Testing for PIK3CA/AKT1/PTEN in Hormone Receptor-Positive HER2-Negative Locally Advanced or Metastatic Breast Cancer

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Contents

1.Objective and scope

This document provides clinical guidance for testing relevant biomarkers for alpelisib and capivasertib eligibility in patients as per NICE recommendations Technology Appraisal (TA816) and (TA11513) to guide treatment decisions for treating hormone receptor-positive, HER2-negative locally advanced or metastatic breast cancer.

2.NICE recommendation for Alpelisib (TA816)

Alpelisib plus fulvestrant is recommended (1) as an option for treating hormone receptor-positive, HER2-negative, PIK3CA-mutated, locally advanced or metastatic breast cancer in adults, only if:
-their cancer has progressed after a CDK4/6 inhibitor plus an aromatase inhibitor

3.NICE recommendation for Capivasertib (TA1063)

Capivasertib, in combination with fulvestrant, is recommended (2) as an option for treating hormone receptor positive, HER2-negative, locally advanced or metastatic breast cancer in adults with:
- recurred or progressed after a CDK 4 and 6 inhibitor plus an aromatase inhibitor
- an alteration in one or more genes in the AKT pathway (PIK3CA, AKT1, or PTEN).

4.Who to test?

Consider testing in all individuals with ER-positive, HER2-negative locally advanced or metastatic breast cancer, including women, trans men, trans women, and non-binary people.

5.When to test?

To allow upfront treatment planning, test at first confirmation of locally advanced or metastatic HR+, HER2- breast cancer. NICE recommend re-biopsy at first presentation of metastatic disease to confirm hormone receptor status, which also provides an opportunity to test for *PIK3CA*/AKT/PTEN variants. Alternatively, consider *PIK3CA/AKT/PTEN* variant testing during first-line (1L) endocrine-based therapy (ahead of anticipated progression) for patients who would be suitable for alpelisib or capivasertib plus fulvestrant treatment.

6.What sample to test?

Ideally, test metastatic tissue from the first relapse biopsy or most recent biopsy. However, samples of bone metastases are not recommended due to poor quality of nucleic acid.
If tissue from the first relapse biopsy or most recent biopsy is not available, archival tissue from the primary tumour can be used.
Formalin-fixed paraffin-embedded (FFPE) samples are acceptable.
The tumour must be confirmed as HR+, HER- prior to requesting *PIK3CA/AKT1/PTEN* genetic testing.
Genomic testing of *PIK3CA/AKT1/PTEN* using circulating tumour DNA (NHSE Cancer Test Directory M3.13) will be available from AWMGS in due course.

7.PIK3CA/AKT/PTEN biomarker test request process

PIK3CA/AKT/PTEN testing is performed by the All Wales Medical Genomics Service (AWMGS).
All requests should be made using the appropriate All Wales Medical Genomics Service (AWMGS) request form which is available at*: www.medicalgenomicswales.co.uk (Health Professional//Which genetic test do you require?//Breast Cancer)* (see appendix). The oncologist should complete the patient demographic information and enter their own name and email address in the appropriate sections before passing the form to the relevant pathology laboratory.

For patients not previously tested for PIK3CA.
AWMGS will require FFPE slides.
Please note: Requests for *PIK3CA/AKT/PTEN* testing should not be made directly to the AWMGS laboratory as samples are not stored here and histopathology services are unavailable in this laboratory.
The referring clinician should contact the relevant histopathology laboratory where the tissue is stored and send a completed AWMGS request form indicating the requirement for *PIK3CA/AKT/PTEN* testing.

- The histopathology laboratory will retrieve the histological specimen and perform slide cutting and tumour assessment.
- The pathology laboratory should complete the remaining fields on the request form and send a paper copy of the form with the prepared slides directly to the AWMGS laboratory within a 5 working day turnaround time.

Histopathological sample preparation requirements for PIK3CA/AKT/PTEN testing
The pathology laboratory storing the patient specimen should prepare the sample as follows before sending the FFPE slides to AWMGS with an appropriately completed request form (see appendix):

- 1 H&E-stained slide with area of highest neoplastic cell content CLEARLY circled.
- 60µM (preferably 6x 10µM) air dried unstained sections mounted on slides.

