AR-V7 Status and CTC Heterogeneity Improve the Prediction of Drug Sensitivity and Patient Outcomes for Taxanes and Approved AR Targeted Therapies

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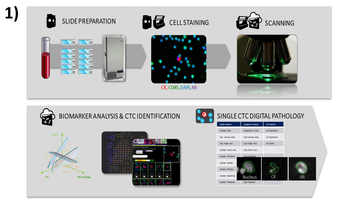
Background

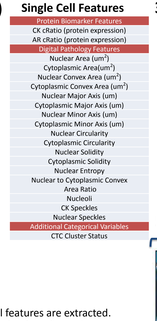
A critical unmet need in the management of mCRPC is how to use currently approved agents to maximize survival for the individual patient. Outcomes to AR signaling inhibitors such as, abiraterone acetate and enzalutamide (AR Tx), and taxane based chemotherapy, the two most commonly used drug classes, vary with line of therapy and sequence of administration, but cross-sensitivity and resistance are not predictable at the individual patient level when a treatment decision is required.

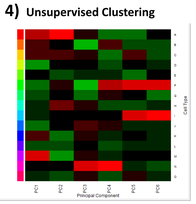
We recently identified a statistically significant therapy interaction between nuclear AR-V7 protein expression in CTCs and improved OS with Taxanes over AR Tx (HR=0.242,p=0.0350)[1] supporting taxane use for AR-V7+ patients. Similarly, high CTC heterogeneity in mCRPC patients also demonstrated a therapy interaction supporting Taxanes over AR Tx (HR=0.302, p=0.0229)[2]. We studied both markers in a single cohort and related the two biomarkers to clinical outcomes.

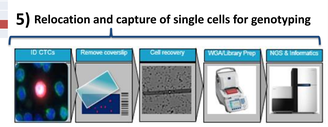
Methods for CTC Detection; Phenotypic, Genomic Characterization, and Heterogeneity Score

191 blood samples from 161 unique patients were analyzed with the Epic Sciences platform. Analysis included digital pathology of 23 discrete phenotypic cell features inclusive of AR and CK expression, and cellular size and shape measures. Single CTCs were characterized, data standardized, features clustered and categorized into 15 phenotypically distinct CTC subtypes. Individual patient samples were then analyzed for the frequency and heterogeneity (Shannon Index) of CTC subtypes and monitored for clinical endpoints. A subset of CTCs (n=741) were individually sequenced and analyzed for clonality and CNV to assess genomic heterogeneity.









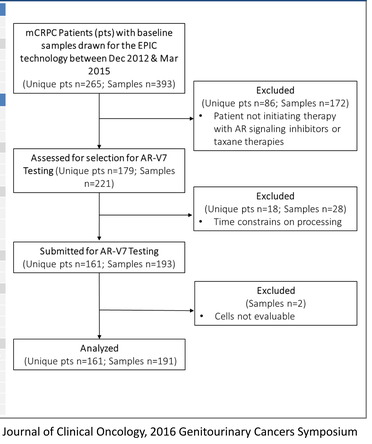
Schematic of Epic CTC Platform CTC enumeration, morphology, and biomarker analyses workflow:

1) Nucleated cells from blood sample placed onto slides and stored in a -80˚C biorepository. Slides are stained with cytokeratin (CK), CD45, DAPI, AR N-term or AR-V7 and scanned. CTC candidates are detected by a multi-parametric digital pathology algorithm followed by human reader confirmation of CTCs and quantification of biomarker expression.
2) CTCs are segmented within the DAPI, CK, and AR channels and single cell features are extracted.
3) CTCs undergo Principle Component Analysis (PCA) removing noise and redundant dimensions, and weighing features with more variance.
4) Machine learning clustering algorithms found 15 CTC subtypes from macro trends in high-dimensional biomarkers across all CTCs from all samples in cohort, and assigned each CTC to 1 of 15 subtypes. Heterogeneity is quantified by counting CTCs per "Cell Type" in each sample, then using a standard Shannon Index to quantify CTC phenotypic diversity per patient sample.
5) Single cells are identified, relocated, captured, and sequenced for genomic correlation.

