ESR1 Biomarker Testing in Metastatic Breast Cancer

Lilly

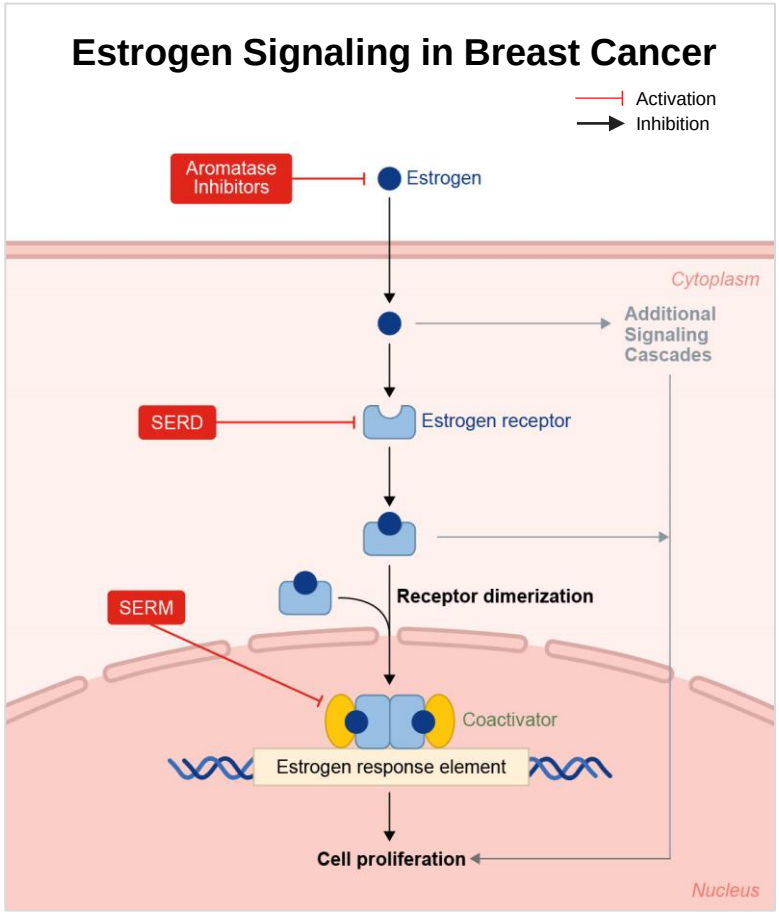
Learning Objectives

By the end of this presentation, you should:

- Understand the role *ESR1* mutations play in mediating resistance to estrogen therapy in ER+, HER2- advanced breast cancer
- Be familiar with guideline recommendations on testing for *ESR1* mutations in ER+, HER2- advanced breast cancer
- Recognize appropriate testing methodologies for identifying *ESR1* mutations

ER+ = estrogen receptor-positive; HER2- = human epidermal growth factor receptor 2-negative.

Targeting the Estrogen Pathway in ER+, HER2- mBC[1]

