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ORIGINAL ARTICLE

Association of location of *BRCA1* and *BRCA2* mutations with benefit from olaparib and bevacizumab maintenance in high-grade ovarian cancer: phase III PAOLA-1/ENGOT-ov25 trial subgroup exploratory analysis

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Background: In the phase III PAOLA-1 study, the addition of maintenance olaparib to bevacizumab in patients with newly diagnosed high-grade ovarian cancer (HGOC) resulted in prolonged progression-free survival (PFS), particularly for homologous recombination deficiency-positive tumors, including those with a BRCA mutation (BRCAm). The magnitude of benefit from olaparib and bevacizumab according to the location of mutation in *BRCA1/BRCA2* remains to be explored.

Patients and methods: Patients with advanced-stage HGOC responding after platinum-based chemotherapy + bevacizumab received maintenance therapy bevacizumab (15 mg/kg q3w for 15 months) + either olaparib (300 mg b.i.d. for 24 months) or placebo. PFS was analyzed in the subgroup of patients with *BRCA1*m/*BRCA2*m according to mutation location in the functional domains of *BRCA1* [Really Interesting Gene (RING), DNA-binding domain (DBD), or C-terminal domain of *BRCA1* (BRCT)] and *BRCA2* [RAD51-binding domain (RAD51-BD); DBD].

Results: From 806 randomized patients, 159 harbored *BRCA1*m (19.7%) and 74 *BRCA2*m (9.2%). *BRCA1*m in RING, DBD, and BRCT domains was detected in 18, 40, and 33 patients, and *BRCA2*m in RAD51-BD and DBD in 36 and 13 patients, respectively. After a median follow-up of 25.5 months, benefit from maintenance olaparib + bevacizumab was observed irrespective of location of BRCAm. The benefit was particularly high for those with *BRCA1*m located in the DBD, with 24-month PFS estimated to be 89% and 15% [olaparib + bevacizumab versus placebo + bevacizumab hazard ratio = 0.08 (95% confidence interval 0.02-0.28); interaction $P = 0.03$]. In *BRCA2*m patients, 24-month PFS rates for those with mutations located in the DBD were 90% and 100% (olaparib + bevacizumab versus placebo + bevacizumab), respectively.

Conclusions: Advanced-stage BRCA-mutated HGOC patients reported PFS benefit from maintenance olaparib and bevacizumab regardless of mutation location. The benefit is particularly high for patients with mutations located in the DBD of *BRCA1*. Mutations located in the DBD of *BRCA2* are also associated with excellent outcome.

Key words: PARP inhibitor, olaparib, ovarian cancer, BRCA mutation, location of mutation, genotype, type of mutation

INTRODUCTION

BRCA1 and *BRCA2* are the two major hereditary breast and ovarian cancer syndrome susceptibility genes, accounting for 5%-7% of breast cancer[1] and 10%-15% of ovarian cancer.[2] They are also associated with increased risk of pancreatic,[3] prostate,[4] serous endometrial,[5] and possibly gastric cancer.[6] The proteins encoded by these genes play

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central roles in maintaining genome integrity by repairing DNA double-strand breaks (DSBs) through homologous recombination, a low error repair mechanism using the sister chromatid as template.[7] Tumors arising in BRCA mutation (BRCAm) carriers are characterized by high genomic instability and subsequent genomic scars, named homologous recombination deficiency or HRD.[8] *BRCA1* and *BRCA2* are two large genes characterized by large central exon 11. BRCA1 is a multifunctional protein that links DNA damage response and DNA repair. It has several functional domains that include: (i) a highly conserved N-terminal Really Interesting New Gene (RING) domain with an E3 ubiquitin ligase activity; (ii) a DNA-binding domain (DBD); and (iii) a C-terminal domain of BRCA1 (BRCT) that facilitates phosphoprotein binding.[7] In contrast, BRCA2 plays a central role in homologous recombination by mediating the recruitment of RAD51 recombinase to DSBs. BRCA2 has three main functional domains: a central domain that contains eight BRC repeats constituting the primary interaction site to RAD51 monomers named RAD51-binding domain (RAD51-BD), a C-terminal highly conserved DBD, and an additional RAD51 interaction site (TR2) that binds to RAD51 filaments.

Murine models with different genomic alterations in *BRCA1* suggested a genotype—phenotype correlation for tumorigenesis.[9,10] For instance, homozygous deletion of *BRCA1* resulted in embryonic lethality in murine models,[11] while mice lacking the second BRCT repeat showed a delayed embryonic lethality[12] and those expressing *BRCA1-Δ11* transcript were viable and developed mammary tumors.[13] Women are more likely to develop breast or ovarian cancer depending on the location of *BRCA1/BRCA2* mutations.[14-16] Women carrying germline pathogenic mutations located in the exon 11 of both genes are at higher risk of developing ovarian than breast cancers, while those with mutations located in the 3' or 5' are more likely to develop breast cancer. Location of mutations is also prognostic in ovarian cancer patients. Retrospective studies showed that women with *BRCA1* mutations located outside exon 11 had better survival than those with mutations located inside exon 11,[17] while in *BRCA2* carriers, those having mutations located in the RAD51-BD (that overlaps with exon 11) had prolonged survival and higher sensitivity to platinum.[18]

The use of inhibitors of poly(ADP-ribose) polymerase-1 (PARPi) for the treatment of HRD tumors, including *BRCA1* or *BRCA2* mutated, is the first clinical translation of the concept of synthetic lethality into cancer care.[19,20] Although developed for all BRCA-inactivated tumors,[21] preclinical studies showed in genetically engineered mouse models that *BRCA1* mutations located in the RING domain led to mammary tumor development characterized by reduced sensitivity to PARPi and platinum.[22,23] *In vitro* inactivation of the DBD of *BRCA2* is associated with increased sensitivity to PARPi and platinum,[24] while large intragenic deletions in *BRCA2* that completely remove the DBD but encode the TR2 domain cause PARPi resistance.[25] Anecdotal observations in clinical trials of PARPi in relapsing ovarian cancer patients selected according to their platinum sensitivity suggested that long-term responders were enriched in *BRCA2* carriers[26] or BRCA structural variants.[27]

