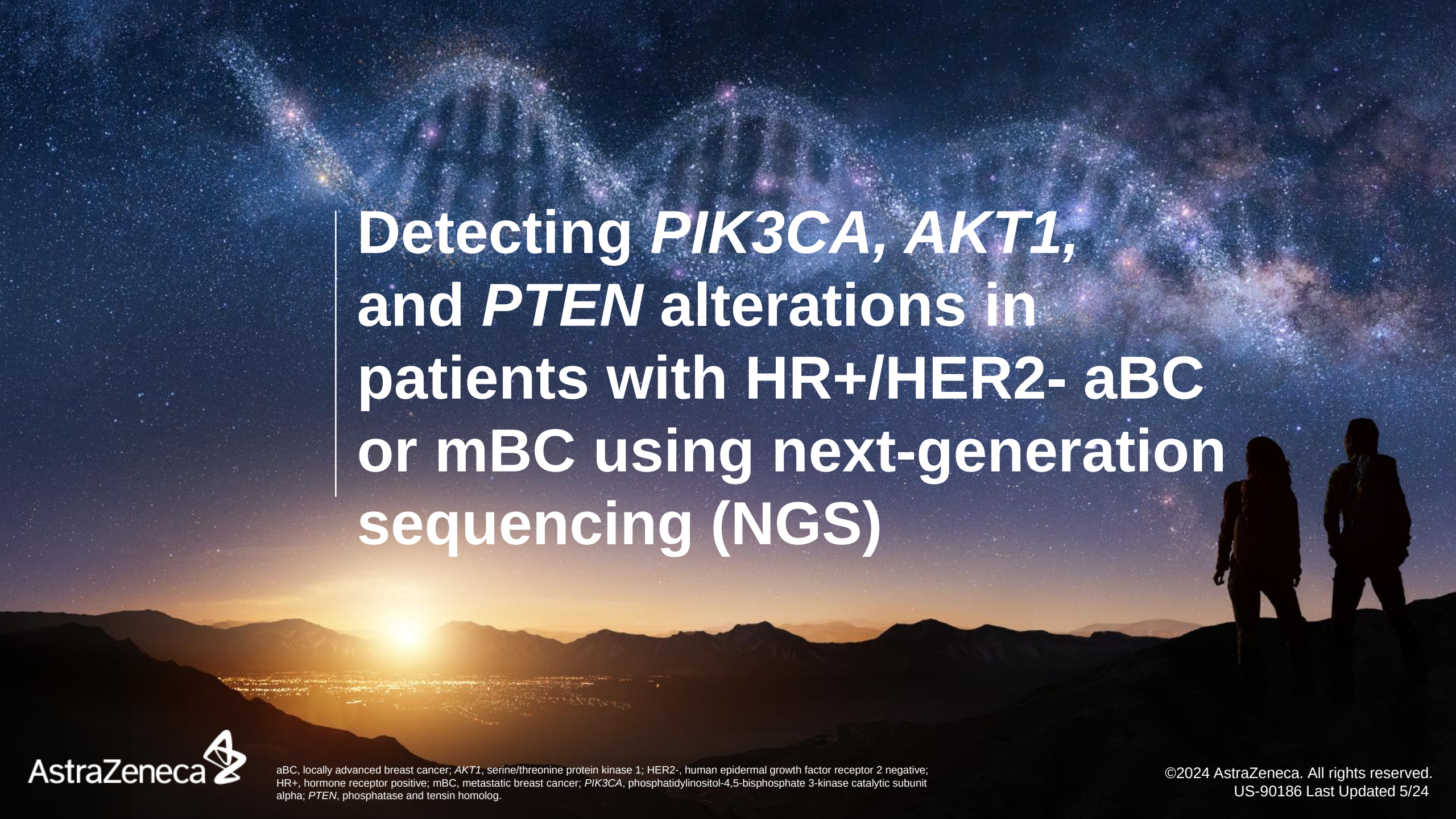

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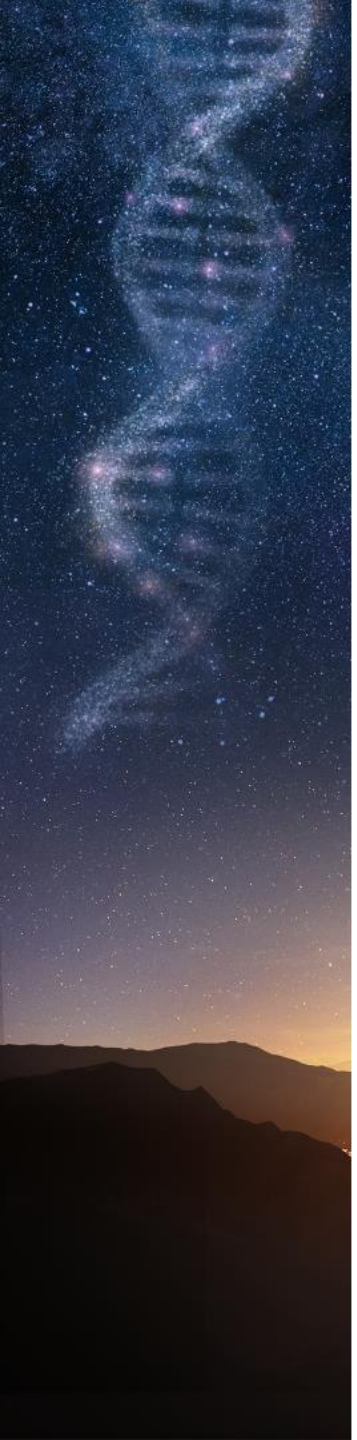
aBC, locally advanced breast cancer; *AKT1*, serine/threonine protein kinase 1; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; mBC, metastatic breast cancer; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.