Patient Demographics

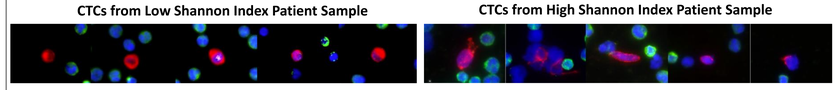
Patient Characteristic	All Patients
Number of Unique Patients	161
Age, Years	68 (45-91)
Primary Treatment	
Prostatectomy	77 (48%)
Radiation	28 (18%)
Brachytherapy	7 (4%)
None	49 (30%)

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%)		53 (27.5%)
Taxane ± other [ADC, exp, combo]	10 (7.7%)	0	<0.0001	10 (5.2%)
AR AND Taxane ± other	30 (23.0 %)	33 (52.4%)		63 (32.6%)
Chemotherapy Status				
Chemo-naïve	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)



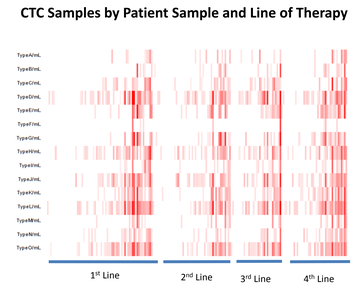
References: 1) Scher et al 2016, JAMA Oncology, Epub 4 June 2016 **2)** Scher et al 2016, Journal of Clinical Oncology, 2016 Genitourinary Cancers Symposium (January 7-9, 2016). Vol 34, No 2_suppl (January 10 Supplement), 2016: 163

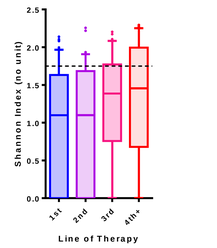
15 CTC Subtypes Defined by Morphology and Protein Enable Quantification of Intra-Patient Cancer Diversity



Mathematical Cell Types

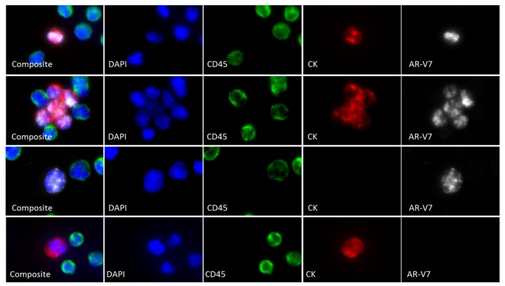
Cell Type	CTCs in Cohort
CellTypeA	83
CellTypeB	176
CellTypeC	272
CellTypeD	1219
CellTypeE	545
CellTypeF	137
CellTypeG	727
CellTypeH	418
CellTypeI	166
CellTypeJ	615
CellTypeK	362
CellTypeL	1307
CellTypeM	143
CellTypeN	148
CellTypeO	610





CTCs Characterized by Epic Nuclear AR-V7 Test

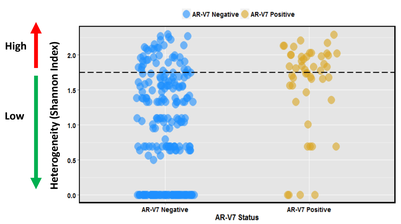
AR-V7 protein is expressed in the nucleus of multiple CTC subtypes including "traditional" CK(+) CTCs **(A)**, CTC Clusters **(B)**, and CK(-) CTCs **(C)**. CTCs not expressing AR-V7 were also found **(D)**.



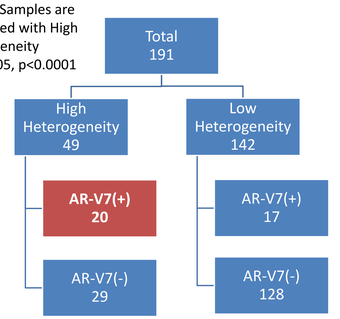
Incidence and Subclonal Contribution of AR-V7(+) CTCs By Line of Therapy in mCRPC Setting			
Line of Tx in mCRPC Setting (Number of Samples)	1st (n=67)	2nd (n=50)	3rd or greater (n=74)
% of Samples with AR-V7 (+) Cells	2 (3%)	9 (18%)	23 (31%)
% of AR-V7(+) CTC In AR-V7 (+) Samples Median (Range)	5.7%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

AR-V7+ Samples Frequently Have High CTC Heterogeneity

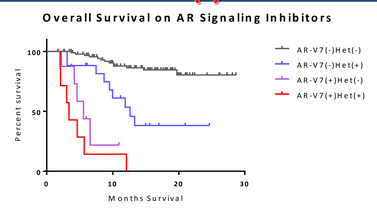
		High CTC Heterogeneity	Low CTC Heterogeneity
		49	142
AR-V7+	34	20 (61%)	14
AR-V7-	157	29	128

