

Characterization of Functional Estrogen Receptor (ER) Dependence via Comprehensive Epigenomic Liquid Biopsy Stratifies Endocrine Therapy (ET) Responders with Metastatic Breast Cancer (MBC)

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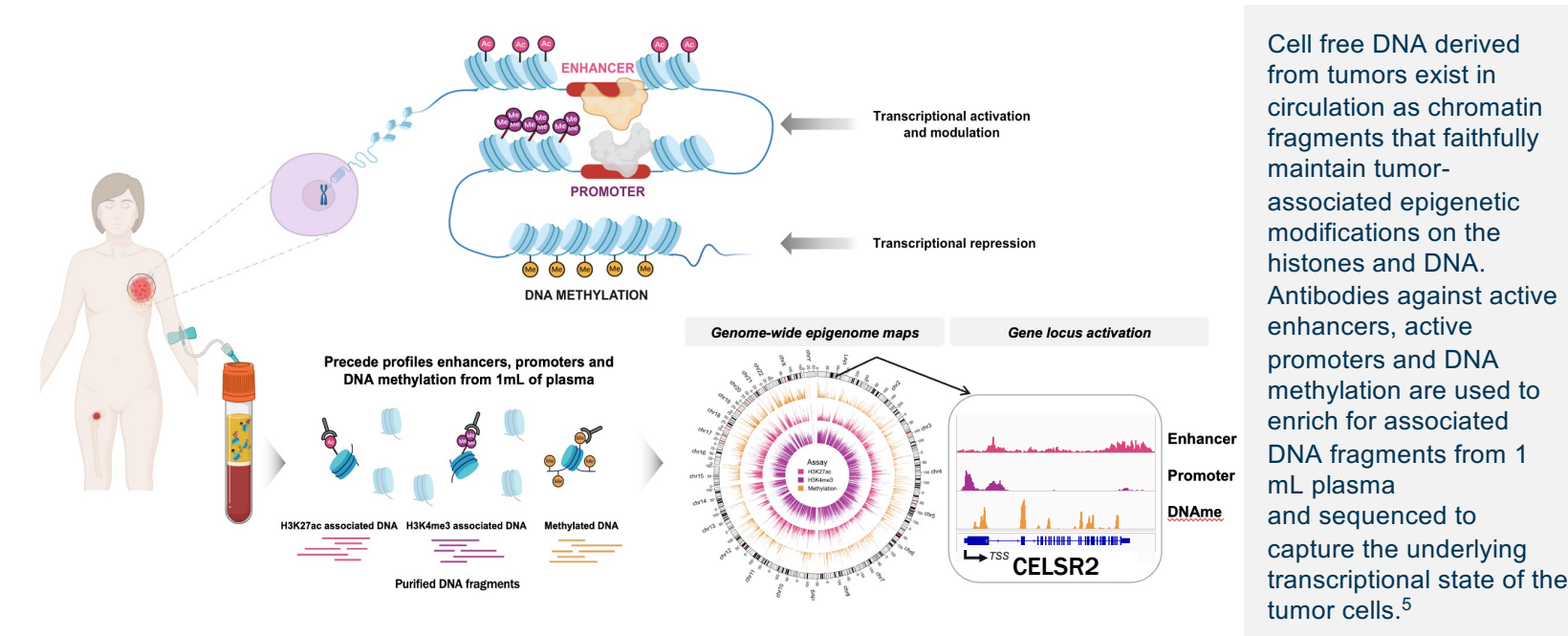
BACKGROUND

- 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.
- Methods to assess ER-pathway activity are crucial to identify patients who will continue to benefit from additional 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.

