# Clinicopathological characteristics and potential impact of previous treatment history in metachronous contralateral breast cancer: Insights from a real-world retrospective study

RITSUKO SASAKI[1,2], KOJI YAMAMOTO[3], KOTARO IIJIMA[1], MASAMI ARAI[1,4], MITSUE SAITO[1] and GORO KUTOMI[1]

[1]Department of Breast Oncology, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan; [2]Department of Breast Surgery, Institute of Science Tokyo, Tokyo 113-8519, Japan; [3]Department of Biostatistics, Yokohama City University School of Medicine, Yokohama, Kanagawa 236-0027, Japan; [4]Department of Clinical Genetics, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan

Received January 30, 2026; Accepted July 31, 2026

DOI: 10.3892/ol.2026.15847

**Abstract.** Metachronous contralateral breast cancer (mCBC) accounts for 40-50% of new secondary cancers in breast cancer survivors. With improved breast cancer treatment outcomes, the number of long-term survivors is increasing. To better understand the clinical features of mCBC in real-world practice, patients who developed mCBC, defined as contralateral second primary breast cancer diagnosed ≥12 months after the first primary breast cancer (FBC), were retrospectively analyzed. Two types of comparisons were conducted: i) A within-patient comparison between FBC and mCBC (n=115) to evaluate paired differences in tumor characteristics; and ii) a between-patient comparison of the mCBC cohort with a unilateral breast cancer (UBC; n=1,877) cohort of patients who remained recurrence-free for ≥5-10 years to assess differences in baseline clinical features and indicators related to hereditary cancer risk, thereby identifying features unique to mCBC. The median age at diagnosis was 48 years for FBC in patients who subsequently developed mCBC and 55 years for UBC, with a significant difference (P<0.001). The median time interval from FBC to mCBC onset was 9 (1-44) years. The stage at diagnosis was significantly earlier in mCBC than in FBC (P<0.001). Among patients with mCBC, prior chemotherapy (CTx) for FBC was associated with lower odds of the luminal subtype relative to the triple-negative subtype compared with no prior CTx, although this association was not statistically significant (P=0.086). The proportion of patients with a first-degree family history was significantly higher in patients with mCBC than in patients with UBC (P=0.025). No significant association was observed between family history and the timing of mCBC onset. Further adequately powered studies are warranted to clarify whether prior CTx influences the molecular subtype of mCBC (P=0.602). The present results highlighted the importance of family history assessment and the need for lifelong surveillance in breast cancer survivors.

*Correspondence to:* Dr Ritsuko Sasaki, Department of Breast Oncology, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
E-mail: rt-sasaki@juntendo.ac.jp

*Abbreviations:* BC, breast cancer; mCBC, metachronous contralateral breast cancer; CTx, chemotherapy; ER, estrogen receptor; ETx, endocrine therapy; FBC, first primary breast cancer; FH, family history; HR, hormone receptor; ILC, invasive lobular carcinoma; N/A, not available; OR, odds ratio; TN, triple-negative; UBC, unilateral breast cancer

*Key words:* mCBC, FH, previous treatment history, within-patient comparison, breast cancer survivors

## Introduction

Metachronous contralateral breast cancer (mCBC) is defined as a second primary breast cancer (BC) diagnosed in the contralateral breast after the first primary BC (FBC) diagnosis. The time interval used to distinguish synchronous contralateral breast cancer from mCBC varies among previous studies, ranging from 3-12 months (1-3), and no consensus has yet been reached regarding the optimal cut-off. In the present study, we defined mCBC as contralateral breast cancer diagnosed at least 12 months after FBC diagnosis. The first 12 months of follow-up after FBC diagnosis were excluded to minimize the potential for misclassification of metastases or undetected synchronous bilateral BC as mCBC (4-6). The annual mCBC risk is approximately 0.3% in the overall population (7-9). These data indicate that 6% of patients with BC develop mCBC within 20 years of FBC onset. Increases in BC incidence coupled with advances in treatment and improved survival (10), have led to an increase in the number of breast cancer survivors at risk of mCBC.

The influence of mCBC diagnosis on survival compared to unilateral BC (UBC) has been investigated in several studies, but with inconsistent results (1,2,11-13). Although there are many confounding factors, such as the interval between diagnoses, age at FBC diagnosis, and BC-specific mortality rate in mCBC, numerous studies have indicated poorer overall survival in mCBC than in UBC (13-17).

Women diagnosed with BC have a two- to six-fold increased risk of developing mCBC compared with women in the general population (18,19). A population cohort database identified multiple mCBC risk factors from which scores were developed (20). A meta-analysis showed that the presence of a germline genetic variant, family history or systemic treatment for FBC were strongly implicated as risk factors for the development of mCBC. Younger age at FBC onset, high body mass index (BMI), lobular histology and hormone receptor-negative status were cited as weak risk factors (21). In addition, results from the WECARE study, a large international multicenter, population-based case-control study nested within a cohort of over 52,500 female BC survivors, revealed that patients with first-degree relatives with young-onset or bilateral BC are at high risk of mCBC, regardless of germline *BRCA1/2*, *CHEK2*, and *ATM* pathogenic variants (22).

mCBC is the most common second primary cancer among women with UBC, accounting for approximately 40-50% of all new second primary cancers (23,24). Furthermore, with improvements in treatment outcomes using perioperative medications in recent years, the number of BC survivors achieving long-term recurrence-free survival is increasing. Therefore, it is important for survivors of BC to understand the nature of mCBC. Previously reported mCBC risk factors were identified using large databases and may differ from those in actual clinical practice in specific details. The results of recent paired genomic analyses of the first and subsequent breast tumors suggest that some subsequent contralateral BCs may represent genetically independent primary lesions rather than recurrences (25). However, evidence specifically evaluating immunophenotypic changes in strictly defined mCBC remains limited (3). Here, we aimed to characterize mCBC from two perspectives: i) a within-patient comparison between FBC and mCBC to evaluate paired differences in tumor characteristics and associations with prior treatment; and ii) a between-patient comparison of the mCBC cohort with a UBC cohort to assess differences in baseline clinical features and hereditary cancer risk, thereby identifying features unique to mCBC.

