ESR1 Biomarker Testing in Metastatic Breast Cancer

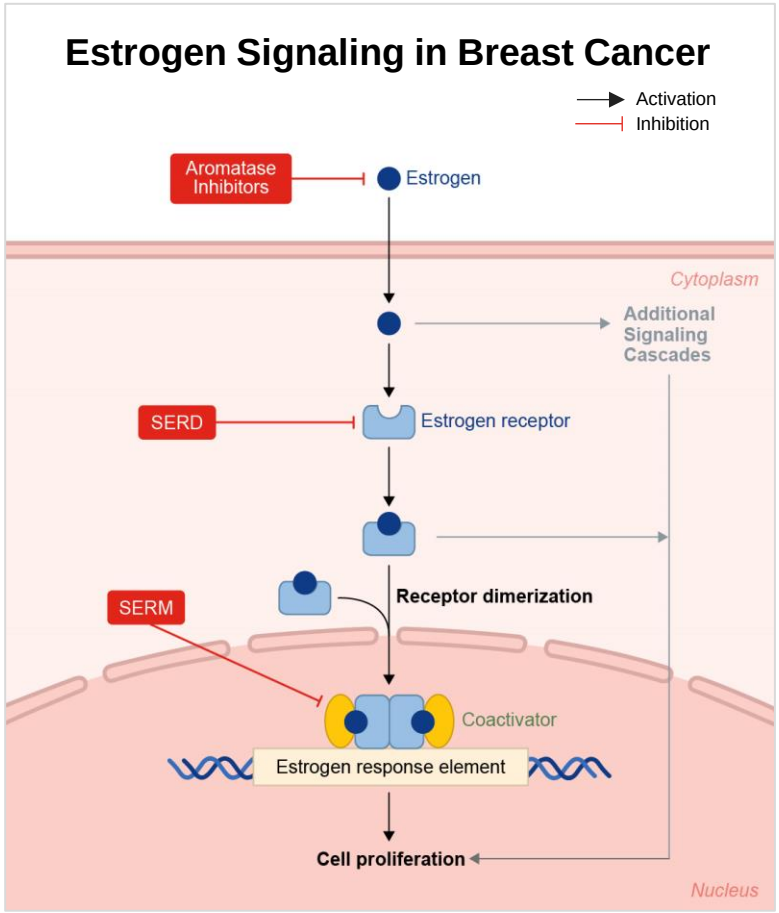
Learning Objectives

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; ESR1 = estrogen receptor 1 gene; HER2- = human epidermal growth factor receptor 2-negative.

