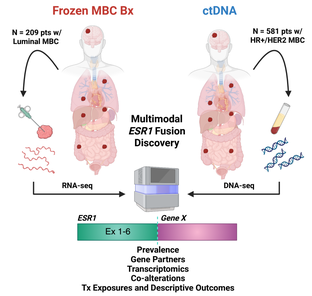
Clinicogenomic characterization and ctDNA detection of *ESR1* fusion positive metastatic breast cancer

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INTRODUCTION



Multimodal Identification of ESR1 Fusions in Metastatic Breast Cancer. Schematic illustrating ESR1 fusion discovery through tumor RNA-seq of fresh-frozen biopsies and ctDNA profiling.

ESR1 fusions are an emerging mechanism of acquired resistance to endocrine therapy (ET) in hormone-receptor positive (HR+) metastatic breast cancer (MBC). These fusions drive estrogen-independence and eliminate binding sites for all approved ER-targeting agents, posing a significant clinical challenge. Non-invasive detection of *ESR1* fusions is limited by diverse gene partners and variable DNA breakpoints, which complicates assembling meaningfully large cohorts.

By integrating high quality RNA-sequencing (RNA-seq), a sensitive, methylation-informed circulating tumor DNA (ctDNA assay), and robust clinical data, we set out to comprehensively characterize *ESR1* fusion-positive disease.

OBJECTIVES

- To define the clinical, genomic, and biological characteristics of *ESR1* fusion–positive HR+ MBC across multiple detection modalities.
- To determine the timing, prevalence, and patterns of *ESR1* fusion acquisition in the metastatic setting.
- To assess impact of treatment exposures and outcomes prior to and after *ESR1* fusion detection.
- To characterize transcriptional programs associated with *ESR1* fusion-driven disease.

METHODS

- Patients with HR+ MBC harboring *ESR1* fusions were retrospectively identified using whole-transcriptome RNA-seq on flash-frozen biopsies and a next-generation methylation-informed ctDNA assay (Guardant360).
- Comprehensive clinicogenomic and outcomes data were abstracted for all fusion-positive cases, including prior therapies, timing of fusion emergence, and responses to subsequent systemic treatments following fusion emergence
- For tumors with RNA-seq data, PAM50 molecular subtype assignment and GSEA were performed to define transcriptional programs associated with *ESR1* fusion-positive disease.

SUPPORT

Saverin BrCa Research Fund

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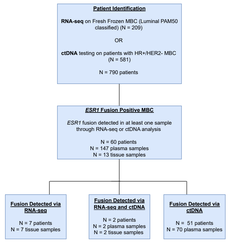
BCRF BREAST CANCER RESEARCH FOUNDATION

AACR American Association for Cancer Research

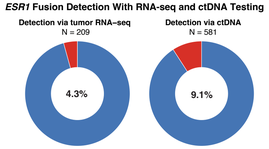
GUARDANT

RESULTS

ESR1 Fusion Discovery



Identification of ESR1 Fusion–Positive Cases. REMARK diagram summarizing identification of 60 patients with *ESR1* fusion-positive HR+ MBC from 790 profiled using tumor RNA-seq, ctDNA testing, or both.



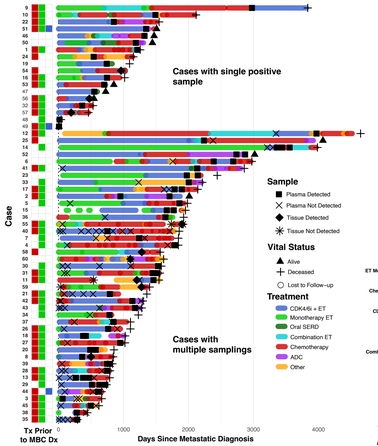
Prevalence of ESR1 Fusions Across Detection Modalities. *ESR1* fusions were identified in 4.3% of tumors profiled by RNA-seq and 9.1% of patients tested by ctDNA.

