The Breast 87 (2026) 104786

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast

Integrating *PIK3CA* Testing into Clinical Practice for Advanced HR+/HER2- Breast Cancer: An Expert Consensus

Carmine De Angelis [a,b,*,1], Dario de Biase [c,d,1], Lorenzo Gerratana [e,f,1], Grazia Arpino [g,h], Giampaolo Bianchini [i,j], Isabella Castellano [k,l], Giuseppe Curigliano [m,n], Lucia Del Mastro [o,p], Alessandra Fabi [q], Nicola Fusco [r,n], Alessandra Gennari [s,t], Valentina Guarneri [u,v], Claudio Zamagni [w], Alberto Zambelli [x,y], Umberto Malapelle [z,1], Fabio Puglisi [e,f,1]

[a] *Department of Breast and Thoracic Oncology, Istituto Nazionale Tumori IRCCS – Fondazione G. Pascale, Via Mariano Semmola, 52, Napoli 80131, Italy*
[b] *Clinical and Translational Oncology, Scuola Superiore Meridionale, Naples, Italy*
[c] *Solid Tumor Molecular Pathology Laboratory, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy*
[d] *Department of Pharmacy and Biotechnology (FaBit), University of Bologna, Bologna, Italy*
[e] *Department of Medical Oncology, Centro di Riferimento Oncologico di Aviano (CRO), National Cancer Institute, IRCCS, Aviano, Italy*
[f] *Department of Medicine, University of Udine, Udine, Italy*
[g] *Dipartimento di Medicina Clinica e Chirurgia Federico II, Naples, Italy*
[h] *Institute for Cancer Research and Molecular Medicine, Temple University, Philadelphia, USA*
[i] *Department of Medical Oncology, IRCCS Ospedale San Raffaele, Milan, Italy*
[j] *Università Vita-Salute San Raffaele, Milan, Italy*
[k] *Pathology Unit, Department of Medical Sciences, City of Health, Turin, Italy*
[l] *Science University Hospital, University of Turin, Turin, Italy*
[m] *European Institute of Oncology, IRCCS, Milan, Italy*
[n] *Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy*
[o] *IRCSS Ospedale Policlinico San Martino, UO Clinica Oncologia Medica, Genoa, Italy*
[p] *Department of Internal Medicine and Medical Specialties (DIMI), Università di Genova, Genoa, Italy*
[q] *Precision Medicine Unit in Senology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[r] *Division of Pathology, European Institute of Oncology IRCCS, Milan, Italy*
[s] *Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy*
[t] *Division of Medical Oncology, Maggiore University Hospital, Novara, Italy*
[u] *Oncology 2, Veneto Institute of Oncology, IOV-IRCCS, Padua, Italy*
[v] *Department of Surgery, Oncology, and Gastroenterology, University of Padua, Padua, Italy*
[w] *IRCCS Azienda Ospedaliero Universitaria di Bologna, Bologna, Italy*
[x] *Oncology Unit, ASST Papa Giovanni XXIII, Bergamo, Italy*
[y] *Department of Medicine and Surgery, Università Degli Studi di Milano Bicocca, Milan, Italy*
[z] *Department of Public Health, University Federico II of Naples, Naples, Italy*

ARTICLE INFO

Keywords:
HR+/HER2− breast cancer
PIK3CA
Liquid biopsy
NGS
Expert consensus

ABSTRACT

Background: Alterations in the PI3K/AKT/mTOR signaling pathway represent a mechanism of resistance to endocrine therapy in advanced HR+/HER2-breast cancer. The identification of activating mutations in *PIK3CA* has gained therapeutic relevance, guiding the use of PIK3CA-targeted inhibitors such as alpelisib and inavolisib.
Methods: A multidisciplinary panel of Italian experts conducted a structured consensus process to develop shared recommendations for molecular testing of *PIK3CA* in advanced HR+/HER2-breast cancer, addressing pre-analytical, analytical, and clinical-therapeutic areas of concern.
Results: A total of 23 out of 29 statements reached complete agreement (100%). The resulting recommendations encompass sample selection, analytical methodologies, sensitivity thresholds, result reporting, and clinical interpretation and integration. Additional guidance is provided for the management of non-canonical gene variants and for other genes involved in the PI3K/AKT/mTOR pathway.

* Corresponding author. Clinical and Translational Oncology (CTO), Scuola Superiore Meridionale Via Mezzocannone, 4 – 80134, Naples, Italy.
E-mail addresses: c.deangelis@ssmeridionale.it, c.deangelis@pec.ssmeridionale.it (C. De Angelis).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.breast.2026.104786
Received 12 February 2026; Received in revised form 1 April 2026; Accepted 9 April 2026
Available online 10 April 2026

Conclusions: This consensus document provides operational and interpretative guidelines aimed at standardizing *PIK3CA*testing and assessing related genes in clinical practice. These recommendations promote a harmonized approach to patient care, in line with the latest scientific evidence.

Abbreviations

AKT	protein kinase B
CDK4/6	cyclin dependent kinases 4/6
cfDNA	cell-free DNA
CI	confidence interval
ClinVar	Clinical Variants
CNV	copy number variations
COSMIC	Catalogue of Somatic Mutations in Cancer
ctDNA	circulating tumor DNA
EDTA	ethylenediaminetetraacetic acid
ERD	estrogen receptor degrader
ESCAT	ESMO Scale for Clinical Actionability of Molecular Targets
ESMO	European Society for Medical Oncology
ESR1	estrogen receptor 1
HGNC	HUGO Gene Nomenclature Committee
HGVS	Human Genome Variation Society
HR	hazard ratio
HR+/HER2	hormone receptor-positive/human epidermal growth factor receptor 2-negative
IHC	immunohistochemistry
LOD	limit of detection
mTOR	mammalian target of rapamycin
NCCN	National Comprehensive Cancer Network
NGS	next-generation sequencing
OncoKB	Oncology Knowledge Base
ORR	objective response rate
OS	overall survival
PFS	progression-free survival
PI3K	phosphoinositide 3-kinase (also known as phosphatidylinositol 3-kinase)
PI3K/AKT/mTOR	phosphatidylinositol 3-kinase/protein kinase B/ mammalian target of rapamycin
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PTEN	phosphatase and tensin homolog
qRT-PCR	quantitative Reverse Transcription Polymerase Chain Reaction
SC	steering committee
SERD	selective estrogen receptor degrader
TCGA	The Cancer Genome Atlas
VAF	variant allele frequency

Glossary

cfDNA	circulating cell-free DNA refers to short DNA fragments released into the bloodstream from normal and pathological cells that can be analyzed for diagnostic, prognostic, and monitoring purposes
ctDNA	circulating tumor DNA is the fraction of cell-free DNA in the bloodstream that originates from tumor cells and carries tumor-specific genetic and epigenetic alterations
LOD	Limit Of Detection is the lowest variant allele fraction of a tumor-derived molecular alteration in ctDNA/cfDNA that an assay can reliably detect with a predefined level of confidence, reflecting the analytical sensitivity of the method
NGS	Next-Generation Sequencing, a high-throughput sequencing technology that enables the simultaneous, massively parallel sequencing of millions of nucleic acid fragments with high sensitivity and scalability

1. Introduction

Breast cancer is one of the most prevalent and widely studied cancers in women worldwide. Over the past few decades, the gradual implementation of precision oncology has profoundly transformed both the prognosis and therapeutic approach for this disease. Within this evolving landscape, the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway–a key regulatory node for cell survival and proliferation–has emerged as a critical axis in breast cancer pathogenesis, progression, and resistance to endocrine and targeted therapies.

