Low Pass Whole Genome Sequencing of Single Circulating Tumor Cells for Detection of Chromosomal Instability (CIN) and Non-Coding Indels Across Multiple Solid Tumors

Angel E. Rodriguez, Jerry Lee, Ramsay Sutton, Rhett Jiles, Yipeng Wang, Mark Landers, Ryan Dittamore

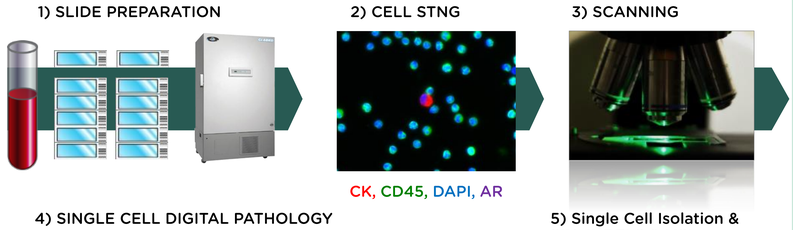
Epic Sciences, 9381 Judicial Dr, Suite 200, San Diego, CA 92121 epicsciences.com

Background

- Mismatch-repair deficiency (MMR-D) has emerged as a robust biomarker to predict patients response to immune checkpoint inhibitors, whereas chromosomal instability could inform response to PARP inhibitors.
- Assessment of MMR-D and CIN in bulk tumor samples is well explored, but limited by sample accessibility and tumor heterogeneity.
- Analysis of ctDNA is challenging for MMR-D and CIN, especially for patients who harbor subclonal genomic alterations, limiting its clinical utility.
- Epic Sciences' CTC platform employs a non-enrichment based approach to provide insight into subclonal heterogeneity.
- Here we present a single CTC genomics assay to simultaneously detect CIN and non-coding indels by using low coverage whole genome sequencing (WGS).

Methods

- Contrived samples were prepared by spiking prostate cancer cell lines, LNCaP, PC3 and VCaP, into healthy donor blood. Red blood cells lysed, nucleated cells deposited onto glass slides and immunofluorescently stained (DAPI, CK, CD45, and Androgen Receptor). Identified cancer cells were individually isolated from the slides, lysed, whole genome amplified (WGA), shotgun library prepared, and low pass whole genome sequenced to ~ 0.1X coverage. Data were analyzed for non-coding indels and large scale transitions LSTs (surrogates for MMR-D and CIN, respectively). Microsatellite instability (MSI) was measured by the Qiagen Type-It MSI PCR kit as per manufacture's protocol on the control cell lines.
- 1998 CTCs from 175 prostate, breast, bladder and lung, renal cancer patients were evaluated for clinical feasibility.

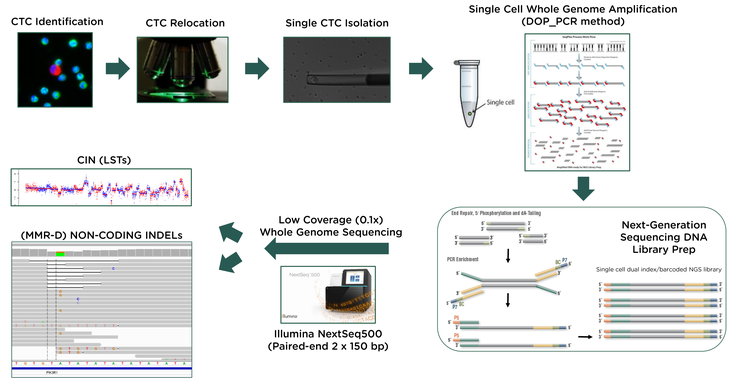


Schematic of Epic CTC platform for CTC identification, single cell sequencing, and INDELS, CIN analyses workflow:

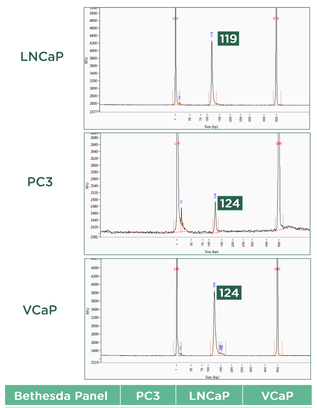
1) Nucleated cells from patient's blood samples or prostate cancer (PCa) cell lines spiked into normal blood donor were deposited onto 10-12 glass slides containing ~3 million cells.
2) Slides were IF-stained and scanned automatically at high speed to visualize cytokeratin (an epithelium marker), AR 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) # of INDELs per Mbp were computed meanwhile 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.