Disclaimers

This presentation is supported by AstraZeneca.

This presentation is intended to provide educational information on testing for *PIK3CA*, *AKT1*, and *PTEN* alterations in patients with locally advanced or metastatic HR+/HER2- breast cancer.

This presentation does not discuss or promote any investigational or approved drugs.

AKT1, serine/threonine protein kinase 1; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.

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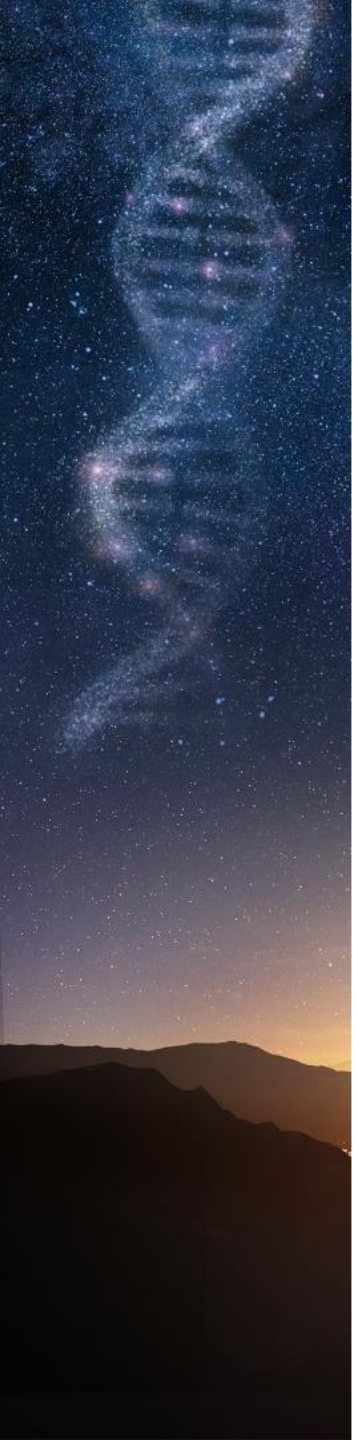

Objectives of this discussion

Recognize the role that the PI3K/AKT/PTEN signaling pathway plays in breast cancer

Understand the role NGS panel testing plays in breast cancer management

Know how to test for *PIK3CA*, *AKT1*, and *PTEN* biomarkers

Discuss common challenges for NGS testing and the importance of looking back for patients who have previously been tested

AKT, serine/threonine protein kinase; *AKT1*, serine/threonine protein kinase 1; NGS, next-generation sequencing; PI3K, phosphoinositide 3-kinase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.

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The PI3K/AKT/PTEN signaling pathway plays a significant role in regulating cell proliferation and survival[1,2]

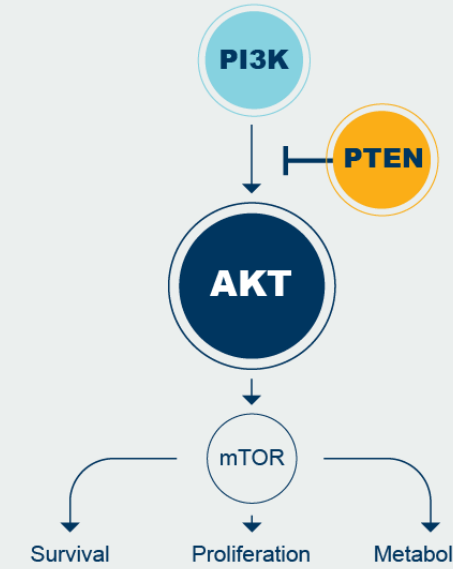
The PI3K/AKT/PTEN pathway regulates various cellular processes, including cell proliferation, cell survival, and cell metabolism[1-4]

In HR+/HER2- breast cancer, alterations in *PIK3CA, AKT1,* and/or *PTEN* may lead to hyperactivation of the PI3K/AKT/PTEN pathway.[1,5,6] These alterations include:

- ***PIK3CA* activating mutations[1]**
- ***AKT1* activating mutations[5]**
- ***PTEN* inactivating alterations[6]**

Abnormal PI3K/AKT/PTEN pathway activation mediates tumor growth and resistance to many breast cancer treatments, including endocrine therapies and CDK4/6is[1,3,7-9]

Overview of the PI3K/AKT/PTEN pathway



Adapted from Paplomata E, O'Regan R. *Ther Adv Med Oncol*. 2014;6(4):154-166.

AKT, serine/threonine protein kinase; *AKT1*, serine/threonine protein kinase 1; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; mTOR, mammalian target of rapamycin; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PI3K, phosphoinositide 3-kinase; *PTEN*, phosphatase and tensin homolog.

