PATHOLOGICA 2025;117:571-579
DOI: 10.32074/1591-951X-1603

Original article

PIK3CA testing in HR+/HER2– metastatic breast cancer: assessing pathology laboratories capacity and needs

Eltjona Mane[1*], Giulia Cursano[1,2,*], Konstantinos Venetis[1,*], Chiara Frascarelli[1,2], Francesco Pepe[3], Mariantonia Nacchio[3], Lucia Palumbo[3], Pasquale Pisapia[3], Elisa De Camilli[1], Isabella Castellano[4], Bruna Cerbelli[5], Leopoldo Costarelli[6], Giulia d'Amati[5], Antonio Rizzo[7], Alfredo Santinelli[8], Cristian Scatena[9], Carmen Criscitiello[2,10], Carmine De Angelis[11], Maria Vittoria Dieci[12,13], Giancarlo Troncone[3], Giuseppe Curigliano[2,10], Giuseppe Viale[1], Elena Guerini-Rocco[1,2,#], Umberto Malapelle[3,#] Nicola Fusco[1,2,#] and the PIK3CA in breast cancer GIPaM/PMMP cooperative group (see Supplementary Materials) *Co-first; #Co-last

[1] Division of Pathology, European Institute of Oncology IRCCS, Milan, Italy; [2] Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy; [3] Department of Public Health, Federico II University of Naples, Naples, Italy; [4] Unit of Pathology, Department of Medical Sciences, City of Health and Science University Hospital, University of Turin, Turin, Italy; [5] Department of Medical-Surgical Sciences and Biotechnologies Sapienza University of Rome, Rome, Italy; [6] Unit of Pathology, San Giovanni-Addolorata Hospital, Rome, Italy; [7] Division of Pathology, Clinical Institute Humanitas Catania, Misterbianco (CT), Italy [8] Unit of Anatomic Pathology, Azienda Sanitaria Territoriale di Pesaro-Urbino, Pesaro, Italy; [9] Division of Pathology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy; [10] Division of New Drugs and Early Drug Development for Innovative Therapies, European Institute of Oncology IRCCS, Milan, Italy; [11] Clinical and Translational Oncology, Scuola Superiore Meridionale, Naples, Italy; [12] Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy; [13] Department of Oncology 2, Veneto Institute of Oncology IOV IRCCS, Padova, Italy

Summary

The management of hormone receptor-positive/HER2-negative (HR+/HER2–) metastatic breast cancer (MBC) relies on molecular testing to inform treatment decisions. *PIK3CA* mutations, present in ~40% of cases, represent a key predictive biomarker for PI3K-pathway–targeted therapies. Despite its clinical relevance, *PIK3CA* testing continues to face challenges related to laboratory organization, standardization, and access. We conducted a nationwide, cross-sectional survey to evaluate current practices and institutional readiness for PIK3CA testing in Italy, in the context of the anticipated expansion of PI3K-targeted therapies, including inavolisib. A total of 118 healthcare professionals from institutions across 15 regions participated, providing data on test availability, laboratory workflows, analytical methodologies, accreditation status, and implementation barriers. Descriptive statistics were used for analysis. Overall, 88.1% of institutions reported the ability to perform PIK3CA testing, with 57.6% offering on-site analysis. Testing was predominantly performed in pathology laboratories (76.5%), followed by molecular biology (16.2%) and genetics laboratories (7.4%). However, 46.6% of institutions lacked formal molecular accreditation, and ISO:15189 certification remained uncommon. Pre-analytical workflows relied mainly on formalin-fixed paraffin-embedded (FFPE) tissue samples (89.7%), with limited routine use of liquid biopsy. Next-generation sequencing (NGS) was the most frequently adopted analytical approach (45.6%), followed by combined NGS and PCR-based strategies (36.8%). Most institutions reported turnaround times of 7–15 days. In conclusion, this updated survey indicates progress in access to PIK3CA testing and consolidation of NGS-based methodologies in Italy. Nevertheless, persistent gaps in accreditation, heterogeneous workflows, and limited integration of liquid biopsy highlight ongoing challenges in standardization and diagnostic equity. Coordinated national strategies will be essential to ensure consistent, high-quality molecular diagnostics in HR+/HER2– MBC.

Key words: PIK3CA testing, molecular diagnostics, HR+/HER2– metastatic breast cancer, next-generation sequencing (NGS), precision oncology

Received: August 13, 2025
Accepted: January 4, 2025

Correspondence
Nicola Fusco
E-mail: nicola.fusco@ieo.it
Umberto Malapelle
E-mail: umbertomalapelle@gmail.com
Elena Guerini-Rocco
E-mail: elena.guierinirocco@ieo.it

How to cite this article: Mane E, Cursano G, Venetis K, et al. *PIK3CA* testing in HR+/HER2– metastatic breast cancer: assessing pathology laboratories capacity and needs

Introduction

Molecular testing is essential to inform clinical decision-making in patients with hormone receptor-positive/HER2-negative (HR+/HER2-) metastatic breast cancer (MBC) [1, 2]. In this patient population, several biomarkers, including *PIK3CA* along with associated pathway alterations (i.e. *AKT1* and *PTEN*), have direct clinical implications [3-7]. Accurate *PIK3CA* testing, however, requires appropriate methodologies, such as real-time (RT)-PCR, droplet digital (dd)PCR, and/or next-generation sequencing (NGS), which should be performed on suitable biological specimens: formalin-fixed paraffin-embedded (FFPE) tissue samples and/or circulating tumor DNA (ctDNA) [8-11]. Regrettably, molecular testing for HR+/HER2- MBC remains particularly challenging. This can be due to the varying levels of coordination between molecular pathology laboratories, tumor boards, and accredited breast centers [12-14].

In 2019, *PIK3CA* became the first biomarker assessed by methods other than immunohistochemistry (IHC) to guide selection of targeted therapy in breast cancer, with alpelisib approved as the corresponding targeted treatment for HR+/HER2- MBC [15-17]. Since then, additional agents targeting phosphoinositide 3-kinase (PI3K) signaling have been approved for clinical use, including capivasertib and inavolisib [18-21]. Mutations in the PI3K pathway are highly prevalent in patients with HR+/HER2- MBC, occurring in approximately 50% of cases, with *PIK3CA* being the most frequently mutated gene (~40% of cases) [22]. Despite their clinical significance, technical challenges remain to ensure precise molecular testing.

