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Exploring the impact of *BRCA1* and *BRCA2* mutation type and location on Olaparib maintenance therapy in platinum-sensitive relapsed ovarian Cancer patients: A single center report

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HIGHLIGHTS

- BRCA1/2 mutation location impacts the outcome of relapsed ovarian cancer patients on olaparib maintenance monotherapy (OMT).
- Patients on OMT with *BRCA1* pathogenic variant (PV) had shorter progression free survival (PFS) compared to *BRCA2* PV.
- Patients on OMT with PV in *BRCA2* or in *BRCA1* DNA binding domain had better PFS compared to PV in *BRCA1* RING domain.
- No significant difference in PFS between patients on OMT with germline or somatic *BRCA1/2* PV was observed.
- Adverse events in patients on OMT were not significantly different between germline and somatic PV in BRCA1/2.

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ABSTRACT

Objective. In patients with platinum-sensitive relapsed ovarian cancer (PSROC) harboring pathogenic/likely pathogenic variants (PV) in *BRCA1* and *BRCA2* genes, olaparib maintenance monotherapy (OMT) is a viable option. Our study aimed to evaluate the impact of different *BRCA1/2* PV in survival outcomes and safety of OMT in *BRCA1/2*-mutated PSROC patients, focusing on the type and location of PV.

Methods. We assessed the outcomes of 100 *BRCA1/2*-mutated PSROC patients treated at our institute, analyzing progression-free survival (PFS) and overall survival (OS). Germline and tumor *BRCA1/2* genotyping was conducted using Illumina's next-generation sequencing (NGS).

Results. PFS and OS were significantly shorter in PSROC patients with PV in *BRCA1* compared to those with PV in *BRCA2* (PFS:14.0 vs. 38.8 months, $p = 0.007$, OS: 21.8 vs. 62.0 months, $p = 0.011$). Notably, there was a significant difference in PFS based on the intragenic location of *BRCA1* PV, with shorter PFS in patients with 1st/2nd relapse, harboring PV in *BRCA1* RING domain compared to those with PV in the DNA binding domain (DBD) and BRCT domains (12.4 vs. 23.0 months, $p = 0.046$). No differences in PFS and OS were observed between patients with germline versus somatic *BRCA1/2* PV (PFS:14.9 vs.19.3, $p = 0.316$, OS: not reached vs. 25.8 months; $p = 0.224$). However, there were significant differences in the reasons for OMT discontinuation between patients with germline and somatic *BRCA1/2* PV, primarily due to adverse side effects.

Conclusions. In summary, the type and location of *BRCA1* and *BRCA2* PV provide additional insight into the expected survival outcomes of olaparib MT in PSROC patients.

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1. Introduction

Genomic instability is one of the main features across human cancers. In cancer cells, the accelerated mutational capacity arises

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Table 1
Clinical characteristics of patients included in the study.

	All patients	Somatic *BRCA1/2* PV	Germline *BRCA1/2* PV	Germline *BRCA1* PV	Germline *BRCA2* PV
Number of patients n (%)	$n = 100$ (100)	$n = 10$ (10)	$n = 90$ (90)	$n = 69$ (69)	$n = 21$ (21)
Follow up (months):					
- estimated median	57	32	57	59	65
- range	(12–86)	(17–79)	(12–86)	(12–86)	(13–86)
Age (years)					
- estimated median	60	69	60	58	63
- range	(31-84)	(56-84)	(31-79)	(39-79)	(31-73)
Histology n (%)					
- High grade serous	99 (99)	10 (100)	89 (99)	68 (99)	21 (100)
- Endometroid	1 (1)	0	1 (1)	1 (1)	0
Line of therapy n (%)					
- 1st relapse	68 (68)	8 (80)	60 (67)	47 (68)	13 (62)
- 2nd relapse	18 (18)	1 (10)	17 (29)	13 (19)	4 (19)
- ≥3rd relapse	14 (14)	1 (10)	13 (14)	9 (13)	4 (19)
Family history n (%)					
- Negative	57 (57)	7 (70)	50 (56)	37 (54)	13 (62)
- Positive	43 (43)	3 (30)	40 (44)	32 (46)	8 (38)

from increased sensitivity to exogenous or endogenous mutagenic agents, defective mechanisms for DNA damage detection and repair, or both [1,2]. Genomic instability, characterized by an increased mutational capacity or mutation rate of the cellular genome, enables cancer cells to acquire a range of mutations, providing them with an advantage for more efficient adaptation to their environment and consequently promoting tumor growth and metastasis.

On the other hand, excessive accumulation of mutations can also cause DNA damage to such an extent that the cell can no longer function normally. This fact is exploited in triggering synthetic lethality. Synthetic lethality is a phenomenon in which the aberration of one gene product allows cell survival, while two simultaneous mutations in two gene products lead to cell death [3]. Cell death by synthetic lethality can also be achieved when a cell carries an inactivating mutation in one gene and the second gene product is inhibited by a pharmacological agent. Therefore, proteins involved in synthetic lethal interactions can serve as specific drug targets for cancer cell elimination.

