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Identifying germline pathogenic variants in breast cancer using tumor sequencing☆

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ABSTRACT

Purpose: To investigate the performance of an in-house tumor sequencing panel to identify patients with breast cancer and a germline pathogenic variant (gPV).
Patients and methods: Retrospective and blinded tumor sequencing analysis in 90 patients with breast cancer and prior germline genetic testing (45 non-carriers and 45 carriers of a gPV) using an in-house panel (VHIO-300). Sensitivity (S), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of tumor sequencing were calculated. A Cohen's kappa coefficient ≥0.80 was predefined as minimum to be reliably acceptable for clinical implementation.
Results: The cohort included 84 women and 6 men with a median age of 48 years (29–84). Tumors of germline carriers were mainly stage II (47 % vs 31 %, P = 0.047), luminal B-like (56 % vs 31 %, p = 0.037) or triple negative (22 % vs 16 %, = 0.037). The in-house tumor panel identified 91 % (40/44) of the gPV. The analysis did not detect any of the 2 patients with germline large rearrangement alterations nor 2 of the 7 patients with intronic variants included. The tumor sequencing panel yielded 7 % of false positive results (ie, genetic alterations suggestive of germline origin). Hence, S was 91 %, Sp 93 % and Cohen's kappa coefficient between tumor and germline testing was 0.84 (95 % CI 0.73–0.95).
Conclusion: Tumor tissue sequencing with our in-house panel demonstrated an acceptable performance to identify patients with breast cancer carriers of a gPV.

1. Introduction

In the era of precision medicine, next generation sequencing has improved molecular diagnosis and led towards tailored therapies in solid tumors, particularly in breast cancer (BC). About 10 % of BC cases are considered hereditary and several genes have been associated to susceptibility, such as *BRCA1, BRCA2, PALB2, ATM, CHEK2, BARD1, RAD51C, RAD51D* and *TP53*.

☆ **List presentation of results:** The results were partially presented at the ESMO Breast 2024 Congress, Berlin, Germany, and at SEOM 2024 Congress, Madrid, Spain.

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Germline testing identifies pathogenic variants (PV) of hereditary origin and have personal and familial implications. When a germline PV (gPV) is detected, the individual is included in a personalized surveillance program according to their cancer risk estimation and risk reduction interventions are offered. Moreover, predictive cascade testing is recommended to relatives to provide preventive and screening programs in carriers.

Assessment of germline cancer susceptibility has traditionally been based on fulfillment of clinical criteria; however, this approach lacks sensitivity and may overlook patients with an unknown family history or those with a *de novo* germline variant. In addition, the identification of a gPV may have therapeutic implications [1]. Olaparib has demonstrated improved outcomes in patients with a pathogenic germline *BRCA1/2* (g*BRCA*) variant [2,3], underscoring the importance of identifying a gPV for preventive strategies and for targeted therapies. Due to the benefit of PARP-inhibitors (PARPi) in BC patients carrying a g*BRCA* PV, the most recent ASCO recommendations advocated for offering germline genetic test to all patients diagnosed with BC at ≤65 years old and to patients with BC diagnosed over 65 years with family history [4].

On the other hand, tumor testing in metastatic BC has increased over the past few years. The ESMO Precision medicine working group recently recommended next-generation sequencing (NGS) in metastatic BC to determine *ESR1* and *PIK3CA/AKT1/PTEN* pathway status as their alterations involve therapeutic implications [5]. Moreover, PARPi have shown benefit in patients with tumor PV in *BRCA1/2* in the phase II TBCRC 048 clinical trial, raising an opportunity for targeted therapies in these patients, beyond germline alterations [6,7].

Germline and tumor genetic tests provide valuable information, but they carry an economic and human-resource cost that could limit their implementation and accessibility. The clinical validity and utility of BC molecular profiling to identify germline pathogenic variants is currently tested in many settings, and optimal panels are debated. Hence, we hypothesize that optimization of tumor genetic sequencing may improve the applicability of collecting molecular information to tailor treatment in patients with BC and diagnose a genetic susceptibility.

We aimed to examine the capacity of our in-house tumor sequencing panel for detecting variants of germline origin in patients with BC.