For patients who have had PIK3CA testing by AWMGS but who now require expanded PIK3CA, AKT1 and PTEN analysis to assess AKT inhibitor response.
Reanalysis requests must be made using the referral form referenced above.
To indicate that you would like reanalysis of a sample previously tested for PIK3CA, please tick 'Previous PIK3CA testing at AWGL (if known) Yes' located under the section for patient and clinician details.

Please send these requests directly to All Wales Genomics Laboratory (address below).

Please note: If RNA NGS for NTRK1/2/3 is required, these referrals will require further FFPE slides; details on sample requirements can be found on the same referral form, but these requests must pass through the relevant pathology laboratory.)

Send samples/forms to:

All Wales Medical Genomics Service
Canolfan Iechyd Genomig Cymru (CIGC)
Cardiff Edge Business Park
Longwood Drive
Whitchurch
Cardiff
CF14 7YU

8.Which PIK3CA/AKT1/PTEN alterations are clinically relevant?

Alpelisib, is a PIK3A inhibitor. PIK3CA activating variants (e.g., H1047R, E545K, E542K) are considered predictive of benefit:
Capivasertib is an AKT inhibitor. . The following genomic alterations are considered predictive of benefit:

- PIK3CA activating variants (e.g., H1047R, E545K, E542K)
- AKT1 activating variants(e.g., E17K)
- PTEN loss-of-function variants or copy number loss

9.Interpreting a PIK3CA/AKT1/PTEN biomarker test result

The All Wales Genomics Laboratory utilises the Illumina TruSight Oncology 500 High Throughput DNA/RNA assay for next generation sequencing using the Illumina NovaSeq 6000TM to identify nucleotide variants and gene rearrangements (fusions) in patient with solid tumours. More information on this service is available here.

Table 1: Trial variants covered by the TSO500DNA breast panel.

Breast Trial Variants		
Codon	**Alpelisib** (SOLAR/BYLieve)- PIK inhibitor	**Capivasertib** (CAPItello/FAKTION)- AKT inhibitor
PIK3CA		
88		Arg88Gln
345		Asn345Lys
420	Cys420Arg	Cys420Arg
542	Glu542Lys	Glu542Lys
545	Glu545**Ala/Gly/Lys/Asp (1635G>T only)**	Glu545**X**
546	Gln546**Arg/Glu**	Gln546**X**
1043		Met1043Val/Ile
1047	His1047**Leu/Arg/Tyr**	His1047**X**
1049		Gly1049Arg
AKT1		
17		Glu17Lys (E17K)
PTEN**		
Any		LoF

The regions included in the TSO500DNA breast panel are: PIK3CA (exons 2, 3, 5, 6, 8, 9, 10, 14, 19, 20, 21 +/-5bp); AKT1 (exons 4, 5, 12 +/-5bp); PTEN (exons 1-9 +/-5bp). These regions include all the hotspot codons listed in table 1 and additional exons have been included in the panel due to their presence in cancerhotspots.org.

****Please note:** the TSO500DNA assay is only validated to detect substitution and small indel variants. Loss of PTEN can also occur by copy number alterations which are not yet validated, so patients with this alteration will not be identified.

The AWMGS report will describe detected variants using HGVS nomenclature. Test interpretation falls into the following categories:

1. Actionable pathway alteration detected

Where a clinically actionable variant has been detected, a statement will be included in the report on the likelihood of response to alpelisib and/or capivasertib. This is based on the clinical trial data referenced in the respective NICE guidance documents (BYLieve (3) and SOLAR-1 (4) for alpelisib, and CAPItello-291 (5) and FAKTION (6) for capivasertib.

a) Likely response to alpelisib and capivasertib. For example:

"Molecular analysis detected PIK3CA c.3140A>G p.(His1047Arg).
Molecular analysis did not detect any variants in AKT1 or PTEN.

The presence of a clinically actionable variant in PIK3CA indicates that this patient is likely to respond to PI3K and AKT inhibitors.
The implication of this result for this patient should be determined in the context of this patient's full clinical details."

b) Likely response to capivasertib, unlikely response to alpelisib. For example:
"Molecular analysis detected AKT1 c.49G>A p.(Glu17Lys).
Molecular analysis did not detect any variants in PIK3CA and PTEN.
The presence of a clinically actionable variant in AKT1 indicates that this patient is likely to respond to AKT inhibitors but is unlikely to respond to PI3K inhibitors.
The implication of this result for this patient should be determined in the context of this patient's full clinical details."