Among the molecular alterations affecting this pathway, activating mutations in the *PIK3CA* gene, which encodes the p110α subunit of PI3K, represent the most frequent event, particularly in hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-) breast cancer. Therefore, these mutations constitute both a therapeutic target and a potential predictive biomarker of response to PI3K inhibition.

Molecular characterization of intracellular signaling pathways has profoundly influenced the management of advanced HR+/HER2-breast cancer. The PI3K/AKT/mTOR pathway, in addition to being subject to mutations in *PIK3CA*, is also commonly altered through mutations in *AKT1*, and loss of function of the tumor suppressor*PTEN* [1–3].

Clinically, the presence of *PIK3CA* mutations has been correlated with an increased likelihood of response to PI3K inhibitors in patients with HR+/HER2-metastatic breast cancer, consolidating the importance of genetic testing for identifying eligible patients for targeted therapy [4].

PIK3CA mutations are detected in approximately 35-40% of HR+/HER2-tumors and, in addition to their potential prognostic value, serve as predictive biomarkers for the use of specific PI3K inhibitors, such as alpelisib and, more recently, inavolisib, in combination with endocrine therapy [5–7].

Advances in next-generation sequencing (NGS) and liquid biopsy techniques have enabled a more comprehensive and dynamic assessment of the tumor mutational profile, even in advanced or treatment-refractory settings. Traditionally, *PIK3CA* testing has relied on DNA extracted from biopsy or surgical tissue, which allows direct typing of mutations with high sensitivity and specificity, provided that the sample is of sufficient quality. However, tissue-based testing has inherent limitations, including intratumoral heterogeneity, the availability of material – particularly in metastatic or relapsed disease – and the inability to capture the temporal molecular evolution of the tumor.

To overcome these limitations, liquid biopsy, especially the analysis of circulating tumor DNA (ctDNA), has emerged as a complementary or alternative approach. This minimally invasive technique enables serial monitoring and offers the potential to reflect the dynamic clinical evolution of the tumor, including the emergence of resistance mechanisms. Recent studies have demonstrated the feasibility of detecting *PIK3CA* mutations in ctDNA, which show good concordance with tissue results

and predictive value for treatment response and prognosis. In accordance with the European Society for Medical Oncology (ESMO) guidelines, testing for circulating mutations (e.g., *PIK3CA* and *ESR1*) is already recommended in selected clinical cases [8].

Despite the availability of international recommendations, substantial variability persists in the application of molecular testing, particularly in preanalytical procedures, sample selection, and reporting standards. To address these critical issues, a national multidisciplinary panel of pathologists, molecular biologists, and oncologists convened to develop a consensus document based on the latest scientific evidence and the collective experiences of Italian reference laboratories.

This article presents 23 expert recommendations aimed at providing practical, harmonized guidelines for the analysis of *PIK3CA* in advanced HR+/HER2-breast cancer, thereby supporting the implementation of personalized diagnostic and therapeutic approaches in clinical practice.

2. Methods

This research project employed a modified Delphi method –a structured, interactive approach designed to achieve expert consensus [9,10], involving a panel of pathologists, molecular biologists, and oncologists specializing in breast cancer to establish a consensus on unresolved issues regarding the role of *PIK3CA* testing in the diagnosis and management of HR+/HER2-metastatic breast cancer.

The panel comprised two supervisors (one oncologist and one pathologist), a steering committee (SC) (two oncologists and one molecular pathologist), and 11 nationally and internationally renowned breast cancer experts affiliated with tertiary care centers. Experts were selected based on predefined criteria, including recognized clinical or research expertise in breast cancer, authorship of peer-reviewed publications, and experience in the management of HR+/HER2− metastatic breast cancer. Efforts were made to ensure geographic diversity. All participants were invited by the supervisors and agreed to participate voluntarily.

During the initial meeting (March 2025), the SC drafted and validated a set of research questions that formed the basis for the literature review. The outputs of these analyses, combined with the SC's clinical experience, formed the basis for the first draft of the statements. A total of 29 preliminary statements were generated and subsequently reviewed by the supervisors.

Between June and September 2025, two Delphi rounds were conducted to evaluate and refine the statements, using an anonymised, blinded electronic voting platform (e-survey) (summary in Supplementary Table 1).

Consensus was defined a priori as at least 80% of responding Delphi panelists rating a statement as 'agree' or 'strongly agree' (score $\geq$7 on a 9-point Likert scale where 1 = no agreement, 9 = complete agreement). Statements that scored a median of 7 or above in the first round without any comments were considered approved. Statements that did not reach this threshold were amended based on participant feedback. Qualitative feedback provided by participants was systematically reviewed by the steering committee. Statements were revised iteratively to improve clarity and address areas of disagreement. Revised statements were redistributed in subsequent rounds for re-evaluation. Amended statements scoring 7 or above in the second round, receiving no further comments, were deemed to have reached consensus. All invited panelists participated in both Delphi rounds.

A virtual consensus meeting was held on October 1, 2025, to discuss the results of the second Delphi round. Statements that did not achieve consensus in the second round were amended in real time as needed before a final round of voting.

All statements that successfully reached a consensus are included in the final list.

No statements were excluded due to lack of consensus among panelists (see Supplementary Table 1). However, during the iterative Delphi process, some statements were removed because they were deemed redundant or overlapping with other statements after refinement and discussion.

A schematic summary of the entire process is presented in Fig. 1, and detailed voting results from each Delphi round are provided in Supplementary Table 1.

3. Results

3.1. Statement 1

Question: In patients with advanced HR+/HER2-breast cancer, what is the clinical relevance of *PIK3CA* mutations in relation to prognosis and response to endocrine therapy?

3.1.1. Recommendation

The presence of *PIK3CA* mutations has been associated with decreased efficacy of endocrine therapy in certain studies, reflecting their role in endocrine resistance. However, with the advent of PI3K pathway inhibitors, these mutations have acquired a positive predictive value, serving as clinically actionable biomarkers that identify patients who are most likely to benefit from targeted therapeutic interventions.

3.1.2. Comment

Activating mutations in *PIK3CA* represent an early event in breast tumorigenesis, conferring a proliferative advantage through AKT and mTOR activation. Although initially associated with endocrine resistance and poor prognosis, the availability of selective PI3K inhibitors, such as alpelisib and inavolisib, has transformed these alterations into clinically actionable predictive biomarkers [1–3]. Their detection is crucial for optimizing patient selection for combination therapies targeting the estrogen receptor and PI3K pathways.

3.2. Statement 2

Question: Which biological matrix should be used for*PIK3CA*testing?