Following the introduction of alpelisib in clinical practice, we previously conducted a nationwide survey to assess the molecular diagnostic landscape in various healthcare settings (e.g., academic centers, general hospitals, hubs, and spokes) [23]. Five years later, with the imminent expansion of PI3K pathway-targeted therapies, a key question arises: has this landscape evolved? The present study specifically aims to capture how *PIK3CA* testing practices in MBC have progressed over time, with a particular focus on changes in molecular pathology laboratory capacity, testing modalities, accreditation status, and integration into clinical workflows.

Materials and methods

Study Design

This cross-sectional survey assessed the practices and capabilities of *PIK3CA* testing for HR+/HER2– MBC in 118 Italian healthcare institutions involved in HR+/HER2– MBC management. The questionnaire covered multiple aspects, including demographic details, test availability, routine practices, laboratory protocols, and barriers to implementation. To ensure comprehensive data collection, questions were primarily open-ended. The study participants were pathologists, geneticists, molecular biologists, and oncologists. The survey was administered online in an anonymized format.

Survey structure

The study employed structured questions to assess four key aspects of *PIK3CA* molecular diagnostic workflows specifically for HR+/HER2- MBC (Supplementary Methods S1). Facility capabilities were evaluated by examining the availability and modality of *PIK3CA* testing within participating centers, including the presence of a dedicated breast unit, in-house testing capacity, laboratory certification status, and frequency of *PIK3CA* testing requests over the past year. Centers that neither conducted nor outsourced the test were asked about their future plans for implementation and the specific barriers preventing adoption. Pre-analytical factors focused on the types of biomaterials used for *PIK3CA* testing, to distinguish between FFPE samples and liquid biopsies. The analytical section explored the technologies employed for mutation detection, including Sanger sequencing, RT-PCR, ddPCR, and NGS, as well as the adaptability of laboratories in applying different methodologies. Post-analytical processes were assessed by identifying the healthcare professionals responsible for test interpretation and reporting, along with the estimated turnaround time for *PIK3CA* test completion. Results were expressed as percentages, with respondents serving as the unit of analysis. All statistical analyses were performed using R Statistical Software version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 118 Institutions across 15 regions in Italy were surveyed regarding the status of *PIK3CA* testing in HR+/HER2– MBC (Fig. 1). These institutions represented a diverse spectrum, encompassing major general hospitals, specialized cancer centers, academic institutions, and smaller regional hospitals.

Testing availability and laboratory practices

Among the 118 surveyed participants, 99 (83.9%) conducted their activities in hospitals with accredit-

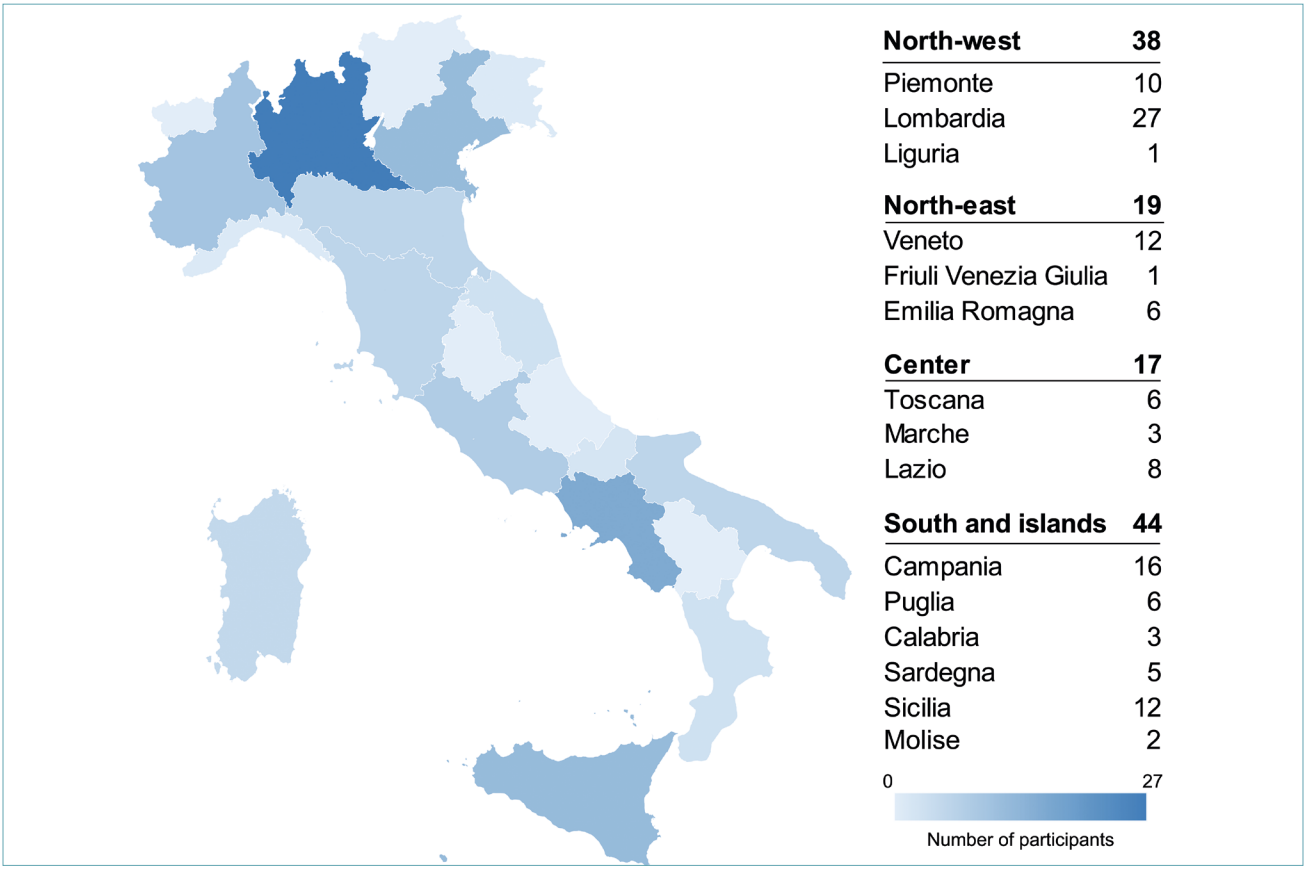

Figure 1. Distribution of 118 institutions across 15 regions in Italy participating in a national survey on *PIK3CA* testing for HR+/HER2- metastatic breast cancer.