An example of synthetic lethality as a form of cancer treatment is inhibition of poly(ADP - ribose) polymerase (PARP) protein activity in homologous recombination-deficient (HRD) tumors [4,5]. The idea is based on the fact that PARP inhibitors prevent single strand breaks (SSB) repair, while *BRCA1* and *BRCA2* are among the key proteins for repair of DNA double strand breaks (DSBs) through homologous recombination (HRR). HRR ensures a high rate of accurate repairs and restoration of the original DNA sequence [6]. In the case of non-functioning *BRCA* genes, HRR is replaced by more error-prone repair mechanisms such as non-homologous end joining (NHEJ) [7]. The accumulation of SSB and eventually DSB triggers apoptosis, leading to cell death. Indeed, PARP inhibitors gained clinical utility in 2014 when both European Medicines Agency (EMA) and United States Food and Drug Administration (FDA) approved the first PARP inhibitor, olaparib, for the maintenance therapy of advanced ovarian cancer patients with inactivating mutations in *BRCA1* or *BRCA2* genes [8,9]. Later different PARP inhibitors were approved in a variety of clinical scenarios in different cancer types (ovarian, breast, prostate and pancreatic cancer). In ovarian cancer, olaparib was EMA approved in 2019 for first line monotherapy in BRCA1/2 mutated patients (SOLO-1) [10] and in 2020 for first line in combination with bevacizumab in HRD patients (PAOLA-1) [11].

However, the question remains whether all inactivating mutations in *BRCA1/2* genes have the same impact in survival outcomes in patients on olaparib therapy. Therefore, the aim of our study was to evaluate the impact of different *BRCA1/2* mutations in survival outcomes as well as safety of olaparib maintenance monotherapy (OMT) in patients with relapsed *BRCA1* or *BRCA2* mutated ovarian cancer who responded to platinum-based chemotherapy, considering the location and type of the *BRCA1* or *BRCA2* mutation.

2. Patients and methods

2.1. Patients

Between 2015 and 2021, 100 patients with platinum-sensitive relapsed ovarian cancer (PSROC) and germline or tumor pathogenic/likely pathogenic variants (PV) in *BRCA1/2* genes were retrospectively enrolled (Table 1). All patients underwent genetic testing for *BRCA1/2* variants at the Institute of Oncology Ljubljana (IOL), Slovenia, following genetic counseling based on established clinical pathways and guidelines [12], and in compliance with the Oviedo Convention. Informed consent was obtained from all participants prior to study participation, conducted in accordance with the Declaration of Helsinki. The study received approval from the National Ethics Committee (permissions no. 100/05/15 and 0120–303/218/3) and the Institutional Ethics Committee (permissions no. ERID-KESOPKR/97 and ERID-EK/136), and was registered in the ISRCTN registry on 24 November 2015 (ISRCTN42408038).

2.2. PARPi (olaparib) therapy

Patients were initially treated with olaparib 2x400mg capsules (following the Study 19 protocol) from 2015 to 2019, and subsequently transitioned to 2x300mg tablets (following the SOLO-2 protocol) after 2019 [13,14]. Cross-over from capsules to tablets adhered to the olaparib label guidelines [15]. Treatment was administered on an outpatient basis with regular follow-ups every 4 weeks for the first 6 months, then every 4–8 weeks for two years, and subsequently every 12 weeks. Each follow-up included clinical and blood examinations, while treatment response was assessed every 12 weeks using abdominal CT and gynecologic US, and adverse events were documented per Common Terminology Criteria for Adverse Events (CTCAE) - ver 5.0 [16]. Response to treatment was evaluated based on Response Evaluation Criteria in Solid Tumors (RECIST) - ver 1.1 [17]. At the time of analysis, 30% of patients had received subsequent chemotherapy following disease progression on olaparib.

2.3. Categorization of pathogenic variants according to location in BRCA1/2

Pathogenic variants (PV) in *BRCA1/2* were categorized according to their location within functional domains based on ClinGen ENIGMA *BRCA1* and *BRCA2* Expert Panel Specifications, ACMG/AMP Variant Interpretation Guidelines Version 1.1.0, and additional criteria from Clark et al. and Zhang et al. [18–20]. PV affecting RNA and protein function were grouped into three categories (A, B, and C) based on their specific locations (Fig. 1, Table 2): Group A included *BRCA1* PV upstream of the