1. Miricescu D, Totan A, Stanescu-Spinu II, et al. *Int J Mol Sci*. 2020;22(1):173. **2.** Robertson JFR, Coleman R, Cheung KL, et al. *Clin Cancer Res*. 2020;26(7):1574-1585. **3.** Martorana F, Motta G, Pavone G, et al. *Front Pharmacol*. 2021;12:662232. **4.** Andrikopoulou A, Chatzinikolaou S, Panourgias E, et al. *Breast*. 2022;63:157-167. **5.** Smyth LM, Zhou Q, Nguyen B, et al. *Cancer Discov*. 2020;10:526-535. **6.** Chen J, Sun J, Wang Q, et al. *Front Oncol*. 2022;12:825484. **7.** Paplomata E, O'Regan, R. *Ther Adv Med Oncol*. 2014;6(4):154-166. **8.** Li H, Prever L, Hirsch E, Gulluni F. *Cancers (Basel).* 2021;13(14):3517. **9.** Dong C, Wu J, Chen Y, et al. *Front Pharmacol*. 2021;12:628690.

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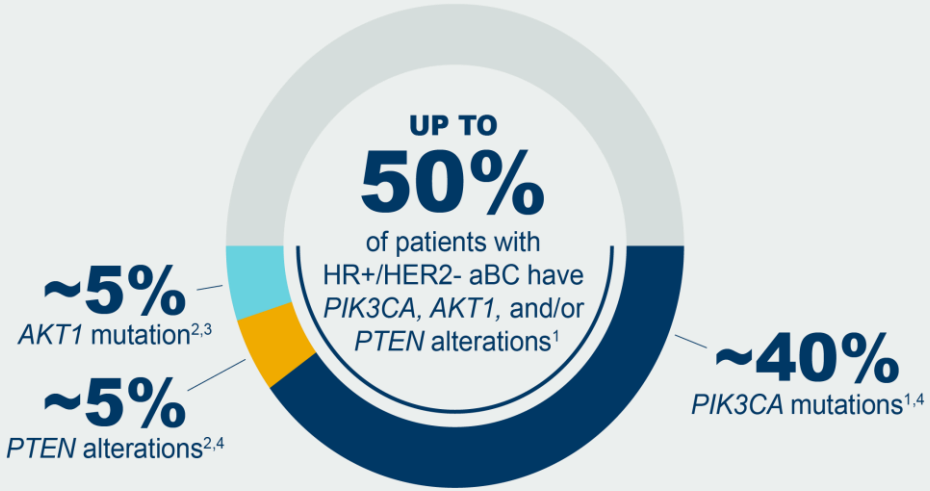
Up to 50% of patients with metastatic HR+/HER2- breast cancer have *PIK3CA*, *AKT1*, and/or *PTEN* alterations[1]

Incidence rates of genomic alterations within the PI3K/AKT/PTEN pathway in HR+ breast cancer



Key biomarkers in the PI3K/AKT/PTEN pathway

Gene	Key genetic event	Impact
PIK3CA[2]	Point mutations of *PIK3CA*	Gain of function of PI3K
AKT1[3]	Point mutation of *AKT1*	Gain of function of AKT
PTEN[4]	Point mutations, large deletions, and genomic rearrangements involving *PTEN*	Loss of function of PTEN

AKT, serine/threonine protein kinase; *AKT1*, serine/threonine protein kinase 1; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PI3K, phosphoinositide 3-kinase; *PTEN*, phosphatase and tensin homolog.

1. Martorana F, Motta G, Pavone G, et al. *Front Pharmacol*. 2021;12:662232. **2.** Miricescu D, Totan A, Stanescu-Spinu II, et al. *Int J Mol Sci*. 2020;22(1):173. **3.** Smyth LM, Zhou Q, Nguyen B, et al. *Cancer Discov*. 2020;10(4):526-535. **4.** Chen J, Sun J, Wang Q, et al. *Front Oncol*. 2022;12:825484.

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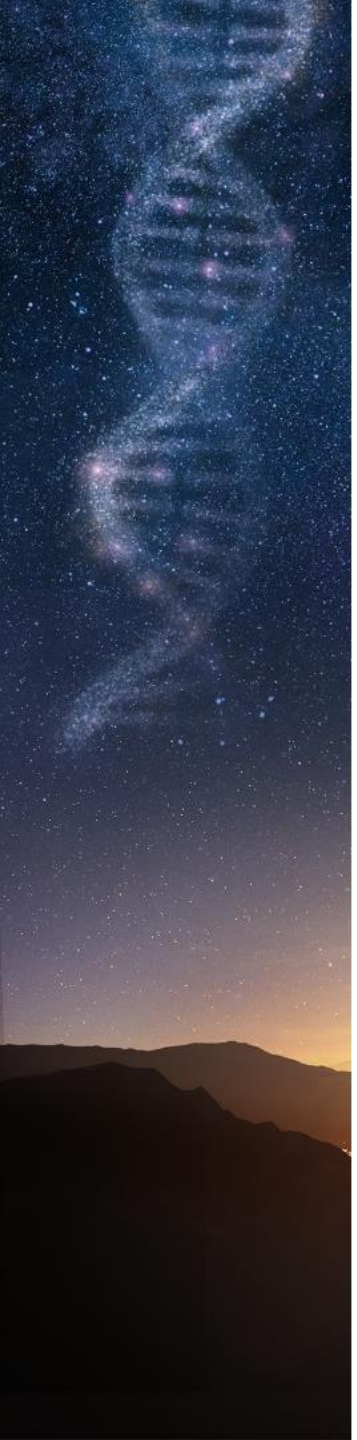