PAOLA-1/ENGOT-ov25 (ClinicalTrials.gov identifier: NCT02477644) is a multicenter, randomized, double-blind, placebo-controlled, phase III trial investigating the benefit of adding olaparib to bevacizumab as maintenance therapy in the advanced-stage [International Federation of Gynecology and Obstetrics (FIGO) stage III-IV] high-grade ovarian cancer (HGOC) patients who had previously received chemotherapy plus bevacizumab as induction therapy in the first line. The addition of olaparib to bevacizumab led to a substantial progression-free survival (PFS) benefit in all-comers, particularly in patients with HRD, including those with *BRCA1/BRCA2* mutations.[28] In the current study, we carried out *post hoc* analyses in the subgroup of patients with *BRCA1/BRCA2* mutations (BRCAm) of the PAOLA-1 trial to determine the predictive value of the location of *BRCA1/BRCA2* mutations on benefit from maintenance with olaparib and bevacizumab.

PATIENTS AND METHODS

Patient selection

This study is a *post hoc* analysis of data from the PAOLA-1 trial, a randomized, double-blind, placebo-controlled, international phase III trial conducted in 11 countries. Protocols were approved by institutional review boards or independent ethics committees of all investigational sites and informed consent was received; the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The design and results of PAOLA-1 have been previously reported;[28] briefly, the study enrolled patients with advanced-stage (FIGO stage III and IV) high-grade carcinoma of the ovary (endometrioid, serous, or other epithelial non-mucinous). Intravenous bevacizumab was initiated in combination with chemotherapy and was continued after randomization as maintenance therapy at a dose of 15 mg/kg every 3 weeks for a total duration of up to 15 months. At least 3 weeks and no more than 9 weeks after the last dose of chemotherapy, patients were randomized in a 2 : 1 ratio to receive 'placebo' or olaparib (300 mg twice daily). 'Placebo' or olaparib was administered for up to 24 months from randomization until disease progression [according to investigators' assessment of imaging based on the modified Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1], death, or unacceptable treatment toxicity.

Genetic analysis

All patients were screened for somatic mutations of *BRCA1* and *BRCA2* as stratification factor. Archival tumor samples were collected from the site pathologist during the screening period and sent to one of five central French academic laboratories selected by the Institut National du Cancer for assessment of tissue *BRCA1/BRCA2* mutation status before patients were randomized. The results of tumor BRCA testing were sent to the study site principal investigator for patient

stratification. Tumor BRCA testing used two different next-generation sequencing methods based on capture or re-sequencing technology based on either hybrid capture or amplicon technology.[29] Retrospective central tumor BRCA testing was conducted using the MyChoice® HRD Plus assay (Myriad Genetic Laboratories, Inc, Salt Lake City, UT) on tumor samples.[28] The central genetic analyses carried out by Myriad were used in the current study. Mutations were reported on transcripts NM_007294.3 (*BRCA1*) and NM_000059.3 (*BRCA2*) on Human Genome hg19. Pathogenic mutations were defined as mutations generating a premature termination codon [nonsense (NS)], frameshift (FS), splicing, or large rearrangements (LR). Missense (MS) variants considered pathogenic by the Breast Cancer Information Core committee were included. For *BRCA1*, the functional domains were defined as follows: (i) RING domain: amino acids (AA) 8-96; DBD: AA 452-1092; BRCT: AA 1646-1736 and 1760-1855.[30,31] Exon 11 corresponded to the region covering AA 224-1366. The functional domains of *BRCA2* were defined as follows: (i) RAD51-BD: AA 900-2000; (ii) DBD: AA 2459-3190.[32] Exon 11 corresponded to the region covering AA 637-2281.

Statistical analysis

This *post hoc* analysis was carried out on the subgroup of BRCA-mutated patients. Efficacy data were analyzed in the intention-to-treat population, i.e. all randomized patients, regardless of the received intervention. PFS was estimated using the Kaplan—Meier method, and log-rank tests were used to assess the differences between the two treatment trial arms. Cox proportional hazards models were used to calculate the hazard ratios (HRs) and their associated 95% confidence intervals (CIs). Interaction terms were introduced in the survival models to test for significant effect modification of treatment efficacy by mutated gene and functional domain. Median follow-up time was determined through the calculation of the reverse Kaplan—Meier estimate. The primary endpoint was PFS, as determined by RECIST version 1.0. by the investigators, and was calculated according to mutated gene (*BRCA1* and *BRCA2*), exon 11 genotype, and mutated domain of *BRCA1* (RING, DBD, BRCT, or other genomic locations) and *BRCA2* (RAD51-BD, DBD, or other genomic locations). Power and sample size were calculated with the R software 'powerSurvEpi' package.[33] The calculations considered the 2 : 1 randomization, an $\alpha = 0.05$, and the event rates observed in each mutational site group. A two-tailed $P < 0.05$ was considered statistically significant, and data were analyzed using the R statistical software (R 4.1.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Cohort characteristics

Of 806 patients who underwent randomization in the PAOLA-1 study, 235 participants harbored *BRCA1/BRCA2* mutations. Two participants (one *BRCA1* and one *BRCA2*) were excluded as they harbored alterations affecting multiple functional domains; 233 women were included in the analysis (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2022.11.003). The clinical and demographic characteristics according to treatment arm are summarized in Table 1. *BRCA1* and *BRCA2* mutations were present in 68.2% ($n = 159$) and 31.8% ($n = 74$) of the patients, respectively. About half of the mutations (52.8%, $n = 123$) were located in exon 11. The most commonly mutated functional domains in *BRCA1* were the DBD (25.2%, $n = 40$), followed by BRCT domain (20.8%, $n = 33$) and RING domain (11.3%, $n = 18$). For *BRCA2,* about half of the mutations were located in the RAD51-BD (48.6%, $n = 36$), followed by DBD (17.6%, $n = 13$). A substantial proportion of the mutations were located outside these functional domains of *BRCA1* and *BRCA2* (42.8%, $n = 68$ and 33.8%, $n = 25$, respectively).