3.2.1. Recommendation

Tumor tissue is the biological material of choice for *PIK3CA* testing; however, liquid biopsy may be a valid alternative method. In the case of a negative liquid biopsy result, a tissue-based analysis is recommended.

3.2.2. Comment

Tumor tissue remains the preferred matrix because it generally provides a higher DNA quantity and quality. Nonetheless, liquid biopsy offers a minimally invasive and repeatable option, particularly when tumor tissue is unavailable or unrepresentative. This approach enables dynamic monitoring of disease evolution and the emergence of resistant clones [6,7]. Given that a negative plasma result does not rule out the presence of *PIK3CA* mutations, whenever possible, tissue confirmation is mandatory in accordance with the international guidelines [11].

3.3. Statement 3

Question: Is it necessary to identify *PIK3CA* variants in both biological matrices (i.e., tissue and liquid biopsy)?

3.3.1. Recommendation

The identification of an activating *PIK3CA*mutation in at least one of the matrices (tissue or liquid biopsy) is sufficient to confirm the presence of molecular alterations.

3.3.2. Comment

The results obtained from tissue and plasma analyses are generally concordant; however, discrepancies may occur due to tumor heterogeneity or differences in analytical sensitivity between methods. The

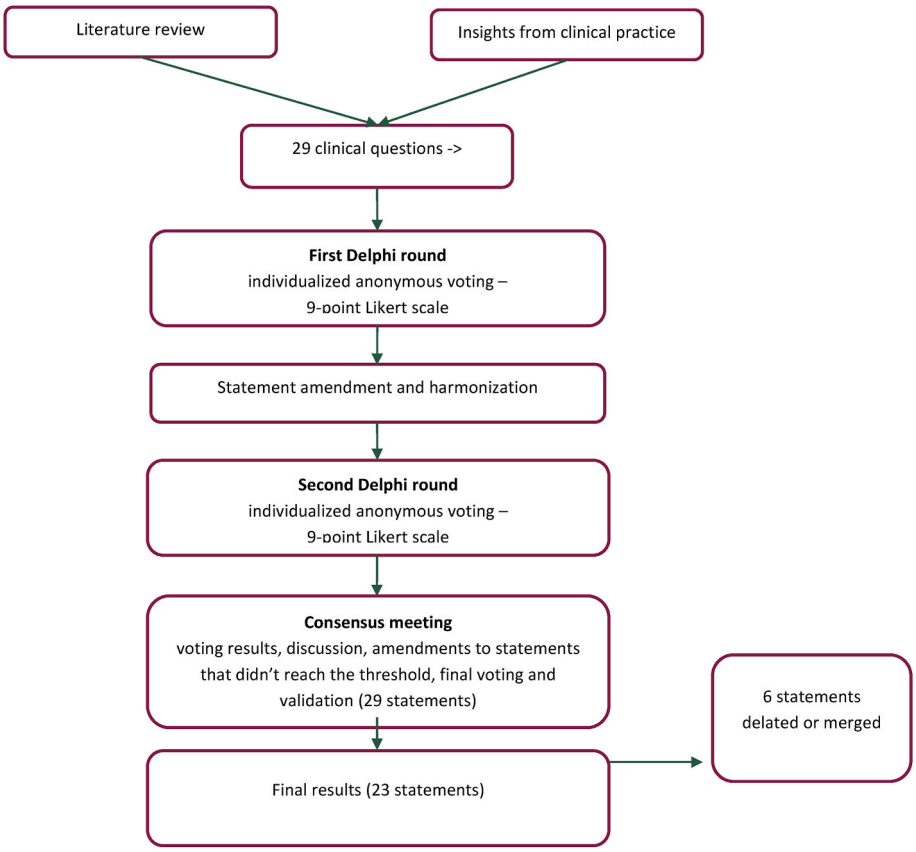

Fig. 1. Consensus flow.

detection of a PIK3CA mutation in either matrix is clinically significant and sufficient to determine eligibility for treatment with PI3K inhibitors [11].

3.4. Statement 4

Question: Which regions of the *PIK3CA* gene should be analyzed?

3.4.1. Recommendation

Although activating mutations occur mainly in exons 9 (E542K, E545K) and 20 (H1047 R/L), activating variants have also been described in exons 1, 2, 5, and 7. Therefore, analytical methods that cover the entire coding sequence of *PIK3CA* (exons 1–20) are preferable.

3.4.2. Comment

Broadening the analytical target is crucial for maximizing the clinical sensitivity of the assay. Non-canonical variants, such as *PIK3CA* p.R88Q or p.N345K, are functionally activating in clinical databases (Catalogue of Somatic Mutations in Cancer (COSMIC), The Cancer Genome Atlas (TCGA), Oncology Knowledge Base (OncoKB) [12–14]. In the registration trials, *PIK3CA* analyses were performed using: i) in the SOLAR-1 trial, real-time PCR kit was used, allowing the detection of 11 *PIK3CA* mutation hotspots [1]; ii) in the INAVO120 study, *PIK3CA* variants were assessed using a sponsor-approved polymerase chain reaction (PCR)-based or next-generation sequencing (NGS) assay [15]; iii) in the CAPItello study, *PIK3CA* variants were assessed using NGS [16]. Although polymerase chain reaction (PCR)-based methods were used in two registration trials, the use of NGS panels covering the entire coding region of *PIK3CA* enables the comprehensive characterization and accurate identification of patients eligible for PI3K inhibitor therapy. Because exon numbering may vary depending on the reference sequence used for alignment (e.g., exon 9 or exon 10, exon 20 or exon 21), all variants should be reported following the HUGO Gene Nomenclature Committee (HGNC) recommendations [17].

3.5. Statement 5

Question: What limit of detection (LOD) is required for implementing *PIK3CA* testing using liquid biopsy?

3.5.1. Recommendation

The assay should ensure an LOD of $\leq$0.5%. In the event of a negative result, testing should be performed on tumor tissue.

3.5.2. Comment

The analytical sensitivity of the liquid biopsy assay is critical for accurate *PIK3CA* testing. A sensitivity threshold of $\leq$0.5% enables the reliable identification of low variant allele frequency (VAF) mutations that may have clinical relevance [18]. Protocol standardization and validation of the assay LOD are essential to minimize false-negative results, particularly in patients with low tumor cell-free DNA (ctDNA) levels.

3.6. Statement 6

Question: Should *PIK3CA* testing be performed on metastatic lesions or primary tumors during tissue analysis?

3.6.1. Recommendation

As*PIK3CA*mutations are early molecular events, testing can be performed on either primary or metastatic samples. However, when available, the analysis of the most recent metastatic sample with adequate tumor cellularity is preferred.

3.6.2. Comment

PIK3CA mutations are generally maintained throughout clonal evolution, with high temporal concordance between primary tumors and metastatic lesions [19,20]. Nonetheless, testing the most recent specimen ensures greater biological representativeness and increases the likelihood of detecting co-occurring mutations associated with acquired therapeutic resistance. In the absence of a recent metastatic sample, primary tumor analysis remains appropriate.