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

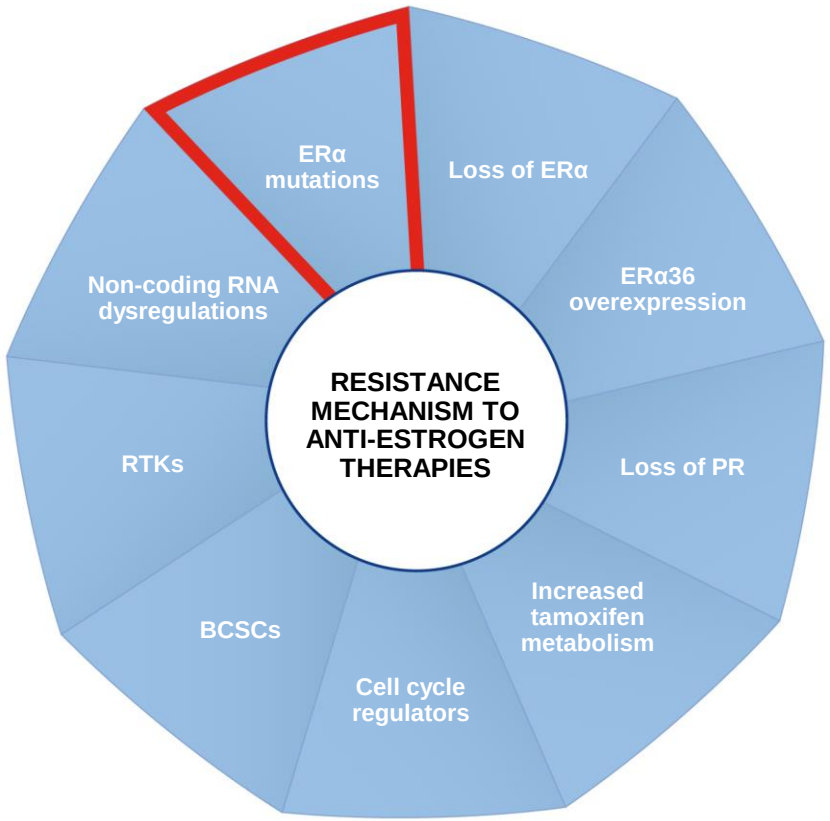


- Breast cancer is the most common cause of cancer mortality in women,[1] with approximately 70% of cases classified as ER+[2]
- In these tumors, signaling through the ER controls multiple proliferation-promoting and anti-apoptotic pathways[2-4]
- ET remains the backbone therapy and is the 1L standard 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; mBC = metastatic breast cancer; 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]

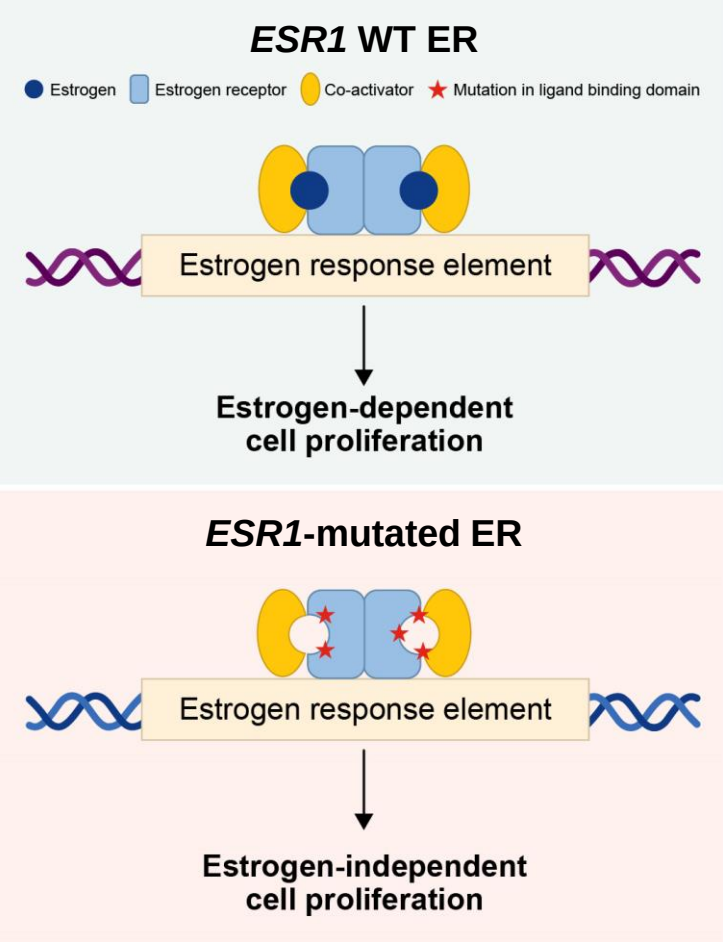


- Despite the utility of 1L ET ± CDK4/6i, patients may still experience progression or recurrence[2]
- After progression or recurrence on ET ± CDK4/6i, no consensus on an optimal treatment strategy in ER+, HER2- mBC has been reached[2-4]
- There are multiple mechanisms of resistance to ET ± CDK4/6i,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

BCSC = breast cancer stem cell; CDK4/6i = cyclin-dependent kinase 4/6 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



Mutated ERα is autoactivated 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