ed breast centers. Compared with the 2023 survey, the current analysis suggests an overall increase in reported institutional capacity for *PIK3CA* testing. While approximately one third of institutions previously reported no access to in-house or outsourced testing, 88.1% (n = 104) of participating centers now report the ability to perform *PIK3CA* analysis, with more than half (57.6%; n = 68) offering on-site testing. Taken together, these findings are consistent with a reduction in previously reported structural limitations to test availability at the institutional level. The organization of testing also appears to have evolved, with pathology laboratories accounting for the majority of in-house analyses (76.5%; n = 52), compared with the more heterogeneous laboratory distribution reported in 2023. However, this increase in reported testing capacity has not been accompanied by a comparable improvement in quality frameworks. Nearly half of institutions (46.6%; n = 55) still operate without formal molecular accreditation, and ISO:15189 certification remains uncommon (3.4%; n = 4), suggesting that gains in access have not yet translated into uniform advances in laboratory standardization (Fig. 2). Institutions currently lacking testing capacity are detailed in Supplementary Figure S1.

Analytical workflow, reporting, and turnaround times

Analysis of pre-analytical practices indicates a change compared with the 2023 survey. Whereas more than half of institutions previously reported the ability to analyze both tissue and liquid biopsy samples, the current survey shows a predominant reliance on FFPE tissue alone (89.7%; n = 61), with combined tissue–plasma testing reported by a smaller proportion of centers (10.3%; n = 7). These findings suggest a more limited routine use of liquid biopsy for PIK3CA testing in current practice. From an analytical perspective, NGS-based approaches remain the most commonly adopted testing strategy. In the current survey, NGS targeted panels were the most frequently reported methodology (45.6%; n = 31), followed by combined NGS and/or PCR-based approaches (36.8%; n = 25). A smaller subset reported the use

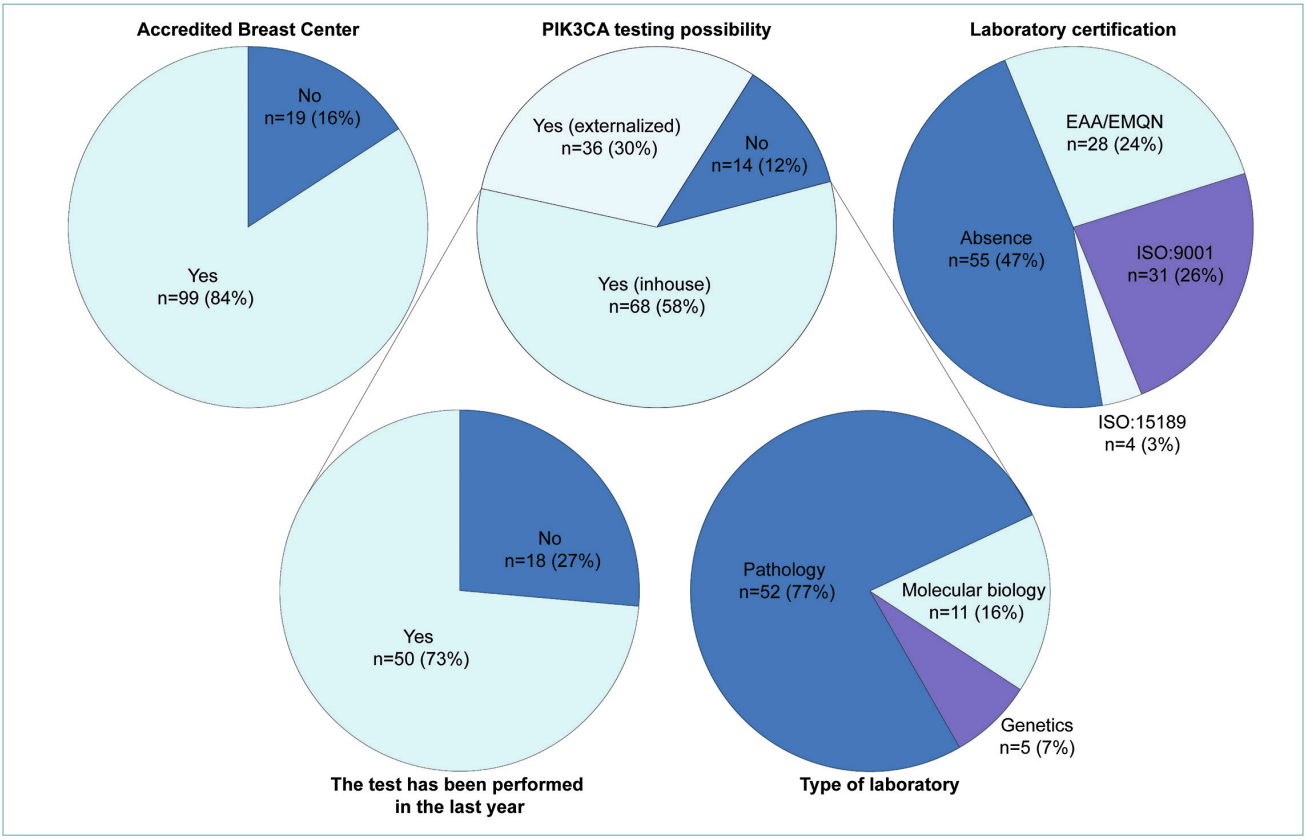

Figure 2. Facility capabilities, testing availability, and laboratory practices.

of NGS and/or PCR-based methods together with direct sequencing (10.3%; n = 7), while PCR-based methods alone accounted for 7.4% (n = 5). While NGS was already prevalent in 2023, the present data are consistent with a continued relative decline in RT-PCR-only workflows. In contrast, post-analytical practices appear largely stable over time. The distribution of report signatories remains similar to that observed in 2023, with persistent heterogeneity in professional responsibility and frequent co-signing models involving molecular biologists and pathologists. Turnaround times also appear largely unchanged, with most institutions reporting a TAT of 7–15 days (64.7%; n = 44), and only a minority exceeding 15 days (2.9%; n = 2), suggesting that increased analytical capacity has not yet been associated with shorter reporting timelines (Fig. 3).

