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Novel Epigenomic Liquid Biopsy Assay to Predict Estrogen Receptor (ER) Status and to Infer ER Pathway Activation in Breast Cancer

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BACKGROUND

- Endocrine therapy (ET) is the cornerstone of treatment for ER-positive (ER+) breast cancer (BC)
- ER+ tumors can become resistant to ET regardless of ER expression, necessitating alternative treatments.
- Blood-based biomarkers to predict ET sensitivity are lacking, limiting non-invasive monitoring and hindering personalized treatment strategies.
- Endocrine therapies, including next generation endocrine agents, are showing efficacy in breast cancers still addicted to the endocrine signaling pathway.
- Methods to assess endocrine activity are crucial to identify which patients will continue to benefit from ET after progression of disease on ET.
- Similarly, these assays may help to understand when disease becomes resistant to ET, and when alternative, non-endocrine therapies are preferred.

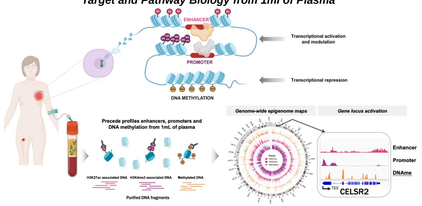
METHODS

- We identified patients with metastatic breast cancer seen at Dana-Farber Cancer Institute (DFCI) from 2012-2023 or from commercial biobanks who had blood samples drawn in Streck tubes within 6 weeks of tumor biopsy with ER scored via immunohistochemistry (IHC).
- ER expression ≥1% was considered positive.
- A novel, multi-analyte, liquid biopsy assay was applied to capture genome-wide epigenomic signals, mapping enhancers, promoters, and DNA methylation data from 1mL of plasma.
- A previously developed, IHC-benchmarked, regularized logistic regression model was used to infer ER status from plasma-based epigenomic profiles (Parsons et al., SABCS 2023).
- We developed an ER pathway activity score from the promoter and enhancer profiles of genes previously associated with ER activity.[2,3]
- The ER activity score was corrected for the circulating tumor DNA (ctDNA) fraction as assessed by low pass whole genome sequencing (iChorCNA algorithm[4]).
- The association between the ER activity score and ER status by IHC was evaluated using the Wilcoxon rank-sum test.
- The ER classifier and the ER activity score were applied to 19 of 43 DFCI samples (44%) all of which had detectable ctDNA as assessed by iChorCNA (>3%). Eleven out of 19 (58%) patients had ER+ BC by IHC (ER IHC range 80-95%).

RESULTS

Figure 1: Comprehensive Epigenomic Profiling

Comprehensive Epigenomic Platform Offers Dynamic Resolution Into Target and Pathway Biology from 1ml of Plasma



Cell free DNA derived from tumors exist in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against active enhancers, active promoters and DNA methylation are used to enrich for associated DNA fragments from 1 mL plasma and sequenced to capture the underlying transcriptional state of the tumor cells.[5]

Patient Cohort & REMARK

		Patients (n=19)
Age at Metastatic Diagnosis: Median (Range)		50 yrs (40-65)
ER Status	ER+	11
	ER-	8
HER2 Status	HER2+	2
	HER2-low	10
	HER2-0	7
ESR1 Status	mESR1	3
	wtESR1	12
	N/A	4
Lines of Metastatic Therapy at Time of Blood Draw: Median (Range)		2 (0-8)
Previous Endocrine Therapy for MBC	≥ 4 lines	3
	< 4 lines	8

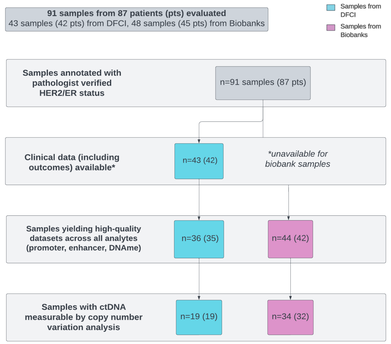
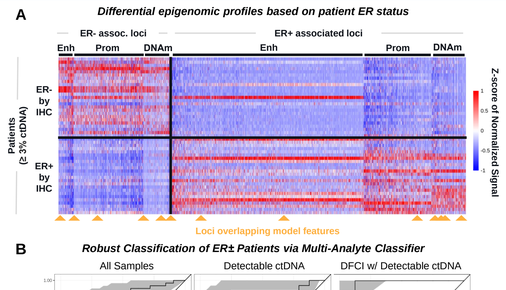


Figure 2: Multi-analyte ER Classifier Robustly Stratifies Patients by ER Status

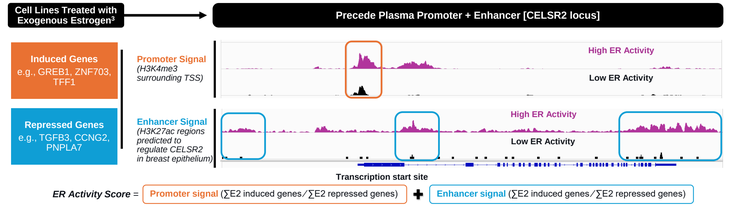


A Heatmap representation of z-scored, ctDNA- and background-normalized counts at differential peaks (DE-seq, FDR < 0.01, log2(fold change) > 1) across ER +/- patients by IHC. Each row represents the signal of an indivual patient, each column represents an enhancer/promoter/DNAm locus.

