, in addition to primary tumor biopsies, providing rationale for expansion of CTC testing by FISH. Herein, we demonstrate a new approach for CTC isolation from EpCAM-high and EpCAM-low cancer specimens, which yields high CTC recovery and facilitates downstream characterization of CTCs by FISH.

Materials & Methods

- We developed a highly sensitive method for CTC characterization by integrating the following platforms: CellSearch® Profile Kit for CTC isolation, immunofluorescent analysis using Laser Scanning Cytometry (LSC) for CTC detection and enumeration, and an automated platform for FISH analysis.
- The efficiency of CTC isolation by CellSearch® Profile Kit was compared to that of standard CellSearch CTC enumeration kit from Veridex.
- CTC FISH for c-MET was performed using the NCI-H1993 lung cancer cells spiked into donor blood.
- CTCs isolated from residual blood of patients with breast and prostate cancers were subjected to FISH for detection of IGF1R, Androgen Receptor (AR) amplification, PTEN deletion and TMPRSS2-ERG fusion.

Results

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

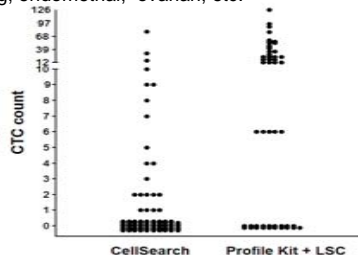
CTC recovery from blood of the same cancer patients was performed using both CellSearch® Profile Kit/LSC and CellSearch® CTC Kit (Table 1).

Patient ID	Primary Diagnosis	Count Per 7.5mL Blood	
		CellSearch® Profile Kit + LSC	CellSearch® CTC Kit
1	HCC	25	0
2	HCC	97	0
3	HCC	27	0
4	Prostate cancer	88	52
5	Prostate cancer	178	48
6	Prostate cancer	47	0
7	Prostate cancer	31	0
8	Prostate cancer	79	1
9	Prostate cancer	0	4
10	Prostate cancer	43	0
11	Prostate cancer	127	1
12	Prostate cancer	75	1

Table 1: In a side-by-side comparison using same patient blood, Profile Kit/LSC method recovered more CTCs as compared with CellSearch® CTC kit.

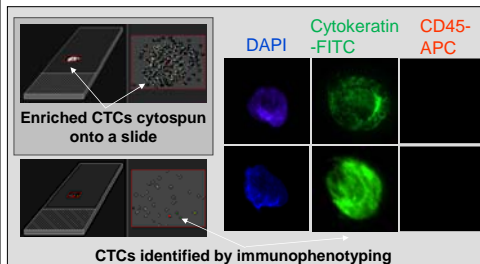
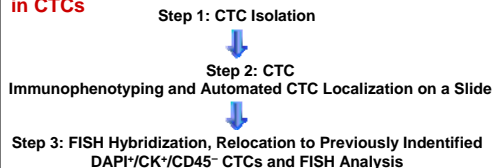
Overall Comparison between CTC Recovery by Profile Kit/LSC Method and Standard CTC Enumeration Kit

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, endometrial, ovarian, etc.



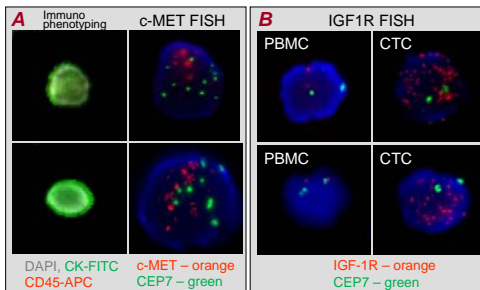
Profile Kit/LSC method recovered a significantly larger number of CTCs compared with CellSearch® CTC Kit ($p < 0.01$). 79 out of 90 (88%) were CTC-negative using standard CTC kit, while only 37% of patients (18 out of 52) were CTC-negative by Profile Kit/LSC method.

FISH – Based Examination of Genetic Alterations in CTCs

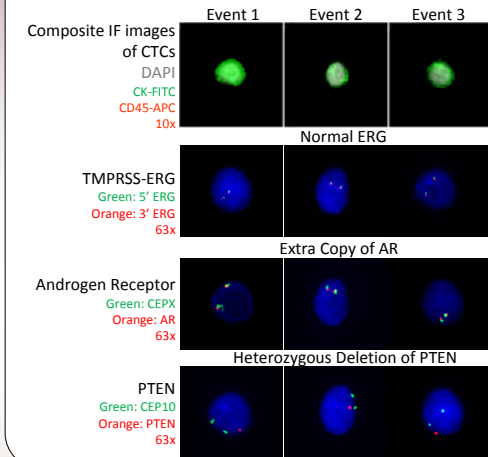


Work flow for CTC identification for FISH and representative immunofluorescence images of CTC immunophenotyping based on Cytokeratin, CD45 and DAPI staining.

c-MET FISH in NCI-H1993 Tumor Cells (c-MET amplification-positive) Spiked into Donor Blood (A). IGF1R FISH in Isolated Breast Cancer CTCs (B).



FISH detection of AR, PTEN and TMPRSS2-ERG in Prostate Cancer CTCs Demonstrates AR Gain and Heterozygous Deletion of PTEN in the Same CTCs



Conclusions

- We developed a new method that offers higher CTC recovery and provides a broader capability for downstream molecular characterization of cancers.
- We integrated the CellSearch® Profile CTC isolation kit, a custom CTC immunophenotyping algorithm, CTC re-location platform and an automated CTC FISH analysis platform. Clinical oncology FISH tests as well as custom developed FISH assays for c-MET, IGF1R, AR, PTEN and TMPRSS2-ERG were run to assess genetic alterations in CTCs.
- Analysis of breast cancer specimens demonstrated the presence of IGF1R amplification in CTCs.
- Analysis of the prostate cancer specimens demonstrated that AR gain and PTEN deletions may occur in the same CTCs.