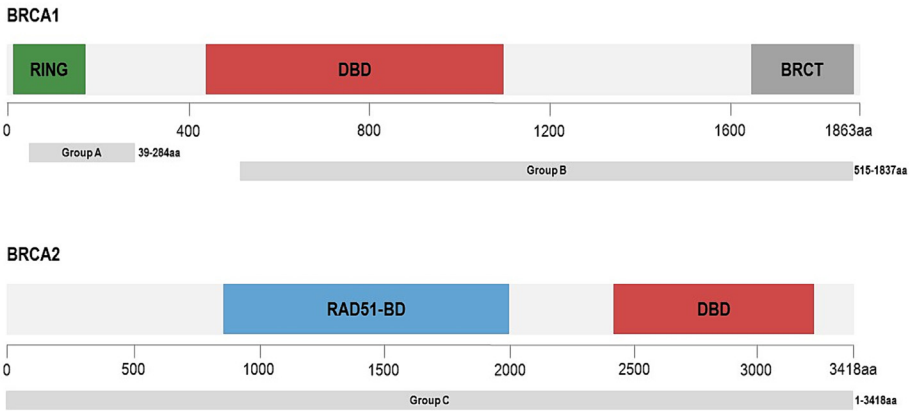


Fig. 1. Graphical presentation of *BRCA1* and *BRCA2* pathogenic variant subgroups and joined groups. DBD – DNA binding domain; RING – *BRCA1* Zn-finger RING domain; BRCT – *BRCA1* C-terminal (BRCT) domain; *RAD51*-BD – *RAD51*-binding domain; aa – amino acid.

DNA binding domain (DBD) (spanning amino acids 39 to 284), Group B encompassed PV within the DBD and downstream (amino acids 515 to 1837), and Group C comprised all *BRCA2* PV. Variants with unclear RNA effects were not categorized. *BRCA1* and *BRCA2* PV were further subdivided into nine subgroups (PV subgroups) based on gene location, variant type, and presumed RNA and protein effects (Table 2, Fig. 1). To minimize confounding factors, the analysis focused only on OMT patients with first or second relapse post-platinum therapy ($N = 86$). Ten patients who discontinued olaparib treatment for reasons other than disease progression or death (other causes for olaparib discontinuation - OCOD) were excluded, resulting in 76 patients (1st/2ndR-noOCOD group) included in the PFS comparison (Table 2).

2.4. DNA isolation

According to the established laboratory protocols genomic DNA was isolated from a whole blood sample using InnuPREP Master Blood Kit (Analytik Jena, Jena, Germany), and from an FFPE-tissue sample using GeneRead DNA FFPE Kit (Qiagen GmbH, Hilden, Germany) or MagMax DNA/RNA kit (ThermoFisher Scientific, Woodward, USA).

2.5. Germline and tumor genotyping

2.5.1. Genotyping for germline alterations

Blood DNA samples from all patients were subjected to targeted next-generation sequencing (NGS) using the Illumina MiSeqDx Sequencing System. The Nextera DNA Library Preparation Kit with either TruSight Cancer Panel or TruSight Hereditary Panel (Illumina, San Diego, USA) was employed, following our previously described protocol [21,22]. Additionally, bidirectional Sanger sequencing [23] and Multiplex Ligation dependent Probe Amplification (MLPA) using SALSA MLPA P002 *BRCA1* or P045 *BRCA2*/CHEK2 kits (MRC-Holland, Amsterdam, Netherlands) were performed according to established laboratory and manufacturer's protocols.

2.5.2. Tumor genotyping

Targeted NGS was performed on 34 selected tumor DNA samples using either the Illumina MiSeqDx or NextSeq 550 Sequencing System. Sequencing utilized the Targeted DNA Repair_2 Gene consortium panel kit – TSCA-BRCA kit (Illumina, San Diego, USA) in 9 cases, or the TruSight Tumor 170-DNA Library Preparation Kit (Illumina, San Diego, USA) in 25 cases, following manufacturer protocols. FFPE tumor tissue samples were prepared and assessed by a pathologist as previously described [24]. Bioinformatics analysis and variant classification followed established procedures [24,25].

2.5.3. Variants description and exon numbering

Pathogenic variants in *BRCA1* and *BRCA2* were described using HGVS nomenclature v15.11 with reference sequences NM_007294.3 for *BRCA1* and NM_000059.3 for *BRCA2*. Exons in *BRCA1* and *BRCA2* were numbered according to historical conventions as outlined in the ClinGen ENIGMA *BRCA1* and *BRCA2* Expert Panel Specifications, or systematically starting from 1 and counting numerically for each exon.

2.6. Statistical analysis

Progression-free survival (PFS) was defined as the duration from the initiation of olaparib treatment to objective disease progression on imaging (according to modified RECIST, version 1.1) or death from any cause [17]. Overall survival (OS) was defined as the duration from the initiation of olaparib treatment to death from any cause. The differences in PFS and OS between patient groups were assessed using the Kaplan-Meier method and log-rank tests. A safety analysis of adverse events (AEs) occurrence between the groups of patients was performed by Chi-square test. Statistical analysis was performed by IBM SPSS software ver. 25, with statistical significance defined as $p < 0.05$.

3. Results

At the Institute of Oncology Ljubljana, 100 patients with platinum-sensitive relapsed ovarian cancer (PSROC) and *BRCA1/2* gene pathogenic variants (PV) received OMT. Of these, 90 had germline PV (69 in *BRCA1* and 21 in *BRCA2*), and 10 had somatic PV (7 in *BRCA1* and 3 in *BRCA2*). Overall, 76 patients had *BRCA1* PV and 24 had *BRCA2* PV (see Supplementary Table 1).

