Supplementary Material

Bridging Biomarker Testing to Biomarker-Driven Targeted Therapy: Real-World *ESR1* Mutation Testing Patterns and Prevalence in ER+, HER2- Metastatic Breast Cancer

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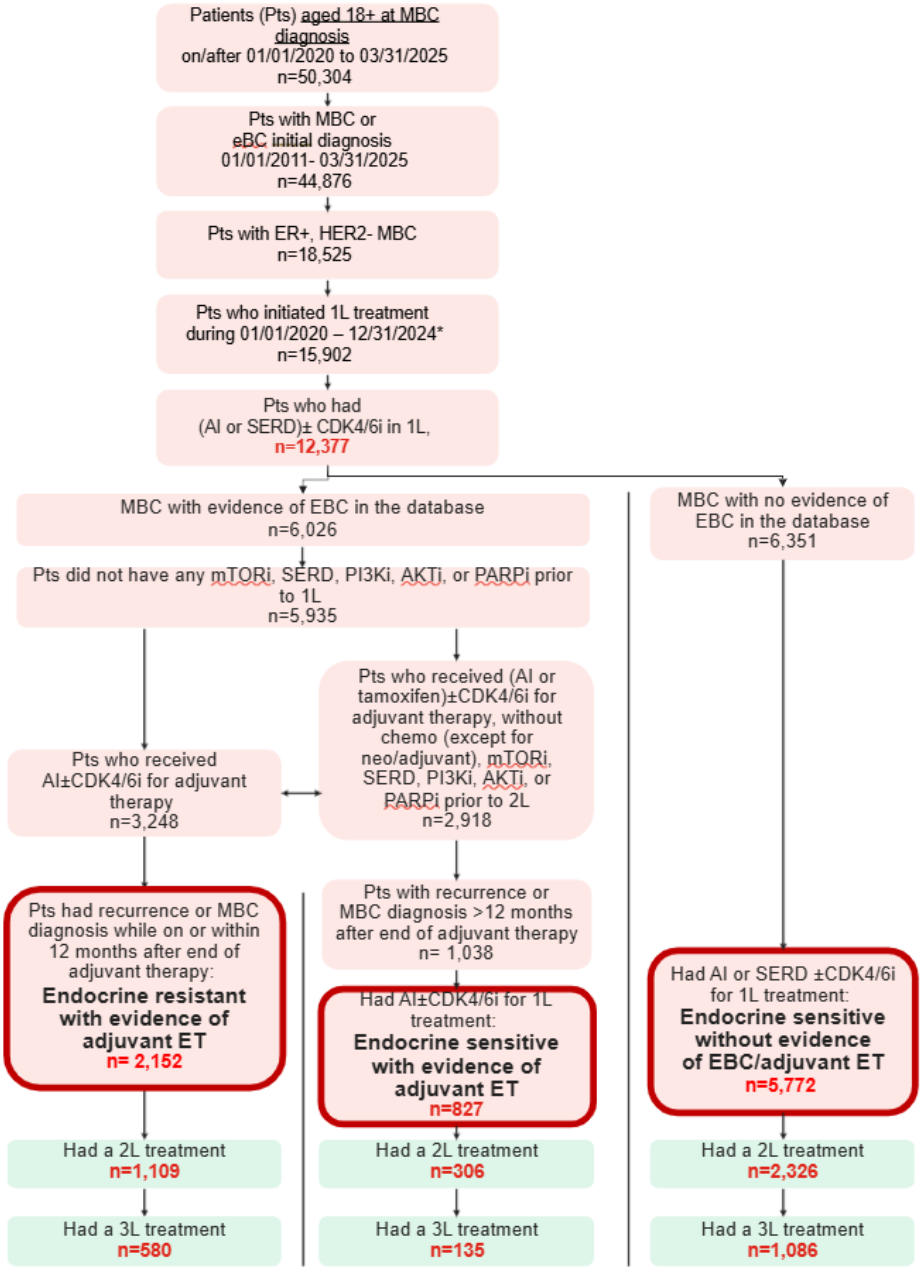
Supplemental Table 1. *ESR1* testing and *ESR1* mutation positive (*ESR1*m+) rates by time windows and disease subgroups

