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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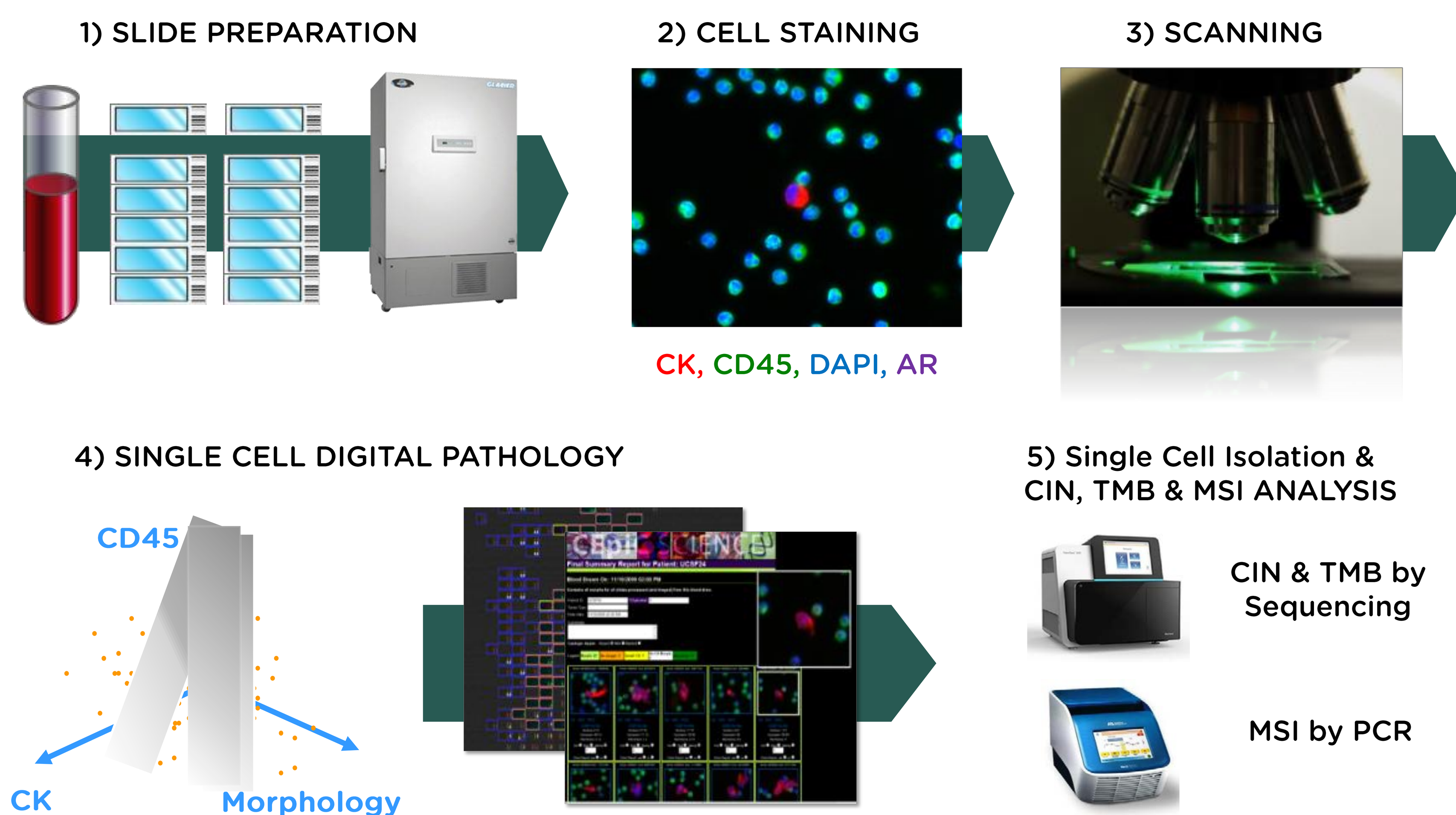
Background

- 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 could 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 (1) TMB, (2) CIN and (3) tumor clonality, paving the way for the development and validation of comprehensive biomarkers of response for immuno-oncology (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.