2. Material and methods

2.1. Study population

Unselected female or male patients with BC diagnosis, all of whom had previously performed a germline genetic test for hereditary breast and ovarian cancer (HBOC) at Vall d'Hebron Institute of Oncology between 2017 and 2022. Selection for germline testing was based on local clinical criteria. All patients had given prior consent for research purposes and the project was approved by the local Ethical Committee.

2.2. Germline and tumor genetic analysis

The genes included in the germline analysis were *BRCA1, BRCA2, PALB2, ATM, CHEK2, RAD51C, RAD51D, MLH1, MSH2* and *MSH6*. The cohort included patients harboring germline large rearrangements (LR) or intronic PV/LPV in the genes analyzed. In our current clinical practice, *TP53, STK11, CDH1* and *PTEN* are only analyzed when patients meet specific phenotypic clinical criteria, which was not applicable to the study population in this case. Overall, 90 patients were included, 45 had a germline pathogenic/likely pathogenic variant (further on referred to as gPV) in at least one of the above genes, and 45 had no gPV identified. A paired tumor sample (primary or metastatic) was obtained, and tumor sequencing was carried out using an in-house panel sequencing. The results of the germline test were blinded during the tumor sequencing analysis. In line with ESMO guidelines, the cut-off values of variant allele fraction (VAF) of 30 % for Single-Nucleotide variants (SNVs) and 20 % for small insertions or deletions were applied for germline analysis recommendation after tumor genetic testing [8,9]. To reduce the likelihood of bias in interpretation of the tumor analysis results, only three samples with founder germline PVs were included (2 with c.68_69del PV in *BRCA1* and 1 with c.658_659del PV in *BRCA2*). The Hereditary Plus OncoKitDx that employs massive high-throughput sequencing (NGS) technology to detect SNVs, small insertions and deletions (INDELs), copy number variations (CNVs) and the presence of large inserts, such as ALU inserts associated with familial cancer had been used for germline testing.

Tumor sequencing was performed with the VHIO-300 panel. DNA was extracted from formalin-fixed paraffin embedded (FFPE) blocks using either the Qiagen AllPrep® DNA/RNA FFPE kit for FFPE-derived samples or using the Maxwell® RSC FFPE Plus DNA Kit (Promega). DNA underwent mechanical fragmentation using a Covaris M220 focused-ultrasonicator, aiming at 150 bp fragment-size, prior to library preparation. Tumor-only hybrid capture-based targeted sequencing was performed using the ISO-accredited VHIO-300 targeted panel. In brief, libraries were prepared using SureSelect XT Human (Agilent) and captured using a customized panel covering exonic regions of 435 genes. Libraries were sequenced in a HiSeq2500 instrument (Illumina), 2 × 100 paired end. Sequencing reads were aligned against the GRCh37 (hg19) reference genome using BWA (v0.7.17), and base recalibrated and indel realigned using GATK (v3.7.0) and abra2 (v2.23), respectively. For mutations, variant calling was performed with VarScan2 (v2.4.3) and Mutect2 (Genome Analysis Toolkit (GATK) v4.1.0.0). Frequent single nucleotide polymorphisms (SNPs) were filtered based on the gnomAD database (allele frequency ≤0.0001). Only variants identified by both callers, with a minimum of 7 supporting reads, and with a minimum VAF of 5 % for SNVs and 10 % INDELs were considered. Variant annotation was performed using publicly available databases (COSMIC, ClinVar, VarSome, OncoKB) and manually curated. Copy number alterations (CNA) were calculated using CNVkit (v0.9.6).

Tumor purity, defined as "the percentage of tumor cells over the total number of cells in the sample, tumor or normal" will be assessed for evaluation of tumor sample quality and according to institutional standards a minimum cellularity of 30 % will be considered a requirement.

2.3. Statistical analysis

A descriptive analysis was conducted for baseline variables in the overall population, the germline positive and the germline negative cohorts. Frequencies and percentages were reported for categorical variables, and median with interquartile range (IQR) for numerical variables. The Wilcoxon test was applied to assess significant differences in numerical variables, and the Chi-square test was used for categorical variables.

