Androgen receptor expression on circulating tumor cells (CTCs) in metastatic breast cancer

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Introduction

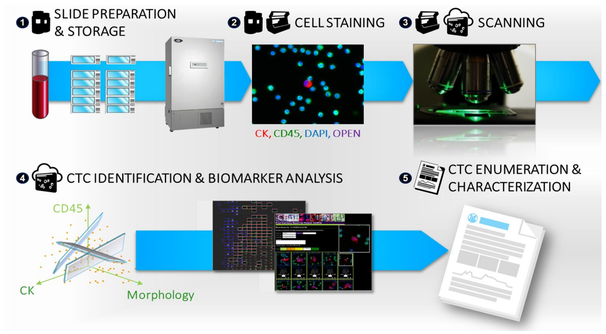
- Androgen receptor (AR) expression is reported in >70% of estrogen receptor-positive (ER+), >60% of HER2-positive (HER2+), and 40-50% of triple-negative (TNBC) breast cancers, respectively.
- Clinical trials of AR-target therapies in breast cancer are ongoing.
- The biology of AR and its role in breast cancer remain unclear.
- The development of predictive and prognostic biomarkers for breast cancer treated with AR targeted therapy is less explored.
- The heterogeneity of CTCs both in phenotype and genotype at the single cell level in prostate cancer has been identified using the Epic Sciences unbiased CTC detection and analysis platform.
- AR expression and heterogeneity in breast cancer CTCs has not been evaluated.

Objectives

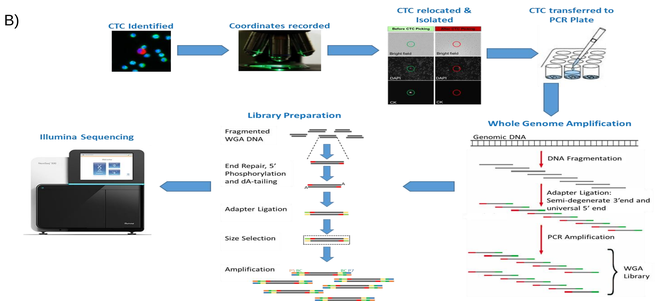
To evaluate the rates of AR expression and heterogeneity along with genomic copy number profiles in CTCs from patients with metastatic breast cancer.

Methods

A)



B)



Epic CTC Assay and Single Cell Copy Number Variation (CNV) Assay

A) Epic Sciences CTC Platform to identify CTCs. Nucleated cells were deposited onto glass slides and stored at -80ºC until stained with a cocktail of antibodies including cytokeratin (CK), CD45, DAPI and a biomarker (AR or ER). Stained slides were scanned and images were analyzed using multi-parametric digital pathology algorithm to determine CTC enumeration and biomarker expression. Identified CTCs were isolated and processed using Epic Sciences Single Cell Genomics CNV assay.

B) Overview of Epic Sciences single cell CTC isolation and genomic analysis. Individual CTCs were isolated and processed for single cell whole genome amplification and NGS library preparation. Sequencing was performed on an Illumina NextSeq500 and CNV analysis was performed.

Patient characteristics and CTC Incidence

Characteristic	No. (%) or Median (range)
No. of patients	59
Age, years	53 (27 - 73)
Tumor Histology	
ER+	25 (42%)
ER+/HER2+	13 (22%)
HER2+	6 (10%)
TNBC	15 (25%)
Primary Treatment	
Prior Treatment	53 (90%)
None	6 (10%)
IBC vs. Non-IBC	
IBC	18 (30.5%)
Non-IBC	41 (69.5%)
Metastatic Sites	
Bone	44 (75%)
Brain	9 (15%)
Liver	26 (44%)
Lung	30 (51%)
Lymph Node	43 (73%)
Skin	9 (15%)
Other	9 (15%)

All 59 patients were tested for AR in CTCs and 6 of 59 patients were tested for ER in CTCs.

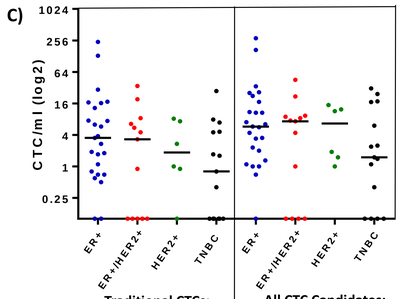
A)

	Traditional CTCs*	All CTC Candidates
Samples tested (n)	59	59
Samples with CTC	76% (45/59)	85% (50/59)
Range (CTC/mL)	0 - 240.3	0 - 282.1
Median (CTC/mL)	1.9	4.4

B)

Tumor Subtype	Traditional CTCs	All Candidate CTCs
ER+ (*n*=25)	92%	96%
ER+/HER2+ (*n*=13)	62%	69%
HER2+ (*n*=6)	83%	100%
TNBC (*n*=15)	60%	73%

C)