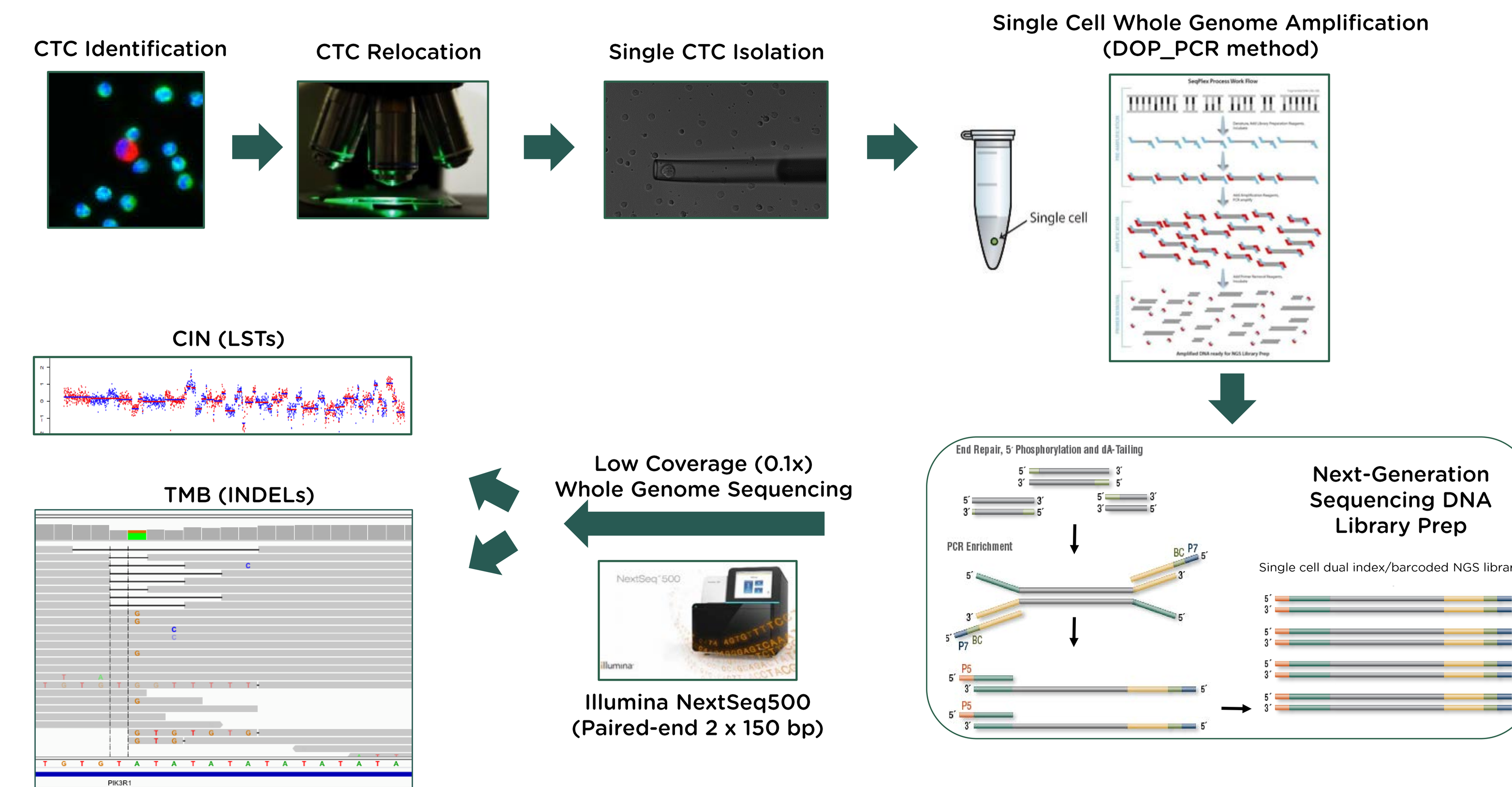


Schematic of Epic CTC platform for CTC identification, single cell sequencing, and TMB, CIN analyses workflow:

