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ORIGINAL ARTICLE

# BRCA functional domains associated with high risk of multiple primary tumors and domain-related sensitivity to olaparib: the Prometheus Study

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Available online xxx

**Background:** Germline pathogenic variants (gPVs) in the breast cancer susceptibility gene *1*/2 (*BRCA1*/2) genes confer high-penetrance susceptibility to breast cancer (BC) and ovarian cancer (OC). Although most female *BRCA* carriers develop only a single *BRCA*-associated tumor in their lifetime, a smaller subpopulation is diagnosed with multiple primary tumors (MPTs). The genetic factors influencing this risk remain unclear. Further, in patients with *BRCA*-mutated tumors, there appears to be a variability in the effectiveness of olaparib treatment.

**Patients and methods:** This real-world, multicenter, observational study aimed to determine whether the location of *BRCA* gPVs within functional domains (FDs) is associated with the development of MPTs and the magnitude of olaparib benefit. The study population comprised consecutive patients with OC who underwent hereditary cancer genetic testing between May 2015 and March 2023. MPT history was assessed based on mutated genes (*BRCA1* or *BRCA2*) and the location of the PVs within the FDs. Clinical outcomes of olaparib first-line maintenance therapy were evaluated according to *BRCA1/2* FD location.

**Results:** The frequency of MPT history in the overall population was 13.3% (118/882), and 20.4% in the *BRCA*-mutated subpopulation (68/333; $P < 0.001$). We observed a significant association between the DNA-binding domain (DBD) FD of *BRCA2* and MPT. Specifically, 55.6% of *BRCA2*-mutated patients with PVs in the DBD had a history of BC as a second tumor. At a median follow-up of 48.5 months (95% confidence interval 10-70 months), the 48-month progression-free survival rates were 100.0% for patients with PVs in DBD, 91.7% for those with PVs in other FDs, and 36.4% for those with PVs in the RAD51-binding domain (RAD51-BD) of *BRCA2* ($P = 0.01$). Results in the *BRCA1* cohort were not statistically significant.

**Conclusions:** The results suggest that the location of PVs within *BRCA* FDs may influence the onset of multiple tumors and the benefit of olaparib in patients with *BRCA*-mutated OC. These findings could be relevant for cancer prevention efforts, particularly given the increasing number of cancer survivors. However, further understanding is needed before these results can inform clinical decisions.

**Key words:** *BRCA*, hereditary breast cancer, hereditary ovarian cancer, multiple tumors, olaparib, second tumor history

## INTRODUCTION

Women carrying germline pathogenic/likely pathogenic variants (gPVs) in breast cancer susceptibility gene 1 (*BRCA1*) and breast cancer susceptibility gene 2 (*BRCA2*) have elevated lifetime risk of developing breast cancer (BC) and/or epithelial ovarian cancer (OC).[1] The estimated cumulative risk is 31%-78% for BC and 10%-63% for OC by the age of 70 years.[2,3]

Although most *BRCA* female carriers, in their lifetime, develop only a *BRCA*-associated tumor, in a smaller subpopulation, multiple primary tumors (MPTs) are diagnosed.[4-6] The few published data on this topic showed that the probability of OC following BC diagnosis is 12.7% for

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*BRCA1* carriers and 6.8% for *BRCA2* carriers,[7] and the likelihood of developing BC following OC in *BRCA1/2* carriers ranges from 3.9% to 10.98%.[8,9] Although important progress has been made in understanding the biological background of heredo-familial tumors,[10] the potential genetic factors associated with the risk of developing MPTs in *BRCA1/2* carriers are widely unknown, and optimal surveillance strategies in these high-risk women have not been defined. Previous data showed the relevance of PV location in estimating the risk of BC or OC.[11,12] According to Rebbeck et al.,[12] women carrying gPVs in the large central exon 11 of both *BRCA1/2* genes were at a higher risk of developing OC, whereas women carrying gPVs located in the 3' or 5' were more likely to develop BC, highlighting the value of *BRCA1* and *BRCA2* PV location in the variation of BC and OC risk.

Poly(ADP-ribose) polymerase (PARP)-inhibitor (PARPi) maintenance therapy has dramatically improved clinical outcomes for *BRCA* mutation carriers, representing the new standard of care in the recurrent and frontline OC setting.[13-15] Despite the known PARPi effectiveness in *BRCA*-mutated tumors, a different degree of benefit seems to exist.[16] Importantly, preclinical and preliminary clinical data suggested that PVs located in specific functional domains (FDs) of *BRCA1* and *BRCA2* genes were associated with reduced or increased sensitivity to PARPi, as previously shown for the DNA damage agent platinum.[16-19] However, real-world data on domain-related PARPi benefits in patients with OC treated with olaparib single agent as maintenance therapy are lacking.

Whether the location of the PVs in the *BRCA1/2* FDs was associated with different clinical outcomes in patients with OC treated with olaparib or bevacizumab as first-line maintenance therapy remains to be investigated. The findings could help to better predict the magnitude of PARPi benefit, identify patient subgroups more or less sensitive to PARPi maintenance therapy, guide the therapeutic choice, and gather important information to overcome drug resistance.

## PATIENTS AND METHODS

### *Study design*

This was a real-world, hospital-based, observational, retrospective study. The primary objective was to investigate whether the position of the PVs in the *BRCA1/2* FDs and/or the PV types were associated with the development of MPTs in patients with epithelial OC carrying *BRCA1/2* germline PVs (preventive purpose).

The second objective of the study was to investigate the magnitude of olaparib or bevacizumab benefit according to PV type and location in the FDs of *BRCA1* and *BRCA2* in patients with high-grade OC (HGOC) in the advanced-stage [International Federation of Gynecology and Obstetrics (FIGO) stage III-IV], who had previously received platinum-based chemotherapy, and were treated with olaparib or bevacizumab as first-line maintenance therapy (therapeutic purpose).

