Prevalence and tissue concordance of BRCA2 copy number loss evaluated by single-cell, shallow whole genome sequencing of circulating tumor cells (CTCs) in castration resistant prostate cancer (CRPC)

Barnett ES[1], Schonhoft J[2], Schultz N[1], Lee J[2], Zaidi S[1], Abida W[1], Carmichael T[1], Dago AE[2], Solit D[1], Wenstrup R[2], Scher HI[1]
MSKCC[1], Epic Sciences[2]

Introduction

- DNA damage-repair alterations occur in ~20-25% of mCRPC tissue samples[1]
- PARP inhibitors are now considered a standard of care for patients with homologous recombination deficiency (HRD), notably LoF mutations and deletions in BRCA2
- Obtaining sufficient tumor material for profiling is difficult due to the osteotropic nature of prostate cancer (PCa)
- Detection of copy number alterations (CNAs) using cell-free DNA (cfDNA) is also challenging due to high background of DNA from healthy cells
- CTCs represent a non-invasive source of genomic material which can be used to detect CNAs in BRCA2 and HRD-related genomic events, such as large scale transitions (LSTs)

Trial Design/Methods

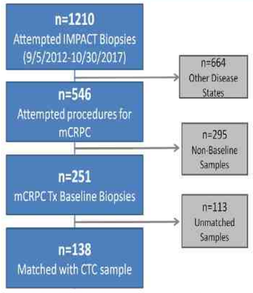


Figure 1. REMARK diagram of Cohort

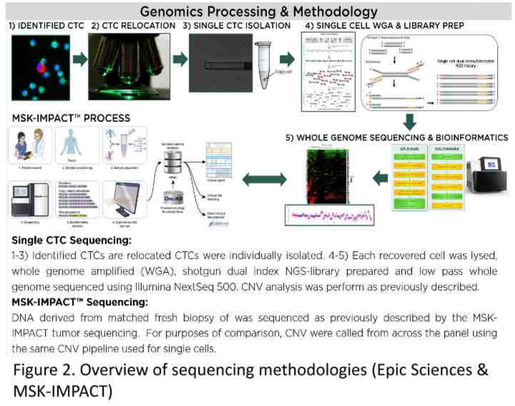


Figure 2. Overview of sequencing methodologies (Epic Sciences & MSK-IMPACT)

- Retrospective analysis of 138 CTC samples collected concurrently with a baseline mCRPC tumor biopsy sent for sequencing using MSK-IMPACT[2] (Figure 1)
- Single-cell shallow WGS on CTCs to assess copy number alterations[3] (blinded to MSK-IMPACT results) (Figure 2)
- Sequencing results were compared for overall concordance and BRCA2 copy number status

Cohort Demographics

Table 1. Demographics

		CTC Sequencing Successful	Tissue Sequencing Successful	Both Sequencing Successful	All Patients
Sample Size n(%)		115 (83.3)	108 (78.3)	92 (66.7)	138 (100)
Age years (range)		68 (47-85)	68 (47-85)	68 (47-85)	67 (47-85)
Laboratory Assessments	PSA ng/dL (range)	28.46 (<0.05-16275.14)	34.21 (<0.05-6905.71)	34.09 (<0.05-4026.23)	28.16 (<0.05-16275.14)
	ALK U/L (range)	120.5 (44-1574)	112 (42-1574)	112 (42-1574)	116.5 (42-1574)
	LDH U/L (range)	246.5 (126-3381)	239 (126-3381)	249.5 (126-3381)	238 (126-3381)
Treatment Exposures	Prior Tx Lines median (range)	1 (0-5)	1 (0-5)	1 (0-5)	1 (0-5)
	Prior ARSi n(%)	73 (63.5)	74 (68.5)	63 (67.4)	85 (61.6)
	Prior Taxane n(%)	33 (28.7)	35 (32.4)	30 (32.6)	38 (27.5)
Disease sites	Bone n(%)	93 (80.9)	83 (76.9)	70 (76.1)	112 (81.2)
	LN n(%)	89 (77.4)	86 (79.6)	72 (78.2)	107 (77.5)
	Liver n(%)	24 (20.9)	26 (24.1)	23 (25.0)	29 (21)
	Lung n(%)	11 (9.6)	11 (10.2)	9 (9.8)	13 (9.4)
	Other n(%)	31 (27.0)	30 (27.8)	27 (29.3)	35 (25.4)

Overall CTC-Tissue Genomic Concordance

- Concordant copy number profiles (>60% tissue similarity of 1+ CTC) were noted in 51% (47/92) matched CTC/tissue pairs (Figure 3A, black circles)