3.7. Statement 7

Question: What is the relevance of the preanalytical phase in the context of liquid biopsy?

3.7.1. Recommendation

The preanalytical phase is crucial and must comply with the best practices defined at the national and international level [21–24].

3.7.2. Comment

Optimal sample handling is essential for preserving cfDNA quality. When standard ethylenediaminetetraacetic acid (EDTA) tubes are used, plasma must be separated within 2 h of blood collection. Using specialized cfDNA preservation tubes extends this window to up to 72 h. Plasma should be stored at −20 °C (for up to one month) or at −80 °C for longer periods [22]. Deviations from these parameters can result in cfDNA degradation and a loss of analytical sensitivity. Therefore, strict adherence to preanalytical standards is indispensable for ensuring the diagnostic reliability of liquid biopsy assays.

3.8. Statement 8

Question: Which analytical method should be used to detect pathogenic variants of *PIK3CA*?

3.8.1. Recommendation

NGS technologies are preferred owing to their superior ability to detect multiple variants and simultaneously analyze other relevant breast cancer biomarkers.

3.8.2. Comment

Compared with other techniques (e.g., quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), Sanger sequencing), NGS provides broader genomic coverage and enables the simultaneous detection of concomitant mutations, including those in the *AKT1* and *PTEN* genes. NGS also offers higher analytical sensitivity and specificity, thereby reducing the risk of false negatives [25,26]. The implementation of validated NGS protocols coupled with interlaboratory quality control represents the gold standard for the molecular characterization of HR+/HER2-breast cancer.

3.9. Statement 9

Question: How should *PIK3CA* variants be reported in molecular pathology reports?

3.9.1. Recommendation

Variants must be described according to the HGNC and Human Genome Variation Society (HGVS) nomenclature, specifying the VAF, functional effect, and level of clinical significance according to the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) [27,28].

3.9.2. Comment

A standardized reporting format is essential for ensuring interpretative consistency and clinical transparency. Including the VAF provides information on the representativeness of the variant and facilitates its clinical interpretation. The ESCAT classification allows grading of the level of clinical evidence associated with the therapeutic target [27,28]. Integrating these elements into the report strengthens communication between molecular laboratories and clinicians, supporting evidence-based therapeutic decision-making.

3.10. Statement 10

Question: At what point in the therapeutic pathway should *PIK3CA* testing be recommended?

3.10.1. Recommendation

PIK3CA testing should be performed in all patients who are candidates for treatment with PI3K pathway inhibitors. [see also Statement 11 for specific recommendations regarding the timing of testing].

3.10.2. Comment

Early molecular testing enables timely therapeutic stratification and prevents delays in the initiation of targeted treatments. According to the ESMO and National Comprehensive Cancer Network (NCCN) guidelines, the assessment of the mutational status of *PIK3CA* is strongly recommended in patients with advanced HR+/HER2-breast cancer who have progressed on endocrine therapy with or without a CDK4/6 inhibitor [21,29]. Incorporating *PIK3CA* testing into the early diagnostic workflow facilitates optimal treatment planning and precision-based care.

3.11. Statement 11

Question: When should *PIK3CA* mutational status be evaluated in relation to the timing of adjuvant endocrine therapy, based on the INAVO120 study?

3.11.1. Recommendation

Testing for *PIK3CA* mutations is recommended for all patients with advanced HR+/HER2-breast cancer who experience disease progression during or up to 12 months after completing adjuvant endocrine therapy.

3.11.2. Comment

The phase III INAVO120 study demonstrated that the combination of inavolisib, fulvestrant, and palbociclib significantly improved progression-free survival and overall survival in *PIK3CA*-mutated patients with early progression after adjuvant endocrine therapy [15]. Consequently, early identification of *PIK3CA* mutations allows the rational implementation of such targeted therapeutic combinations as first-line treatment for metastatic disease.

3.12. Statement 12

Question: In which patients is molecular profiling of the PI3K/AKT/mTOR pathway recommended based on the type and sequence of endocrine therapy?

3.12.1. Recommendation

Testing for genetic alterations in the PI3K/AKT/mTOR pathway (including *PIK3CA*, *AKT1*, and *PTEN*) should be performed in patients with advanced HR+/HER2-breast cancer who experience disease progression during or after treatment with aromatase inhibitors and CDK4/6 inhibitors.

3.12.2. Comment

Progression of combination therapy with CDK4/6 inhibitors represents a crucial moment for molecular re-evaluation. Alterations in *PIK3CA*, *AKT1*, and *PTEN* contribute to endocrine resistance, enabling access to targeted therapeutic strategies [30–32]. The use of multigene NGS panels enables the simultaneous analysis of these alterations, optimizing the clinical and predictive utility of molecular testing.

3.13. Statement 13

Question: In the event of disease progression, should molecular testing of the PI3K/AKT/mTOR pathway be repeated on a new sample (tumor tissue or liquid biopsy) if the previous analyses did not reveal significant alterations?

3.13.1. Recommendation

In the absence of previously detected alterations, repeat molecular testing on a new tumor tissue or liquid biopsy sample at the time of disease progression may be beneficial to reassess eligibility for targeted therapy.

3.13.2. Comment

The dynamic evolution of metastatic breast cancer justifies molecular retesting of patients with disease progression. Under therapeutic pressure, new alterations in *PIK3CA* or *AKT1* may emerge over time [33]. Repeated testing facilitates the identification of novel clonal subpopulations, supporting an individualized approach to treatment planning.

3.14. Statement 14

Question: Is the detection of an activating *PIK3CA* mutation in an archived sample sufficient for eligibility for PI3K pathway inhibitor therapy?

3.14.1. Recommendation

Documentation of an activating *PIK3CA* mutation detected at any point during the patient's disease course is sufficient to consider the patient eligible for PI3K pathway inhibitor treatment [34–36].

3.14.2. Comment

PIK3CA mutations are genetically stable events maintained throughout tumor evolution and remain clinically actionable regardless of when they are identified [37]. Consequently, prior test results retain therapeutic relevance and can inform treatment decisions, avoiding unnecessary repeat testing in the absence of evidence of clonal heterogeneity.

3.15. Statement 15

Question: In patients with advanced HR+/HER2-breast cancer and activating *PIK3CA* mutations who experience progression during or within 12 months after adjuvant endocrine therapy, which treatment combination is preferable?

3.15.1. Recommendation

In patients with activating *PIK3CA* mutations and appropriate clinical characteristics, the combination of fulvestrant, palbociclib and inavolisib represents the first-line treatment option.