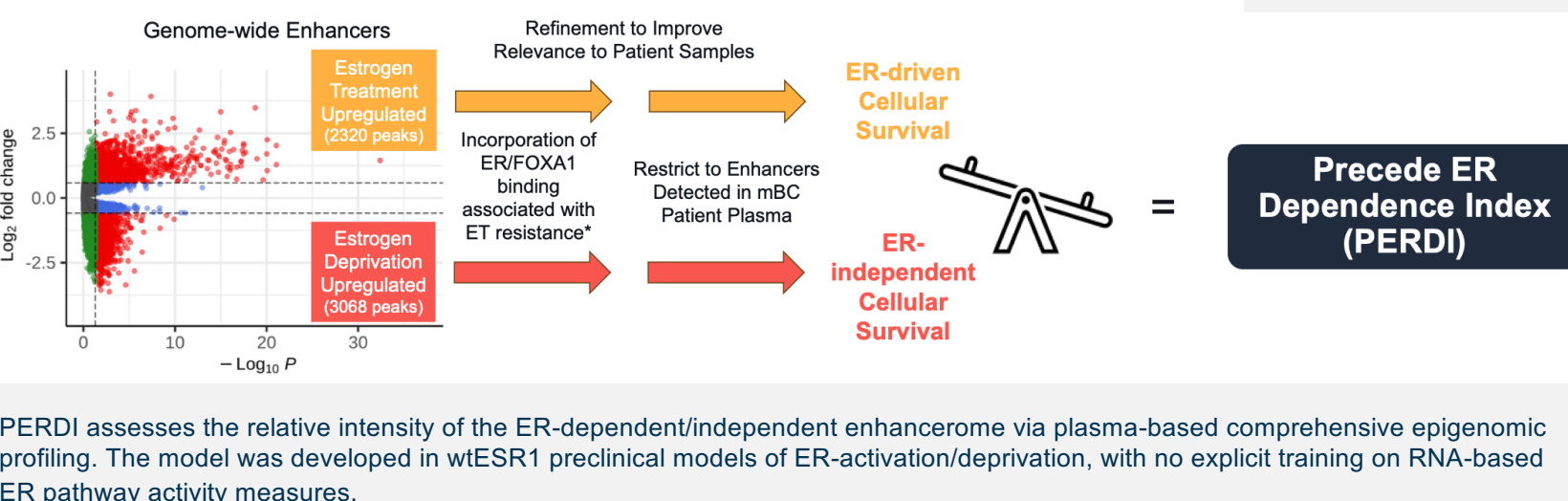
METHODS

- We identified 122 patients with metastatic breast cancer (MBC) enrolled in the EMBRACE research study at Dana-Farber Cancer Institute (DFCI) who received 1-2 lines of ET and had blood collected +/- 30 days of switch from first to second-line (1L to 2L) or from second to third line therapy (2L to 3L).
- A multi-analyte, liquid biopsy assay was applied to capture genome-wide epigenomic signals, mapping enhancers, promoters, and DNA methylation data from 1mL of plasma.
- Samples that did not meet quality thresholds (n=16; 9.5%) or with tumor fraction <0.5% (n=48; 33%) were excluded.
- We applied the Precede ER dependence index (PERDI) (Beagan, SABCS 2024) that quantifies relative activities of ER-driven vs estrogen-starvation-induced enhancers and classified samples as PERDI-high or -low using a predefined threshold from an independent ET-treated cohort.
- We inferred tumor gene expression estimates via our breast cancer-specific gene expression models (Nguyen, AACR 2025) and combined them to quantify the activity of additional pathways of interest.
- We investigated the association between PERDI and time-to-next-treatment (TTNT). A conditional landmark approach defined TTNT beginning from 30 days after the start of the treatment of interest (ET or non-ET) until the start of the next treatment line, considering only treatment switch for tumor progression as events.
- Time-dependent ROC curves were used in the prespecified primary analysis investigating the association between PERDI and early progression on ET, combining patients who received 2L or 3L ET (n=57). Kaplan Meier methods and Cox proportional hazard models were used in additional analyses.

PERDI score



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



PERDI assesses the relative intensity of the ER-dependent/independent enhancerome via plasma-based comprehensive epigenomic profiling. The model was developed in wESR1 preclinical models of ER-activation/deprivation, with no explicit training on RNA-based ER pathway activity measures.