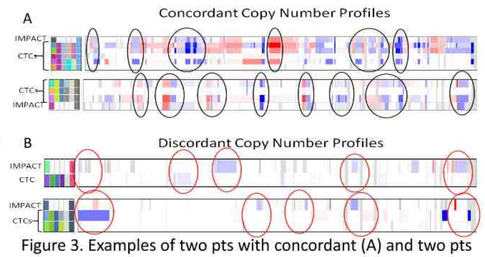
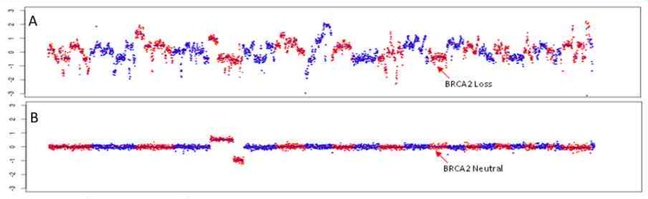


Figure 3. Examples of two pts with concordant (A) and two pts with discordant (B) CTC copy number profiles.

Prevalence of BRCA2 Loss in CTCs vs Tissue

Figure 4. examples of CTC copy number profiles with (A) and without (B) BRCA2 loss



- BRCA2 Loss was detected in 21% of sequenced tissue samples and 50% of CTC samples (Figures 4A & 5)
- A median of 46% (range 4%-100%) of CTCs harbor BRCA2 loss in samples which a BRCA2 loss was detected

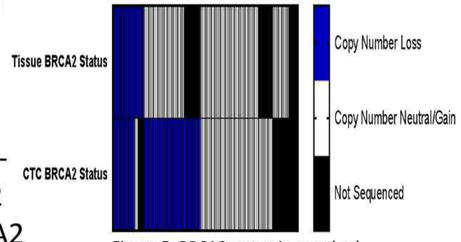


Figure 5. BRCA2 status in matched tissue and CTC samples

- BRCA2 loss was detected in CTCs in 46/92 (50%) of cases where both assays were successful: 16/19 (84%) tissue-positive and 30/73 (41%) tissue-negative cases (PPA=84%, NPA=59%) (Figure 5)

Large Scale Transitions as a Marker of HRD

- LSTs, chromosomal breakages that generate chromosomal gains or losses of 10 Mb or more, are indicative of HRD (High LST profile depicted in Figure 4A)
- CTCs with BRCA2 loss (n=220) had a significantly higher number of LSTs as compared to BRCA2 neutral (n=565) CTCs ($p<0.0001$) (Figure 6)

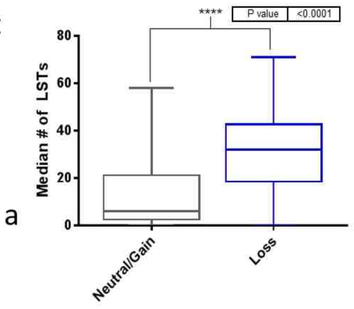


Figure 6. # of LSTs by BRCA2 Status

BRCA2 Loss and Co-occurring Alterations

- Co-occurring CNAs were assessed in 5 genes relevant to PCa (AR, MYC, TP53, RB1, PTEN)
- All assessed alterations were significantly more prevalent in BRCA2 loss CTCs (n=220) compared to BRCA2 neutral/gain (n=565) (all $p<0.0001$) (Figure 7)

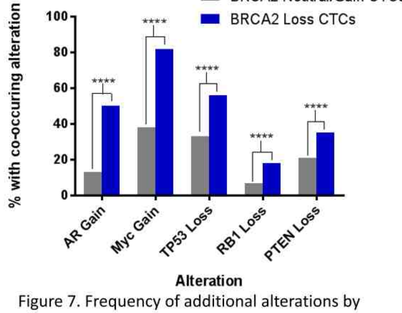


Figure 7. Frequency of additional alterations by BRCA2 status

Conclusions

- Epic Sciences single-cell CTC sequencing assay can detect BRCA2 loss in a majority of cases which tissue sequencing detected the loss and numerous instances which it did not
- CTCs with detected BRCA2 loss have a significantly higher number of LSTs and co-occurring CNAs, indicative of HRD
- Single-cell CTC sequencing can potentially be utilized, alone or in conjunction with ctDNA sequencing, to detect actionable alterations in BRCA2 and HRD-related genes to predict sensitivity to PARPi

References

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2. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. *J Mol Diagn.* 2015;17(3):251-264. doi:10.1016/j.jmoldx.2014.12.006
3. Greene SB, Dago AE, Leitz LJ, et al. Chromosomal Instability Estimation Based on Next Generation Sequencing and Single Cell Genome Wide Copy Number Variation Analysis. *PLoS One.* 2016;11(11):e0165089. Published 2016 Nov 16. doi:10.1371/journal.pone.0165089