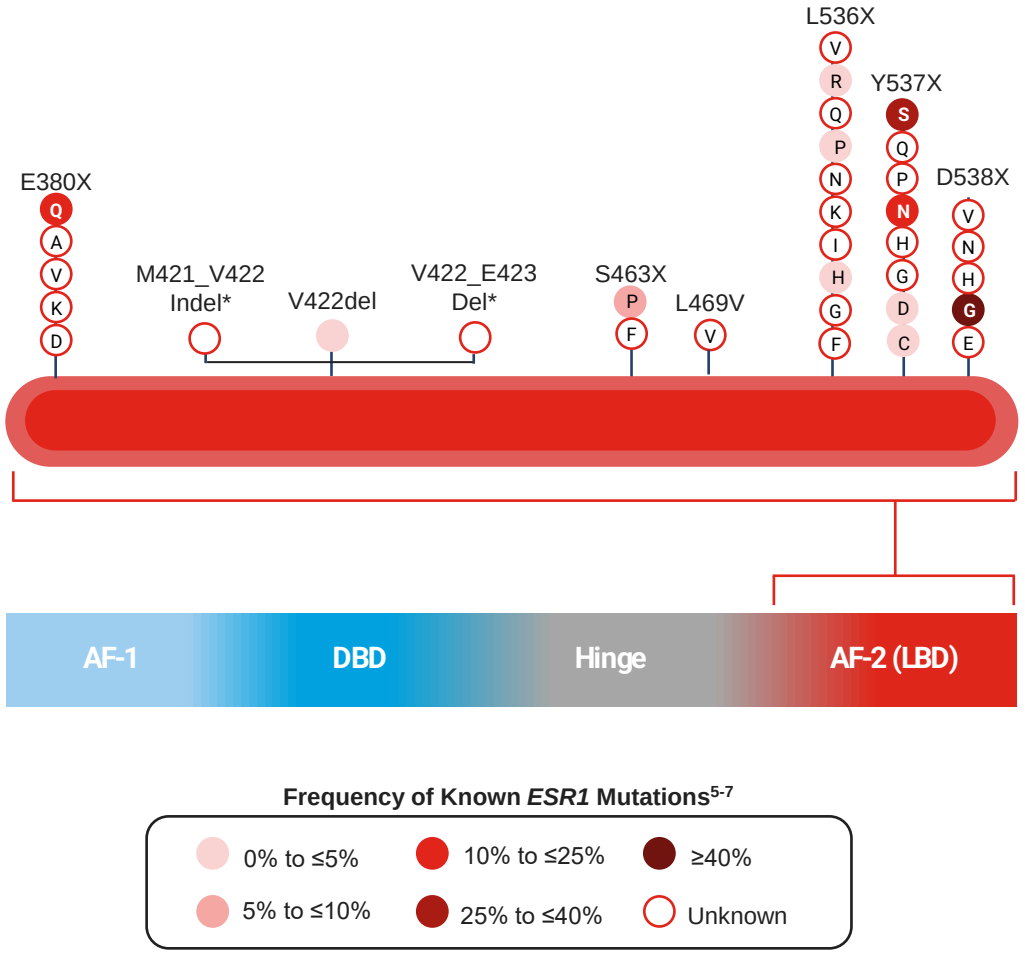
ER = estrogen receptor; SERCA = selective estrogen receptor covalent antagonists; WT = wildtype.

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.