Distribution of mutations in BRCA1 and BRCA2

The distribution of type of mutations was different between *BRCA1* and *BRCA2* (Fisher's exact test, $P = 0.01$). Specifically, we found among *BRCA1* mutations 85 FS (53.5%), 18 MS (11.3%), 38 NS (23.9%), 8 LR (5%), and 10 splicing mutations (6.3%) (Figure 1A and Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.11.003). The majority of *BRCA2* mutations were FS ($n = 43$, 58.1%) or NS ($n = 27$, 36.5%). There were no LR and only two MS (2.7%) mutations among *BRCA2* cases. Most MS and splicing mutations were located in the RING or BRCT domains of *BRCA1* or the DBD of *BRCA2* (Figure 1B). Mutations located in *BRCA1* DBD and *BRCA2* RAD51-BD were exclusively NS or FS.

Benefit of olaparib and bevacizumab according to mutated gene and exon 11

Median follow-up was 25.5 months. PFS rates at 24 months and median PFS according to the location of mutations are presented in Table 2. No differences in efficacy of olaparib + bevacizumab were observed according to which gene was mutated [*BRCA1*, HR = 0.26 (95% CI 0.16-0.41); *BRCA2*, HR = 0.22 (0.09-0.54); interaction $P = 0.64$; Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.11.003]. Similarly, patients with or without mutations located in exon 11 of both genes derived benefit from olaparib + bevacizumab [exon 11, HR = 0.2 (0.11-0.36); non-exon 11, HR = 0.41 (0.22-0.75)]. Although exon 11 patients tended to benefit more from the combination, the difference in the magnitude of benefit was not significant (interaction $P = 0.14$; Supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2022.11.003).

Benefit of olaparib and bevacizumab according to the location of mutations in BRCA1

Differences in PFS were observed when comparing subgroups according to the location of mutations in the different domains of *BRCA1* (Figure 2; Table 2). Specifically, patients with *BRCA1* mutation located in the DBD derived greater benefit from olaparib + bevacizumab than any

Table 1. Patient characteristics

	Placebo ($n = 76$)	Olaparib ($n = 157$)	Overall ($n = 233$)
Age			
Mean (SD)	56.2 (9.91)	57.4 (9.26)	57.0 (9.47)
Median (min, max)	56.0 (35.0, 82.0)	57.0 (37.0, 77.0)	57.0 (35.0, 82.0)
Primary tumor location, *n* (%)			
Fallopian	4 (5.3)	15 (9.6)	19 (8.2)
Ovary	68 (89.5)	134 (85.4)	202 (86.7)
Peritoneal	4 (5.3)	8 (5.1)	12 (5.2)
ECOG, *n* (%)			
0	58 (77.3)	114 (74.0)	172 (75.1)
1	17 (22.6)	40 (26.0)	57 (24.9)
Mutated gene, *n* (%)			
BRCA1	47 (61.8)	112 (71.3)	159 (68.2)
BRCA2	29 (38.2)	45 (28.7)	74 (31.8)
FIGO stage, *n* (%)			
III	50 (65.8)	113 (72.0)	163 (70.0)
IV	26 (34.2)	44 (28.0)	70 (30.0)
Histology, *n* (%)			
Other	5 (6.6)	8 (5.1)	13 (5.6)
Serous	71 (93.4)	149 (94.9)	220 (94.4)
Surgery, *n* (%)			
No surgery	6 (7.9)	5 (3.2)	11 (4.7)
Initial	45 (59.2)	83 (52.9)	128 (54.9)
Interval (after neoadjuvant chemotherapy)	25 (32.9)	69 (43.9)	94 (40.3)
Residual macroscopic disease, *n* (%)			
No	22 (31.4)	49 (32.2)	71 (32.0)
Yes	48 (68.6)	103 (67.8)	151 (68.0)
Response after surgery/chemotherapy, *n* (%)			
CR/NED	56 (73.7)	126 (80.3)	182 (78.1)
PR	20 (26.3)	31 (19.7)	51 (21.9)
CA-125 normalization, *n* (%)			
No	10 (13.2)	16 (10.2)	26 (11.2)
Yes	66 (86.8)	141 (89.8)	207 (88.8)
Tumor content			
Mean (SD)	60.5 (21.6)	59.0 (21.7)	59.5 (21.6)
Median (min, max)	66.0 (1.00, 94.6)	60.5 (1.00, 96.7)	62.9 (1.00, 96.7)
Mutation type, *n* (%)			
FS	43 (56.6)	85 (54.1)	128 (54.9)
MS	7 (9.2)	13 (8.3)	20 (8.6)
NS	21 (27.6)	44 (28.0)	65 (27.9)
LR	1 (1.3)	7 (4.5)	8 (3.4)
Splicing	4 (5.3)	8 (5.1)	12 (5.2)
Location of mutation, *n* (%)			
BRCA1			
RING	5 (10.6)	13 (11.6)	18 (11.3)
DBD	16 (34.0)	24 (21.4)	40 (25.2)
BRCT	9 (19.1)	24 (21.4)	33 (20.8)
Other locations	17 (36.2)	51 (45.5)	68 (42.8)
BRCA2			
RAD51-BD	15 (51.7)	21 (46.7)	36 (48.6)
DBD	3 (10.3)	10 (22.2)	13 (17.6)
Other locations	11 (37.9)	14 (31.1)	25 (33.8)
Exon 11			
No	29 (38.2)	81 (51.6)	110 (47.2)
Yes	47 (61.8)	76 (48.4)	123 (52.8)