B ROC curves assessing performance of regularized logistic regression model to classify ER IHC status (positive vs negative) of (left) all biobank and DFCI samples, (center) samples with detectable ctDNA by iChor, and (right) only the DFCI samples drawn in Streck tubes within 6 weeks of tumor biopsy with HER2/ER pathologist assessment .

C The summed weights of the negative (left) and positive (right) coefficients in the ER model, stratified by analyte. Genes of known biological importance found proximal to enhancers/promoters are highlighted.

Figure 3: Development of Plasma Based Epigenomic ER Activity Score Enables Potential Patient Stratification Based on Endocrine Signaling



A Quantification of the enhancer/promoter signals surrounding differentially expressed genes upon E2 treatment to generate an epigenomic-based 'ER activity score'.

B Epigenomic ER activity score displays a relationship with ctDNA% (iChor) in ER+, but not ER-, samples (left). The linear trend between tumor fraction and activity score is regressed out to produce the ctDNA-corrected ER activity score (center). Elevated promoter signals at estrogen-induced genes are visible in high scoring samples across a range of ctDNA% (right).

C HER2- (IHC) patients display lower ctDNA-corrected activity scores than HER2+ patients (left). The signal at enhancers and promoters of genes such as GREB1, ZNF703, and IGFBP4 drives in part the higher activity scores of ER+/HER2- patients (right, top). Conversely the signal at enhancers and promoters of genes such as TGFB3, CCNG2, and PNPLA7 drives in part the lower activity scores of HER2+ patients (right, bottom).

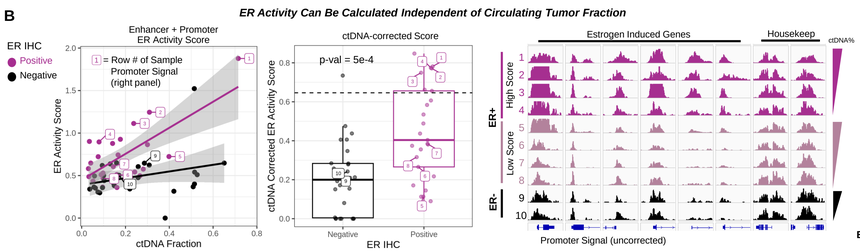
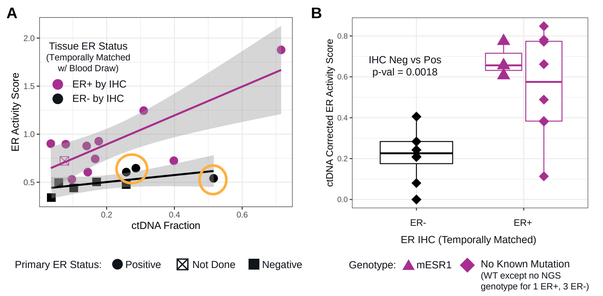


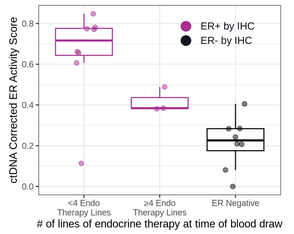
Figure 4: ER Activity Score Tracks with ESR1 Mutation Status and ER IHC Switches



A ER activity scores are significantly higher in ER+ (IHC) patients within the DFCI cohort compared to ER- patients. This includes 3 patients who were ER+ on primary biopsy but had switched to ER- by the time of blood draw (yellow circle). Patients with ER-loss have low activity scores and are properly classified as ER-.

B Patients found to have a mutation in the ESR1 gene displayed consistently high activity scores compared to the wider score ranges of patients where mutations were not detected or not annotated.

Figure 5: ER Activity Scores by Lines of Endocrine Therapy



Patients with ≥4 Lines of Endocrine Therapy Display Lower ER Activity Scores

ctDNA-corrected ER activity scores stratified by number of endocrine therapy lines each patient had received at the time of blood draw.

ER activity score from ER- patients shown as a control.

CONCLUSIONS

- In this retrospective study profiling 1mL plasma from 87 patients with metastatic breast cancer, we applied a blood-based epigenomic classifier trained on enhancer, promoter, and DNA methylation profiles to assess both ER status and ER pathway activation.
- An IHC-benchmarked, cross validated logistic regression model reliably classified patients by ER status from plasma-based epigenomic profiles (AUC 0.88, samples with detectable ctDNA).
- We utilized the enhancer and promoter signatures of genes with differential expression upon estrogen exposure to develop a novel 'ER activity score' and infer ER signaling.
- Patients with mutations in the ESR1 gene and/or fewer prior rounds of endocrine therapies displayed elevated ER activity scores.
- With further validation training on patient responses to next-gen endocrine agents this minimally invasive assay could become a valuable tool for guiding treatment selection in ER+ breast cancer patients.

REFERENCES: 1) Parsons et al., *SABCS*, 2023; 2) Li et al., *BioRXiv*, 2023, https://estrogene.org/; 3) Guan et al., *Cell*, 2019; 4) Adalsteinsson et. al., *Nature Communications*, 2017 5) Baca et. al., *Nature Medicine*, 2023

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