Pembrolizumab in combination with bevacizumab and oral cyclophosphamide in heavily pre-treated platinum-resistant ovarian cancer

Angeliki Andrikopoulou, Michalis Llontos, Efthymia Skafida, Konstantinos Koutsoukos, Kleoni Apostolidou, Maria Kaparelou, Angeliki Rouvalis, Garyfallia Bletsa, Meletios-Athanasiou Dimopoulos, Flora Zagouri

ABSTRACT
Objective Immune checkpoint inhibitors have been widely implemented in the treatment of solid tumors. Combinations of immune checkpoint inhibitors with chemotherapy, anti-vascular endothelial growth factor (VEGF) compounds, and poly-adenosine diphosphate-ribose polymerase inhibitors (PARP) are under evaluation in ovarian cancer. We aim to explore the efficacy of pembrolizumab in combination with bevacizumab and oral cyclophosphamide in patients with recurrent epithelial ovarian cancer.

Methods This was a retrospective study of all patients who received pembrolizumab in combination with bevacizumab and oral cyclophosphamide for recurrent platinum-resistant heavily pre-treated ovarian cancer in the Oncology Unit of Alexandra University Hospital from January 2021 to July 2022.

Results Median age at diagnosis was 56 years (SD 9.2; range 37–72). All patients were diagnosed with high-grade serous ovarian carcinoma. Initial disease stage was International Federation of Gynecology and Obstetrics (FIGO) IIIC in most cases (11/15, 73%). Patients were heavily pre-treated with a median of six (range 4–9) prior lines of systemic therapy. All patients experienced disease progression on first-line platinum-based chemotherapy, and median progression-free survival on first-line treatment was 22 months (95% CI 10.6 to 33.4). Patients received a median of four cycles of pembrolizumab in combination with bevacizumab and cyclophosphamide (range 3–20). Overall response rate was 13% (2/15) and disease control rate was 33% (5/15) with two patients achieving partial response and three patients achieving stable disease. Median progression-free survival was 3.5 months (95% CI 1.3 to 5.7) and the 6-month progression-free survival rate was 20%. Treatment was well tolerated with no dose-limiting toxicities.

Conclusion We showed that the combination of pembrolizumab with bevacizumab and oral cyclophosphamide is an effective alternative in heavily pre-treated patients with ovarian cancer who have otherwise limited treatment options.

INTRODUCTION
Ovarian cancer is the eighth most common malignancy in women and the seventh most common cause of cancer-related mortality, accounting for 207,252 deaths worldwide in 2020.1 Epithelial ovarian cancer comprises the majority of malignant ovarian neoplasms (about 90%). Although the treatment landscape of ovarian cancer has changed through the years, prognosis remains poor. Around two-thirds of ovarian cancer patients are diagnosed at an advanced stage (III/IV) while 5-year survival is as low as 39% for stage III epithelial ovarian cancer and 17% for stage IV epithelial ovarian cancer. Novel treatment strategies have extended the survival rates; however, most patients (65–80%) recur within the first 5 years after first-line chemotherapy. Response rates to subsequent chemotherapy remain low, especially in the platinum-resistant population who relapse within 6 months after first-line therapy (15%).2 Recently, the AURELIA study established the combination of bevacizumab with chemotherapy by demonstrating an almost doubling of progression-free survival (3.4 vs...
Immune checkpoint inhibitors have been widely implemented in solid tumors. Anti-programmed death 1 (PD1)/programmed cell death ligand 1 (PD-L1) compounds demonstrated only modest activity as monotherapy in advanced recurrent ovarian cancer.\(^{8-10}\) The KEYNOTE-100 Phase II study demonstrated a median progression-free survival of 2.1 months and an overall response rate of 8% to pembrolizumab monotherapy in patients with advanced recurrent ovarian carcinoma.\(^{8}\) Accordingly, the JAVELIN study reported an overall response rate of 9.6% to treatment with avelumab in heavily pre-treated ovarian cancer patients who had received a median of three prior lines of treatment, while the overall response rate was 15% for nivolumab.\(^{9,10}\) Apart from the platinum-resistant setting, the ATALANTE trial explored the addition of immunotherapy to platinum-based chemotherapy and anti-vascular endothelial growth factor (VEGF) treatment at platinum-sensitive relapse,\(^{11}\) but again, no significant prolongation of progression-free survival was noted. To enhance the anti-tumor effect of immunotherapy in ovarian cancer, multiple studies were designed to explore combinations of immune checkpoint inhibitors with chemotherapy (JAVELIN 200, IMAgyn050), anti-VEGF compounds (GOG3015/ENGOT OV39, NCT02873962, EORTC-1508), and PARP inhibitors (ATHENA GOG3020/ENGOT Ov45, KEYNOTE-162, MEDIOLA). Although these combinations appear promising, especially in heavily pre-treated patients, neither immune checkpoint inhibitors as monotherapy nor in combination have yet been approved.

