# Convergent genomic evolution from early to lethal HR+/HER2- metastatic breast cancer

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## INTRODUCTION

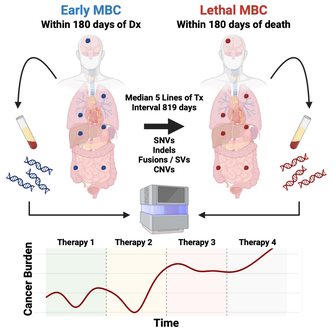



*Fig 1. Study Design.* Serial ctDNA profiling from patients with HR+/HER2– MBC from paired Early and Lethal MBC timepoints to characterize genomic evolution driving terminal progression.

Despite therapeutic advances, hormone receptor-positive/HER2-negative (HR+/HER2-) metastatic breast cancer (MBC) remains incurable and is a leading cause of cancer mortality. **Genomic characterization of MBC has historically relied on samples collected early in the disease course**; however, a lethal phenotype—characterized by accelerated disease progression and profound therapeutic resistance—is clinically well-recognized. The biological mechanisms driving this terminal transition remain largely unknown.

**Leveraging a carefully selected cohort of patients with highly sensitive circulating tumor DNA (ctDNA) profiling across this disease continuum, we aimed to characterize genomic alterations driving lethal HR+/HER2- MBC.**

## OBJECTIVES

- To profile longitudinal genomic evolution across the early-to-lethal disease continuum in HR+/HER2- MBC
- To identify resistance-associated alterations and pathways that converge across patients despite therapeutic and clinical heterogeneity
- To define the degree of mutational heterogeneity and genomic instability that shape terminal therapeutic resistance

## METHODS

- Serial plasma samples, linked to detailed clinical annotation, were collected from patients with MBC enrolled in the EMBRACE cohort study. We included patients with HR+/HER2- subtype who had paired plasma collection within 180 days of MBC diagnosis (**Early MBC**), within 180 days of death (**Lethal MBC**), with at least 365 days between early and lethal samplings.
- Plasma-derived ctDNA was analyzed using a >700-gene liquid biopsy assay (Guardant360) that assesses single nucleotide variants (SNVs), indels, large genomic rearrangements (LGR), fusions, copy number alterations (CNAs), and a methylation-derived tumor fraction (TF) score.
- To limit variation due to tumor shed, comparison of somatic alterations were restricted to patients with TF > 1% at both time points. Significance of enrichment was determined using paired McNemar tests with FDR corrections.

**SUPPORT**

Saverin BrCa Research Fund

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BCRF BREAST CANCER RESEARCH FOUNDATION

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## RESULTS

### Lethal MBC Cohort

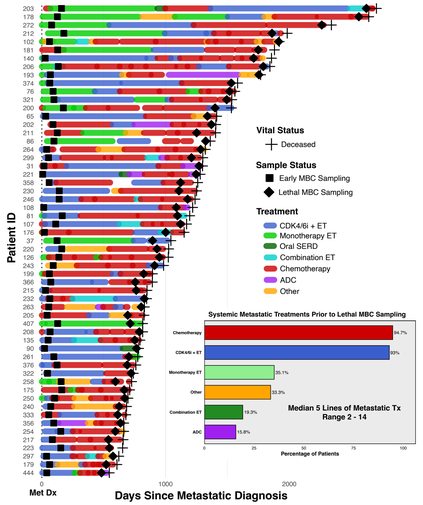
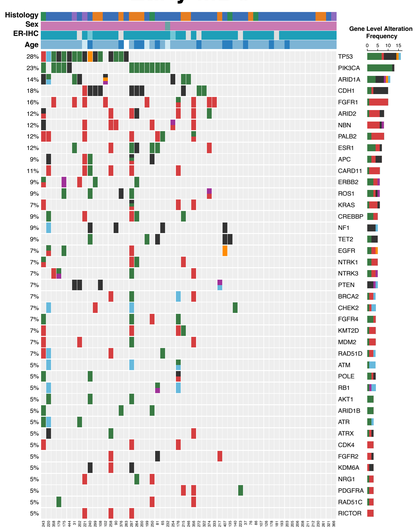



*Fig 2. Lethal MBC cohort overview.* Treatments for each patient (n = 57) showing therapies received, sampling timepoints for Early and Lethal MBC, and overall survival from metastatic diagnosis. Bar plot summarizes the proportion of metastatic treatments administered prior to the Lethal MBC sampling. Median 5 lines of metastatic therapy (range 2–14), median interval of 819 days between Early and Lethal sample (Range 393-2464), MBC Dx to Early MBC sampling median 85 days (Range 7-178), Lethal MBC sampling to Death median 71 days (Range 5-153)

### Early MBC




### Lethal MBC




*Fig 3. Clinically actionable and biologically relevant alterations expand in lethal MBC.* Genomic profiles of early and lethal HR+/HER2– MBC demonstrate increased frequency and diversity of top 40 alterations (*OncoKB* Level 1–4 clinically actionable genes, other breast cancer drivers) including *TP53*, *ESR1*, and DNA repair genes and cell cycle regulators. Basic clinicopathological features highlighted above.

