### Serial genotypic characterization of circulating tumor cells (CTCs) in patients with metastatic castration resistant prostate cancer (mCRPC) undergoing treatment with abiraterone acetate (abi) or enzalutamide (enza)

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#### Background:
While enza and abi have substantially improved outcomes for patients (pts) with mCRPC, de novo and acquired resistance mutations are increasingly recognized.

#### Methods:
Pts receiving abi or enza in the course of routine clinical care were consented for blood collection at weeks 0, 4, 8 and 12 of therapy, and at the time of progression (based on Prostate Cancer Working Group 3 [PCWG3] criteria). CellSearch was used for CTC enumeration; individual cells were isolated and subsequently classified for EpCAM and C45 positivity. RNA sequencing (RNA-seq) was performed on pools of up to 10 CTCs.

#### Results:
Amongst 36 pts enrolled, median age was 71 (range, 54-84) and median PSA was 21.9 ng/dL (range, 0-918.3). Regarding treatment, 21 pts received abi and 15 received enza. By PCWG3 criteria, 23 pts met the definition of progression on abi or enza. Mean/median CTC count was 158/5 (IQR 25%-75%, 0-15). On RNA-seq of CTCs collected at the time of progression, AR was the most mutated gene followed by ATRX, GNAS, FOXA1, KMT2A and CNOT1. Several deleterious mutations in the DNA damage response genes were noted including frameshift mutations in PRKDC, MSH2 and MLHI. Differential gene expression analysis between abi/enza sensitive and abi/enza resistant samples revealed 2100 differentially regulated genes in drug-resistant CTCs. Ingenuity pathway analysis was used to identify pathways altered due to differential regulation of these genes. Among these pathways, TGFβ and CCEFN1 signaling were found to be significantly up-regulated in drug resistant CTCs. In vitro enza-resistant models will be presented, offering validation of our clinical findings.

#### Conclusions:
RNA-seq of CTCs representing abi/enza sensitive and resistant states can identify potential mechanisms of resistance. Therapies targeting the downstream signaling mediated by CCND1, such as CDK4/6 inhibitors (e.g., palbociclib or ribociclib), could avert resistance. Targeting TGFβ, another putative mediator of resistance, may be warranted.

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Sumanta Kumar Pal

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