## Materials and methods

*Study design and patient selection*. We retrospectively studied patients diagnosed with mCBC, defined as contralateral BC occurring at least 12 months after the initial diagnosis of FBC, who underwent radical mastectomy at our hospital between January 2010 and December 2019. To ensure treatment homogeneity and facilitate accurate classification of contralateral BCs, only patients who underwent mastectomy were included; those treated with breast-conserving surgery were excluded because of potential confounding from adjuvant radiotherapy and difficulty in distinguishing new primaries from ipsilateral recurrences. Patients with distant metastasis at the time of mCBC diagnosis were excluded. For the control group, patients with UBC who underwent radical resection during the same period and remained recurrence-free for at least 5-10 years postoperatively were selected.

*Data collection*. We interviewed patients about their medical history, hormone use, and detailed family history of cancer (a first- or second-degree relative with breast or ovarian cancer) during visits to the outpatient department. Family histories were obtained to screen for hereditary breast and ovarian cancer syndrome. Patients whose mother, sister, or daughter had cancer were classified as having a first-degree family history, whereas those whose grandmother, aunt or half-sister had cancer were classified as having a second-degree family history. There were no cases of male BC cases among the study participants or the family history. Data on treatment and tumor characteristics, including subtype, were obtained from medical records.

*Pathological assessment*. Two experienced pathologists performed the pathological examinations at our hospital. The tumor grade was determined on the basis of the modified Bloom-Richardson histological grading system. For the Ki67 labelling index, a hot spot was chosen under 200x magnification and cells positive for nuclear Ki67 were semi-quantitatively evaluated. Estrogen and progesterone receptor statuses were assessed semi-quantitatively by immunohistochemistry and were reported positive when more than 1% of the nuclei of cancer cells showed staining. Human epidermal growth factor receptor 2 (HER2) was classified as positive if more than 10% of the tumor cells showed strong staining of the entire cell membrane or if HER2/neu gene amplification was confirmed by fluorescence in situ hybridization. The molecular subtype classification was applied only to invasive BC. Invasive FBC cases diagnosed before 2008 without documented HER2 status were classified as not available (N/A), and HER2 status was not inferred retrospectively. When HER2 immunohistochemistry was performed using archived formalin-fixed paraffin-embedded tissue from FBC at the time of mCBC diagnosis, the documented result was used for subtype classification.

*Statistical analysis*. Age at initial BC diagnosis was compared between the mCBC cohort at the time of FBC diagnosis and the UBC cohort using the unpaired Student t-test. Categorical clinicopathological parameters were compared between the mCBC and UBC cohorts using the $\chi^2$ test or Fisher's exact test. Paired differences between FBC and mCBC in terms of pathological stage, histological type, tumor grade and molecular subtype were evaluated using McNemar $\chi^2$ test. Associations between prior FBC treatment and the four mCBC subtypes were evaluated using the multinomial logistic regression analysis. Given that treatment selection is stage dependent and that disease stage is associated with tumor biology, models were adjusted for stage to minimize confounding factors and estimate the independent association between prior treatment and mCBC subtype. As an additional exploratory analysis, the four subtypes were combined into luminal (HR+/HER2– and HR+/HER2+) and non-luminal (HR–/HER2+ and HR–/HER2–) categories, and the stage-adjusted binary logistic regression analysis was performed to evaluate associations with prior chemotherapy and prior endocrine therapy. To determine whether family history influences the time interval between FBC and mCBC, log rank and Kaplan-Meier methods were used. Most Statistical analyses were performed using EZR version 1.40 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (26), a graphical user interface for Rcmdr version 2.5-1 running on R version 3.5.2 (The R Foundation for Statistical Computing,

Vienna, Austria). Multinomial and binary logistic regression analyses were performed using R version 4.0.3 (The R Foundation for Statistical Computing, Vienna, Austria). Graphs were generated using GraphPad Prism 8 software (GraphPad Software, Inc. CA, USA). The Sankey diagram was generated using SankeyMATIC (web-based application; https://sankeymatic.com/; accessed December 2025). A two-sided *p*-value of less than 0.05 was considered to indicate a statistically significant difference.

*Ethics statements*. This study was conducted in accordance with the principles of the Declaration of Helsinki and its amendments, and it was approved by the Ethics Committee of Juntendo University Hospital (number: H19-0289). Opt-out informed consent was obtained from all participants.

## Results

*Clinicopathological characteristics of mCBC: Within-patient comparison with FBC and between-patient comparison with UBC*. In total, 115 patients were included in the mCBC group, and 1,877 patients were included in the control group. A comparison of clinicopathological factors between FBC and mCBC in 115 patients is shown in Table I. The median age at diagnosis was 48 years (range: 24-84) for FBC and 62 years (range: 36-86) for mCBC. The median interval between FBC and mCBC was 9 years (range: 1-44 years). Stage 0/I (early stage) was more frequent than stage II/III (advanced stage) mCBC (P=0.003). Furthermore, to evaluate the effectiveness of postoperative follow-up, we compared the clinicopathological stages at diagnosis between FBC and mCBC. The stage at diagnosis was significantly earlier in mCBC than in FBC. Overall, 33% (28/84) of the patients had advanced-stage FBC, but were later diagnosed with early-stage mCBC, whereas 8% (7/84) had early-stage FBC but were later diagnosed with advanced-stage mCBC (P<0.001, Fig. 1). When restricted to patients with advanced-stage FBC at diagnosis, 77.8% (28/36) had early-stage mCBC. Conversely, when restricted to patients with early-stage FBC at diagnosis, 14.6% (7/48) had an advanced stage mCBC. The frequency of undergoing chemotherapy (CTx) was significantly lower in mCBC than in FBC (P=0.006). Meanwhile, no statistically significant differences were observed in the histological type, tumor grade, or subtype (P=0.232, P=0.214, and P=0.270, respectively). Paired subtype combinations in FBC and mCBC are listed in Table SI and are visualized using a Sankey diagram (Fig. 2).

Fig. 3 shows the age distribution at diagnosis in the FBC and mCBC groups. Additionally, we compared the age distribution of patients (n=1877) who underwent radical surgery for UBC at our hospital during the same period. The median ages at diagnosis were 48 and 55 years in the FBC and UBC groups, respectively (P<0.001). Those younger than 40 years of age accounted for 23.5% (27/115) of the FBC cases, which was higher than the 7.5% (140/1877) of the UBC cases (P=0.003). Regarding menopausal status at the time of diagnosis, most patients with FBC were premenopausal (67.0%, 77/115), compared with approximately one-third of the patients with UBC (35.9%, 674/1,877). The remaining patients were postmenopausal, accounting for 33.0% (38/115) in the FBC group and 64.1% (1,203/1,877) in the UBC group.