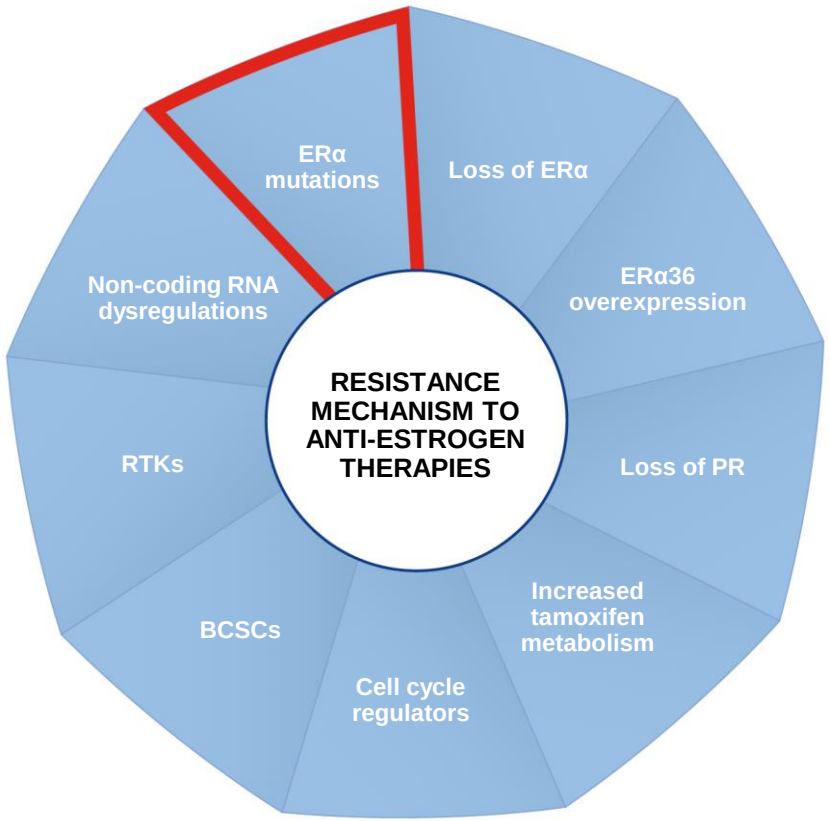


- Breast cancer is the most common cause of cancer mortality in women,[1] with approximately 70% of cases classified as ER+[2]
- In these tumors, signaling through the ER controls multiple proliferation-promoting and anti-apoptotic pathways[2-4]
- ET remains the backbone therapy and is the 1L standard for ER+, HER2- mBC[5]
- The 3 main classes of approved ER-targeted therapies, which lead to inhibition of proliferation and cell survival, are AIs, SERMs, and SERDs[2,6,7]

AI = aromatase inhibitor; ET = endocrine therapy; SERD = selective estrogen receptor degrader; SERM = selective estrogen receptor modifier; 1L = first line.

1. Misganaw M, et al. *PLoS One*. 2023;18(1):e0279656. 2. Le Romancer M, et al. *Endocr Rev.* 2011;32(5):597-622. 3. Shanle EK, Xu W. *Adv Drug Deliv Rev*. 2010;62(13):1265-1276. 4. Williams MM, et al. *Cell Death Dis*. 2018;9(2):21.
5. Gradishar WJ, et al. *J Natl Compr Canc Netw*. 2023;21(6):594-608. 6. Chen YC, et al. *Expert Opin Investig Drugs*. 2022;31(6):515-529. 7. Patel HK, Bihani T. *Pharmacol Ther.* 2018;186:1-24.

Resistance to Estrogen Therapy in ER+, HER2-, mBC[1]

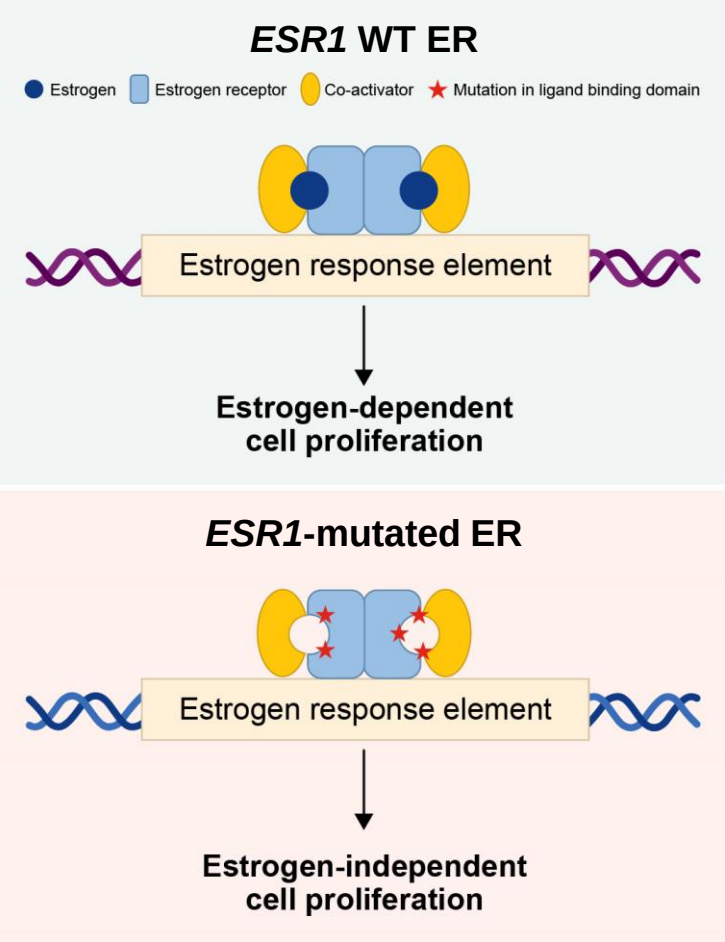


- Despite the utility of 1L ET ± CDK4/6i, patients may still experience progression or recurrence[2]
- After progression or recurrence on ET ± CDK4/6i, no consensus on an optimal treatment strategy in ER+, HER2- mBC has been reached[2-4]
- There are multiple mechanisms of resistance to ET ± CDK4/6i, including mutations in RTKs or the estrogen receptor ERα[1-3]