The clinical and genetic data were prospectively collected in a genetic information management system, designed to collect and update the genetic and clinical information of patients undergoing genetic testing over time. The data were subsequently retrospectively analyzed.

### *Study population*

The study population included a consecutive series of patients with histologically confirmed diagnosis of epithelial OC at age $\geq$18 years who were undergoing hereditary cancer genetic testing between May 2015 and March 2023 as part of routine clinical care.

All included patients had a known genetic testing result. Women lacking information on genetic testing and/or clinicopathological information on primary tumors will be excluded from this study. Patients with OC harboring only *BRCA1/BRCA2* tumor PVs were also excluded from the study.

The study was conducted according to good clinical practice and has been designed with the ethical principles laid down in the Declaration of Helsinki on human experimentation. The study protocol was approved by the Ethics Committee of the University Hospital AOUP 'Paolo Giaccone', Palermo, Italy (Comitato Etico Palermo 1; Protocol Information: "Prometheus" Study, approval number: 0423-02112023) and by the Institutional Review Board of the other participating center.

### *Procedures*

**Predisposition gene mutation screening.** Predisposition gene mutation screening in the study population was assessed as part of routine clinical care. The eligibility for genetic counseling and testing was in agreement with international and national guidelines, and clinically available risk assessment tools, taking into account the personal and family history of cancer: age at diagnosis, MPTs, number of affected relatives, and molecular characteristics of tumors.[20,21] Genetic data, demographic information, personal/family history of the tumor, and clinicopathological information on OC were extracted from medical and pathology reports for clinical use.

Germline testing was carried out using next-generation sequencing analysis on peripheral blood samples from patients with epithelial ovarian cancer; PVs and likely pathogenic variants (LPVs) identified by next-generation sequencing were validated using Sanger sequencing according to the local manufacturers' protocols (see Supplementary Materials, available at https://doi.org/10.1016/j.esmoop.2024.104076).

**Genetic variant classification and interpretation.** The detected *BRCA1/BRCA2* gene variants were locally categorized according to criteria developed by the Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium (https://enigmaconsortium.org/) and the International Agency for Research on Cancer recommendations.[22] The gene variants were classified into five classes: benign (class I), likely benign (class II), variants of uncertain significance (class III), likely pathogenic (class IV), and pathogenic (class V).[23] The databases used were BRCA

Exchange, LOVD, VarSome, and ClinVar.[24] The detected variants were named based on the recommendations for the description of sequence variants supplied by the Human Genome Variation Society.[25]

The presence/absence of MPT history was evaluated according to: (i) mutated gene (*BRCA1* or *BRCA2*); (ii) PV/LPV location (FDs); (iii) *BRCA1/2* PVs or likely PVs (classes IV and V) type.

The *BRCA1* FDs were classified as (i) really interesting new gene [RING; amino acids (AAs) 8-96]; (ii) DNA-binding domain (DBD; AAs 452-1092); (iii) BRCA1 C terminus (BRCT; AAs 1646-1736 and 1760-1855); and (iv) other location. The *BRCA2* FDs were classified as (i) RAD51-binding domain (RAD51-BD) (AAs 900-2000); (ii) DBD (AAs 2459-3190); and (iii) others.

The *BRCA1/2* PV/LPV types were classified as (i) nonsense; (ii) frameshift; (iii) missense; and (iv) splicing or large rearrangements.

**Outcome measures.** The disease control (progression disease, stable disease, partial response, complete response) according to RECIST version 1.1, progression-free survival (PFS) to treatment, and overall survival (OS) were assessed. Computed tomography, magnetic resonance imaging scans, and laboratory tests were carried out following standard local procedures. The primary objective was to assess the PFS. The OS was also investigated.

### *Statistical considerations*

Descriptive analyses were used to assess patients' characteristics. The differences between subgroups on the prevalence of gene variants and the clinicopathological characteristics of tumors were evaluated by Student's *t*-test and chi-square test. PFS was defined as the time from the start of therapy to progression or death from any cause. OS was calculated from the start of treatment to death from any cause. The analysis of PFS and OS between groups was estimated using the Kaplan—Meier method and compared using a log-rank test. We censored those patients without progression or death at their last follow-up. The relative hazards for each group were estimated with a Cox proportional hazards model. *P* values $<0.05$ were considered statistically significant. Statistical analyses were conducted using IBM SPSS Statistic Software, Version 28.0 (IBM Corporation, Armonk, NY). The graphs were created with Microsoft Excel software.

## RESULTS

### *Study population*

**Genetic landscape.** A total of 1004 patients with OC were included in the study. Among these, 122 were subsequently excluded from the analysis as they were carriers of germline PVs in no-*BRCA1/2* genes ($n = 41$), or variants of uncertain significance ($n = 81$).

Among the 882 patients with OC included in the analysis, 549 had *BRCA1/2* wild-type (WT) genetic testing (named WT cohort), whereas 333 were carriers of germline PVs in *BRCA1/2* genes (named *BRCA* cohort): 225 (67.6%) and 107 (32.1%) patients presented with *BRCA1* and *BRCA2* PVs, respectively, with only 1 patient with OC showing a double-heterozygosity for *BRCA1* and *BRCA2* genes (0.3%; Table 1).

***BRCA1/2* status and MPT history.** The frequency of MPT history in the overall OC population was 13.3% (118 patients): 68 (20.4%) and 50 (9.1%) patients in the *BRCA* and WT cohort, respectively ($P < 0.00001$). No differences in personal second tumor frequency between *BRCA1* and *BRCA2* PV carriers were observed [MPT in *BRCA1* versus *BRCA2* PV/LPV carriers: 46 (20.4%) versus 22 (20.2%) patients, $P = 0.13$] (Figure 1A and B).