*Association of previous treatment history with changes in the subtype distribution*. As no significant differences in subtype distribution between FBC and mCBC were observed in the initial analysis (P=0.270, Table II), we further examined the distribution of the mCBC subtype, considering the possible effects of previous treatments for FBC. Only patients with invasive FBC and mCBC were included in this analysis.

There was no significant difference in the ratio of the subtypes between patients with invasive mCBC who had received endocrine therapy for FBC (prior ETx) and those who had never received ETx (never ETx) (P=0.312) (Table II). By contrast, the subtype distribution was significantly different between patients with and without prior CTx (P=0.016) (Table II). Therefore, we compared the subtypes according to stage and treatment using the multinomial logistic regression analysis. Among mCBC patients with prior CTx, the luminal subtype was associated with lower odds than the triple-negative (TN) subtype (odds ratio [OR]=0.3 vs. 1, 95% CI: 0.07-1.19), compared with untreated patients (Table II). However, this association was not statistically significant (P=0.086). No significant differences in subtype distribution were found among patients with mCBC who underwent prior ETx. To further address the limited sample size across the four subtype categories, we performed an additional exploratory binary analysis (Table SII). After adjustment for stage, prior CTx showed numerically higher odds of non-luminal mCBC, although this association was not statistically significant (OR=2.02, 95% CI: 0.63-6.63, P=0.238). Prior ETx showed no significant association with non-luminal mCBC (OR=1.25, 95% CI: 0.41-3.83, P=0.698). Moreover, the median time intervals to the onset of mCBC according to prior therapy were 8.5 (1-33) years for prior ETx and 8.5 (1-24) years for prior CTx, which was comparable to the 9 years for the overall cohort.

*Comparison of family history and genetic factors between mCBC and UBC*. We compared breast and ovarian cancer family histories between 115 patients with mCBC and 1,877 patients with UBC (Fig. 4). Overall, patients in the mCBC group had a distinctly higher family history than those in the UBC group (34.8% vs. UBC 19.0%; P=0.016). Moreover, the influence of family history became even more apparent in patients with mCBC (24.4%) when only first-degree relatives were considered compared with those with UBC (11.1%) (P=0.025). There was no significant difference between the two groups in terms of a second-degree family history (10.4% and 7.9%, respectively, P=0.806).

Next, we examined the differences in the duration of mCBC onset according to family history among patients with mCBC. Fig. 5A shows a comparison of the time interval from FBC to mCBC onset among patients with no family history and those with first-, and second-degree family histories. There was no significant difference in the time from FBC to mCBC onset according to the family history (P=0.602). Furthermore, we compared the proportion of patients first diagnosed with BC at our institution in the past ten years who developed mCBC according to their family history. Considering that the median time for developing mCBC was approximately 9 years, the 10 years for which UBC could be followed-up in this study was insufficient to determine whether the incidence of mCBC

Table I. Comparison of clinicopathological characteristics between FBC and mCBC.

| Characteristics | FBC (n=115) | mCBC (n=115) |
|---|---|---|
| Median age, years (range) | 48 (24-84) | 62 (36-86) |
| Time period of FBC diagnosis, calendar year, n (%) | | |
| 1968-1999 | 29 (25.2) | - |
| 2000-2009 | 49 (42.6) | - |
| 2010-2018 | 37 (32.2) | - |
| Interval from FBC to mCBC (years), n (%) | | |
| 1-5 | - | 36 (31.3) |
| 6-10 | - | 35 (30.5) |
| 11-20 | - | 22 (19.1) |
| 21-50 | - | 22 (19.1) |
| mCBC detection method, n (%) | | |
| Self-examination[a] | - | 18 (15.7) |
| Follow-up screening[b] | - | 97 (84.3) |
| Pathological stage, n (%) | n=84 | n=115 |
| 0 | 22 (26.2) | 37 (32.2) |
| IA | 26 (31.0) | 52 (45.2) |
| IIA/B | 31 (36.8) | 21 (18.3) |
| IIIA/B | 5 (6.0) | 5 (4.3) |
| N/A | 31 | 0 |
| Histological type (invasive)[c], n (%) | n=69 | n=78 |
| IBC-NST | 58 (84.1) | 59 (75.6) |
| Others[d] | 11 (15.9) | 19 (24.4) |
| Tumor grade, n (%) | n=50 | n=115 |
| High | 6 (12.0) | 23 (20.0) |
| Low/intermediate | 44 (88.0) | 92 (80.0) |
| Subtype (invasive)[c], n (%) | n=73 | n=78 |
| $HR^+$ $HER2^-$ | 50 (68.5) | 51 (65.4) |
| $HR^+$ $HER2^{+e}$ | 4 (5.5) | 11 (14.1) |
| $HR^-$ $HER2^{+e}$ | 6 (8.2) | 4 (5.1) |
| $HR^-$ $HER2^-$ | 13 (17.8) | 12 (15.4) |
| N/A | 20 | 0 |
| Adjuvant treatment (invasive)[c,f], n (%) | n=73 | n=78 |
| ETx | 54 (74.0) | 62 (79.5) |
| CTx | 39 (53.4) | 21 (26.9) |
| Trastuzumab | 4 (5.5) | 11 (14.1) |

[a]Self-palpation and/or nipple discharge. [b]Mammography and/or ultrasound. [c]Stage 0 cases were excluded. For variables restricted to invasive carcinoma, n denotes cases with available data; denominators vary because missing data differed across variables and did not always involve the same patients. [d]Three lobular carcinomas were included in FBC and four in mCBC. [e]FBC cases diagnosed before routine HER2 testing was introduced were classified as N/A unless the HER2 status was available from retrospective testing performed at the time of mCBC diagnosis. The N/A rows do not include percentages and show the number of unavailable cases. [f]Denominators are invasive cases with available data, including patients who received multiple treatments. Percentages for adjuvant treatments do not add up to 100%. FBC, first primary breast cancer; mCBC, metachronous contralateral breast cancer; IBC-NST, invasive breast carcinoma of no special type; N/A, not available; HR, hormone receptor; ETx, endocrine therapy; CTx, chemotherapy.

was directly dependent on family history. We compared the three groups as shown in Fig. 5A. The analysis included 1,913 cases, comprising 36 mCBC and 1877 UBC cases. The results showed that the incidence of mCBC within a certain period was not significantly different among the three groups (Fig. 5B, no family history, mCBC vs. UBC: 28 vs. 1519; first degree family history: 5 vs. 208; second degree family history: 3 vs. 150, P=0.680).

