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ESR1 mutations drive resistance to *CDK4/6* inhibitors in ER+ Breast Cancer

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INTRODUCTION

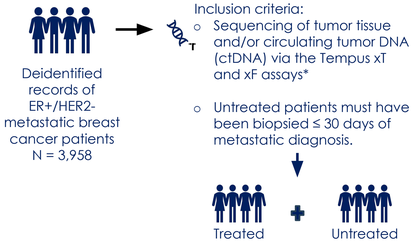
- Alterations in *ESR1* are major drivers of resistance to antiestrogen therapy in ER+ breast cancer.
- However, it remains unclear whether *ESR1* mutations also drive resistance to *CDK4/6* inhibitors (CDK4/6i).

KEY TAKEAWAYS

- *ESR1* alterations are **enriched following treatment with CDK4/6i** in a cohort of 3,958 patients with ER+/HER2- metastatic breast cancer.
- ESR1 alterations **directly promote resistance to CDK4/6i alone or CDK4/6i + antiestrogens** in ER+ breast cancer cells in vitro and in vivo.
- *ESR1* alterations **induce basal-like gene expression signatures** which may be linked to CDK4/6i resistance.

METHODS

Patient data



*Tempus xT is a targeted, tumor/normal-matched DNA panel that detects single-nucleotide variants (SNVs), insertions and/or deletions (indels), and copy number variants (CNVs) in 648 genes, as well as chromosomal rearrangements in 22 genes with high sensitivity and specificity. Tempus xF is a targeted liquid biopsy DNA panel that identifies SNVs and indels in 105 genes, CNVs in six genes, and chromosomal rearrangements in seven genes.

Breast Cancer Cell Culture

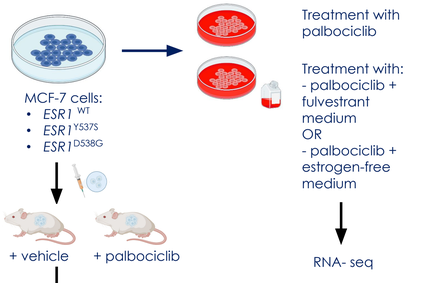


Table 1. Patient Demographics

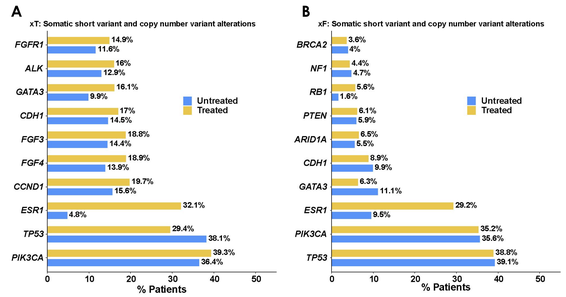
	Overall N = 3,958[1]	Untreated N = 1,003[1]	Treated N = 2,955[1]	p-value[2]
Age at Diagnosis	57 (47, 65)	58 (48, 67)	56 (46, 64)	<0.001
Unknown	311	72	239	
Gender				0.7
Female	3,905 (99%)	991 (99%)	2,914 (99%)	
Male	52 (1.3%)	12 (1.2%)	40 (1.4%)	
Unknown	1	0	1	
Race				0.7
White	2,012 (77%)	443 (76%)	1,569 (77%)	
Black or African American	280 (11%)	71 (12%)	209 (10%)	
Other Race	196 (7.5%)	41 (7.0%)	155 (7.6%)	
Asian	106 (4.1%)	25 (4.3%)	81 (4.0%)	
American Indian or Alaska Native	13 (0.5%)	3 (0.5%)	10 (0.5%)	
Native Hawaiian or Other Pacific Islander	6 (0.2%)	0 (0%)	6 (0.3%)	
Unknown	1,345	420	925	
Ethnicity				0.053
Not Hispanic or Latino	1,390 (87%)	330 (90%)	1,060 (86%)	
Hispanic or Latino	209 (13%)	37 (10%)	172 (14%)	
Unknown	2,359	636	1,723	
Hormone Status				<0.001
ER+, PR+, HER2-	3,206 (81%)	708 (71%)	2,498 (85%)	
ER+, PR-, HER2-	647 (16%)	271 (27%)	376 (13%)	
ER+, HER2-	105 (2.7%)	24 (2.4%)	81 (2.7%)	
Months from Metastatic Disease to Sample Collection	19 (1, 42)	0 (0, 1)	29 (15, 52)	<0.001
Assay				<0.001
xF	2,138 (54%)	253 (25%)	1,885 (64%)	
xT	1,820 (46%)	750 (75%)	1,070 (36%)	
Treated with abemaciclib	707 (18%)	0 (0%)	707 (24%)	<0.001
Treated with ribociclib	416 (11%)	0 (0%)	416 (14%)	<0.001
Treated with palbociclib	2,272 (57%)	0 (0%)	2,272 (77%)	<0.001