In the subgroup of patients with OC with MPT history, the most frequently detected tumor was BC (96; 81.5%): 39 (40.6%), 22 (22.9%), and 35 (36.6%) BCs were diagnosed in *BRCA1* PV carriers, in *BRCA2* PV carriers, and in the *BRCA* WT subgroup, respectively. Other sites of second tumor were endometrium (9; 7.6%), melanoma (4; 3.4%), thyroid cancer (2; 1.7%), lung cancer (2; 1.7%), renal cell carcinoma (1; 0.8%), cholangiocarcinoma (1; 0.8%), and other sites (3; 2.5%), including 1 head and neck tumor, 1 chondrosarcoma, and 1 uterine leiomyosarcoma (Table 2).

**Age at first and second tumor diagnoses.** No differences in the median age at OC diagnosis between the 'No-MPT' and 'MPT' subgroups were observed [57 years old (range 19-84) versus 57 (range 23-80), respectively ($P = 0.46$)] (Figure 1C).

However, statistical differences at OC onset were found according to the mutated gene: women carriers of *gBRCA1* PVs developed OC earlier than women carriers of *BRCA2* PVs, regardless of a second primary tumor history. In detail, in the 'No-MPT' subgroup, *BRCA1* PV carriers developed OC 5 years before *BRCA2* PV carriers [53 years (range 25-82) versus 58 years (range, 28-81 years), respectively ($P = 0.001$)]; by contrast, in the 'MPT' subgroup, *BRCA1* PV carriers developed OC 8 years before *BRCA2* PV carriers [54 years (range 23-75 years) versus 62 years (range 37-78 years), respectively ($P = 0.01$)] (Figure 1D).

Concerning the interval between the first and the second tumor diagnoses, the OC diagnosis occurred with a median of 5 years after the first tumor diagnosis [median age at OC diagnosis, 57 years (range 23-80 years) versus median age at other tumor diagnoses, 52 years (range 19-77 years); Table 2].

In the 'MPT' subgroup, endometrioid histology was more frequently detected when compared with 'no-MPT' patients [19/118 (16.2%) versus 54/764 (7.1%)], respectively; $P = 0.01$; Table 1].

### *Distribution of PVs in the BRCA1 and BRCA2 functional domains*

We explored the distribution of PVs in the FDs of *BRCA1* (RING, DBD, BRCT, and others) and *BRCA2* genes (RAD51-BD, DBD, and others), according to the personal history of the MPTs (Table 3).

In the *BRCA1*-mutated OC group, we noticed that 21 (9.3%), 33 (14.7%), 61 (27.1%), and 97 (43.1%) patients

**Table 1. Patient and tumor characteristics of the study population, showing patients with OC with a personal second tumor history versus patients with OC without a personal second tumor history**

| Characteristics | Personal second tumor history, n (%) | | | No personal second tumor history, n (%) | | | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | Total | *BRCA1/2* | *BRCA1/2* wild type | Total | *BRCA1/2* | *BRCA1/2* wild type | |
| ***BRCA1/2* status** | 118 (13.4) | 68 (57.6) | 50 (42.4) | 764 (86.6) | 265 (34.7) | 499 (65.3) | — |
| **Age at OC diagnosis, median (range)** | 57 (23-80) | 56 (23-78) | 57 (25-80) | 57 (19-84) | 54 (25-82) | 60 (23-84) | 0.46 |
| **Histology** | | | | | | | 0.01 |
| HGSC | 95 (80.5) | 64 (94.1) | 31 (62.0) | 641 (83.9) | 242 (91.3) | 399 (79.9) | |
| LGSC | 3 (2.5) | 0 (0.0) | 3 (6.0) | 6 (0.8) | 3 (1.1) | 3 (0.6) | |
| Mucinous | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (0.3) | 0 (0.0) | 2 (0.4) | |
| Endometrioid | 19 (16.2) | 3 (4.4) | 16 (32.0) | 54 (7.1) | 9 (3.4) | 45 (9.1) | |
| Clear cell | 1 (0.8) | 1 (1.5) | 0 (0.0) | 11 (1.4) | 2 (0.8) | 9 (1.8) | |
| Other/NA | 0 (0.0) | 0 (0.0) | 0 (0.0) | 50 (6.5) | 9 (3.4) | 41 (8.2) | |

BRCA, breast cancer susceptibility gene; HGSC, high-grade serous carcinoma; LGSC, low-grade serous carcinoma; NA, not available; OC, ovarian cancer.
[a]Personal second tumor versus no personal second tumor (total).

showed PVs in the RING FD, DBD, BRCT, and other FDs, respectively, whereas data on FDs from 13 patients (5.8%) were not available. Although we observed a very low number of PVs in the RING FD of the 'MPTs' (2/46, 4.3%) when compared with the 'no-MPTs' subgroup (19/179, 10.6%), the distribution of PVs within the *BRCA1* FDs was not statistically significant to discriminate patients with OC with or without MPT history ($P = 0.8$). Conversely, a significant difference was observed in the *BRCA2*-mutated OC group with 43 (40.2%), 9 (8.4%), and 51 (47.7%) patients showing PVs in the RAD51-BD FD, DBD, and other FDs, respectively. Data on 4 (3.7%) patients were missing. Notably, we observed that among patients with OC in the 'MPT' subgroup, 5 out of 22 (22.7%) individuals had PVs in the DBD FD, whereas in the 'no-MPT' group, only 4 out of 85 (4.7%) individuals had PVs in the same DBD FD of *BRCA2* ($P = 0.003$). Finally, 55.6% of *BRCA2*-mutated patients with OC carrying PVs in the DBD FD presented with a second tumor history (Table 3 and Figure 2A-C).

### *BRCA1/2* PV type and second primary tumors

We also explored the distribution of *BRCA1/2* PV types (nonsense; frameshift; splicing, large rearrangements, and missense) according to MPT history. In the 'MPTs' subgroup, the number of missense PVs was lower, whereas the number of nonsense PVs was higher when compared with the 'No-MPTs' group ['MPT' group: nonsense 15 (32.6%); frameshift 26 (56.5%); splicing, large rearrangements 2 (4.4%), and missense 3 (6.5%); 'no-MPTs' group: nonsense 61 (23.1%); frameshift 140 (53%); splicing, large rearrangements 17 (6.5%), and missense 46 (17.4%); $P = 0.01$; Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.104076)].

