Prevalence and tissue concordance of BRCA2 copy number loss evaluated by single-cell, shallow whole genome sequencing of circulating tumor cells (CTCs) in castration resistant prostate cancer (CRPC)

Barnett ES1, Schonhoff J3, Schultz N1, Lee J2, Zaidi S1, Abida W1, Carmichael T1, Dago AE2, Solit D1, Wenstrup R2, Scher HI1

MSKCC1, Epic Sciences2

Introduction

•DNA damage-repair alterations occur in ~20-25% of mCRPC tissue samples1
•PARP inhibitors are now considered a standard of care for patients with homologous recombination deficiency (HRD), notably LOF mutations and deletions in BRCA2
•Obtaining sufficient tumor material for profiling is difficult due to the osteotropic nature of prostate cancer (PCa)
•Detection of copy number alterations (CNAs) using cell-free DNA (cfDNA) is also challenging due to high background of DNA from healthy cells
•CTCs represent a non-invasive source of genmic material which can be used to detect CNAs in BRCA2 and HRD-related genomic events, such as large scale transitions (LSTs)

Trial Design/Methods

•Retrospective analysis of 138 CTC samples collected concurrently with a baseline mCRPC tumor biopsy sent for sequencing using MSK-IMPACT2 (Figure 1)
•Single-cell shallow WGS on CTCs to assess copy number alterations3 (blinded to MSK-IMPACT results) (Figure 2)
•Sequencing results were compared for overall concordance and BRCA2 copy number status

Cohort Demographics

<table>
<thead>
<tr>
<th>Table 1. Demographics</th>
<th>CTC Sequencing</th>
<th>Tissue Sequencing</th>
<th>Both Sequencing</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>115 (84.8)</td>
<td>108 (78.5)</td>
<td>92 (66.7)</td>
<td>138 (100)</td>
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<tr>
<td>Laboratory Assessments</td>
<td></td>
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<tr>
<td>PSA (ng/mL) (Range)</td>
<td>28.46 (0.05-5679.14)</td>
<td>34.21 (0.03-6595.72)</td>
<td>39.06 (0.05-4626.23)</td>
<td>38.16 (0.05-6575.14)</td>
</tr>
<tr>
<td>Prostate Volume (ml)</td>
<td>125 (64.7)</td>
<td>125 (64.7)</td>
<td>125 (64.7)</td>
<td>125 (64.7)</td>
</tr>
<tr>
<td>PSA Density (ng/mL/mL)</td>
<td>246.6 (1.26-3381)</td>
<td>249 (126-3381)</td>
<td>249 (126-3381)</td>
<td>249 (126-3381)</td>
</tr>
<tr>
<td>Treatment Exposures</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Prior Taxane (n%)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Prior Androgen (n%)</td>
<td>7 (3.7)</td>
<td>7 (3.7)</td>
<td>7 (3.7)</td>
<td>7 (3.7)</td>
</tr>
<tr>
<td>Prior Tamoxifen (n%)</td>
<td>9 (8.0)</td>
<td>9 (8.0)</td>
<td>9 (8.0)</td>
<td>9 (8.0)</td>
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<tr>
<td>Disease status</td>
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<td></td>
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<tr>
<td>LST (n%)</td>
<td>12 (9.8)</td>
<td>12 (9.8)</td>
<td>12 (9.8)</td>
<td>12 (9.8)</td>
</tr>
<tr>
<td>Other (n%)</td>
<td>31 (27.0)</td>
<td>30 (27.3)</td>
<td>27 (20.3)</td>
<td>35 (25.4)</td>
</tr>
</tbody>
</table>

Overall CTC-Tissue Genomic Concordance

•Concordant copy number profiles (>60% tissue similarity of 1+ CTC) were noted in 51% (47/92) matched CTC/tissue pairs (Figure 3A, black circles)

Prevalence of BRCA2 Loss in CTCs vs Tissue

•BRCA2 Loss was detected in 21% of sequenced tissue samples and 50% of CTC samples (Figures 4A & S)
•A median of 46% (range 4%-100%) of CTCs harbor BRCA2 loss in samples which a BRCA2 loss was detected
•BRCA2 loss was detected in CTCs in 46/92 (50%) of cases where both assays were successful: 16/19 (84%) tissue-positive and 30/73 (41%) tissue-negative cases (PPA=84%, NPA=59%) (Figure 5)

Large Scale Transitions as a Marker of HRD

•LSTs, chromosomal breakages that generate chromosomal gains or losses of 10 Mb or more, are indicative of HRD (High LST profile depicted in Figure 4A)
•CTCs with BRCA2 loss (n=220) had a significantly higher number of LSTs as compared to BRCA2 neutral (n=565) CTCs (p<0.0001) (Figure 6)

BRCA2 Loss and Co-occurring Alterations

•Co-occurring CNAs were assessed in 5 genes relevant to PCa (AR, MYC, TP53, RB1, PTEN)
•All assessed alterations were significantly more prevalent in BRCA2 loss CTCs (n=220) compared to BRCA2 neutral/ gain (n=565) (all p<0.0001) (Figure 7)

Conclusions

•Epic Sciences single-cell CTC sequencing assay can detect BRCA2 loss in a majority of cases which tissue sequencing detected the loss and numerous instances which it did not
•CTCs with detected BRCA2 loss have a significantly higher number of LSTs and co-occurring CNAs, indicative of HRD
•Single-cell CTC sequencing can potentially be utilized, alone or in conjunction with cfDNA sequencing, to detect actionable alterations in BRCA2 and HRD-related genes to predict sensitivity to PARPI

References

1. Figure 5. BRCA2 status in matched tissue profiles and CTC samples

2. Figure 1. REMARK diagram of Cohort

3. Figure 2. Overview of sequencing methodologies (Epic Sciences & MSK-IMPACT)