3.1. Clinical characteristics, PFS and OS of all PSROC patients after the treatment with olaparib

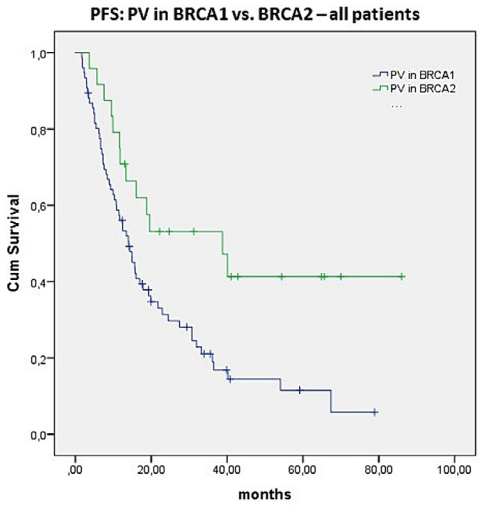
Table 1 presents the clinical characteristics of PSROC patients. Among them, 85 had abdominal relapse only, 8 had relapse outside the abdomen only, and 7 had both. Patients with germline *BRCA1/2* PV were significantly younger than those with somatic *BRCA1/2* PV (59 years vs. 69 years; $p = 0.001$).

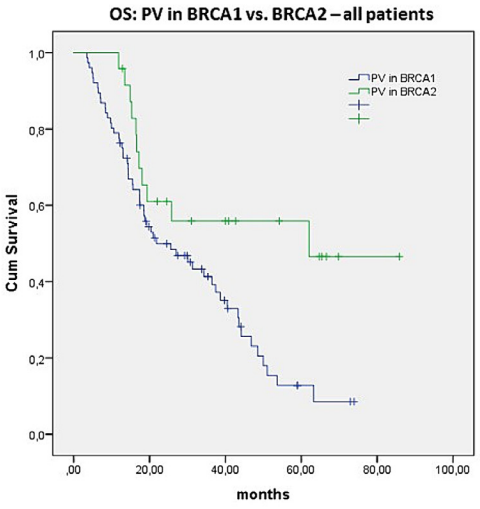
The estimated median PFS for all 100 patients was 15.6 months (range: 1.7 to 86 months). At the analysis time, 74 patients had disease progression or had died (Supplementary Fig. 1). The estimated median PFS did not significantly differ between patients with somatic *BRCA1/2* PV compared to those with germline *BRCA1/2* PV (19.3 vs. 14.9 months respectively, $p = 0.316$, Chi-Square = 1.006) (Supplementary Fig. 2). At the time of analysis, 60% of patients with somatic *BRCA1/2* PV and 75.6%

Table 2
Description of *BRCA1* and *BRCA2* groups and subgroups of pathogenic variants detected in all platinum-sensitive relapsed ovarian cancer patients.

Group	Sub-group	Description of subgroup of pathogenic variant according to functional domains (PV-subgroup)	PV in our cohort (AA -aminoacid no.)	Systematic exon numbering (historical exon numbering)[1]	No. of patients included in the study	No. of patients with 1st/2nd relapse	No. of patients with 1st and 2nd relapse without OCOD
NA	NA	frameshift or start loss variants in ***BRCA1* Zn-finger RING domain** before AA48:					
		- splice variant in *BRCA1* Zn-finger RING domain (AA1–101)	AA1–27	2 (2)	1	1	1
		- frameshift variant in *BRCA1* Zn-finger RING domain (AA1–101)	AA41	3 (3)	1	0	0
A	1	missense variants in *BRCA1* Zn-finger RING domain (AA1–101)	AA39–64	3–4	10	9	8
	2	splice variant in *BRCA1* Zn-finger RING domain (AA1–101)	AA44–71	4 (5)	1	0	0
	3	large in-frame deletion in *BRCA1* involving RING domain,	AA45-AA224	in-frame deletion of 4–9 (5–10)	11	8	7
	4	truncating variants in *BRCA1* after RING domain including *RB1* binding site (BS) (AA102–394)	AA282–284,	start of ex 10 (11)	13	11	11
B	5	truncating variants in *BRCA1* DNA binding domain (DBD) including RAD50/MRE11/NBS- BS, RAD51-BS and in NLS1/NLS2 (AA395–1092)	AA515–1006	sec. q of ex10 (11)	20	19	18
	6	truncating variants in *BRCA1* after DBD including PALB2 -BS and ATM phosphorylation sites (AA1093–1650)	AA1121–1453	last q of ex 10–12 (11—13)	10	10	7
	7	truncating or missense variant in BRCA1 in *BRCA1* C-terminal (BRCT) domain (AA1651–1863)	AA1712–1837	17–23 (18–24)	6	6	5
NA	NA	large deletion in *BRCA1* after RING and DBD domain	AA1112–1863	last q of ex 10–23 (11–24)	2	2	2
C	8	truncating variant in *BRCA2* including RAD51-BD and up to DBD (AA1–2402)	AA588–2133	10–11 (10—11)	17	15	13
	9	truncating variant in *BRCA2* in DBD (AA2403–3186)	AA2602–2984	17–23 (17–23)	7	4	3
		large deletion in *BRCA2*	AA2919–3418	22–27 (22–27)	1	1	1
	sum				100	86	76

OCOD - other cause for olaparib discontinuation.; BS – binding site; DBD- DNA binding domain; ex – exon; q – quartile; sec.-second; NA - not assigned - pathogenic variants that could not be assigned to any of group.– one frameshift and one splice PV before possible alternative start codon (unknown effect on RNA - whether it undergoes nonsense mediated decay or transcription from alternative start codon occurs, and two large deletions, with unclear effect on RNA).