NGS testing can detect numerous actionable biomarkers in patients with metastatic breast cancer[1,2]

National Comprehensive Cancer Network® (NCCN®) recommended biomarker testing for recurrent unresectable (local or regional) or stage IV (M1) disease[1]

	Biomarkers	NGS	PCR	FISH/ISH	IHC	Germline Sequencing
Biomarkers associated with FDA-approved therapies[1-3]	*PIK3CA*	✔	✔			
	AKT1	✔				
	PTEN	✔				
	ESR1	✔	✔			
	NTRK fusion	✔	✔	✔		
	MSI-H/dMMR	✔	✔		✔	
	TMB-H	✔				
	RET-fusion	✔				
	PD-L1[3]				✔	
	HER2 (HER2 IHC 1+ or 2+/ISH negative)			✔	✔	
	Germline *BRCA1/2*					✔
Emerging biomarkers[1]	*HER2* activating mutations	✔				
	Somatic *BRCA1/2* mutations	✔				
	Germline *PALB2*					✔

AKT1, serine/threonine protein kinase 1; *BRCA1/2*, breast cancer susceptibility gene 1/2; dMMR, DNA mismatch repair; *ESR1*, estrogen receptor 1; FDA, US Food and Drug Administration; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; ISH, in situ hybridization; HER2, human epidermal growth factor receptor 2; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; *NTRK*, neurotrophic tropomyosin receptor kinase; *PALB2*, partner and localizer of *BRCA2*; PD-L1; programmed death-ligand 1; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; *RET*, rearranged during transfection; TMB-H, tumor mutational burden-high.
 2. Henry LN, Somerfield MR, Dayao Z, et al. *J Clin Oncol.* 2022;40(27):3205-3221. **3.** Erber R, Hartmann A. *Breast Care (Basel).* 2020;15(5):481-490.

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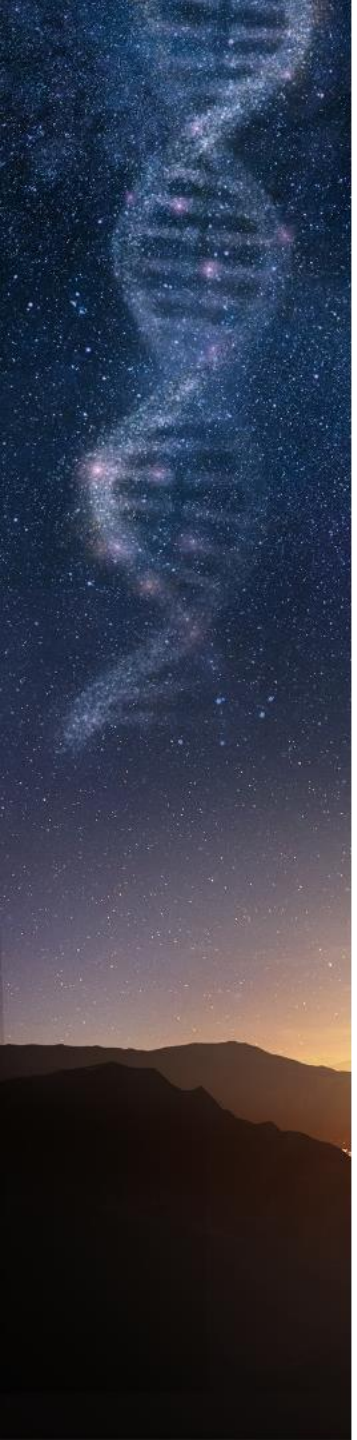

NGS can be performed using tissue or liquid biopsy samples

Tissue biopsy sample

- Use metastatic or archival primary tumor samples*[1]
- For metastatic biopsies, ensure there is adequate tumor tissue for NGS testing, as well as other necessary tests[1]
- Ensure the minimum tumor fraction within the selected areas is 10-20%[2]
- If there is inadequate tissue, NGS testing can be done using plasma-based ctDNA[3]

Liquid biopsy sample

- Use freshly drawn peripheral blood
- Weaker sensitivity compared to tissue-based assays, but may reflect tumor heterogeneity more accurately[4]
- If a liquid biopsy is negative, tumor tissue testing is recommended[4]

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommends use of tissue and/or liquid biopsy for NGS tests[4]

*As bone is the most common site of metastases in breast cancer, bone biopsies may be used. Osseous tissues require decalcification with agents such as EDTA.[5] Some commercial companies may not accept decalcified samples, please confirm with your lab or vendor when sending metastatic bone samples for NGS testing.

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ctDNA, circulating tumor DNA; EDTA, ethylenediaminetetraacetic acid; NGS, next-generation sequencing.

