Characterizing circulating tumor cell (CTC) enumeration and phenotypic profiling in blood with synchronously obtained tissue in metastatic breast cancer (MBC) in TBCRC /AURORA US

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Background

- In MBC, CTC enumeration is highly prognostic for both progression free survival (PFS) and overall survival (OS).
- Multiparametric technologies enable expanded phenotypic profiling of CTCs, including ER and HER2 assessment. Limited information is available on the concordance of CTC ER and HER2 levels compared to synchronously obtained tissue.
- We assessed the prognostic performance of CTC enumeration and phenotypic profiling of ER and HER2 on CTCs with tissue across subtypes at diagnosis of MBC or at time of progressing disease.

Methods

- The prospective, multi-institutional Aurora study enrolled pts with MBC of any subtype either at diagnosis of metastatic disease or at time of progression on any line of systemic therapy. Pts underwent a single time metastatic site biopsy, consented to archival primary tissue collection, and donated blood samples serially every 3-4 months until disease progression.
- CTCs, from blood collected at study entry, were identified as nucleated, EpCAM+/cytokeratin+/CD45- cells using an automated imaging system (RareCyte, Seattle). ER and HER2 expression levels were measured by mean fluorescence intensity (MFI), with thresholds above 80 (ER) and 160 (HER2) considered positive. MFI variability was assessed with coefficient of variation (CV). Comparisons of ER and HER2 expression were made to total RNAseq from paired metastatic tissue biopsies.
- PFS and OS were calculated from the time of study entry to last follow up or death.

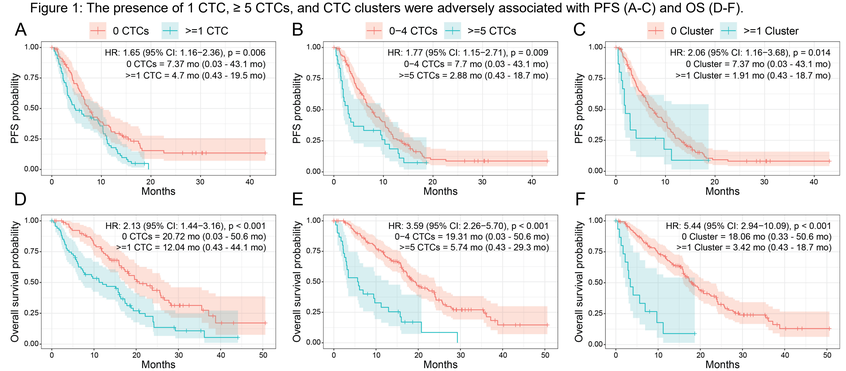
Patient Demographics

		Overall Patients N = 154	Patients with 0 CTCs n = 86	Patients With ≥1 CTC(s) n = 68
Vital status	Deceased	101	48	53
Gender	Female	151	84	67
	Male	2	1	1
	Missing	1	1	0
Race	White	131	72	59
	Black or African American	16	11	5
	Other/ Unknown	6	2	4
Age (yrs)	Median Age at met Diagnosis	56.0 (27-88)	55.9 (30-85)	56.0 (27-88)
Primary Tumor Subtype	ER+	104	54	50
	HER2+	22	14	8
	TNBC	18	11	7
	Missing	10	8	2
Metastatic Tumor Subtype	ER+	89	51	38
	HER2+	14	7	7
	TNBC	29	15	14
	Missing	22	13	9
# of Txs Prior to CTC Blood Draw	0	51	33	18
	1	29	9	20
	2	21	14	7
	3+	53	30	23
Last Metastatic Tx Before Aurora Blood Collection	Received 0 Treatments	51	34	17
	ADC	15	8	7
	Chemotherapy	55	28	27
	Endocrine therapy	59	28	31
	HER2	7	3	4
	Immunotherapy	8	4	4
	No Treatment Information	9	5	4
	Targeted therapy	5	4	1

CTCs were highly prognostic for both PFS and OS

- At least 1 CTC was identified in 68/154 pts (44%), 30 (19%) had ≥ 5 CTCs, and 15 (9.7%) had CTC clusters (groups of 2 or more CTCs). The median CTC number per sample was 0 (range 0-877).
- No associations were identified between CTCs and histology or presence of visceral metastases.