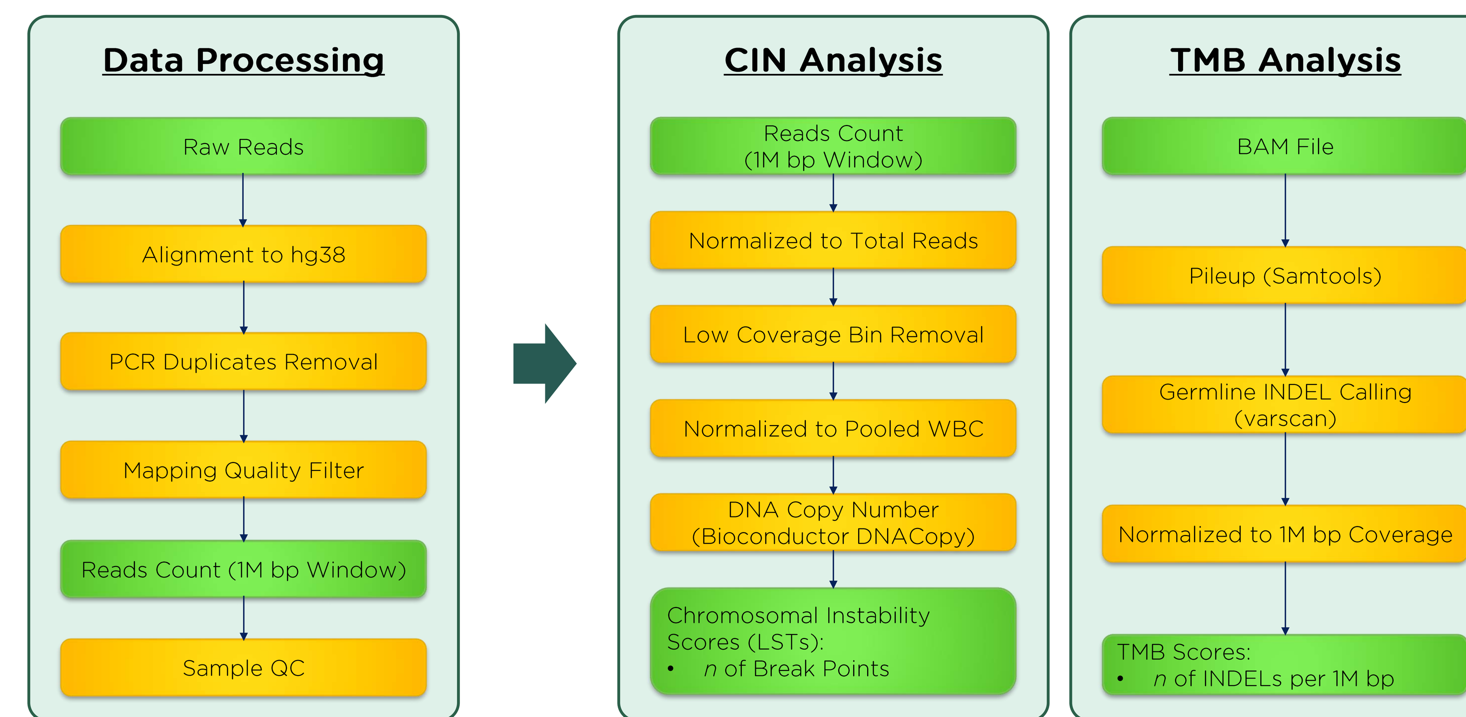
1) Nucleated cells from patient's blood samples or prostate cancer (PCa) cell lines spiked into normal blood donor were deposited onto 10-12 glass slides containing ~3 million cells.
2) Slides were IF-stained and scanned automatically at high speed to visualize cytokeratin (an epithelium marker), AR and CD45 (leukocyte exclusion marker), while DAPI was used as nuclear counterstain.
3) CTC identification based on (DAPI+; CK+; CD45-) phenotype was achieved using a multi-parametric digital pathology algorithm. Subsequently, relocated CTCs were individually isolated.
4) Each recovered cell was lysed, whole genome amplified (WGA), shotgun dual index NGS-library prepared and low pass whole genome sequenced using Illumina NextSeq 500.
5) TMB was measured as # of INDELs per Mbp and CIN was measured as large scale transitions (# of breakpoints for DNA segments larger than 10MB). For MSI validation, the QIAGEN Type-it microsatellite PCR kit was used to evaluate four different *loci* (BAT26, BAT25, D2S123, and D5S346).

Reference: Chromosomal Instability Estimation Based on Next Generation Sequencing and Single Cell Genome Wide Copy Number Variation Analysis. Greene SB, Dago AE, et al. PLoS One. 2016 Nov 16;11(11):e0165089.

