Multi-level analysis of prostate cancer circulating tumor cells allowing IHC-based identification, 6-parameter fluorescence phenotyping, and individual cell molecular analysis

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Background
Analysis of circulating tumor cells (CTC) allows non-invasive investigation of prostate cancer biology and response to treatment. The primary level of analysis is the CTC count, which has been demonstrated to be prognostic of outcome. Deeper characterization of CTC phenotype and pertinent biomarkers can confirm cancer lineage and identify drug targets or drug-resistance markers. Single cell analysis of individual CTCs can provide genomic insight into cancer heterogeneity. RareCyte has developed AccuCyte® - CyteFinder® (AC-CF), an integrated technology platform for highly sensitive visual identification and rare cell recovery in blood by both immunohistochemistry (IHC) and immunofluorescence (IF) staining. Recently we have developed technology allowing 6-marker assays for broader phenotypic analysis.

Methods
Normal human whole blood samples were spiked with prostate cancer lines as model CTCs (mCTCs). Blood samples from University of Washington patients with advanced prostate cancer were collected under an IRB-approved protocol. Blood was processed using AccuCyte and the nucleated cell fraction was collected and spread onto microscope slides. Slides were stained on an automated stainer using (1) an IHC assay for cytokeratin, (2) a standard 4-wavelength IF assay (DAPI, CD45, cytokeratin and EpCAM) or (3) a 6-parameter IF assay using SYTOX-Orange (nuclear stain), cytokeratin, EpCAM, androgen receptor (AR), prostate-specific membrane antigen (PSMA) and CD45. An assay for AR variant 7 (ARv7) was applied to samples with mCTCs with the ARv7 splice variant. Percent recovery of IHC-stained slides (by blinded pathologist review) was compared to IF-stained slides (by CyteFinder image analysis). Individual IF-stained CTCs were retrieved after on-slide visual identification and re-visualized after dispensing for confirmation. Whole genome amplification (WGA) of retrieved cells was performed, followed by X- and Y-chromosome amplified DNA PCR (using AccuCyte protocol). The splice variant ARv7 was substituted for AR to demonstrate expression of this protein in spike-in samples using an IF assay for cytokeratin and other lineage-specific markers.

Results
There was strong linear correlation between IF and IHC counts of mCTCs over a range of ~25 - 100 cells/ml (R2 = 0.89). The 6-parameter IF assay was successfully applied to mCTC and clinical samples. AR and PSMA were co-expressed in the majority of epithelial-marker positive clinical CTCs. The ARv7 assay identified mCTCs that express the splice variant. Individual IHC-stained mCTCs spiked into female donor blood were demonstrated to be male after WGA and PCR.

Conclusions
• Light microscopy of cytokeratin IHC on mCTCs approximated IF identification
• 6-parameter phenotyping of prostate cancer CTCs is feasible and allows identification of pertinent lineage-specific markers
• IHC-stained cells can be individually retrieved from slides for genome amplification and molecular analysis

Shockwave microfluidics technology for phenotyping circulating tumor cell clusters

1. IHC and immunofluorescence staining of spike-in PC3 cells

2. Comparison of immunofluorescence and IHC recovery of spike-in CTCs

3. CytePicker retrieval of IHC-stained cells for molecular analysis

4. Prostate cancer patient CTC clusters – composite images