Single Cell Analysis of AR N terminal, AR C terminal and the AR-V7 Splice Variant in the CTCs of metastatic Castration Resistant Prostate Cancer (mCRPC) Patients

James Kelvin1, David Lu1, Jessica Louw1, Davin Packer1, Richard Bambury2,3, Dana Rathkopf4,5, Nicole Schreiber2, Ryan Brennan1, Natalie Prigzohn2, David Brown1, Rachel Krupa1, Adam Jendrisak1, Lyndsey Dugan1, Edward Swangren1, Mark Landers1, Florence Lee1, Martin Fleisher2, Dena Martinucci2, Ryan Dittamore1, Howard J. Scher1

1 Epic Sciences, Inc., San Diego, CA 2 Sidney Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan-Kettering Cancer Center, New York, NY 3 Department of Medicine, Weill Cornell Medical College, New York, NY

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 but not to taxane chemotherapy. Alterations to the ligand binding domain (LBD) measured through the loss of the AR C terminus in bone and CTCs have also been associated with resistance to A & E but not to taxanes. Together, measurements of AR-V7 and/or AR N & C termini (AR N & C) may provide clinical utility in therapy selection between A & E or taxanes.

Previous work showed high CT CTC phenotypic heterogeneity in patients resistant to A & E. Understanding if AR-V7 is the predominant driver alteration of resistance in mCRPC patients is critical to the development of novel AR N terminal targeted therapies. A key limitation to assessing AR-V7 mRNA in CTCs is the pre-analytic robustness of measurements in live cells (>2h to processing, combined with 59 cycles of qPCR).

To address both questions, we developed an AR-V7 rabbit MAb if assay to assess single CTCs utilizing samples from mCRPC patients and intra-patient sensitivity to these agents. The same samples were assessed with an AR N BC IF test to understand driver mechanisms in context of other AR negative CTC subtypes.

Methods

Fifty patients tested samples were collected at time of metastatic disease diagnosis of 3rd generation substrains (2D, E2, Alkaline substrate CT) and Cabazitaxel (3D). Outcomes were recorded as Sensitivity (S), Pattern 1 and 2, or Resistant (R). Pattern 5 (AR) Samples were processed utilizing the Epic Sciences platform.

AR-V7 in CTCs Predicts Resistance to AR Tx but not to Taxane Chemotherapy

Patient Demographics

**AR-V7 in CTCs Predicts Resistance to AR Tx but not to Taxane Chemotherapy**

Patient Line of Therapy

<table>
<thead>
<tr>
<th>Line</th>
<th>AR-V7 Positive</th>
<th>AR-V7 Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**AR-V7 Represents a Subset of Total AR LBD Alterations**

- AR-V7 mRNA expression in CTCs of mCRPC patients assessed through the Epic Sciences platform is compatible with diagnostic sensitivity (median 27 hours from close to processing) and specificity for AR-V7 mRNA detected in AR-V7 positive AR intact normal.
- AR-V7 prevalence increased with increased exposure to systemic therapy. The low median percentage (15%) of AR-V7 CTCs in patients who have in at least 1 AR-V7 CTC shows disease heterogeneity and suggests that AR-V7 alone may not be the primary driver of disease progression.
- AR-V7 presence was highly specific to insensitivity of AR directed therapy, and AR-V7 negativity was associated with response to parathyroidectomy.
- The high number of patients with AR-V7 AR C terminal loss of less than 50% suggests alternative or co-existing AR LBD alterations: what rates associate with a specific clinical phenotype or outcome is uncertain.
- Utilization of an AR C terminal loss assay may enable the identification of patients most likely to benefit from novel AR targeted terminal therapies, particularly in the 2nd and 3rd treatment decision point where the degree of heterogeneity is less than after additional lines of therapy.

**Conclusions**

- AR-V7 expression in CTCs of mCRPC patients assessed through the Epic Sciences platform is compatible with diagnostic sensitivity (median 27 hours from close to processing) and specificity for AR-V7 mRNA detected in AR-V7 positive AR intact normal.
- AR-V7 prevalence increased with increased exposure to systemic therapy. The low median percentage (15%) of AR-V7 CTCs in patients who have in least 1 AR-V7 CTC shows disease heterogeneity and suggests that AR-V7 alone may not be the primary driver of disease progression.
- AR-V7 presence was highly specific to insensitivity of AR directed therapy, and AR-V7 negativity was associated with response to parathyroidectomy.
- The high number of patients with AR-V7 AR C terminal loss of less than 50% suggests alternative or co-existing AR LBD alterations: what rates associate with a specific clinical phenotype or outcome is uncertain.
- Utilization of an AR C terminal loss assay may enable the identification of patients most likely to benefit from novel AR targeted terminal therapies, particularly in the 2nd and 3rd treatment decision point where the degree of heterogeneity is less than after additional lines of therapy.

**Support:** MSKCC SPORE in Prostate Cancer (PS01), the Department of Defense Prostate Cancer Research Program (PC051382), the P50 National Cancer Institute 5P50 CA92629, the Department of Defense Breast Cancer Research Program (BC1201).