BRCT, C-terminal domain of *BRCA1*; CR, complete response; DBD, DNA-binding domain; ECOG, Eastern Cooperative Oncology Group; FIGO, International Federation of Gynecology and Obstetrics; FS, frameshift; LR, large rearrangement; max, maximum; min, minimum; MS, missense; NED, no evidence of disease; NS, nonsense; PR, partial response; RAD51-BD, RAD51-binding domain; RING, Really Interesting New Gene; SD, standard deviation.

other location in *BRCA1* [HR = 0.08 (0.02-0.28); interaction $P = 0.03$; Figure 2A]. Patients with *BRCA1* mutation located in the RING [HR = 0.38 (0.07-2.13); interaction $P = 0.40$; Figure 2B], BRCT [HR = 0.55 (0.2-1.56); interaction $P = 0.09$; Figure 2C], or other locations [HR = 0.24 (0.11-0.51); interaction $P = 0.92$; Figure 2D] showed consistent benefit from olaparib + bevacizumab.

Benefit of olaparib and bevacizumab according to the location of BRCA2 mutations

We found that only 1 out of the 13 patients with *BRCA2* DBD mutations had a recurrence, with both treatment arms showing excellent outcomes, despite presenting with advanced-stage HGOC (100% versus 90% 24-month PFS for

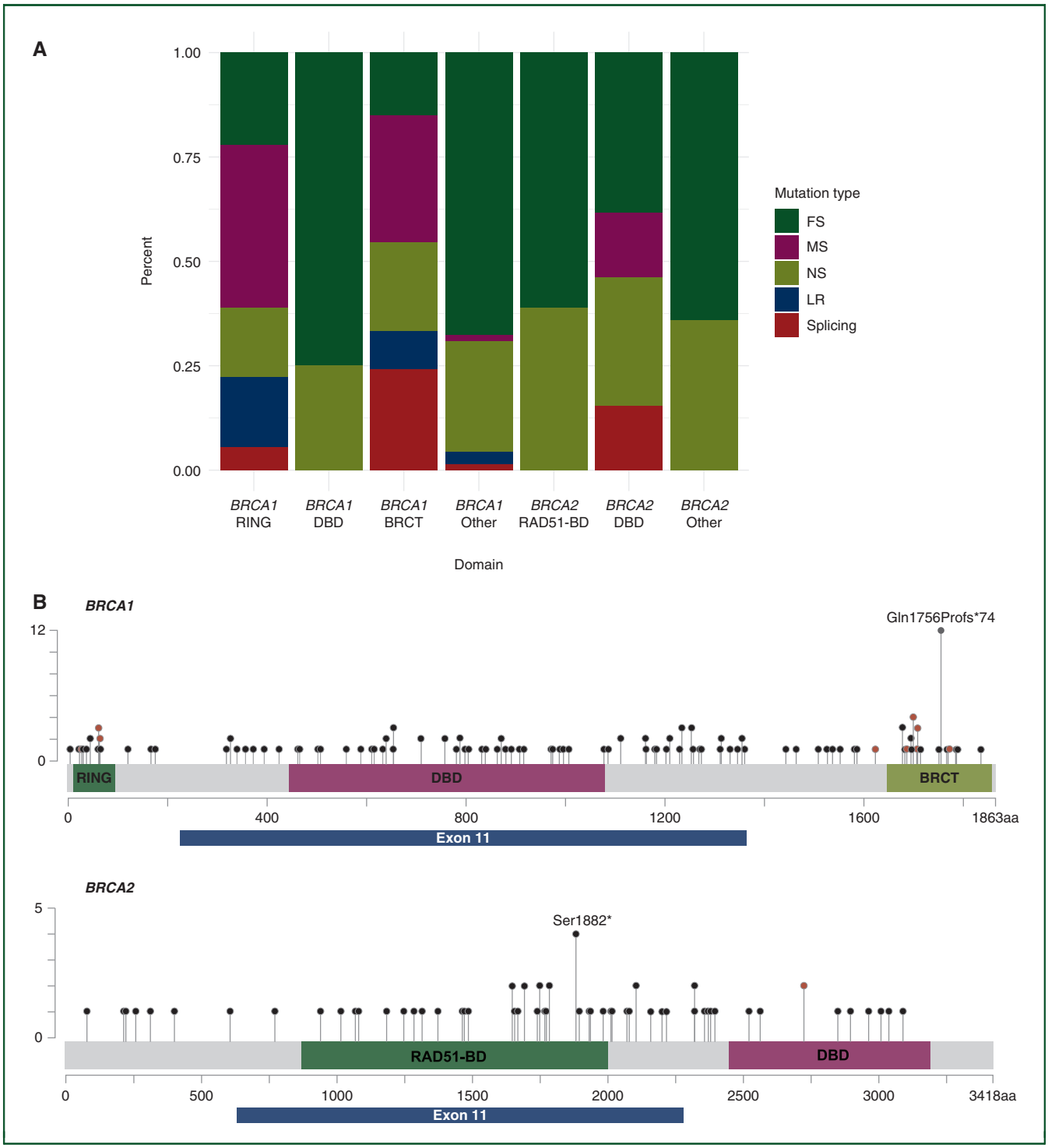

Figure 1. Distribution of mutations in *BRCA1* and *BRCA2*. (A) Graphical summary of *BRCA1* and *BRCA2* mutations. Mutation plot was generated using cBioPortal MutationMapper tool. Black = nonsense and frameshift mutations, brown = missense mutations. (B) Type of mutations in *BRCA1* and *BRCA2*.
BRCT, C-terminal domain of *BRCA1;* DBD, DNA-binding domain; FS, frameshift; LR, large rearrangement; MS, missense; NS, nonsense; RAD51-BD, RAD51-binding domain; RING, Really Interesting New Gene.

placebo + bevacizumab and olaparib + bevacizumab, respectively; Figure 3A). A clinical benefit of olaparib + bevacizumab was observed in patients harboring mutations located in the *BRCA2* RAD51-BD [HR = 0.31 (0.11-0.92); interaction $P = 0.39$; Figure 3B], as well as in those carrying mutations in genomic locations other than DBD and RAD51-BD [HR = 0.09 (0.01-0.75); interaction $P = 0.29$; Figure 3C].