Figure 1: The presence of 1 CTC, ≥ 5 CTCs, and CTC clusters were adversely associated with PFS (A-C) and OS (D-F).



High intra-patient heterogeneity in ER and HER2 MFI observed in CTCs with higher ER-MFI observed in ER+ pts

- Across all subtypes, the median CTC ER-MFI across was 36.2 (range 4.2-10,016.6) and the median HER2-MFI was 139.6 (range 4.2-2480.5). We observed high intra-patient heterogeneity of ER-MFI (CV: 0.65 [0.34-1.25]; Fig 2A) and HER2-MFI (CV: 0.79 [0.42-1.54]; Fig 2B).
- Pts with clinical ER+ MBC had a higher median ER-MFI compared to clinical TNBC (111.9 vs 68.6, p = 0.019); Fig 3.
- However, 11 pts (26.8%) with clinical ER+ MBC had all CTCs with an ER-MFI <80. Lower ER expression was observed in CTCs collected after prior exposure to a SERD (median ER-MFI: post-SERD [n=8] 27 vs post-aromatase inhibitor [n=4] 252, p<2.22e-16).

Fig 2: MFI for each pt is plotted on a log scale. Each black circle represents a unique CTC. The red diamond represents the median MFI across the CTCs for a specific pt. The dashed line represents the MFI threshold for staining to be present for ER (2A, >160) and HER2 (2B, >80). The samples are subcategorized on the x-axis according to the clinical ER and HER2 expression values.

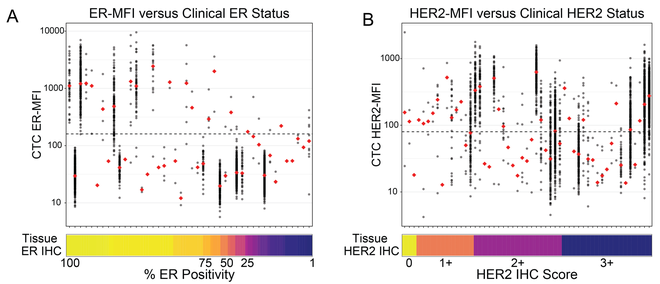
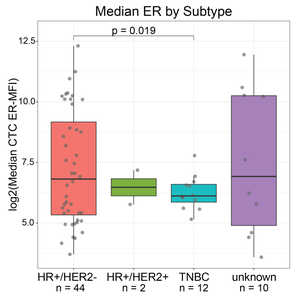
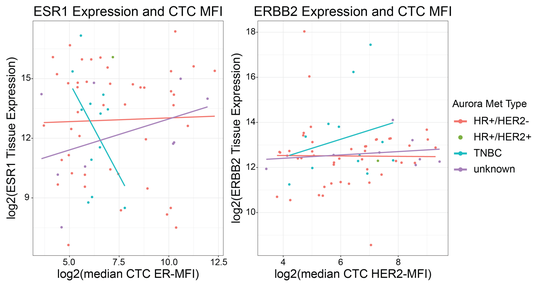


Fig 3 (right): The median ER-expression of all CTCs for each individual patient is represented as a block dot and each column represents a different clinical subtype based on the metastatic biopsy results.



No correlations observed between CTC ER-MFI or HER2-MFI and tissue *ESR1* or *ERBB2* RNA

Fig 4: In the 66 pts with CTCs and paired metastatic tissue with RNA-seq available, we plot the *ESR1* expression (A) or *ERBB2* expression (B) on the y-axis vs the median CTC ER (or HER2 MFI on the x-axis. Legend notes the clinical receptor subtype.



Conclusions

- CTCs are strongly predictive of PFS and OS.
- CTC ER- and HER2-MFI were highly heterogeneous without clear correlation with bulk tissue RNA expression.
- The clinical impact of discordant clinical receptor status and CTC receptor status warrants further exploration.

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