Of the patients with mCBC, 8 of the 115 (7%) underwent genetic testing after genetic counseling, with two cases of a g*BRCA1* pathogenic variant and one case of a g*PTEN* pathogenic variant (Cowden disease). In addition, 4 patients with

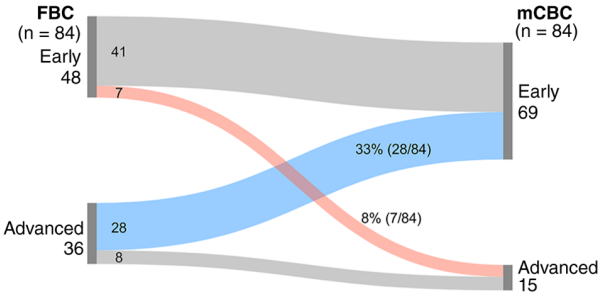


Figure 1. Sankey diagram showing the clinicopathological stage distributions for FBC and mCBC, and the patient-level stage differences between them. The diagram was created using SankeyMATIC. Numbers indicate the number of patients. Differences in onset stages between FBC and mCBC are shown as percentages. Early stage, 0/I; advanced stage, II/III. FBC, first primary breast cancer; mCBC, metachronous contralateral breast cancer.

mCBC (3.5%) had concomitant ovarian cancer, and 3 of them had first-degree relatives with a family history.

## Discussion

Our real-world retrospective findings reinforce several key characteristics of mCBC, offering valuable insights for the counseling and long-term care of BC survivors. One is the characteristically younger age of onset for FBC than for UBC, especially in patients younger than 40 years of age. This reflects the cumulative nature of BC risk, as the probability of developing mCBC increases with increased survival (27). Moreover, we observed a higher prevalence of a first-degree family history in patients with mCBC than in those with UBC, which may reflect an underlying genetic susceptibility. However, in our cohort, a family history was not associated with a shorter interval to mCBC, possibly because of follow-up limitations.

The time interval between FBC and the onset of mCBC was 9 years in this study, which was relatively long compared with approximately 6 years in most previous studies (12,28). A higher proportion of mCBCs was diagnosed at an earlier stage than the initial FBCs: one-third of patients with advanced-stage FBC were later diagnosed as having early-stage mCBC (Fig. 1), suggesting the usefulness of regular medical checkups (84.4% of mCBCs were detected by screening, Table I). Additionally, the CTx rate decreased in patients with mCBC, partly because of fewer advanced-stage cases at the time of mCBC diagnosis. However, this finding should be interpreted with caution as it might be a result of lead-time bias. Liquid biopsy-based surveillance, including circulating tumor DNA monitoring, is emerging as a promising approach for minimal residual disease detection and the early identification of molecular relapse in early BC; however, its clinical utility for detecting mCBC remains unestablished (29). Regarding the histological type, invasive lobular carcinoma (ILC) was previously identified as a risk factor for developing mCBC (30); however, subtype-specific comparisons by histology were inconclusive in our cohort because of the small number of ILC cases. Prior treatment for FBC may influence not only the risk of developing mCBC but also its biological phenotype, including receptor profile and treatment resistance.

In our cohort, we could not directly quantify the prophylactic effect of systemic therapy on the absolute incidence of mCBC because our study design was not population-based. However, when we considered the entire systemic treatment history of FBC, we observed a shift in the distribution of mCBC subtypes according to prior CTx exposure. Among women who developed mCBC, despite not reaching conventional statistical significance, those with a history of CTx had lower odds of presenting with luminal, rather than TN, mCBC than untreated patients. By contrast, ETx history was not associated with an apparent shift in the mCBC subtype distribution; most within-patient transitions in our Sankey diagram were luminal-to-luminal, and our a priori hypothesis that prior ETx would be associated with a lower proportion of luminal mCBC was not supported. The additional exploratory binary analysis showed numerically higher odds of non-luminal mCBC with prior CTx, whereas the estimate for prior ETx was closer to the null. However, neither association was statistically significant, and given the limited sample size, wide CIs, and binary classification that did not distinguish HER2 status, these findings should be interpreted cautiously as exploratory.

These findings align with those of large population-based studies, including the WECARE study (31) and a recent meta-analysis (21), showing that adjuvant CTx and ETx reduce the overall risk of metachronous contralateral disease and that these protective effects are driven by a marked reduction in HR-positive mCBC, with attenuation beyond 10 years after the index diagnosis. By contrast, the benefit appears less pronounced for HR-negative tumors, and some studies have reported that long-term tamoxifen (≥5 years) more than halves the risk of ER-positive contralateral tumors while increasing the risk of ER-negative contralateral tumors by approximately four-fold (32). Other studies have reported subtype/receptor shifts after systemic therapy, including luminal-to-non-luminal switching after neoadjuvant CTx and ER-negative outgrowth in endocrine-resistant recurrence (33,34).

The mechanisms underlying the apparent enrichment of non-luminal mCBC among CTx-exposed patients remain uncertain and may include preferential suppression of hormone-sensitive precursor lesions, underlying host factors, or treatment-related effects. For example, germline *BRCA1* pathogenic variants are associated with both an elevated CBC risk and a predominance of ER-negative/triple-negative breast cancer tumors (21), and could contribute to non-luminal enrichment in selected high-risk subgroups.

Given the long interval between FBC and mCBC in many patients, it is plausible that any early prophylactic effect of ETx on ER-positive contralateral disease was attenuated by the time mCBC emerged, which is consistent with studies that ETx-associated risk reduction is time-limited and most pronounced within several years of therapy completion (35,36).

Some germline genetic variant carriers are at high risk for mCBC (37), and the WECARE study further reported that family history confers an increased mCBC risk even among non-carriers (22). In the present study, 24.4% of patients with mCBC had first-degree relatives with breast cancer, which was significantly higher than that in patients with UBC (11.1%). This finding highlights the practical value of systematic, interview-based family-history assessments for risk stratification and survivorship counseling. In our cohort, 3.5% of the patients with mCBC had concurrent ovarian cancer. Three-quarters of