The performance of the panel in identifying the presence or absence of germline mutations was evaluated using sensitivity (S), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV). These metrics provide a comprehensive assessment of the panel's ability to correctly classify individuals as carriers or non-carriers of germline mutations. To evaluate the concordance between tumor molecular analysis and germline testing in identifying pathogenic germline mutations, Cohen's kappa (κ) coefficient was used.

The following considerations were adapted for the sensitivity and specificity analysis: A true positive result required a germline variant to be identified in the previous germline testing and in the tumor test. A result was categorized as false negative if the tumor test did not detect the PV/LPV previously detected in the germline test. A true negative result required the absence of a PV/LPV in both the tumor test and the germline test. We considered a false positive result when the tumor test detected a PV/LPV in an HBOC gene suggestive of a germline origin, but the germline genetic test was negative (so, the variant was classified as of somatic origin).

All statistical analyses were performed using R software version 4.2.2. Results were considered statistically significant if $p < 0.05$ and for

S, Sp, PPV, NPV, and κ, a value greater than 0.80 was considered clinically relevant.

3. Results

3.1. Patient characteristics

Median age of BC diagnosis was 48 years (29–84) and all but six, were women. Most of the patients (61 %) were premenopausal at diagnosis. The majority were invasive BC of no special type (87 %), with an overrepresentation of HER2-negative luminal tumors by immunohistochemistry; 30 % luminal A-like and 43 % luminal B-like. Most of the patients were diagnosed with stage I (38 %) or stage II (39 %) BC. More than half of the patients (54 %) had a first- and/or second-degree relative affected with BC.

The main characteristics of the cohort are presented in Table 1. Patients who carried a gPV were diagnosed at higher stages (stage I: 24 % vs 51 %, stage II: 47 % vs 31 %, stage III 18 % vs 7 % and stage IV: 11 % vs 11 %; p = 0.047) and had higher incidence of luminal B-like and triple negative tumors (Luminal A: 20 % vs 40 %, luminal B HER2 negative: 56 % vs 31 %, luminal HER2 positive: 0 % vs 11 %, HER2 positive: 2 % vs 2 % and triple negative 22 % vs 16 %; p = 0.037) in comparison with patients with a negative germline genetic test. Also, they were more likely to have a first- and/or second-degree relative affected with BC.

3.2. Correlation of germline–tumor results

Two tumor samples could not be sequenced due to insufficient tumor sample availability, one from a carrier of a germline PV in *PALB2* and one from a patient with a negative result in the germline genetic test (Fig. 1). Out of 44 samples from patients with germline PV, the tumor testing detected the PV in 40 patients. These variants were considered true positives. The following four variants were not identified by the tumor panel (false negatives): c.902-19_1065 + 869del1052 in *ATM*, c.793-1G > A in *CHEK2*, deletion of exons 3 and 4 of *CHEK2* and c.8332-13T > G in *BRCA2* (Table 2). After unblinding the results, the variants were identified in the revised sequencing results. An ad-hoc analysis shown that the intronic variant c.8332-13T > G in *BRCA2* had not been identified because it was outside the variant reading area of the in-house sequencing panel, and the intronic variant c.793-1G > A in *CHEK2* was not detected because the region was poorly covered with the panel. The two germline large rearrangements included (c.902-19_1065 + 869del1052 in *ATM*, and deletion of exons 3 and 4 of *CHEK2*) were not identify by tumor sequencing. Hence, the technique demonstrated a lack of sensitivity in identifying the two large rearrangements included. The reanalysis of the two large rearrangements (c.902-19_1065 + 869del1052 in *ATM* and deletion of exons 3 and 4 of *CHEK2*) showed that the proportion of reads was lower in those exons and compatible with the deletions. Overall, as described in Table 3, more than 80 % of the gPV of the BC genes were detected, except for *CHEK2* (50 %). Reversely, 3 PVs of germline origin presented in the tumor sequencing testing with a VAF lower than 30 % (one in *BRCA1* at 6.17 %, one in *BRCA2* at 20.9 % and one in *CHEK2 at* 23.57 %) that would have been misinterpreted of somatic origin in the absence of germline testing.