Benefit of olaparib and bevacizumab according to mutation type

Since some mutations, for instance those located in *BRCA1* DBD and *BRCA2* RAD51-BD, were exclusively NS or FS, it raises the question of whether the type of mutation, rather than its location, is responsible for an enhanced benefit from olaparib + bevacizumab. None of the mutation types

Table 2. PFS according to the location of mutations in *BRCA1* and *BRCA2*

	Region (AA)	Median PFS, placebo (95% CI)	Median PFS, olaparib (95% CI)	24-month PFS, placebo (95% CI)	24-month PFS, olaparib (95% CI)	Placebo events (cases)	Olaparib events (cases)	HR (95% CI)	*P*
Gene									
BRCA1 ($n = 159$)		17.6	36	0.2 (0.11-0.39)	0.7 (0.61-0.79)	34 (47)	37 (112)	0.26 (0.16-0.41)	<0.001
BRCA2 ($n = 74$)		22.2	NR	0.5 (0.34-0.73)	0.84 (0.73-0.96)	17 (29)	7 (45)	0.22 (0.09-0.54)	0.001
Functional domain of *BRCA1*									
RING ($n = 18$)	8-96	11	27.4	0.5 (0.19-1)	0.66 (0.43-1)	2 (5)	6 (13)	0.38 (0.07-2.13)	0.273
DBD ($n = 40$)	452-1092	16	NR	0.15 (0.04-0.51)	0.89 (0.76-1)	14 (16)	4 (24)	0.08 (0.02-0.28)	<0.001
BRCT ($n = 33$)	1646-1736	19.9	29.6	0.3 (0.1-0.88)	0.59 (0.42-0.84)	6 (9)	10 (24)	0.55 (0.2-1.56)	0.265
Other ($n = 68$)	1760-1855	16.6	37.2	0.18 (0.05-0.59)	0.67 (0.55-0.82)	12 (17)	17 (51)	0.24 (0.11-0.51)	<0.001
Functional domain of *BRCA2*									
RAD51-BD ($n = 36$)	900-2000	21.7	NR	0.33 (0.15-0.75)	0.74 (0.57-0.97)	10 (15)	5 (21)	0.31 (0.11-0.92)	0.034
DBD ($n = 13$)	2459-3190	NR	NR	1 (1-1)	0.9 (0.73-1)	0 (3)	1 (10)	NC	NC
Other ($n = 25$)		24	NR	0.55 (0.32-0.94)	0.93 (0.8-1)	7 (11)	1 (14)	0.09 (0.01-0.75)	0.025
Exon 11 mutation									
Yes ($n = 123$)		17.6	37.2	0.24 (0.14-0.43)	0.78 (0.68-0.89)	34 (47)	18 (76)	0.2 (0.11-0.36)	<0.001
No ($n = 110$)		19.9	NR	0.45 (0.3-0.68)	0.7 (0.6-0.81)	17 (29)	26 (81)	0.41 (0.22-0.75)	0.004

AA, amino acid; BRCT, C-terminal domain of *BRCA1;* CI, confidence interval; DBD, DNA-binding domain; HR, hazard ratio; NC, not calculated; NR, not reached; PFS, progression-free survival; RAD51-BD, RAD51-binding domain; RING, Really Interesting New Gene.

were associated with an effect modification of the olaparib + bevacizumab benefit (all interaction $P > 0.01$) (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.11.003, and Figure 4). A stratified analysis cannot be carried out due to the small sample size.

Power calculations

We sought to determine whether, despite the low number of participants, the sample size of each location of mutation subgroup was sufficient to detect an HR similar to that of the DBD subgroup of *BRCA1*. For the group of patients with a *BRCA1* DBD mutation, the power for detecting an HR = 0.08 with an $\alpha = 0.05$, and considering the 2 : 1 randomization, the available sample size (olaparib $n = 24$, placebo $n = 16$) and the observed event rates (olaparib 4/24, placebo 14/16), was estimated to be 99%. We then calculated the power of detecting a similar effect size to that of the *BRCA1* DBD mutation, i.e. HR = 0.08, in the other groups defined according to mutation site (*BRCA1*: RING, BRCT, Other; *BRCA2*: RAD51-DB, Other) using the same α but considering the sample size and the event rate of each group. All groups had a statistical power above 90% except the '*BRCA2* Other' group (power = 76%). In all the other groups, considering their event rates and a power of 80% with an alpha=0.05, there was a sufficient sample size to detect an HR similar to that of the *BRCA1* DBD, with the exception of the '*BRCA2* Other' group (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.11.003).

DISCUSSION

Sensitivity to DNA damage agents such as platinum salts and PARPi relies on inactivation of both alleles of BRCA tumor suppressor genes. Biallelic loss of BRCA through loss of heterozygosity in tumors occurs in virtually all BRCA-mutated ovarian cancer cases,[34,35] but is less systematic in other BRCA-associated cancers.[36-38] Thus, ovarian cancer is a unique model to explore the predictive value of BRCA genotypes for response to platinum salts and PARPi. In this *post hoc* subgroup analysis of the PAOLA-1 trial, we found that all women with *BRCA1/BRCA2* mutations derive benefit from maintenance therapy with olaparib and bevacizumab, regardless of location of mutations in both genes, but with different degrees of magnitude.

