Access to prescribing information (via QR code) and adverse event reporting can be found at the end of this document, on page 6.

# Genomic testing for PI3K/AKT pathway alterations in breast cancer

**An introduction and overview of *PIK3CA/AKT1/PTEN* alterations and their detection in hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-) advanced breast cancer (aBC) for healthcare professionals involved in the testing and treatment pathways.**

## Why is the PI3K/AKT pathway important in breast cancer?

*PI3K/AKT/PTEN signalling has an important role in cell proliferation, metabolism and survival, and aberrant activation of this signalling pathway contributes to the pathogenesis of many cancer types, including breast cancer (BC), (Figure 1).*[1]

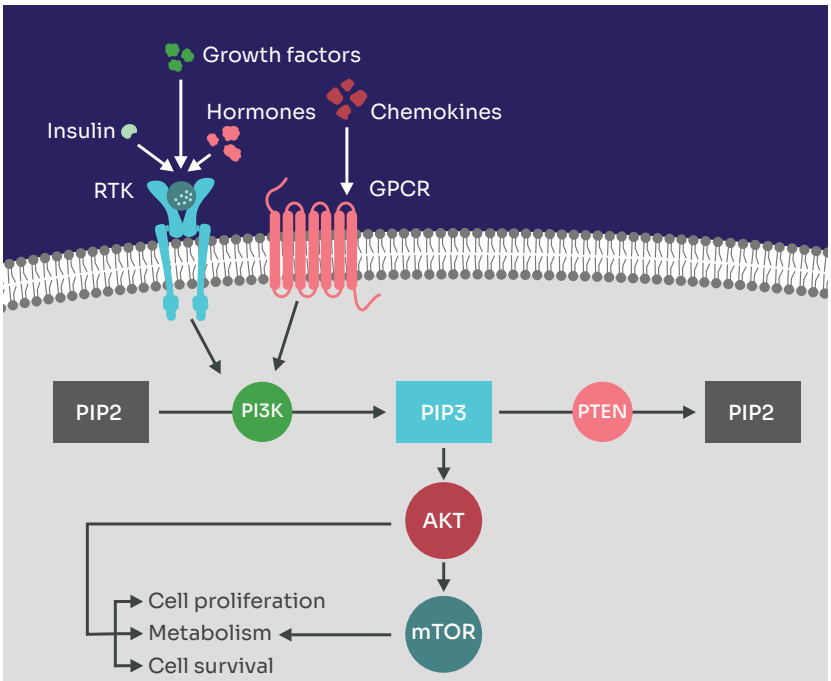
**Aberrant hyperactivation of the PI3K/AKT pathway occurs in >70% of HR+ BC cases.**[2] **Both activating and loss-of-function alterations can lead to dysregulation and hyperactivation of the pathway.**[3–5]

- **Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)** is a key pathway component, transducing signals from activated receptor proteins embedded within the cell membrane to intracellular molecules at the top of the signalling cascade. **Activating** mutations in the ***PIK3CA* gene** (which encodes the PI3K subunit α protein) are the most common genetic alterations in the PI3K/AKT pathway in BC. They can lead to the expression of a constitutively active form of the PI3K protein (Table 1) which drives signalling even in the absence of stimulus from a cell-surface receptor[4,6]

- **AKT serine/threonine kinases (AKT)** (AKT1, AKT2, AKT3) are the downstream effectors of the pathway. **Activating** mutations in the ***AKT1*** gene are known to result in expression of an AKT protein form with aberrant activation (Table 1)[4,6] which stimulates uncontrolled gene expression

- In contrast to the stimulatory roles of **PI3K** and **AKT**, **phosphatase and tensin homologue (PTEN)** plays a controlling role and downregulates signalling via the PI3K/AKT pathway by converting PIP3 back to PIP2. Consequently, loss-of-function alterations in ***PTEN***, which can take many forms and occur throughout the gene, can also lead to pathogenic PI3K/AKT pathway activation[6]

### Figure 1. The PI3K/AKT pathway in breast cancer[7–9]




AKT=AKT serine/threonine kinase; GPCR=G protein-coupled receptor; mTOR=mechanistic target of rapamycin; PI3K=phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP2=phosphatidylinositol 4,5-bisphosphate; PIP3=phosphatidylinositol (3,4,5)-trisphosphate; PTEN=phosphatase and tensin homologue; RTK=receptor tyrosine kinase.