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Study Cohort & REMARK diagram

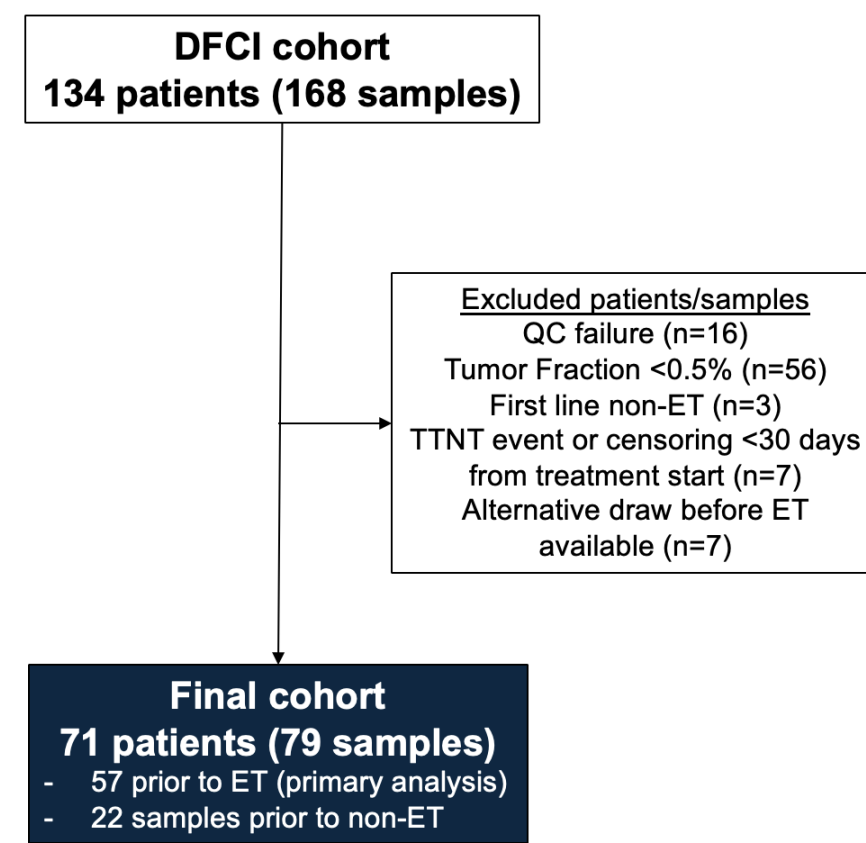
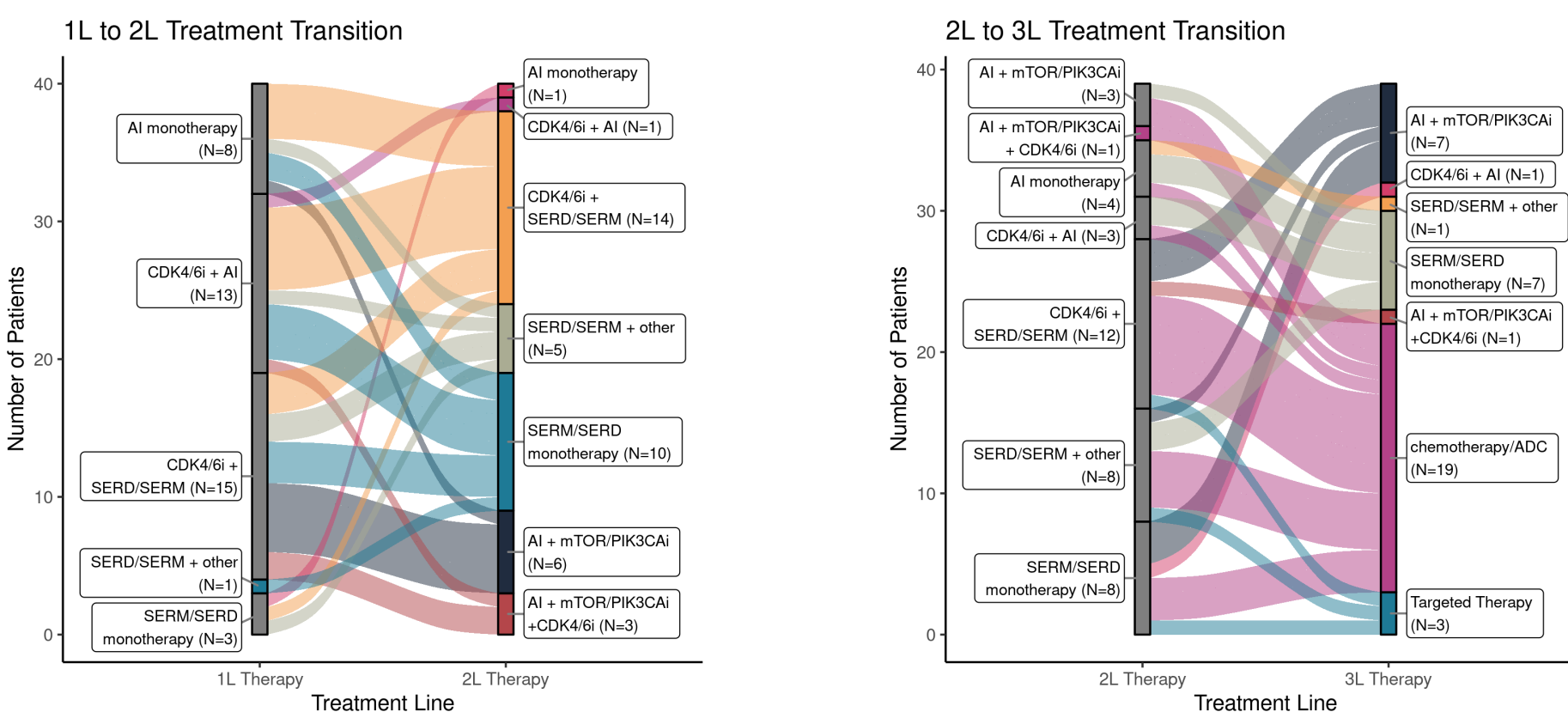