Among the 44 patients with negative results in the germline genetic test, the tumor sequencing detected a PV/LPV of potential germline origin with a VAF above 30 % in 3 samples. These variants were c.8023A > G (VAF 35 %) and c.8695C > T (VAF 58 %) in *BRCA2,* and c.5193+1G > T in *BRCA1* (VAF 45 %). The germline analyses were revised in these 3 samples and the threshold of detection was reduced to 5 % aiming to identify potential mosaicisms. The revised analysis did not identify any germline PV in these 3 cases; therefore, the results were classified as false positives (Table 2).

Additionally, in a tumor sample of a patient with a gPV in *PALB2*, another PV in *PALB2* was identified in the tumor testing with a VAF of 11 %, suggesting that this variant was acting as the second hit. In another case, the tumor analysis reported a PV in the *FH* gene that was suggestive of germline origin, but this was classified as a variant of uncertain significance in the germline analysis.

Finally, additional PV of tumor origin in other genes were detected in the tumor samples of patients with a gPV, such as a PV in *TP53* in 60 % of *BRCA1* gPV tumors (6/10), 29 % of *BRCA2* gPV (5/17) tumors and 100 % of *RAD51C/D* gPV tumors (2/2). PVs in *PTEN/PI3K/AKT* signaling pathway were identified in 7 tumors (1 from a gPV *BRCA1* carrier, 1 from a gPV *BRCA2* carrier, 2 from a gPV *PALB2* carriers, 2 from a gPV *ATM* carriers, and 1 from a gPV *MSH6* carrier). The main characteristics of BC tumors and tumoral findings are summarized in Table S1.

In summary, the in-house tumor sequencing panel failed to identify gPV in 4 patients with verified gPV (4/44 false negative) and 3 tumor PV were falsely reported as of potential germline origin (3/44 false positive), among 88 patients with both tumor tissue and germline results. The statistical analysis yielded a sensitivity of 91 %, specificity 93 %, PPV 93 % and NPV 91 %. The Cohen's kappa coefficient was 0.84 (95 % CI 0.73–0.95). A summary of the classification of the results applied in the statistical analysis is presented in Table S2.

Table 1
Patient and tumor characteristics of the patients included in the analysis.

	Total (90 p)	Germline positive testing	Germline negative testing	
Female	84 (93 %)	41 (9 %)	43 (96 %)	0.39
Median age	48 y (29–84)	48 y (30–84)	49 y (29–79)	0.40
Menopausal status	67 p (80 %)	26 (63 %)	29 (67 %)	0.51
Tumor characteristics				
Stage at diagnosis				
Stage I	34 (38 %)	11 (24 %)	23 (51 %)	0.047
Stage II	35 (39 %)	21 (47 %)	14 (31 %)	
Stage III	11 (12 %)	8 (18 %)	3 (7 %)	
Stage IV	10 (11 %)	5 (11 %)	5 (11 %)	
Histology				
Ductal	78 (87 %)	41 (91 %)	37 (82 %)	0.686
Lobular	7 (8 %)	2 (3,9 %)	5 (11 %)	
Ductolobular	2 (2 %)	1 (2 %)	1 (2 %)	
Other	4 (4 %)	2 (4 %)	2 (4 %)	
Subtype				
Luminal A	27 (30 %)	9 (20 %)	18 (40 %)	0.037
Luminal B HER2 negative	39 (43 %)	25 (56 %)	14 (31 %)	
Luminal HER2 positive	5 (4,8 %)	0 (0 %)	5 (11 %)	
HER2 positive non-luminal	2 (1,9 %)	1 (2 %)	1 (2 %)	
Triple negative	17 (19 %)	10 (22 %)	7 (16 %)	
Other cancer diagnosis	6 (6,7 %)	5 (11 %)	1 (2 %)	0.09
BC FDR and/or SDR	49 (54 %)	30 (67 %)	19 (42 %)	0.019

4. Discussion

In this single institution analysis, we report a good rate of detection of germline pathogenic variants through tumor sequencing analysis of breast tumors with our in-house panel. The results demonstrate a good performance of tumor sequencing panel with high sensitivity and specificity, both above 90 %, and an overlap between germline and tumor sequencing of 0.84.