Adapted from: Miricescu D, et al. *Int J Mol Sci.* 2021;22:170.

## Table 1. Frequency of *PIK3CA*/*AKT1*/*PTEN* alterations in HR+/HER2-aBC[9-12]

| Gene | Type of Alteration | Frequency in HR+/HER2- aBC[13-35] |
|---|---|---|
| *PIK3CA* | Activating mutation | ~40% |
| *AKT1* | Activating mutation | ~5% |
| *PTEN* | Loss-of-function mutation / structural variant | ~5% |

**Aberrant PI3K/AKT pathway activation is known to confer resistance to multiple therapies in BC,** including CDK4/6 inhibitors (CDK4/6is), endocrine therapies (ETs) and chemotherapy.[2,5]

- Thus, **inhibition of the PI3K/AKT pathway is a therapeutic target of interest** to overcome resistance to treatment with CDK4/6is and ETs and delay time to chemotherapy
- Genomic variants in the *PIK3CA*, *AKT1*, and PTEN genes are associated with pathogenic over-activation of the signalling pathway.

# When should *PIK3CA*/*AKT1*/*PTEN* alteration testing be carried out in the breast cancer diagnostics pathway?

- Testing for the presence of *PIK3CA*/*AKT1*/*PTEN* alterations should be a **routine aspect of the biomarker status assessment** performed on all cases of HR+/HER2- aBC[39-42]
- The latest guidelines recommend testing for presence of *PIK3CA* or *AKT1* activating mutations or *PTEN* alterations for all cases of HR+/HER2- aBC, to guide treatment options for patients who have progressed on a first-line (1L) aBC therapy (Table 2)[40-43]

**For timely decision-making regarding potential second-line (2L) treatment options, it is advisable to assess the biomarker status for *PIK3CA/AKT1/PTEN* alterations at the diagnosis of HR+/HER2- advanced breast cancer (aBC). However, if the biomarker status has not been established, conducting testing upon disease progression remains an acceptable approach.[44]**

## Table 2. Clinical guidelines for *PIK3CA*/*AKT1*/*PTEN* alteration testing

| Recommended biomarker testing for recurrent unresectable (local or regional) or stage IV (M1) disease in HR+/HER2-patients | | | | |
|---|---|---|---|---|
| | | | NHS England[70] | |
| Alteration | ESMO (Precision Medicine Working Group) HR+/HER2-aBC[36,63] | ASCO (expert panel) aBC[40,64] | NGS tissue bopsy | NGS (liquid biopsy) disease Progression |
| *PIK3CA* | NGS (tissue or plasma) | NGS (tissue, plasma or otherwise) | M3.6 (test code) | M3.13 (test code) |
| *AKT1* | NGS (tissue or plasma) | NGS (tissue, plasma or otherwise) | M3.6 (test code) | M3.13 (test code) |
| *PTEN* | NGS (tissue or plasma) | NGS (tissue, plasma or otherwise) | M3.6 (test code) | M3.13 (test code) |
| *ESR1m* | NGS (tissue or plasma) | NGS (tissue, plasma or otherwise) | | M3.13 (test code) |
| *gBRCA 1/2m* | NGS (tissue or plasma)* | NGS (tissue, plasma or otherwise) | | R444.1 (test code) Germline only |

*Tumour tissue testing detects both somatic and germline BRCA mutations but does not distinguish between them. Reflex germline test is required to determine if the mutation is inherited.[71,72] Triage according to UKCCG guidance and proposed framework.[74,75]