### An untenable heterogeneity in Lethal MBC

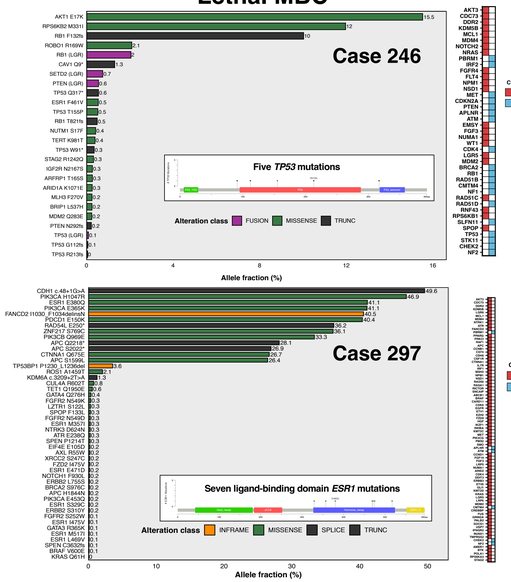



*Fig. 7. Genomic heterogeneity in lethal HR+/HER2– MBC.* Individual somatic alterations from lethal ctDNA samples of two representative cases (246 and 297) are shown with allele fractions and alteration classes. Right panels display sample copy-number events. Multiple independent *TP53* truncations (Case 246) and clustered *ESR1* ligand-binding–domain mutations (Case 297) highlighted to show extent of heterogeneity.

### Tumor fraction from Early to Lethal MBC

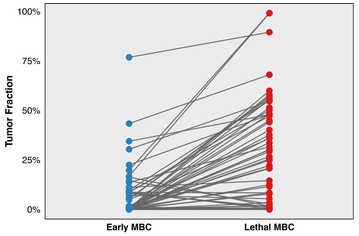



*Fig 4. Tumor fraction increases in lethal disease.* ctDNA tumor fraction significantly increased from Early to Lethal MBC (paired Wilcoxon signed-rank test, $p < 0.001$) reflecting rising tumor burden coinciding with ineffective therapies towards the end of life.

### Somatic alterations enriched in Lethal MBC

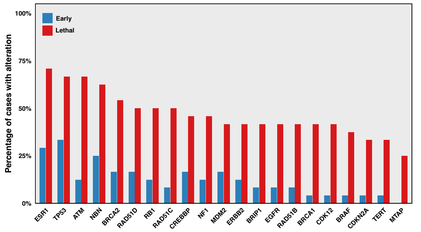



*Fig 5. Paired enrichment analysis of 24 cases with tumor fraction >1% at both draws.* Bar plot showing gene-level alteration prevalence in Early (blue) and Lethal (red) MBC samples. Genes shown reached FDR-adjusted significance for enrichment in Lethal MBC vs Early MBC (McNemar test, $p\text{-}adj < 0.25$).

### Variant types among enriched Lethal MBC alterations

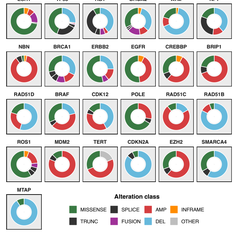



*Fig 6. Distribution of alteration classes among significantly enriched genes in lethal disease.* Each donut represents the composition of variant types observed per enriched somatic gene, highlighting extensive CNVs.

## CONCLUSIONS

- Longitudinal ctDNA profiling demonstrates substantial genomic evolution from Early to Lethal HR+/HER2– MBC.
- Resistance-driving alterations—particularly affecting DNA repair, cell-cycle control, and endocrine or growth-factor signaling—become markedly enriched in terminal disease, reflecting convergent pressure toward shared resistance pathways despite considerable interpatient heterogeneity.
- Copy-number variation emerge as a dominant form of acquired evolution in Lethal MBC, suggesting progressive structural genomic instability near end of life.
- Late-line alterations are often absent in early metastatic disease, showcasing the value of serial profiling and a need for methods that identify clinically meaningful changes in clonal architecture over time.
- The breadth of resistance mechanisms and heterogeneity suggest traditional therapies are unlikely to achieve durable control in late line settings—highlighting a critical need for (1) eliminating cancers before the emergence of this untenable heterogeneity and (2) developing more innovative therapies that remain effective despite it.

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