Comparing tumor molecular profiling and germline testing has previously shown promising results. In a cohort of 1040 patients with selected solid tumors, 101 (9.7 %; 95 % CI, 8.1 %–11.7 %) were identified to have actionable germline mutations that lacked clinical indication for germline testing and would have been otherwise missed [10]. In an analysis of the OlympiAD trial, with a known population of *gBRCA* mutations, tumor profiling also identified the *gBRCA* mutations [1]. Vice versa, in a large cohort of 21333 patients with solid tumors, tumor-only

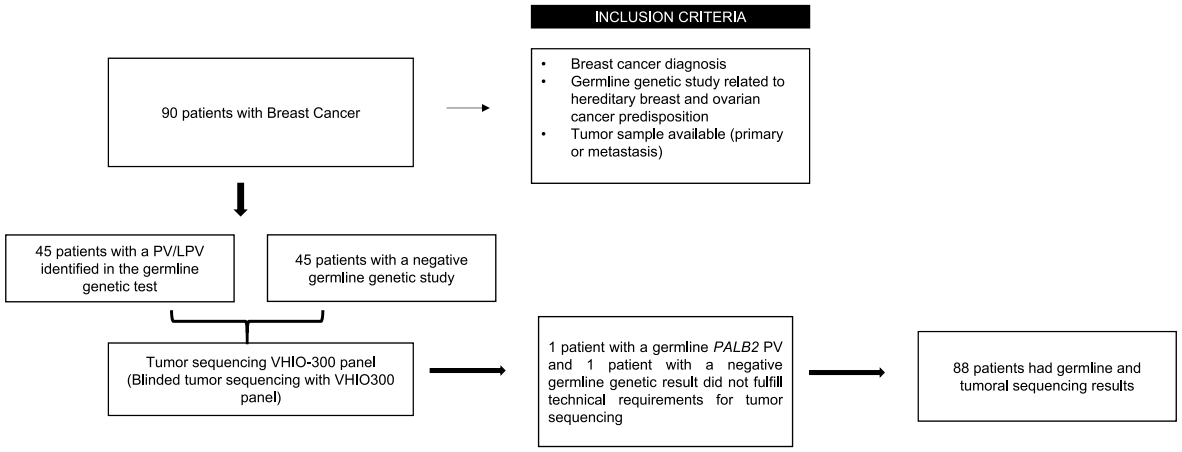

Fig. 1. CONSORT diagram.
PV/LPV: pathogenic variant/likely pathogenic variant.

Table 2
Description of variants classified as false negatives and false positives.

Germline PV/LPV not detected by tumor sequencing (FN)				
Gene	Variant	Type of variant	BC Tumor	Other PV/LPV in tumor testing
ATM	c.902-19_1065 + 869del1052	Large rearrangement	Luminal B HER2 negative	*GATA3* mt
CHEK2	deletion of exons 3 and 4	Large rearrangement	Luminal B HER2 negative	*ARID1A*, *FOXA1*, *MAP3K1* mt
CHEK2	c.793-1G > A	Intronic variant affecting splicing	Luminal A	None
BRCA2	c.8332-13T > G	Intronic variant affecting splicing	Luminal B HER2 negative	None

PV/LPV in HBOC genes detected in tumoral sequencing not having a germline origin (FP)					
Gene	Variant	Type of variant	VAF	BC Tumor	Other PV/LPV in tumor testing
BRCA1	c.5193+1G > T	Intronic variant affecting splicing	45.32 %	Triple-negative	*TP53* mt
BRCA2	c.8695C > T	Point mt (Nonsense)	57.66 %	Luminal B HER2 negative	*PIK3CA* mt
BRCA2	c.8023A > G	Point mt (Missense)	35.19 %	Luminal A	*PIK3CA* mt
CHEK2	c.341G > A	Point mt (Nonsense)	5.20 %	Luminal A	*PIK3CA* mt

PV: Pathogenic variant. LPV: Likely pathogenic variant. FN: False negative. FP: False positive. BC: Breast cancer. Mt: mutation.

sequencing with the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Targets (MSK-IMPACT) assay, detected germline variants in 89.5 % [11]. Despite the overall acceptable sensitivity for identification of pGV, tumor-only sequencing demonstrated lower performance in identifying germline copy number variants, intronic variants and insertions.