1. Schwartzberg L, Kim ES, Liu D, et al. *Am Soc Clin Oncol Educ Book*. 2017;37:160-169. **2.** Chen H, Luthra R, Goswami RS, et al. *Cancers (Basel)*. 2015;7(3):1699-1715. **3.** Foundation Medicine. FoundationOne®Liquid CDx. Technical Information. Accessed April 17, 2024. https://www.foundationmedicine.com/sites/default/files/media/documents/2023-11/P190032_S011_F1LCDx%20Technical%20Label%5B76%5D.pdf. **4.** Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.2.2024. © National Comprehensive Cancer Network, Inc. 2024. All rights reserved. Accessed April 17, 2024. To view the most recent and complete version of the guideline, go online to NCCN.org. **5.** Washburn E, Tang X, Caruso C, et al. *Hum Pathol.* 2021;117:108-114.

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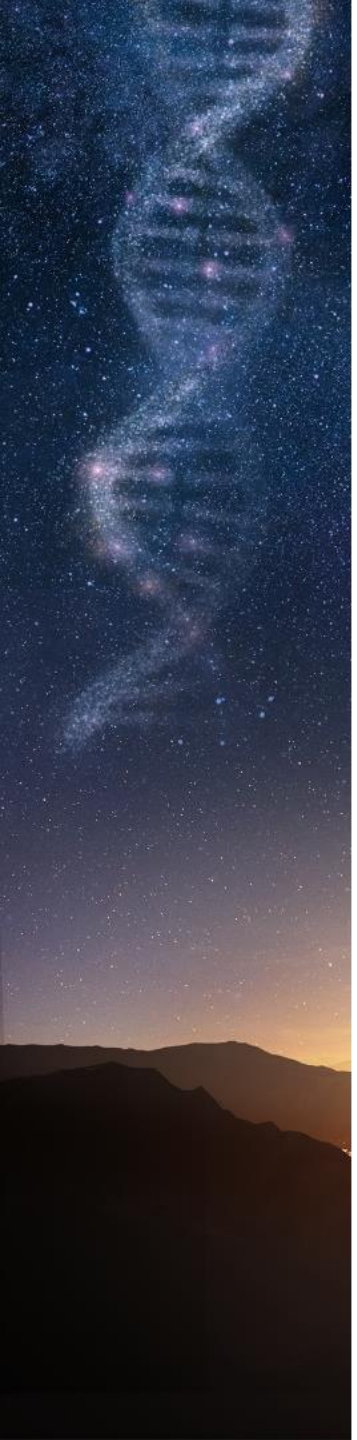

The NCCN Guidelines® recommend NGS testing for *PIK3CA, AKT1,* and *PTEN* alterations[1]

Why?[1]

PIK3CA, AKT1, and *PTEN* are recognized as actionable biomarkers in metastatic HR+/HER2- breast cancer

Whom?[1]

Patients with metastatic HR+/HER2- breast cancer

How?[1]

NGS panel testing

When?[1]

At metastatic diagnosis

NGS panel testing can also be ordered after treatment progression

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AKT1, serine/threonine protein kinase 1; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.
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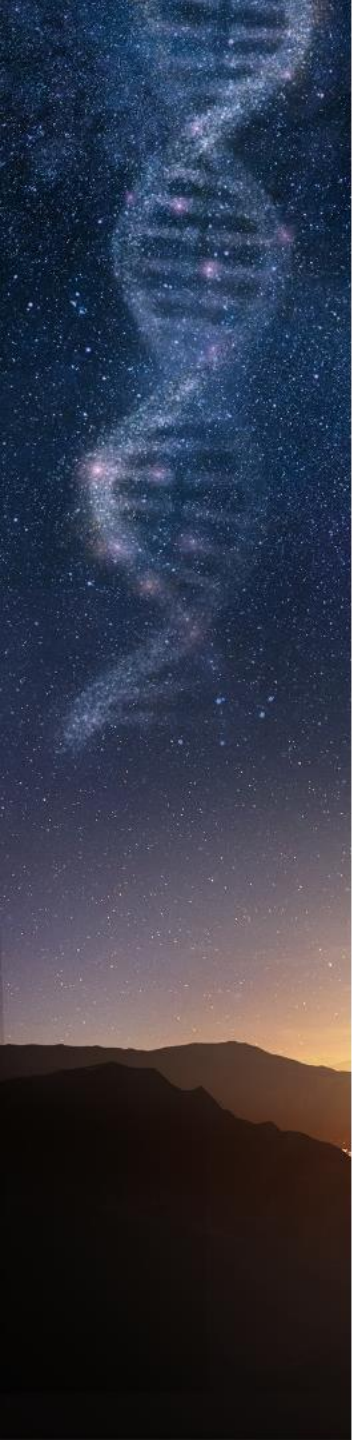

Incorporate NGS into the diagnostic pathway to ensure *PIK3CA*, *AKT1*, and *PTEN* alterations are identified

Genetic counselors | Pathologists | Nurse/advanced practitioners | Oncologists | Interventional radiologists

1 Deciding to order NGS panel

NGS panel testing can be performed in-house or via a commercial testing lab, depending on your institution.

For in-house panels, ensure that *PIK3CA*, *AKT1*, and *PTEN* are included.

Common NGS panel test options +

2 Obtaining the sample

It is helpful to plan for NGS at initial biopsy of metastatic disease.*[1]

Tumor fraction should be 10-20%.[2]

3 Sample processing

When using tissue, samples should be formalin-fixed paraffin-embedded (FFPE), fresh or frozen.[1,3]

Standard fixation methods should be used to preserve the nucleic acid integrity.[4]

Assess if DNA yield is sufficient for a test and perform a DNA quality check.[5]

*The decision to perform additional biopsies for NGS after the initial patient workup can be made on a case-by-case basis; this should be discussed with the multidisciplinary team.