# How to test

## How should testing for *PIK3CA*/*AKT1*/*PTEN* alterations be performed?

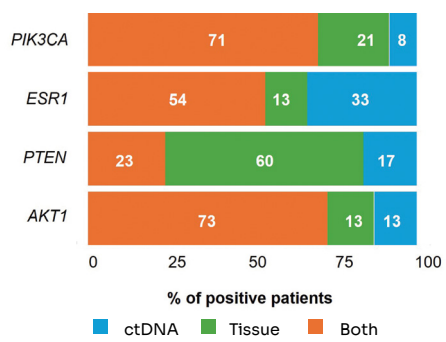
- The prevailing objective of testing for *PIK3CA*/*AKT1*/*PTEN* alterations is to reliably determine biomarker status for as many patients as possible, and hence enable the best decisions to be made regarding the care provided to them.[41,42]
- Ideally, **testing should be performed on samples of tumour tissue obtained by biopsy at the point at which a case of HR+/HER2- aBC is diagnosed.**[44]
- If a sample of tissue from the biopsy at diagnosis of metastatic disease is not available, tissue from an archival sample (such as from a primary tumour resection), or liquid biopsies can be utilised. Current evidence indicates that *PIK3CA* alterations are generally stable from early to metastatic disease, with only a small percentage of cases gaining or losing alterations.[46] The stability of *AKT1* and *PTEN* alterations over time is less well known.[47-55]
- **In order to gain insight into the alteration status of all three genes of interest, analysis of samples using next-generation sequencing (NGS) technology is preferred** (Figure 2).[42] This technique is theoretically capable of detecting all possible actionable gene alterations, although in practice, sensitivity and specificity will vary between assay systems.[56]
- Other genomic analysis technologies such as polymerase chain reaction (PCR) can also be used.[42,57] These tests are only capable of detecting the presence of a limited range of pre-specified molecular alterations,[56] and are hence unlikely to correctly identify all samples which harbour actionable variants, particularly those with *PTEN* loss-of-function alterations which can be highly variable and structurally complex in their nature. Consequently, use of this form of test risks incorrect determination of biomarker status and may in turn lead to suboptimal treatment decisions being made.[58]

### Figure 2. Testing pathway for *PIK3CA*/*AKT1*/*PTEN* analysis[59,60]

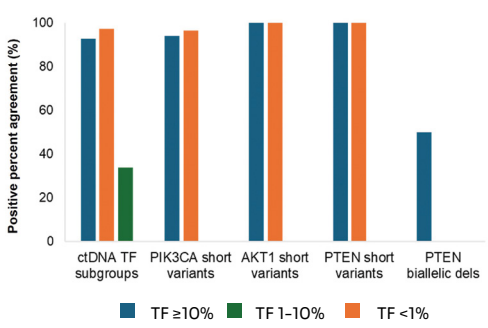

- Testing all tumours for biomarker status is recommended at diagnosis of metastatic disease.[64]
- Liquid biopsy provides a less invasive and effective alternative to tissue biopsy.
- ***Positive ctDNA*** results are ***actionable without the need for tissue confirmation***[65-67]. However, ***negative ctDNA outcomes should be verified with a tissue biopsy*** [68,69]. For confirmatory tissues testing, it is preferable to use most recent biopsy sample or tissue from metastatic diagnosis. In the absence of recent biopsy, archived or early samples can be used since relevant mutations appear early in tumorigenesis (NCCN, ESMO,ASCO).

### Rates of concordance between tumour and ctDNA testing vary between different genomic alterations[72,73]

**Between tumour and ctDNA in aBC, adapted from Tsai C, et al 2024**




**Between liquid and tissue NGS, adapted from Vasan N, et al 2024**



# Where to test

## Genomic testing provisions in UK[45,70]

### England:

- National Genomic Test Directory (England) : https://www.england.nhs.uk/publication/national-genomic-test-directories/