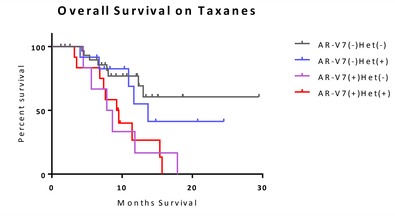


AR-V7+ Samples are associated with High Heterogeneity OR=6.305, p<0.0001



High Heterogeneity is Associated with Increased Risk of Death in AR-V7(-) Pts Treated with AR Tx but Not Taxanes





Biomarker		Hazard Ratio of Overall Survival vs. AR-V7, Low CTC Heterogeneity			
AR-V7	Heterogeneity	ARS TX (n)	ARS Tx (HR)	Taxane (n)	Taxane (HR)
Positive	High	8	22.7, p < 0.0001	12	3.32, p = 0.0068
Positive	Low	8	12.7, p < 0.0001	6	4.00, p = 0.0051
Negative	**High**	**17**	**4.72, p < 0.0001**	**12**	**1.40, p = 0.55**
Negative	Low	95	n/a	33	n/a

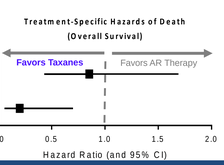
AR-V7+/HET+ Patients Have Reduced Risk of Death on Taxanes

Patients with AR-V7(+) CTCs and **high heterogeneity** have **improved OS times with taxanes**

A Comparison of Treatment-Specific Effects of Biomarkers					
Study	Samples	Comparison: Taxane vs. ARSi	n Positive	Hazard Ratio: Overall Survival	p-value
Scher et al 2016, JAMA Oncology[1]	191	AR-V7(+) patients	34	0.24 (1.0 to 0.57)	**0.035**
Scher et al 2016, ASCO GU[2]	221	High Heterogeneity patients	56	0.30 (0.10 to 0.85)	**0.023**
This study	191	AR-V7 and High Heterogeneity (double positive) patients	20	0.20 (0.057 to 0.70)	**0.012**

Multivariate Feature	HR	p-value
Both Biomarkers (positive vs. negative)	1.39	0.46
3rdline or later (1=yes, 0=no)	1.88	0.064
Visceral Mets (1=yes, 0=no)	1.63	0.17
PSA pre-therapy (1 is >37.7, 0 is <=37.7)	1.55	0.22
LDH pre-therapy (1 is >250, 0 is <=250)	1.73	0.10
Albumin pre-therapy (1 is >4, 0 is <=4)	0.77	0.36
Hgb pre-therapy (1 is >12, 0 is <=12)	0.47	0.039
Alk pre-therapy (1 is >130, 0 is <=130)	1.40	0.30
Treatment (1 = Taxane, 0 = ARS)	1.17	0.65
Both Biomarkers : Taxane Interaction	**0.20**	**0.012**

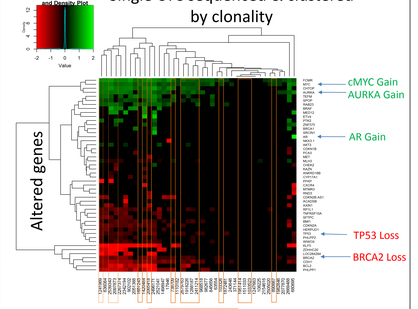
AR-V7 : Therapy Interaction: Multivariable Cox PH Model		
	Comparison	Hazard Ratio (95% CI)
AR-V7 & Heterogeneity Status & Therapy	AR-V7 (+) & Het(+): Taxane vs AR	0.20 (0.057 to 0.70)
	All Samples In Cohort	0.85 (0.43 to 1.69)



Genomic Complexity & Heterogeneity of an AR-V7+/HET+ Patient

AR-V7+/HET+ patient genomic analysis:
37% (15/41) CTCs sequenced from a single patient blood draw are AR-V7+

Gene alterations associated with AR signaling independent pathways are found in both AR-V7 (+) and AR-V7 (-) CTCs include cMYC, AURKA, TP53, BRCA2.



Conclusions

- Nuclear AR-V7 protein expression and high phenotypic heterogeneity, when analyzed separately and in combination, predict poor outcomes on AR Tx alone or in combination.
- AR-V7 nuclear positivity is strongly associated with high phenotypic heterogeneity (OR= 6.3, p<0.0001).
- Patients with both nuclear AR-V7 and high heterogeneity have the worst outcome on AR Tx (HR=22.7, p<0.0001) and the strongest prediction of benefit from taxane therapy vs. ARS by hazards of death reduction (HR=0.20, p=0.011).
- Single CTC sequencing highlights high genomic complexity with multiple drivers of disease progression in AR-V7/HET+ disease.
- Prospective validation of these biomarkers is ongoing.

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