AKT1, serine/threonine protein kinase 1; NGS, next-generation sequencing; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.

1. Schwartzberg L, Kim ES, Liu D, Schrag D. *Am Soc Clin Oncol Educ Book*. 2017;37:160-169. **2.** Chen H, Luthra R, Goswami RS, et al. *Cancers (Basel).* 2015;7(3):1699-1715. **3.** Schmid S, Jochum W, Padberg B, et al. *ESMO Open.* 2022;7(5):100570. **4.** FoundationOne®CDx. Specimen Instructions. Accessed April 17, 2024. https://assets.ctfassets.net/w98cd481qyp0/6qYLg8jUuEYEvUytoBz8p6/f7764c8e3fcadda9ec1374fe26bb999e/F1CDx_Specimen_Instructions.pdf. **5.** Gonzalez D, Mateo J, Stenzinger A, et al. *J Pathol Clin Res*. 2021;7(4):311-325.

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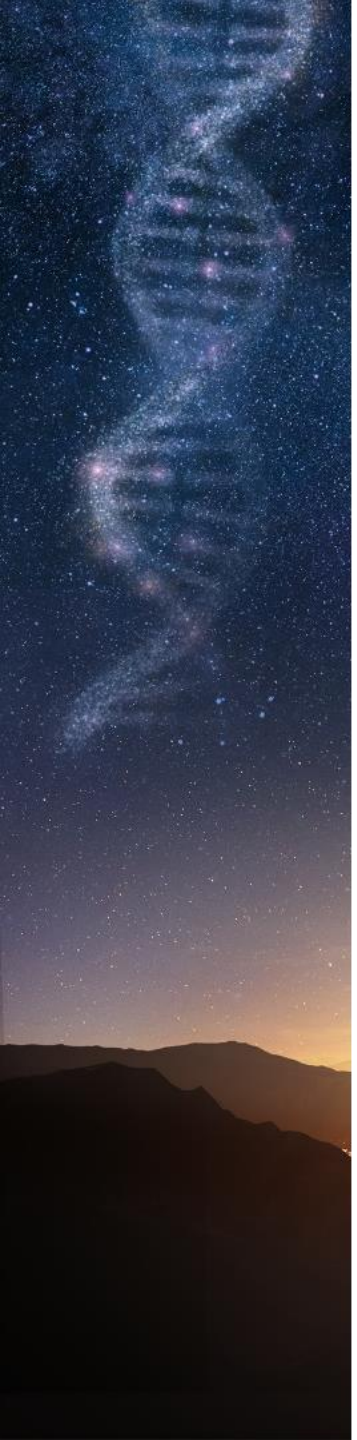


Incorporate NGS into the diagnostic pathway to ensure *PIK3CA*, *AKT1*, and *PTEN* alterations are identified (cont'd)

Genetic counselors | Pathologists | Nurse/advanced practitioners | Oncologists | Interventional radiologists

4 Analysis

For send-out tests, NGS will be performed by a commercial testing lab.[1]

For in-house tests, NGS workflow and analysis will be managed by the pathologists and/or geneticists.[1]

5 Interpreting the results

Biomarkers are classified according to their clinical significance[1,2]:

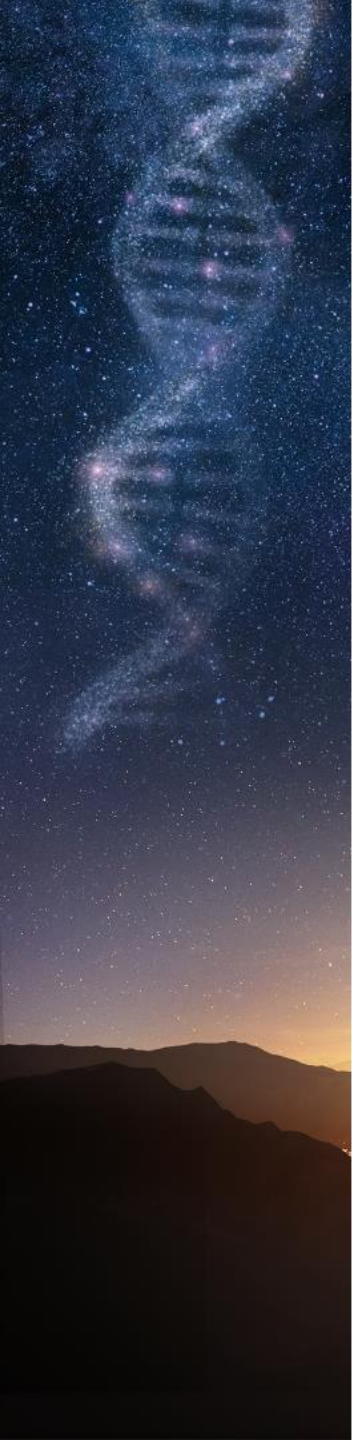
- Actionable biomarkers detected
- Potentially actionable biomarkers detected
- No actionable biomarkers detected

6 Clinical implications

Examine the NGS report to detect any biomarker alterations and be informed on any clinical implications that may arise.[3]

AKT1, serine/threonine protein kinase 1; NGS, next-generation sequencing; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.