2. No actionable pathway alteration detected

Due to the data from the CAPItello-291 trial (5), it is important to note that in the instance where no actionable variant is detected, patients may still respond to capivasertib. The report may read:
"Molecular analysis did not detect any clinically actionable variants in PIK3CA, AKT1, or PTEN.
Therefore, this patient is unlikely to respond to PI3K inhibitors and treatment with AKT inhibitors is not recommended by NICE.
The implication of this result for this patient should be determined in the context of this patient's full clinical details."

3. Poorly characterised variant detected

Due to the nature of next generation sequencing, there is a possibility testing will detect a variant outside of the expected variants seen in clinical trials. In this instance, an investigation of the variant will be carried out to determine the impact the variant may have on targeted therapies, i.e. alpelisib and capivasertib. Reports will therefore be specific to that variant. It is important to note that the absence of a variant from a clinical trial is not necessarily an indicator for response, but more so an indicator of variants that more commonly occur in breast cancer cohorts and were detected in the trial. Unfortunately, there is little data or evidence for variants outside of these trials at present, and so therapeutic significance of these variants is likely to be uncertain

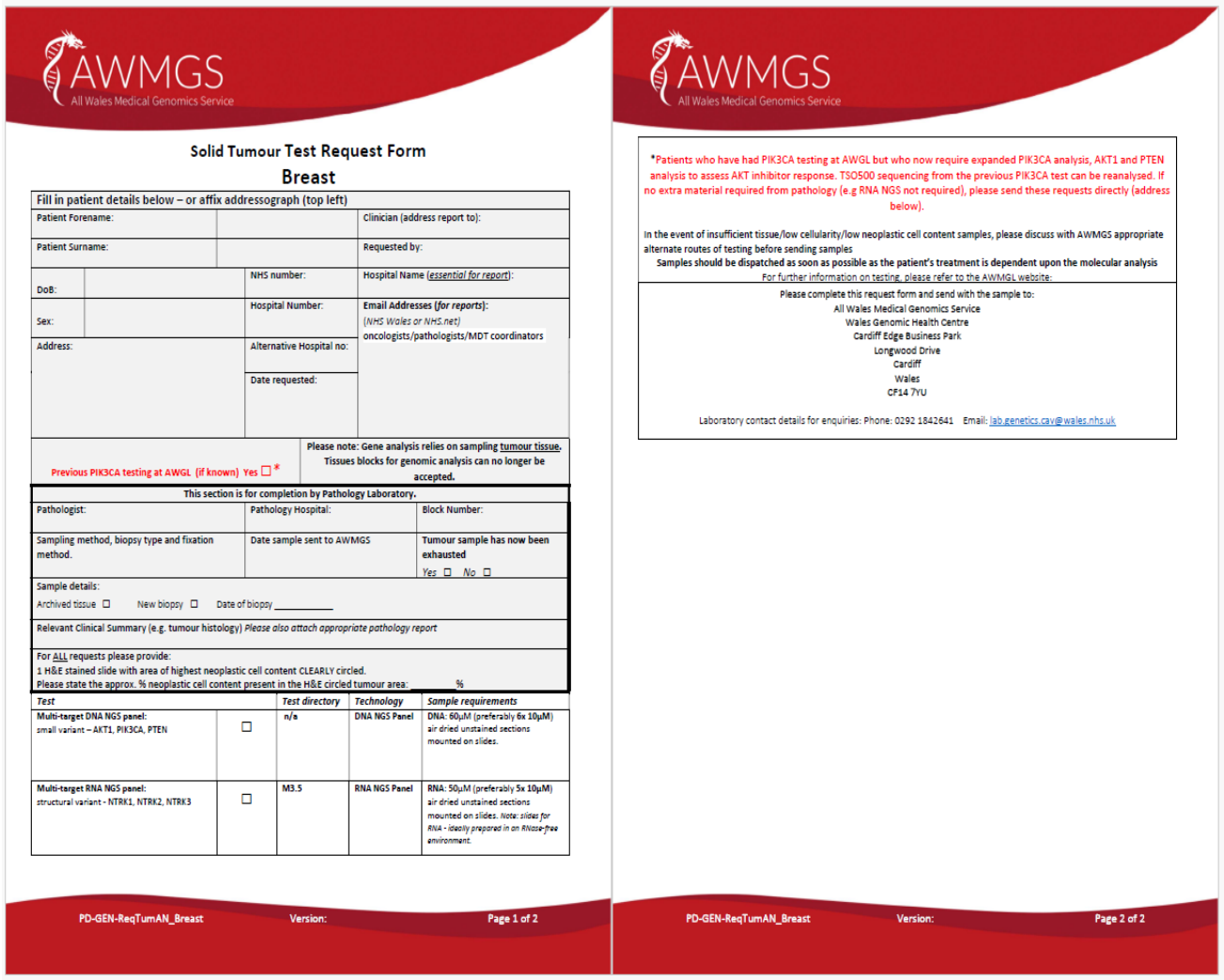
Consent for genetic testing and DNA storage is assumed when a test request and samples are received.