BCSC = breast cancer stem cell; i = inhibitor; PR = progesterone receptor; RTK = receptor tyrosine kinase.

1. Ozyurt R, Ozpolat B. *Cancers*. 2022;14(21):5206. 2. Gradishar WJ, et al. *J Natl Compr Canc Netw.* 2023;21(6):594-608. 3. Al-Qasem AJ, Alves CL, Ditzel HJ. *Cancers (Basel).* 2021;13(21):5397. 4. Zhou FH, et al. *Front Cell Dev Biol.* 2023;11:1148792.

ESR1 Mutations Mediate Resistance to ET in ER+, HER2- mBC



ESR1-mutated ER autoactivates, even in the absence of estrogen, leading to constitutive ER signaling

- ERα is encoded by **the _ESR1_ gene**
- Rarely mutated or altered in primary breast cancer, *ESR1* mutation is a mechanism of acquired resistance to ET
  - Approximately 20%-40% of patients who have received AI for mBC develop *ESR1* mutations
- *ESR1* mutations predict poor response to single-agent AI and can blunt response to combination therapies using AI
- Novel SERDs, SERMs and SERCAs may offer more effective treatment options

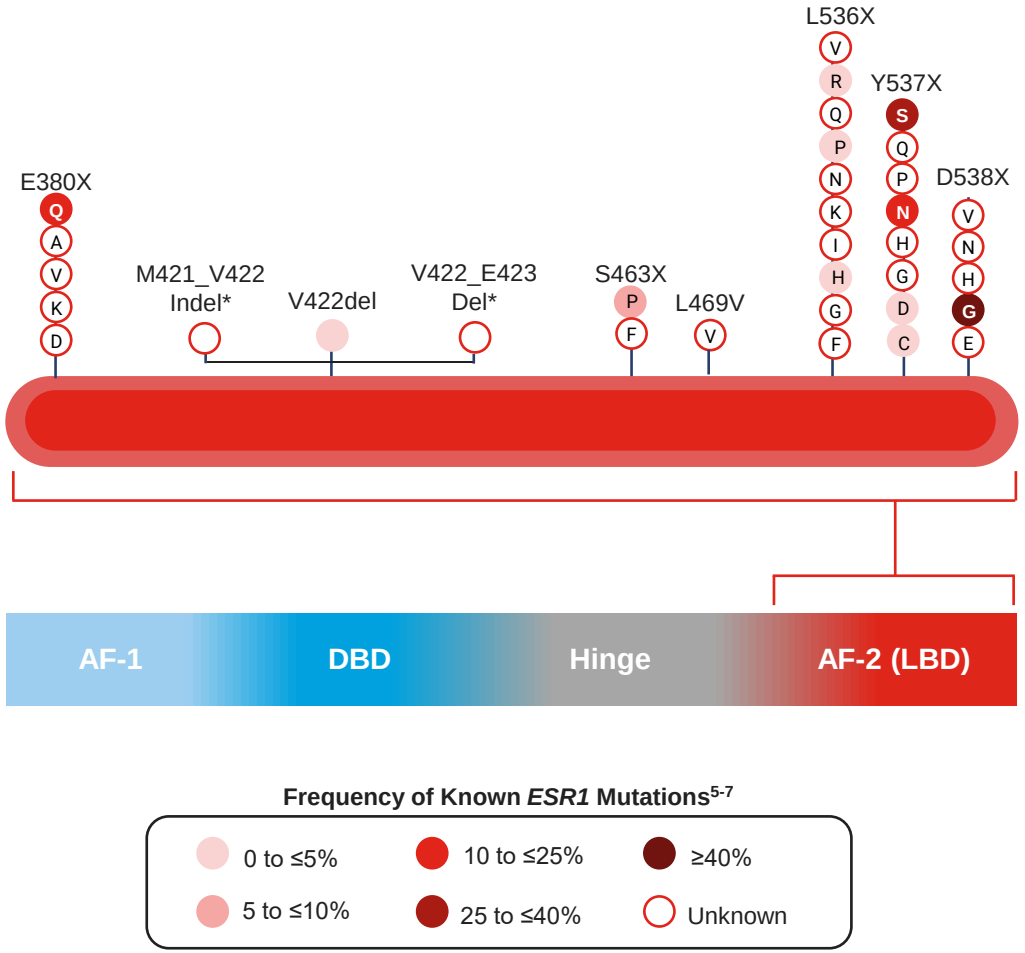
mBC = metastatic breast cancer; SERCA = selective estrogen receptor covalent antagonists; WT = wildtype.