1. Schmid S, Jochum W, Padberg B, et al. *ESMO Open*. 2022;7(5):100570. **2.** Chakravarty D, Johnson A, Sklar J, et al. *J Clin Oncol*. 2022;40(11):1231-1258. **3.** FoundationOne®CDx. Technical Information. Accessed April 17, 2024. https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019S006C.pdf.



Addressing common challenges can help improve the NGS testing procedure

	Inadequate volume of tissue	Poor stabilization of tissue
Addressing the challenge	Proactive communication with interventional radiologists (or the performing proceduralist) on the need for additional tissue for molecular profiling	Ensure the use of standardized and quality-controlled 10% neutral phosphate-buffered formalin[3]
	Tissue samples can be divided into 2 blocks — one for histology testing and one for molecular testing — to help conserve tissue	Check formalin pH before and routinely during use[3]
	If feasible, implement rapid on-site evaluation (ROSE)[1,2]	Ensure optimal time in formalin (6-36 hours — optimal time may differ due to tissue type)[3]

NGS, next-generation sequencing.

1. da Cunha Santos G, Ko HM, Saieg MA, et al. *Cancer Cytopathol*. 2013;121(1);4-8. **2.** Schmidt RL, Walker BS, Cohen MB. *PLoS One*. 2015;10(8):e0135466. **3.** Compton CC, Robb JA, Anderson MW, et al. *Arch Pathol Lab Med*. 2019;143(11):1346-1363.

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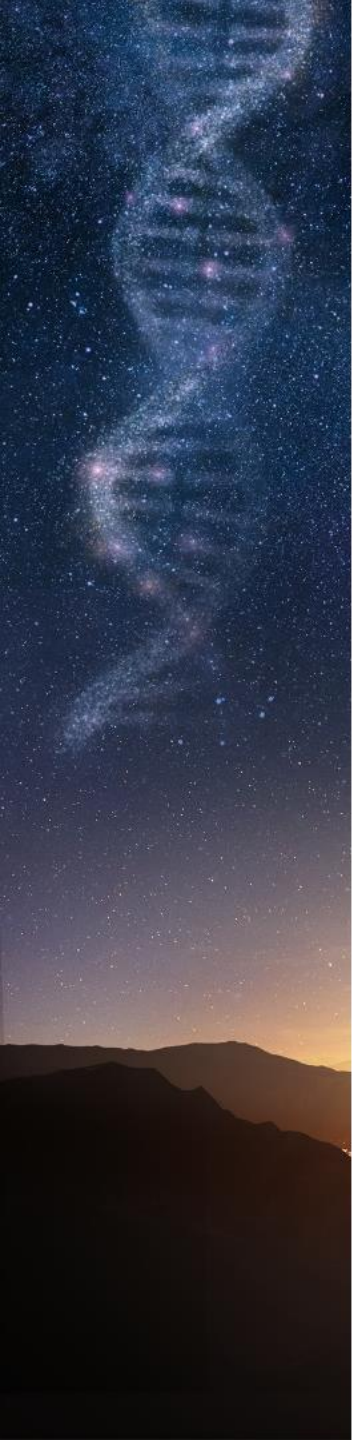
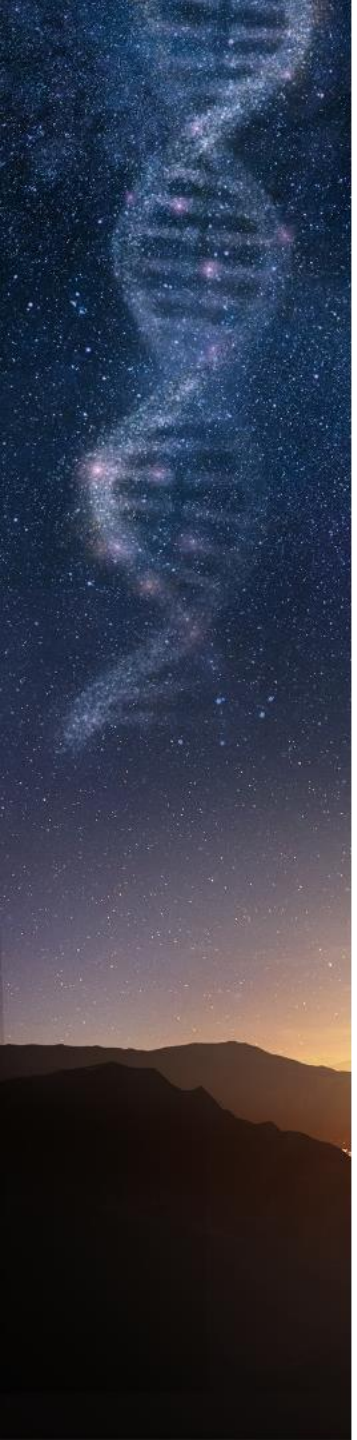


The importance of looking back at old NGS reports

Today	Future
Ensure you look back at patients' old NGS reports to identify *PIK3CA, AKT1,* and *PTEN* alterations	If an NGS panel test is ordered prior to treatment progression, ensure relevant stakeholders are looking back to check on previously ordered NGS test results

AKT1, serine/threonine protein kinase 1; NGS, next-generation sequencing; *PIK3CA,* phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.



