Intra-patient genomic heterogeneity of single circulating tumor cells (CTCs) associated to phenotypic CTC heterogeneity in metastatic castrate resistant prostate cancer (mCRPC)

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Background

Analysis of somatic genomic alterations in primary tumors is often used to define mutational status and guide therapeutic decisions. Selective pressures can lead to clonal selection and tumor evolution resulting in intra-tumor clonal heterogeneity. Circulating tumor cells (CTCs) in mCRPC pts have demonstrated phenotypic heterogeneity in size, shape, cytokeratin (CK) and androgen receptor (AR) expression[1], which can be associated with resistance to androgen receptor signaling targeted therapies (ARS Tx)[2]. To understand if there is an underlying genomic heterogeneity leading to phenotypic heterogeneity, we performed NGS whole genome copy number variation (CNV) analysis at the single CTC level in the context of patients progressing in mCRPC and baseline to a change in therapy. We assessed the CTCs for driver somatic alterations, phenotypic features, phenotypic heterogeneity and subsequent therapeutic resistance.

Methods

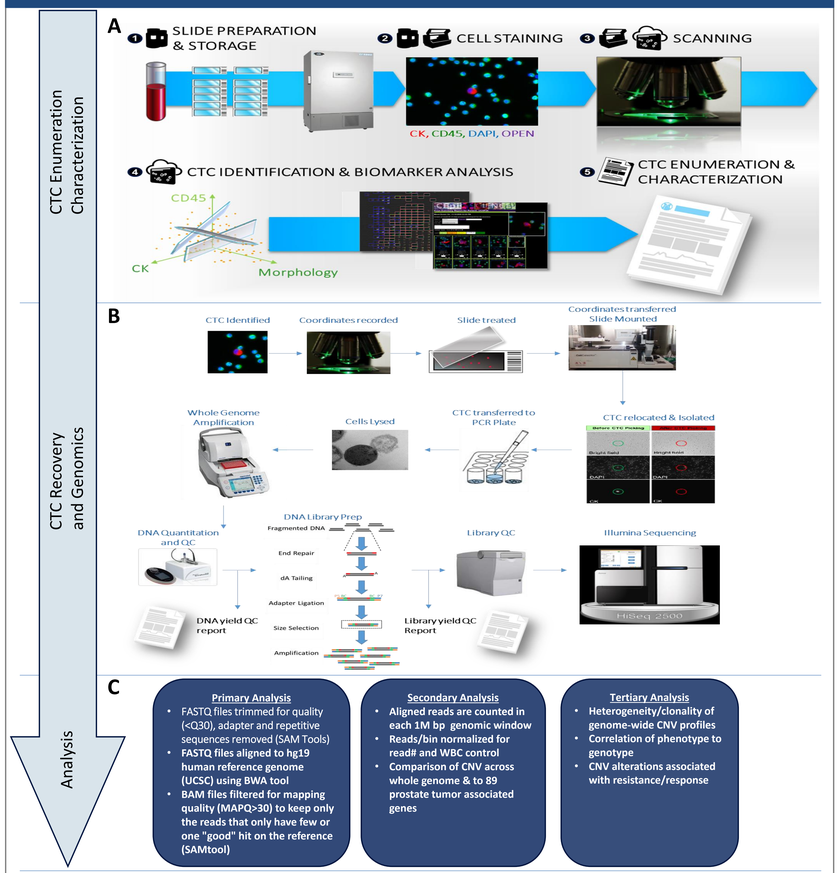


Figure 1. Methods
A. Description of standard Epic CTC analysis process (top). **B.** Description of the CTC recovery and genomic profiling workflow (middle). **C.** Description of the bioinformatics analysis for the whole genome CNV assay (bottom).

Table 1. Description of the CTC morphological and molecular features analyzed during the Epic CTC analysis (right).

Table 1. Phenotypic Features

Nuclear Features	Cytoplasmic Features	Cell Features
Nuclear Area	Cytoplasmic Area	AR Expression
Nuc. Convex Area	Cyto Convex Area	CK Expression
Nuc Major Axis	Cyto Major Axis	N/C Ratio
Nuclear Minor Axis	Cyto Minor Axis	
Nuclear Circularity	Cyto Circularity	
Nuclear Solidity	Cyto Solidity	
Nuclear Entropy	Cyto Entropy	
Nuclear Speckling	Cyto Speckling	
Nucleoli Presence	Cyto Presence	

