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 morphologic 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 CCR 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

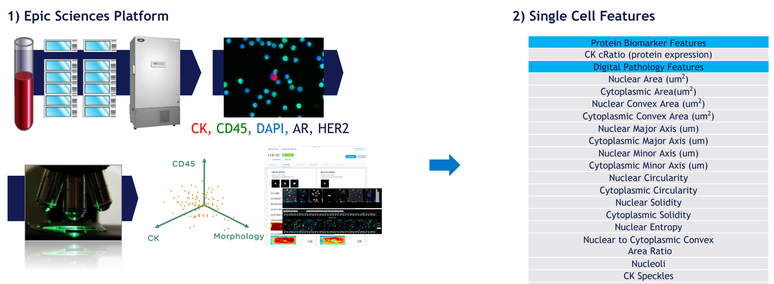
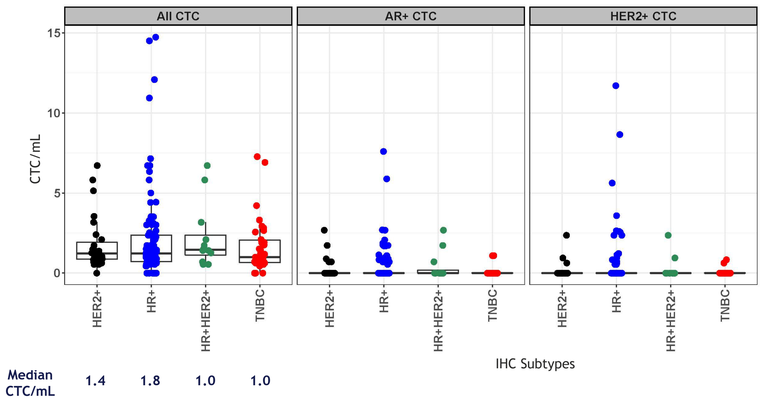


FIGURE 2. CTC BURDEN BY DISEASE SUBTYPE



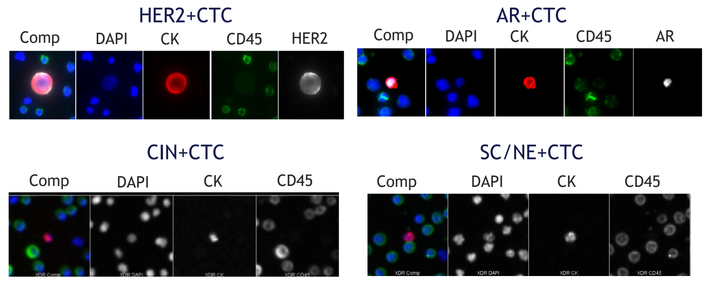
AR, androgen receptor; CTC, circulating tumor cell; HR, hormone receptor; TNBC, triple-negative breast cancer

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

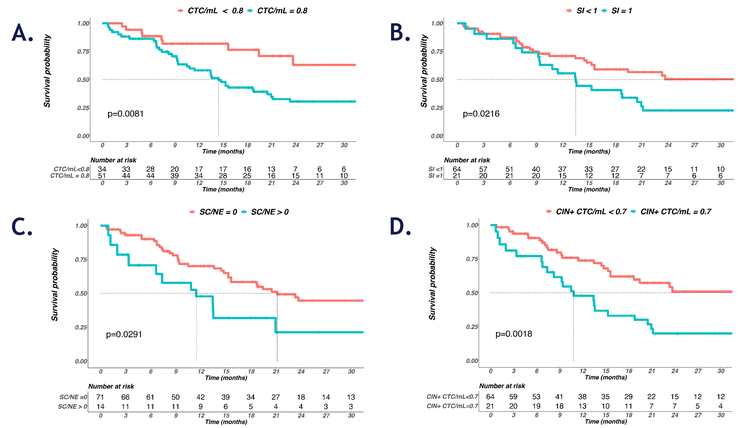
AR, androgen receptor; CIN, chromosomal instability; CTC, circulating tumor cell; HR, hormone receptor; het, heterogeneity; MBC, metastatic breast cancer; SI, Shannon Index; SC/NE, small cell/neuroendocrine

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

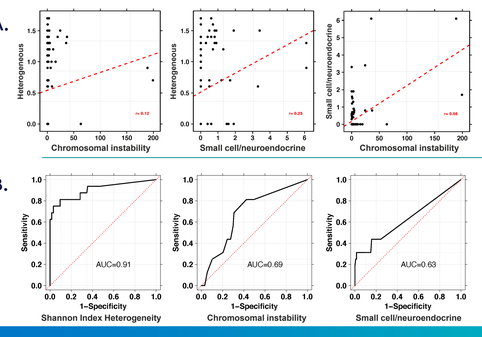


Kaplan-Meier plot for OS in patients with or without CTCs in 3 mL of blood tested **B)** Kaplan-Meier plot for patients with high CTC heterogeneity (SI≥1 vs <1) **C)** Kaplan-Meier plot for patients for OS in patients with or without SC/NE CTCs **D)** Kaplan-Meier plot for patients CIN+ CTCs ≥0.7/mL 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



A. CTC heterogeneity as measured by the Shannon Index correlated weakly with SC/NE and CIN+ phenotypes. CIN+ and SC/NE+ CTCs did not correlate with each other demonstrating they are independent CTC phenotypes.

B. HER2+ CTCs strongly correlated with a higher SI(ii) but not SC/NE+ (i), or CIN+ (iii). AR+ CTCs did not correlate with any of the CTC phenotypes (data not shown).

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.