Circulating tumor cell (CTC) transcriptional profiling identifies diverse CTC phenotypes in metastatic breast cancer

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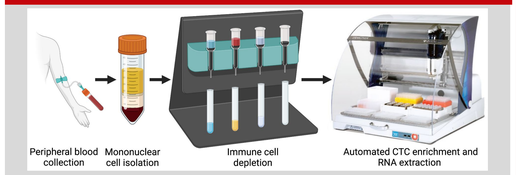
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Background

- Breast cancer is a heterogeneous disease with multiple clinically and transcriptionally defined subtypes with differing biology and prognosis.
- Understanding the role of subtype switching in treatment response and resistance has been limited by the challenges of obtaining serial tissue biopsies from patients.
- Liquid biopsies, including circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs), are a source of tumor-derived material shed into the blood of patients with cancer that require only a peripheral blood draw, making them uniquely suited for longitudinal sampling to evaluate for subtype evolution in metastatic breast cancer.
- Here, we report the development of a targeted gene expression assay for genes associated with estrogen and HER2 signaling as well as luminal and basal identity in breast cancer CTCs, to address this need.

Methods



- 15mL peripheral blood was collected from a pilot cohort of patients with metastatic breast cancer (n=67) enrolled in an IRB-approved liquid biopsy collection protocol. Following immune cell depletion, CTC enrichment and RNA extraction was performed utilizing the automated mTAE platform[2,3].
- CTC RNA underwent pre-amplification followed by qRT-PCR[4] for the following genes of interest:

Category	Gene(s)
Epithelial cell identity	EpCAM, KRT18, KRT19
Estrogen signaling	ESR1, ESR2, PGR. TFF1, TFF3, FOXA1, IGFBP4, PTGES, CDH1, CA12, AGR2
HER2 & other growth factors	ERBB2, GRB7, FGFR1, FGFR4
Basal differentiation	CDH3, KRT5, KRT14
Immune cell content control	CD45
Housekeeping genes	RPLP0, POLR2A

- Gene expression from CTCs isolated from 15mL blood were calculated as 33-Ct values. Epithelial content score was calculated by summing expression of epithelial cell identity genes. Estrogen signaling score was calculated by summing expression of the estrogen signaling genes. Samples with EpCAM expression>0 were classified as CTC positive, samples with epithelial content score>10 were classified as high CTC content, and high content samples with CD45 expression <5 were classified as high CTC purity.

References

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CTC detection across breast subtypes and disease status

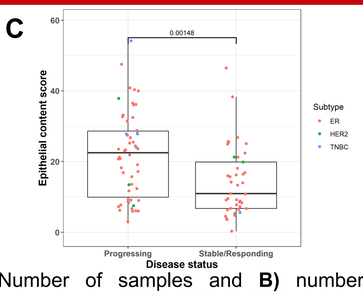
A

Characteristic	Total cohort	ER positive	TNBC	HER2	Stable/ Responding	Progressing
No. of samples	131	113	7	11	61	64
CTCs detected	98 (74.8%)	86 (76.1%)	7 (100%)	5 (45.5%)	41 (67.2%)	54 (84.4%)
High content	64 (48.9%)	55 (48.7%)	5 (71.4%)	4 (36.4%)	22 (36%)	40 (62.5%)
High purity	41 (31.3%)	35 (31%)	3 (42.9%)	3 (42.9%)	14 (22.9%)	25 (39%)

B

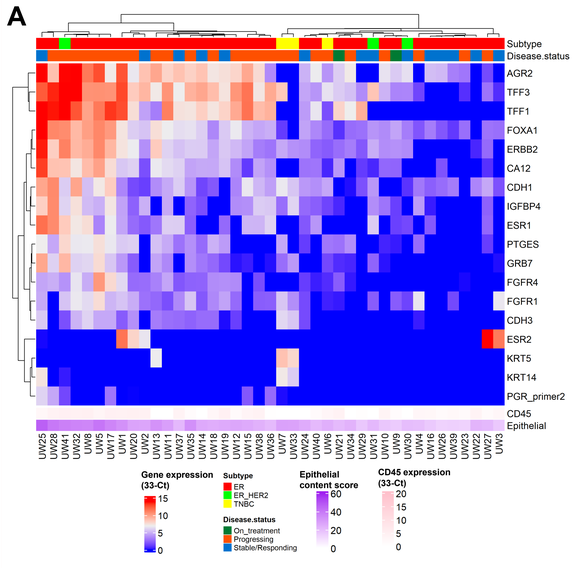
Characteristic	Total cohort	ER positive	TNBC	HER2	Stable/ Responding	Progressing
No. of patients	67	58	4	5	39	39
CTCs detected	57 (85.1%)	50 (86.2%)	4 (100%)	3 (60%)	29 (74.4%)	35 (89.7%)
High content	40 (59.7%)	33 (56.9%)	3 (75%)	3 (60%)	17 (43.6%)	29 (74.4%)
High purity	30 (44.8%)	25 (43.1%)	3 (75%)	2 (40%)	12 (30.8%)	21 (53.8%)