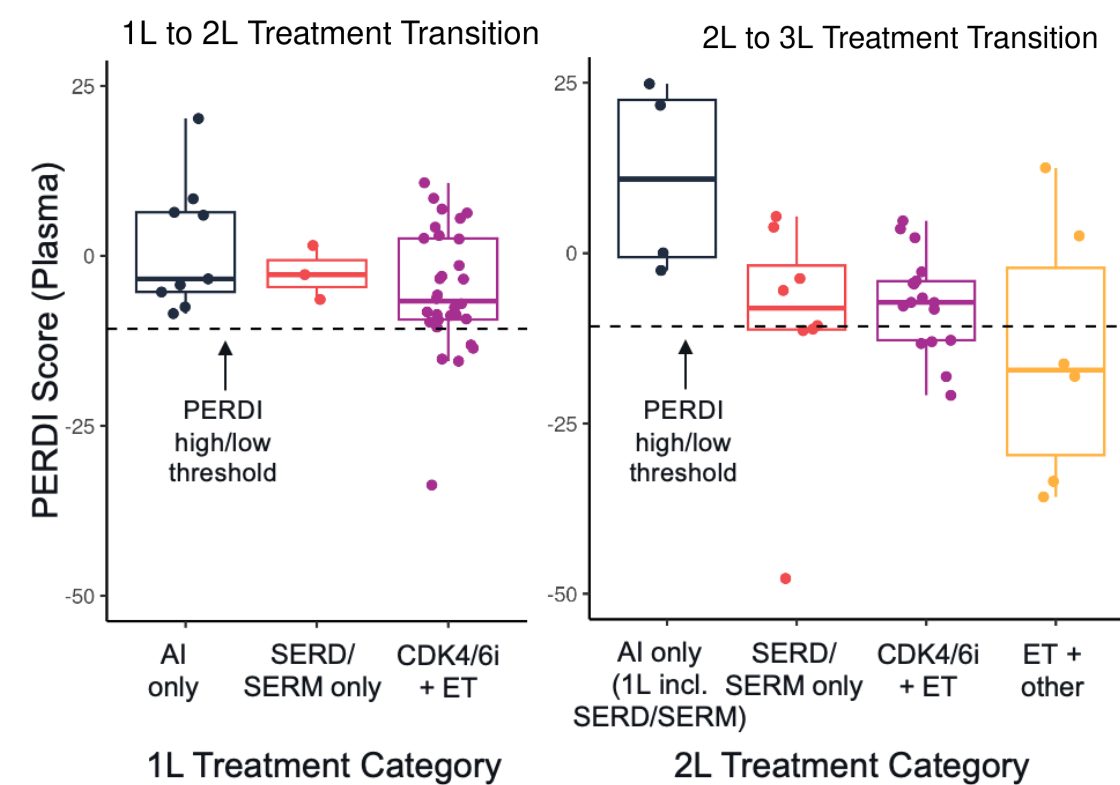
Table 1: Patient characteristics, including individuals with blood draws before switching to ET (primary analysis cohort, N=71)