### Outcome analysis: the magnitude of olaparib and bevacizumab benefit

***BRCA1* versus *BRCA2*.** We evaluated the survival outcome of patients with advanced-stage HGOC responding after platinum-based chemotherapy and receiving maintenance therapy with olaparib or bevacizumab, according to the *BRCA1*- or *BRCA2*-mutated gene. Outcome data were available for 130 patients. The median follow-up was 48.5 months [95% confidence interval (CI) 10-70 months]. The PFS rates at 48 months and the median PFS according to *BRCA1* and *BRCA2* status are presented in Figure 3.

In the cohort of 71 patients with *BRCA1* PVs, the PFS rate at 48 months was 26.8% (median PFS 27 months; 95% CI 20.1-33.1 months). During the follow-up, a total of 52 PFS events (recurrence or death) were observed (50.8%). Sixteen events occurred in the group of 26 patients treated with olaparib (61.5%), whereas 36 events occurred in the group of 45 patients treated with bevacizumab (80.0%). When PFS between the two groups was compared, patients on olaparib maintenance showed more favorable PFS than those in the bevacizumab maintenance group, although the difference was not statistically significant (48-month PFS 38.5% versus 20.0%, respectively; median PFS 36.0 months, 95% CI 26.1-45.9 months for olaparib maintenance; median PFS 24.0 months, 95% CI 21.1-26.9 months for bevacizumab maintenance; $P = 0.05$; Figure 3A).

In the cohort of 59 patients with *BRCA2* PVs, the PFS rate at 48 months was 49.2% (median PFS 37 months; 95% CI 30.1-43.8 months). During the follow-up, a total of 30 PFS events (recurrence or death) were observed (50.8%). Seven events occurred in the group of 28 patients treated with olaparib (25%), and 23 events occurred in the group of 31 patients treated with bevacizumab (74.2%). When PFS between the two groups was compared, patients on olaparib maintenance showed more favorable PFS than those in the bevacizumab maintenance group (48-month PFS 75.0% versus 25.8%, respectively; median PFS 40.0 months, 95% CI 34.3-46.0 months for olaparib maintenance; median PFS 25.0 months, 95% CI 19.9-30.0 months for bevacizumab maintenance; $P < 0.001$; Figure 3B).

Therefore the magnitude of olaparib benefit compared with bevacizumab was greater in the *BRCA2* PV carriers.

**Olaparib/bevacizumab benefits according to FDs.** Differences in PFS were observed when comparing subgroups according to PV location in the different FDs of *BRCA1* and *BRCA2*. Greater benefit from olaparib was observed in

