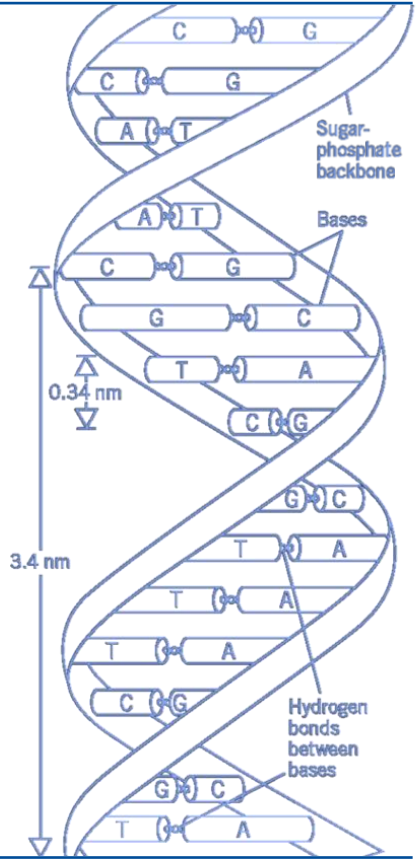
OVERVIEW OF THE PI3KINASE PATHWAY AND *PIK3CA* MUTATION TESTING



This is a medical resource for scientific information and is intended for healthcare providers practicing in the United States.

Current as of May 2024

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OBJECTIVES

The information provided will allow for:

1. Understanding of the PI3K pathway and *PIK3CA* mutations and their role in oncogenesis

2. Discussion of *PIK3CA* mutation testing
    a. Testing methodologies
    b. Current guidelines for *PIK3CA* testing

PI3K=phosphatidylinositol 3-kinase; PIK3CA=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

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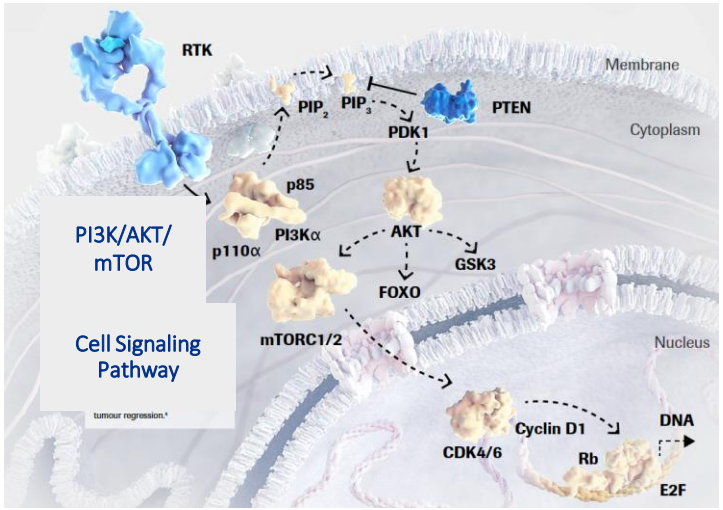
THE PI3K/AKT/mTOR CELL SIGNALING PATHWAY DRIVES MANY CELL PROCESSES AND IS FREQUENTLY ALTERED IN CANCER