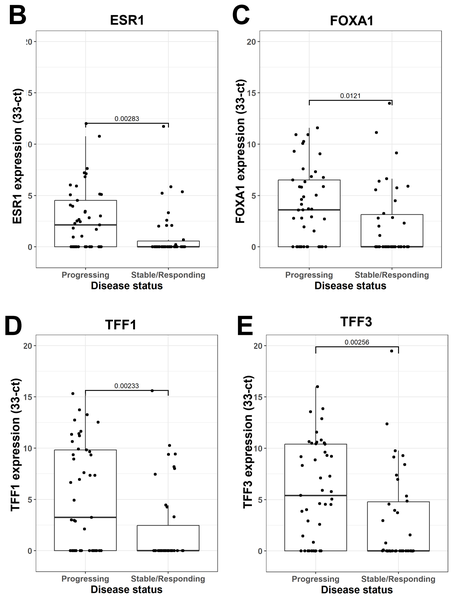
C



A) Number of samples and **B)** number of patients with any CTCs (EpCAM>0), high CTC content (Epithelial content score > 10) and high CTC purity (Epithelial score >10, CD45 <5) are presented across breast cancer subtypes and clinical disease status at time of CTC collection. **C)** Epithelial content by disease status across breast cancer subtypes.

Hierarchical clustering reveals differential estrogen signaling in ER+ CTCs



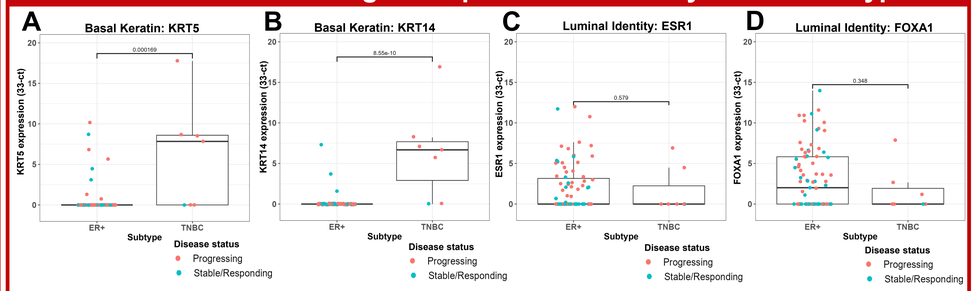


A) Hierarchical clustering of high purity CTC samples (Epithelial content score > 10, CD45 <5) reveals a subset of clinically progressing ER+ samples with low estrogen signaling that cluster with TNBC samples as well as a subset of clinically responding ER+ samples with high estrogen signaling; however, basal keratin expression (KRT5, KRT14) is restricted to the TNBC samples. **B-E)** In ER+ samples with detectable CTCs (EpCAM>0), estrogen signaling gene expression was higher on average in patients with clinically progressing versus stable/responding disease, but there was significant heterogeneity in estrogen signaling gene expression in both groups.