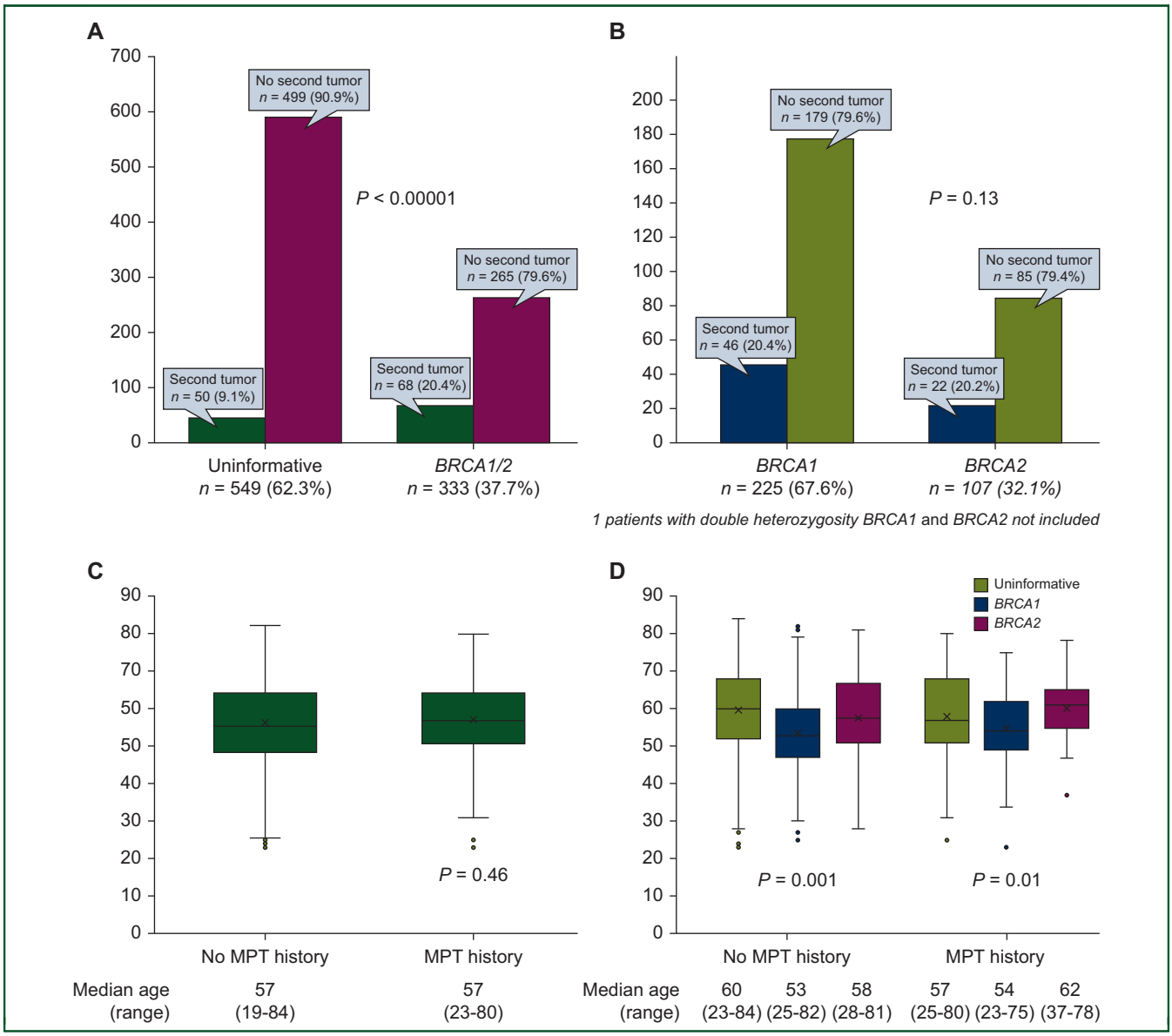


**Figure 1. (A) Breast cancer susceptibility gene (*BRCA*) status and a second primary tumor history in the study population; (B) *BRCA1* or *BRCA2* germline pathogenic variant in the subpopulation of patients with *BRCA*-mutated ovarian cancer (OC).** (C) The median age at OC diagnosis showing patients with a second tumor history compared with patients without a second tumor history; (D) The median age at second tumor diagnosis versus OC diagnosis.
MPT, multiple primary tumor.

patients with *BRCA1* or *BRCA2* PVs in the DBD FDs, followed by other locations. In the *BRCA1* cohort, the 48-month PFS rates were 69.2%, 42.9%, 33.3%, and 20.0% for patients with PVs in the DBD FD, other FDs, RING, and BRCT subgroups, respectively ($P = 0.04$; Figure 3C). In the *BRCA2* cohort, the 48-month PFS rates were 100.0%, 91.7%, and 36.4% for patients with PVs in the DBD FD, other FDs, and RAD51-BD subgroups, respectively ($P = 0.01$; Figure 3D).

While patients with OC carrying a *BRCA1* PV located in the DBD FD were highly sensitive to olaparib maintenance therapy, those with a similar mutation in the same DBD FD of *BRCA1* had a higher risk of relapse when treated with bevacizumab maintenance therapy. The 48-month PFS rates were 30.0%, 33.3%, 60.0%, and 9.1% for patients with PVs in the DBD FD, other FDs, RING, and BRCT subgroups, respectively ($P = 0.002$; Figure 3E).

In the *BRCA2* cohort, the 48-month PFS rates were 66.7%, 21.4%, and 20.0% for patients with PVs in the DBD FD, Other FDs, and RAD51-DB subgroups, respectively ($P = 0.4$; Figure 3F).

Therefore the greater clinical benefit of olaparib maintenance was observed in patients harboring mutations located in the *BRCA2* DBD FD (*BRCA2* carriers on olaparib maintenance, hazard ratio 0.27, 95% CI 0.75-0.96; *BRCA1* carriers on olaparib maintenance, hazard ratio 0.91, 95% CI 0.57-1.46).


https://doi.org/10.1016/j.esmoop.2024.104076

**Table 2. The second tumor sites in the OC population**

| Second tumor site | Total, *n* (%); median age (range) | *BRCA1*, *n* (%) | *BRCA2*, *n* (%) | *BRCA1/2* wild type, *n* (%) |
|---|---|---|---|---|
| All cancers | 118; 52 (19-77) | 46 (38.8) | 22 (18.8) | 50 (42.4) |
| Breast cancer | 96 (81.5); 51.5 (32-77) | 39 (40.6) | 22 (22.9) | 35 (36.5) |
| Thyroid cancer | 2 (1.7); 52.5 (50-55) | 2 (100) | 0 (0) | 0 (0) |
| Melanoma | 4 (3.4); 36 (19-53) | 3 (75) | 0 (0) | 1 (25) |
| Renal cancer | 1 (0.8); 57 (57) | 1 (100) | 0 (0) | 0 (0) |
| Lung cancer | 2 (1.7); 56 (51-61) | 0 (0) | 0 (0) | 2 (100) |
| Cholangiocarcinoma | 1 (0.8); 57 (57) | 0 (0) | 0 (0) | 1 (100) |
| Endometrium | 9 (7.6); 52 (31-55) | 1 (11.1) | 0 (0) | 8 (88.9) |
| Other[a] | 3 (2.5); 65 (49-73) | 0 (0) | 0 (0) | 3 (100) |

BRCA, breast cancer susceptibility gene; OC, ovarian cancer.
[a]Head and neck carcinoma, chondrosarcoma, and uterine leiomyosarcoma.

## DISCUSSION

It is widely known that women carrying deleterious variants in *BRCA1* or *BRCA2* susceptibility genes are at increased lifetime risk of developing BC and/or OC. More recently, associations with risks for other cancers have been also suggested, including pancreatic cancers, stomach cancers, gallbladder cancers, renal cancers, uterine cancers, and melanoma.[26,27] Although in most *BRCA* carriers only one diagnosis occurs, affected individuals are at a risk of developing MPTs over time.[5,7-9] Genetic or clinical factors that can increase this risk, beyond the diagnosis at a younger age and a family history of cancer, are unknown. Given the lack of clear guidelines for the management of *BRCA* mutation carriers previously diagnosed with a *BRCA*-associated tumor, identifying specific genetic predisposition factors is essential for prevention efforts and personalized risk-reducing strategies.[28]

We questioned whether the location of the PVs in the FDs of *BRCA1* and *BRCA2* genes had an impact on the development of MPTs. Results from our analysis on a large-scale cohort of patients with OC indicate that germline PVs in the DBD FD of the *BRCA2* gene were associated with a greater number of patients developing double primary tumors, mainly BC. Thus 55.6% of patients with *BRCA2*-mutated OC harboring gPVs in the DBD FD showed a second primary tumor history. The reason for this finding remains speculative. Although DBD represents the most evolutionarily conserved domain of the *BRCA2*, its function is less defined than others.[29] Recent research in mouse cell lines and purified human BRCA2 protein missing the DBD domain showed that, after treatment with different DNA-damaging agents, the absence of the BRCA2 DBD leads to significant sensitization to ionizing radiation inducing double-strand break, replication disruption by olaparib, and DNA inter-strand crosslinks by cisplatin. Importantly, *BRCA2* variants missing DBD were defective in architectural changes and rearrangements linked with impaired homologous recombination function.[29] Other preclinical studies have shown that PVs in *BRCA2* DBD weaken assembly with the partner protein DSS1, resulting in BRCA2 oligomers being excluded from the cell nucleus, and an impaired homologous recombination repair.[30,31] All these intriguing observations indicate that *BRCA2* DBD is specifically important for homologous recombination activity and dynamic localization at the sites of DNA breaks. We speculate that this key role of the DBD domain in the efficient response to DNA damage through mechanisms involving mobility, conformation rearrangements, and interaction with partners during DNA repair could represent the genetic background leading to the increased susceptibility to develop a broad spectrum of double primary malignancies over time.[29-31]

