Validation of AR-V7 as a Treatment-Specific Biomarker at Decision Points in Management of Castration-Resistant Prostate Cancer

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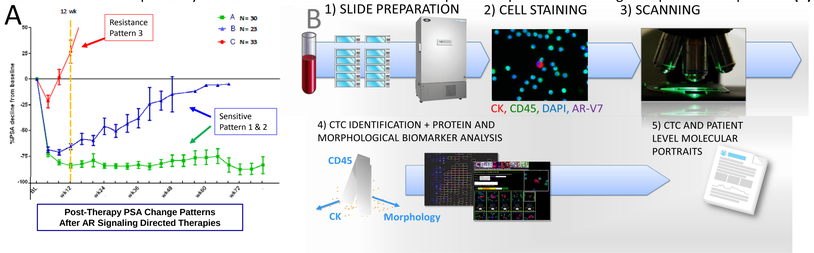
Background

Androgen receptor signaling directed therapies (AR Tx), including Abiraterone Acetate + Prednisone (A) and Enzalutamide (E), prolong survival in patients with mCRPC and are FDA approved. The presence of the splice variant AR-V7 mRNA in EpCAM selected CTCs has been prospectively linked to resistance to A & E[1] but not to taxane chemotherapy (T)[2]. AR-V7 may provide clinical utility in therapy selection between A & E or T.

A key limitation to the predictive value of an AR-V7 mRNA assay in CTCs is the analytical validation of the measurement of low frequency and labile mRNA in CTCs, and to be able to meet diagnostic workflows amenable to community practices. Separately, EpCAM based CTC isolation and capture methods will not detect EpCAM(-) cells, potentially leading to under-sampling of the AR-V7 biomarker. We developed an AR-V7 immunofluorescent test for use in fixed single CTCs that is inclusive of all CTC subtypes which was evaluated in blood samples from men with progressive mCRPC patients in need of a change in therapy. The context of use is to inform the selection of treatment. The Epic CTC platform was designed for global diagnostic workflows with a rapid turnaround time. The focus was the association between the pre-therapy detection of nuclear localized AR-V7(+) CTCs and objective clinical outcomes following treatment with the most frequently, approved drug classes for management of mCRPC: AR Tx and taxanes. The goal is the development of predictive biomarkers for use at the point a treatment decision is needed that will enable broad adoption from global clinical sites.

Methods

193 mCRPC patient blood samples were collected prior to starting Abiraterone (44); Enzalutamide (81), Docetaxel (46) Cabazitaxel (13), and Paclitaxel (2). PSA Outcomes were recorded as Sensitive (S): Patterns **1** and **2**, or Resistant (R): Pattern **3** **(A)**[6]. Patients were monitored for up to 2.3 yrs to assess rPFS and OS outcomes. Samples were processed utilizing the Epic Sciences platform **(B)**.



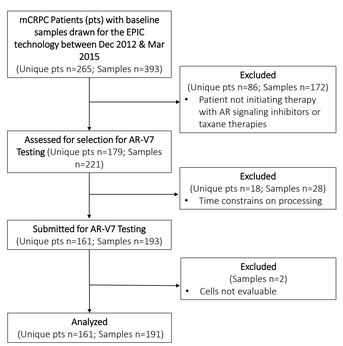
Schematic of Epic CTC Platform CTC enumeration, morphology, biomarker, & FISH analyses workflow:

1) Nucleated cells from blood sample placed onto slides and stored in a -80°C biorepository
2) Slides stained with cytokeratin (CK), CD45, DAPI, AR-V7
3) Slides scanned
4) CTC candidates detected by a multi-parametric digital pathology algorithm
5) Human reader confirmation of CTCs & quantitation of biomarker expression

