Development and analytic validation of an ARv7 / PSMA dual biomarker circulating tumor cell assay

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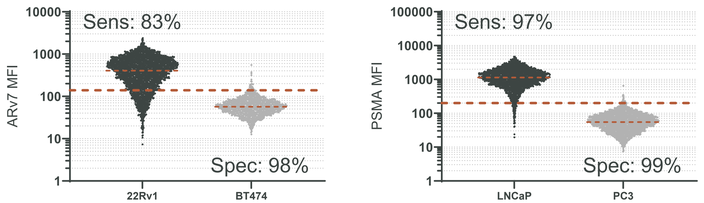
#7486

BACKGROUND

Prostate cancer drug targets and biomarkers that are not assessable using plasma cfDNA may be investigated on circulating tumor cells (CTCs) obtained via liquid biopsies. In this study we describe the development and analytic validation of a novel dual biomarker assay to quantify ARv7 and PSMA expression on CTCs. The assay was applied to nine blood samples from patients with Stage IV prostate cancer to demonstrate clinical feasibility.

ASSAY SENSITIVITY AND SPECIFICITY



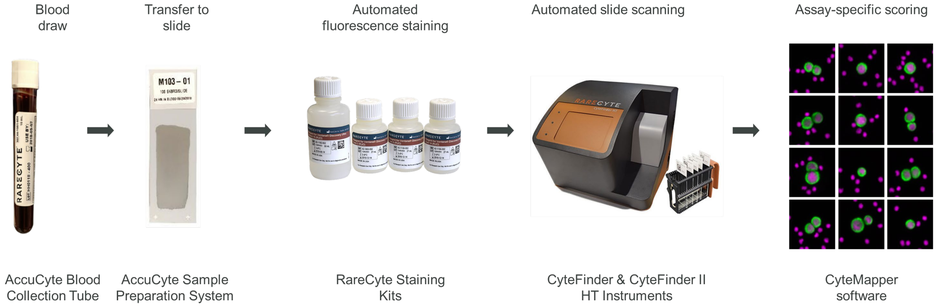
Dual Biomarker Assay Performance. Aggregate data of over 1000 model CTCs from 15 tested slides in each population, run on 3 separate days. Known positive (dark grey) and negative (light grey) biomarker-expressing cell lines are spiked into healthy normal donor blood. These spike-in slides are then processed to slides using AccuCyte, stained, and analyzed in the same workflow as a clinical sample. These spike-ins are used to establish preliminary positivity thresholds (copper dashed line) and determine the Sensitivity and Specificity of the assay.

PROSTATE CLINICAL SAMPLE RESULTS

Patient #	#CTCs	ARv7 %+	PSMA %+
#1	1	100	0
#2	384	96.5	0
#3	3	33.3	33.3
#4	4	75	75
#5	2	100	0
#6	1	100	0
#7	4	75	25
#8	86	27.9	98.8
#9	9	77.8	11.1

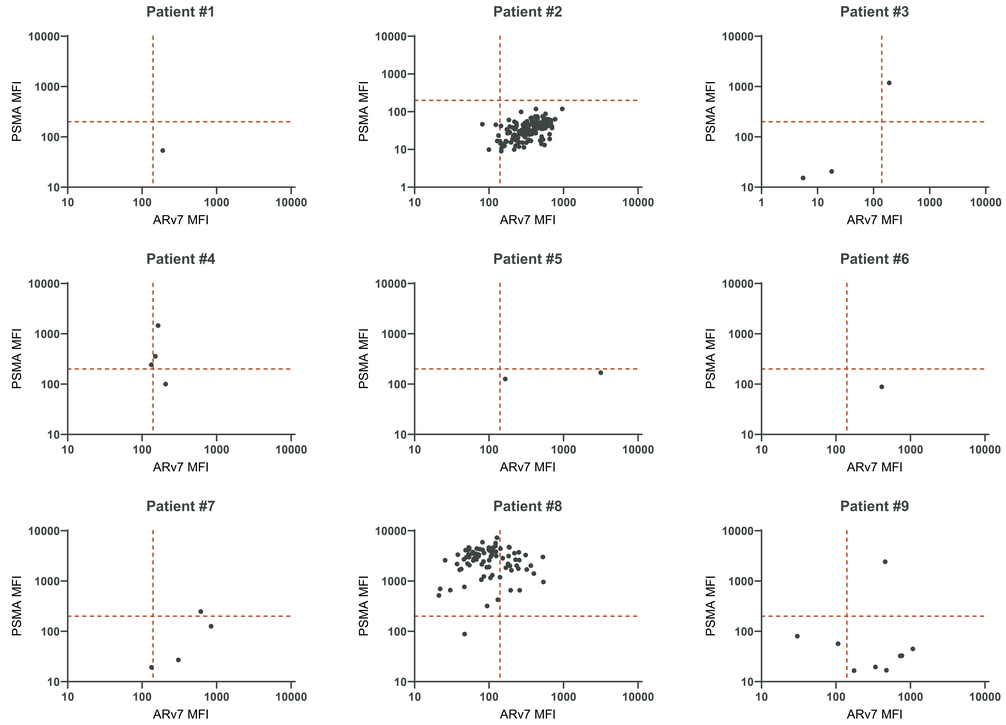
Table of prostate cancer sample results. CTC enumeration and percent biomarker positivity for each sample are tabulated.