Patient Summary

Table 2. Patient demographics

Characteristic	No. (%) or Median (range)
Number of Baseline Samples (unique patients)	17 (15)
Age, years	68 (52 – 91)
Primary Treatment	
Prostatectomy	8 (47%)
Radiation	6 (35%)
None	3 (18%)
Hormone Therapies	
1 - 2 lines	2 (12%)
3 lines	10 (59%)
≥ 4 lines	5 (29%)
Chemo-naïve	6 (35%)
Chemo-exposed	11 (65%)
Metastatic Disease	
Bone	17 (100%)
Lymph Node	12 (71%)
Liver	1 (6%)
Lung	2 (12%)
Other Soft Tissue	2 (12%)
Laboratory Measures	
PSA, ng/mL	159.20 (3.91 – 1479.07)
Hgb, (g/dl)	10.2 (7.0 – 13.1)
ALK, (unit/L)	180 (51 – 1096)
LDH, (unit/L)	294 (154 – 964)
ALB, (g/dl)	4.1 (3.3 – 4.6)
Cell Search CTC, (cells/7.5mL)	80 (0 – >200)

Table 3. CTCs/patient

Sample ID	IF Marker	CTC/mL	# CTCs Sequenced	# CTCs Passed QC	# CTCs w/ CNVs	# CTCs w/o CNVs	CTC Characterized For Morphological Features
1	AR	102	33	30	10	20	Yes
2	AR	44	12	12	2	10	Yes
3	AR	8	12	11	5	6	Yes
4	AR	24	17	16	14	2	Yes
5	AR	31	26	22	3	19	Yes
6	AR	14	20	17	5	12	Yes
7	AR	14	19	17	4	13	Yes
8	AR	5	4	4	3	1	Yes
9	AR	22	13	12	1	11	Yes
10	AR	102	47	44	4	40	Yes
11	AR	6	10	9	1	8	Yes
12	PSMA	30	12	12	8	4	No
13	PSMA	41	20	16	9	7	No
14	PSMA	35	5	4	2	2	No
15	PSMA	136	73	62	25	37	No
16	PSMA	6	5	5	2	3	No
17	PSMA	38	22	22	8	14	No

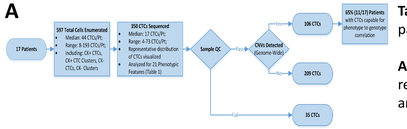


Figure 2.
CTCs were characterized in blood samples from mCRPC patients at baseline prior to 2nd-7th lines of therapeutic intervention.

Table 2. Patient demographics (left).

Table 3. CTCs per patient (right).

A. Flow chart representing CTCs analyzed.

CNV in CTCs

Figure 3. CNV Alterations vs. CTC Phenotype
A. Histogram summarizing the number of CNVs observed across all 1M bp windows/CTC in 17 baseline mCRPC patient samples. 106/315 CTCs in total had CNV alterations in whole genome.
B. Bar chart comparing the total number of CNV alterations (red=deletions, green= amplifications) called from the analysis of 68 CTCs that had CNVs occurred in windows containing prostate specific tumor genes (n=89).

- **Prostate specific tumor suppressor /oncogenes CNV detected in 68/315 CTCs**
- **Most common gene amplifications; AR, PTK2, NDRG1, c-MYC, YWHAZ**
- **Most common gene deletions; PTPRJ, RAB23, KLF5, RB1, BRCA2, ATM**

CTC Genotype to Phenotype Correlation

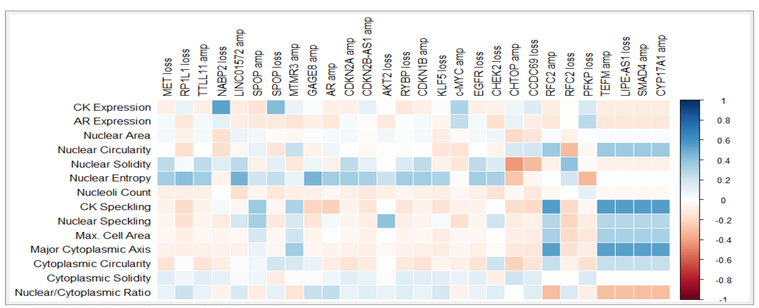
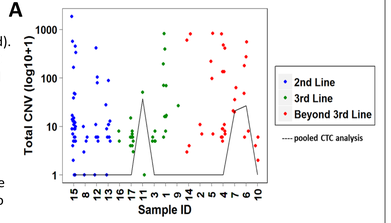


Figure 4. CNV Alterations vs. CTC Phenotype
Correlation matrix describing the association of observed CTC phenotypic features (rows = cell phenotypic features) with 89 prostate and tumor specific CNV alterations (columns) using 43 CTCs. The degree of correlation is depicted in both positive(blue) and inverse (red) relationships.