	Median progression free survival (months)				Median overall survival (months)			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
1- PV in BRCA1	14.0	1.7	10.7	17.3	21.8	5.9	10.3	33.4
2- PV in BRCA2	38.8	14.3	10.7	66.9	62.0	36.6	0.000	133.8
Overall	15.6	1.7	12.3	18.9	27.0	7.0	13.2	40.8

Kaplan-Meier - Medians for Survival Time. Median follow up was 57 months.

Fig. 2. Progression free survival (PFS) and overall survival (OS) in all platinum sensitive relapsed ovarian cancer (PSROC) patients included in the study ($n = 100$) according to PV in BRCA1 or BRCA2.

with germline *BRCA1/2* PV had experienced disease progression or death.

Patients with *BRCA1* PV ($N = 76$) had a significantly shorter estimated median PFS compared to those with *BRCA2* PV ($N = 24$) (14.0 months vs. 38.8 months respectively, Chi-square = 7.255, $p = 0.007$). At the time of analysis, 61 patients (80%) with *BRCA1* PV and 13 (54%) with *BRCA2* PV had experienced disease progression or death (Fig. 2).

The estimated median OS for all patients was 27.0 months. At the analysis time, 66 patients (66%) had died (Supplementary Fig. 1). Median OS did not significantly differ between patients with somatic and germline *BRCA1/2* PV (not reached vs. 25.8 months; Chi-Square = 1.48, $p = 0.224$) (Supplementary Fig. 2). At the time of analysis, 4 patients (40%) with somatic *BRCA1/2* PV and 62 (68.9%) with germline *BRCA1/2* PV had died.

OS according to *BRCA1* or *BRCA2* PV is presented in Fig. 2. Patients with germline or somatic *BRCA1* PV had a significantly shorter estimated median OS (21.8 months) compared to those with germline or somatic *BRCA2* PV (62.0 months) (Chi-Square = 6.420, $p = 0.011$). At the time of the analysis, 55 patients (72%) with *BRCA1* PV had died compared to 11 (46%) with *BRCA2* PV.

3.2. PFS among PSROC patients with first and second relapse after platinum-based chemotherapy stratified by different groups of PV in BRCA1/2 genes

From the initial cohort of 100 PSROC patients, only those with first and second relapses after platinum-based chemotherapy were included ($N = 86$). Additionally, 10 patients, with 1st or 2nd relapse, who discontinued olaparib treatment for reasons other than disease progression or death (OCOD) were excluded. The remaining 76 patients (1st/2ndR-noOCOD group), categorized into groups A, B, and C, and various PV-subgroups, were included in the PFS comparison (Table 2 and Supplementary Table 1).

The estimated median PFS for the 76 patients in the 1st/2ndR-noOCOD group was 18.8 months (95% confidence interval 12.1–25.5 months) (see Supplementary Fig. 3 A).

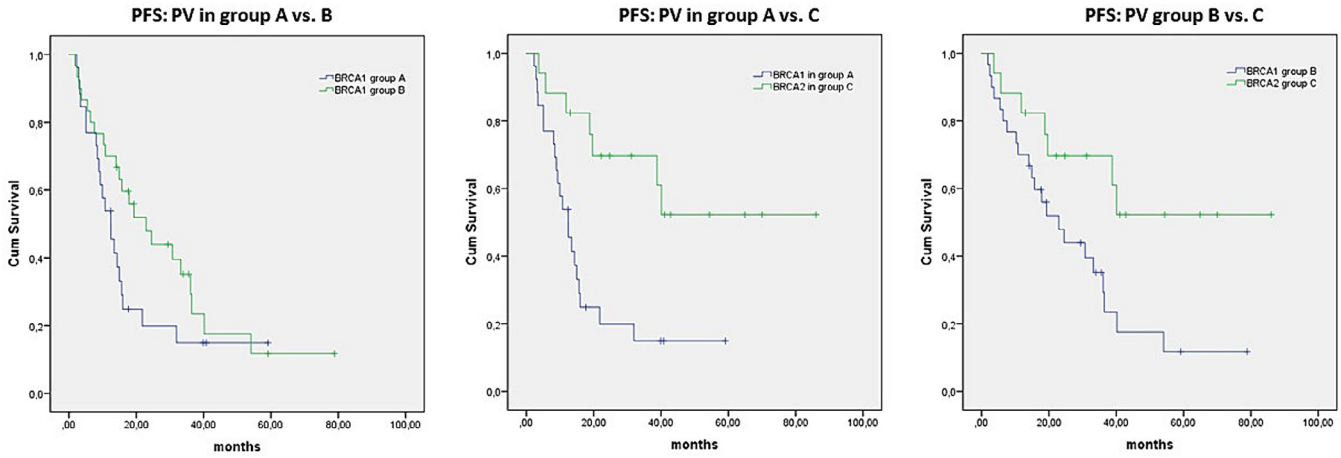
Among the 1st/2ndR-noOCOD group, the estimated median PFS was significantly shorter for 59 patients with *BRCA1* PV compared to 17 patients with *BRCA2* PV (14.9 months vs. not reached, Chi Square 8.408, $p = 0.004$) (Supplementary Fig. 3B).