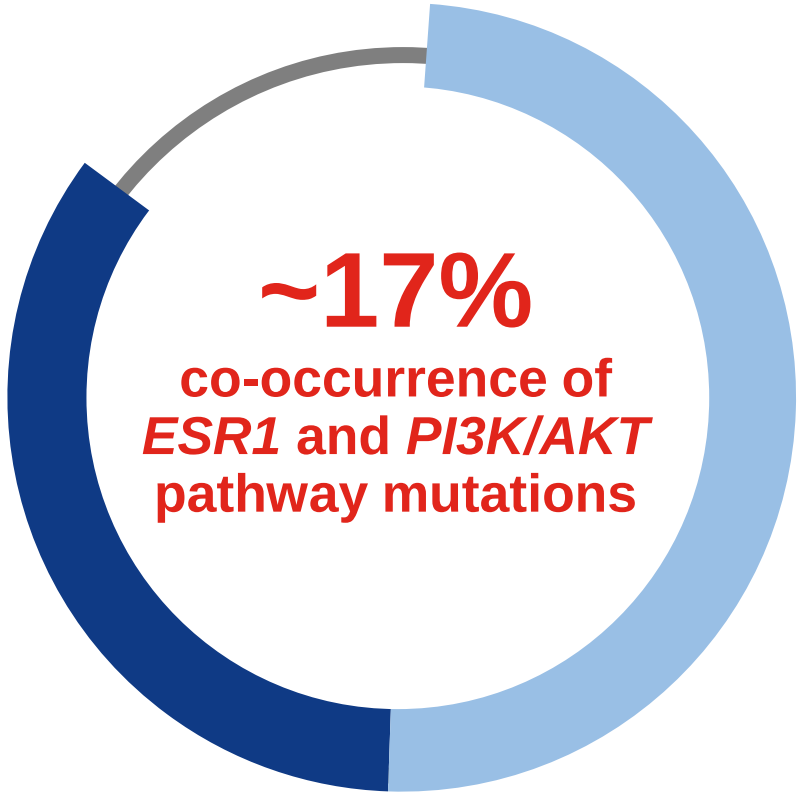
Co-Occurrence of *ESR1* and *PI3K/AKT* Pathway Mutations

- **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 progression following prior lines of ET 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
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

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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.
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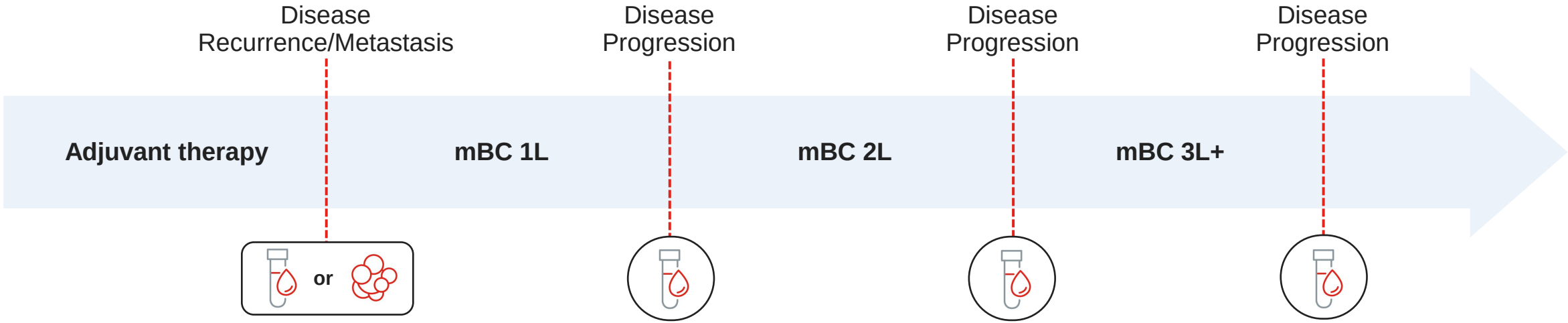
To view the most recent and complete version of the guidelines, go online to NCCN.org.

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



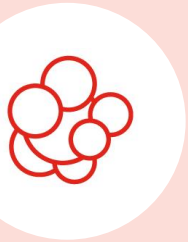
Testing for *ESR1* mutations should be performed on blood or tissue obtained ***at the time of progression.***[1]

- Alterations in *PIK3CA*, *AKT1*, and *PTEN* are likely driver mutations, not resistance mutations, and should be present in the primary tumor[1-4]
- Re-biopsy 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

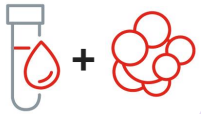
Longer TAT, limited tissue quantity/quality, invasive, tumor heterogeneity not always captured, re-biopsy not always feasible

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