Previous studies have investigated the prognostic value of BRCAm in patients with ovarian cancer. Ovarian cancer patients carrying a germline BRCAm have a longer survival than non-carriers, especially when *BRCA2* is affected.[39,40] We previously showed that germline *BRCA2* mutations located in the RAD51-BD, but not the DBD, were associated with prolonged survival.[18] Our study had several limitations, including the fact that it was retrospective and included only women who had been addressed for genetic counseling. Among *BRCA1* mutation carriers, those with mutations located outside exon 11 have better survival than non-carriers and *BRCA1* carriers with mutations located inside exon 11.[17] The deleterious effect of mutations located in exon 11 of *BRCA1* was linked to the expression of the *BRCA1-Δ11q* splicing variant in cancer cells that promotes partial resistance to platinum. For *BRCA2* carriers, we previously showed that those with mutations located in the RAD51-BD have prolonged platinum-free interval and survival.[18] Since most patients with HGOC are treated with platinum salts, the prognostic value of BRCAm most likely reflects sensitivity to these agents. It is not excluded that type of mutation may be a confounder or an effect modifier of the association between the mutation location and benefit from olaparib + bevacizumab. While the analysis by mutation type did not identify a significant interaction, a larger sample size is needed to allow an analysis with adequate statistical power that considers both location and type of mutation.

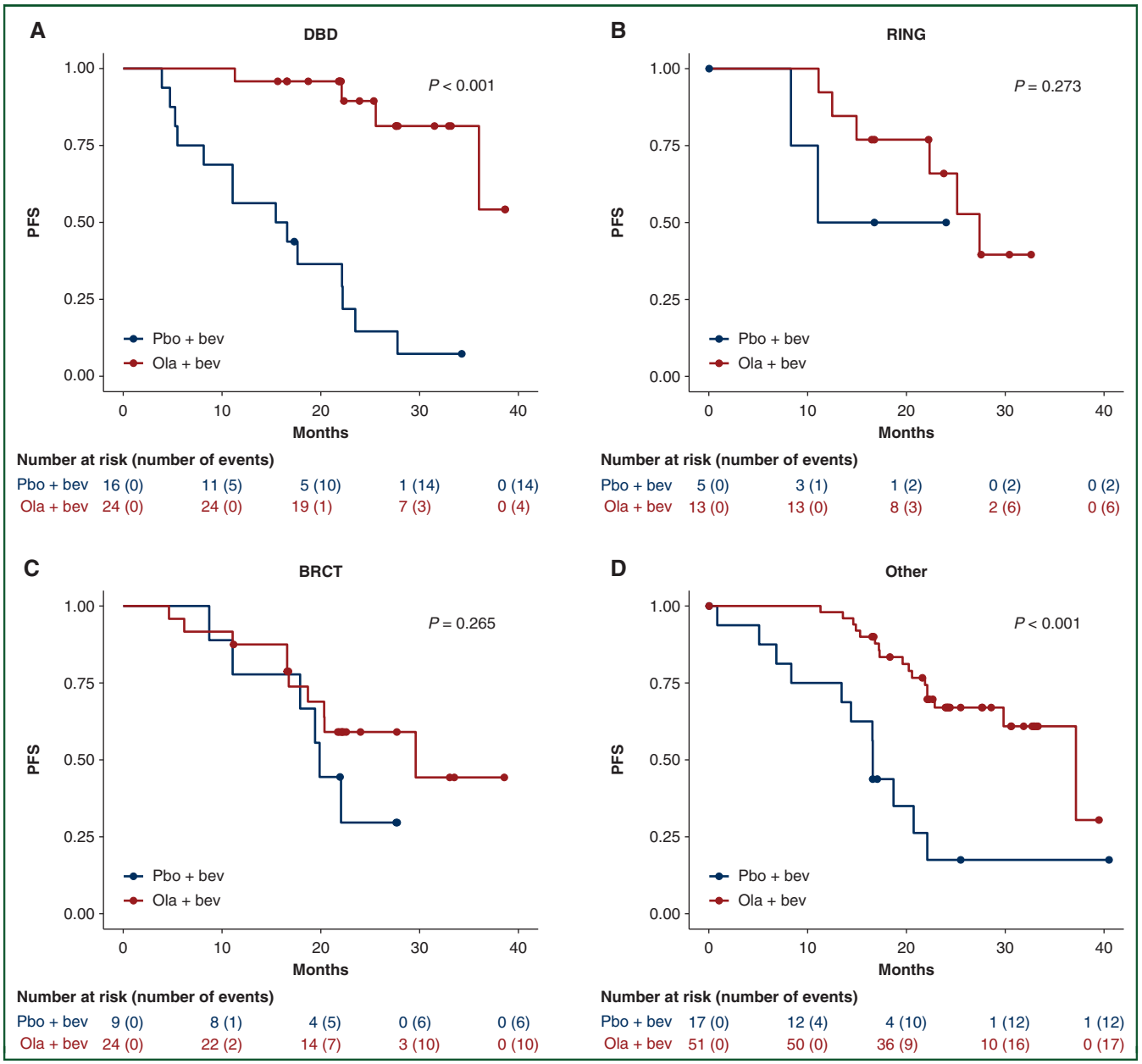

Figure 2. PFS according to the location of mutations in *BRCA1*. (A) DBD. (B) RING. (C) BRCT. (D) Other locations.
Bev, bevacizumab; BRCT, C-terminal domain of *BRCA1*; DBD, DNA-binding domain; Ola, olaparib; Pbo, placebo; PFS, progression-free survival; RING, Really Interesting New Gene.

An intriguing observation in the current study was the excellent outcome of women with mutations located in the DBD of *BRCA2* with only 1 out of the 13 patients with stage III-IV HGOC relapsing, suggesting an extreme sensitivity to platinum. The DBD is the most conserved part of the BRCA2 protein, from fungi to humans,[41] but its function is less clear than other more 'recent' BRCA2 regions.[24] Biochemistry and microscopic studies highlighted the importance of this domain for conformation rearrangements of BRCA2 and its interaction with DNA repair partners.[24,42,43] *In vitro*, deletion of DBD but not the C-terminal domain of *BRCA2* similarly leads to increased sensitization to platinum salts and PARPi.[24] Studies investigating the distribution of secondary mutations of *BRCA2* that restore the open reading frame of the protein, a common mechanism of secondary resistance to platinum and PARPi in patients,[44-48] showed that reversion mutations in *BRCA2* exhibit a position dependence.[46,49] Specifically, reversions of pathogenic mutations located in the C-terminus of *BRCA2* (CDS position >7617) that contains the DBD are very rare, a 'desert', suggesting that pathogenic mutations located in this domain are less able to be reverted by secondary mutations, a potential explanation for the excellent prognosis of *BRCA2* DBD mutations.[46] Extensive genomic analyses of paired biopsies at baseline and when patients acquired resistance to PARPi are warranted for a better comprehension of these mechanisms of resistance in patients.