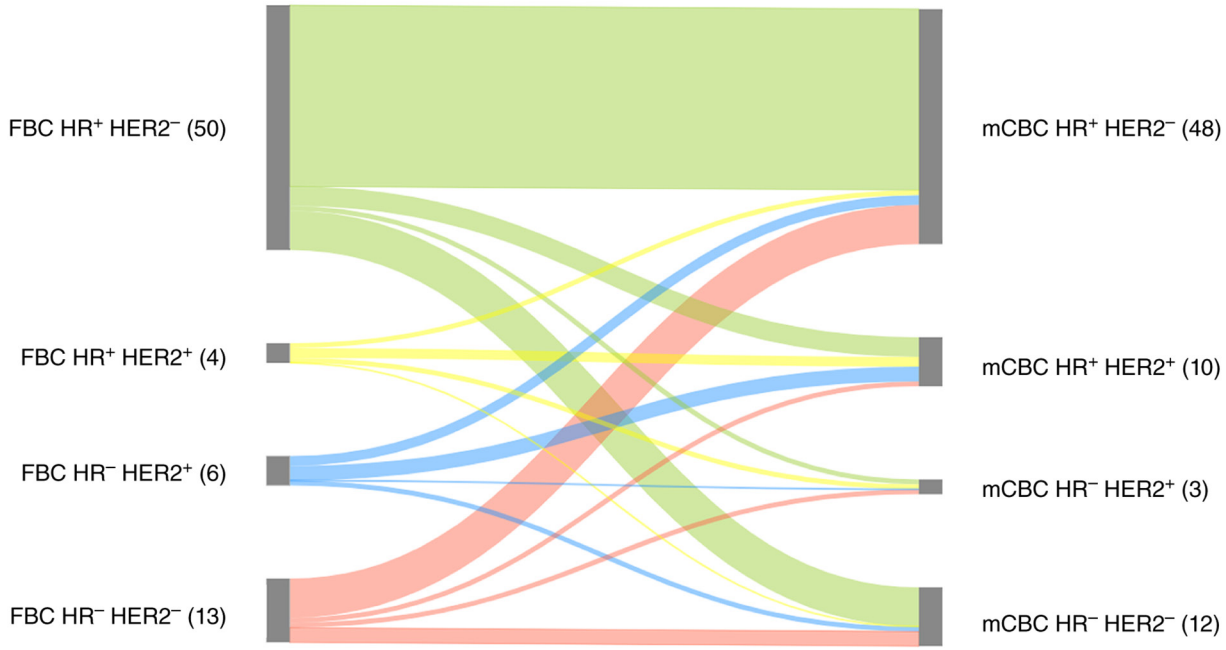


Figure 2. Sankey diagram illustrating the distribution of paired subtype combinations between FBC and mCBC. Numbers indicate the number of patients. HER2 status was based on available pathological records. FBC cases diagnosed before 2008 without documented HER2 results were not included in this analysis. In some patients, HER2 status of the FBC was re-evaluated using archived formalin-fixed paraffin-embedded tissue at the time of mCBC diagnosis, and these documented results were included. FBC, first primary breast cancer; HR, hormone receptor; mCBC, metachronous contralateral breast cancer.

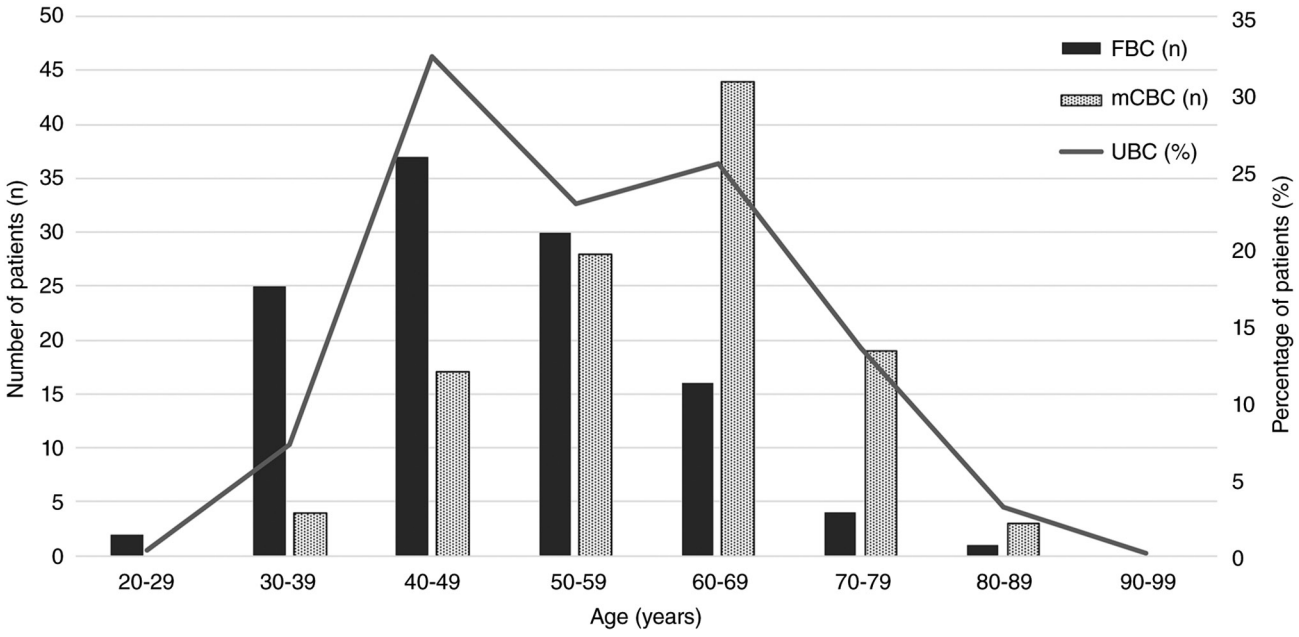


Figure 3. Age distribution at disease onset among patients with FBC, those with mCBC and those with UBC who underwent radical surgery during the same period. FBC, first primary breast cancer; mCBC, metachronous contralateral breast cancer; UBC, unilateral breast cancer.

the patients had a first-degree relative with a history of breast or ovarian cancer. The proportion of patients with mCBC who had concurrent ovarian cancer in our study was higher than the approximately 1% in the general Japanese population (38), although this comparison should be regarded with caution as it is based on an unmatched external population. Our results support the importance of informing BC survivors with a family history of risk for mCBC and ovarian cancer, including genetic testing options.

