Analytic validation of an assay to detect androgen receptor splice variant ARv7 protein expression on circulating tumor cells from prostate cancer patients

Daniel Campton[1], Edward Lo[1], Lillian Costandy[1], Brady Gardner[1], Ryan Huston[1], Jeffery L. Werbin[1], Kyla Teplitz[1], Daniel E. Sabath[2], Alisa C. Clein[2], Celestia S. Higano[2], Tanisha Mojica[3], Kristin Province[3], Eric P. Kaldjian[1], Arturo B. Ramirez[1], Tad George[1]

[1]RareCyte, Inc., Seattle, WA, USA; [2]University of Washington, Seattle, WA, USA; [3] Seattle Cancer Care Alliance, Seattle, WA, USA.

RARECYTE

Seattle Cancer Care Alliance

POSTER 5384

BACKGROUND

Circulating tumor cells (CTCs) can provide information on drug target expression, response to therapy, and disease prognosis from a non-invasive blood draw. Presence of the androgen receptor splice variant ARv7 in prostate cancer cells is associated with resistance to second generation anti-androgen therapies. We report here the analytical validation of an immunofluorescence assay for characterization of ARv7 protein expression on CTCs using the RareCyte platform.

METHODS

Blood samples from healthy normal donors spiked with positive and negative cell lines for ARv7 expression were processed using the AccuCyte® Sample Preparation System. Slides were stained by immunofluorescence using an automated slide staining system and the RarePlex® ARv7 CTC Panel Kit comprised of a nuclear dye, anti-CD45 antibody to exclude white blood cells, cocktailed antibodies to cytokeratin (CK) and epithelial cell adhesion molecule (EpCAM), and an ARv7 antibody. Stained slides were imaged with the CyteFinder® Instrument. CTCs were identified using machine learning-based algorithms and confirmed by user review. Mean fluorescence intensity (MFI) measurements were used as a metric for ARv7 expression on confirmed CTCs. Analytic validation studies of the ARv7 CTC assay were performed using 22RV1 (ARv7-high), LNCaP (ARv7-low), and BT-474 (ARv7-negative) cell lines. Performance studies included accuracy, sensitivity, specificity, repeatability, and inter-stainer run coefficient of variation. Performance metrics for CTC recovery were calculated on spike-in and clinical samples. For a gold standard comparison, the number of CTCs found in the ARv7 assay was compared to the number of CTCs found with the CTC detection assay.

RESULTS

An ARv7 MFI threshold that segregated negative and positive cell lines was statistically defined. This threshold identified 83% of 22RV1 cells as positive for ARv7, 98% of BT-474 cells as negative, with an overall accuracy of 90%. When the assay was applied to clinical prostate cancer samples, staining with proper nuclear localization was observed. CTC recovery was at least as high with the ARv7 assay as with the base CTC detection assay.

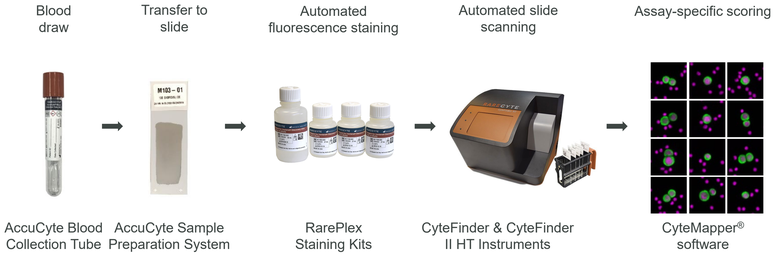

Figure 1. The RareCyte ARv7 CTC assay workflow. Blood was collected into AccuCyte Blood Collection Tubes. Nucleated blood cells were processed to slides using the density-based AccuCyte Sample Preparation System. Slides were stained with the RarePlex ARv7 CTC Panel Kit using the Leica® BOND RX automated slide staining system. Slides were scanned using the CyteFinder II Instrument and images were analyzed using CyteMapper® software and analysis tools. CTCs were analyzed by a trained reviewer and CTC ARv7 status was determined with a fluorescence intensity threshold.

	ARv7-	ARv7+	
	BT-474	22Rv1	LNCaP
Test Positive (MFI > 100)	24	832	245
Test Negative (MFI ≤ 100)	976	168	755
Specificity	***0.976***		
Sensitivity		***0.832***	***0.245***
Accuracy		***0.904***	***0.611***

Table 1. Summary of Analytic Validation results. Sensitivity, specificity, and accuracy calculated on ARv7 positive and negative cell lines using an MFI cutoff of 100.

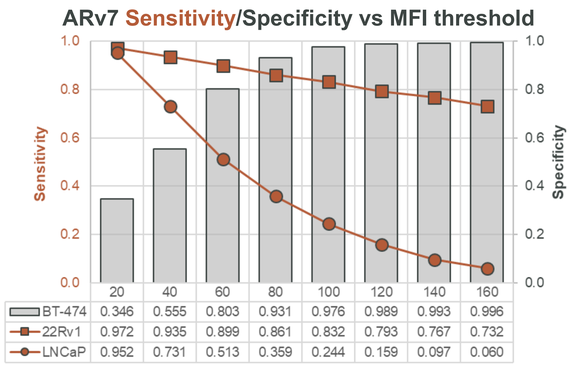

Figure 2. Sensitivity/specificity of ARv7 detection on cell lines. Method for determining ARv7 MFI intensity threshold. Sensitivity is graphed in two curves, one for cell line 22RV1 (ARv7-high) and one for LNCaP (ARv7-low). Bars and right axis values indicate Specificity for the biomarker-negative cell line BT-474. An MFI cutoff of 100 was selected that achieved a minimum Specificity value of 0.9. A cell with an MFI value greater than the 100 MFI threshold constitutes a positive test with the ARv7 assay for validation studies.