Activating mutations in *PIK3CA* are the primary dysregulating event



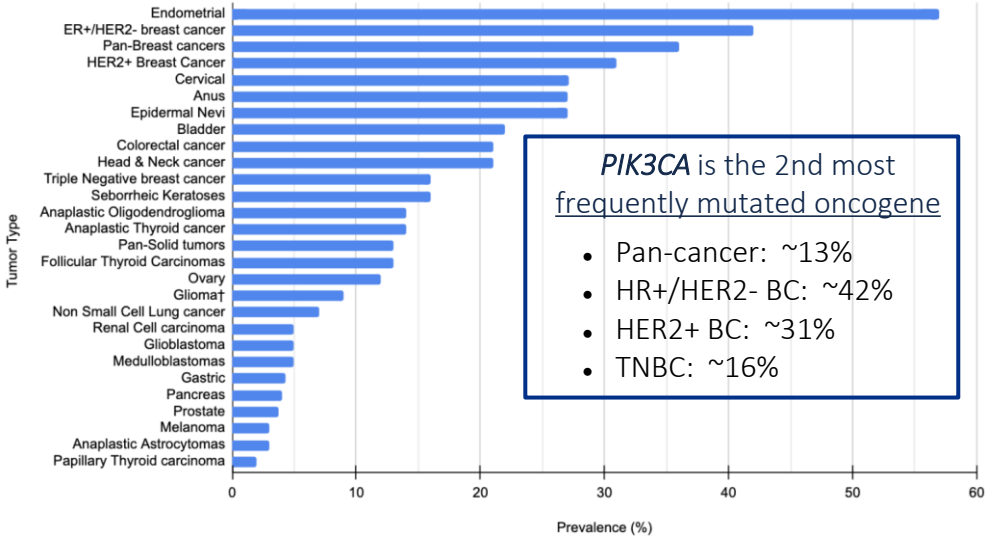
- The PI3Kα enzyme is made up of p110α catalytic and p85 regulatory protein subunits[1,2]
- p110α is encoded by the *PIK3CA* gene[1,2]
- Various mutations in the *PIK3CA* gene can activate the PI3K enzyme[2]
- Hyperactivation of the PI3K/AKT/mTOR signaling pathway has been shown to promote both *de novo* and acquired resistance to hormone therapy in ER-positive breast cancer cell lines and xenograft models[3]

AKT=protein kinase B; CDK=cyclin-dependent kinase; ER=estrogen receptor; FOXO=forkhead box O; GSK3=glycogen synthase kinase 3; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; mTOR=mammalian target of rapamycin; PDK1=phosphoinositide-dependent protein kinase-1; PI3K=phosphatidylinositol 3-kinase; PI3Kα=phosphatidylinositol 3-kinase alpha; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PIP2=phosphatidylinositol (4,5)-bisphosphate; PIP3=phosphatidylinositol (3,4,5)-trisphosphate; PTEN=phosphatase and tensin homolog; Rb=retinoblastoma protein; RTK=receptor tyrosine kinase.

1. Katso R, et al. Annu Rev Cell Dev Biol. 2001;17:615–75. 2. Zhao L, Vogt PK. Oncogene. 2008;27:5486–5496. 3. Sabnis G, et al. Clin Cancer Res. 2007;13(9):2751.
References for image: Vasan N, et al. Ann Oncol. 2019;30(Suppl 10):x3-x11; Mosele F, et al. Ann Oncol. 2020;31(3):377-386; Miller TW, et al. Breast Cancer Res. 2011;13(6):224; Hong R, et al. Cancer Res. 2018;78(4 Suppl):PD4-14; Edgar K, et al. Cancer Res. 2020;80(4 Suppl):P3-11-23.

ACTIVATING *PIK3CA* MUTATIONS HAVE BEEN OBSERVED IN MANY TUMOR TYPES

Prevalence of *PIK3CA* gain-of-function mutations across a subset of tumor types*



PIK3CA is the 2nd most frequently mutated oncogene

- Pan-cancer: ~13%
- HR+/HER2- BC: ~42%
- HER2+ BC: ~31%
- TNBC: ~16%

*Plotted *PIK3CA* mutation prevalence rates based on cited literature. Variability likely exists across studies due to factors such as sample size and testing methods.†IDH-mutant glioblastoma.
BC=breast cancer; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; IDH=isocitrate dehydrogenase; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TNBC=triple-negative breast cancer.

