Increased phenotypic depth for automated visual identification of biomarkers on circulating tumor cells by cocktailting epithelial markers EpCAM and cytokeratin

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Background
Circulating tumor cells (CTCs) are rare cells shed into the bloodstream by tumors. These cells have the potential to metastasize and spread the cancer. AccuCyte® - CyteFinder® system is a platform for automated visual identification and retrieval of rare cells from blood by immunofluorescence (IF). RareCyte has developed AccuCyte® - CyteFinder® system, a platform for automated visual identification and retrieval of rare cells from blood by immunofluorescence (IF). RareCyte has also developed a 4-color standard panel for epithelial CTC detection (CTC-4 panel) in which EpCAM and CK are placed in individual fluorescence channels, allowing for identification of CTCs that express EpCAM and/or CK. Combined with recent expansion of CyteFinder imaging capabilities from 4 to 6 channels, this 4CTC panel can be combined with 2 additional markers for deeper phenotypic analysis. Given that our platform does not require co-expression of EpCAM and CK, RareCyte also developed a 3-color panel for CTC identification (CTC-3 panel) that combines detection reagents for EpCAM and CK detection reagents within a single channel, thus enabling 3 extra markers for deeper phenotyping. We tested feasibility of epithelial marker cocktailting with an assay to detect androgen receptor (AR) in prostate CTCs.

Methods
Normal human whole blood samples were spiked with prostate or breast cancer lines as model CTCs (mCTCs). Blood samples from University of Washington patients with advanced cancer were collected under an IRB-approved protocol. Blood was processed onto microscope slides using the AccuCyte sample processing system. Slides were then stained on an automated stainer using either the CTC-4 panel or CTC-3 panels combined with one or two or three additional biomarkers relevant to the mCTC. After development using spike-in models, the CTC-3 + AR assay was applied to a patient sample.

Sample preparation and analysis workflow
CTCs were stained on the stainer and analyzed on the AccuCyte system, with images of identified CTCs being collected on slides for further analysis. Images were then analyzed by the CyteFinder system to confirm the presence of AR.

Results
No CTCs were identified in non-spiked normal donor blood samples (not shown). In spike-in models, the epithelial cocktail CTC-3 panel performed as well as the non-cocktail CTC-4 panel in identifying CTCs (not shown).

A 4-parameter CTC-3 + AR assay demonstrated AR expression in LNCaP and 22RV1 mCTCs but not in PC3 mCTCs, confirming specificity of AR staining. When the assay was applied to a blood sample from a prostate cancer patient with known high CTC count, 160 CTCs per ml were identified; of these, 114 were observed to be AR-positive (71%).

A 5-parameter CTC-3 + AR / Ki-67 assay demonstrated AR-V7 expression in 22RV1 mCTCs but not in LNCaP or PC3 mCTCs, confirming specificity of AR-V7 staining.

A 6-parameter CTC-3 + ER / Her2 / Ki-67 assay demonstrated consistent with reported phenotype and AR expression in CTCs.

Conclusions
Combining the epithelial markers cytokeratin and EpCAM into a single wavelength cocktail is feasible for identifying CTCs, allowing up to three additional channels for phenotypic investigation. In a patient with advanced prostate cancer with high CTC count, there was high incidence (71%) of AR expression in identified CTCs.