

# Circulating tumor cells (CTCs), CTC heterogeneity and distinct morphological CTC phenotypes predict worse survival in metastatic breast cancer (MBC)

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## BACKGROUND

- The presence of CTCs has been shown to be a poor prognostic factor in patients with metastatic breast cancer (MBC).
- We have previously demonstrated that CTC morphologic heterogeneity, distinct phenotypes of chromosomal instability (CIN), and small cell/neuroendocrine-like (SC/NE) morphology each independently confer a poor prognosis for metastatic prostate cancer patients.
- In this study, we report a comprehensive analysis of CTC presence, heterogeneity, and specific morphological phenotypes (CIN, SC/NE) observed in MBC patients.

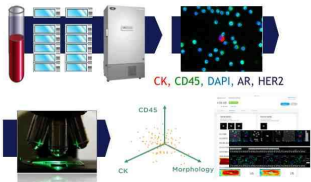
## METHODS

- A total of 148 blood samples from 90 patients with MBC whose disease was progressing on therapy were enrolled for CTC analysis using the Epic Sciences platform
- Total CTCs were quantified per sample from approximately 3 mL of blood
- CTC morphology phenotypes (heterogeneity [Scher et al Cancer Res 2017], small cell/neuroendocrine [SC/NE; Brown et al Cancer 2021] and chromosomal instability [CIN+; Schonhoft et al Cancer Res 2020]) were quantified as previously described (see schematics below).

## FIGURE 1. METHODS:

### EPIC SCIENCES PLATFORM IDENTIFIES CELL SUBTYPES & PHENOTYPES

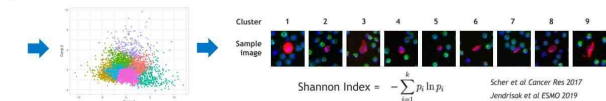
#### 1) Epic Sciences Platform



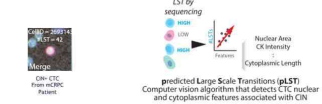
#### 2) Single Cell Features

Protein Biomarker Features
CK Ratio (protein expression)
Signal-to-noise ratio
Nuclear Area (µm <sup>2</sup> )
Cytoplasmic Area (µm <sup>2</sup> )
Nuclear Convex Area (µm <sup>2</sup> )
Cytoplasmic Convex Area (µm <sup>2</sup> )
Nuclear Major Axis (µm)
Cytoplasmic Major Axis (µm)
Nuclear Minor Axis (µm)
Cytoplasmic Minor Axis (µm)
Nuclear Circularity
Cytoplasmic Circularity
Nuclear Solidity
Cytoplasmic Solidity
Nuclear Entropy
Nuclear to Cytoplasmic Convex Area Ratio
Nucleoli
Nuclear Speckles

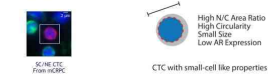
#### 3) Unsupervised Clustering



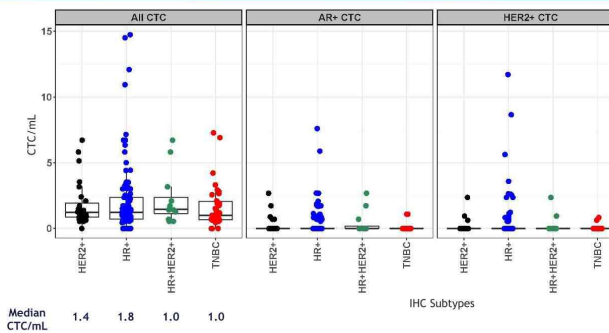
#### CTC Chromosomal Instability (CIN)



#### SC/NE CTC



## FIGURE 2. CTC BURDEN BY DISEASE SUBTYPE



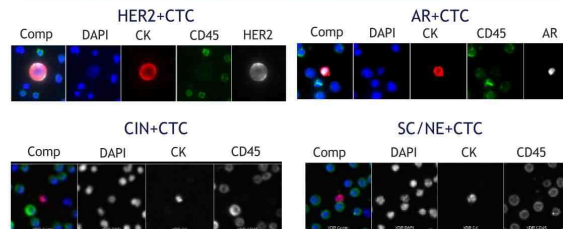
AR, androgen receptor; CTC, circulating tumor cell; HR, hormone receptor; TNBC, triple-negative breast cancer

