**Harnessing the power of liquid biopsy for understanding mechanisms of PD-L1/PARP1-targeted therapy resistance in metastatic castration-resistant prostate cancer**

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Background:

Metastatic castration-resistant prostate cancer (mCRPC) is a class of advanced prostate cancer with a high mortality which is largely due to the frequent emergence of therapy resistance. Combination therapy that targets PD-L1 and PARP1 initially showed promising results, yet most patients suffered from disease progression within a year. Liquid biopsy, such as circulating-tumor DNA (ctDNA), offers tremendous opportunities for interrogating the genomic impacts without invasive tissue sampling. Therefore, we hypothesized that mCRPCs that failed to respond to the combination therapy carried distinct genomic and immunological profiles, which were captured in plasma ctDNA and circulating immune cell genomes and could be computationally resolved using whole-genome sequencing (WGS).

Methods:

To address this question, we obtained plasma cell-free DNA (cfDNA), a mixture of ctDNA and other tissue-derived DNA, from 38 individuals treated with the PD-L1/PARP1-targeted combination therapy (NCT02484404). WGS (median coverage: 139x) was performed using cfDNA and fragmented buffy coat DNA as germline control. Various computational approaches were used to identify germline and somatic single-nucleotide variants (SNVs), indels, copy-number variants (CNVs), and structural rearrangements, which were then individually curated and used to determine cfDNA tumor fraction. To infer immunological activities, buffy coat-derived genome sequences of T/B cell receptor genes were used to infer clonotypes using MIXCR.

Results:

Through various computational strategies, we observed a negative association between baseline cfDNA tumor fraction and therapy response. Even though predisposed *BRCA2* alterations predicted favorable outcomes as expected, oncogenic mutations in *TP53* and MAPK signaling pathway were negatively associated with therapy response. It was also noted that the abundance of tandem duplications associated with therapy failures. Despite lacking correlation with therapy response, *CDK12* genetic deficiencies and their associated tandem duplication phenotypes were identified from three patients who displayed clinical signs of response and subsequent disease progression. Genes duplicated encode cell cycle regulators and growth-promoting kinases, which may contribute to the disease progression within *CDK12*-deficient tumors. Patients with favorable response also had a greater reservoir of T cell clonotypes defined by CDR3 sequence diversity from the buffy coat DNA, which often implicated a stronger antigenic recognition. Copy-number losses of MHC genes were associated with some individuals with poor therapy outcomes and low clonotype diversity. Transcriptomic profiling of the buffy coat is currently being performed to identify critical immune determinants of therapy outcome.

Conclusions:

Plasma cfDNA carries tumor-derived genomic information that informs whole-body disease burdens. Consistent to their protective/cytotoxic effects under PARP inhibition, baseline genetic deficiencies associated with DNA damage repair are differentially correlated with PD-L1/PARP1-targeted therapy response. The diversity of T cell clonotypes inferred using buffy coat genomes also predict positive therapy outcomes.

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