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Highly sensitive and specific detection of Cytokeratin Positive and Negative Circulating Tumor Cells

Veena M. Singh, MD, Jason Alter, PhD, Deanna Fisher, and Julie Ann Mayer, PhD

Biocept Inc, San Diego CA

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Completing the Answer™

Introduction

An important limitation of tissue biopsies is their inability to be used to measure disease progression and treatment longitudinally. Clinicians and researchers believe Circulating tumor cells (CTCs) can be an alternative, cost-effective monitoring tool and that there is a critical role for real-time assessment[1-3]. Circulating tumor cells (CTCs) are a rare biological event (~1 CTC/billion cells) and rare cell detection requires platforms to demonstrate sensitive and specific capture. Additionally there is a broad phenotypic range of CTCs comprising cytokeratin positive and cytokeratin negative CTCs. The ability to identify both sub-sets is important for biomarker correlation as well as identifying potential differing clinical outcomes for CTC variants. Clinical application of CTCs in a more comprehensive and sensitive manner requires enhanced methodology that can efficiently enrich, capture, and subsequently improve the detection rates of CTCs. CTC detection and enumeration can provide significant information and lead to earlier disease detection and faster, more appropriate treatment: switching patients from ineffective medications. The Biocept Target Selector™ assays allow for real-time, longitudinally treatment monitoring.

Methods

Peripheral blood from 494 samples were collected in CEE-Sure™ blood collection tubes consisting of patients with metastatic breast, lung, colorectal and prostate cancer (N=93), from healthy donors with no history of cancer (N=326) and analytical samples obtained by spiking healthy donor blood with cancer cell lines (N=75). The buffy coat layer was incubated with primary antibody cocktail followed by biotinylated secondary antibody. Cells were stained with a pan-cytokeratin mixture, CD45, Pan-CTC marker, and DAPI. CTC enumeration analysis was undertaken and classified either as cytokeratin positive (CK+/CD45-/DAPI+) or cytokeratin negative (PanCTC+/CD45-/DAPI+).

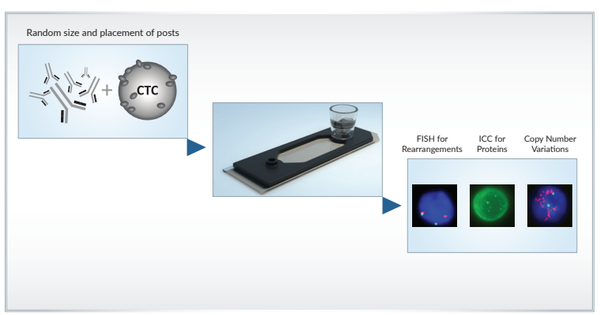

Figure 1. Workflow of the Target Selector™ CTC Platform Assays

Results

CEE-Sure™ Blood Collection Tubes

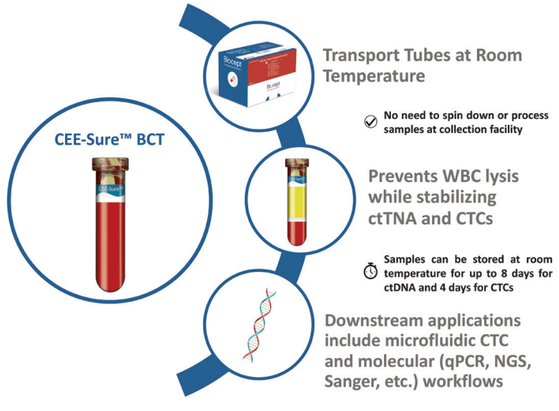

Figure 2. Schematic Illustrating the benefits of the CEE-Sure™ Blood Collection Tubes

Data Table

Target Selector™ CTC Detection Assay Overall Summary of Passing Performance		
STUDY		RESULTS
Accuracy		92.00%
Precision (%CV)	Intra-Assay	19.10%
	Inter-Assay	17.70%
Analytical Specificity		100.00%
Clinical Specificity		93.00%
Clinical Sensitivity		82.00%
Limit of Detection		One (1) CTC
Reportable Range		Detection of CTCs were linear over the reportable range of 0 to 2094 tumor cells

Table 1. Target Selector™ CTC Detection Assay Overall Performance Summary

Results

Variety of Cell Types Captured

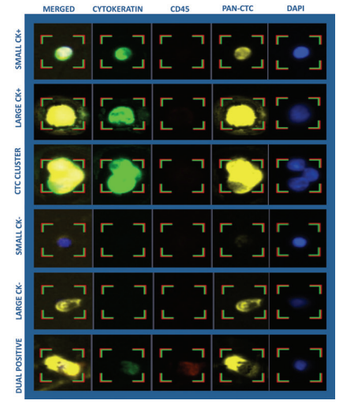

Figure 3. Types of cells captured in the microfluidic channels

LOD/Linearity

A dilution series was performed to evaluate test linearity across 30 samples. Linear regression analysis was performed over the 30 samples and yielded a slope of 1.0006, an intercept of 2.3322, and an R^2 = 1.0 (R = 1.0). The analysis of the data demonstrated that the detection of CTCs were linear over the reportable range of 0 to 2094 tumor cells.

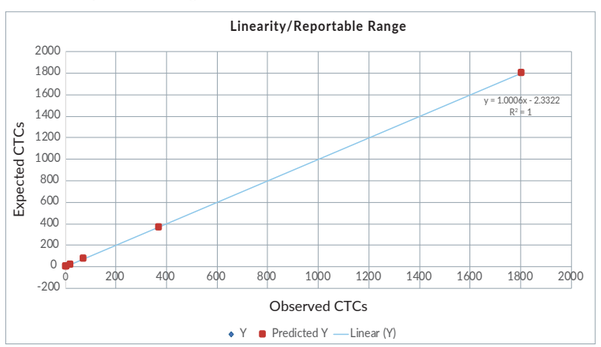

Figure 4. Linear Regression analysis of 30 samples ranging from 0 to 2094 CTCs

Summary

Overall Summary Performance

- 92% concordance for Clinical Accuracy and 100% for Analytical Specificity was obtained.
- CTC detection was linear over a reportable range of 0 to 2094 CTCs with a % CV of 19%.
- Based on the linearity/reportable range data above, one (1) CTC per 8.0 mL can be detected by the Target Selector™ CTC platform resulting in a limit of detection of one (1) CTC in a channel.
- No CTCs were observed in any of the healthy donor samples.
- There was broad variability observed with regards to CTC sizes ranging from small CTCs equivalent to small lymphocytes (7-10 µm) to CTCs with diameters several fold larger.

Conclusions

- The Target Selector™ CTC detection assay has demonstrated highly specific and sensitive CTC capture both for epithelial and non-epithelial sub-sets.
- Hence the ability to capture and characterize a broader range of CTCs unlike other CTC technologies that identify only epithelial CTCs or utilize sized based selection would be beneficial for subsequent biomarker analysis and clinical outcomes assessment.

References

1. Aktas B, Tewes M, Fehm T, et al. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res. 2009;11(4):1-9.
2. Venesio T, Siravegna G, Bardelli A, Sapino A. Liquide biopsies for monitoring temporal genomic heterogeneity in breast and colon cancers. Pathobiol 2018;85:146-154.
3. Lecharpentier A, Vielh P, Perez-Moreno P, et al. Detection of circulating tumour cells with a hybrid (epithelial/mesenchymal) phenotype in patients with metastatic non-small cell lung cancer. Br J of Cancer. 2011;105:1338–1341.