METHODS



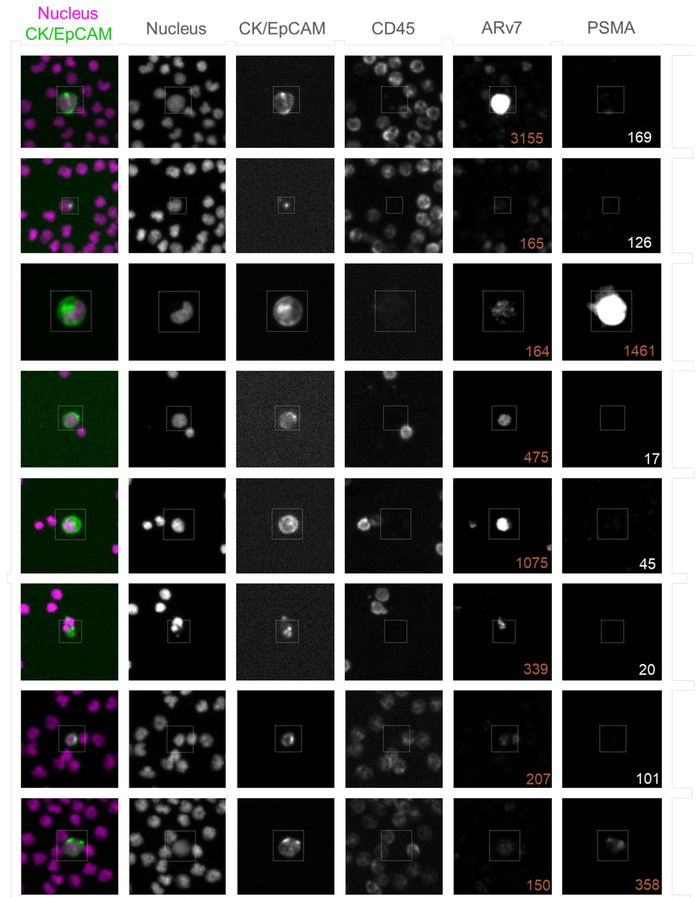
RareCyte platform workflow. After blood is collected into AccuCyte® blood collection tubes, nucleated cell are separated from plasma and red blood cells using the AccuCyte Sample Preparation System, an unbiased, density-driven separation process and spread to slides. These slides are tested with assay-specific RareCyte staining kits using the Leica BOND RX® automated slide staining system. Slides are scanned using the CytoFinder® Instrument and images are analyzed using CytoMapper® software and analysis tools. CTCs are confirmed by a trained reviewer, and cell biomarker status is determined by a fluorescence intensity threshold.

BIVARIATE ANALYSIS OF BIOMARKER MFI FROM INDIVIDUAL CTCS



Bivariate analysis of PSMA and ARv7 expression on individual CTCs. Each grey dot represents the PSMA and ARv7 Mean Fluorescence Intensity (MFI) on an individual CTC from each sample. Copper dashed lines indicate positivity thresholds for PSMA (MFI=200) and ARv7 (MFI = 140).

PROSTATE CANCER SAMPLE CTC IMAGES



Representative images of CTCs identified from prostate cancer samples. Images are all displayed with consistent parameters. Numbers indicate biomarker MFI within each channel shown. Copper text indicates cell MFI is above preliminary positivity threshold for maximum accuracy determined from model cell line data. White text indicates cell MFI is below positivity threshold.

CONCLUSIONS

- A novel ARv7/PSMA dual biomarker assay was developed and validated
- Assay demonstrates high accuracy, sensitivity, and specificity for ARv7 and PSMA based on model cell lines
- Application to clinical samples shows utility of this assay in detecting and classifying biomarker positivity on CTCs from late-stage prostate cancer
- This assay presents an exciting opportunity for evaluating prostate cancer patient tumor phenotypes through liquid biopsies