The estimated median PFS of patients categorized into A, B, and C groups is detailed in Table 3. Median PFS was shorter in group A

Table 3
Estimated median PFS in the groups A, B, and C among 76 platinum sensitive ovarian cancer patients with first and second relapse (after platinum-based chemotherapy), excluding those who discontinued treatment for reasons other than disease progression or death (1st/2nd R-noOCOD group).

Group	Median Estimate (month)	Std. error (month)	95% Confidence Interval		No. of patients included*	No. of events	Live patients without progression	
			Lower bound	Upper bound			No.	Percent
A	12,4	2,1	8,2	16,6	26	21	5	19.2%
B	23.0	5.3	12.7	33.2	30	22	8	26.7%
C	NR	.	.	.	17	7	10	58.8%

A – BRCA1 PV upstream of BRCA1 DNA binding domain (DBD) (AA1-AA394); B – BRCA1 PV in DBD and downstream (AA395–1863); C – PV in BRCA2; NR-not reached. *Three patients from 1st/2nd R-noOCOD group were not assigned to any of A, B, C group and were not included in this table statistics. Event = progression of disease or death. Median PFS in group A was shorter compared to group B but the difference was not statistically significant (Chi Square = 2.522, p = 0.112). Patients in group C had a statistically significantly longer PFS (not reached) compared to those in groups A (Chi Square = 9.639, p = 0.002) and B (Chi Square = 5.470, p = 0.019).



	Test of equality of survival distributions for the A and B.			Test of equality of survival distributions for the A and C.			Test of equality of survival distributions for the B and C.		
	Chi-Square	df	Sig.	Chi-Square	df	Sig.	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	2.522	1	0.112	9.639	1	0.002	5.470	1	0.019

A – BRCA1 PV upstream of BRCA1 DNA binding domain (DBD) (AA1-AA394);
B – BRCA1 PV in DBD and downstream (AA395-1863);
C – PV in BRCA2; 8- truncating variant in BRCA2 including RAD51-BD and up to DNA binding domain (DBD) (AA1-2402);
Event= progression of disease or death.
Median follow up was 44 months.

Fig. 3. Progression free survival (PFS) in platinum sensitive ovarian cancer patients with first and *sec*ond relapse (after platinum-based chemotherapy) and excluding those who discontinued treatment for reasons other than disease progression or death (1st/2ndR-noOCOD group). Comparison between groups (A, B, C). Three patients from 1st/2ndR-noOCOD group were not assigned to any of A, B, C group and were not included in the statistics.

(12.4 months) compared to group B (23.0 months), though not statistically significant (Chi Square = 2.522, $p = 0.112$). Patients in group C had a significantly longer PFS (not reached) compared to those in groups A (Chi Square = 9.639, $p = 0.002$) and B (Chi Square = 5.470, $p = 0.019$) (Table 3, Fig. 3).

The estimated median PFS of patients with 1st/2nd relapse without OCOD in *BRCA1/2* subgroups is provided in Supplementary Table 2. Across all subgroups, patients with in-frame deletions in the *BRCA1* RING domain (subgroup 3) had a significantly shorter PFS compared to those with PV in *BRCA1* DBD or downstream of DBD (subgroups 5 to 9) (Supplementary Table 3).

Patients in subgroups combining missense PV and large in-frame deletions in the *BRCA1* RING domain (subgroups 1 and 3) demonstrated a significantly shorter estimated median PFS (12.4 months, 95% confidence interval 9.2–15.7 months) compared to patients in subgroups with PV in *BRCA1* DBD or downstream of DBD (subgroups 5, 6, and 7) (23.0 months, 95% confidence interval 12,7–33.2 months) (Chi Square = 3.992, $p = 0.046$).

3.3. Adverse events in all PSROC patients after olaparib treatment

Adverse events (AE) related to olaparib treatment are detailed in Supplementary Table 4. Common events included anemia (73%), increased creatinine levels (65%), nausea (63%), and fatigue (57%). Most events were mild or moderate (grade 1 or 2), with no significant differences between germline and somatic *BRCA1/2* PV.

Overall, 21% of patients experienced serious adverse events (SAE) (grade 3 or 4), such as grade 3 anemia (10%), grade 3 neutropenia (5%), grade 4 neutropenia (1%), and grade 3 nausea/vomiting (5%). No SAE-related deaths occurred, and differences between germline and somatic *BRCA1/2* PV were not significant.