Patient Demographics

Patient Characteristic	All Patients			
Number of Unique Patients	161			
Age, Years	68 (45-91)			
Primary Treatment				
Prostatectomy	77 (48%)			
Radiation	28 (18%)			
Brachytherapy	7 (4%)			
None	49 (30%)			
Sample Characteristic	**Pre-AR Therapy**	**Pre-Taxane Therapy**	**P-value**	**All Samples**
Number of Baseline Samples	130	63	N/A	193
Age, years	68.5 (45 – 87)	68 (48 – 91)	0.4190	68 (45 – 91)
Blood Age, hours	25 (2 – 78)	27 (1 – 51)	0.2563	26 (1 - 78)
Treatment Decision*				
1st	56 (43.1%)	11 (17.4%)		67 (34.7%)
2nd	40 (30.8%)	10 (15.9%)	<0.0001	50 (25.9%)
3rd or later	34 (26.1%)	42 (66.7%)		76 (39.4%)
Prior Exposure to Life-Prolonging Therapies				
None	56 (43.1%)	11 (17.5%)		67 (34.7%)
AR only	34 (26.2%)	19 (30.1%)	<0.0001	53 (27.5%)
Taxane ± other [ADC, evp, combo]	10 (7.7%)	0		10 (5.2%)
AR AND Taxane ± other	30 (23.0 %)	33 (52.4%)		63 (32.6%)
Chemotherapy Status				
Chemo-naive	90 (69%)	30 (48%)	0.0045	120 (62%)
Chemo-exposed	40 (31%)	33 (52%)		73 (38%)
Metastatic Disease				
Bone Only	39 (30%)	19 (30%)	1.0	58 (30%)
Lymph Node (LN) Only**	21 (16%)	2 (3%)	0.0084	23 (12%)
Bone & LN	51 (39%)	18 (29%)	0.1542	69 (36%)
Bone & Visceral ± LN**	19 (15%)	24 (38%)	0.0004	43 (22%)
Laboratory Measures Pre-Therapy: Median (range)				
PSA, ng/mL	28.0 (0.1 – 2454.5)	99.5 (0.1 – 3728.2)	<.0001	37.7 (0.1 – 3728.2)
Hgb, (g/dl)	12.4 (7.0 – 15.0)	11.6 (8.2 – 14.5)	0.0052	12.1 (7.0 – 15.0)
ALK, (unit/L)	99 (25 – 2170)	181 (49 – 1816)	<.0001	111 (25 – 2170)
LDH, (unit/L)	208 (123 – 1293)	251.5 (141 – 1004)	0.0006	220 (123 – 1293)
ALB, (g/dl)	4.2 (3.4 – 4.9)	4.2 (3.1 – 4.9)	0.7982	4.2 (3.1 – 4.9)
AR-V7 Test: Total CTCs/mL	1.77 (0 – 441.3)	4.35 (0 – 601.5)	0.0040	2.38 (0 – 601.5)

Note: 17 patients received both AR and Taxane for ≥ 1 occurrences. P-values from Wilcoxon Rank Sum test for continuous parameters and Fisher's Exact Test for categorical parameters.
Note: AR Therapies include: Abiraterone Acetate, Enzalutamide, and ARN-509. Taxane therapies include: docetaxel, cabazitaxel, and paclitaxel.
*only includes SOC life-prolonging therapies and experimental therapies patient was exposed to after standard ADT and development of mCRPC disease and prior to initiation on the baseline therapy
**includes patients with other soft tissue disease

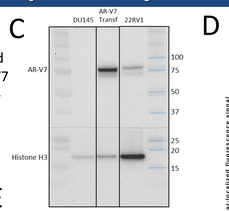


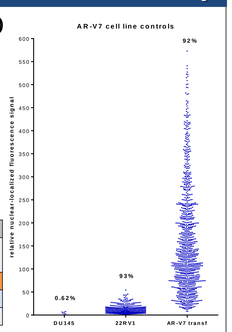
References: 1) Antonarakis, Emmanuel S., et al. *New England Journal of Medicine* 371.11 (2014): 1028-1038. **2)** Antonarakis, Emmanuel S., et al. J Clin Oncol 33, 2015 (suppl 7; abstr 138). **3)** Efstathiou, E., et al. *Annals of Oncology* 2014 (25 suppl 4) **4)** Bambury, Richard M., et al. *Annuals of Oncology* 2014 (25 suppl 4) **5)** Scher, Howard I., et al ASCO GU Abstract #147 **6)** Scher, Howard I., et al. Cancer J. 2013 Jan-Feb;19(1):43-9

Cell-level and Patient-level Specificity of AR-V7 Antibody

Analytical Validation: Specificity of AR-V7 Detection
The specificity of AR-V7 protein detection in single prostate cancer cell line cells spiked into whole blood was corroborated by single-cell mRNA analyses **(C, D)**. The requirement for AR-V7 protein signal localization in the nucleus is consistent with AR-V7-mediated ligand-independent proliferation in preclinical models. Nuclear AR-V7 localization was required to be considered AR-V7+ in all patient samples.

Specificity of AR-V7 in Patient Samples
Parallel AR Assays (N-terminal and AR-V7) were utilized, and the incidence of CTCs positive for either biomarker on sister slides is shown to the right. Comparison of stained sister slides per sample with different assays is subject to some stochastic differences in CTC incidence across slides in samples. **(E)**. However, the much greater incidence of AR N-terminal CTCs is consistent with previous studies on AR-V7 prevalence in relation to total *AR* gene product.