Table 1. Prevalence of CTCs and CTC phenotypes in MBC

Breast Cancer Subtypes	N	CTC > 0 % samples	AR+CTCs % samples	HER2+CTC % samples	Het+SI ≥ 1 % samples	CIN+ ≥ 0.7/mL % samples	SC/NE+ % samples
All	148	91	19	14	32	28	19
HR+/HER2-	82	90	24	18	33	39	22
HR+/HER2+	21	100	19	14	29	19	19
HR-/HER2+	7	86	14	0	29	0	14
TNBC	38	90	5	8	32	16	13

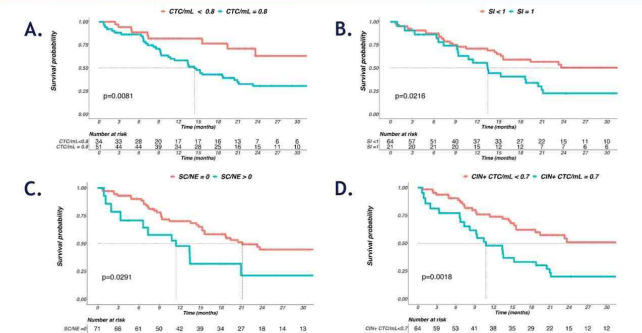
AR, androgen receptor; CIN, chromosomal instability; CTC, circulating tumor cell; HR, hormone receptor; het, heterogeneity; MBC, metastatic breast cancer; SI, Shannon Index; SC/NE, small cell/neuroendocrine

## FIGURE 3. REPRESENTATIVE IMAGES OF CELL TYPES OBSERVED



Each of the 4 panels above show representative cell images from the same field of view for each of the cell types analyzed. Top left: HER2+CTC; Top right: AR+CTC; Bottom left: CTC with representative CIN+ features; Bottom right: CTC with representative SC/NE+ features (see features described in methods). Examples of the cell phenotypes that comprised the heterogeneity analysis are shown in the methods figure. AR: androgen receptor; CIN: chromosomal instability; CK: cytokeratin; Comp: composite; DAPI, 4',6-diamidino-2-phenylindole; SC/NE, small cell/neuroendocrine.

## FIGURE 4. CTCs & CTC PHENOTYPES PREDICT SHORTENED SURVIVAL IN MBC

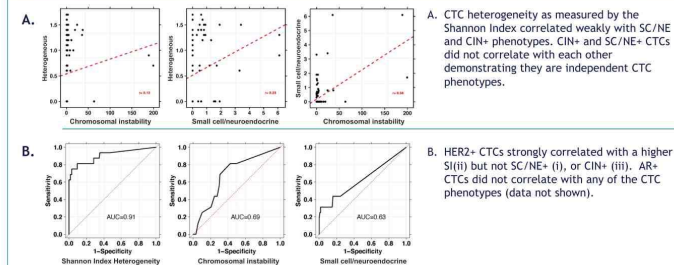


Kaplan-Meier plot for OS in patients with or without CTCs in 3 mL of blood tested B) Kaplan-Meier plot for patients with high CTC heterogeneity (SI ≥ 1 vs < 1) C) Kaplan-Meier plot for patients for OS in patients with or without SC/NE CTCs D) Kaplan-Meier plot for patients CIN+ CTCs ≥ 0.7/mL vs < 0.7/mL. CIN, chromosomal instability; CTC, circulating tumor cell; SC/NE, small cell/neuroendocrine; SI, Shannon Index.

Table 2. Overall survival of patients with and without distinct CTC phenotypes

	Het+	CIN+	SC/NE			
	SI < 1	SI ≥ 1	< 0.7/mL	≥ 0.7/mL	0	≥ 1
Median OS (mos)	24	13.5	24	10.8	21.2	11.5
P value	.022	.002	.029			

## FIGURE 5. CORRELATION OF OBSERVED CTC PHENOTYPES WITH EACH OTHER



## CONCLUSIONS

- Epic Sciences imaging analysis of CTCs identifies heterogeneous disease and characterizes distinct CTC morphological phenotypes that predict for shortened survival in MBC patients.
- SC/NE CTCs represent a breast cancer subtype which requires further characterization and may necessitate a novel therapeutic approach.
- Longitudinal characterization of CTC phenotypes across the MBC continuum could guide decision-making by monitoring CTC presence, heterogeneity, and specific morphologies.