Discussion

Mutational analysis of *PIK3CA*, a key predictive biomarker in HR+/HER2– MBC, has gained significant clinical relevance with the development of novel targeted therapies for *PIK3CA*-mutated tumors [24, 25]. These advances reinforce the need for molecular diagnostic workflows that are not only technically robust but also standardized, timely, and equitably accessible [26, 27]. Building on our previous nationwide assessment conducted in 2023, the present study provides an updated overview of institutional readiness for single-biomarker *PIK3CA* testing in Italy, with a specific focus on changes in testing capacity, analytical practices, and organizational frameworks over time.

A key finding is the overall increase in reported institutional capacity to perform *PIK3CA* testing. Compared with the 2023 survey, in which approximately one-third of institutions reported no access to either in-house or outsourced testing, the majority of participating centers now report the ability to perform the analysis, with more than half offering on-site testing. These findings suggest a progressive reduction in previously reported structural barriers to test availability. At the same time, the organization of testing appears to have become more centralized within pathology laboratories, which now account for the majority of in-house analyses, compared with the more heterogeneous

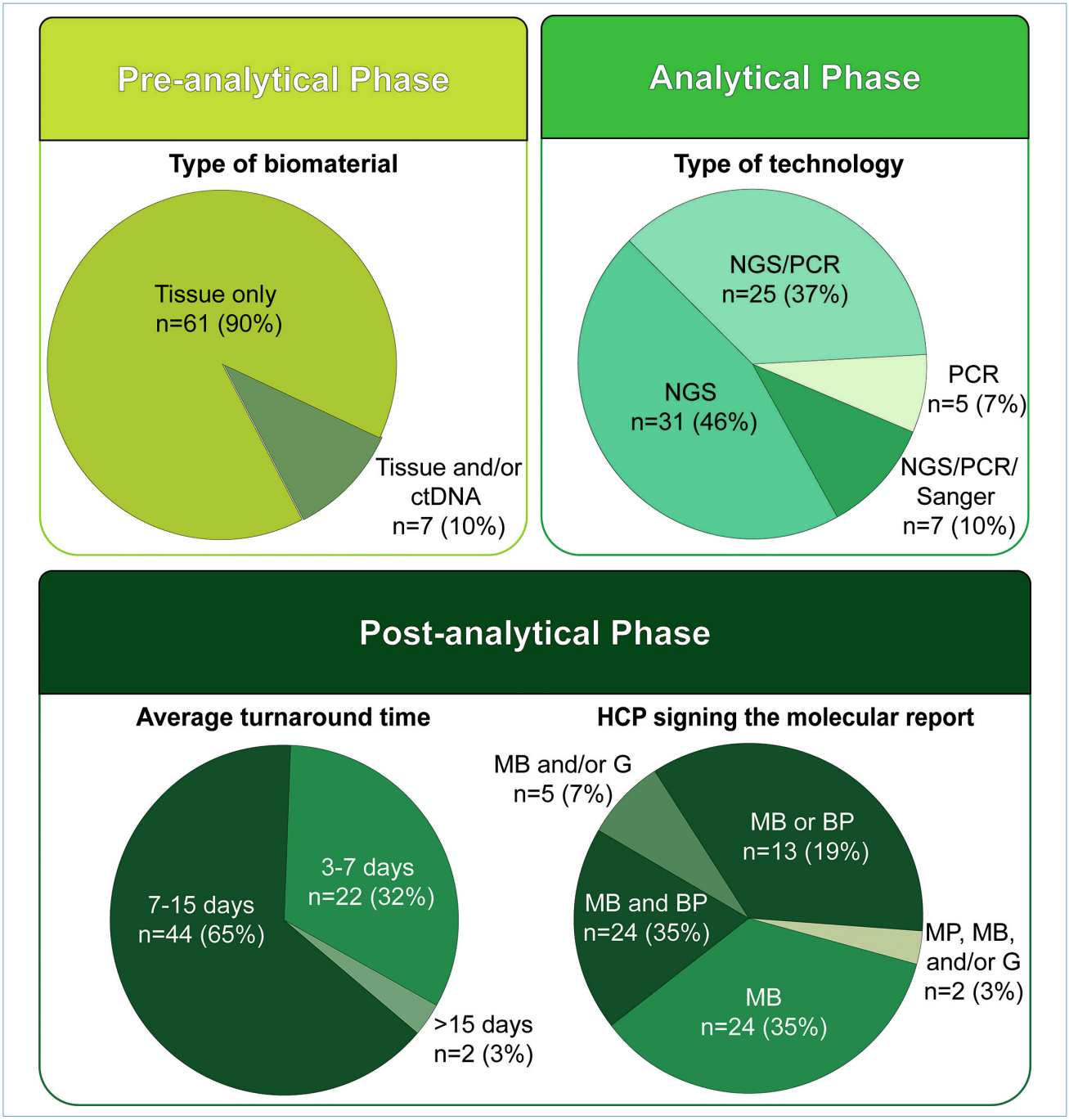

Figure 3. Overview of *PIK3CA* testing strategies from a national survey of 118 institutions. Pie charts depict the biomaterials used (pre-analytical phase), testing technologies (analytical phase), turnaround time, and report signatories (post-analytical phase), highlighting practices across all procedural stages. MB, molecular biologist; BP, breast pathologist; MP, molecular pathologist; G, geneticist.