The rationale for immunotherapy administration in ovarian carcinoma is based on the immunogenicity and the presence of tumor-infiltrating T lymphocytes (TILs). In 2003, Zhang et al associated the presence of intratumoral T cells with a 3.9-fold increase in progression-free survival and a 2.8-fold increase in overall survival compared with tumors that contained no T cells.\(^{12}\) Immune checkpoint inhibitors could enhance tumor infiltration with immune cells and remove immunosuppressive aspects of the ovarian tumor microenvironment. However, epithelial ovarian cancer has traditionally been considered scarcely immunogenic and studies evaluating immune checkpoint inhibitors in platinum-resistant ovarian cancer report contradicting results.\(^{13,14}\) The addition of anti-VEGF treatment to immunotherapy could prove to be more effective than either treatment regimen alone. Indeed, the ovarian tumor microenvironment consists of different types of stromal cells embedded in the omental extracellular matrix, such as myeloid-derived suppressor cells, tumor-associated macrophages, adipocytes, cancer-activated fibroblasts, and resident and infiltrating immune cells, including regulatory T cells.\(^{15}\) VEGF and other angiogenic factors like angiopoietin-2 that seep from the tumor microenvironment facilitate the extravasation and dissemination of cancer cells.\(^{16}\) Apart from enabling metastasis, VEGF enhances the suppressive function of regulatory T cells, inhibits the antigen presentation by dendritic cells and consequently the activation of cytotoxic T lymphocytes.\(^{16}\) Overall, the consequence of vascular abnormality is a compromised cytotoxic T lymphocyte-mediated anti-cancer immune response and an immunosuppressive tumor microenvironment. Of note, PD-1/PD-L1 pathway is often upregulated both on tumor cells and on tumor-infiltrating cytotoxic T lymphocytes, rendering them dysfunctional or ‘exhausted’. These interactions of PD-1/PD-L1 and VEGF pathways provide the rationale for combining anti-VEGF regimens with immune checkpoint inhibitors.

A phase II study by Zsiros et al was recently published, exploring the efficacy of pembrolizumab combined with bevacizumab and cyclophosphamide in recurrent ovarian cancer.\(^{17}\) We have applied this treatment regimen in our institution in heavily pre-treated patients with platinum-resistant recurrent ovarian carcinoma. Herein, we retrospectively report the efficacy data of this combination in a real-world setting.

METHODS

Eligibility criteria

We retrospectively identified all patients with advanced/metastatic high-grade serous ovarian carcinoma treated with the combination of pembrolizumab with bevacizumab and oral cyclophosphamide in the Oncology Unit of Clinical Therapeutics Department of University of Athens in Alexandra General Hospital from January 2021 to July 2022. The administration of this treatment regimen was approved by the Greek National Organization for Healthcare Services. The study has been performed in accordance with the 1964 Helsinki Declaration and has been approved by the Institutional Review Board of Alexandra University Hospital. Written informed consent was obtained from all patients included in this study. Patients received intravenous pembrolizumab at a dose of 200 mg, intravenous bevacizumab at a dose of 7.5 mg/kg every 3 weeks, and 50 mg oral cyclophosphamide once daily during the treatment cycle until disease progression or unacceptable toxicity.

Clinical information

Medical records of patients with high-grade serous ovarian carcinoma who were treated with this combination were retrospectively collected. Clinicopathological characteristics were extracted from the patients’ files including: age at diagnosis, International Federation of Gynecology and Obstetrics (FIGO) stage at diagnosis, histological subtype, grade, debulking status, performance status (ECOG score), progression-free survival on first-line chemotherapy, number of previous chemotherapy lines, type of maintenance treatments received, BRCA mutation status, number of pembrolizumab/bevacizumab/cyclophosphamide cycles administered, best response to pembrolizumab/bevacizumab/cyclophosphamide, disease progression, progression-free survival on pembrolizumab/bevacizumab/cyclophosphamide, and overall survival. Disease progression was evaluated by treating physicians based on imaging assessment with computerized tomography scans by response evaluation criteria in solid tumors version 1.1 criteria. Optimal debulking was defined as maximum residual tumor <1 cm in diameter after debulking surgery. Patients who had not experienced disease progression at the time of database closure (1 August 2022) were censored at the date of last follow-up.
Statistics
Descriptive statistics were used to assess clinicopathological parameters of the patients. Progression-free survival was calculated from the initiation of treatment with pembrolizumab, bevacizumab, and cyclophosphamide until disease progression or last follow-up. Overall survival was calculated from the initiation of treatment until death or last follow-up. Statistical analysis was performed with SPSS 24.0 statistical software. In accordance with the journal’s guidelines, we will provide our data for independent analysis by a team selected by the editorial board, for the purposes of additional data analysis, or for the reproducibility of this study in other centers if requested.