Brett JO, et al. *Breast Cancer Res*. 2021;23(1):85.

Clinical Impact of *ESR1* Mutations

Most identified mutations are found in the LBD.[1]

- The most frequent mutations occur at Y537 and D538, but ≥51 have been identified[1]
  - Unique *ESR1* alterations may still occur
- More than half of patients with *ESR1*-mutated mBC have ≥1 *ESR1* mutation on different alleles[2,3]
  - Polyclonal activating mutations have poor prognosis relative to monoclonal[1]
- Mutations have different clinical implications for patients[1,2]
  - Compared to D538G, Y537S has greater resistance to traditional estrogen deprivation and some new SERMs and SERDs
  - D538G produces greater metastatic potential, especially to the liver[4]



*The frequency of this specific mutation is presumed to be unknown as it could not be verified in the current literature.
AF = activation function; DBD = DNA-binding domain; LBD = ligand-binding domain.

1. Dustin D, Gu G, Fuqua SAW. *Cancer*. 2019;125(21):3714-3728. 2. Bardia A, et al. *J Clin Oncol.* 2021;39(12):1360-1370. 3. Wang P, et al. *Clin Cancer Res*. 2016; 22(5):1130-1137. 4. Brett JO, et al. *Breast Cancer Res*. 2021;23(1):85. 5. Corné J, et al. *Clin Chim Acta*. 2023;545:117366. 6. Kingston B, et al. *Cancer Discov*. 2024;14(2):274-289. 7. Grinshpun A, et al. *Biochim Biophys Acta Rev Cancer*. 2023;1878(1):188830.

Co-Occurrence of *ESR1* and *PI3K/AKT* Pathway Mutations

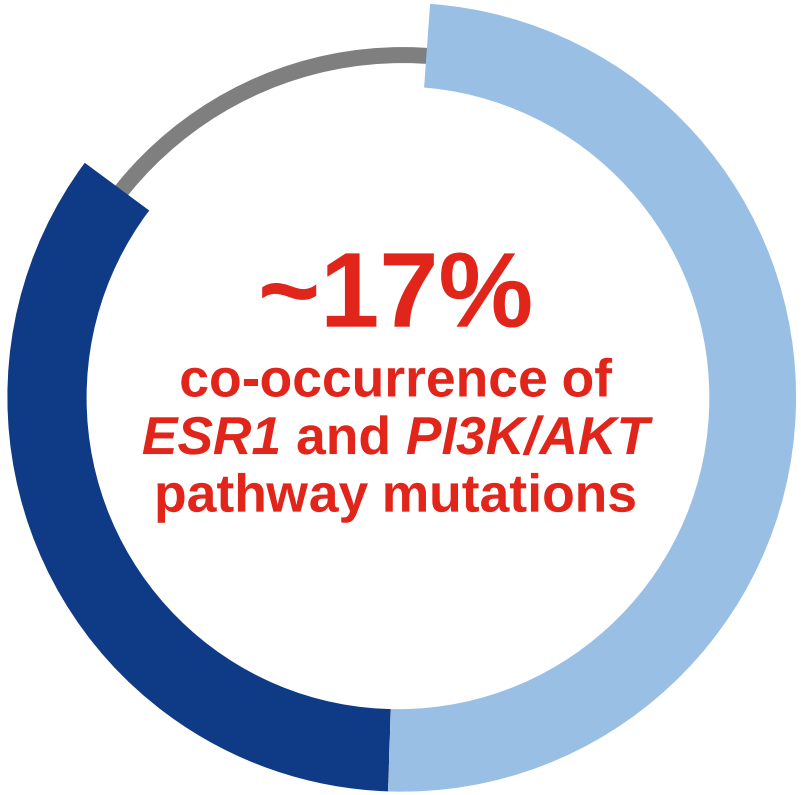
- **38%-55%** of patients with mBC have a genomic alteration in *PIK3CA*, *AKT1,* and *PTEN* at the time of 1L treatment
  - The frequency of *ESR1* mutations increases from <5% in *de novo* mBC to >40% after ET
- After 2 lines of therapy, the co-occurrence of *PI3K/AKT* and *ESR1* mutations have been found to be as high as **~17%**
- Mutations in *ESR1* and the *PI3K/AKT* pathway confer resistance to SOC ET and inform the use of appropriate therapy