In our tumor sequencing pipeline, intronic locations were included in the probe set design, although these regions are sometimes difficult to profile due to high complexity and presence of repetitive elements [12]. On the other hand, large rearrangements are not simple to call using exome-targeting hybrid capture panels because they will affect a small number of exons and ideally would require WGS approaches. Our re-analysis after matching with the germline alterations led to identification of missed alterations and further improvement of the pipeline to detect them. The detection of large rearrangements is mainly influenced by library insert size, as NGS libraries from FFPE samples typically have short inserts due to DNA fragmentations caused by formalin fixation. One potential approach to enhance detection would be the use of long-read sequencing technologies, which are less affected by DNA fragmentation.

An expert consensus on the acceptable cut-off of false negative gPV results from tumor sequencing panels has not been established. Whether a cut-off above 90 %, correlating to a high Cohen's coefficient, could be acceptable to their use as a proxy of germline testing remains to be agreed. Nonetheless, our level of accuracy through tumor sequencing appears to be valuable to consider it an screening tool to identify patients with potential gPV, that would otherwise not be identified by current clinical criteria. Indeed, nowadays 9 % of mutation carriers do not fulfill clinical criteria for germline testing [13]. The tumor sequencing panel did not identify 9 % of gPV due to factors related to the panel itself, which is similar to previous reports [1,11]. However, analysis of the technical limitations that prompted the overlook of these gPV has thereafter improved the analytical performance and pipeline of our tumor testing technology. Nevertheless, in other settings germline testing should not be excluded after a negative tumor sequencing in patients with BC if there is a clinical suspicion of carrying a germline pathogenic variant.

In patients with BC, especially in the metastatic setting, tumor sequencing is currently being performed to identify biomarkers of sensitivity and resistance to targeted therapies, such as *ESR1*, *PIK3CA*, and HER2 [5]. Tumor sequencing of HBOC-associated genes could be a tool to identify variants of germline origin in patients with metastatic BC undergoing tumor sequencing. This is particularly relevant when deciding which patients with luminal HER2-negative BC warrant germline testing. Many germline carriers remain unidentified and there are not enough health care resources to provide genetic counseling and germline testing for all patients with a luminal HER2-negative phenotype.

In addition, despite targeted therapies for patients with BC and tumor *BRCA1* or *BRCA2* pathogenic variants have yet not been approved by any regulatory agency, there is biological and clinical evidence that somatic mutations in these genes are likely to show biallelic inactivation and be a good biomarker for targeted therapies [14]. Thus, tumor sequencing of HBOC-associated genes could be a pathway to screen patients for targeted therapies and candidates for reflex germline testing (Fig. 2).

Our study had some limitations. For instance, all patients fulfilled

Table 3
Detection of germline variants with tumor sequencing by type of variant. All gPV (31) were identified for *BRCA1, BRCA2, PALB2* genes, except one (c.8332-13T > G, *BRCA2*). More than 80 % of the gPV were detected for each gene except for *CHEK2* (50 %). 100 % of single nucleotid exonic gPV were identified, 71 % of intronic gPV and no large rearrangement (GR) were identified (0/2).

Gene	gPV (%)	Samples sequenced (%)	gVP identified with VHIO300	SNVs included (%)	SNVs identified by VHIO300 (%)	Intronic gPV included (%)	Intronic gPV identified with VHIO300 (%)	LR included (%)	LR identified with VHIO300 (%)
BRCA1	10 (22 %)	10 (100 %)	10 (100 %)	7 (70 %)	7 (100 %)	3 (30 %)	3 (100 %)	0 (0 %)	–
BRCA2	17 (38 %)	17 (100 %)	16 (94 %)	15 (88 %)	15 (100 %)	2 (12 %)	1 (50 %)	0 (0 %)	–
PALB2	5 (11 %)	4 (80 %)	4 (100 %)	4 (100 %)	4 (100 %)	0 (0 %)	–	0 (0 %)	–
ATM	6 (13 %)	6 (100 %)	5 (83 %)	4 (67 %)	4 (100 %)	1 (17 %)	1 (100 %)	1 (17 %)	0 (0 %)
CHEK2	4 (9 %)	4 (100 %)	2 (50 %)	2 (50 %)	2 (100 %)	1 (25 %)	0 (0 %)	1 (25 %)	0 (0 %)
RAD51C	1 (2 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	0 (0 %)	–	0 (0 %)	–
RAD51D	1 (2 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	0 (0 %)	–	0 (0 %)	–
MSH6	1 (2 %)	1 (100 %)	1 (100 %)	1 (100 %)	1 (100 %)	0 (0 %)	–	0 (0 %)	–