References: Gavgani F, et al. Int J Mol Sci. 2018;19(12):3931; Martínez-Sáez O, et al. Breast Cancer Res. 2020;22(1):45; Voutsadakis I. J Clin Med. 2021;10(2):220; Mills SZ, et al. JAMA Oncol. 2016;2(12):1565-1573; Hafner C, et al. Proc Natl Acad Sci USA. 2007;104(33):13450-13454; Voutsadakis I. Clin Colorectal Cancer. 2021;20(3):201-215; Qiu W, et al. Int J Cancer. 2008;122(5):1189–1194; Broderick DK, et al. Cancer Res. 2004;64(15):5048–5050; Santarpia L, et al. J Clin Endocrinol Metab. 2008;93(1):278–284; Wang Y, et al. J Clin Endocrinol Metab. 2007;92(6):2387–2390; Levine DA, et al. Clin Cancer Res. 2005;11(8):2875–2878; Brito C, et al. Clin Med Insights Oncol. 2022;16; Singh N, et al. Cancer Res. 2023;83(7):923; Li VSW, et al. BMC Cancer. 2005;5(29); Witkiewicz AK, et al. Nat Commun. 2015;6:6744; Herberts C, et al. European Urology. 2020;78(6):834-844; Omholt K, et al. Melanoma Research. 2006;16(2):197-200; Abubaker J, et al. J Clin Endocrinol Metab. 2008;93(2):611–618.

METASTATIC HR+/HER2- BREAST CANCER PATIENTS WITH TUMORS HARBORING *PIK3CA* MUTATIONS HAVE A WORSE PROGNOSIS

In a meta-analysis of 11 trials, ***PIK3CA* mutation** was associated with:[1]

- **Shorter median PFS**: difference -1.8 months (95% CI: -3.4, -0.1, I^2=35%, N=3,219)
- **Shorter median OS**: difference -8.4 months (95% CI: -13.4, -3.5, I^2=58%, N=1,545)

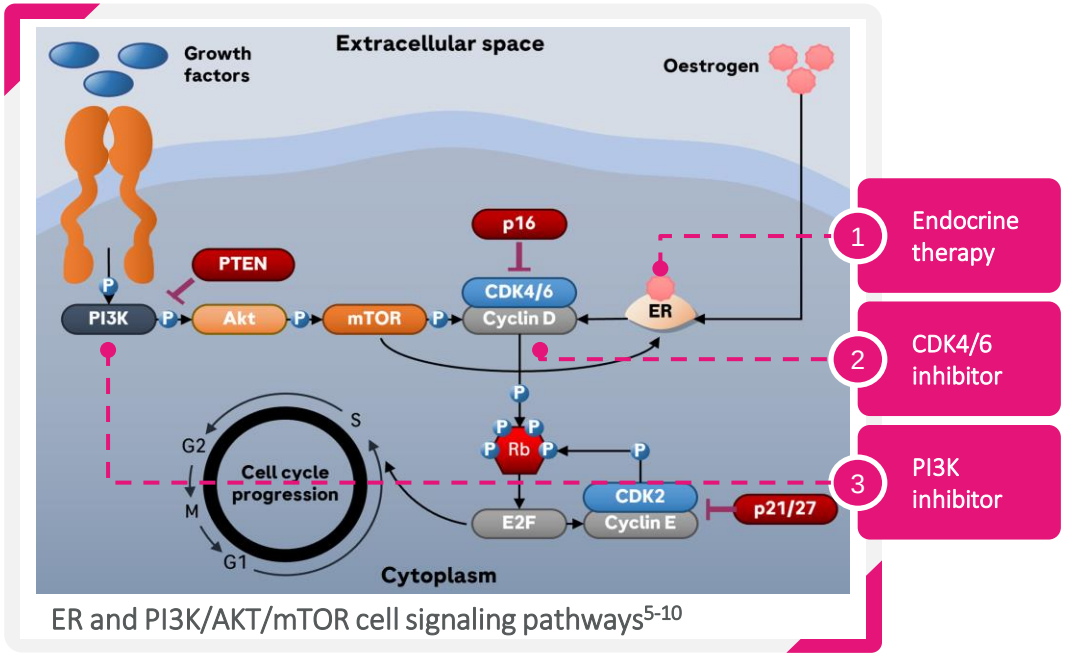
Methods:[1] Meta-analysis of 3,219* patients from 33 study arms across 11 randomized clinical trials of patients with HR+/HER2– mBC. Trials identified via systematic literature review. Trial arms with PI3K-targeted therapies were excluded. Meta-regression analysis was used to estimate the association between *PIK3CA* status and PFS and OS.