3.15.2. Comment

In the double-blind, randomized phase III INAVO120 trial, the combination of the PI3Kα inhibitor inavolisib with palbociclib and fulvestrant demonstrated a significant and clinically meaningful benefit compared with placebo plus palbociclib and fulvestrant in patients with PIK3CA-mutated, HR+/HER2– locally advanced or metastatic breast cancer who had relapsed during or within 12 months after the completion of adjuvant endocrine therapy. The study met its primary endpoint, showing a statistically significant improvement in progression-free survival (PFS) with a hazard ratio (HR) of 0.42 (95% confidence interval [CI], 0.32–0.55), as well as a significant overall survival (OS) benefit (HR 0.67, 95% CI, 0.48–0.94). Moreover, the objective response rate (ORR) was 62.7% in the inavolisib group compared with 28% in the control arm ($p < 0.001$) [15]. Based on these data, inavolisib received approval from the U.S. Food and Drug Administration (FDA) in October 2024, followed by the National Medical Products Administration (NMPA) of China in March 2025, and the European Commission in July 2025; parallel regulatory reviews have been completed or are ongoing in other countries, including Canada, Switzerland, and Australia. Collectively, these results and the corresponding regulatory approvals establish inavolisib plus palbociclib and fulvestrant as a new standard of care for patients with PIK3CA-mutated, endocrine-resistant advanced breast cancer.

3.16. Statement 16

Question: In the presence of a previously documented *ESR1* mutation, is testing of the PI3K/AKT/mTOR pathway recommended for accurate therapeutic stratification?

3.16.1. Recommendation

PIK3CA testing is recommended even in the presence of an *ESR1* mutation, as these two alterations confer distinct and non-mutually exclusive mechanisms of endocrine resistance.

3.16.2. Comment

Mutations in *ESR1* and *PIK3CA* confer endocrine resistance via distinct resistance pathways. The former mediates ligand-independent activation of the estrogen receptor, whereas the latter promotes cytoplasmic signaling that enhances cell survival and proliferation [33,34]. In HER+/HER2-metastatic breast cancer, co-mutations in both *genes* are detected in approximately 10 to 15% of cases; however, this overlap may be higher in patients who received multiple lines of endocrine therapy due to the rising prevalence of *ESR1* mutations under selective treatment pressure. [35,36]. In this complex setting, subsequent treatment is increasingly guided by longitudinal molecular profiling. Oral selective estrogen receptor degraders (SERDs), such as elacestrant, imlunestrant, and giredestrant, provide a biomarker-driven option when an *ESR1* mutation is detected. Conversely, AKT inhibitors such as capivasertib may represent an alternative for cancers harboring alterations in the PI3K/AKT/PTEN pathway, irrespective of *ESR1* status [37]. Notably, the prognostic impact of concurrent *ESR1* and *PIK3CA* alterations remains unclear, providing a rationale for combined therapeutic strategies, such as next-generation SERDs plus PI3K inhibitors. However, no clinical data currently support either sequential targeting of the PI3K/AKT pathway or its combination with SERDs; accordingly, these strategies remain investigational and warrant evaluation in dedicated clinical trials.

3.17. Statement 17

Question: In cases of discordant *PIK3CA* results between tumor tissue and liquid biopsy, which result should guide clinical decision-making?

3.17.1. Recommendation

In the presence of discordant results, positive findings, regardless of the biological matrix, should be considered clinically valid for therapeutic decision-making.

3.17.2. Comment

Discrepancies between tissue and plasma results may reflect intratumoral heterogeneity or insufficient ctDNA levels rather than analytical errors. A positive result in either matrix is clinically significant and sufficient to define eligibility for PI3K inhibitor therapy [38,39]. This approach maximizes clinical sensitivity and minimizes the risk of excluding potentially responsive patients.

3.18. Statement 18

Question: What is the role of the *PIK3CA* mutation VAF, detected in tissue or ctDNA, in predicting response to PI3K pathway inhibitors?

3.18.1. Recommendation

There is no validated VAF threshold value for determining eligibility for treatment, and even low-frequency activating mutations are considered clinically relevant.

3.18.2. Comment

Several studies have evaluated the correlation between VAF and response to PI3K inhibitors without identifying a universal predictive threshold [40,41]. Low-frequency *PIK3CA* mutations may reflect minor resistant subclones with therapeutic implications. Consequently, any validated activating mutation, irrespective of its allelic frequency, should be deemed actionable, in accordance with the ESMO guidelines.

3.19. Statement 19

3.19.1. Question

Do non-canonical *PIK3CA* variants (located outside exons 9 and 20) justify the use of PI3K pathway inhibitors in advanced HR+/HER2- breast cancer?

3.19.2. Recommendation

The use of PI3K pathway inhibitors is warranted in the presence of activating *PIK3CA* mutations, irrespective of their location in the canonical or non-canonical regions.

3.19.3. Comment

Non-canonical variants such as p.R88Q, p.N345K, and p.C420R have been recognized as functionally activating by international databases (OncoKB, Clinical Variants (ClinVar)) and can drive activation of the PI3K/AKT/mTOR pathway [16,42]. Therefore, therapeutic decision-making should be guided by the functional classification of the variant rather than its exon position. The use of NGS facilitates the identification of less common but clinically relevant mutations.

3.20. Statement 20

Question: Is menopausal status a criterion for determining the eligibility of *PIK3CA* testing in patients with advanced HR+/HER2- breast cancer?

3.20.1. Recommendation

Menopausal status does not restrict *PIK3CA* testing, which should be recommended according to disease stage. In premenopausal patients, the use of PI3K inhibitors must be accompanied by ovarian suppression.

3.20.2. Comment

PIK3CA mutations are clinically relevant, independent of menopausal status. However, ovarian suppression is required in premenopausal patients to ensure effective endocrine deprivation when using PI3K inhibitors [42]. This approach aligns the efficacy and safety outcomes with those observed in postmenopausal patients.

3.21. Statement 21

Question: In addition to *PIK3CA*, is it possible to analyze alterations in *AKT1*and *PTEN*?

3.21.1. Recommendation

Using an NGS panel that includes *PIK3CA*, *AKT1,* and *PTEN*, the mutational status of all three genes can be determined within a single analysis.

3.21.2. Comment

The PI3K/AKT/mTOR pathway is regulated by multiple molecular nodes, and concomitant alterations in *AKT1* (e.g., E17K) or loss of PTEN may influence therapeutic sensitivity [31,43]. Combined analysis of these genes provides a more comprehensive molecular profile, supporting the identification of subgroups that may benefit from combined targeted drug therapy.

3.22. Statement 22

Question: Can all PTEN alterations be identified using NGS?

3.22.1. Recommendation

The loss of PTEN protein expression cannot be directly assessed using NGS. However, NGS can detect mutations and copy number variations (CNVs) in PTEN.

3.22.2. Comment

NGS provides information on the genomic status of *PTEN* but does not capture its protein expression. PTEN protein loss often results from epigenetic or post-transcriptional mechanisms that must be evaluated using immunohistochemistry (IHC) [43]. The integration of NGS and IHC data offers the most accurate assessment of PTEN status and its correlation with the response to PI3K pathway inhibitors.

3.23. Statement 23

Question: Is it possible to analyze PTEN protein loss using liquid biopsy?

3.23.1. Recommendation

PTEN loss can be partially assessed in liquid biopsy by NGS analysis of cfDNA for mutations or gene copy loss, but loss of protein expression cannot be directly determined.

3.23.2. Comment

Current technologies do not facilitate the direct assessment of protein expression in plasma. However, cfDNA analysis can detect genomic events such as inactivating mutations or copy number loss (CNV), providing indirect indications of gene function [35]. Therefore, a comprehensive assessment of PTEN status should integrate liquid biopsy findings with tissue-based evaluation, preferably by IHC.