Age at MBC Diagnosis: Median (Range)	57.6 (33.1-83.8)
Race	
White	51 (89.5%)
African American	2 (3.5%)
Asian or Pacific Islander	1 (1.8%)
Other	2 (3.5%)
Stage at Diagnosis	
0-III	41 (71.9%)
IV	15 (26.3%)
Unknown	1 (1.8%)
Metastatic sites at diagnosis: Median (Range)	2 (1-5)
Age at Blood Draw: Median (Range)	59.9 (34.9-86.5)
Timing of blood draw collection	
1L to 2L	40 (70.2%)
2L to 3L	17 (29.8%)
CDK4/6 inhibitors prior to blood draw	40 (70.2%)
Duration of ET prior to blood draw	
>=18 months	21 (36.8%)
<18 months	36 (63.2%)
Visceral disease at time of blood draw	31 (54.4%)



Of the 57 samples collected prior to ET, 40 were drawn before starting 2L and 17 before starting 3L therapy. Next-line ET and non-ET regimens are reported in the Sankey plot.

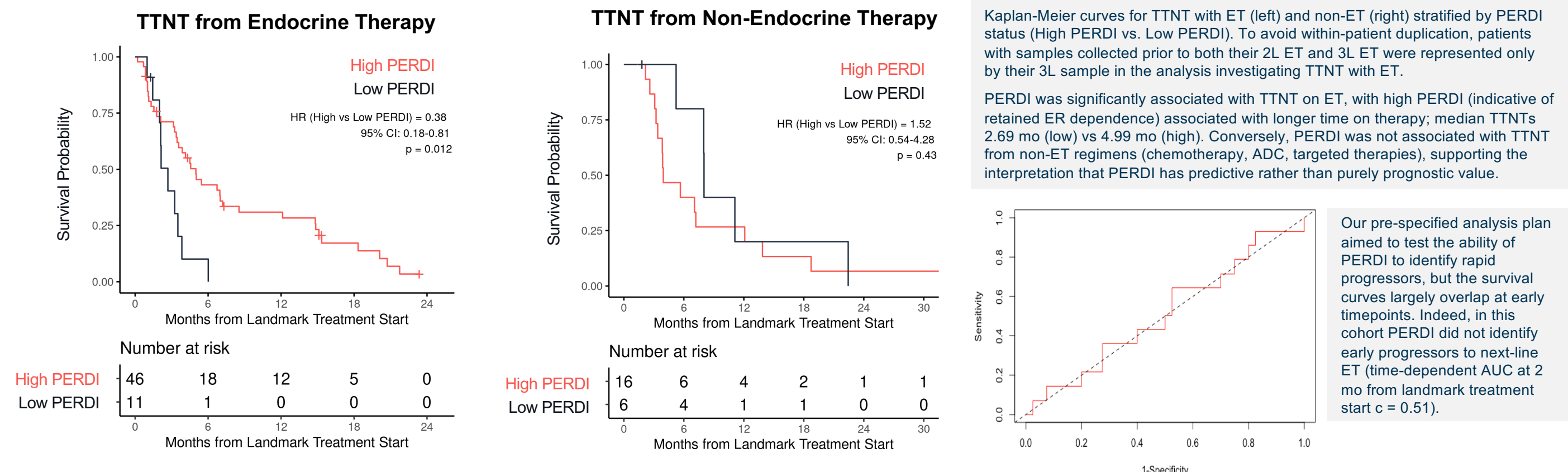
Prior exposure to CDK4/6i is associated with low PERDI classification