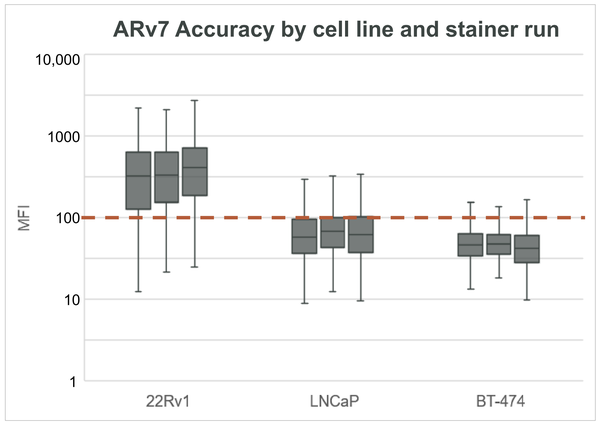

Figure 3. Accuracy of ARv7 detection on cell lines by stainer run. Distribution of ARv7 MFI for stainer run 1 (left), 2 (center), and 3 (right) for each cell line. Threshold dotted line at MFI=100 is used to determine biomarker expression status on a per-cell basis.

Sample			Count	ARv7+ %		mCTC ARv7 MFI	
mCTC Spike-in	Replicates	Stainer Run	mCTC	Mean	CV	Mean	CV
22Rv1	7	1	310	80.6%	10.9%	422	17.9%
	7	2	557	84.3%	4.7%	442	16.3%
	7	3	607	85.3%	7.2%	516	19.7%
	Inter-stainer Mean			***83.4%***		***460***	
	Inter-stainer CV			***3.0%***		***10.8%***	
LNCaP	7	1	738	22.4%	61.0%	75	17.5%
	7	2	788	24.3%	27.4%	78	15.3%
	7	3	792	26.0%	34.4%	74	17.1%
	Inter-stainer Mean			***24.3%***		***76***	
	Inter-stainer CV			***7.5%***		***2.3%***	
BT-474	7	1	673	3.6%	96.0%	51	7.8%
	7	2	649	3.8%	64.8%	51	12.3%
	7	3	599	3.1%	69.8%	46	19.3%
	Inter-stainer Mean			***3.5%***		***49***	
	Inter-stainer CV			***9.8%***		***5.3%***	

Table 2. Inter-stainer run mean and CV. ARv7 MFI and CV shown for each cell type and stainer run. Each run consisted of 7 slide replicates. ARv7 percent positivity was determined using an MFI cutoff of 100.

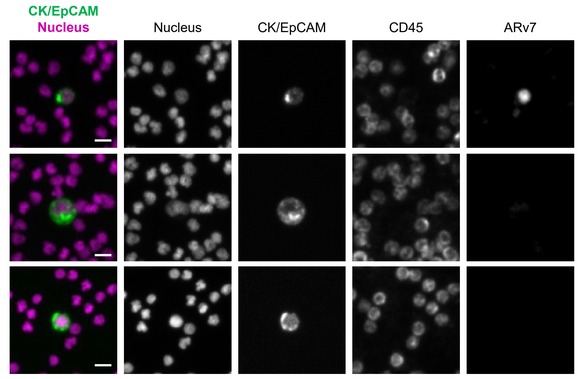

Figure 4. mCTC stained for ARv7. Representative images of mCTC identified with the ARv7 Panel Kit. Scale bars represent 5 μm.

Patient	Indication	Gold Standard	ARv7 Assay
1	Prostate	1	1
2	Prostate	0	0
3	Prostate	3	4
Total		***4***	***5***

Table 3. Clinical comparison results. Results of a small clinical comparison study comparing the number of CTCs counted using the ARv7 Staining Kit and a validated CTC detection assay (Gold Standard).

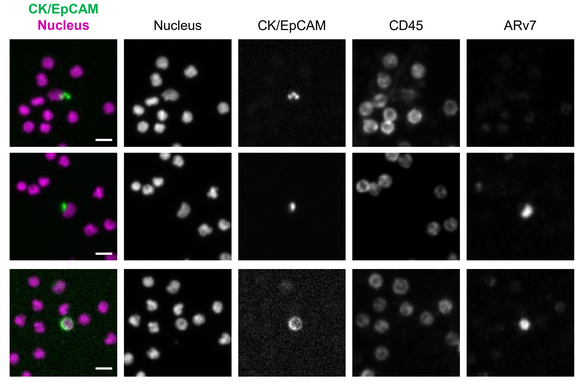

Figure 5. Clinical CTC stained for ARv7. Representative images of clinical CTCs obtained by staining slides from prostate cancer patients with the ARv7 Staining Kit, with status indicated, and with ARv7 MFI shown on respective images. Scale bars represent 5 μm.

CONCLUSIONS

An ARv7 assay was developed and validated which shows good sensitivity, specificity, accuracy, and repeatability in discerning between ARv7 positive and negative cell lines when spiked into healthy normal blood. CTC detection was comparable to a gold standard assay when applied to mCTC studies and clinical samples. ARv7 positive and negative CTCs were identified from clinical samples based upon the statistical cutoffs determined via mCTC studies. While ARv7 positive CTCs were detected in this clinical comparison, a larger clinical study will be required to determine the clinical cut off for ARv7. ARv7 presence has been found to predict resistance to anti-androgen therapies. Identifying ARv7 on CTCs may enable the use of a liquid biopsy assay to predict drug response. The ARv7 Staining Kit is also compatible with RarePlex 488 Developer Kit for addition of a second biomarker.