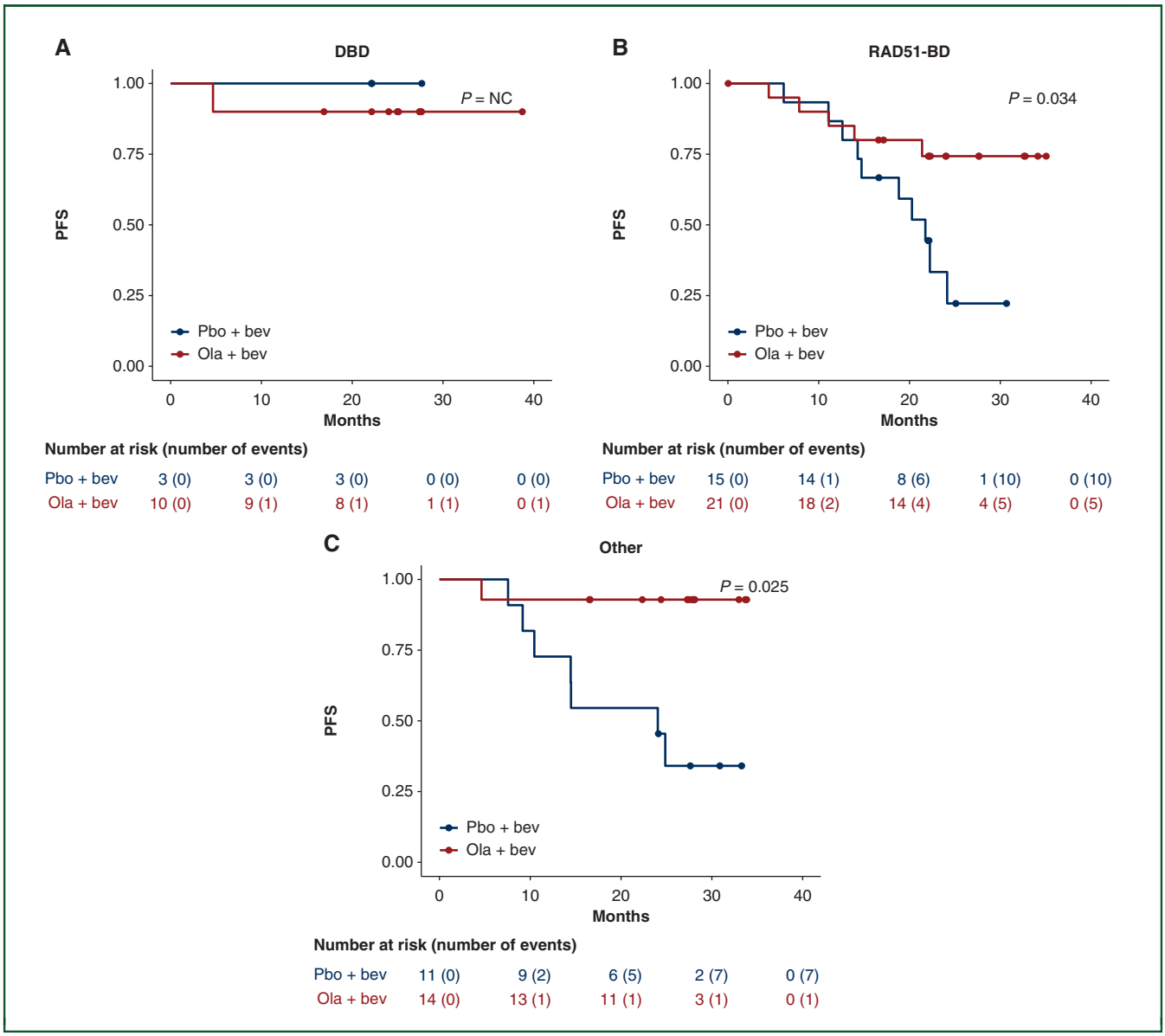

Figure 3. PFS according to the location of mutations in *BRCA2*. (A). DBD. (B) RAD51-BD. (C) Other locations.
Bev, bevacizumab; DBD, DNA-binding domain; NC, not calculated; Ola, olaparib; Pbo, placebo; PFS, progression-free survival; RAD51-BD, RAD51-binding domain.

Our study revealed that patients with mutations located in the DBD of *BRCA1* receiving maintenance with bevacizumab and placebo had a high risk of relapse with a median PFS of 16 months, comparable with the PFS of women without BRCAm in the PAOLA-1 trial.[28] This observation suggests that these patients might be less sensitive to platinum salts than patients with other *BRCA1*m tumors. The DBD of *BRCA1* is located within exon 11. Our observation is consistent with a previous report showing that HGOC patients carrying *BRCA1* mutations located in the exon 11 are less sensitive to platinum salts and have similar survival to that of non-carriers.[17] Intriguingly, we found that these patients were extremely sensitive to olaparib and bevacizumab maintenance therapy, with an HR of 0.08, suggesting that mutations located in the DBD of *BRCA1* might not lead to cross-resistance between platinum salts and PARPi. Biochemistry and functional studies are warranted to formally investigate this hypothesis.