Patient samples were stratified by therapy line at the end of which the sample was collected. The dashed horizontal line indicates the PERDI threshold (-10.75) set based on previous studies (unpublished), separating high PERDI (ER-active, above) from low PERDI (below). Patients who received prior CDK4/6i + ET combinations trend toward lower PERDI scores, consistent with selection for ER-independent disease following more intensive ET exposure.

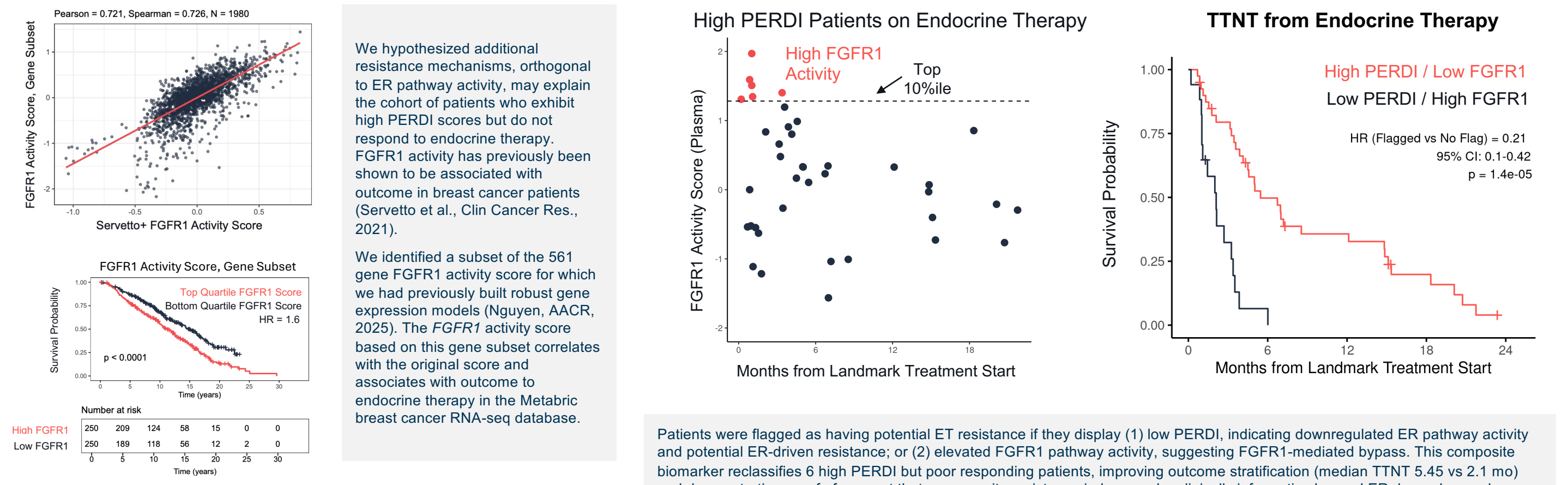
RESULTS

PERDI is associated with benefit from Endocrine Therapy



Kaplan-Meier curves for TTNT with ET (left) and non-ET (right) stratified by PERDI status (High PERDI vs. Low PERDI). To avoid within-patient duplication, patients with samples collected prior to both their 2L ET and 3L ET were represented only by their 3L sample in the analysis investigating TTNT with ET. PERDI was significantly associated with TTNT on ET, with high PERDI (indicative of retained ER dependence) associated with longer time on therapy; median TTNTs 2.69 mo (low) vs 4.99 mo (high). Conversely, PERDI was not associated with TTNT from non-ET regimens (chemotherapy, ADC, targeted therapies), supporting the interpretation that PERDI has predictive rather than purely prognostic value. Our pre-specified analysis plan aimed to test the ability of PERDI to identify rapid progressors, but the survival curves largely overlap at early timepoints. Indeed, in this cohort PERDI did not identify early progressors to next-line ET (time-dependent AUC at 2 mo from landmark treatment start = 0.51).

Plasma-based FGFR1 activity score identifies PERDI-high patients with poor response to Endocrine Therapy