Clinical Characteristics

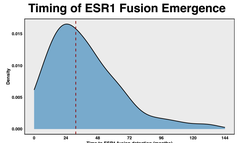
	ESR1 Fusion Detected Cohort
Number of patients	60
Median age at metastatic diagnosis (met dx)	57 (30-74)
Stage at Initial dx (%)	
0 (DCIS)	3 (5%)
1	12 (20%)
2	19 (32%)
3	15 (25%)
4	10 (17%)
Unknown	1 (1%)
Race (%)	
Caucasian	52 (87%)
Hispanic or Latino	3 (5%)
African American	3 (5%)
Asian or Pacific Islander	2 (3%)
Histology at initial diagnosis (%)	
DCIS	3 (5%)
Invasive Ductal (IDC)	46 (77%)
Invasive Lobular (ILC)	4 (7%)
Micropapilary	1 (1%)
Mixed (IDC and ILC)	3 (5%)
Unknown	3 (5%)
Histologic grade at initial diagnosis (%)	
II	27 (45%)
III	25 (42%)
Unknown	8 (13%)
Vital Status (Alive, %)	10 (17%)
Gender (%)	
Female	59 (98%)
Male	1 (2%)
HER2 receptor status at met dx (%)	
Negative	60 (100%)
Prior lines of therapy before first fusion detected, median (%)	5 (1-15)
Prior ADC	7 (12%)
Prior Chemo	52 (87%)
Prior CDK 4/6 Inhibitor	53 (88%)
Prior ET (any)	60 (100%)
Prior lines of therapy before first fusion detected by sample type, median	
Plasma	4 (1-11)
Tissue	4 (2-13)
Median number of samples	2 (1-11)
Median overall survival (days)	283 (4-1103)

Cohort Characteristics of ESR1 Fusion-Positive HR+ MBC. Subset of clinical, pathological, and treatment features of the 60 patients with *ESR1* fusion-positive MBC—including stage, histology, prior therapies, sample counts, and overall survival.

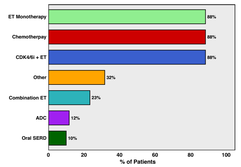
ESR1 Fusion Emergence In Late Line Setting



Longitudinal Treatment Course and Sampling in ESR1 Fusion–Positive MBC. Swimmer plot showing systemic therapies, plasma and tissue fusion testing results, and survival outcomes— illustrating extensive prior endocrine exposure and late emergence of *ESR1* fusions during metastatic disease course

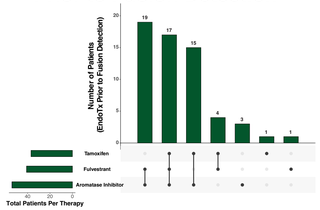


Timing of ESR1 Fusion Emergence in HR+ Metastatic Breast Cancer. Density plot showing that *ESR1* fusions are typically detected late in the metastatic course, with a **median time to fusion detection of 34.1 months** after MBC diagnosis.



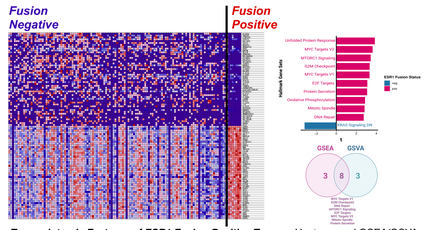
Therapies Administered Prior to ESR1 Fusion Detection. Bar plot showing the percentage of ESR1 fusion-positive patients who received each major class of systemic therapy before fusion emergence, including endocrine therapies, CDK4/6 inhibitors, chemotherapy, and antibody–drug conjugates.

Endocrine Therapy Backbones Prior to Fusion Detection



Endocrine Therapies Received Prior to ESR1 Fusion Detection. Upset plot summarizing pre-fusion exposure to endocrine agents (aromatase inhibitors, tamoxifen, and fulvestrant), including the number of patients treated with each agent and the intersections of therapies received.

Hyperproliferative Transcriptomic Program in *ESR1* Fusion MBC



Transcriptomic Features of ESR1 Fusion-Positive Tumors. Heatmap and GSEA/GSVA pathway analyses show Luminal B classified *ESR1* fusion-positive tumors cluster distinctly vs other Luminal B MBCs with enrichment for proliferation-associated programs (including MYC, E2F, G2M checkpoint, mitotic spindle, and MTORC1 signaling).

