Multi-parametric liquid biopsy analysis of circulating tumor cells (CTCs), cell-free DNA (cfDNA), and cell-free RNA (cfRNA) in metastatic castrate resistant prostate cancer (mCRPC)

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Background: Molecular profiling of prostate cancer using liquid biopsies such as CTC capture and cell-free nucleic acid analysis is advancing rapidly. These techniques yield informative yet distinct datasets, and additional insights may be gained by simultaneously interrogating multiple liquid biopsy components to construct a more comprehensive molecular disease profile. Our laboratory founded a multi-platform Liquid Biopsy Core at the USC Norris Comprehensive Cancer Center (NCCC), and we have conducted an initial proof of principle study aimed at piloting this multi-parametric approach.

Methods: Blood was drawn under an IRB-approved protocol with informed consent from 21 mCRPC patients encountered at USC NCCC. Samples were analyzed simultaneously for the following: CTC enumeration and single CTC and matched white blood cell (WBC) capture for whole genome amplification (WGA) and low-pass copy number variation (CNV, CellSearch and DEPArray platforms); CTC DNA and matched cfDNA for somatic single nucleotide variant (SSNV) analysis using targeted AmpliSeq NGS (Cynvenio Liquid Biopsy and Ion S5 platforms); plasma cfRNA extraction (Qiagen) and qRT-PCR for AR, AR-V7, and PCA3 (Liquid Genomics). When available, liquid biopsies were compared with matched tumor molecular profiles (e.g. FoundationOne).

Results: Fifteen of 21 patients (71%) had detectable CTCs by CellSearch (range: 1-421/7.5mL, median: 16/7.5mL). Thirteen of 21 patients (62%) had detectable SSNVs in CTC DNA and/or matched cfDNA, including mutations in *TP53*, *PIK3CA*, *HRAS*, and *EGFR*. Matched CTC DNA and cfDNA demonstrated both shared and distinct SSNVs. A majority of these mutations were present in matched tumor profiles, but some were exclusive to the liquid biopsies. Copy number analysis of single CTCs was performed in 3 of the patients, and all 3 had CNVs in multiple cancer relevant genes (amplifications: *AR*, *MYC*, *BCL6*, *SOX2*, *STAT4*, *TERC*, *CCND1*, *SRD5A1*, *SRD5A3*, *TMPRSS2*, *ZBTB10*, *PCA3*; deletions: *KLF5*, *CHD1*, *BRCA2*, *ATM*, *FANCI*, *NKX3.1*, *RB1*). CNV profiles in single CTCs overlapped with matched solid tumors but also contained new and distinct gains and losses suggestive of heterogeneity and clonal evolution. Plasma PCA3 and AR expression was detected in 18 of 21 (86%) and 19 of 21 (90%) cfRNA samples, respectively, and AR-V7 was detected in 2 of 21 cfRNA samples, both from patients who had progressed on abiraterone and docetaxel. Second time points are being collected and analyzed in a subset of patients.

Conclusions: In this pilot cohort, simultaneous multi-parametric profiling was feasible for CTC DNA mutations and copy number variations as well as matched plasma cfDNA mutations and cfRNA gene expression. These disease-specific molecular profiles were highly concordant with tumor tissues, but they also contained new, potentially actionable alterations that were unique to CTC DNA or cfDNA in matched samples. Expanded studies will build upon this multi-parametric approach to enhance and optimally leverage liquid biopsies for molecularly directed patient management.

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