Comprehensive genomic profiling at the time of diagnosis/recurrence and at the start of each line of therapy can identify actionable mutations and help optimize treatment selection

SOC = standard of care.

Bhave MA, et al. *Breast Cancer Res Treat*. 2024. 10.1007/s10549-024-07376-w. Epub ahead of print.

After ≥2 lines of therapy



~33%-38%
***ESR1* mutation**

~46%-55%
***PIK3CA* or *AKT* mutation or *PTEN* alteration**

The NCCN Guidelines® Recommend Comprehensive Genomic Profiling at Diagnosis of mBC

The NCCN Guidelines currently recommend testing the following biomarkers in mBC:

Biomarkers with FDA-approved therapies	Testing method
PIK3CA-activating mutations	NGS or PCR
AKT1-activating mutations	NGS or PCR
PTEN alterations	NGS or PCR
ESR1 mutations	NGS or PCR (blood)
NTRK fusion	NGS, FISH, or PCR
MSI-H/dMMR	NGS, IHC, or PCR
TMB-H	NGS
RET fusion	NGS

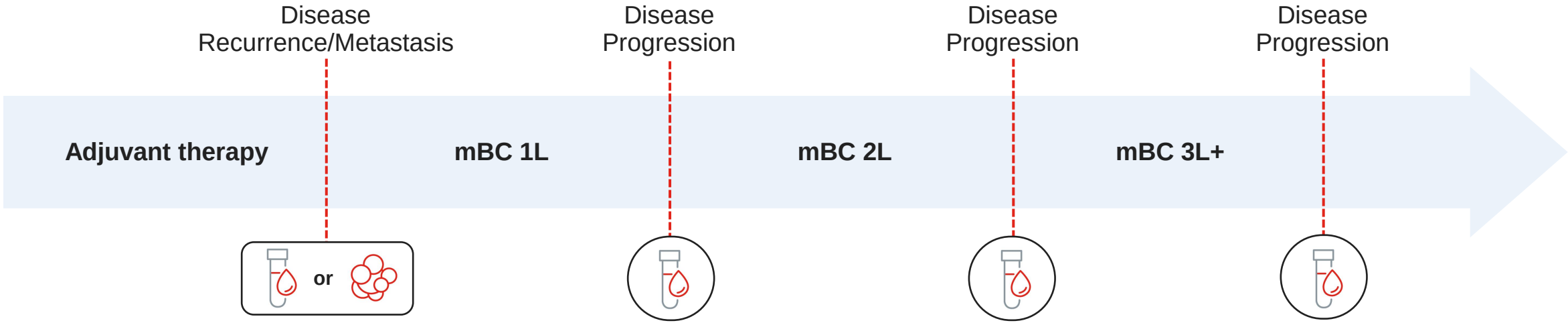
NGS can detect all guideline-recommended biomarkers

☐ Breast cancer–specific biomarker ☐ Pan-tumor biomarker

NCCN makes no warranties of any kind whatsoever regarding their content, use, or application and disclaims any responsibility for their application or use in any way.
dMMR = DNA mismatch repair; FDA = US Food and Drug Administration; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; MSI-H = high microsatellite instability; NCCN = National Comprehensive Cancer Network; NGS = next-generation sequencing; PCR = polymerase chain reaction; TMB-H = high tumor mutational burden.
Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.6.2024 © National Comprehensive Cancer Network, Inc 2024. All rights reserved. Accessed October 16, 2024.
To view the most recent and complete version of the guidelines, go online to NCCN.org.