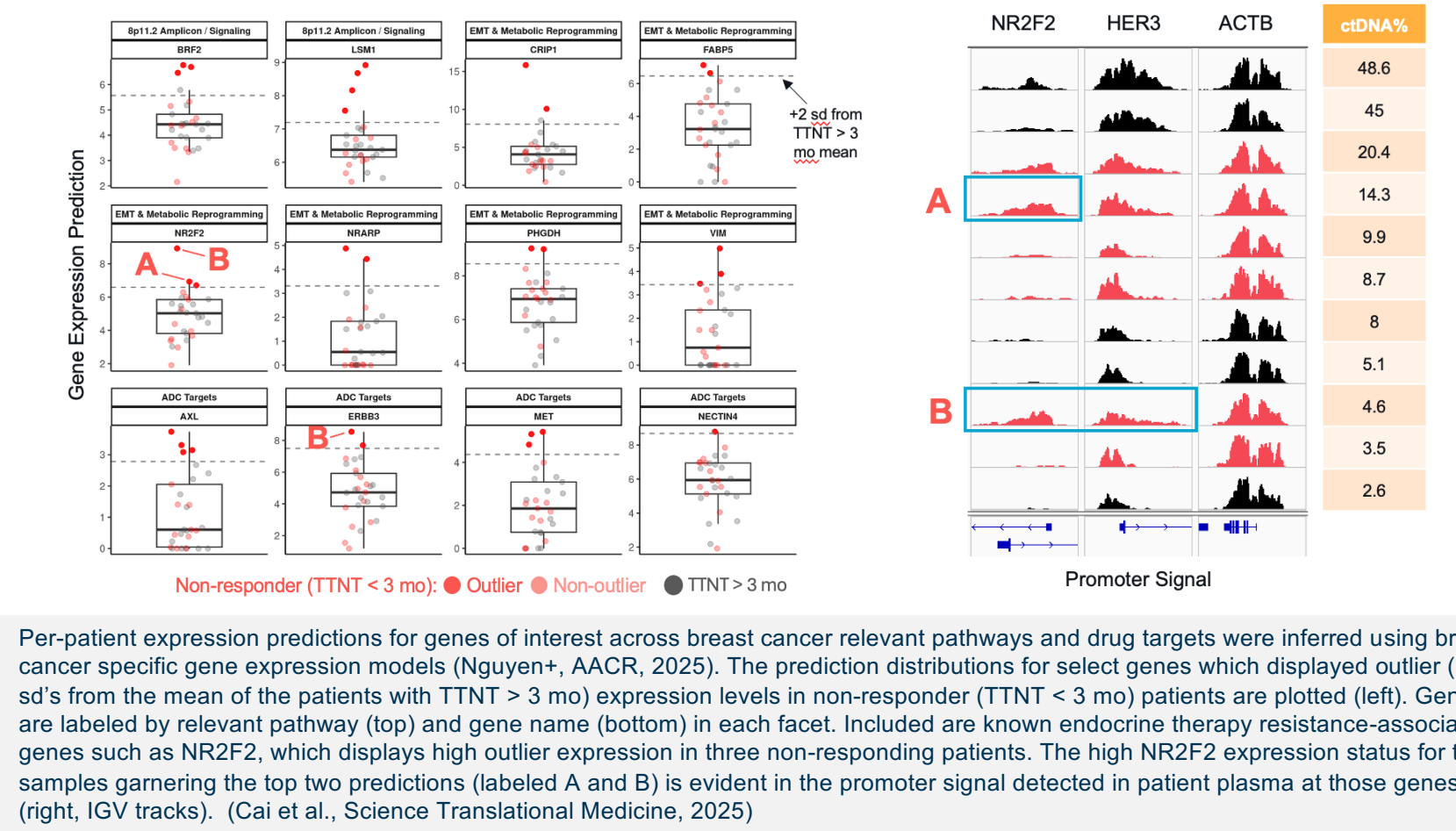


We hypothesized additional resistance mechanisms, orthogonal to ER pathway activity, may explain the cohort of patients who exhibit high PERDI scores but do not respond to endocrine therapy. FGFR1 activity has previously been shown to be associated with outcome in breast cancer patients (Servetto et al., Clin Cancer Res., 2021). We identified a subset of the 561 gene FGFR1 activity score for which we had previously built robust gene expression models (Nguyen, AACR, 2025). The FGFR1 activity score based on this gene subset correlates with the original score and associates with outcome to endocrine therapy in the Metabric breast cancer RNA-seq database.

Patients were flagged as having potential ET resistance if they display (1) low PERDI, indicating downregulated ER pathway activity and potential ER-driven resistance; or (2) elevated FGFR1 pathway activity, suggesting FGFR1-mediated bypass. This composite biomarker reclassifies 6 high PERDI but poor responding patients, improving outcome stratification (median TTNT 5.45 vs 2.1 mo) and demonstrating proof of concept that a composite resistance index may be clinically informative beyond ER dependence alone.

Prior exposure to CDK4/6i is associated with low PERDI classification

Elevated plasma-based expression predictions for resistance, drug target genes in select rapid progressing patients