We also explored the domain-related olaparib or bevacizumab effectiveness in newly diagnosed patients with HGOC treated with platinum-based chemotherapy and olaparib or bevacizumab first-line maintenance, administered according to clinical indication and medical choice. Despite the clinical effectiveness of PARPi, drug resistance is a growing clinical problem in the advanced setting. We observed that patients with OC whose germline PVs were located within the DBD FD of the *BRCA2* gene had prolonged PFS to olaparib maintenance, but not to bevacizumab maintenance, compared with patients with OC harboring mutations in other *BRCA1* or *BRCA2* FDs.

This observation is consistent with preclinical data in cell lines showing that loss of DBD *BRCA2*, but not other domains, induced a marked sensitization to DNA-damaging agents, including olaparib.[29]

**Table 3. Distribution of germline PVs in the *BRCA1* and *BRCA2* functional domains in the subpopulations of patients with OC with and without a personal second tumor history**

| Distribution | Total, *n* (%) | Personal second tumor history, *n* (%) | No personal second tumor history, *n* (%) | *P* value |
|---|---|---|---|---|
| ***BRCA1* FDs** | | | | |
| Total | 225 | 46 (20.4) | 179 (79.6) | — |
| RING | 21 (9.3) | 2 (4.3) | 19 (10.6) | **0.8** |
| DBD | 33 (14.7) | 5 (10.9) | 28 (15.6) | |
| BRCT | 61 (27.1) | 10 (21.7) | 51 (28.5) | |
| Others | 97 (43.1) | 16 (34.8) | 81 (45.3) | |
| Missing | 13 (5.8) | 13 (28.3) | 0 (0.0) | |
| ***BRCA2* FDs** | | | | |
| Total | 107 | 22 (20.6) | 85 (79.4) | — |
| RAD51-BD | 43 (40.2) | 6 (27.3) | 37 (43.5) | **0.003** |
| DBD | 9 (8.4) | 5 (22.7) | 4 (4.7) | |
| Others | 51 (47.7) | 7 (31.8) | 44 (51.8) | |
| Missing | 4 (3.7) | 4 (18.2) | 0 (0.0) | |

BRCA, breast cancer susceptibility gene; BRCT, BRCA1 C terminus; DBD, DNA-binding domain; FD, functional domain; OC, ovarian cancer; PV, pathogenic variant; RAD51-BD, RAD51-binding domain; RING, really interesting new gene.

