Multi-parameter immunofluorescence assays for detection of androgen receptor, androgen receptor variant 7 and PSMA in prostate circulating tumor cells

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Background
There is great interest in the non-invasive investigation of expression of androgen receptor (AR) and the splice variant AR-V7 in prostate cancer. RareCyte has commercialized a platform for automated visual identification of circulating tumor cells (CTCs) by immunofluorescence (IF) that enables 6-parameter assessment. We developed a set of prototype CTC assays using tyramide amplification to detect AR and AR-V7 individually and in combination with prostate membrane-specific antigen (PSMA). Using a novel method, we also developed an assay to identify AR and AR-V7 simultaneously.

Methods
Normal human whole blood samples were spiked with prostate cancer lines PC3, LNCaP, and 22RV1 as model CTCs. Prostate cancer blood from a patient with advanced and known high CTC count were collected under an IRB-approved protocol. Blood was processed onto microscope slides and stained with CTC detection assays incorporating 4 core parameters (nuclear dye, CD45, pan-cytokeratin, EpCAM) plus the following markers: (1) AR; (2) AR-V7; (3) AR and PSMA; (4) AR-V7 and PSMA; (5) AR and AR-V7. An antibody denaturing process was used to sequentially amplify two rabbit monoclonals for the AR and AR-V7 assay. No-primary (diluent only) staining controls were run to confirm success of the denaturing step.

Results
Staining of spike-in CTC models confirmed the reported AR and AR-V7 phenotype of the cell lines, supporting the specificity of the assays. When applied to an equivalent volume of the patient sample, the assays identified a mean of 36 CTCs. In the assays staining AR, the mean percent of AR+ CTCs was 70% and for the assays staining AR-V7, the percent of AR-V7+ CTCs was 30%. In the assay for both AR and AR-V7, 10 of 38 CTCs were ARV7+ (26%); all of these were AR+; 15 CTCs were only AR+ (39%).

Conclusions
We have developed multi-parameter assays for detection of AR, AR-V7 and PSMA in prostate CTCs, including the first assay to simultaneously visualize AR and AR-V7. The assays have been validated in prostate cancer CTC models. The percentages of AR and AR-V7-positive CTCs identified in a patient sample with the combined AR and AR-V7 assay are consistent with the percentages observed when AR and AR-V7 are detected in independent assays, supporting the accuracy of the simultaneous detection assay. All patient sample AR-V7-positive CTCs were also AR-positive, supporting the specificity of AR-V7 staining.

Yao Sun, Daniel Campton, Arturo Ramirez and Eric Kaldjian are employees of RareCyte. Other authors declare no conflicts of interest.

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