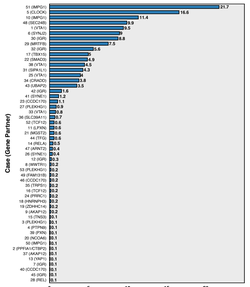
Descriptive Treatment Patterns and Outcomes Following Fusion Detection

Regimen Class	Total Patients	Total Regimen Treatments	Median Lines Prior to Fusion Detection	Median Time on Treatment (ToT)	Median Time to Next Treatment (TTNT)
Chemotherapy	37	64	4.5	2.4	3.2
ADC	12	15	4	3.4	3.7
CDK4/6i + ET	11	15	4	2.1	2.5
Combination ET	5	5	3	1.5	1.8
Oral SERD	3	3	2	1.1	5.2*
Monotherapy ET	2	2	3	1.4	4.3*

Therapies Received After ESR1 Fusion Detection. Descriptive summary of regimen classes administered post-fusion, including total patients treated, total treatment courses given, and corresponding median prior lines of therapy classes prior to fusion, and time-on-therapy durations (in months).

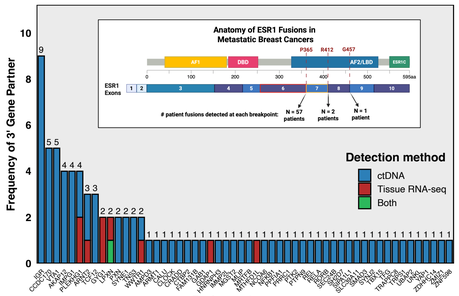
*TTNT in Oral SERD and Monotherapy ET groups inflated due to censoring in one patient who was lost to follow up with no subsequent therapy annotated and one patient who transitioned to hospice for a period of time before death.

Allele Fractions of *ESR1* Fusion Positive Cases



Distribution of ESR1 Fusion Variant Allele Fractions. Bar plot showing range of *ESR1* fusion VAFs across individual cases (max VAF / case), demonstrating substantial variability in detectable allele fraction (n = 53 cases detected via ctDNA).

Anatomy and 3' Partners of *ESR1* fusions



ESR1 Fusion Breakpoints and 3' Partner Diversity. Schematic of *ESR1* exons highlighting recurrent breakpoint positions with bar plot of 3' fusion partner frequencies—demonstrating a few recurrent partners and a long tail of rare, n-of-1 events. All breakpoints were identified within the ligand binding domain (LBD) of *ESR1*.

Co-Alterations in *ESR1* Fusion Positive Cases



Genomic Landscape of ESR1 Fusion–Positive MBC. Oncoprint showing co-occurring somatic alterations (OncoKB clinically actionable and breast-cancer related genes) across *ESR1* fusion-positive cases.

CONCLUSIONS

- *ESR1* fusions can be a clinically meaningful, yet historically difficult-to-detect mechanism of acquired endocrine therapy resistance. Here, using multiple modalities on later line MBC samples, we identify *ESR1* fusions in 4–10% of HR+/HER2– samples; with highest yield from next-generation ctDNA profiling.
- Fusion allele fractions were lower and more variable than canonical drivers, consistent with late, subclonal emergence. Many carried VAFs <0.5% suggesting low-level abundance and unclear clinical significance.
- *ESR1* fusions frequently co-occurred with other *ESR1* alterations including amplifications and point mutations, supporting a multimodal, on-target resistance architecture.
- Fusion partners were highly heterogeneous, with a few recurrent partners and a long tail of n-of-1 events.
- All patients received extensive endocrine therapy before fusion detection—including many progressing on CDK4/6 inhibition—indicating emergence after cumulative exposure to multiple lines of combination ET.
- Transcriptomic profiling of samples with RNA-level evidence of *ESR1* fusions were uniformly Luminal B and enriched for proliferative programs (G2M, E2F, MYC, mTOR1C, mitotic spindle).
- Clinically, post-fusion outcomes were poor, with limited ToT for both combination ET and oral SERDs—although the cohort was generally heavily pre-treated at time of fusion detection.
- As next-generation SERDs more effectively suppress *ESR1* point mutations, a subset of activating *ESR1* fusions may emerge as a more dominant mechanism of endocrine escape—highlighting a need to build larger cohorts to better understand their unique biology and clinical behavior.

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