laboratory distribution observed previously. This trend likely reflects growing consolidation of molecular diagnostics within pathology-driven workflows [28]. Although participating institutions were distributed across multiple Italian regions, geographic differences were explored only descriptively. Given the regional organization of the Italian National Health Service, variability in reimbursement policies, laboratory accreditation,

and access to advanced molecular technologies may contribute to territorial inequities in *PIK3CA* testing. Centers lacking in-house testing capabilities or formal certification may experience longer turnaround times or rely on external referrals, potentially delaying treatment initiation. Such regional disparities may disproportionately affect patients treated in peripheral or resource-limited settings, underscoring the need for national diagnostic networks, standardized quality frameworks, and centralized referral pathways to ensure equitable access to precision oncology. In practical terms, reported barriers likely include limited availability of dedicated molecular personnel, lack of in-house sequencing platforms in smaller centers, absence of formal outsourcing agreements, and region-specific reimbursement constraints. Organizational fragmentation between pathology, molecular biology, and clinical teams may further contribute to delays or inefficiencies in test implementation, particularly in low-volume or peripheral institutions.

Despite these improvements in reported access, important challenges persist. In particular, the expansion of testing capacity has not been accompanied by a parallel increase in formal laboratory accreditation. A substantial proportion of institutions continue to operate without recognized molecular certifications, and ISO:15189 accreditation remains uncommon. This finding, consistent with but not improved over the 2023 survey, highlights an ongoing gap between technical availability and quality assurance frameworks. Given the central role of molecular testing in guiding therapeutic decisions, this mismatch underscores the need for further efforts to harmonize accreditation and quality standards across institutions [29-35]. Importantly, while accreditation provides an essential framework for quality assurance, this survey did not assess laboratory-level internal validation procedures or assay performance metrics (e.g. sensitivity and specificity), which are critical determinants of analytical reliability in molecular diagnostics.

The choice of biomaterial represents another area in which current practice appears to differ from that reported previously. While the 2023 survey documented widespread availability of combined tissue and liquid biopsy testing, the present analysis shows a predominant reliance on FFPE tissue samples, with a smaller proportion of centers reporting routine use of plasma-based assays. Although FFPE tissue remains a reliable and widely accepted substrate for *PIK3CA* mutation analysis, the more limited use of liquid biopsy observed in the current survey suggests that its integration into routine clinical workflows remains variable. This may reflect technical constraints, sensitivity considerations, or differences in local expertise and infrastructure, rather than a lack of clinical interest. As liquid biopsy technologies continue to evolve and their clinical utility becomes more clearly defined, particularly in scenarios where tissue sampling is challenging, their role in *PIK3CA* testing warrants continued evaluation [36-39].

From an analytical standpoint, the present data confirm the central role of NGS in *PIK3CA* testing. While NGS was already widely adopted in 2023, the current findings are consistent with further consolidation of standardized NGS-based approaches and a relative decline in RT-PCR-only workflows. This trend suggests increasing methodological homogenization across laboratories, although some degree of heterogeneity persists, particularly in centers that combine multiple techniques. Differences in access to instrumentation and local resource availability may continue to influence the choice of analytical strategies, with potential implications for diagnostic equity [40-42].

In contrast, post-analytical practices appear largely unchanged over time. The distribution of report signatories remains heterogeneous, with continued involvement of molecular biologists, pathologists, and multidisciplinary co-signing models, similar to what was observed in 2023. Turnaround times for PIK3CA testing also appear broadly stable, with most institutions reporting results within 7-15 days. These findings suggest that increases in analytical capacity have not yet translated into measurable reductions in reporting timelines, likely reflecting organizational and workflow-related constraints rather than analytical limitations alone [43, 44].

This study has several limitations that should be considered when interpreting the findings. First, the data reflect only the responses of institutions that chose to participate in the survey, introducing a potential selection bias. It is likely that centers with established or developing molecular diagnostics were more inclined to respond, possibly overestimating national readiness for *PIK3CA* testing. Second, given that inavolisib is not yet approved, the clinical demand for *PIK3CA* testing remains limited. As a result, current testing practices may not fully reflect future capacities or priorities once the therapy becomes available. Additionally, the survey relied on self-reported data, which may be subject to inaccuracies or inconsistencies in how respondents interpreted and reported their laboratory capabilities, workflows, and timelines. It is also important to acknowledge that the survey was not designed to capture detailed information on internal validation procedures or assay performance characteristics, such as sensitivity and specificity, as it focused on institutional organization and workflow rather than analytical validation. As a result, aspects of quality control at the

individual assay level could not be evaluated. Finally, the survey did not include patient-level clinical data (e.g. time to treatment initiation or access to PI3K-targeted therapies) which are crucial components of quality molecular diagnostics and are essential to fully assess the downstream impact of diagnostic capacity on clinical decision-making and patient care. Future real-world studies integrating molecular diagnostic data with clinical outcomes and treatment timelines will be critical to quantify the downstream impact of diagnostic infrastructure on patient care.

In conclusion, this updated nationwide survey indicates progress in the availability and organization of PIK3CA testing across Italian institutions since 2023, particularly in terms of institutional access and consolidation of NGS-based methodologies (45-48). However, persistent gaps in accreditation, continued heterogeneity in post-analytical practices, and variable integration of liquid biopsy highlight areas where further improvement is needed. To address these barriers, coordinated efforts by scientific societies and healthcare stakeholders should prioritize the development of formal laboratory networks with predefined referral pathways, shared validation protocols, and accredited outsourcing models for centers lacking in-house capabilities. Additional measures may include targeted funding for molecular infrastructure, harmonized regional reimbursement policies, and structured educational programs aimed at strengthening interdisciplinary collaboration between pathologists, molecular biologists, and clinicians.

