PIK3CA Testing for Patients with HR+/HER2- Advanced Breast Cancer

What role does PIK3CA mutation play in breast cancer?

Mutations in the PIK3CA gene can act as cancer drivers and are found in ~40% of hormone receptor-positive (HR+) breast cancer cases. They are the most common mutations in HR+ breast cancer and have been associated with a poor prognosis.

Why should I test for PIK3CA mutation in my HR+/HER2- breast cancer cases?

Until 2019, there was no targeted therapy available for patients with advanced or metastatic HR+/HER2- breast cancer. PIQRAY® (alpelisib) is the first and only FDA-approved targeted therapy for patients with HR+/HER2- advanced or metastatic breast cancer who have progressed on endocrine therapy and whose tumors have a PIK3CA mutation. Only patients with a PIK3CA mutation are eligible for biomarker-driven therapy with alpelisib.

Is there a companion diagnostic test for PIQRAY?

Yes, QIAGEN's therascreen® PIK3CA RGQ PCR is an FDA-approved companion diagnostic test. NeoGenomics provides this test under the name PIK3CA Mutation CDx and it is available through an exclusive Companion Diagnostic Testing Program.

What is the Companion Diagnostic Testing Program?

The Companion Diagnostic Testing Program is offered exclusively through NeoGenomics and is designed to ensure access to testing for patients who may be eligible for alpelisib. Enrolled patients may receive one PIK3CA Mutation CDx by tissue and/or plasma (blood) test at no cost. Repeat tests for monitoring purposes are not accepted in this program. The Companion Diagnostic Testing Program is sponsored by Novartis Pharmaceuticals Corporation.

What if no tissue is available? Can I submit blood or plasma for liquid biopsy testing?

PIK3CA Mutation CDx – Plasma testing is appropriate when no primary or metastatic breast tumor tissue is available, or the only available tissue is from a tumor in the bone and therefore unsuitable for molecular testing. Tissue is the recommended specimen type in all other cases.

Clients who require plasma testing for patients with available tissue specimens may order plasma testing concurrently with tissue testing, or plasma testing followed by tissue testing if the plasma result is negative. The Companion Diagnostic Testing Program will cover testing for one such plasma and tissue specimen pair.

How do I order the test and access the Companion Diagnostic Testing Program?

The ordering health care provider must complete and sign the appropriate PIK3CA Mutation CDx Test Request Form (for either Tissue or Plasma) and submit it to NeoGenomics, or complete the information and attestation in NeoGenomics' online ordering system. Completion of this form is required and submission also enrolls the patient in the Companion Diagnostic Testing Program.

The Test Request Form for Tissue is available here. If tests in addition to PIK3CA Mutation CDx are required on the submitted tissue specimen, please also complete a separate standard, printed NeoGenomics test requisition or create a new accession in online ordering.

The Test request Form for Plasma is available directly from Client Services at 866-776-5907, option 3, after verbal review of specimen processing requirements.

What are the specimen requirements for tissue?

NeoGenomics offers PIK3CA testing on formalin-fixed paraffin-embedded (FFPE) tumor resection or biopsy samples. Breast tumor tissue (either primary or metastatic) is required; non-breast tumors are not accepted for this test. The paraffin block is preferred. Alternatively, send 1 H&E slide plus 6–12 unstained slides for core needle biopsy (or 5–10 slides for resection) cut at 5 microns. Please use positively-charged slides and 10% NBF fixative. Do not use zinc fixatives or send decalcified specimens.

What are the specimen requirements for plasma (liquid biopsy)?

Requirements for collection, processing, and shipping plasma samples are very precise to maximize detection of circulating tumor DNA (ctDNA) in the patient's plasma. NeoGenomics will provide a custom shipping container,

collection tubes, and detailed instructions after telephone review and prequalification of their account. Requirements include:

- Processing of blood specimens within 4 hours of collection
- High-speed centrifuge and ability to transfer plasma to new tubes
- Low-temperature freezer (-70°C to -90°C)
- Access to dry ice for shipment to NeoGenomics

Please call Client services at **866-776-5907, option 3**, to prequalify before scheduling your patient's blood draw.

What is the positive yield of the plasma test?

Overall, there is a chance of approximately 80–90% that a plasma sample result will be negative. About 60% of results are true negatives as the PIK3CA mutation frequency in tissue is approximately 40%. Plasma specimens tested in the SOLAR-1 study showed correlation with mutation-positive paired tumor specimens in approximately 55% of cases. Other studies support specimen handling and low or absent tumor DNA shedding as significant contributors to discrepancies between positive tissue and negative plasma status.

The low expected positive rate in plasma reinforces the importance of testing tissue whenever tissue is available.

Who should get tested, and when?

The test is intended to identify PIK3CA mutation in HR+/HER2- male or post-menopausal female breast cancer patients with advanced or metastatic disease that progressed on or after endocrine therapy. Testing is appropriate when patients present with metastases. Tissue testing is preferred over plasma testing.

Which HR/HER2 biomarker profiles qualify?

Participation in the Companion Diagnostic Testing Program is available to patients whose tumors are IHC positive for either or both estrogen receptor (ER) and progesterone receptor (PgR or PR) AND negative for HER2 by IHC, ISH, or FISH.

What are technical details of the test?

PIK3CA Mutation CDx runs on the QIAGEN RotorGene Q MDx platform using the therascreen® PIK3CA RGQ PCR Kit. This is a real-time qualitative PCR assay for the detection of 11 mutations in the PIK3CA gene using genomic DNA extracted from FFPE breast tumor tissue or DNA extracted from cell-free peripheral blood plasma. Mutations detected are in exon 7 (C420R), exon 9 (E542K, E545A, E545D [c.1635G>T only], E545G, E545K, Q546E, Q546R), and exon 20 (H1047L, H1047R, H1047Y).

When can I expect the results?

Results are typically ready in 7 days from the time the specimen is received at NeoGenomics lab and will be sent to the ordering physician's office or laboratory by their preferred method of delivery. Please add at least 6 days if NeoGenomics is retrieving tissue from another location.

What if I get a bill?

No patient, health care program, or beneficiary shall be billed for this mutation test. If you should receive a bill, please contact the NeoGenomics Billing Department at **866-776-5907, option 2**.

Who should I contact if I have a question?

If you have questions about our testing process, please contact NeoGenomics Client Services at **866.776.5907** or **client.services@neogenomics.com**.

Further information about PIQRAY and PIK3CA is available at www.us.piqray.com and www.pik3ca-cdx.com.

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