This study has several limitations. It was retrospective and conducted at a single institution, with a small number of mCBC cases, particularly for the treatment-stratified subtype analyses. This resulted in broad CIs for the estimated ORs. Therefore, the findings should be interpreted cautiously and regarded as hypothesis-generating. Our inclusion criteria were restricted to patients who underwent mastectomy; thus, generalizability of these findings may be limited. This restriction was intended to improve classification accuracy, as

Table II. Comparison of subtype distributions between FBC and mCBC and associations of prior FBC treatment with mCBC subtype.

| Patient categories | $HR^+$ $HER2^-$ | $HR^+$ $HER2^+$ | $HR^-$ $HER2^+$ | $HR^-$ $HER2^-$ | P-value |
|---|---|---|---|---|---|
| FBC total, n (%) (n=73) | 50 (68.5) | 4 (5.5) | 6 (8.2) | 13 (17.8) | 0.270[a] |
| mCBC total, n (%) (n=78) | 51 (65.4) | 11 (14.1) | 4 (5.1) | 12 (15.4) | |
| mCBC with prior ETx for FBC, n (%) (n=35) | 24 (68.6) | 3 (8.6) | 2 (5.7) | 6 (17.1) | 0.312[b] |
| mCBC without prior ETx for FBC, n (%) (n=43) | 27 (62.8) | 8 (18.6) | 2 (4.7) | 6 (14.0) | |
| mCBC with prior CTx for FBC, n (%) (n=27)[c] | 15 (55.6) | 4 (14.8) | 1 (3.7) | 7 (25.9) | 0.016[b] |
| mCBC without prior CTx for FBC, n (%) (n=42)[c] | 30 (71.4) | 5 (11.9) | 3 (7.1) | 4 (9.5) | |
| mCBC: Prior ETx for FBC (with vs. without)[d] | | | | | |
| Odds ratio | 0.93 | 0.38 | 0.97 | 1 | |
| 95% CI | 0.26, 3.28 | 0.07, 2.17 | 0.10, 9.38 | | |
| P-value | 0.904 | 0.274 | 0.976 | | |
| mCBC: Prior CTx for FBC (with vs. without)[d] | | | | | |
| Odds ratio | 0.30 | 0.47 | 0.19 | 1 | |
| 95% CI | 0.07, 1.19 | 0.08, 2.83 | 0.01, 2.47 | | |
| P-value | 0.086 | 0.407 | 0.203 | | |

[a]McNemar's chi-squared test. [b]Fisher's exact test. [c]A total of 9 patients with invasive mCBC were excluded from the chemotherapy-stratified analysis because their prior chemotherapy history after FBC was unavailable. [d]Multinomial logistic regression analysis with the $HR^-$ $HER2^-$ subtype as the reference category and adjusted for stage. The first two rows show the subtype distributions of FBC and mCBC among patients with available subtype data, irrespective of treatment history. Rows 3-6 show the mCBC subtype distributions according to prior ETx and CTx for FBC (with vs. without), assessed separately. The bottom section shows the results of separate multinomial logistic regression analyses comparing the odds of each mCBC subtype relative to the $HR^-$ $HER2^-$ subtype between patients with and without prior ETx or CTx for FBC. HR, hormone receptor; ETx, endocrine therapy; CTx, chemotherapy; FBC, first primary breast cancer; mCBC, metachronous contralateral breast cancer; vs., versus.

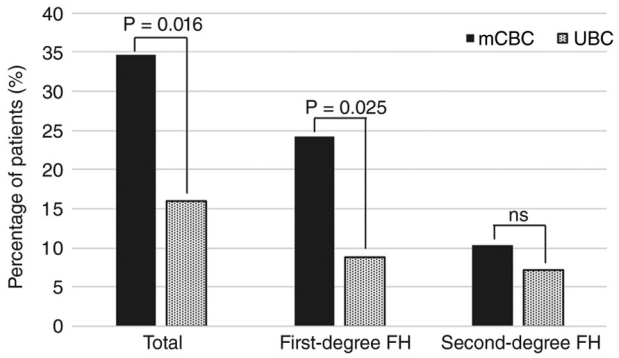


Figure 4. Comparison of FH between mCBC and UBC. The percentages of patients with a FH of breast or ovarian cancer are shown for the mCBC (n=115) and UBC (n=1,877) groups. FH was categorized into three groups: Total, first-degree and second-degree. Comparisons between the mCBC and UBC groups were performed using Pearson's $\chi^2$ test based on separate 2x2 contingency tables for the presence or absence of any FH in first- and/or second-degree relatives (Total), first-degree FH and second-degree FH, respectively. FH, family history; mCBC, metachronous contralateral breast cancer; UBC, unilateral breast cancer; ns, not significant.

breast-conserving therapy cases introduce challenges in identifying and interpreting mCBC owing to the need to distinguish new primary tumors from ipsilateral breast tumor recurrence and to account for the influence of postoperative radiotherapy. Although menopausal status at FBC/UBC diagnosis was included when available, other established or potential risk factors for contralateral BC, including BMI, reproductive factors, detailed radiotherapy exposure, and genetic predisposition, were not comprehensively available in this retrospective dataset. Further, the comparisons were exploratory and unadjusted; therefore, residual confounding factors such as age, menopausal status, breast density, and other unmeasured risk factors cannot be excluded. The UBC control cohort was defined as recurrence-free for at least 5-10 years and followed for up to 10 years postoperatively, according to institutional rules. Therefore, selection and follow-up constraints may have influenced the comparisons and limited the assessment of long-term outcomes and time-to-onset patterns. For example, we did not observe an association between family history and earlier onset of mCBC within the available follow-up period, which may reflect the limited observation time and statistical power. Finally, treatment effects should be interpreted cautiously given potential residual confounding by treatment indication and baseline tumor biology, some patients received both ETx and CTx, complicating the attribution of subtype patterns to a single treatment modality and introducing potential bias related to mixed treatment effects.

In conclusion, our evaluation of paired differences in tumor characteristics and their associations with prior treatment between FBC and mCBC suggests that further adequately powered studies are needed to clarify whether prior chemotherapy for FBC is associated with the molecular subtypes of mCBC. A higher proportion of mCBCs in our cohort were diagnosed at an earlier stage than the initial FBCs, suggesting that regular postoperative checkups and patient education may contribute to the earlier detection of contralateral tumors.