gPV: germline Pathological Variant, LR: large rearrangements, SNV: Single Nucleotide.

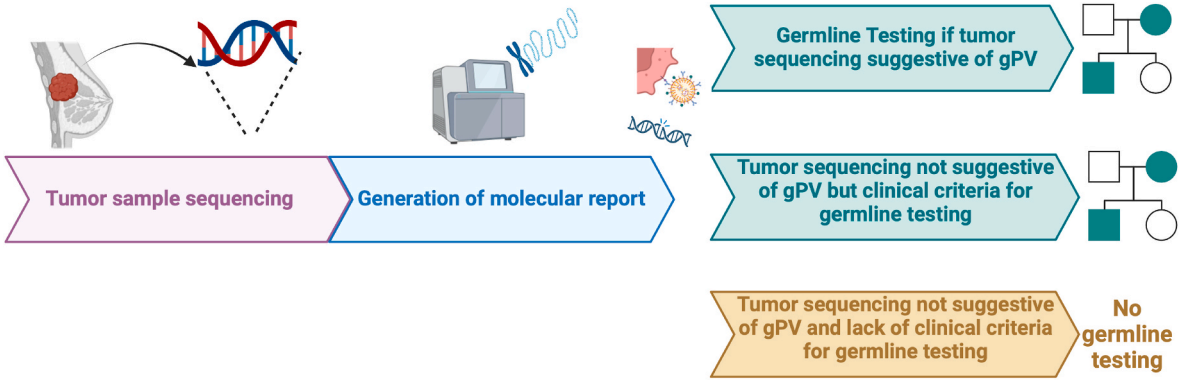

Fig. 2. Two-step pathway for identification of patients with germline pathogenic variants (gPV) based on tumor sequencing: reflex germline testing if a tumor alteration is found, or if negative tumor sequencing but suspicious clinical criteria.

clinical criteria for germline testing, thus they represent a population with increased likelihood of gPV. Therefore, the generalizability of this approach to a population of all comers is uncertain. However, sequencing analysis and interpretation of the results were performed blinded, i.e. without knowledge of which patients had verified gPV/LPV, which might overcome this limitation.

Detecting the main limitations in gPV identification allows for incorporating measures to improve them. Currently, if there is a personal/family history suggestive of gPV, we recommend a germ cell study regardless of the tumor result. In the absence of personal/family history, the VHIO300 panel allows the presence of a gPV to be ruled out with high reliability.

CRediT authorship contribution statement

Mara Cruellas: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andri Papakonstantinou:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Adrià López-Fernández:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Ester Castillo:** Writing – review & editing, Writing – original draft, Software, Resources, Investigation, Formal analysis, Data curation. **Judit Matito:** Writing – review & editing, Software, Resources, Investigation, Formal analysis, Data curation. **Marina Gómez:** Writing – review & editing, Resources, Investigation, Data curation. **Alejandra Rezqallah:** Writing – review & editing, Investigation, Data curation. **Sharela Vega:** Writing – review & editing, Investigation, Data curation. **Víctor Navarro:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Maite Torres:** Writing – review & editing, Software, Resources, Investigation, Data curation. **Alejandro Moles-Fernández:** Writing – review & editing, Writing – original draft, Software, Resources, Investigation, Data curation. **Cristina Saura:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization. **Ana Vivancos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Conceptualization. **Judith Balmaña:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Mafalda Oliveira:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Ethical approval

The project was approved by the local Ethical Committee on August 26, 2022 with the following number project: PR(AG)273/2022.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2025.104439.

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