[1] Median (IQR); n (%)

[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

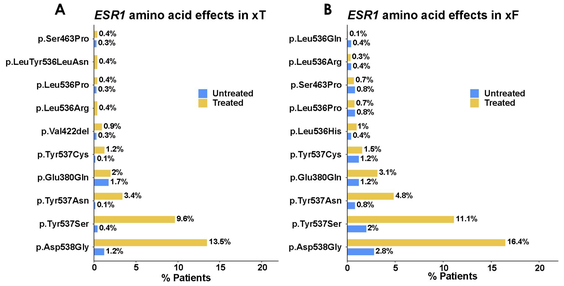
RESULTS

Figure 1. Somatic Mutations



Ten most frequent genes with pathogenic/likely pathogenic (P/LP) somatic short variant or copy number alterations in **(A)** xT and **(B)** xF.

Figure 2. *ESR1* Amino Acid Effects



Ten most frequently occurring ESR1 amino acid effects in **(A)** xT and **(B)** xF

Figure 1. *ESR1* Alterations Promote Resistance to Palbociclib ± Antiestrogens in vitro and in vivo

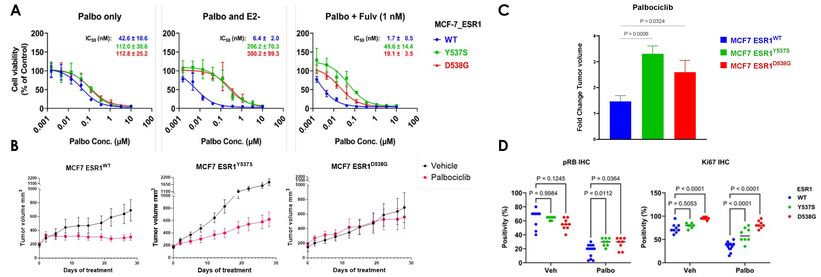


Figure 3. (A) MCF7_ESR1^{WT}, MCF7_ESR1^{Y537S} and MCF7_ESR1^{D538G} cells were treated with 9 concentration of palbociclib ± estrogen deprivation (E2-) or 1 nM fulvestrant. After 6 days of treatment, cell viability was measured by the CyQUANT assay. **(B)** Tumor growth of MCF7_ESR1^{WT} (n=12), MCF7_ESR1^{Y537S} (n=8), or MCF7_ESR1^{D538G} (n=8) xenografts in ovariectomized nude mice. Mice were treated with vehicle or 50mg/kg palbociclib p.o. for 4 weeks. **(C)** Comparison of the fold change in tumor volume at the end of palbociclib treatment of tumors described in **(B)**. **(D)** Quantification of IHC staining of the tumors in (**B**). Data represent mean ± SD; statistical analysis was conducted using One-way ANOVA with a Dunnett's post-hoc test.

Figure 4. *ESR1*-mutant Cells are Associated with Upregulation of Cell Cycle-related and Basal Gene Signatures

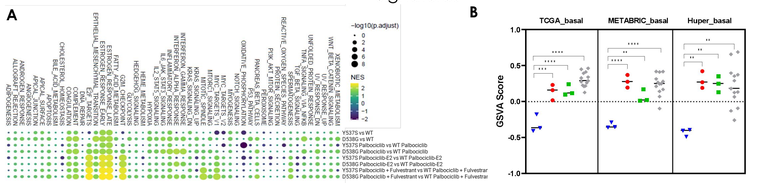


Figure 4. (A) RNA-seq based gene set enrichment analysis (GSEA) of Hallmark pathways significantly up- or down-regulated in ESR1-mutant vs. WT cells after 48h treatment with vehicle (DMSO) or 200nM palbociclib ± estrogen deprivation or 10 nM fulvestrant. **(B)** Basal gene signature enrichment analysis was performed on RNA-seq data from vehicle-treated cells from (**A**). Previously published basal gene signature gene lists were used to calculate GSVA scores.[1] Statistical analysis was conducted using One-way ANOVA with a Dunnett's multiple comparison test.

1. Li, Z., McGinn, O., Wu, Y., Bahreini, A., Priedigkeit, N.M., Ding, K., Onkar, S., Lampenfeld, C., Sartorius, C.A., Miller, L., et al. (2022). ESR1 mutant breast cancers show elevated basal cytokeratins and immune activation. Nat Commun 13, 2011. 10.1038/s41467-022-29498-9

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