	Endocrine resistant MBC	1L endocrine sensitive MBC	Endocrine sensitive/de novo MBC
	n (%)	n (%)	n (%)
Total number of patients	**n=2152**	**N=827**	**n=5772**
Ever received at least 1 *ESR1* test during the study period	1316 (61.2)	418 (50.5)	2486 (43.1)
***ESR1*m+[2]**	**375 (28.5)**	**68 (16.3)**	**484 (19.5)**
Number of *ESR1* tests received			
1	817 (62.1)	261 (62.4)	1504 (60.5)
2	306 (23.3)	101 (24.2)	676 (27.2)
3	121 (9.2)	37 (8.9)	195 (7.8)
4+	72 (5.5)	19 (4.6)	111 (4.5)
Mean (SD) number of *ESR1* tests per patient	1.6 (1.0)	1.6 (1.0)	1.6 (1.0)
Total number of patients who initiated 1L	**n=2152**	**N=827**	**n=5772**
At 1L initiation (90 days prior and 28 days after 1L start date)[1]	778 (36.2)	248 (30.0)	1131(19.6)
***ESR1*m+[2]**	**192 (24.7)**	**17 (6.9)**	**24 (2.1)**
Received AI +/- CDK4/6 inhibitor in 1L[3]	118 (25.9)	197 (29.1)	1024 (19.7)
***ESR1*m+[2]**	**12 (10.2)**	**12 (6.1)**	**19 (1.9)**
Received SERD+/- CDK4/6 inhibitor in 1L[4]	531 (39.6)	n/a	n/a
***ESR1*m+[2]**	**151 (28.4)**	n/a	n/a
Within 1L (29 days after 1L start and 91 days prior to 2L start)[1]	232 (15.2)	83 (12.7)	669 (15.1)
***ESR1*m+[2]**	**53 (22.8)**	**7 (8.4)**	**69 (10.3)**
Total number of patients who initiated 2L	**n=1109**	**N=306**	**n=2326**
At 2L initiation (90 days prior and 28 days after 2L start date)[1]	360 (32.5)	104 (34.0)	668 (28.7)
***ESR1*m+[2]**	**122 (33.9)**	**29 (27.9)**	**216 (32.3)**
Received AI +/- CDK4/6 inhibitor in 1L[3]	74 (30.0)	89 (35.3)	593 (28.8)
***ESR1*m+[2]**	**16 (21.6)**	**27 (30.3)**	**199 (33.6)**
Received SERD +/- CDK4/6 inhibitor in 1L[4]	222 (33.4)	n/a	n/a
***ESR1*m+[2]**	**88 (39.6)**	n/a	n/a
Within 2L (29 days after 2L start and 91 days prior to 3L start)[1]	53 (8.9)	23 (11.7)	164 (11.5)
***ESR1*m+[2]**	**20 (37.7)**	**8 (34.8)**	**36 (22.0)**
Patients with testing at 1L initiation who initiated 2L	**n=385**	**N=92**	**n=462**
Patients with re-testing at 2L	146 (37.9)	44 (47.8)	190 (41.1)
Total number of patients who initiated 3L	**n=580**	**N=135**	**n=1086**
At 3L initiation (90 days prior and 28 days after 3L start date)[1]	141 (24.3)	34 (25.2)	313 (28.8)
***ESR1*m+[2]**	**53 (37.6)**	**13 (38.2)**	**132 (42.2)**
Received AI +/- CDK4/6 inhibitor in 1L[3]	29 (24.0)	34 (28.3)	273 (28.2)
***ESR1*m+[2]**	**13 (44.8)**	**13 (38.2)**	**116 (42.5)**
Received SERD +/- CDK4/6 inhibitor in 1L[4]	83 (23.5)	n/a	n/a
***ESR1*m+[2]**	**29 (34.9)**	n/a	n/a
Within 3L (29 days after 3L start and 91 days prior to 4L start)[1]	20 (6.5)	7 (10.0)	53 (9.2)
***ESR1*m+[2]**	**2 (10.0)**	**1 (14.3)**	**8 (15.1)**

[1]Testing periods are not mutually exclusive such that, at line initiation and within lines of therapy have overlapping for window definition. See Supplemental Figure 1.
[2] Positive rates = (n positive/n tested) x 100%
[3]Sensitiviy analysis among patients with ER+, HER2− MBC who received an AI+/- CDK4/6 inhibitor in 1L. Endocrine sensitive/de novo patients were excluded from the sensitivity analyses if they only had a CDK4/6i in their 1L treatment.
[4]Sensitiviy analysis among patients with ER+, HER2− MBC who received a SERD +/- CDK4/6 inhibitor in 1L. No patients with secondary endocrine resistant MBC had a SERD 1L. To be in the de novo MBC group, patients could not have had a SERD in 1L.

