Examination of the additive value of CTC biomarkers of Heterogeneity (Het) and Chromosomal Instability to Nuclear-localized (nl) AR-V7+ CTCs in prediction of poor outcomes to Androgen Receptor signaling inhibitor (ARSi) in Metastatic Castration Resistant Prostate Cancer (mCRPC)

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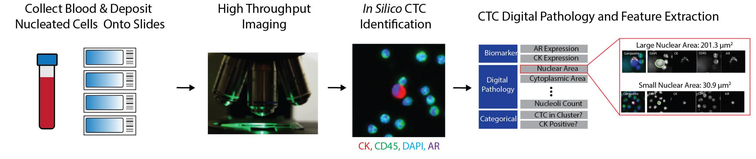
BACKGROUND

- AR Signaling Inhibitors (ARSi) are the standard first line therapy for men with mCRPC based on a high response rate and proven survival benefit. In the 2nd line or greater line setting, taxanes or a 2nd ARSI are most frequently used, but determining who will respond to one class over the other can not be predicted based on clinical parameters alone or based on the response to the first line agent.
- Androgen receptor (AR) splice variants lack portions of the ligand binding domain that activate signaling independent of ligand. The coded exon of AR variant 7 (AR-V7) codes for a protein that can be detected in circulating tumor cells (CTC) and predicts for resistance to ARSi.
- The Epic Sciences Nuclear Localized AR-V7 (nlAR-V7) test detects the coded protein of the variant in CTCs which was shown to predict for an improved survival for patients with nlAR-V7 positive CTCs who were treated with a taxane vs. an ARSi. The test is covered by Medicare, included in the NCCN guidelines, and NYS approved.
- nlAR-V7 positivity does not explain all resistance. The prevalence was previously found to be 3%, 18%, and 31% in the 1st, 2nd and 3rd Lines respectively (Scher et al., 2016 JAMA Oncol).

The Epic Sciences Functional Cell Profiling (FCP) Platform

CTC Identification using the Epic Sciences platform



Study Design And Goals

CONSORT

236 2nd Line mCRPC Patients From 3 Centers (MSK, LHS, ICR) From 2013-2017

- 119 Blood Samples Taken Prior to Starting an ARSi
- 117 Blood Samples Taken Prior to Starting a Taxane

↓

CTC nlAR-V7 Testing from 2013-2017

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Analysis of additional Epic biomarkers
1) CTC Heterogeneity (Shannon Index)
2) CTC Chromosomal Instability (pLST)

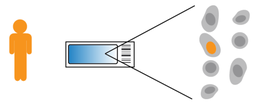
PATIENT DEMOGRPAHICS

Patient Characteristics	Next Tx = ARSi	Next Therapy = Taxane
Blood Samples, N		
Unique Patients	112	106
Unique Samples	119	117
Therapy Line - no. (%)		
Pre 2nd Line	72 (60.50%)	27 (23.1%)
Pre 3rd+ Line	47 (39.50%)	90 (76.9%)
Sites of Metastases - no. (%)		
Lymph Node Only	15 (12.6%)	6 (5.1%)
Bone Only	33 (27.8%)	20 (17.1%)
Lung Only	1 (0.8%)	0 (0.0%)
Multiple	70 (58.9%)	82 (70.1%)
Baseline Variables, median (min, max)		
Patient Age, years	69 (40,87)	70 (48,85)
PSA ng/mL	32.55 (0.82,1516)	138.3 (0.06,1627)
ALB g/L	4.1 (2.4,46)	4.1 (2.9,45)
ALK U/L	95.5 (42,1040)	134 (43,1816)
HGB g/dL	12.2 (7.1,136)	11.4 (8,151)
LDH U/L	203 (101,2115)	246.5 (141,1487)
WBC 10^6 cells/mL	6.2 (2.3,49.2)	6.8 (2.3,15.6)

Main Question: What is the additive value of CTC Heterogeneity and Chromosomal Instability (CIN) to nuclear localized AR-V7 in predicting ARSi resistance for 2nd Plus Line mCRPC patients?

The Epic Sciences CTC Based Biomarkers of ARSi Resistance

Biomarker 1: CTC Nuclear Localized AR-V7 (nlAR-V7)

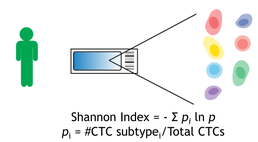


Biomarker+ Definition ≥ 1 AR-V7+ CTC

Prevalence by Tx Line in This Study	
Pre 2nd	Pre 3rd+
19/99 (19.2%)	38/137 (27.8%)

CTC nlAR-V7 has been found to predict ARSi resistance in three independent studies, two of which were blinded (Scher et al., JAMA Oncol 2016, Scher et al., JAMA Oncol 2018, Armstrong et al., JCO 2019)

Biomarker 2: CTC Heterogeneity

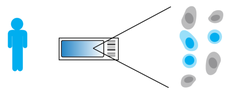


Biomarker+ Definition Shannon Index > 1.5

Prevalence by Tx Line in This Study	
Pre 2nd	Pre 3rd+
28/99 (28.3%)	47/137 (34.3%)