Acknowledgements

The Authors would like to thank Medica Editoria for providing logistic supporto to the survey, and all colleagues involved in the educational program, including Drs. Maria Addesso, Caterina Aiello, Gian Paolo Andreoletti, Valeria Aveta, Claudia Lucia Bonanno, Elena Boschiroli, Elisa Canova, Anna Maria Carillo, Sara Casella, Beatrice Casini, Ivana Cerillo, Sofia Chiatamone Ranieri, Maria Pia Cocca, Mara Compagno, Federica Ferrante, Graziano Crudele, Maria Luisa D'Aponte, Domenico De Judicibus, Maria Caterina De Nisi, Leonarda Di Candia, Loren Duda, Raffaella Fabbri, Diletta Favero, Elda Feyles, Thelema Filippini, Giuliana Finocchiaro, Elisa Forti, Laura Maria Freschi, Elena Gallo, Jessica Gasparello, Giovanna Giliberti, Maria Ingenito, Eleonora Lauri, Lorella Laurini, Araceli Yamila Leonardelli, Crocifissa Lo Cunsolo, Valentina Nunziella Lombardo, Manuela Lunghi, Laura Manotti, Francesca Marciano, Marta Mariano, Roberta Marozzo, Simona Massa, Giuseppe Mastropasqua, Pasquale Mellone, Martina Moretti, Margherita Mula, Giovanni Napolitano, Gennaro Palumbo, Konstantinos Partsinevelos, Barbara Pertoldi, Natalia Pinto, Concetta Maria Porretto, Paola Possanzini, Giulia Racaniello, Claudia Rivello, Silvia Maria Rossi, Dario Sabbatinfilio, Filippa Scialfa, Paola Francesca Silenzi, Gaia Spagnuolo, Valeria Stecconi, Michela Stumpo, Federico Tallarigo, Enisse Hornela Tassie, Francesca Federica Tava, Francesca Romana Tofanetti, Irene Tornabene, Barbara Vanni, Matteo Villa, Luca Viviano, Caterina Zanella, Carla Angela Zavattero, and Martina Zichi. Konstantinos Venetis was supported by the Fondazione Umberto Veronesi, Mariia Ivanova by the IEO-Monzino Foundation. The content of the manuscript is solely the work of the authors. Final proofreading, including grammar and syntax review, was assisted by digital tools—ChatGPT-4 and Grammarly (version 6.8.263)—used exclusively to support clarity and readability.

Conflicts of interest statement

The authors disclose the following relationships with industry, unrelated to the present study: Bruna Cerbelli has received support from AstraZeneca, Novartis, MSD, and Roche; Caterina Chiappetta from Johnson & Johnson; Carmen Criscitiello from Pfizer, Roche, MSD, Novartis, Lilly, Seagen, Gilead, and Daiichi Sankyo; Giuseppe Curigliano from Merck, Ellipses Pharma, Roche/Genentech, Pfizer, Daiichi Sankyo, AstraZeneca, Novartis, Lilly, Foundation Medicine, Samsung, Seagen, Menarini, Gilead Sciences, Exact Sciences, Bristol Myers Squibb, Boehringer Ingelheim, GlaxoSmithKline, Guardant Health, Veracyte, Celcuity, Hengrui Therapeutics, and Blueprint Medicines; Giulia Cursano from Veracyte and Sysmex; Giulia d'Amati from AstraZeneca, MSD, Daiichi Sankyo, Lilly, Roche, and Novartis; Carmine De Angelis reports advisory roles for Roche, Lilly, Novartis, AstraZeneca, Pfizer, Gilead, Daiichi Sankyo, and GSK, speaker honoraria from Roche, Lilly, Novartis, Pfizer, and GSK, travel grants from Gilead, and institutional research support from Novartis, Gilead, and Daiichi Sankyo; Dario de Biase from Boehringer Ingelheim, Eli Lilly, Menarini Stemline, AstraZeneca, and GSK; Nicola Fusco 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; Elena Guerini-Rocco from AstraZeneca, Exact Sciences, GSK, Novartis, Roche, and Thermo Fisher Scientific; Mariia Ivanova from Agilent Technologies; Francesco Pepe from Menarini and Roche; Antonio Rizzo from AstraZeneca; Maria Vita Sanò from Novartis, AstraZeneca, Daiichi Sankyo, Eli Lilly, Pfizer, MSD, Gilead, and Istituto Gentili; Cristian Scatena from Bristol Myers Squibb, AstraZeneca,

Daiichi Sankyo, Gilead, and Menarini; Konstantinos Venetis from MSD, Roche, AstraZeneca, and Johnson & Johnson; and Giuseppe Viale from Roche, AstraZeneca, Daiichi Sankyo, Pfizer, Agilent, Eli Lilly, and Gilead. These companies had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and/or in the decision to publish the results. All other authors declare no potential conflicts of interest.

Funding

This work was partially supported by the Italian Ministry of Health through Ricerca Corrente 5×1000 funds; the Italian Ministry of Innovations via the Sustainable Growth Fund – Innovation Agreements under the Ministerial Decree of December 31, 2021, and the Director›s Decree of November 14, 2022 (2nd Call), Project No.: F/350104/01-02/X60; and the Italian Ministry of University and Research (MUR) 2023 through the "Future Artificial Intelligence Research – FAIR" program, PE0000013, CUP D53C22002380006, within the National Recovery and Resilience Plan (PNRR), Mission 4, Component 2, Investment 1.3 – funded by the European Union – NextGenerationEU. Project: "AIDH – Adaptive AI Methods for Digital Health.

Authors contribution

Conceptualization: U.M., N.F. Methodology: K.V., E.G-R., U.M., N.F. Investigation: E.M., G.Curs., K.V., E.G-R. Data curation: E.M., C.F., F.P., M.N., L.P., P.P., E.D-C. Formal analysis: E.M., G.Cursa,. Writing—original draft: E.M., G.Cursa., K.V., N.F. Writing—review & editing: All Authors. Supervision: E.G-R., U.M., N.F. All authors have read and agreed to the published version of the manuscript.

Ethical consideration

This study collected aggregated information on institutional practices related to PIK3CA testing in HR+/HER2– metastatic breast cancer. No patient-level data, biological specimens, or identifiable personal/institutional information were collected. Participation was voluntary, non-remunerated, and responses were recorded anonymously. Formal Ethics Committee approval and written informed consent were not required for this type of research. The study was conducted in accordance with the principles of the Declaration of Helsinki and relevant data protection regulations.