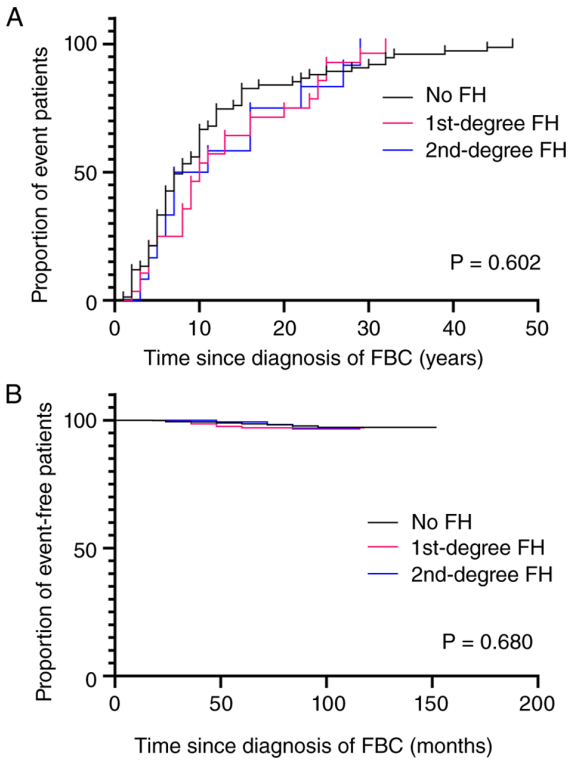


Figure 5. Kaplan-Meier curve showing the time interval between FBC and mCBC. (A) Comparison of the time interval from FBC to mCBC onset in three groups: No, first-degree and second-degree FH. (B) Proportion of event-free patients after 2010. The analysis included 1,913 cases, comprising 36 mCBC and 1,877 UBC cases. Patients were divided into three groups according to FH status. The numbers of mCBC vs. UBC cases, respectively, were as follows: No FH, 28 vs. 1,519; first-degree FH, 5 vs. 208; and second-degree FH, 3 vs. 150. Kaplan-Meier curves were compared using the log-rank test. FBC, first primary breast cancer; mCBC, metachronous contralateral breast cancer; FH, family history; UBC, unilateral breast cancer.

Furthermore, because a substantial proportion of patients develop mCBC more than 10 years after FBC surgery, with a maximum of 44 years, we believe that lifetime follow-up is indicated for this population. Moreover, our between-patient cohort comparison of mCBC and UBC confirmed the usefulness of taking a family history to provide BC survivors with appropriate risk management information.

## Acknowledgements

The authors would like to thank Dr Katrin Ishii-Schrade (Juntendo University, Tokyo, Japan) for English language editing.

## Funding

The present study was supported by the Japan Society for the Promotion of Science Grants-in-Aid for Young Scientists (grant nos. JP19K16780 and 23K14661).

## Availability of data and materials

The data generated in the present study are not publicly available due to patient privacy and ethical restrictions but may be requested from the corresponding author.

## Authors' contributions

RS designed the study, analyzed and visualized the data, and wrote the paper. KY performed the statistical analysis. KI collected the clinical data and contributed to the interpretation of the clinicopathological findings. MA, MS and GK advised on the study design and contributed to the assessment of the statistical analyses and interpretation of the results. RS and KI confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

## Ethics approval and consent to participate

The present study was conducted in accordance with the principles of the Declaration of Helsinki and its amendments, and was approved by the Ethics Committee of Juntendo University Hospital (approval no. H19-0289; Tokyo, Japan). Opt-out informed consent was obtained from all participants.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Park BK, Schneider J and Suh Y: Survival analysis for patients with metachronous contralateral breast cancer: Insights from a retrospective study. Oncol Lett 28: 390, 2024.
2. Qiu R, Zhao W, Yang J, Shen Y, Wang B, Li P, Zhao A, Tian Q, Zhang M, Yi M, *et al*: Comparative analysis of outcomes and clinicopathological characteristics of synchronous and metachronous contralateral breast cancer: A study of the SEER database. J Breast Cancer 22: 297-310, 2019.
3. Li C, Lyu Z, Wang Z, Hao C, Huang Y and Song F: The changes of subtype markers between first and second primary breast cancers. Cancer Med 12: 13649-13660, 2023.
4. Nichols HB, Berrington de Gonzalez A, Lacey JV Jr, Rosenberg PS and Anderson WF: Declining incidence of contralateral breast cancer in the United States from 1975 to 2006. J Clin Oncol 29: 1564-1569, 2011.
5. Kheirelseid EA, Jumustafa H, Miller N, Curran C, Sweeney K, Malone C, McLaughlin R, Newell J and Kerin MJ: Bilateral breast cancer: Analysis of incidence, outcome, survival and disease characteristics. Breast Cancer Res Treat 126: 131-140, 2011.
6. Sakai T, Ozkurt E, DeSantis S, Wong SM, Rosenbaum L, Zheng H and Golshan M: National trends of synchronous bilateral breast cancer incidence in the United States. Breast Cancer Res Treat 178: 161-167, 2019.
7. Kramer I, Schaapveld M, Oldenburg HSA, Sonke GS, McCool D, van Leeuwen FE, Van de Vijver KK, Russell NS, Linn SC, Siesling S, *et al*: The influence of adjuvant systemic regimens on contralateral breast cancer risk and receptor subtype. J Natl Cancer Inst 111: 709-718, 2019.
8. Wong SM, Freedman RA, Sagara Y, Aydogan F, Barry WT and Golshan M: Growing use of contralateral prophylactic mastectomy despite no improvement in long-term survival for invasive breast cancer. Ann Surg 265: 581-589, 2017.
9. Xiong Z, Yang L, Deng G, Huang X, Li X, Xie X, Wang J, Shuang Z and Wang X: Patterns of occurrence and outcomes of contralateral breast cancer: Analysis of SEER data. J Clin Med 7: 133, 2018.
10. Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Gavin A, Visser O and Bray F: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer 103: 356-387, 2018.