Details on treatment modifications due to adverse events are in Supplementary Table 5. Overall 28% of patients had olaparib dose reductions, with no significant differences by PV status (germline vs. somatic). By analysis time, 75% of patients discontinued olaparib; notably, more with germline *BRCA1* PV (84%) compared to germline *BRCA2* (52%, $p = 0.015$).

Reasons for olaparib discontinuation differed significantly by PV status ($p = 0.026$). Among patients with somatic *BRCA1/2* PV, all discontinued due to disease progression, none due to SAE. Among patients with germline PV, 78% discontinued due to disease progression. All patients who discontinued olaparib due to SAE had germline *BRCA1/2* PV.

4. Discussion

PARP inhibitors (PARPi) have shown greater efficacy in cancer cases with altered homologous recombination genes [14,26–28]. However, questions remain regarding the efficacy of PARPi and the relationship between survival outcomes and type/location of mutations (PV) in *BRCA1/2* genes, including germline and somatic mutations, large deletions, insertions, frameshift, missense or nonsense PV. Our study aimed to evaluate the impact of different *BRCA1/2* PV in survival outcomes in patients receiving OMT and safety of OMT in relapsed *BRCA1/2* mutated ovarian cancer patients who responded to platinum-based chemotherapy, considering the *BRCA1/2* PV location and type. The study was limited to clinical observations and did not investigate the molecular mechanisms underlying PARP inhibitors' action.

Our first olaparib treatments began in November 2015 with 50 mg capsules (Study 19 protocol) [13], and transitioned to 150 mg tablets by the end of 2019 (SOLO 2 protocol) [14]. This transition from capsules to tablets was conducted based on an adaptive trial, Study 24 [15]. Estimated median PFS for patients treated with olaparib maintenance in relapsed *BRCA1/2* mutated ovarian cancer was 15.6 months, longer than Study 19's 11.2 months but shorter than SOLO-2s 19.1 months [14]. These differences can be attributed to the varying proportions of *BRCA2* PV among the studies (Study 19: 20%; SOLO2: 30%; our study: 24%).

Limited data exists comparing the predictive value of somatic versus germline *BRCA1/2* PV in advanced ovarian cancer patients treated with PARPi [12,29–31]. Until 2019, PARPi treatment was based on positive germline *BRCA1/2* PV, leading to a low number of patients with somatic PV in studies. Consequently, until 2019, ovarian cancer patients in our hospital were only tested for germline PV. For this reason, 90% of patients in our study had germline PV, and 10% had somatic PV, making it difficult to draw definitive conclusions about OMT survival outcomes between germline and somatic *BRCA1/2* PV.

Comparison of PFS between patients with germline and somatic *BRCA1/2* PV showed no significant differences (14.3 months vs. 19.3 months; $p = 0.294$), aligning with Study 19 findings, which reported similar hazard ratios for germline and somatic BRCA PV [32]. Similar results were observed in the NOVA trial with niraparib and ARIEL 3 trial with rucaparib [29,31]. However, significant differences in PFS were noted between *BRCA1* and *BRCA2* PV (germline or somatic). At analysis time, 80% of patients with *BRCA1* PV had disease progression or had died, compared to 54% of *BRCA2* PV patients, resulting in a significantly shorter median PFS for patients with *BRCA1* PV ($p = 0.007$). This aligns with prior data showing better responses to chemotherapy and better survival for patients with *BRCA2* mutated ovarian cancer [33].

Our study's OS was inferior to that reported in Study 19 and SOLO-2 trials [13,14,34]. Possible reasons for this discrepancy are that in our study only 30% of patients received subsequent chemotherapy after disease progression compared to 61% in Study 19 and 40% in SOLO-2, and a shorter follow-up period in our study (57 months) compared to Study 19 (78 months) and SOLO-2 (65 months).

Similarly to PFS, there was no statistically significant difference in OS between patients with germline and somatic *BRCA1/2* PV. This lack of significance was likely due to the small number of patients with somatic *BRCA1/2* PV. However, patients with *BRCA1* PV had significantly shorter median OS compared to those with *BRCA2* PV (21.8 vs. 62.0 months; $p = 0.011$). This is consistent with higher 5-year survival rates for patients with *BRCA2* PV after platinum based chemotherapy, which is indicator of HRD and response to olaparib treatment [33]. The lower OS in our study likely results from the high proportion of patients with *BRCA1* PV (76%), reflecting the higher frequency of *BRCA1* PV in the Slovenian population compared to Western Europe and the USA [35,36].

The most common AE of OMT were anemia, creatinine level increase, nausea, and fatigue, mostly mild or moderate. Severe adverse events (SAE) affected 21% of patients, including anemia (10%), neutropenia (6%), and vomiting (5%), with no deaths attributed to SAE. These results align with those from Study-19 and SOLO-2 trials [13,14]. There were no significant differences in SAE between patients with germline and somatic *BRCA1/2* PV, nor in olaparib dose reductions, which occurred in 28% of patients. However, significant differences were noted in OMT discontinuation between germline and somatic *BRCA1/2* PV ($p = 0.026$), with all six patients with somatic PV discontinuing OMT due to disease progression and none due to AE, compared to 78% of patients with germline PV discontinuing OMT due to disease progression and 15% due to AE.