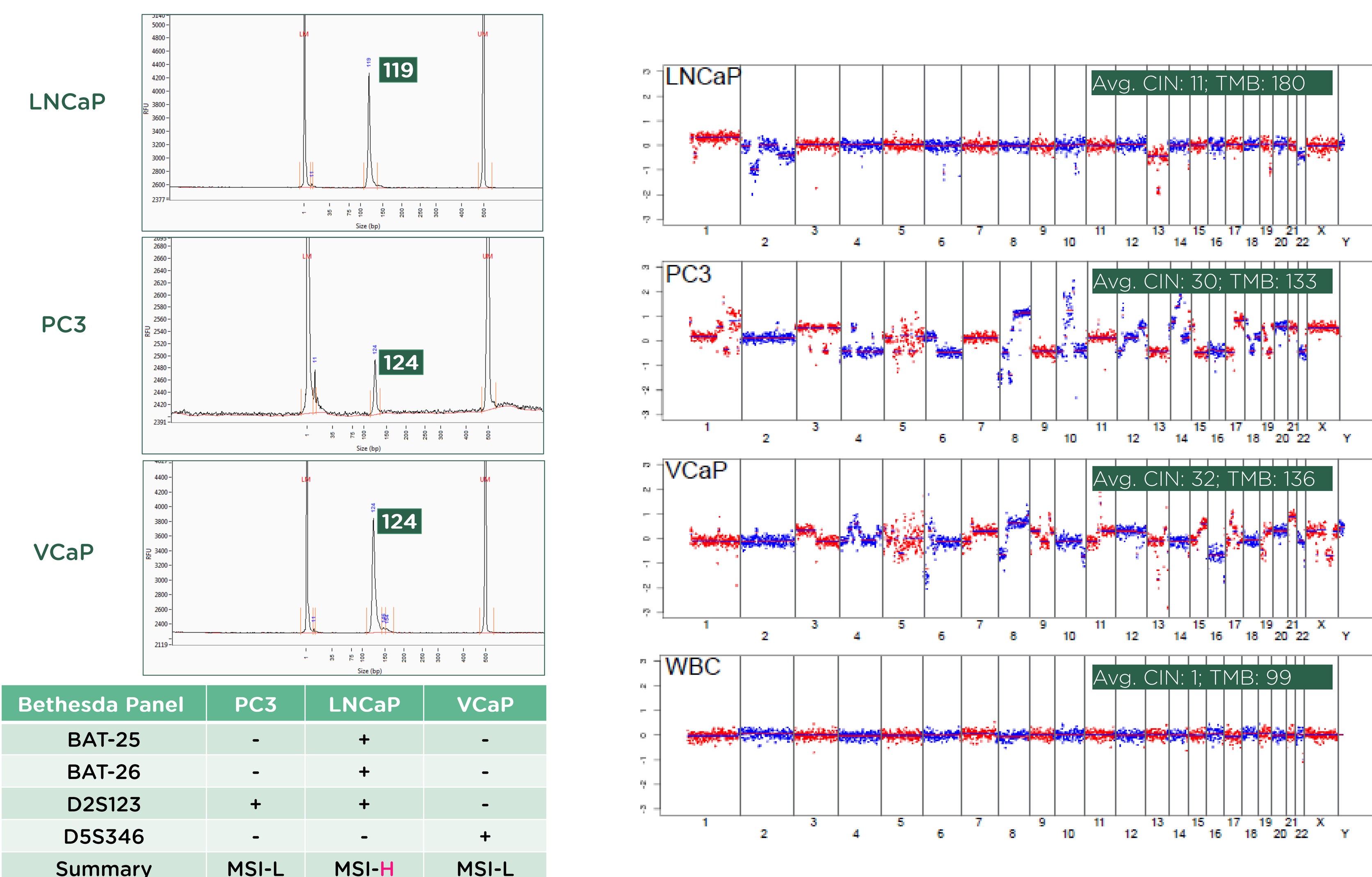
Single Cell Genomics Workflow



Single Cell CIN & TMB Bioinformatics Pipeline

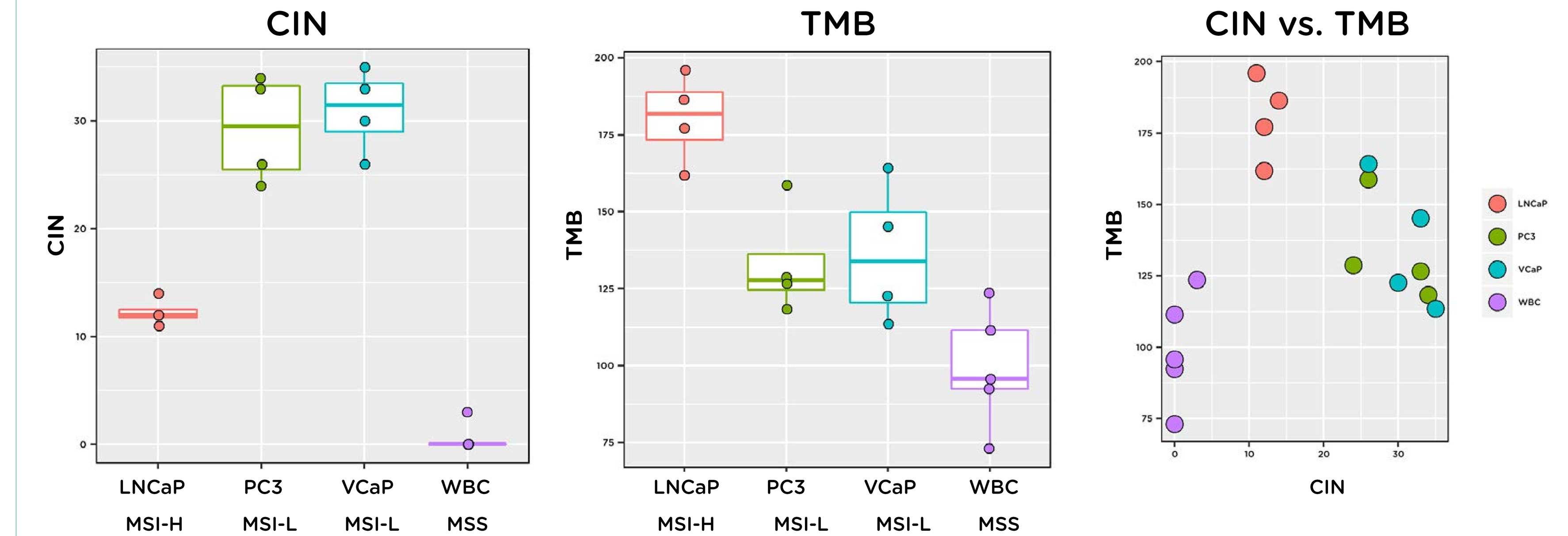


Single Cell MSI Validation, CIN & TMB