- **28 CNVs have significant correlations with phenotypic features**
- **Nuclear Entropy is the morphology feature that has the strongest correlation with CNV alterations**

Intra-Patient CTC Heterogeneity

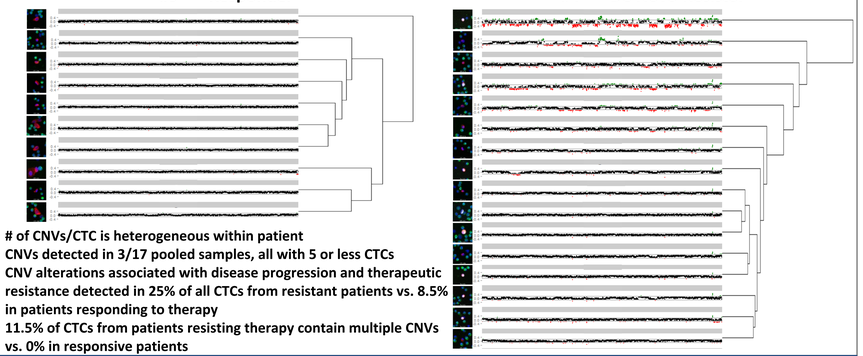
Figure 5. Intra-patient CTC Heterogeneity
Intra-patient genomic heterogeneity (multiple clonal populations) was observed across 47% of patients across all lines of therapy 2nd (red), 3rd (green) or 3rd + (red).
A. Dot plot (right) showing the number of observed CNV alterations for each CTC within a single patient. The solid line depicts the number of alterations detected from a simulated pooled CTC sample (merged BAM files).
B. The table below depicts the number of distinct clonal populations (K-means clustering) and the % of CTCs detected harboring CNV co-occurring in windows with specific prostate tumor associated genes for patients prior to resisting or responding to AR Tx or Taxane therapy.
C. Shown below are hierarchical clustered CNV plots illustrating the genome-wide CNV profiles and cell images of each CTC sequenced from a patient responding to ARS Tx (left) and patient progressing on AR Tx (right).



B

Response	Drug	Line of Tx	Sample ID	# of CTCs sequenced	%of patient CTCs with CNV alteration: AR Amp	PTEN loss	c-Myc Amp	AURKA amp	RB loss	BRCA2 loss	ATM loss
Resistance	AR Tx	2nd	12	12	16.67%		16.67%	8.33%			
		2nd	13	16	6.25%		6.25%				
		3rd	1	30	3.33%	3.33%	16.67%	3.33%	6.67%	6.67%	3.33%
		4th	2	12	8.30%						
		4th	3	11							
		4th	14	4			25.00%	25.00%	25.00%	25.00%	
		5th	4	16	75.00%				12.50%	12.50%	18.75%
		5th	5	22	4.55%		9.09%				
		6th	6	17			11.76%		5.88%	5.88%	
	Taxane	2nd	15	62		2.04%					
		6th	7	17					5.88%	5.88%	
Response	AR Tx	2nd	8	4	25.00%						
		3rd	11	9	11.11%						
		3rd	9	12							
	Taxane	3rd	16	5							
		3rd	17	22							
		7th	10	44							

C



- **# of CNVs/CTC is heterogeneous within patient**
- **CNVs detected in 3/17 pooled samples, all with 5 or less CTCs**
- **CNV alterations associated with disease progression and therapeutic resistance detected in 25% of all CTCs from resistant patients vs. 8.5% in patients responding to therapy**
- **11.5% of CTCs from patients resisting therapy contain multiple CNVs vs. 0% in responsive patients**

Conclusions

- ✓ CTCs from progressing mCRPC patients can harbor multiple genomic alterations per cell
- ✓ CNV alterations were strongly associated with CTC phenotypic features, but not with CTC/mL count
- ✓ Intra-patient CTC clonal heterogeneity is higher in patients who went on to resist therapy with multiple subclonal drivers of therapeutic resistance
- ✓ DNA repair genes BRCA & ATM were often found to be deleted in patients resisting therapy, identifying potentially actionable non-point mutation based alterations
- ✓ Single CTC sequencing through a liquid biopsy provides a platform for assessing selection and evolution of clonal subtypes through therapeutic monitoring
- ✓ Utilization of single CTC genomic data and genomic heterogeneity to associate to clinical endpoint will require prospective validation

References

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