References

1 Gennari A, André F, Barrios CH, Cortés J, de Azambuja E, DeMichele A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Annals of Oncology. 2021;32(12):1475-95.

2 Sukhun SA, Temin S, Barrios CH, Antone NZ, Guerra YC, Chavez-MacGregor M, et al. Systemic Treatment of Patients With Metastatic Breast Cancer: ASCO Resource–Stratified Guideline. JCO Global Oncology. 2024(10):e2300285.

3 Curigliano G, Castelo-Branco L, Gennari A, Harback N, Criscitiello C, Trapani D. ESMO metastatic breast cancer living guideline v1.1 May 2023 [Original citation]. Ann Oncol. 2023;32(12):1475-95.

4 Senkus E, Delaloge S, Domchek SM, Conte P, Im SA, Xu B, et al. Olaparib efficacy in patients with germline BRCA-mutated, HER2-negative metastatic breast cancer: Subgroup analyses from the phase III OlympiAD trial. Int J Cancer. 2023.

5 Heeke AL, Xiu J, Elliott A, Korn WM, Lynce F, Pohlmann PR, et al. Actionable co-alterations in breast tumors with pathogenic mutations in the homologous recombination DNA damage repair pathway. Breast cancer research and treatment. 2020;184(2):265-75.

6 Henry NL, Somerfield MR, Dayao Z, Elias A, Kalinsky K, McShane LM, et al. Biomarkers for Systemic Therapy in Metastatic Breast Cancer: ASCO Guideline Update. Journal of Clinical Oncology. 2022;0(0):JCO.22.01063.

7 Bardia A, Cortés J, Bidard FC, Neven P, Garcia-Sáenz J, Aftimos P, 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(19):4299-309.

8 Fusco N, Malapelle U. Next-generation sequencing for PTEN testing in HR+/HER2- metastatic breast cancer. Crit Rev Oncol Hematol. 2025:104626.

9 Zeng Z, Yi Z, Xu B. The biological and technical challenges facing utilizing circulating tumor DNA in non-metastatic breast cancer patients. Cancer Lett. 2025;616:217574.

10 Guerini-Rocco E, Venetis K, Cursano G, Mane E, Frascarelli C, Pepe F, et al. Standardized molecular pathology workflow for ctDNA-based ESR1 testing in HR+/HER2- metastatic breast cancer. Crit Rev Oncol Hematol. 2024;201:104427.

11 Varga Z, Maccio U. Molecular pathology in breast disease: diagnostic, prognostic, and therapeutic tools. Virchows Arch. 2024;484(2):247-61.

12 Boscolo Bielo L, Guerini Rocco E, Crimini E, Repetto M, Lombardi M, Zanzottera C, et al. Molecular tumor board in patients with metastatic breast cancer. Breast cancer research and treatment. 2025;210(1):45-55.

13 Aristei C, Tomatis M, Antonio P, Marotti L, Cardoso MJ, Cheung KL, et al. Treatment and outcomes in breast cancer patients: A cross section study from the EUSOMA breast centre network. Eur J Cancer. 2024;196:113438.

14 Weiss A, Lopez NE, Ward EP, Lee KC, Caudle AS, Dickson-Witmer D, et al. Challenges in Clinical Trial Implementation: Results from a Survey of the National Accreditation Program of Breast Centers (NAPBC). Annals of surgical oncology. 2019;26(13):4364-71.

15 Fusco N, Malapelle U, Fassan M, Marchio C, Buglioni S, Zupo S, et al. PIK3CA Mutations as a Molecular Target for Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer. Front Oncol. 2021;11:644737.

16 Markham A. Alpelisib: First Global Approval. Drugs. 2019;79(11):1249-53.

17 André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor–Positive Advanced Breast Cancer. New England Journal of Medicine. 2019;380(20):1929-40.

18 Hao C, Wei Y, Meng W, Zhang J, Yang X. PI3K/AKT/mTOR inhibitors for hormone receptor-positive advanced breast cancer. Cancer Treat Rev. 2025;132:102861.

[19] Turner NC, Im SA, Saura C, Juric D, Loibl S, Kalinsky K, et al. Inavolisib-Based Therapy in PIK3CA-Mutated Advanced Breast Cancer. N Engl J Med. 2024;391(17):1584-96.

[20] Turner Nicholas C, Oliveira M, Howell Sacha J, Dalenc F, Cortes J, Gomez Moreno Henry L, et al. Capivasertib in Hormone Receptor–Positive Advanced Breast Cancer. New England Journal of Medicine. 2023;388(22):2058-70.

[21] Dilawari A, Buturla J, Osgood C, Gao X, Chen W, Ricks TK, et al. US Food and Drug Administration Approval Summary: Capivasertib With Fulvestrant for Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Locally Advanced or Metastatic Breast Cancer With PIK3CA/AKT1/PTEN Alterations. J Clin Oncol. 2024;42(34):4103-13.

[22] Schagerholm C, Robertson S, Toosi H, Sifakis EG, Hartman J. PIK3CA mutations in endocrine-resistant breast cancer. Scientific Reports. 2024;14(1):12542.

[23] Pepe F, Venetis K, Cursano G, Frascarelli C, Pisapia P, Vacirca D, et al. PIK3CA testing in hormone receptor-positive/HER2-negative metastatic breast cancer: real-world data from Italian molecular pathology laboratories. Pharmacogenomics. 2024;25(3):161-9.

[24] Hossain MT, Hossain MA. Targeting PI3K in Cancer Treatment: A Comprehensive Review with Insights from Clinical Outcomes. Eur J Pharmacol. 2025:177432.

[25] Asnaghi R, Antonarelli G, Battaiotto E, Castellano G, Guidi L, Izzo D, et al. An update on promising and emerging protein kinase B/AKT inhibitors for breast cancer. Expert Opin Pharmacother. 2025;26(3):235-47.