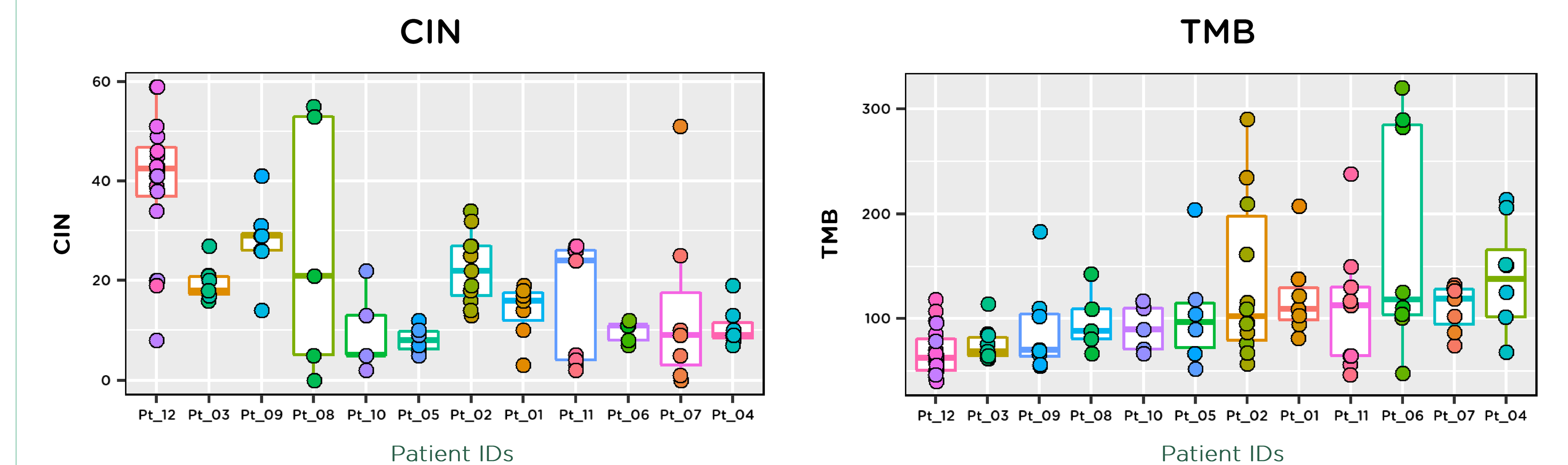


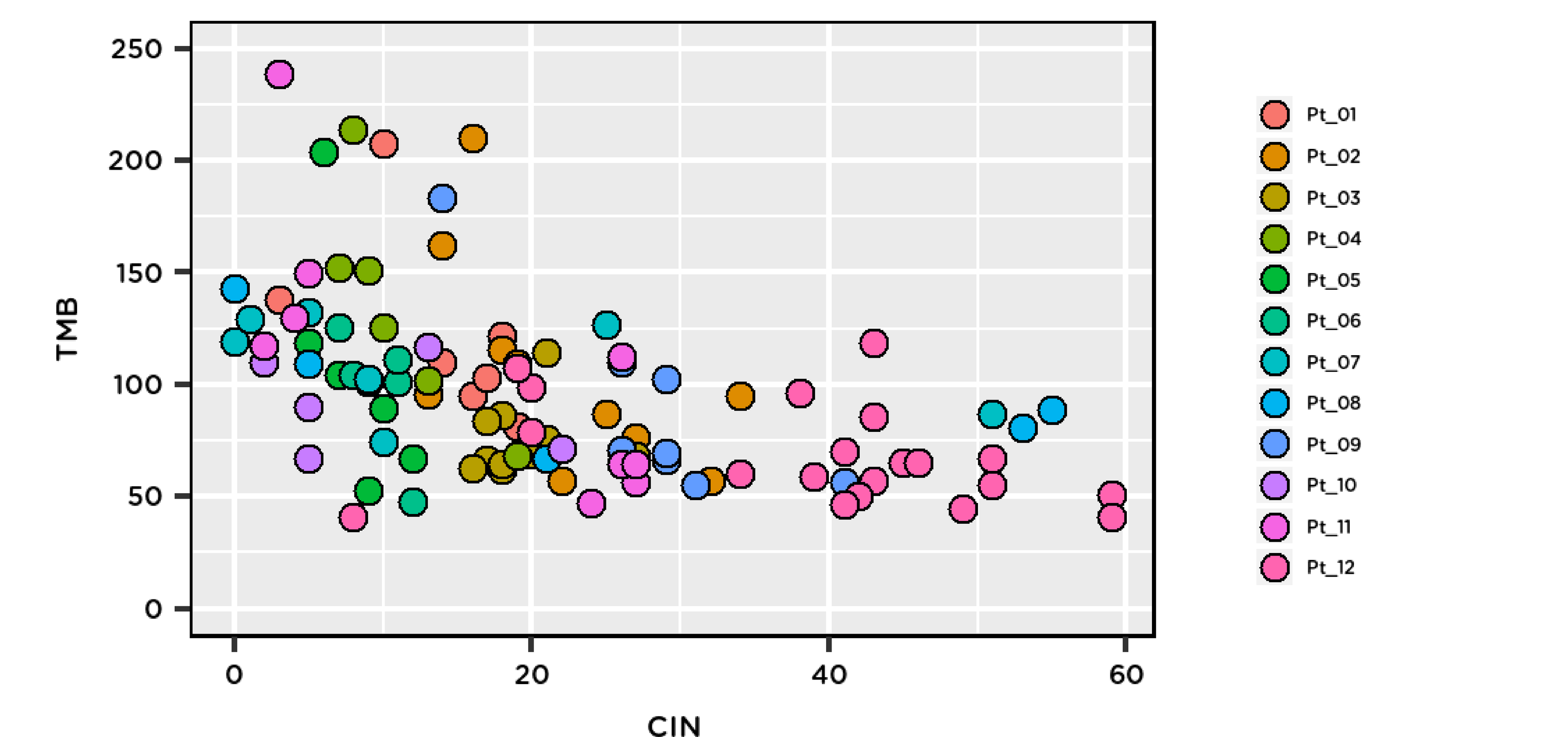
Bethesda Panel	PC3	LNCaP	VCaP
BAT-25	-	+	-
BAT-26	-	+	-
D2S123	-	+	-
D5S346	-	-	+
Summary	MSI-L	MSI-H	MSI-L

CIN and TMB Scores of PCa Cell Lines and WBCs



CIN and TMB Scores of mCRPC Patients





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 intra-patient 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.