Our study further investigated the relationship between OMT survival outcome and the type/location of *BRCA1/2* PV, monitoring PFS among three groups (A, B, and C) regardless of germline or somatic origin. To minimize potential influences on PFS, only patients with 1st and 2nd relapse after platinum based therapy were analyzed, excluding those who discontinued OMT for reasons other than progression (1st/2ndR-noOCOD group).

Shortest PFS was observed in group A, subgroups including missense mutations, large in-frame deletions in the RING domain and the truncating variants at very beginning of exon 11 (upstream DBD) of *BRCA1*. For PV in subgroups 1 and 3, it is assumed, that with the exception of the RING domain, the rest of the protein is preserved. This is consistent with mouse model studies showing less sensitivity to PARPi for *BRCA1* RING domain mutations [37] and in vitro studies indicating PARPi resistance in cells with *BRCA1* RING domain mutations [38,39].

Patients with truncating BRCA1 PV at the start of exon 11 (subgroup 4) had a short PFS, similar to those in subgroups 1 and 3. In contrast, patients with PV in the BRCA1 DBD or downstream (subgroups 5, 6, 7) had significantly longer PFS than those with missense or in-frame PV in the BRCA1 RING domain (subgroups 1 and 3). Notably, our study found better median PFS for subgroup 6 compared to subgroups 5 and 7, though not statistically significant (Supplementary Tables 2 and 3).

Longest PFS, in patients receiving OMT, was observed in patients with PV in *BRCA2*. Patients with *BRCA2* PV with mutations in the gene's first half, specifically in the RAD51-BD region upstream of the DBD (subgroup 8), had shorter PFS compared to those with PV in the DBD region (subgroup 9). No disease progression was observed in the latter group during the observation period. To our knowledge no such data had been published yet for olaparib maintenance monotherapy in any line of ovarian cancer treatment. PAOLA-1 trial results showed the association of location of *BRCA1* and *BRCA2* mutations with survival outcomes, but in different treatment setting – 1st line treatment and maintenance treatment with olaparib/bevacizumab in combination, showing the best survival outcome in patients with RAD51-BD and DBD *BRCA2* PV, while the clinical benefit in comparison to placebo group was greatest in patients with PV in DBD of BRCA1 [40].

Our study is among the first to investigate the role of *BRCA1/2* mutation type and location in predicting olaparib maintenance monotherapy treatment outcomes for relapsed ovarian cancer patients. Limitations of our study are, its retrospective nature and relatively small cohort. Since the comparison of survival outcomes in our study could not be compared to control group (placebo), the clinical benefit of OMT in PSROC patients could not be assessed.

In conclusion, this study identifies specific mutation subgroups that may influence survival outcomes, suggesting potential avenues for personalized therapy. PSROC patients receiving OMT with *BRCA1* PV had significantly shorter PFS and OS compared to those with *BRCA2* PV. AE associated with OMT were not significantly different between patients with germline and somatic PV. Nonetheless, the study's limitations, underscore the necessity for further research to validate these findings and optimize treatment strategies for ovarian cancer patients with diverse *BRCA1/2* mutations.

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Availability of data and materials

Data supporting reported results are available upon request.

Ethics approval and consent to participate

The study was approved by National Ethics Committee (permission no. 100/05/15 on 27 July 2015 and permission no. 0120–303/218/3 on 13 June 2018) and the Institutional Ethics Committee of the Institute of Oncology Ljubljana (permission no. ERID-KESOPKR/97 on 17 September 2015 and permission no. ERID-EK/136 on 21 May 2018), and registered at ISRCTN registry on 24 November 2015 (ISRCTN42408038). Individual patient consent was waived for this study as it was a retrospective study, the research involved no risk to the subjects, and the institutional informed consent forms for treatment included: consent for the use of patient's data, materials, and/or test results for research purposes.

Patient consent for publication

Not applicable.

CRediT authorship contribution statement

Erik Škof: Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Vida Stegel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Vita Šetrajčič Dragoš:** Writing – review & editing, Visualization, Resources, Investigation, Formal analysis, Data curation. **Ana Blatnik:** Writing – review & editing, Resources, Investigation, Formal analysis. **Brigita Gregorič:** Writing – review & editing, Resources, Investigation, Formal analysis. **Petra Škerl:** Writing – review & editing, Resources, Investigation, Formal analysis. **Gašper Klančar:** Writing – review & editing, Resources, Investigation, Formal analysis. **Anja Zagožen Klasinc:** Writing – review & editing, Resources, Investigation, Formal analysis. **Alenka Bombač:** Writing – review & editing, Resources, Investigation, Formal analysis. **Mateja Krajc:** Writing – review & editing, Resources, Investigation, Formal analysis. **Srdjan Novaković:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2024.08.012.

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