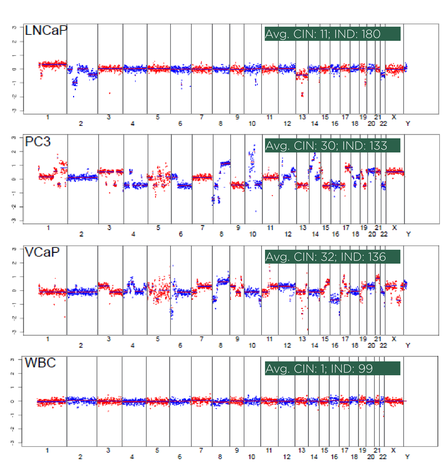
Single Cell Genomics Workflow



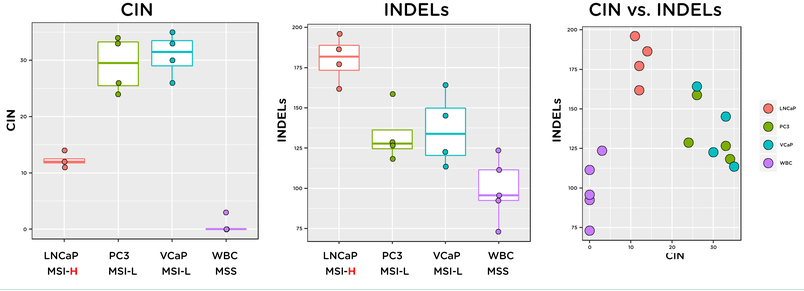
Single Cell MSI Validation, CIN & INDELs



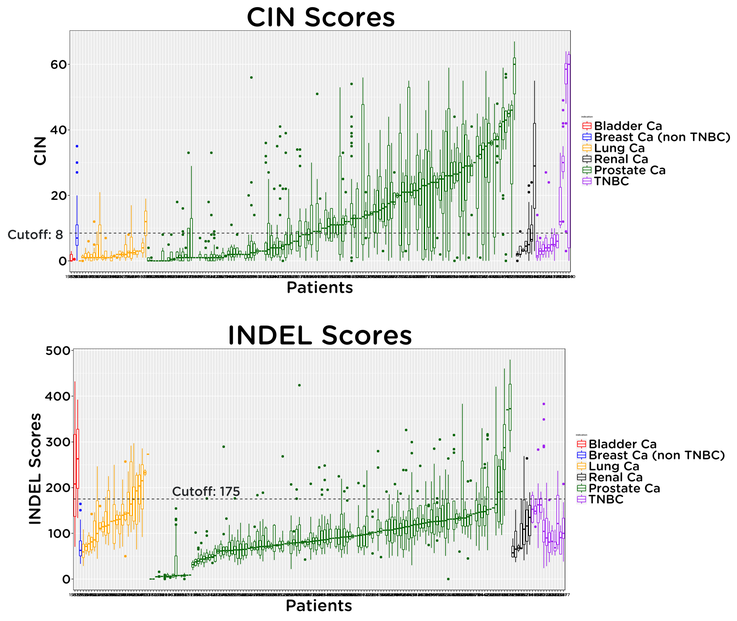
Bethesda Panel	PC3	LNCaP	VCaP
BAT-25	-	+	-
BAT-26	-	+	-
D2S123	+	+	-
D5S346	-	-	+
Summary	MSI-L	MSI-H	MSI-L

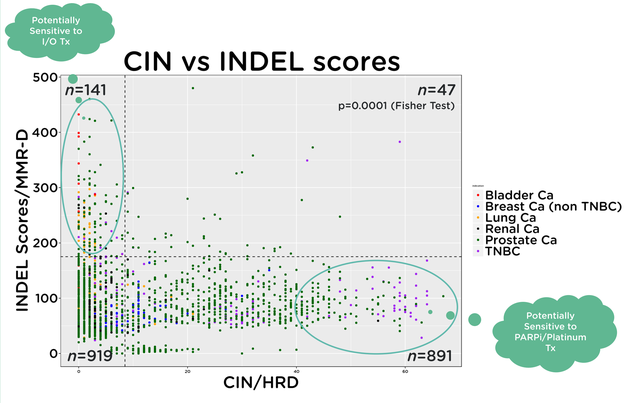


CIN and INDEL Scores of Prostate Ca Cell Lines



CIN and INDEL Scores of CTCs from Multiple Tumors





Indications	# of Pts	# of CTCs
Bladder Cancer	2	14
Breast Cancer (non TNBC)	1	80
Lung Cancer	24	120
Prostate Cancer	129	1574
Renal Cancer	7	63
TNBC	12	147
Total	175	1998

Conclusions

- We demonstrate the feasibility of detecting Indel Scores and CIN simultaneously at the single cell level using Epic Sciences CTC Platform.
- Inter- and intra-patient heterogeneity was observed in this large patient cohort.
- MMR-D and HRD seem to be mutually exclusive events driving tumor genome evolution, however we find patients where both MMR-D and HRD tumor subclones co-occur.
- Studies are on-going to validate the potential correlations of Indels as a surrogate of MMR-D/checkpoint inhibitor, and CIN with PARPi response.