*Of the 3,219 patients, 1,386 were *PIK3CA*-mutated and 1,833 were wild-type.
HR=hormone receptor; HER2=epidermal growth factor receptor 2; mBC=metastatic breast cancer; OS=overall survival; PFS=progression-free survival; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PI3K=phosphatidylinositol 3-kinase.
1. Fillbrunn M, et al. BMC Cancer. 2022;22(1):1002.

CROSSTALK BETWEEN THE PI3K/AKT/mTOR AND ER PATHWAYS PROVIDES A RATIONALE FOR COMBINATION THERAPIES

PI3K/AKT/mTOR and ER pathway crosstalk

- There is an important crosstalk between the ER and PI3K/AKT/mTOR pathways, highlighted by the high frequency of *PIK3CA* mutations (~40%) in patients with HR+ BC[1]
- Activation of the PI3K/AKT/mTOR pathway may promote resistance to endocrine therapy in ER+ BC[2,3]
- Targeting the ER, CDK4/6 and PI3K/AKT/mTOR pathways in combination could extend treatment benefit and reverse/delay the development of treatment resistance[2–4]



ER and PI3K/AKT/mTOR cell signaling pathways[5-10]

AKT=protein kinase B; BC=breast cancer; CDK=cyclin-dependent kinase; ER=estrogen receptor; mTOR=mammalian target of rapamycin; P=phosphate; PI3K=phosphatidylinositol 3-kinase; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN=phosphatase and tensin homolog; Rb=retinoblastoma protein.

1. Vasan N, et al. Ann Oncol. 2019;30:x3–x11; 2. Burris III HA. Cancer Chemother Pharmacol. 2013;71:829–842. 3. Presti D, Quaquarini E. Cancers. 2019;11:1242. 4. Cortés J, et al. Cancer Treat Rev. 2017;61:53–60. 5. Brufsky AM, Dickler MN. Oncologist. 2018;23:528. 6. Anderson EJ, et al. Int J Breast Cancer. 2020;2020:3759179 7. Miller TW, et al. J Clin Oncol. 2011;29:4452–4461. 8. LoRusso PM, et al. J Clin Oncol. 2016;34:3803–3815. 9. Martínez-Sáez O, et al. Breast Cancer Res. 2020;22:45. 10. Fillbrunn M, et al. BMC Cancer. 2022;22(1):1002.

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PIK3CA MUTATION SUMMARY

- PI3K, AKT, and mTOR are major drivers in the PI3K/AKT/mTOR intracellular signaling pathway[1]
- *PIK3CA* is the gene that encodes the catalytic subunit of the alpha isoform of PI3K (p110α).[2-3] *PIK3CA* mutations lead to PI3K pathway hyperactivation and promotes resistance to ET in ER+ BC[4-6]
- *PIK3CA* mutations are a negative prognostic factor in ER+ BC[7]
- There is an important crosstalk between the ER and PI3K/AKT/mTOR pathways. Targeting the ER, CDK4/6 and PI3K/AKT/mTOR pathways in combination could extend treatment benefit and reverse/delay the development of treatment resistance[8-11]
- Sensitive and comprehensive methods are needed to identify patients with tumors that harbor any one (or more) activating *PIK3CA* mutations[12]

AKT=protein kinase B; BC=breast cancer; CDK=cyclin-dependent kinase; ER=estrogen receptor; ET=endocrine therapy; mTOR=mammalian target of rapamycin; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PI3K=phosphatidylinositol 3-kinase

1. Cantrell DA. J Cell Sci. 2001;114(8):1439–1445. 2. Katso R, et al. Annu Rev Cell Dev Biol. 2001;17:615–75. 3. Zhao L, Vogt PK. Oncogene. 2008;27(41):5486–5496. 4. Miller TW, et al. J Clin Invest. 2010;120(7):2406–2413. 5. Burris III HA. Cancer Chemother Pharmacol. 2013;71(4):829–842. 6. Presti D, Quaquarini E. Cancers. 2019;11(9):1242. 7. Fillbrunn M, et al. BMC Cancer. 2022;22(1):1002. 8. Vasan N, et al. Ann Oncol. 2019;30(Suppl 10):x3–x11. 9. Burris III HA. Cancer Chemother Pharmacol. 2013;71(4):829–842. 10. Presti D, Quaquarini E. Cancers. 2019;11(9):1242. 11. Cortés J, et al. Cancer Treat Rev. 2017;61:53–60. 12. Martínez-Sáez O, et al. Breast Cancer Res. 2020;22(1):45.