RESULTS
Baseline characteristics
A total of 15 patients were evaluated. Median age at disease diagnosis was 56 years (SD 9.2; range 37–72) (Table 1). All patients were diagnosed with high-grade serous ovarian carcinoma. Initial disease stage was FIGO IIIC in eleven cases (73%) and IIIB, IIC, IC, and IVB in one case each (7%). The majority of patients underwent primary debulking (80%) except for three cases (20%) where interval debulking surgery after neoadjuvant chemotherapy was performed. Debulking was optimal (absence of residual disease or residual disease >1 cm) in two-thirds of cases (10/15, 67%). All cases had been previously analysed for the presence of BRCA1/2 mutations. BRCA1 mutations were reported in four cases (27%), a somatic mutation in two cases, and germline mutations in the other two cases. Microsatellite instability was evaluated in three cases only, and all of these tumors were microsatellite stable. Patients were heavily pre-treated with a median of six (range 4–9) prior lines of systemic therapy. Prior bevacizumab and PARP inhibitor therapy was received by 73% (11/15) and 87% (13/15) of patients, respectively. All patients have experienced disease progression on first-line platinum-based chemotherapy and median progression-free survival was 22 months (95% CI 10.6 to 33.4) on first-line chemotherapy (online supplemental figure 1). Two patients had primary platinum-resistant disease and progressed within 6 months after the completion of first-line chemotherapy. A median of three (range 1–6) prior lines of chemotherapy were administered after platinum resistance was determined. Eight patients have died since the beginning of the study. Median overall survival was 85 months (95% CI 49.1 to 120.9) (online supplemental figure 2).

Efficacy
Patients received a median of four cycles of pembrolizumab in combination with cyclophosphamide and bevacizumab (range 3–20). Best response was partial response in two cases, stable disease in three cases, and progressive disease in the remaining cases (10/15, 67%) (Table 2). Overall response rate was 13% (2/15) while disease control rate was 33% (5/15). Median progression-free survival with pembrolizumab, cyclophosphamide, and bevacizumab was 3.5 months (95% CI 1.3 to 5.7) and 6-month progression-free survival rate 20% (Figure 1). Of note, duration of response was longer than 1 year in the two patients who achieved partial response (14 months and 18 months). Both patients were BRCA1/2 wild-type and in one microsatellite instability was stable. Interestingly, patients with longer overall survival also demonstrated a longer progression-free survival with pembrolizumab, cyclophosphamide, and bevacizumab.

DISCUSSION
Summary of main results
We found that the combination of pembrolizumab, oral cyclophosphamide, and bevacizumab offered a progression-free survival of...
3.5 months (95% CI 1.3 to 5.7), an overall response rate of 13%, and a disease control rate of 33% in heavily pre-treated ovarian cancer patients who had received a median of six prior lines of chemotherapy. Interestingly, the patients who responded to treatment showed a durable response of over 1 year (14 months and 18 months) that is quite unlikely in this chemoresistant population.

Results in the context of published literature
Recently, a phase II trial of this combination published by Zsiros et al introduced this treatment regimen in the recurrent platinum-resistant setting.17 The authors reported an overall response rate of 47.5% (19/40), with three complete responses (3/40, 7.5%) and a progression-free survival of 10.0 months (90% CI 6.5 to 17.4). However, the study by Zsiros et al also enrolled patients with platinum-sensitive disease (10/40, 25%) and the mean number of prior lines of chemotherapy was 3.4 for all patients.17 In our study, all patients were platinum-resistant and more heavily pre-treated with a median of six prior lines of treatment. In accordance with our results, median progression-free survival was 7.6 months (90% CI 5.7 to 10.3) in patients with platinum-resistant disease in the phase II trial.17 These survival rates suggest an encouraging alternative for this population for whom therapeutic alternatives are limited.