Conclusions

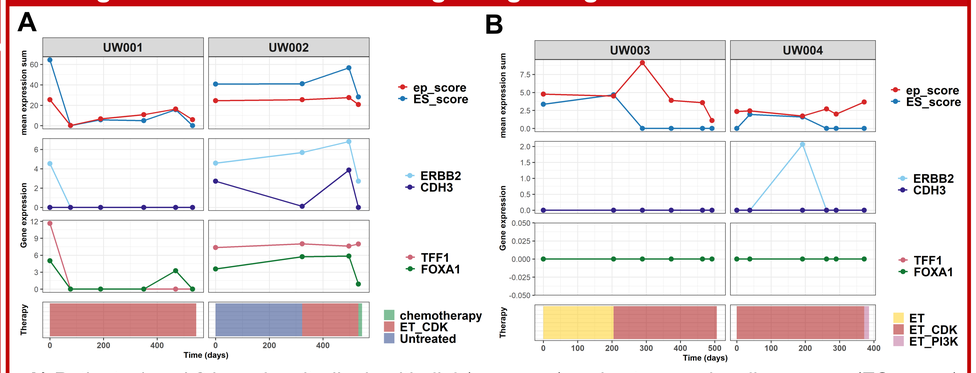
- In a pilot cohort of 67 patients with metastatic breast cancer, we have demonstrated the feasibility of detecting gene expression relevant to breast cancer transcriptional subtypes via liquid biopsy circulating tumor cell gene expression profiling.
- As expected, patients with clinically progressing disease on current treatment had higher rates of CTC detection, but our assay demonstrated relatively high sensitivity even in patients with stable disease, with CTCs detected in 67.2% of samples.
- In CTCs from patients with ER+ breast cancer, heterogeneity in estrogen signaling was detected, including patients with persistent estrogen signaling in the setting of clinically responding disease or low estrogen signaling in the setting of clinically progressing disease, suggesting that this assay could be utilized to monitor response to anti-estrogen therapy and evolving endocrine resistance in this patient population.
- High estrogen signaling on treatment was associated with shorter progression free and overall survival.

CTC luminal and basal gene expression differs by clinical subtype



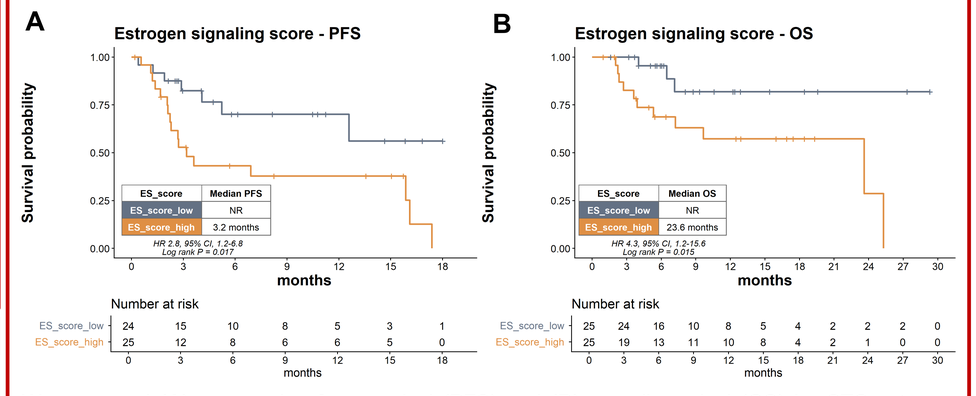
A-B) Expression of basal keratins (KRT5, KRT14) is significantly higher in TNBC CTCs compared to ER+ CTCs **C-D)** Expression of luminal identity associated genes (ESR1, FOXA1) had a trend towards higher expression in ER+ CTCs compared to TNBC CTCs.

Longitudinal evolution of estrogen signaling in ER+ CTCs on treatment



A) Patients 1 and 2 have longitudinal epithelial (ep_score) and estrogen signaling scores (ES_score) that are concordant over time, both while on the same treatment (patient 1), and across different treatments (patient 2). **B)** In contrast, patients 3 and 4 exhibit diverging epithelial and estrogen signaling scores over time consistent with emergence of a population of CTCs with low estrogen signaling, in both cases during treatment with a CDK4/6 inhibitor.

High CTC estrogen signaling is associated with shorter survival



We assessed **(A)** progression free survival (PFS) and **(B)** overall survival (OS) by CTC estrogen signaling score in patients with ER+ disease and detectable CTCs. We observed significant differences in PFS (HR 2.8, 95% CI 1.2-6.8, log rank P=0.017) and OS (HR 4.3; 95% CI 1.2-15.6; log rank P=0.015) when stratified by high (>median) versus low (<median) estrogen scores.

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Disclosures

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