Developed in a large cohort of mCRPC patients, CTC Shannon Index > 1.5 was found to associate with ARSi resistance (Scher *et al.*, 2017 Cancer Res) and was later blindly validated as part of the PROPHECY trial (*Armstrong et al.*, 2019 J Clin Oncol)

Biomarker 3: CTC Chromosomal Instability (CIN)



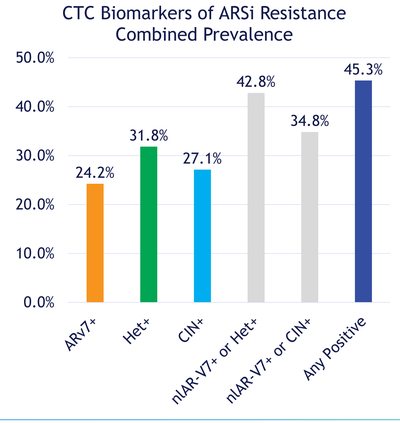
Biomarker+ Definition pLST+ CTC > 3/mL

Prevalence by Tx Line in This Study	
Pre-2nd	Pre-3rd+
20/99 (20.2%)	44/137 (32.2%)

predicted #**L**arge **S**cale **T**ransitions (pLST) is a computer vision algorithm that detects CTC image features associated with LSTs/CIN (Scher *et al.*, ASCO 2016)

CTC pLST is a surrogate measure of single cell chromosomal instability (CIN) and was recently found to associate with ARSi resistance in a large cohort of mCRPC patients (Scher et al., ASCO GU 2019). Blinded validation is underway.

CTC Heterogeneity and CIN Identify an Additional ~20% of Patients Predicted to be Resistant To 2nd Line ARSi

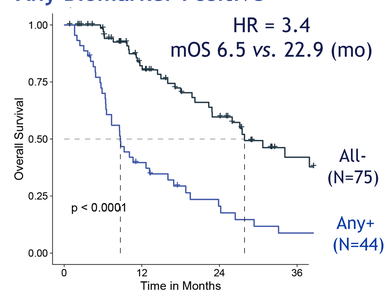


Prevalence of All CTC Biomarker Combinations Pre-2nd Plus Line

Biomarker Status			Prevalence (N= 236)
nlAR-V7+	Het+	CIN+	Count (%)
+	+	+	29 (12.3%)
-	+	-	25 (10.6%)
-	+	+	19 (8.1%)
+	-	-	16 (6.8%)
+	-	+	10 (4.2%)
-	-	+	6 (2.5%)
+	+	-	2 (0.9%)
			Sum = 45.3%

CTC Chromosomal Instability and Heterogeneity Identify ARSi Non-Responders In Addition to CTC nlAR-V7

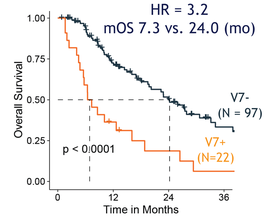
Any Biomarker Positive



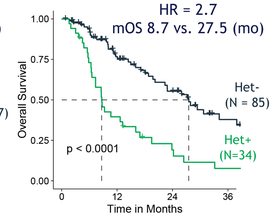
Analysis of ARSi Treated Patients Only (N=119) for any biomarker (Left) or by individual biomarker (Bottom).

The combination of CTC nlAR-V7, Het, and CIN identify non-responders in 44/119 (37%) of patients that started a 2nd Line AR Signaling Inhibitor

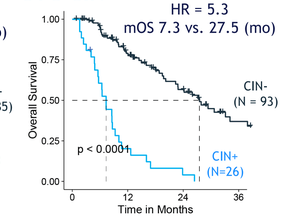
nlAR-V7



CTC Heterogeneity



CTC CIN



Combined CTC nlAR-V7, Heterogeneity, and CIN Pre-2nd Line Provides Additive Prognostic Value to Baseline Clinical Variables

Prediction of ARSi resistance by single and combined use of CTC nlAR-V7, Heterogeneity, and pLST prior to 2nd Line therapy initiation

Biomarker	Patient Group	N	Univariate HR (OS)	*P*	Multivariate HR (OS)‡	*P*
nlAR-V7+	All ARSi	119	3.2	<0.001	3.6	<0.001
Het+	All ARSi	119	2.7	<0.001	1.9	0.02
CIN+	All ARSi	119	5.3	<0.001	4.6	<0.001
Any+	All ARSi	119	3.4	<0.001	2.6	<0.001
Het+	V7- ARSi	97	2.5	<0.001	1.5	0.05
CIN+	V7- ARSi	97	4.9	<0.001	4.2	0.001
Any+	V7- ARSi	97	2.8	<0.001	2.4	0.02

‡Each biomarker listed was used in a Cox PH Model that included baseline clinical variables, LDH, Line of Therapy, Presence of Liver or Lung Mets, Age, Hb. Total CTC counts did not add additional prognostic information.

CONCLUSIONS

Addition of CTC Het (Shannon Index) and CTC CIN (pLST) biomarkers to CTC nlAR-V7 identifies an additional 22% of mCRPC pts (45% of total) that are predicted to have poor survival to AR signaling inhibitors.

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