Per-patient expression predictions for genes of interest across breast cancer relevant pathways and drug targets were inferred using breast cancer specific gene expression models (Nguyen+, AACR, 2025). The prediction distributions for select genes which displayed outlier (>2 sd's from the mean of the patients with TTNT > 3 mo) expression levels in non-responder (TTNT < 3 mo) patients are plotted (left). Genes are labeled by relevant pathway (top) and gene name (bottom) in each facet. Included are known endocrine therapy resistance-associated genes such as NR2F2, which displays high outlier expression in three non-responding patients. The high NR2F2 expression status for the samples garnering the top two predictions (labeled A and B) is evident in the promoter signal detected in patient plasma at those genes (right, IGV tracks). (Cai et al., Science Translational Medicine, 2025)

CONCLUSIONS

- We observed a significant association between PERDI, a plasma-based measurement of tumor dependence on ER pathway activity, and benefit from endocrine therapy.
- PERDI is not prognostic for outcome to non-endocrine therapies.
- Exploratory analysis integrating resistance pathways beyond ER further identified pts with high PERDI but ET resistance.
- Many patients displayed elevated FGFR1 activity levels; integration of FGFR1 activity measurements with PERDI improved outcome stratification.
- Additional patients were predicted to be expressing high levels of known resistance marker genes such as NR2F2.
- Upon further clinical validation, this blood based comprehensive epigenomic assay could become a valuable tool to guide therapeutic decisions for the ER+/HER2- MBC population, or reveal actionable biology to guide subsequent therapy selection in the ET resistant population.