1L, first-line; 2L, second-line; 3L, third-line; 4L, fourth-line; AI, aromatase inhibitor; CDK, cyclin-dependent kinase; ER, Estrogen receptor; *ESR1m*, estrogen receptor 1 mutation; HER2− , human epidermal growth factor receptor 2 negative; MBC, metastatic breast cancer; SERD, selective estrogen receptor degrader.

Supplemental Figure 1. Cohort attrition for patients with ER+, HER2− MBC from the Flatiron Health Research Database

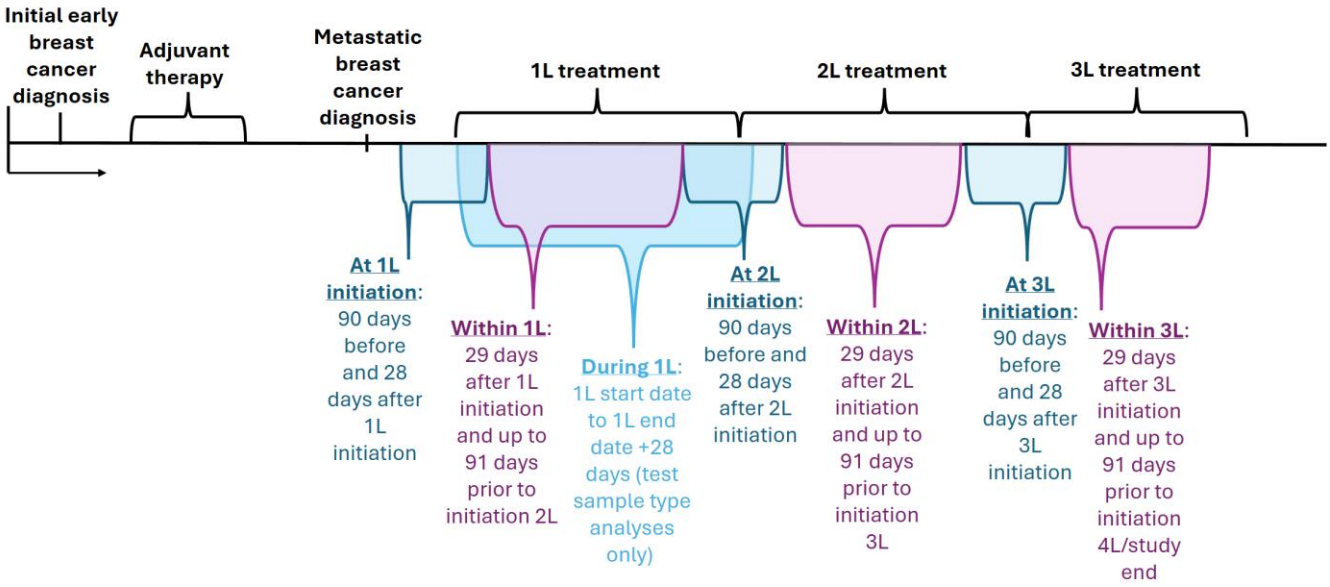


*3 months prior to the data cut #Some patients could fall into either category at this node.

1L, first-line therapy; 2L, second-line therapy; 3L, third-line therapy; AI, aromatase inhibitor; AKTi, AKT (protein kinase B) inhibitor; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; EBC, early breast cancer; ER, estrogen receptor-positive; *ESR1*, estrogen receptor 1; ET, endocrine therapy; HER2−, human epidermal growth factor receptor 2-negative; MBC, metastatic breast cancer; mTORi, inhibitor; Pts, patients; PARPi, poly(ADP-ribose) polymerase inhibitor; PI3Ki, phosphatidylinositol 3-kinase inhibitor; SERD, selective estrogen receptor degraders.

