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 or greater line setting, taxanes or a 2nd ARSi are most frequently used, but determining who will respond to one class over the other cannot 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 (nAR-V7) test detects the coded protein of the variant CTCs which was found to predict for an improved survival for patients with nAR-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.
- nAR-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

PATIENT DEMOGRAPHICS

CTC Biomarkers of ARSi Resistance

Biomarker: CTC Nuclear Localized AR-V7 (nAR-V7)

Prevalence by Tx Line in This Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-2nd</th>
<th>Pre-3rd</th>
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<td>44/199 (22.9%)</td>
<td>38/137 (27.8%)</td>
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CTC nAR-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

Shannon Index = p_i / Σ p_i

CTC Heterogeneity and CIN Identify an Additional ~20% of Patients

Biomarker+ CTC Het (Shannon Index) + CIN

Prevalence by Tx Line in This Study

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<th>Group</th>
<th>Pre-2nd</th>
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<td>28/104 (27.1%)</td>
<td>47/131 (36.0%)</td>
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Developed in a large cohort of mCRPC patients, CTC Shannon index > 1.5 was found to associate with ARSi resistance in a large cohort of mCRPC patients (Scher et al., ASCO 2016) and was later blindly validated as part of the PROPECHY trial (Armstrong et al., 2019 J Clin Oncol).

Biomarker 3: CTC Chromosomal Instability (CIN)

Prevalence by Tx Line in This Study

<table>
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<tr>
<th>Group</th>
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<th>Pre-3rd</th>
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<tr>
<td>20/99 (22.9%)</td>
<td>44/137 (32.5%)</td>
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CTC Chromosomal Instability and Heterogeneity Identify ARSi Non-Responders In Addition to CTC nAR-V7

Any Biomarker Positive

nAR-V7+ HR = 3.4

mOS 6.5 vs. 22.9 (mo)

Analysis of ARSi Treated Patients Only

Any+ (N=119)

Any+ (N=44)

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

CTC Chromosomal Instability and Heterogeneity and CIN Pre-2nd Line Provides Additive Prognostic Value to Baseline Clinical Variables

Combined CTC nAR-V7, Heterogeneity, and CIN Pre-2nd Line

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

Biomarker Patient Group N Univariate HR (95% CI) P Multivariate HR (95% CI) P

nAR-V7+ All ARSi 119 3.2 <0.001 1.6 <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

Het+ All ARSi 119 3.4 <0.001 2.6 <0.001

CIN+ All ARSi 119 2.5 <0.001 1.5 0.05

Het+ V7- ARSi 97 2.5 <0.001 1.6 <0.001

CIN+ V7- ARSi 97 4.9 <0.001 4.2 0.001

Het+ V7 ARSi 97 2.4 <0.001 2.4 0.02

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

CONCLUSIONS

Addition of CTC Het (Shannon Index) and CIN CTC pLST biomarkers to CTC nAR-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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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?