9.References

1. https://www.nice.org.uk/guidance/ta816
2. https://www.nice.org.uk/guidance/ta11513/
3. Rugo HS, Lerebours F, Ciruelos E, et al. Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. Lancet Oncol. 2021;22(4):489-498. doi:10.1016/S1470-2045(21)00034-6

4. André F, Ciruelos EM, Juric D, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol. 2021;32(2):208-217. doi:10.1016/j.annonc.2020.11.011
5. Turner NC, Oliveira M, Howell SJ, et al. Capivasertib in Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2023;388(22):2058-2070. doi:10.1056/NEJMoa2214131
6. Jones RH, Casbard A, Carucci M, et al. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive breast cancer (FAKTION): a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol. 2020;21(3):345-357. doi:10.1016/S1470-2045(19)30817-4

10.Appendix: Solid Tumour Request form (breast)



AWMGS
All Wales Medical Genomics Service

Solid Tumour Test Request Form
Breast

Fill in patient details below – or affix addressograph (top left)			
Patient Forename:			Clinician (address report to):
Patient Surname:			Requested by:
DoB:		NHS number:	Hospital Name (*essential for report*):
Sex:		Hospital Number:	**Email Addresses (*for reports*):** *(NHS Wales or NHS.net)* oncologists/pathologists/MDT coordinators
Address:		Alternative Hospital no:	
		Date requested:	

Previous PIK3CA testing at AWGL (if known) Yes ☐ *

Please note: Gene analysis relies on sampling tumour tissue. Tissues blocks for genomic analysis can no longer be accepted.

This section is for completion by Pathology Laboratory.

Pathologist:	Pathology Hospital:	Block Number:
Sampling method, biopsy type and fixation method.	Date sample sent to AWMGS	**Tumour sample has now been exhausted** *Yes* ☐ *No* ☐

Sample details:
Archived tissue ☐ New biopsy ☐ Date of biopsy _____

Relevant Clinical Summary (e.g. tumour histology) *Please also attach appropriate pathology report*

For ALL requests please provide:
1 H&E stained slide with area of highest neoplastic cell content CLEARLY circled.
Please state the approx. % neoplastic cell content present in the H&E circled tumour area: _____%

Test		*Test directory*	*Technology*	*Sample requirements*
Multi-target DNA NGS panel: small variant – AKT1, PIK3CA, PTEN	☐	n/a	DNA NGS Panel	DNA: 60µM (preferably 6x 10µM) air dried unstained sections mounted on slides.
Multi-target RNA NGS panel: structural variant - NTRK1, NTRK2, NTRK3	☐	M3.5	RNA NGS Panel	RNA: 50µM (preferably 5x 10µM) air dried unstained sections mounted on slides. *Note: slides for RNA - ideally prepared in an RNase-free environment.*

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*Patients who have had PIK3CA testing at AWGL but who now require expanded PIK3CA analysis, AKT1 and PTEN analysis to assess AKT inhibitor response. TSO500 sequencing from the previous PIK3CA test can be reanalysed. If no extra material required from pathology (e.g RNA NGS not required), please send these requests directly (address below).

In the event of insufficient tissue/low cellularity/low neoplastic cell content samples, please discuss with AWMGS appropriate alternate routes of testing before sending samples

Samples should be dispatched as soon as possible as the patient's treatment is dependent upon the molecular analysis

For further information on testing, please refer to the AWMGL website:

Please complete this request form and send with the sample to:
All Wales Medical Genomics Service
Wales Genomic Health Centre
Cardiff Edge Business Park
Longwood Drive
Cardiff
Wales
CF14 7YU

Laboratory contact details for enquiries: Phone: 0292 1842641 Email: lab.genetics.cav@wales.nhs.uk

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