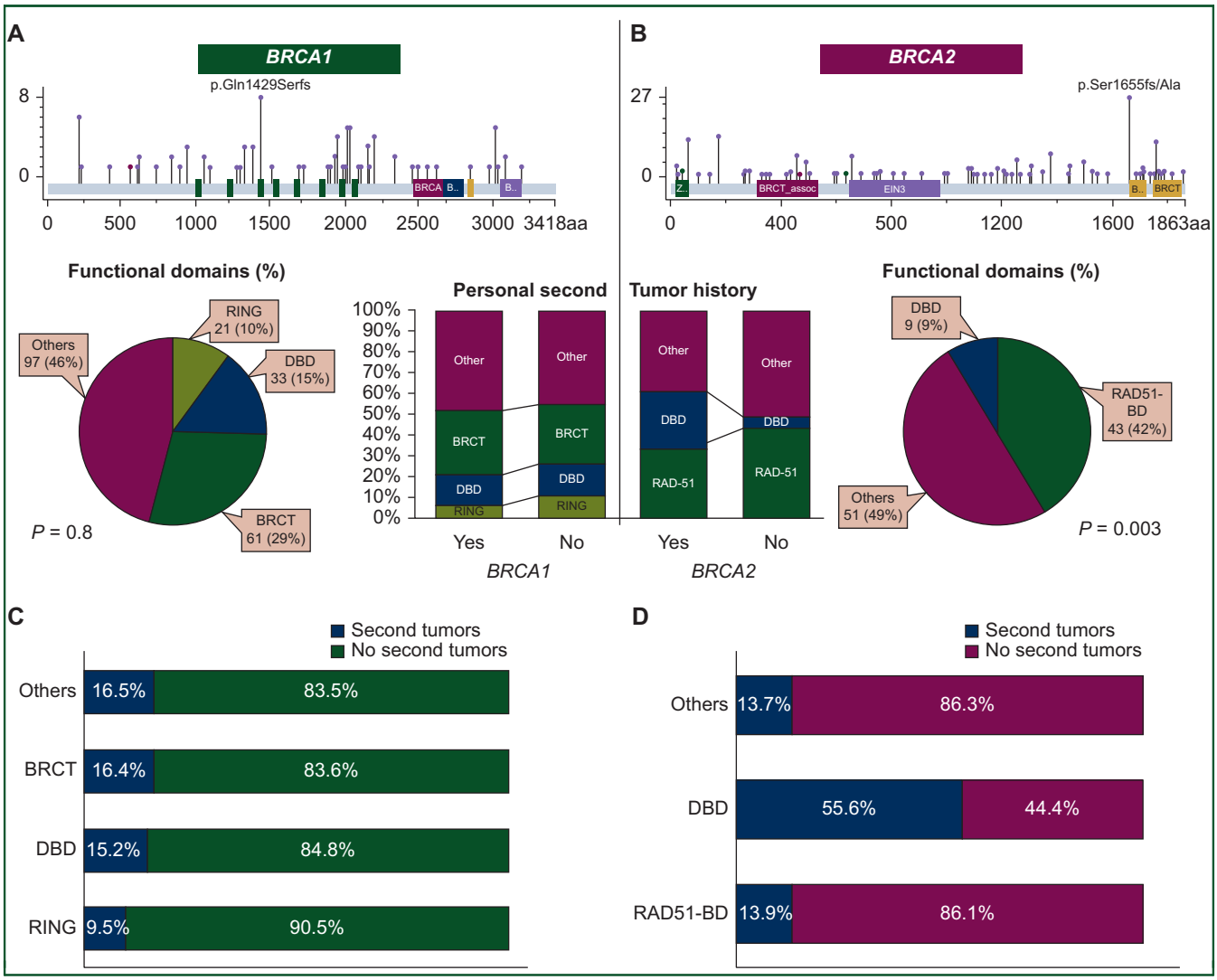


**Figure 2. Distribution of pathogenic variants (PVs) in the breast cancer susceptibility gene 1 (*BRCA1*) and *BRCA2* functional domains.** *BRCA1* functional domains (FDs) were (i) really interesting new gene [RING; amino acids (AAs) 8-96]; (ii) DNA-binding domain (DBD; AAs 452-1092); (iii) *BRCA1* C terminus (BRCT; AAs 1646-1736 and 1760-1855); and (iv) other location. *BRCA2* FDs were (i) RAD51-binding domain (RAD51-BD) (AAs 900-2000); (ii) DBD (AAs 2459-3190); and (iii) others.

Interestingly, a previous study suggested that the position of *BRCA2* PVs affects the propensity of the mutated allele to acquire reversion mutations.[32] Reversion mutations that restore the native reading frame of *BRCA* genes and homologous recombination are the main cause of PARPi resistance in the clinical setting.[33,34] Reversions of PVs in the region encoding the C terminus of *BRCA2*, which contains the DBD, were very rare compared with the region encoding the N-terminus domain of *BRCA2*.[32] This observation suggested that PVs in this 'desert' section of the gene is less able to be reverted through a secondary mutation, and the patients are at a lower risk of developing resistance via reversion. These data are consistent with previous reports showing the importance of the DBD for efficient homologous recombination, and the high degree of amino acid conservation in this domain.[29-32]

Recent clinical data are also published on this topic. In the *post hoc* subgroup analysis of the PAOLA-1 trial investigating the benefit of olaparib and bevacizumab according to the location of *BRCA* PVs, an excellent outcome for patients with OC with PVs located in the *BRCA2* DBD FD at 24 months was found.[35] In this exploratory analysis, women harboring PVs in the *BRCA2* RAD51-BD or located in other FD than DBD and RAD51-BD derive benefit from maintenance therapy with olaparib plus bevacizumab, compared with placebo plus bevacizumab.[35] However, it is important to emphasize that the study population differed from the current research, as they received maintenance therapy with either bevacizumab and olaparib or bevacizumab and a placebo. Therefore, data on the benefits of domain-related PARP inhibitors in patients with OC treated with olaparib as a single-agent maintenance therapy were lacking.

The current study has several strengths, including the large sample size. Importantly, to our knowledge, this is the first cohort study to highlight the role of PV location within *BRCA* FDs in the onset of the second primary tumor in patients with OC. Identifying genetic predisposing factors for MPTs is strategically relevant for cancer prevention efforts, especially given the growing number of cancer survivors who remain at elevated oncological risk throughout