Multiple studies have been designed to explore the combination of immune checkpoint inhibitors with chemotherapeutic regimens in ovarian carcinoma. Liao et al evaluated the combination of pembrolizumab with low-dose carboplatin in platinum-resistant ovarian cancer.18 This study demonstrated clinical efficacy in heavily pre-treated patients with a median progression-free survival of 4.6 months (95% CI 4.3 to 5.0). A total of 10.3% (95% CI 2.2% to 27.4%) of patients had partial response and 51.7% (95% CI 32.5% to 70.6%) had stable disease.18 The TOPACIO/Keynote-162 study explored the combination of pembrolizumab with the PARP inhibitor niraparib in patients with platinum-resistant/refractory recurrent ovarian carcinoma regardless of BRCA status.19 Median progression-free survival was 3.4 months (95% CI 2.1 to 5.1) and overall response rate was 18% (90% CI 11% to 29%).19 Lee et al reported an overall response rate of 26.1% and a median progression-free survival of 5.6 months in platinum-resistant patients treated with pembrolizumab and pegylated liposomal doxorubicin.20 NRG-GY003 phase II study investigated nivolumab as monotherapy or in combination with ipilimumab in 100 platinum-resistant ovarian cancer patients.21 Although less tolerated, the combination of nivolumab and ipilimumab resulted in increased overall response rate (31.4% vs 12.2%), longer progression-free survival (3.9 vs 2.0 months), and longer overall survival (28.1 vs 21.8 months). In accordance with these studies, the combination of pembrolizumab in combination with bevacizumab and oral cyclophosphamide showed promising activity in a heavily pre-treated population.

Only four patients in our study harbored somatic BRCA1 mutations, therefore, a correlation between BRCA status and response to immunotherapy cannot be safely extracted. There is evidence that BRCA-mutated ovarian cancer is associated with higher PD-L1 expression and higher tumor mutational burden. Indeed, BRCA1/2-mutated tumors demonstrated significantly higher neoantigen loads than the homologous recombination, regarding proficient tumors (51.0 vs 37.5; p=0.001), higher number of CD3+TILs, and higher PD-1 and PD-L1 expression in intraepithelial and peritumoral lymphocytes.22 These characteristics may render BRCA-mutated

![Kaplan-Meier curve illustrating progression-free survival (PFS) on treatment with pembrolizumab, cyclophosphamide, and bevacizumab.](image-url)
tumors more susceptible to immunotherapy. However, the IMagyn050 and JAVELIN 100 trials did not confirm this association. Further subanalysis of studies with immune checkpoint inhibitors should evaluate the contribution of BRCA mutations in sensitivity to immunotherapy.

**Strengths and weaknesses**

Our study has some limitations. First, all of the patients enrolled were heavily pre-treated, platinum-resistant, and had received all of the approved standard-of-care treatment lines, and thus represent a group that may be chemoresistant. Response rates are extremely low in this population and achievement of disease control is quite unlikely. Furthermore, patients with platinum-resistant recurrent ovarian carcinoma were considered eligible irrespective of PD-L1 or microsatellite stability status. KEYNOTE-028 showed that PD-L1-positive patients have an improved response rate of 11.5% (95% CI 2.4% to 30.2%) and a median progression-free survival of 1.9 months (95% CI 1.8 to 3.5). KEYNOTE-100 confirmed this increased response rate in patients with PD-L1, with a combined positive score (CPS) ≥10 (overall response rate 17.1% (CPS ≥10) vs 5% CPS<1 vs 10.2% CPS ≥1–10). It is possible that response to immunotherapy may differ between patients with different PD-L1 or microsatellite stability status. In addition, this study was a retrospective single-institutional study lacking a comparison arm to compare the efficacy of this new combination versus standard-of-care regimens. Finally, this novel combination has been recently introduced, so the sample size is relatively limited. More patients are needed to extract a safe conclusion. Currently, data on the use of pembrolizumab, bevacizumab, and cyclophosphamide in combination have emerged only from phase II studies. Phase III trials should be designed to evaluate the clinical efficacy of this combination compared with the standard-of-care treatment regimens in heavily pre-treated recurrent ovarian carcinoma.

**Implications for practice and future research**

Larger case series that include information regarding BRCA mutation and homologous recombination deficiency profile are needed to identify the ideal candidates for immunotherapy. Our findings suggest that immunotherapy combined with other agents like anti-VEGF and immunomodulatory compounds can result in durable clinical benefit even in heavily pre-treated patients who are not expected to respond to subsequent lines of treatment. Other combinations under evaluation in platinum-resistant recurrent ovarian cancer include durvalumab plus pegylated liposomal doxorubicin, nivolumab with bevacizumab and rucaparib (NCT02873962), weekly pacitaxel with pembrolizumab (NCT02440425), pembrolizumab with bevacizumab and pegylated liposomal doxorubicin (PEMBOV, NCT03596281). The combination of pembrolizumab with bevacizumab and oral cyclophosphamide should also be evaluated in prospective phase III studies.

**CONCLUSION**

This study demonstrates that the combination of pembrolizumab with bevacizumab and oral cyclophosphamide is an effective alternative in heavily pre-treated ovarian cancer patients. Phase III trials should be designed to evaluate this combination prospectively in recurrent platinum-resistant ovarian cancer.
Original research

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