Guidelines Recommend Genomic Profiling at Recurrence or Progression on ET in ER+, HER2- mBC[1-4]

To Identify Actionable Biomarkers and Potential Resistance Mechanisms, Like *ESR1* Mutations



Testing for *ESR1* mutations should be performed on blood or tissue obtained ***at the time of progression.***[1]

- Alterations in *PIK3CA*, *AKT1*, and *PTEN* are likely driver mutations, not resistance mutations, and should be present in the primary tumor[1-4]
- Rebiopsy for tissue samples may not always be available, and ctDNA analysis may provide greater sensitivity for *ESR1* mutations[2,4]
- Literature reports an overall **concordance rate** for *ESR1* mutation between **matched tissue and plasma samples** ranging from 40% to 100%, although the majority of the data come from small series[5]

ctDNA = circulating tumor DNA; 2L = second-line; 3L = third-line.

1. Burstein HJ, et al. *J Clin Oncol*. 2023;41(18):3423-3425. 2. Clatot F, et al. *Oncotarget*. 2016;7(46):74448-74459. 3. Al-Qasem AJ, Alves CL, Ditzel HJ. *Cancers (Basel)*. 2021;13(21):5397. 4. Lone SN, et al. *Mol Cancer*. 2022;18;21(1):79. 5. Urso L, et al. *Front Oncol*. 2021;11:625636.

Identifying Driver Mutations to Inform Treatment Decisions: Liquid vs Tissue Biopsies[1,2]

Advantages

Histopathology information, assessment of DNA and non-DNA biomarkers

Disadvantages

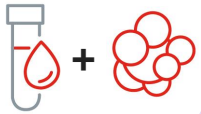
Longer TAT, limited tissue quantity/quality, invasive, tumor heterogeneity not always captured, re-biopsy not always feasible

Advantages

Rapid TAT, minimally invasive, repeatable over time, better capture of tumor heterogeneity

Disadvantages

Non-DNA biomarkers are not evaluable, low tumor fraction, presence of mutations from sites other than the target lesion, negative results necessitate confirmation via tissue-based testing



Advantages

All included in ctDNA and tissue testing leading to the identification of more unique actionable biomarkers

Disadvantages

Potential increased costs[3]

TAT = turnaround time.

1. Mazzitelli C, et al. *Diagnostics.* 2023;13(7):1241. 2. Iams WT, et al. *JAMA Network Open*. 2024;7(1):e2351700. 3. Rivers Z, et al. Abstract presented at San Antonio Breast Cancer Symposium; December 10-14, 2024.

Testing for *ESR1* Mutations: PCR-Based vs NGS-Based Assays[1-3]

NGS-Based Assays

- NGS hybrid capture and amplicon assays
- Good to high sensitivity
- Compatible with liquid and tissue biopsy samples
- Detect **any** *ESR1* mutation
- **Include** other biomarkers like *PIK3CA*, *AKT1,* and *PTEN*

PCR-Based Assays

- ddPCR and qPCR assays
- Good to very high sensitivity
- Compatible with liquid and tissue biopsy samples
- Detect **only predetermined** *ESR1* mutations
- **May not** include other biomarkers

Mutations commonly detected by PCR assays cover **>90%** of *ESR1* mutations frequently observed in patients.[1,4-6] However, these assays do not include unknown mutations or other biomarkers.[1,3]

Commercial PCR- and NGS-based assays to test for *ESR1* mutations are available.[7]

ddPCR = droplet digital PCR; qPCR = quantitative PCR.

1. Dustin D, et al. *Cancer*. 2019;125(21):3714-3728. 2. Brett JO, et al. *Breast Cancer Res.* 2021;23(1):85. 3. Raei M, et al. *BMC Cancer*. 2024;24(1):908. 4. Corné J, et al. *Clin Chim Acta*. 2023;545:117366. 5. Kingston B, et al. *Cancer Discov*. 2024;14(2):274-289. 6. Grinshpun A, et al. *Biochim Biophys Acta Rev Cancer*. 2023;1878(1):188830. 7. Mazzitelli C, et al. *Diagnostics (Basel).* 2023;13(7):1241.

Conclusions

- *ESR1* mutations have a substantial impact on clinical outcomes in ER+, HER2- mBC through mediating resistance to estrogen therapies
- Guidelines advise that testing for *ESR1* mutations should be performed at each instance of disease recurrence or progression
- There are commercial assays available to test for common *ESR1* mutations