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THREE TESTS ARE APPROVED BY THE FDA TO DETECT *PIK3CA* MUTATIONS[1]

Currently, 3 tests are FDA-approved to detect *PIK3CA* mutations in patient specimens[1]

- NGS-based test for tissue specimens
- NGS-based test for blood plasma specimens
- PCR test for tissue or blood plasma specimens

Different factors may be considered in determining which test is most appropriate for a patient:

- NGS tests may be more comprehensive for detecting activating *PIK3CA* mutations[2]
- PCR is a sensitive test method that can be used to detect the most prevalent *PIK3CA* mutations in a patient specimen[3]
- PCR tests optimized for tissue may miss PIK3CA mutations (false negatives) in plasma ctDNA specimens[3]

ctDNA=circulating tumor DNA; NGS=next-generation sequencing; PCR=polymerase chain reaction; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

1. FDA. List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools). Available at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools. Accessed April 29, 2024; FDA. 2. Martínez-Sáez O, et al. Breast Cancer Res. 2020;22(1):45. 3. Qiagen Therascreen *PIK3CA* RGQ PCR plasma assay performance data. Available at https://www.qiagen.com/us/products/diagnostics-and-clinical-research/oncology/therascreen-solid-tumor/therascreen-pik3ca-rgq-pcr-kit-us. Accessed April 29, 2024.

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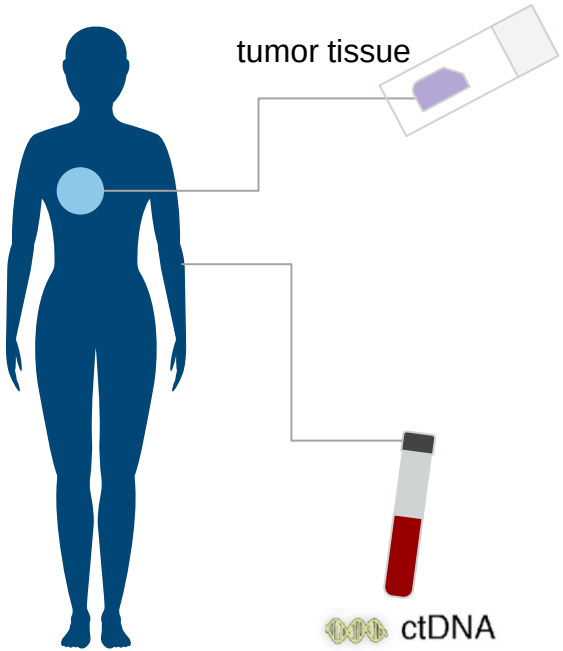
PIK3CA MUTATION TESTING IS CURRENTLY RECOMMENDED FOR INVASIVE HR+/HER2- BREAST CANCER[1]



NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Breast Cancer

- Upon initial workup for recurrent/metastatic disease, comprehensive germline and somatic profiling are recommended to identify candidates for targeted therapies.[1]
- For HR-positive/HER2-negative, recurrent unresectable or metastatic breast cancer, assess for *PIK3CA* mutations on tumor tissue or ctDNA in peripheral blood (liquid biopsy) to identify candidates for targeted treatment.
- Tissue-based assays have greater sensitivity, but ctDNA may reflect tumor heterogeneity more accurately. If one specimen is negative for actionable biomarkers, testing on the alternative specimen can be considered.

ctDNA=circulating tumor DNA; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; NCCN=National Comprehensive Cancer Network® (NCCN®); NGS=next-generation sequencing; PCR=polymerase chain reaction; *PIK3CA*=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V2.2024.© National Comprehensive Cancer Network, Inc. 2024. All rights reserved. Accessed March 11, 2024. To view the most recent and complete version of the guideline, go online to NCCN.org. 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.