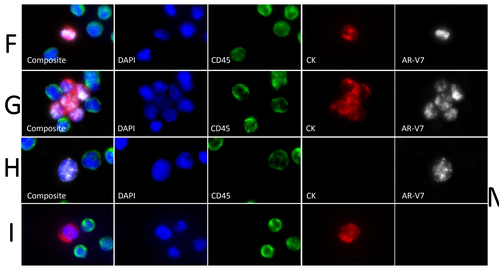


E

Patient Status Per Biomarker			
	AR N-terminal(+)	AR N-terminal(-)	Total
AR-V7(+)	30	4	34
AR-V7(-)	72	85	157
Total	102	89	191

Prevalence and Frequency of AR-V7 CTC Positivity Increases by Line of Therapy

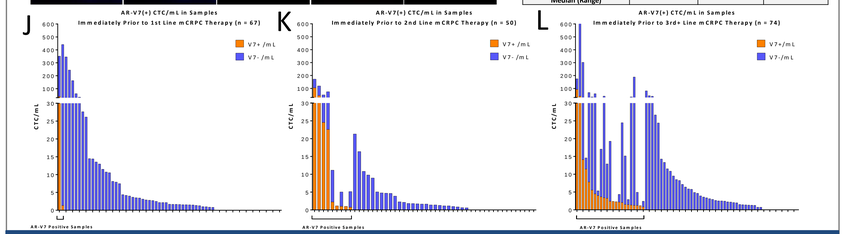
AR-V7 Is Expressed in Multiple CTC Subtypes AR-V7 nuclear expression was found on a variety of CTC subtypes, including "traditional" CK(+) single CTCs **(F)**, CTC clusters **(G)**, and CK(-) CTCs **(H)**. CTCs not expressing AR-V7 were also found **(I)**.



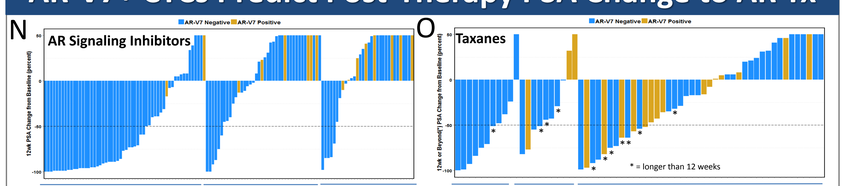
Prevalence and Frequency of AR-V7 CTC Positivity Increases by Line of Therapy
Most of the CTCs detected from 191 evaluable samples were AR-V7(-) **(J-M)**. AR-V7(+) CTCs were detected in 34 samples with AR-V7(+) CTC burden ranging from 0.74-105/mL (median 2.4/mL), The frequency of AR-V7(+) CTC detection increased by line of therapy **(J-L)**, ranging from 3% (2 of 67 samples) prior to first-line therapy, 18% (9 of 50 samples) prior to second-line therapy, and 31% (23 of 74 samples) prior to third or subsequent lines of therapy **(M)** ($p < 0.0001$).

M

Incidence and Subclonal Contribution of AR-V7(+) CTCs By Line of Therapy in mCRPC Setting			
Line of Tx in mCRPC Setting (n Samples)	1st (n=67)	2nd (n=50)	3rd or greater (n=74)
Samples with AR-V7(+) CTCs (% of Samples Positive)	2 of 67 (3%)	9 of 50 (18%)	23 of 74 (31%)
In Samples with AR-V7(+) CTCs: Percent of CTCs AR-V7(+) Median (Range)	5.7% (0.3%-11.2%)	38% (14.3%-100%)	21% (0.5%-100%)

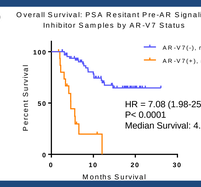


AR-V7+ CTCs Predict Post-Therapy PSA Change to AR Tx



Of the 128 samples from patients treated with AR Tx, 47 (37%) showed sensitive posttherapy PSA change and 81 (63%) had resistant posttherapy PSA change. None of the 47 with sensitive posttherapy PSA changes had AR-V7(+) CTCs (0%, 95% CI: 0.0%–9.41%). In contrast, of the 81 with resistant PTPC, 16 had AR-V7(+) CTCs (20%, 95% CI: 12.1%–30.4%) prior to therapy **(N)**. This was not the case for pre-taxane samples, where posttherapy PSA changes were not significantly different by AR-V7 status. **(O)**.