11. Langballe R, Frederiksen K, Jensen MB, Andersson M, Cronin-Fenton D, Ejlertsen B and Mellemkjaer L: Mortality after contralateral breast cancer in Denmark. Breast Cancer Res Treat 171: 489-499, 2018.
12. Mruthyunjayappa S, Zhang K, Zhang L, Eltoum IA, Siegal GP and Wei S: Synchronous and metachronous bilateral breast cancer: Clinicopathologic characteristics and prognostic outcomes. Hum Pathol 92: 1-9, 2019.
13. Pan B, Xu Y, Zhou YD, Yao R, Wu HW, Zhu QL, Wang CJ, Mao F, Lin Y, Shen SJ and Sun Q: The prognostic comparison among unilateral, bilateral, synchronous bilateral, and metachronous bilateral breast cancer: A meta-analysis of studies from recent decade (2008-2018). Cancer Med 8: 2908-2918, 2019.
14. Jobsen JJ, van der Palen J, Ong F, Riemersma S and Struikmans H: Bilateral breast cancer, synchronous and metachronous; differences and outcome. Breast Cancer Res Treat 153: 277-283, 2015.
15. Shi YX, Xia Q, Peng RJ, Yuan ZY, Wang SS, An X, Cao Y, Tan YT, Jin Y, Cai XY, *et al*: Comparison of clinicopathological characteristics and prognoses between bilateral and unilateral breast cancer. J Cancer Res Clin Oncol 138: 705-714, 2012.
16. Vichapat V, Garmo H, Holmqvist M, Liljegren G, Wärnberg F, Lambe M, Fornander T, Adolfsson J, Lüchtenborg M and Holmberg L: Tumor stage affects risk and prognosis of contralateral breast cancer: Results from a large Swedish-population-based study. J Clin Oncol 30: 3478-3485, 2012.
17. Wang C, Hu K, Deng L, He W, Fang F, Tamimi RM and Lu D: Increased risk of breast cancer-specific mortality among cancer survivors who developed breast cancer as a second malignancy. BMC Cancer 21: 491, 2021.
18. Hartman M, Czene K, Reilly M, Bergh J, Lagiou P, Trichopoulos D, Adami HO and Hall P: Genetic implications of bilateral breast cancer: A population based cohort study. Lancet Oncol 6: 377-382, 2005.
19. Kurian AW, McClure LA, John EM, Horn-Ross PL, Ford JM and Clarke CA: Second primary breast cancer occurrence according to hormone receptor status. J Natl Cancer Inst 101: 1058-1065, 2009.
20. Giardiello D, Hauptmann M, Steyerberg EW, Adank MA, Akdeniz D, Blom JC, Blomqvist C, Bojesen SE, Bolla MK, Brinkhuis M, *et al*: Prediction of contralateral breast cancer: External validation of risk calculators in 20 international cohorts. Breast Cancer Res Treat 181: 423-434, 2020.
21. Akdeniz D, Schmidt MK, Seynaeve CM, McCool D, Giardiello D, van den Broek AJ, Hauptmann M, Steyerberg EW and Hooning MJ: Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. Breast 44: 1-14, 2019.
22. Reiner AS, Sisti J, John EM, Lynch CF, Brooks JD, Mellemkjaer L, Boice JD, Knight JA, Concannon P, Capanu M, *et al*: Breast cancer family history and contralateral breast cancer risk in young women: An update from the women's environmental cancer and radiation epidemiology study. J Clin Oncol 36: 1513-1520, 2018.
23. Evans HS, Lewis CM, Robinson D, Bell CM, Moller H and Hodgson SV: Incidence of multiple primary cancers in a cohort of women diagnosed with breast cancer in Southeast England. Br J Cancer 84: 435-440, 2001.
24. Hayat MJ, Howlader N, Reichman ME and Edwards BK: Cancer statistics, trends, and multiple primary cancer analyses from the surveillance, epidemiology, and end results (SEER) program. Oncologist 12: 20-37, 2007.
25. Van Alsten SC, Love MI, Calhoun BC, Butler EN, Pfefferle AD, Kirk EL, Halloran CS, Zipple IW, Perou CM, Carey LA, *et al*: Identifying locally recurrent versus second primary breast cancer: Genomic versus clinical criteria from carolina breast cancer study phase III. Cancer Epidemiol Biomarkers Prev 35: 311-320, 2026.
26. Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 48: 452-458, 2013.
27. Ghosh R, Pfeiffer RM, Roberts S, Gierach GL and Dallal CM: Adjuvant endocrine therapy and risk of contralateral breast cancer: A systematic review and meta-analysis of observational studies. Cancer Causes Control 36: 107-126, 2025.
28. Liederbach E, Piro R, Hughes K, Watkin R, Wang CH and Yao K: Clinicopathologic features and time interval analysis of contralateral breast cancers. Surgery 158: 676-685, 2015.
29. Schlam I, Tolaney SM, Lin NU, Parsons H and Morganti S: Circulating tumor DNA in early breast cancer: A review. JAMA Oncol 12: 773-784, 2026.
30. Reiner AS, Lynch CF, Sisti JS, John EM, Brooks JD, Bernstein L, Knight JA, Hsu L, Concannon P, Mellemkjaer L, *et al*: Hormone receptor status of a first primary breast cancer predicts contralateral breast cancer risk in the WECARE study population. Breast Cancer Res 19: 83, 2017.
31. Langballe R, Mellemkjær L, Malone KE, Lynch CF, John EM, Knight JA, Bernstein L, Brooks J, Andersson M, Reiner AS, *et al*: Systemic therapy for breast cancer and risk of subsequent contralateral breast cancer in the WECARE study. Breast Cancer Res 18: 65, 2016.
32. Li CI, Daling JR, Porter PL, Tang MT and Malone KE: Adjuvant hormonal therapy for breast cancer and risk of hormone receptor-specific subtypes of contralateral breast cancer. Cancer Res 69: 6865-6870, 2009.
33. Schaapveld M, Visser O, Louwman WJ, Willemse PH, de Vries EG, van der Graaf WT, Otter R, Coebergh JW and van Leeuwen FE: The impact of adjuvant therapy on contralateral breast cancer risk and the prognostic significance of contralateral breast cancer: A population based study in the Netherlands. Breast Cancer Res Treat 110: 189-197, 2008.
34. Hanker AB, Sudhan DR and Arteaga CL: Overcoming endocrine resistance in breast cancer. Cancer Cell 37: 496-513, 2020.
35. Pan H, Gray R, Braybrooke J, Davies C, Taylor C, McGale P, Peto R, Pritchard KI, Bergh J, Dowsett M, *et al*: 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N Eng J Med 377: 1836-1846, 2017.
36. Gierach GL, Curtis RE, Pfeiffer RM, Mullooly M, Ntowe EA, Hoover RN, Nyante SJ, Feigelson HS, Glass AG and Berrington de Gonzalez AB: Association of adjuvant tamoxifen and aromatase inhibitor therapy with contralateral breast cancer risk among US women with breast cancer in a general community setting. JAMA Oncology 3: 186-193, 2017.
37. Copson ER, Maishman TC, Tapper WJ, Cutress RI, Greville-Heygate S, Altman DG, Eccles B, Gerty S, Durcan LT, Jones L, *et al*: Germline BRCA mutation and outcome in young-onset breast cancer (POSH): A prospective cohort study. Lancet Oncol 19: 169-180, 2018.
38. Zhang Y, Luo G, Li M, Guo P, Xiao Y, Ji H and Hao Y: Global patterns and trends in ovarian cancer incidence: age, period and birth cohort analysis. BMC Cancer 19: 984, 2019.