4. Conclusion

This Italian Delphi consensus provides an evidence-based and harmonized framework for the assessment and clinical implementation of *PIK3CA* testing in patients with HR+/HER2-advanced breast cancer. The expert panel reached a strong agreement on the pivotal role of *PIK3CA* mutations as predictive biomarkers for response to PI3K pathway inhibitors, reflecting the transformation of these alterations from markers of endocrine resistance to actionable therapeutic targets. Early molecular testing is recommended to guide treatment selection and optimize the use of treatment combinations such as fulvestrant, palbociclib, and inavolisib, which have demonstrated significant clinical benefits in patients with *PIK3CA*-mutated endocrine-resistant disease.

From a diagnostic perspective, NGS is the preferred methodology for *PIK3CA* analysis because of its superior sensitivity, broader genomic coverage, and ability to detect both canonical and noncanonical activating variants. Liquid biopsy is a valid and minimally invasive alternative to tissue testing, particularly when tumor samples are unavailable or inadequate. However, tissue remains the reference matrix, and negative plasma results require confirmatory tissue analysis in accordance with current ESMO recommendations. The presence of an activating *PIK3CA* mutation in either tissue or plasma is sufficient to define eligibility for PI3K inhibitor therapy, irrespective of the variant allele

frequency or exon localization.

Comprehensive NGS panels, including *PIK3CA*, *AKT1*, and *PTEN*, are strongly endorsed to capture the spectrum of alterations driving PI3K/AKT/mTOR pathway activation. The integration of genomic and IHC data to assess PTEN protein loss enhances the biological interpretation of the results and supports personalized treatment planning. The consensus also highlights the importance of analytical validation, adherence to preanalytical standards for cfDNA handling, and standardized reporting following the HGNC and HGVS recommendations, as well as the ESCAT guidelines to ensure diagnostic reliability and clinical transparency.

Overall, this national consensus translates the current scientific evidence and regulatory guidance into a unified set of practical recommendations for the molecular characterization of HR+/HER2-breast cancer. By promoting methodological consistency, comprehensive genomic profiling, and evidence-based interpretation, these statements aim to optimize patient selection for PI3K-targeted therapies and foster precision oncology in clinical practice.

Continued collaboration between molecular pathologists and oncologists, along with prospective real-world data collection, is essential to refine these recommendations and confirm their clinical impact in diverse healthcare settings.

CRediT authorship contribution statement

Carmine De Angelis: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Dario de Biase:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Lorenzo Gerratana:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Grazia Arpino:** Writing – review & editing, Validation, Methodology, Conceptualization. **Giampaolo Bianchini:** Writing – review & editing, Validation, Methodology, Conceptualization. **Isabella Castellano:** Writing – review & editing, Validation, Methodology, Conceptualization. **Giuseppe Curigliano:** Writing – review & editing, Validation, Methodology, Conceptualization. **Lucia Del Mastro:** Writing – review & editing, Validation, Methodology, Conceptualization. **Alessandra Fabi:** Writing – review & editing, Validation, Methodology, Conceptualization. **Nicola Fusco:** Writing – review & editing, Validation, Methodology, Conceptualization. **Alessandra Gennari:** Writing – review & editing, Validation, Methodology, Conceptualization. **Valentina Guarneri:** Writing – review & editing, Validation, Methodology, Conceptualization. **Claudio Zamagni:** Writing – review & editing, Validation, Methodology, Conceptualization. **Alberto Zambelli:** Writing – review & editing, Validation, Methodology, Conceptualization. **Umberto Malapelle:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Fabio Puglisi:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Formal analysis, Conceptualization.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interests

CDA: Advisory role for Roche, Lilly, Novartis, AstraZeneca, Pfizer, Gilead, Daiichi Sankyo and GlaxoSmithKline and speaker honoraria from Roche, Lilly, Novartis, Pfizer, and GlaxoSmithKline; travel grants from Gilead and research support (to the institution) from Novartis, Gilead and Daiichi Sankyo.

DdB: Personal fees, unrelated to the present work, as an invited speaker for AstraZeneca, GlaxoSmithKline (GSK), Eli Lilly, Pfizer, and Menarini Stemline.

LG: Personal fees from AstraZeneca, Daiichi Sankyo, Eli Lilly, GlaxoSmithKline, Incyte, Novartis, Pfizer, Merck Sharp & Dohme, Menarini Stemline, and AbbVie.

GA: Consulting fees from Astra Zeneca, Daiichi Sankyo, Eli Lilly, Eisai, Pierre Fabre, Roche, Pfizer, Seagen; honoraria from Astra Zeneca, Daiichi Sankyo, Eli Lilly, Eisai, Exact Science, Gilead, Menarini, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Seagen.

GB: Consultancy for Roche, AstraZeneca, Menarini, Novartis, Eli Lilly, MSD, Daiichi Sankyo, Seagen, Tethis and Gilead; payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Roche, AstraZeneca, Menarini, Novartis, Eli Lilly, Pfizer, Daiichi Sankyo, MSD, Gilead, Seagen, and Takeda; support for attending meetings and/or travel from Roche, Novartis, AstraZeneca, Menarini, Eli Lilly, Pfizer, MSD, Daiichi Sankyo, Takeda and Gilead; advisory board activities for Roche, AstraZeneca, Menarini, Novartis, Eli Lilly, Pfizer, Helsinn, MSD, Daiichi Sankyo, Gilead, Seagen, Exact Science; research grant from Gilead (institutional funding).

IC: Nothing to disclose.

GC: Honoraria for speaker engagements from Roche, Seattle Genetics, Novartis, Lilly, Pfizer, Foundation Medicine, NanoString, Samsung, Celltrion, BMS, and MSD; honoraria for consultancy from Roche, Seattle Genetics, and NanoString; honoraria for participation in advisory boards from Roche, Lilly, Pfizer, Foundation Medicine, Samsung, Celltrion, and Mylan; honoraria for writing engagements from Novartis and BMS; and honoraria for participation in the Ellipsis Scientific Affairs Group. GC has also received institutional research funding for conducting phase I and II clinical trials from Pfizer, Roche, Novartis, Sanofi, Celgene, Servier, Orion, AstraZeneca, Seattle Genetics, AbbVie, Tesaro, BMS, Merck Serono, Merck Sharp & Dohme, Janssen-Cilag, Philogen, Bayer, Medivation, and Medimmune.

LDM: Personal fees for advisory board membership from AstraZeneca, Daiichi Sankyo, Eli Lilly, Exact Sciences, Menarini Stemline, Novartis, Pfizer, Roche, Gilead, Olema, Novartis; personal fees as an invited speaker for AstraZeneca, Daiichi Sankyo, Eli Lilly, Pfizer, Gilead, Menarini Stemline, Novartis, Roche.

AF: Consultant or advisor for Roche, Lilly, Novartis, AstraZeneca, Pfizer, Seagen, Gilead, MSD, Menarini Stemline, Dompè Biotech; speaker honoraria from AstraZeneca, Roche, Lilly, Novartis, Gilead, Pfizer, Daiichi Sankyo Exact Sciences; institutional research support from AstraZeneca and Roche.