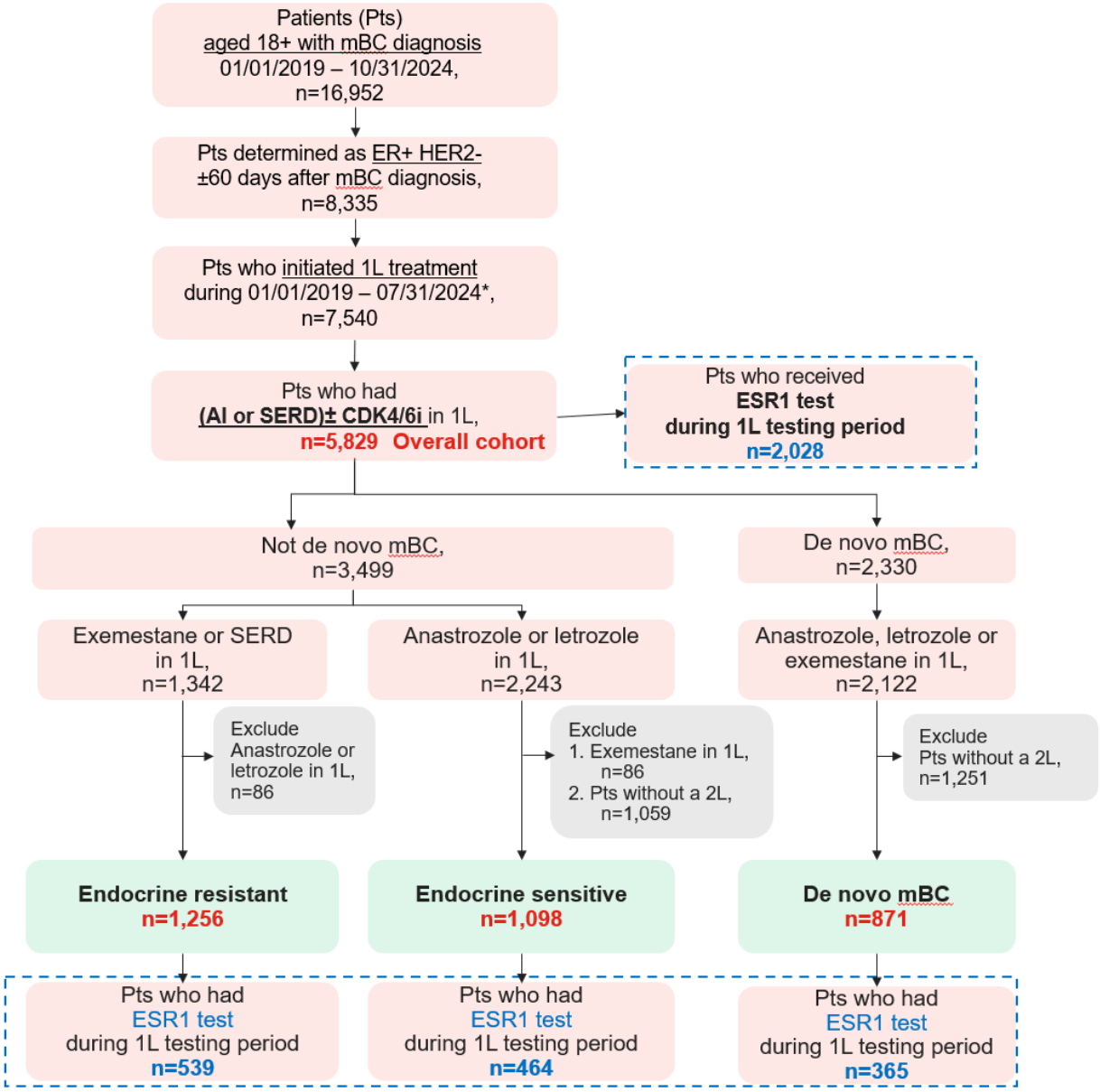
AI: anastrozole, letrozole, exemestane; SERD: fulvestrant, elacestrant; PI3Ki: alpelisib; mTORi: everolimus; AKTi: capivasertib; PARPi: olaparib, talazoparib; CDK4/6i: abemaciclib, palbociclib, ribociclib; HER2+ treatment: trastuzumab, pertuzumab, lapatinib, neratinib, tucatinib.

Supplemental Figure 2. Timeline of testing windows for patients with ER+, HER2− MBC



1L, first-line therapy; 2L, second-line therapy; 3L, third-line therapy; ER, estrogen receptor-positive; HER2−, human epidermal growth factor receptor 2-negative; MBC, metastatic breast cancer.

Supplemental Figure 3. Cohort attrition for patients with ER+, HER2− metastatic breast cancer from the Flatiron Health Research Breast Enhanced Data Mart dataset



*3 months prior to the data cut 10/31/2024

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