Comprehensive Single Cell Analysis of Tumor Mutation Burden (TMB), Chromosomal Instability (CIN) and Microsatellite Instability (MSI) on Circulating Tumor Cells (CTCs)

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**Background**

- Durable clinical responses have been attained with PARPi and immune checkpoint inhibitors, exploiting tumors harboring HR and MMR deficiencies respectively.
- Currently, the field lacks robust and validated biomarkers that can predict response to these agents in heterogeneous metastatic disease.
- Genomic-based methods detecting HR and MMR deficiencies (e.g. BRCA and MSI status) from bulk tumor tissue, and more recently ctDNA, lack the sensitivity required to dissect tumor genomic heterogeneity common in metastatic disease, compromising clinical biomarker performance and utility.
- To circumvent this, we utilized the Epic Sciences CTC detection and single cell DNA sequencing to develop a unique assay to simultaneously assess 3 (TMB, CIN and MSI) tumor clonality, paving the way for the development and validation of comprehensive biomarkers of response for immunotherapy (IO) and PARPi agents.

**Methods**

- Contrived samples were prepared by spiking three well characterized prostate cancer (PCa) cell lines, LNCaP, PC3 and VCaP, into normal blood donor.
- Clinical samples from metastatic castration resistant prostate cancer (mCRPC) patients were included to explore potential clinical feasibility.

1) **SLIDE PREPARATION**
2) **CELL STAINING**
3) **SCANNING**
4) **SINGLE CELL DIGITAL PATHOLOGY**
5) **Single Cell Isolation & CIN, TMB & MSI ANALYSIS**

**Conclusions**

- Our cell line data indicates that genome-wide INDEL burden detected at low coverage correlates with MSI status and may be a valuable surrogate to identify patients with MMR deficiency.
- CIN and TMB scores from single CTCs are inversely correlated, suggesting that MMR and HRD are likely mutually exclusive events driving tumor evolution and disease progression.
- Inter- and intrapatient tumor genomic heterogeneity was commonly observed, suggesting that CIN and TMB scores might be underestimated using tumor tissue or ctDNA for analysis.
- Overall, we demonstrated the feasibility of using a simple blood based test to quantify TMB, CIN, and protein expression on single CTCs, in a cost effective manner, providing the framework to develop and validate a comprehensive biomarker of response for IO and PARPi agents in future clinical studies.