Key takeaways

- *PIK3CA*, *AKT1*, and *PTEN* are actionable biomarkers — the NCCN Guidelines recommend testing for these alterations for patients with HR+/HER2- metastatic breast cancer[1]
- Up to 50% of patients with HR+/HER2- metastatic breast cancer have *PIK3CA*, *AKT1*, and/or *PTEN* alterations[2]
- NGS panel testing can detect actionable biomarkers including *PIK3CA*, *AKT1*, and *PTEN* alterations[1]
- Encourage those in your practice to look back at previous NGS reports/results to ensure *PIK3CA*, *AKT1*, and *PTEN* alterations are identified for all patients with advanced breast cancer

Ensure *PIK3CA*, *AKT1*, and *PTEN* alteration status are identified through NGS testing for all patients with HR+/HER2- metastatic breast cancer.

AKT1, serine/threonine protein kinase 1; HER2-, human epidermal growth factor receptor 2 negative; HR+, hormone receptor positive; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog.
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Most commercially available NGS options include *PIK3CA*, *AKT1*, and *PTEN* alterations

Send-out vs in-house	NGS test panel name	Number of genes	Sample requirements	PI3K/AKT/PTEN pathway biomarkers detected
Send-out	FoundationOne®CDx[1,2]	324	>20% tumor nuclei	*PIK3CA*, *AKT1*, and *PTEN* are detected
	NeoGenomics NeoTYPE® Breast Tumor Profile[3,4]	54		
	NeoGenomics Neo Comprehensive™ Solid Tumor[5]	517		
	Tempus xT[6]	648		
	Caris Life Sciences® Molecular Intelligence® Tumor Profiling[7]	23,000+		
	Labcorp OmniSeq INSIGHT®[8,9]	523	>10% minimum tumor nuclei	
	Quest Diagnostics™ Solid Tumor Core Panel[10]	49	>20% tumor nuclei (>10% minimum)	
In-house	Illumina® TruSight™ Oncology 500[11]	523	40 ng	
	Thermo Fisher Oncomine™ Precision Assay GX[12]	50	10 ng	
	Thermo Fisher Oncomine™ Focus Assay[13]	52	10 ng	*PIK3CA* and *AKT1* are detected

AKT, serine/threonine protein kinase; *AKT1*, serine/threonine protein kinase 1; NGS, next-generation sequencing; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PI3K, phosphoinositide 3-kinase; *PTEN*, phosphatase and tensin homolog.

1. FoundationOne®CDx. Technical Information. Accessed April 17, 2024. https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019S006C.pdf. **2.** FoundationOne®CDx. Specimen Instructions. Accessed April 17, 2024. https://www.foundationmedicine.com/sites/default/files/media/documents/2023-10/F1CDx_Specimen_Instructions%20%283%29.pdf. **3.** Neo Genomics. NeoTYPE® Breast Tumor Profile Test Catalog. Accessed April 17, 2024. https://neogenomics.com/sites/default/files/NeoGenomicsTestCatalog.pdf. **4.** Neo Genomics. Solid Tumor NGS Specimen Requirements. Accessed April 17, 2024. https://neogenomics.com/test-menu/neo-comprehensive-solid-tumor. **5.** Neo Comprehensive – Solid Tumor. Test Menu. Accessed April 17, 2024. https://neogenomics.com/test-menu/neo-comprehensive-solid-tumor. **6.** Tempus Oncology. Specimen Guidelines for Providers. Accessed April 17, 2024. https://www.tempus.com/wp-content/uploads/2022/09/Tempus-Onco_Specimen-Guidelines.pdf. **7.** Caris Life Sciences®. Specimen Preparation Instructions. Accessed April 17, 2024. https://www.carislifesciences.com/wp-content/uploads/2020/08/TN0252-v8_Specimen_Prep_Instructions_hi-rez.pdf. **8.** OmniSeq INSIGHT®. Intended Use & Performance Specifications. Accessed April 17, 2024. https://oncology.labcorp.com/sites/default/files/2022-04/OmniSeq-INSIGHT-INTENDED-US-PERFORMANCE-SPECS.pdf. **9.** OmniSeq INSIGHT®. Gene List. Accessed April 17, 2024. https://oncology.labcorp.com/sites/default/files/2023-03/OmniSeq_gene_list_DX_SS_L26488-1222-2.pdf. **10.** Quest Diagnostics™ Solid Tumor Core Panel Test Detail. Accessed April 17, 2024. https://testdirectory.questdiagnostics.com/test/test-detail/93234/solid-tumor-core-panel?q=93234&cc=MASTER. **11.** TruSight™ Oncology 500 and TruSight Oncology 500 High-Throughput. Accessed April 17, 2024. https://www.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-data-sheet-m-gl-00173/trusight-oncology-500-and-ht-data-sheet-m-gl-00173.pdf. **12.** Thermo Fisher Scientific. Oncomine™ Precision Assay. Accessed April 17, 2024. https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FCSD%2FFlyers%2Foncomine-precision-assay-flyer.pdf. **13.** Thermo Fisher Scientific. Oncomine™ Focus Assay. Accessed April 17, 2024. https://www.thermofisher.com/uk/en/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology/oncomine-focus-assay.html.