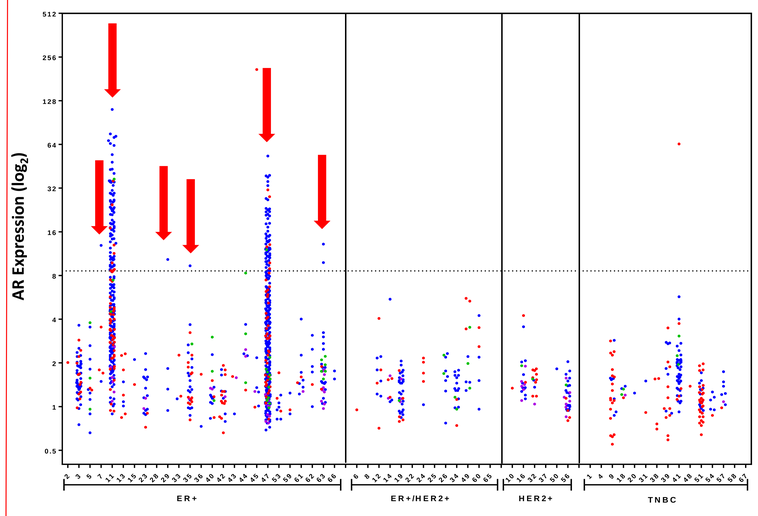
Traditional CTCs: CK+ CTC , Clusters

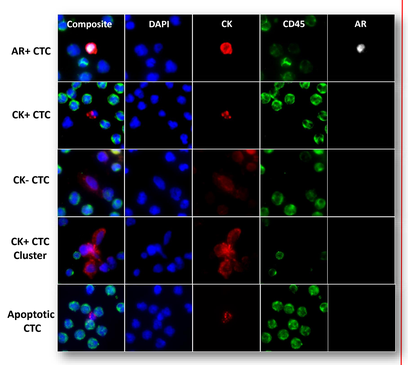
All CTC Candidates: CK+ CTC, Clusters, CK- CTC, Apoptotic CTC

- CTCs were detected in 50 patients (84.7%).
- Six of 59 patients (10%) had AR+ CTCs.

Definition of CTC Types	
Traditional CTCs	cells CK(+), CD45(-), intact DAPI, and are generally larger and morphologically distinct from surrounding WBCs.
CTC Clusters	two or more adjacent CTCs, containing at least one traditional CTC, with shared cytoplasmic boundaries.
CK(-) CTCs	CK(-), CD45(-), with DAPI intact.
Apoptotic CTCs	CK(+), CD45(-) with a DAPI pattern of chromosomal condensation and/or nuclear fragmentation/blebbing that is consistent with the classic definition of apoptosis.

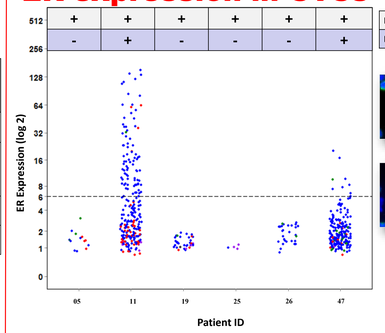
Heterogeneity of AR Expression and CTC Subtypes

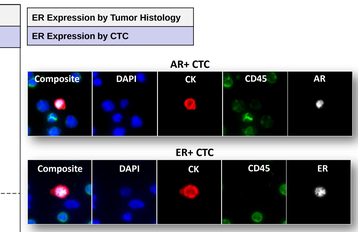




CTC Subpopulation	Patients with CTC (*n*=50)
CK+	88% (44/50)
CK-	32% (16/50)
CK+ Clusters	32% (16/50)
Apoptotic	90% (45/50)

ER expression in CTCs



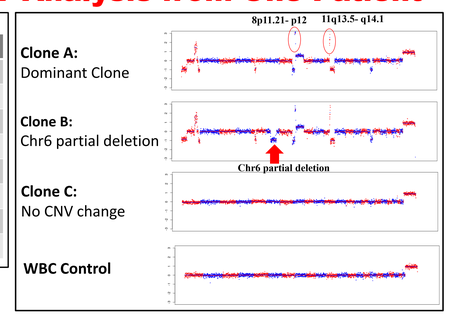


- Two patients with ER+ CTCs had also AR+ CTC

Single CTC CNV Analysis from One Patient

CTCs Sequenced

Staining	Cell Categories	*n*
AR Staining	AR (+)	6
	AR (-)	16
ER Staining	ER (+)	15
	ER (-)	14
HER2 Staining	HER2 (+)	6
	HER2 (-)	21
	WBC	1
	Total CTCs	79



- CTCs from one patient were stained by IHC by a single cell level.
- Each CTC was tested for CNV analysis.
- Three of 6 AR+ CTCs (50%) had no significant CNV (Clone C).
- All HER2+ and ER+ CTCs (*n*=21) had significant CNV (Clones A or B).
- Amplification of chr8p11-p12 was previously reported to be correlated with poor patient outcomes.[1]
- Amplification of chr11q13-q14 was previously reported to be less frequently in breast cancer.[2]

1. Turner-Ivey B. et al. Neoplasia. 2014 Aug;16(8):644-55.
2. Keilty D. et al., PLoS One. 2013 Dec 19;8(12):e81740.

Conclusion

- The Epic Sciences non-enriching or selecting platform can detect AR expression and heterogeneity of CTCs in breast cancer.
- AR+ CTC profiles are being investigated as a method to identify patients who might benefit from AR-targeted therapy.
- The heterogeneity of intra-patient CTC AR expression leads us to a novel hypothesis that patients with AR+ CTCs might have heterogeneous disease with multiple drivers.
- Further studies are warranted to allow serial monitoring of changes in AR and to investigate the clinical applicability of AR+ CTCs and their heterogeneity.

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