NF: Honoraria for consulting, advisory role, speaker bureau, travel, and/or research grants from Abbvie, Alira Health, AstraZeneca, Daiichi Sankyo, Epredia, Exact Sciences, Gilead, GSK, Leica Biosystems, Lilly, Menarini Group, Merck, MSD, Novartis, Pfizer, Roche, Sakura, Sysmex, ThermoFisher, and Veracyte.

AG: Consultancy/advisory role/speaker bureau: AstraZeneca, Daichii-Sankyo, Eisai, Gentili, Gilead, Pfizer, Novartis, Organon, Seagen, Lilly, Roche, MSD, Exact Sciences; Non-profit research support: AIRC, Italian Association for Cancer Research, MIUR, Dept of Excellence, LILT Novara, University of Piemonte Orientale, Italian Ministry of Health, EraNET Transcan; Scientific Board in IBCSG; Membership/affiliation: LILT, FUV.

VG: Personal fees for advisory board membership for AstraZeneca, Daiichi Sankyo, Eli Lilly, Exact Sciences, Menarini Stemline, Novartis, Pfizer, Roche, Gilead; Personal fees as an invited speaker for AstraZeneca, Daiichi Sankyo, Eli Lilly, Pfizer, Exact Sciences, Gilead, Menarini Stemline, Novartis, Roche, and Abbvie.

CZ: Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Daiichi-Sankyo, AstraZeneca, Roche, Novartis, Pfizer, Lilly, AstraZeneca, Daiichi-Sankyo, MSD, Eisai, GSK, Menarini Stemline, ExactSciences, Gilead; support for attending meetings and/or travel for Roche, Novartis, Pfizer, AstraZeneca, Daiichi-Sankyo, MSD, Eisai, GSK, Gilead; participation on a Data Safety Monitoring Board or Advisory Board for Roche, Novartis,

Pfizer, Lilly, AstraZeneca, Daiichi-Sankyo, MSD, Eisai, GSK, Menarini Stemline, ExactSciences, and Gilead.

AZ: Personal fees for advisory board membership for AstraZeneca, Daiichi Sankyo, Eli Lilly, Exact Sciences, Menarini Stemline, Novartis, Pfizer, Roche, Gilead; personal fees as an invited speaker for AstraZeneca, Roche, Exact Sciences, Gilead, Menarini Stemline, and Novartis.

UM: Personal/consulting or speakers' bureau fees from AstraZeneca, Amgen, Boehringer Ingelheim, Diaceutics, Diatech, Eli Lilly & Company, GlaxoSmithKline, Hedra, Janssen Biotech, Merck, Merck Sharp & Dohme, Novartis, Roche Health Solutions Inc., and ThermoFisher Scientific outside the submitted work.

FP: Honoraria for advisory boards, activities as a speaker, travel grants, research grants: Amgen, AstraZeneca, Bayer, Daiichi Sankyo, Celgene, Eisai, Eli Lilly, Exact Sciences, Gilead, Ipsen, Italfarmaco, Menarini. MSD, Novartis, Pierre Fabre, Pfizer, Roche, Seagen, Takeda, Viatris; Research funding: AstraZeneca, Eisai, and Roche.

Acknowledgements

Editorial assistance was provided by Editamed srl.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2026.104786.