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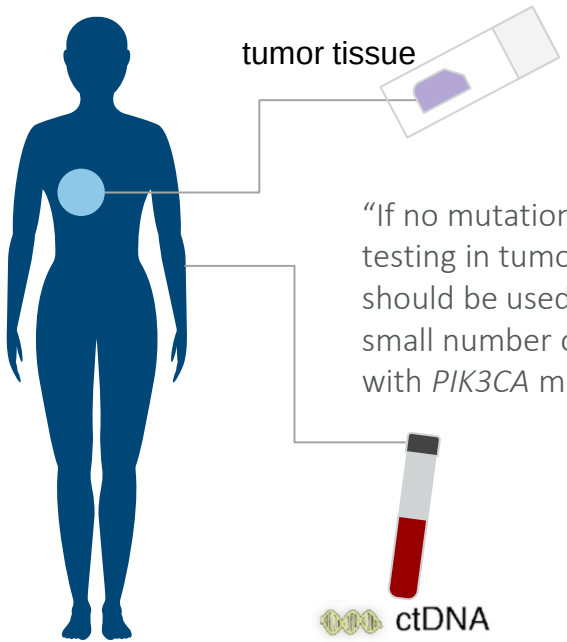
PIK3CA MUTATION TESTING IS CURRENTLY RECOMMENDED FOR LOCALLY RECURRENT OR METASTATIC HR+/HER2- BREAST CANCER BY ASCO[1]



Test can be performed on either tissue and/or plasma ctDNA[1]

"If no mutation is found in ctDNA, testing in tumor tissue, if available, should be used as this will detect a small number of additional patients with *PIK3CA* mutations"

ASCO Biomarkers for Systemic Therapy in Metastatic Breast Cancer: ASCO Guideline Update (June 2022)

- "Patients with locally recurrent unresectable or metastatic HR+/HER2- breast cancer who are candidates for a treatment regimen that includes a PI3K inhibitor and a hormonal therapy should undergo testing for *PIK3CA* mutations using NGS of tumor tissue or ctDNA in plasma…"

ASCO=American Society of Clinical Oncology; ctDNA=circulating tumor DNA; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; NGS=next-generation sequencing; PI3K=phosphatidylinositol 3-kinase; PIK3CA=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

1. Henry NL, et al. Biomarkers for Systemic Therapy in Metastatic Breast Cancer: ASCO Guideline Update. J Clin Oncol. 2022 Sep 20;40(27):3205-3221. doi: 10.1200/JCO.22.01063. Epub 2022 Jun 27.

PIK3CA MUTATION TESTING SUMMARY

- *PIK3CA* mutation testing can be performed using tumor tissue or plasma specimens[1]
- *PIK3CA* mutation testing can occur via NGS or PCR[1]
    - Current FDA-approved NGS tests may be more comprehensive than PCR[2,3]
- Current guidelines recommend testing for *PIK3CA* mutations in patients with locally recurrent unresectable or metastatic HR+/HER2- breast cancer to inform treatment decisions[4,5]

HR=hormone receptor; HER2=human epidermal growth factor receptor 2; PCR=polymerase chain reaction; NGS=next-generation sequencing; PIK3CA=phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

1. FDA. List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools). Available at https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools.. Accessed April 29, 2024. 2. Martínez-Sáez O, et al. Breast Cancer Res. 2020;22(1):45. 3. Qiagen Therascreen PIK3CA RGQ PCR plasma assay performance data. Available at https://www.qiagen.com/us/products/diagnostics-and-clinical-research/oncology/therascreen-solid-tumor/therascreen-pik3ca-rgq-pcr-kit-us. Accessed April 29, 2024. 4. Burnstein HJ, et al. J Clin Oncol. 2023;41(18):3423-3425. 5. Henry NL, et al. J Clin Oncol. 2022;40(27):3205-3221.

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