https://doi.org/10.1016/j.esmoop.2024.104076

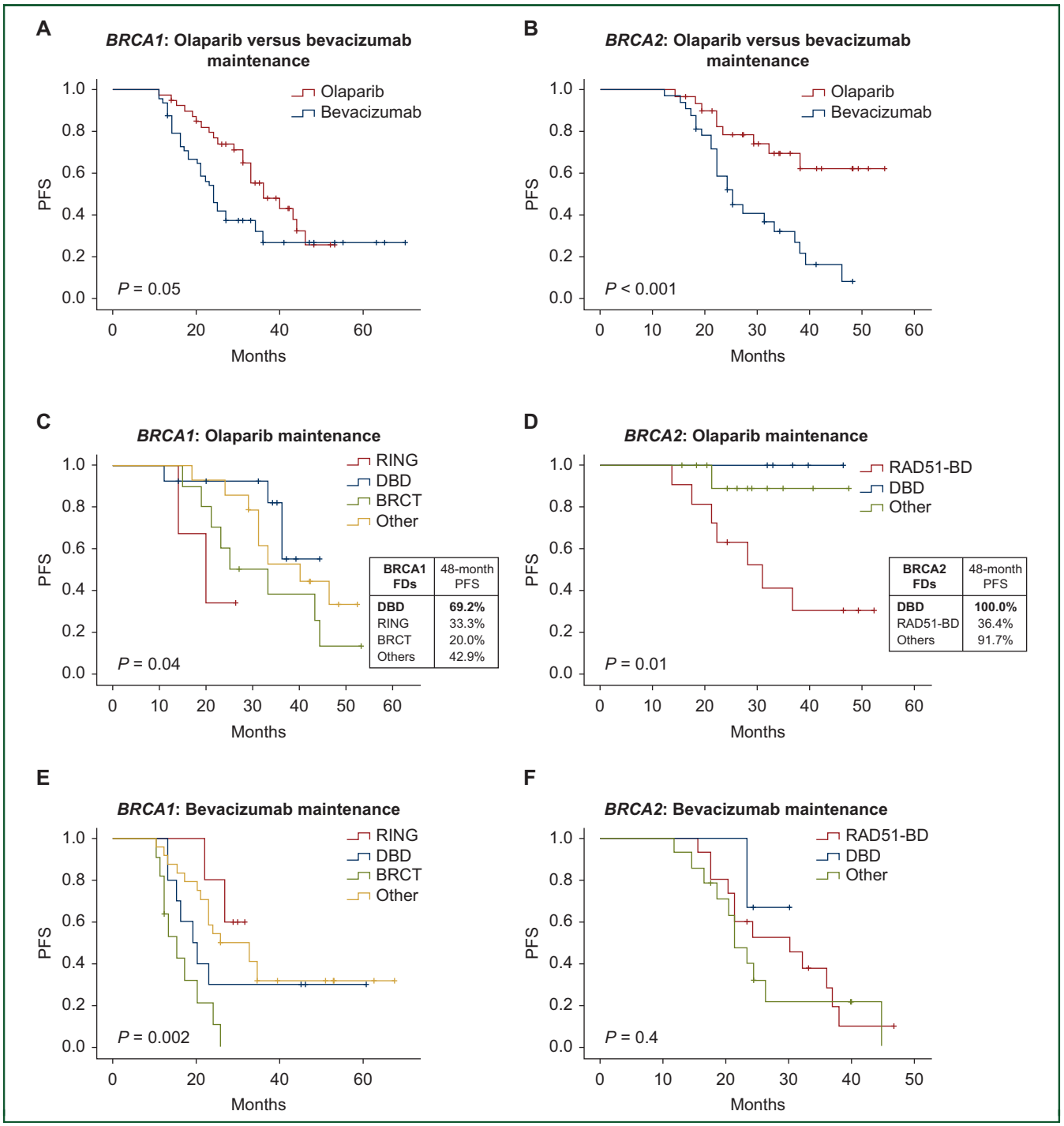


**Figure 3. The magnitude of olaparib versus bevacizumab benefit in patients with ovarian cancer (OC) harboring (A) *BRCA1* germline pathogenic variants (gPVs) or (B) *BRCA2* gPVs.** The magnitude of olaparib maintenance benefit according to functional domains (FDs) in patients with (C) *BRCA1*-mutated or (D) *BRCA2*-mutated OC. The magnitude of bevacizumab maintenance benefit according to FDs in patients with (E) *BRCA1*-mutated or (F) *BRCA2*-mutated OC.
BRCA, breast cancer susceptibility gene; BRCT, BRCA1 C terminus; DBD, DNA-binding domain; PFS, progression-free survival; RAD51-BD, RAD51-binding domain; RING, really interesting new gene.

their lives. The median time window of 5 years between the first and second tumor diagnoses in our population can provide valuable insights for developing clinical strategies and optimizing individualized cancer risk management guidelines.

The data from the current study expand our understanding of the cancer spectrum associated with the development of multiple tumors in *BRCA1/2* carriers. Very few studies have evaluated this risk, focusing primarily on the risk of BC after a diagnosis of OC. In our OC population, although BC remains the most frequently diagnosed second tumor, cases of melanoma, cholangiocarcinoma, renal cancers, lung cancers, thyroid cancers, and endometrial carcinoma were also observed. Notably, a key finding with

potential clinical implications was the relatively high number of patients with OC who developed a second tumor localized to the endometrium. Interestingly, these patients were predominantly those without *BRCA* mutations, indicating the potential involvement of other unrecognized genes in the development of both ovarian and endometrium cancer in the same patients.[36-38] Another relevant observation is the significantly higher incidence of endometrioid histology in patients with OC with an MPT history compared with those without an MPT history. Notably, most of these patients belonged to the *BRCA* WT subgroup (84.2%). Previous research has highlighted the heterogeneous composition of endometrioid ovarian carcinoma, which includes a subset with a hypermutable tumor phenotype associated with mismatch repair deficiency or DNA polymerase epsilon (*POLE*) mutation, similar to endometrial carcinoma.[39] This heterogeneous genetic and genomic profile underscores the importance of understanding the underlying biological drivers which could have critical clinical and therapeutic implications.

We acknowledge several limitations in our study. First, the retrospective nature of the data analysis. However, despite its retrospective design, the inclusion of consecutive patients with OC with a significant median follow-up allowed us to have a comprehensive view of MPT history in our cohort, especially in terms of age at tumor onset. This is particularly relevant given that OC is typically diagnosed later than BC in women who are *BRCA* PV carriers.[7] Second, environmental factors and other genetic and epigenetic events may act as modifiers of the known genetic background, further influencing cancer development.[40] Data on the modifying effects of these potential factors are currently lacking and remain poorly understood. Third, the sample size. Although this study included over a thousand patients with OC, the small number of patients in each *BRCA1* and *BRCA2* FD subgroup necessitates validation of our findings in larger populations.

### *Conclusions*

Results from our analysis provide preliminary evidence that *BRCA1/2* PVs located in specific FDs may be associated with an increased likelihood of developing MPTs. Our data revealed a significant association between PVs in the DBD FD of *BRCA2* and the occurrence of BC—OC in the same individuals. For this subpopulation, targeted screening programs and/or risk-reducing strategies could be proposed in the future to optimize cancer risk management and improve long-term survival.

Furthermore, our study on domain-related sensitivity to olaparib or bevacizumab showed that not all *BRCA1* and *BRCA2* carriers exhibit the same sensitivity to first-line maintenance therapy. The response may depend on the location of PVs within the FDs of the BRCA protein. In our population, we observed improved outcomes with olaparib maintenance therapy in patients with OC whose germline PVs were located within the DBD FD of the *BRCA2* gene.

Further understanding of these findings will aid in shaping preventive strategies and facilitating personalized therapeutic approaches.

## ACKNOWLEDGEMENTS

The authors acknowledge the use of the cBioPortal tool, used in this manuscript for visualizing cancer genetic data.

## FUNDING

LI, GB, and AR were supported by the Piano Nazionale di Ripresa e Resilienza (PNRR) project - Italian Network of Excellence for Advanced Diagnosis (INNOVA); PNC-E3-2022-23683266; PNC-HLS-DA (C43C22001630001).

## DISCLOSURE

The authors have declared no conflicts of interest.

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