The RING domain of *BRCA1* has an E3 ubiquitin ligase activity and is required for interaction with BARD1. This domain is essential for tumor suppression and tumorigenesis. We found that the magnitude of benefit from maintenance therapy with olaparib and bevacizumab in women with *BRCA1* mutations located in the RING domain was lower than in those with mutations in the DBD. This observation is consistent with preclinical data using genetically engineered mouse models of mammary tumors showing that mutations affecting the RING domain of BRCA1 were less sensitive to PARPi than those with deletion of exon 5-13, which contains the DBD.[17,22,23] In these studies, early stop codon at AA 24 ($BRCA1^{185delAG}$) was expected to lead to a small N-terminal BRCA1 protein. Instead,

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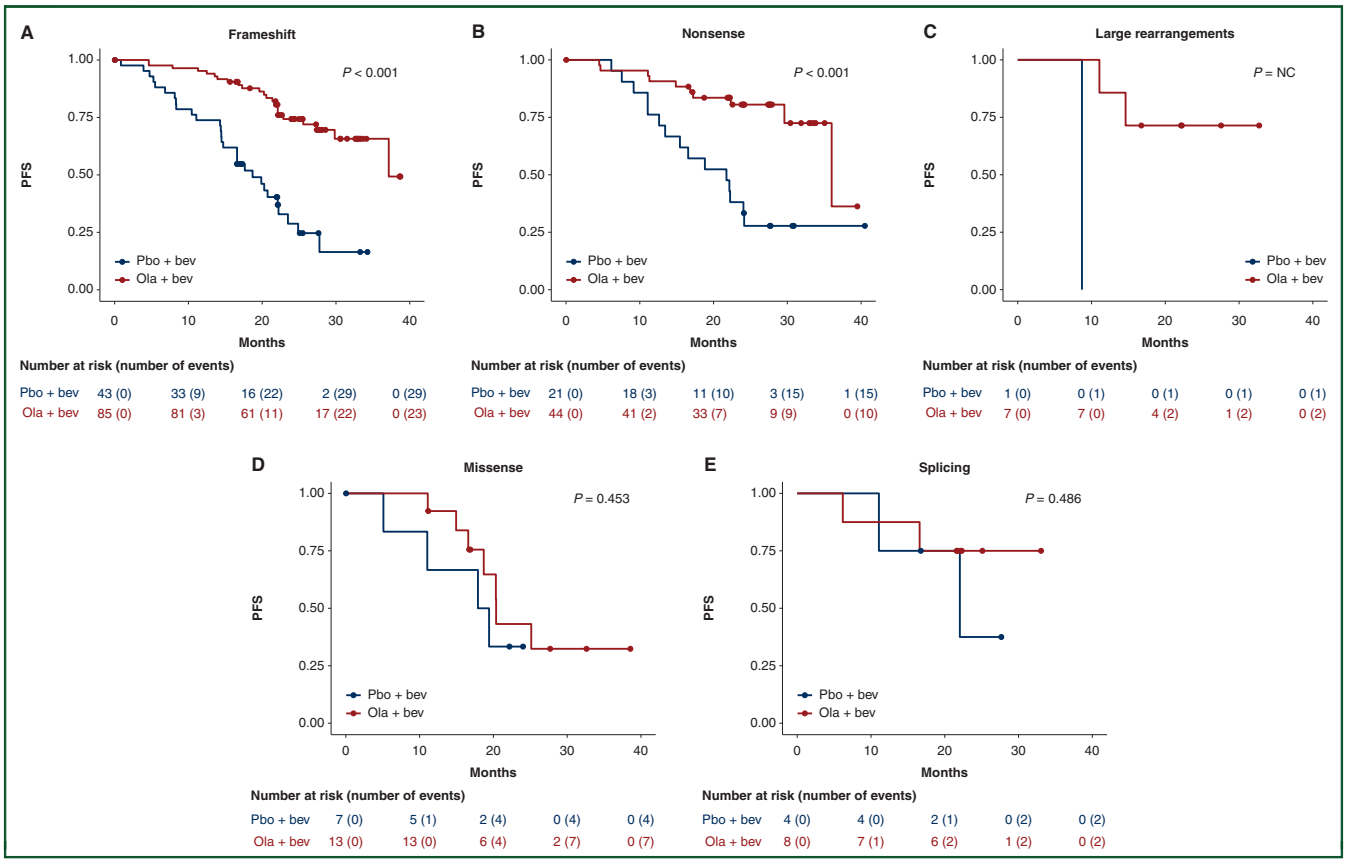

Figure 4. PFS according to type of mutation. (A) Frameshift. (B) Nonsense. (C) Large rearrangements. (D) Missense. (E) Splicing. Bev, bevacizumab; NC, not calculated; Ola, olaparib; Pbo, placebo; PFS, progression-free survival.

a nearly full-length BRCA1 protein (RING-less) was observed, indicating an alternative initiation of translation downstream of the FS mutation. This truncated BRCA1 protein mediates resistance to DNA damage agents (PARPi and platinum) through its residual activity in the DNA damage response, in line with our observation.[23,50]

To our knowledge, our study is the first to highlight the importance of this functional domain for response to PARPi in *BRCA1*. Yet, our exploratory analysis should be taken with caution because of the low number of patients per subgroup, in a *post hoc* analysis, preventing sufficient statistical power to draw definitive conclusions. These results need to be confirmed by meta-analyses of HGOC randomized trials with PARPi maintenance in first-line and relapsing setting,[51-56] as well as in real-life databases.[57] Moreover, biochemistry and functional studies are warranted to investigate the biological mechanisms underlying such domain-related sensitivity to PARPi.

The recent approval of PARPi in several other carcinomas led to the increase of systematic screening for *BRCA1* and *BRCA2* tumor mutations, beyond ovarian cancers. Large real-world cohorts will help gather new information on the genotype—phenotype correlation. Furthermore, the recent development of high-throughput base editing screens could be exploited to retrospectively test all *BRCA1/2* variants found in these trials, enabling functional interrogation on an unprecedented scale.[58-60] Such functional characterization of nucleotide variants will uncover their phenotype and consequences on cell biology, guiding the development of precision medicine in gynecological cancers.

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