[26] Fusco N, Malapelle U, Rolfo C. Role of the International Society of Liquid Biopsy (ISLB) in establishing quality control frameworks for clinical integration. Crit Rev Oncol Hematol. 2025:104619.

[27] Pepe F, Russo G, Venetis K, Scimone C, Palumbo L, Nacchio M, et al. Harmonization trial on ESR1 testing strategies in ER+/HER2- breast cancer patients: an Italian experience. J Liq Biopsy. 2025;9:100314.

[28] Vigliar E, Iaccarino A, Sciortino M, De Luca C, Malapelle U, Bellevicine C, et al. Molecular predictive testing in precision oncology: The Italian experience. Cancer Cytopathol. 2020;128(9):622-8.

[29] Fusco N, Jantus-Lewintre E, Serrano MJ, Gandara D, Malapelle U, Rolfo C. Role of the International Society of Liquid Biopsy (ISLB) in establishing quality control frameworks for clinical integration. Critical Reviews in Oncology/Hematology. 2025:104619.

[30] Buchta C, Marrington R, De la Salle B, Albarede S, Badrick T, Bietenbeck A, et al. Behind the scenes of EQA - characteristics, capabilities, benefits and assets of external quality assessment (EQA). Clin Chem Lab Med. 2025.

[31] Linko S, Boursier G, Bernabeu-Andreu FA, Dzneladze N, Vanstapel F, Brguljan PM, et al. EN ISO 15189 revision: EFLM Committee Accreditation and ISO/CEN standards (C: A/ISO) analysis and general remarks on the changes. Clin Chem Lab Med. 2025.

[32] Dufraing K, Fenizia F, Torlakovic E, Wolstenholme N, Deans ZC, Rouleau E, et al. Biomarker testing in oncology - Requirements for organizing external quality assessment programs to improve the performance of laboratory testing: revision of an expert opinion paper on behalf of IQNPath ABSL. Virchows Arch. 2021;478(3):553-65.

[33] van Krieken JH, Siebers AG, Normanno N. European consensus conference for external quality assessment in molecular pathology. Ann Oncol. 2013;24(8):1958-63.

[34] Schneider F, Maurer C, Friedberg RC. International Organization for Standardization (ISO) 15189. Ann Lab Med. 2017;37(5):365-70.

[35] McDevitt T, Durkie M, Arnold N, Burghel GJ, Butler S, Claes KBM, et al. EMQN best practice guidelines for genetic testing in hereditary breast and ovarian cancer. Eur J Hum Genet. 2024;32(5):479-88.

[36] Park L, Thompson SL, Roose J, Lu Y, Chaki M, Lam C, et al. Testing patterns and prevalence of PIK3CA, AKT1, and PTEN alterations among patients (pts) with HR+/HER2- metastatic breast cancer (mBC) in the US. Journal of Clinical Oncology. 2024;42(16_suppl):1041-.

[37] Vollbrecht C, Hoffmann I, Lehmann A, Merkelbach-Bruse S, Fassunke J, Wagener-Ryczek S, et al. Proficiency testing of PIK3CA mutations in HR+/HER2-breast cancer on liquid biopsy and tissue. Virchows Arch. 2023;482(4):697-706.

[38] Suppan C, Graf R, Jahn S, Zhou Q, Klocker EV, Bartsch R, et al. Sensitive and robust liquid biopsy-based detection of PIK3CA mutations in hormone-receptor-positive metastatic breast cancer patients. Br J Cancer. 2022;126(3):456-63.

[39] Kim E, Dhillon PK, Lambert P, Cheng A, Basho RK. PIK3CA testing and treatment patterns among patients with metastatic breast cancer in US community clinical practice. Journal of Clinical Oncology. 2024;42(16_suppl):e13059-e.

[40] Bravaccini S, Mazza M, Maltoni R. No more disparities among regions in Italy: recent approval of genomic test reimbursability for early breast cancer patients in the country. Breast Cancer Res Treat. 201. Netherlands: © 2023. The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature.; 2023. p. 1-3.

[41] Giorgi Rossi P, Djuric O, Navarra S, Rossi A, Di Napoli A, Frova L, et al. Geographic Inequalities in Breast Cancer in Italy: Trend Analysis of Mortality and Risk Factors. Int J Environ Res Public Health. 2020;17(11).

[42] Ferraris G, Coppini V, Ferrari MV, Monzani D, Grasso R, Pravettoni G. Understanding Reasons for Cancer Disparities in Italy: A Qualitative Study of Barriers and Needs of Cancer Patients and Healthcare Providers. Cancer Control. 2024;31:10732748241258589.

[43] Ma A, O'Shea R, Wedd L, Wong C, Jamieson RV, Rankin N. What is the power of a genomic multidisciplinary team approach? A systematic review of implementation and sustainability. Eur J Hum Genet. 2024;32(4):381-91.

[44] Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19(1):4-23.

[45] Fusco N, Pruneri G, Pagni F, Malapelle U. Molecular Testing in Solid Tumors: Best Practices from the Molecular Pathology and Precision Medicine Study Group of the Italian Society of Pathology (PMMP/SIAPeC): Shaping Excellence in Molecular Diagnostics. Pathologica-Journal of the Italian Society of Anatomic Pathology and Diagnostic Cytopathology. 2025;117.

[46] Marchiò C, Berrino E, Scarpino S, Alì G, Bellomo SE, Buglioni S, et al. Part I–Pre-analytical phase. Pathologica-Journal of the Italian Society of Anatomic Pathology and Diagnostic Cytopathology. 2025;117.

[47] Guerini-Rocco E, Venetis K, Bessi S, Buglioni S, Chiappetta C, de Biase D, et al. Part II–Analytical phase. Pathologica-Journal of the Italian Society of Anatomic Pathology and Diagnostic Cytopathology. 2025;117.

[48] Buglioni S, Seminati D, Bruno R, Casini B, Gristina V, Guerini-Rocco E, et al. Part III–Post-analytical phase. Pathologica-Journal of the Italian Society of Anatomic Pathology and Diagnostic Cytopathology. 2025;117.