AR-V7+ Patients Have Poorer Outcomes Among PSA Resistant Patients

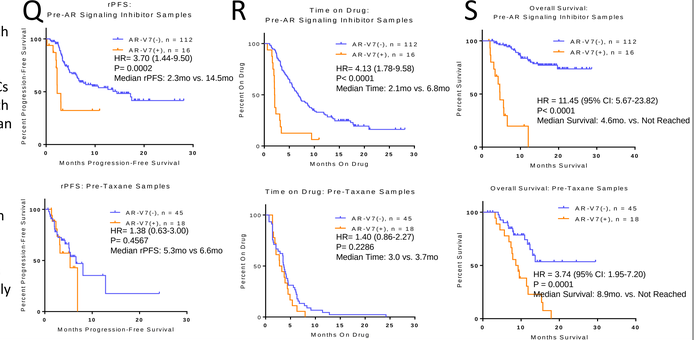


Multivariable Cox Proportional Hazard Analysis of Predictors of Overall Survival	
Effect	**P-value**
Line of Therapy (3rd or later vs 1st/2nd)	0.6889
Visceral Metastases Pre-Therapy	0.3958
LDH Pre-therapy (>250 vs ≤250 U/L)	0.0641
Patient Age (>65 vs ≤65 years)	0.6931
Hemoglobin (>12 vs ≤12 g/dL)	0.0236
AR-V7 Status	**<.0001**

A subset analysis of the pre-AR Tx samples with PSA-resistant profiles (n = 81) showed dramatically worse overall survival with pretherapy AR-V7(+) CTCs relative to those without (median 4.6 months vs. not reached, $p < 0.0001$)

Presence of AR-V7+ CTCs Predicts Response to AR Tx in All Time-To-Event Measures

Samples from patients treated with AR signaling inhibitors with pre-therapy AR-V7+ CTCs were associated with worse outcomes than those without AR-V7+ CTCs in radiographic progression free survival **(Q)**, time on therapy **(R)** and overall survival **(S)**. Pre-taxane samples were not significantly different by AR-V7 status.



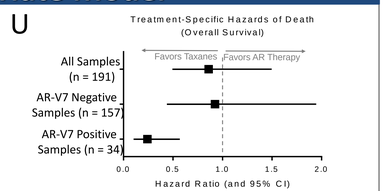
AR-V7+ Patients Exhibit Improved OS with Taxanes over AR Tx in Multivariate Model

T

Multivariable Cox Proportional Hazard Analysis of Predictors of Overall Survival	
Effect	**P-value**
Line of Therapy (3rd or later vs 1st/2nd)	0.1555
Liver and/or Lung Metastases Pre-Therapy	0.0031
LDH Pre-therapy (>250 vs ≤250 U/L)	0.0055
Patient Age (>65 vs ≤65 years)	0.0074
Hemoglobin (>12 vs ≤12 g/dL)	0.0067
Therapy	0.8363
AR-V7 Status	**< 0.0001**
AR-V7 Therapy Interaction	**0.0350**

A Cox PH model incorporating line of therapy, presence of visceral metastases, lactate dehydrogenase, patient age, hemoglobin, therapy type, and AR-V7 status was developed. The result showed that AR-V7 status remained the most significant factor ($p < 0.0001$) among all pretherapy clinical measures **(T)**, and that AR-V7(+) patients had more favorable survival times on taxanes relative to AR Tx, while AR-V7(-) patients did not **(U)**.

U



AR-V7 : Therapy Interaction: Multivariable Cox PH Model		
	Comparison	**Hazard Ratio (95% CI)**
AR-V7 Status & Therapy	AR-V7(+): Taxane vs AR	**0.242** (0.103 to 0.569)
	AR-V7(-): Taxane vs AR	0.924 (0.440 to 1.946)

Conclusions

- AR-V7 IF expression in CTCs of mCRPC patients assessed through the Epic Sciences platform is compatible with diagnostic workflows (median 24 hours from blood draw to processing).
- AR-V7 prevalence increases with increased exposure to systemic therapy AR-V7 ($p < 0.0001$) but still represents a minority population of total CTCs suggestive of disease heterogeneity.
- AR-V7 expression on Epic CTCs is 100% specific (100% PPV) to de novo PSA resistance, and predicts shorter time on drug (HR=4.61, p<0.0001), shorter rPFS (HR=2.92, p=0.0002), and shorter OS (HR=11.44 , p<0.0001) of patients receiving AR Tx.
- AR-V7 expression is not associated with PSA resistance, time on drug, or rPFS in taxane treated patients.
- The detection of nuclear AR-V7 positive CTCs in men with progressive mCRPC supports the selection of taxane based therapy over AR directed therapies. Doing so was associated with a 76% reduction in the risk of death (HR=0.242, p=0.035) relative to the use of AR directed therapy in this retrospective analysis. Prospective validation is planned.

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