| Test Code | Test Name | Target Gene(s) [essential] | Target Gene(s) Clinical trials | Test Scope | Further Eligibility Criteria Essential targets only |
|---|---|---|---|---|---|
| **M3.6** | Multi-target NGS panel - small variant *(PIK3CA, AKT1, PTEN)* | *PIK3CA, AKT1, PTEN* (SNV & CNV) | SNV: BRAF, ERBB2, MET exon 14 skipping CNV: ERBB2, MET | Small variant detection, copy number variant detection | **Molecular assesment will aid diagnosis** or management for ER positive, HER 2 negative advanced /metastatic BC after endocrine therapy plus CDK 4/6 inhibitor |
| **M3.13** | Multi-target ctDNA NGS panel - small variant *(ESR1,PIK3CA, AKT1, PTEN)* | *ESR1,PIK3CA, AKT1, PTEN* (SNV & CNV) | N/A | Small variant detection, copy number variant detection | **AT PROGRESSION** To guide treatment decisions in patients with ER positive, HER2 negative advanced/ metastatic BC that are progressing on 1L treatment with an aromatase inhibitor plus CDK 4/6 inhibitor |

**ctDNA testing in England** is provided by the North Thames, North West and South East Genomic Laboratory Hubs. Please refer to individual websites for further details. GLH regional maps and QR codes provided on the next page

### Wales:

- All Wales Genomic Medicine Service

  https://medicalgenomicswales.co.uk/index.php/en/download-services

| Clinical Indication Name | Test Scope | Technology | Target Genes |
|---|---|---|---|
| **Breast Cancer** | Multi-target NGS panel -small variant | TSO 500 NGS | *PIK3CA exons 2,3,5,6,8,9, 10 ,14,19, 20& 21 +/-5bp*<br>*AKT1 (exons 4,5,12 +/-5bp)*<br>*PTEN (exons 1-9 +/- 5bp)* |

### Northern Ireland:

- Testing as per National Genomic test Directory, provided via https://belfasttrust.hscni.net/service/laboratory-services/clinical-genetics/common-referrals-and-information-for-patients-professionals/

### Scotland:

- Currently no genomic testing available for *PIK3CA, AKT1, PTEN*. For updated testing information see: https://www.genomics.nhs.scot/test-directories/cancer/

Where solid tumour NGS panel testing identifies any variants which may require further expert clinical discussion, referrers are encouraged to consider bringing these to their regional GLH aligned Genomic Tumour Advisory Board **(GTAB)** meetings.

# Testing roadmap for UK

- Central and South GLH
- East GLH
- North East and Yorkshire GLH
- North Thames GLH
- North West GLH
- South East GLH
- South West GLH
- Scotland
- Wales
- Northern Ireland

Image adapted from NHS England Genomic Laboratory Hubs.[45]

Please note: In some areas there may be local testing hubs. Check locally for test request forms.

Referrals for testing will be triaged by the local genomics services to ensure the most appropriate test is performed. For your local genomics services, please consult the regional map and QR codes.

Below links direct to external websites. AstraZeneca is not responsible for the content or the privacy policy of any third-party websites.

Central and South GLH

East GLH

North East and Yorkshire GLH

North Thames GLH

North West GLH

South East GLH

South West GLH

Scotland

Wales

Northern Ireland

Reach out to us at **AZDiagnostics@astrazeneca.com** if you would like to discuss AKT pathway testing in more details.

## The importance of looking back:

- Check if patients who were previously tested with NGS have a positive *PIK3CA* mutation, *AKT1* mutation or *PTEN* alteration.
- Looking back at the NGS reports might provide additional information on *AKT1/PTEN* status.
- Consult with your genomic testing provider if further information on *AKT1/PTEN* status can be made available from the previous NGS test.

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### Abbreviations

1L= First line; 2L= second line; aBC=Advanced breast cancer; AKT= AKT serine/threonine kinase; BC=breast cancer;CDK4/6i= CDK4/6 inhibitor; CDx= companion diagnostic; dels= deletions; ESMO= European Society for Medical Oncology;ET=endocrine therapy; HER2=human epidermal growth factor receptor 2; HR=hormone receptor; NCCN=National Comprehensive Cancer Network; NGS=next-generation sequencing; PCR=polymerase chain reaction; PI3K=phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN=phosphatase and tensin homologue; RUO=research use only; SNV=single nucleotide variant; TF=Tumor Fraction.

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