References

[1] André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-mutated, hormone receptor–positive advanced breast cancer. N Engl J Med 2019;380:1929–40. https://doi.org/10.1056/NEJMoa1813904.
[2] Browne IM, André F, Chandarlapaty S, et al. Optimal targeting of PI3K-AKT and mTOR in advanced oestrogen receptor-positive breast cancer. Lancet Oncol 2024 Apr;25(4):e139–51. https://doi.org/10.1016/S1470-2045(23)00676-9. Erratum in: Lancet Oncol. 2024 Jun;25(6):e234. doi: 10.1016/S1470-2045(24)00219-5.
[3] Fribbens C, O'Leary B, Kilburn L, et al. Plasma ESR1 mutations and the treatment of estrogen receptor-positive advanced breast cancer. J Clin Oncol 2016 Sep 1;34(25):2961–8. https://doi.org/10.1200/JCO.2016.67.3061.
[4] De Mattos-Arruda L. PIK3CA mutation inhibition in hormone receptor-positive breast cancer: time has come. ESMO Open 2020;5:e000890. https://doi.org/10.1136/esmoopen-2020-000890.
[5] Xi J, Ma CX, O'Shaughnessy J. Current clinical utility of circulating tumor DNA testing in breast cancer: a practical approac. JCO Oncol Pract 2024;20:1460–70.
[6] Loizidis S, Stanciu MA, Ignatiadis M. Liquid biopsy in breast cancer: promises and challenges. Curr Opin Oncol 2025;37:512–21.
[7] Gennari A, André F, Barrios CH, et al., ESMO Guidelines Committee. ESMO clinical practice guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021;32:1475–95. https://doi.org/10.1016/j.annonc.2021.09.019.
[8] Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO precision medicine working group. Ann Oncol 2022;33:750–68. https://doi.org/10.1016/j.annonc.2022.05.520.
[9] Research Gate. Delphi method: techniques and applications. In: Linstone HA, Turoff M, editors. (PDF) the Delphi method: techniques and applications; 2002. https://doi.org/10.2307/3150755. [Accessed 10 January 2026].
[10] Habibi A, Sarafrazi A, Sedigheh I. Delphi technique theoretical framework in qualitative research. IJES 2014;3:8–13.
[11] Dumbrava EE, Call SG, Huang HJ, et al. PIK3CA mutations in plasma circulating tumor DNA predict survival and treatment outcomes in patients with advanced cancers. ESMO Open 2021 Oct;6(5):100230. https://doi.org/10.1016/j.esmoop.2021.100230.
[12] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4.
[13] Tate JG, Bamford S, Jubb HC, et al. COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res 2019;47:D941–7. https://doi.org/10.1093/nar/gky1015.
[14] Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. JCO Precis Oncol 2017:00011. https://doi.org/10.1200/PO.17.00011. PO.17.
[15] Turner NC, Im SA, Saura C, et al. Inavolisib-based therapy in PIK3CA-Mutated advanced breast cancer. N Engl J Med 2024 Oct 31;391(17):1584–96. https://doi.org/10.1056/NEJMoa2404625.
[16] Landrum MJ, Chitipiralla S, Kaur K, et al. ClinVar: updates to support classifications of both germline and somatic variants. Nucleic Acids Res 2024. https://doi.org/10.1093/nar/gkae1090. gkae1090.
[17] Bruford EA, Braschi B, Denny P, et al. Guidelines for human gene nomenclature. Nat Genet 2020;52:754–8. https://doi.org/10.1038/s41588-020-0669-3.
[18] Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American society of clinical oncology and college of American pathologists joint review. J Clin Oncol 2018;36:1631–41. https://doi.org/10.1200/JCO.2017.76.8671.
[19] Park J, Cho SY, Chang ES, et al. Analysis of PIK3CA mutation concordance and frequency in primary and different distant metastatic sites in breast cancer. Cancer Res Treat 2023 Jan;55(1):145–54. https://doi.org/10.4143/crt.2022.001.
[20] Rosin J, Svegrup E, Valachis A, et al. Discordance of PIK3CA mutational status between primary and metastatic breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat 2023;201:161–9. https://doi.org/10.1007/s10549-023-07010-1.
[21] National comprehensive cancer network (NCCN) guidelines, accessed 9 January 2026. Breast Cancer. Online version 2.2026. Available at: https://www.nccn.org/guidelines/guidelines-detail?id=1419.
[22] Marchiò C, Berrino E, Scarpino S, et al. Part I - pre-analytical phase. Pathologica 2025;117(Suppl.1):S5–17. https://doi.org/10.32074/1591-951X-1215.
[23] Burstein HJ, DeMichele A, Somerfield MR, et al. Biomarker testing and endocrine and targeted therapy in metastatic breast cancer expert panels. Testing for *ESR1* mutations to guide therapy for hormone receptor-positive, human epidermal growth factor receptor 2-Negative metastatic breast cancer: ASCO guideline rapid recommendation update. J Clin Oncol 2023;41:3423–5. https://doi.org/10.1200/JCO.23.00638.
[24] Trapani D, Martins-Branco D, Curigliano G, et al., ESMO Guidelines Committee. Updated treatment recommendations for systemic treatment: from the ESMO metastatic breast cancer living guideline. Ann Oncol 2025;36:1414–8. https://doi.org/10.1016/j.annonc.2025.07.017.
[25] Mohd Zuhdi NF, Siddig A, Mohd Nafi SN, et al. Next-generation sequencing in breast cancer: current clinical applications and future directions. Ann Med 2025;57:2569989. https://doi.org/10.1080/07853890.2025.2569989.
[26] Venetis K, Pepe F, Munzone E, et al. Analytical performance of next-generation sequencing and RT-PCR on formalin-fixed paraffin-embedded tumor tissues for PIK3CA testing in HR+/HER2- breast cancer. Cells 2022;11:3545. https://doi.org/10.3390/cells11223545.
[27] Mateo J, Chakravarty D, Dienstmann R, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO scale for clinical actionability of molecular targets (ESCAT). Ann Oncol 2018;29:1895–902. https://doi.org/10.1093/annonc/mdy263.
[28] Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO precision medicine working group. Ann Oncol 2024;35:588–606. https://doi.org/10.1016/j.annonc.2024.04.005.
[29] Dieci MV, Vernaci G, Colangelo C, et al. PIK3CA mutations and first-line outcomes in endocrine-resistant HR+/HER2- metastatic breast cancer: a multicentric real-world study. Eur J Cancer 2025;234:116194. https://doi.org/10.1016/j.ejca.2025.116194.
[30] Saatci O, Huynh-Dam KT, Sahin O. Endocrine resistance in breast cancer: from molecular mechanisms to therapeutic strategies. J Mol Med (Berl) 2021;99:1691–710. https://doi.org/10.1007/s00109-021-02136-5.
[31] de Moraes FCA, Sano VKT, Pereira CRM, et al. Effects of AKT inhibitors for PIK3CA/AKT1/PTEN-Altered advanced or metastatic breast cancer: a meta-analysis of randomized clinical trials. Clin Breast Cancer 2025;25:391–400.e15. https://doi.org/10.1016/j.clbc.2025.03.011.
[32] Carnevale MG, Colciago RR, De Santis MC, et al. Advancing breast cancer therapy in the era of molecular diagnostics. Breast 2025;82:104488. https://doi.org/10.1016/j.breast.2025.104488.
[33] Gerratana L, Davis AA, Velimirovic M, et al. Interplay between ESR1/PIK3CA codon variants, oncogenic pathway alterations and clinical phenotype in patients with metastatic breast cancer (MBC): comprehensive circulating tumor DNA (ctDNA) analysis. Breast Cancer Res 2023 Oct 2;25(1):112. https://doi.org/10.1186/s13058-023-01718-0.
[34] Tolaney SM, Toi M, Neven P, et al. Clinical significance of PIK3CA and ESR1 mutations in circulating tumor DNA: analysis from the MONARCH 2 study of abemaciclib plus fulvestrant. Clin Cancer Res 2022;28:1500–6. https://doi.org/10.1158/1078-0432.CCR-21-3276.
[35] Bhave MA, Quintanilha JCF, Tukachinsky H, et al. Comprehensive genomic profiling of ESR1, PIK3CA, AKT1, and PTEN in HR(+)HER2(-) metastatic breast cancer: prevalence along treatment course and predictive value for endocrine therapy resistance in real-world practice. Breast Cancer Res Treat 2024;207:599–609. https://doi.org/10.1007/s10549-024-07376-w. Erratum in: *Breast Cancer Res Treat.* 2024; 207:611–614. doi: 10.1007/s10549-024-07427-2.
[36] Bardia A, Cortés J, Bidard FC, et al. Elacestrant in ER+, HER2- metastatic breast cancer with ESR1-Mutated tumors: subgroup analyses from the phase III EMERALD trial by prior duration of endocrine therapy plus CDK4/6 inhibitor and in clinical subgroups. Clin Cancer Res 2024;30:4299–309.
[37] Turner NC, Oliveira M, Howell SJ, Dalenc F, Cortes J, Gomez Moreno HL, et al. Capivasertib in hormone receptor-positive advanced breast cancer. N Engl J Med 2023;388:2058–70. https://doi.org/10.1056/NEJMoa2214131.
[38] Schiavone ML, Scarpitta R, Ravera F, et al. Liquid biopsy in breast cancer: redefining precision medicine. J Liq Biopsy 2025 Jul 16;9:100312. https://doi.org/10.1016/j.jlb.2025.100312.
[39] Malik S, Zaheer S. The impact of liquid biopsy in breast cancer: redefining the landscape of non-invasive precision oncology. J Liq Biopsy 2025 May 21;8:100299. https://doi.org/10.1016/j.jlb.2025.100299.

[40] Hutchinson KE, Chen JW, Savage HM, et al. Multiple PIK3CA mutation clonality correlates with outcomes in taselisib + fulvestrant-treated ER+/HER2-, PIK3CA-mutated breast cancers. Genome Med 2023 Apr 26;15(1):28. https://doi.org/10.1186/s13073-023-01181-8.

[41] Schagerholm C, Robertson S, Toosi H, et al. PIK3CA mutations in endocrine-resistant breast cancer. Sci Rep 2024;14:12542. https://doi.org/10.1038/s41598-024-62664-1.

[42] Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. JCO Precis Oncol 2017 Jul:00011. https://doi.org/10.1200/PO.17.00011. 2017:PO.17.

[43] Fusco N, Guerini-Rocco E, Castellano I, Malapelle U. Immunohistochemistry for PTEN testing in HR +/HER2- metastatic breast cancer. Virchows Arch 2025 Sep 11;487(4):727–32. https://doi.org/10.1007/s00428-025-04249-5.