Liquid Biopsy Molecular Signatures of Hormone Therapy Resistance in Men with Metastatic Castration-Resistant Prostate Cancer (mCRPC)

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Background: We demonstrated in the multicenter prospective PROPHECY trial that men with mCRPC who have circulating tumor cells (CTCs) that express AR-V7 have very poor outcomes when treated with enzalutamide or abiraterone. However, many men lack AR-V7, and additional molecular alterations likely contribute to resistance. Here, we determined whether baseline or post-treatment DNA alterations in CTCs from AR-V7 negative men with mCRPC could provide clinical utility in predicting outcomes with these hormonal therapies.

Methods: We analyzed whole-genome copy number alterations (CNA) using array comparative genomic hybridization (aCGH) in CTCs from blood of 48 high-risk mCRPC men treated with enzalutamide or abiraterone, including 45 baseline and 28 progression samples. We focused our analysis only on men who tested AR-V7 negative by the Epic Sciences nuclear protein assay. Longitudinal somatic exome mutations in CTCs before and after progression on abi/enza were detected using whole-exome sequencing (WES) in a subset of 11 men with mCRPC and compared to matched germline DNA. Mutations detected in CTC DNA were functionally validated in COSMIC. Findings were associated with clinical outcomes, comparing those men who benefit from therapy (progression free survival, or PFS, >6 mo) vs. those who do not (PFS<3 mo).

Results: We observed broad heterogeneity of copy number alterations between patients; common genomic alterations were identified in CTCs from 48 men included gain in KDM6A (44%), FOXA1 (44%), and AR (38%), and loss in ZFHX3 (57%), BRCA1 (30%) and PTEN (25%). Men who had clinical benefit to abi/enza (n=25, median PFS 10 mo) were more likely to have CTCs with genomic gains of ATM, NCOR2, or PTEN, or loss of BRAF, ABL1, or NCOR1. Likewise, men who did not benefit from abi/enza

(n=16, PFS median 2 mo) had CTCs with more genomic alterations than responding men (median 20 vs. 14, p=0.01), and were enriched for gain of BRCA2, APC, KDM5D, MYCN, CYP11B1, BRD4, and AR, and loss of PTEN, CHD1, ERG, and NCOR2. After progression on abi/enza, we observed clonal evolution of CTCs harboring gain of ATM, FOXA1, UGT2B17, KDM6A, CYP11B1, MYC, APC, EZH2, and NCOR2, and loss of NCOR1, PXN, ZFHX3, ERG, and RUNX2. Exome mutation analysis of CTC DNA detected various COSMIC-validated non-synonymous mutations in progressed or non-responding patients' CTC DNA with enrichment of pathogenic mutations in TP53 (55 vs 27% at baseline), AKAP9 (36 vs 9%), CDK12, KMT2D, and BRAF (each 36 vs 18%), and BRD4 and SPOP (each 18 vs 0%).

Conclusions: We demonstrate that specific CTC genomic profiles associated with WNT, DNA repair, epigenetic, AR signaling, and lineage plasticity pathways are associated with worse clinical outcomes in AR-V7 negative men with mCRPC treated with abiraterone or enzalutamide. Further mechanistic and validation studies are planned.

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