Multi-level analysis of circulating tumor cells in advanced prostate cancer using AccuCyte® – CyteFinder®

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Background
Analysis of circulating tumor cells (CTC) allows non-invasive investigation of cancer biology and response to treatment at several levels. The primary level is the CTC count, which has been demonstrated to be prognostic of outcome in multiple cancer types. Characterization of CTC phenotype and pertinent biomarkers, including drug targets, is a second level. Single cell molecular/genomic analysis of CTCs provides a third level. RareCyte has developed AccuCyte® – CyteFinder® (AC-CF), an integrated technology platform for highly sensitive visual identification and retrieval of rare cells in blood. The AccuCyte kit comprehensively collects the nucleated cell fraction of the blood and transfers it to a microscope slide compatible with automated immune-staining and other slide-based tissue tests. CyteFinder is a highly precise digital scanning fluorescence microscope with image analysis software for multi-parameter visual characterization and integrated mechanical isolation of single cells for genomic analysis.

Methods
Model CTC (mCTC) prostate cancer lines were used to assess capture efficiency and limit of detection. Blood samples from University of Washington (UW) patients with advanced prostate cancer were used to compare blinded CTC counts with the FDA-cleared CellSearch® system using 4-channel immunofluorescence. An additional set of 45 samples from 30 advanced prostate cancer patients from UW patients were analyzed using AC-CF at RareCyte’s laboratory. Prototypic 6-channel prostate cancer marker-specific panels were developed. Individual prostate CTCs were retrieved from slides.

Results
Capture efficiency of mCTCs was over 90%, with limit of detection of 1 cell in 7.5 mL. Enumeration performance of AC-CF matched or exceeded that of CellSearch, depending on levels of EpCAM expression in mCTC cell lines, with higher expressing lines closely matching CellSearch counts and lower expressing lines exceeding CellSearch counts. Of the 30-patients, 9 had no CTCs, 7 had 1 – 3, 3 had 4 – 9, and 11 had 10 or more CTCs. Large CTC clusters were identified in two samples. A 6-channel IF assay using SYTOX-Orange (DNA stain), cytokeratin, EpCAM, androgen receptor (AR), PSA and CD45 was successfully applied to mCTC and clinical samples. An assay for AR variant 7 (ARV7) identified mCTCs known to express the ARV7 splice variant. AR and PSA were expressed in the majority of epithelial-marker positive CTCs. Individual CTCs were retrieved after on-slide visual identification and re-visualized after placement in flat-bottom PCR tubes for confirmation.

Conclusions
AccuCyte – CyteFinder is a highly sensitive, comprehensive technology for the identification of CTCs. AC-CF identifies equivalent or greater numbers of CTCs in advanced prostate cancer patients than CellSearch. The majority of advanced prostate cancer patients evaluated had identifiable CTCs. 6-parameter multiplex phenotyping of prostate cancer CTCs is feasible and individually identified cells can be isolated for molecular analysis. Additional studies in bone marrow have been designed to confirm that CD45 (+), cytokeratin (+), EpCAM (+) cells are bone fide tumor cells distinct from resident marrow cells.

Low EpCAM mCTCs found by AC – CF

Prostate cancer biomarker panels
A. 6-channel panel (LnCAP cells) B. AR v7 (22RV1 cells)
C. 6-channel panel (patient CTC cluster) Composite image

Integrated individual cell retrieval
A. Prostate CTC before / after picking
B. Serial picking of a prostate CTC cluster

CTC counts in 30-patient prostate cancer cohort

CTC count 0 1-3 4-9 10+ Large cell clusters (>50+)
Patients (N=30) 9 7 3 11 2
Fraction 30% 23% 10% 37% 7%

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Laboratory Workflow
A. Density enrichment B. Automated staining C. Image analysis

Add Blood to AccuCyte® Tube and Float
Count Input Cells Spikes Cells into Blood
Concentrate Sample 10µL (optional) @ 5000RPM
Apply CyteBead Recovery Media
Add/Remove Fluid
Plate CytePicker™ into Tube
Add Collection Plate for Bulk Cell Collection
Develop from Bulk Cell
Add Adhesion Fluid
Sync Sample Onto It Eilles
Prepare for Image/capture on Automated Slide Stamper
Automated Image Capture and Analysis
CyteFinder® Patterns
Resultant and Classify Cytosignature
Remove Software