Gynecologic Oncology Reports 53 (2024) 101374

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Pembrolizumab with bevacizumab and cyclophosphamide for the treatment of recurrent ovarian clear cell carcinoma: A case series

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ARTICLE INFO

Keywords:
Clear cell ovarian cancer
Immunotherapy
ARID1A
Recurrent ovarian cancer
Fallopian tube carcinoma
Peritoneal carcinoma

ABSTRACT

Introduction: Treatment for recurrent ovarian clear cell carcinoma (OCCC) is clinically challenging as response rates to traditional chemotherapy are low, and recurrence rates are high. Immunotherapy has shown promise for this ovarian cancer (OC) subtype, and tumor molecular testing allows for the identification of a patient population that might benefit most from this treatment. We describe the clinical course and somatic genomic testing of 4 patients who received pembrolizumab for recurrent OCCC concurrent with a combination of bevacizumab and/or cyclophosphamide.

Methods: All patients with OCCC treated with immune checkpoint inhibitors (ICI) within a single health system between 2018 and 2023 (excluding those on clinical trials) were identified via retrospective chart review.

Results: Four patients were included. The average age at diagnosis was 56.5 years, and the number of prior treatments ranged from 1 to 6. All patients received pembrolizumab combined with either bevacizumab and/or cyclophosphamide. All patients (n = 3) who received pembrolizumab and bevacizumab experienced a partial response. Responses were durable, ranging from 6 to 15 months. Somatic genomic testing results demonstrated microsatellite stability and low tumor mutational burden in all patient tumors, and 3 had AT-Rich Interaction Domain 1A gene (ARID1A) mutations. Notably, two patients had treatment-limiting toxicities, one with presumed immune-mediated grade 2 myocarditis, and another with grade 5 hepatitis.

Conclusions: Pembrolizumab, combined with bevacizumab and cyclophosphamide, is a promising treatment option for patients with recurrent OCCC, though careful risk assessment and counseling regarding toxicities is necessary to maximize the safety and efficacy of this treatment regimen. Prospective studies are needed for validation.

1. Introduction

Ovarian clear cell carcinoma (OCCC) is an aggressive histologic subtype of epithelial ovarian cancer (OC) with a poor prognosis. This subtype is chemotherapy-resistant, with response rates as low as 11 % to first-line therapy and 1–9 % to subsequent treatment (Gadducci et al., 2021). There is a critical need for novel therapies for this patient population.

Immunotherapy has recently revolutionized the treatment of multiple cancer types and is approved for patients with solid tumors with microsatellite instability (MSI) or high tumor mutational burden (TMB). Per the National Comprehensive Cancer Network (NCCN) guidelines, immune checkpoint inhibitors (ICI) can be considered for treatment of recurrent OC with these characteristics. However, case reports and small series suggest that there may be a role for ICI in OCCC even when these biomarkers are not present (Calo et al., 2023; Sia et al., 2022; Zhao and Jiang, 2022). In OCCC, certain mutations, such as in the AT-Rich Interaction Domain 1A gene (ARID1A), have recently been identified as potential biomarkers associated with response to immunotherapy (Jiang et al., 2020; Kuroda et al., 2021). Furthermore, studies suggest that the addition of antiangiogenic therapy could also enhance tumor response to ICI by normalizing the composition of immune cells within the tumor microenvironment (Fukumura et al., 2018).

Here, we present a case series of 4 patients with recurrent OCCC who received treatment with pembrolizumab in combination with either bevacizumab and/or cyclophosphamide to contribute to the growing body of literature demonstrating potential effectiveness of ICI in this population.

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https://doi.org/10.1016/j.gore.2024.101374
Received 26 February 2024; Received in revised form 19 March 2024; Accepted 20 March 2024
Available online 2 April 2024

2. Materials and Methods

After obtaining Institutional Review Board approval, we identified all patients with OCCC who received immunotherapy outside of a clinical trial between April 2018 and April 2023 within the Johns Hopkins Health System. A query of the Johns Hopkins pharmacy was performed with the following criteria: (Gadducci et al., 2021) ICD-10 code diagnosis of C56, C57, or C48 (malignant neoplasm of ovary, unspecified fallopian tube, or retroperitoneum and peritoneum, respectively); (Calo et al., 2023) immunotherapy infusion order (pembrolizumab, dostarlimab, nivolumab, cemiplimab, atezolizumab, or durvalumab). Charts were manually reviewed, and detailed data on cancer diagnosis, stage, and treatment history, immunotherapy regimen and toxicities, somatic genomic testing results, best treatment response, and best treatment outcome were abstracted. Results are reported descriptively given the small number of patients.

3. Results

Four patients met inclusion criteria. The intended treatment regimen for all patients was pembrolizumab (200 mg every 3 weeks), bevacizumab (15 mg/kg every 3 weeks) and oral cyclophosphamide (50 mg daily) based on Phase II data demonstrating efficacy of this combination in OC (Zsiros et al., 2021). However, Patient 2 did not receive bevacizumab due to metastatic involvement of the colon and Patient 3 declined cyclophosphamide due to opposition to cytotoxic chemotherapy (Table 1).

3.1. Case 1

Patient 1 underwent upfront complete cytoreduction of Stage IIIC OCCC at age 57 followed by adjuvant platinum chemotherapy and radiation. She experienced recurrence 14 months later and subsequently received 5 sequential lines of cytotoxic therapy, progressing through all of them. She had a treatment holiday for 6 months, during which she continued to have progression of disease, with the development of pulmonary nodules that became increasingly symptomatic. Genomic testing demonstrated microsatellite stability (MSS), low TMB and a mutation in ARID1A (Table 2). She then started treatment with bevacizumab, cyclophosphamide, and pembrolizumab. A computed tomography (CT) scan after 3 cycles demonstrated decreased size of thoracic metastases and her pulmonary symptoms resolved (Fig. 1, Panel B). After cycle 5 she developed grade 4 autoimmune hepatitis and grade 2 myocarditis requiring inpatient admission and was treated with maximum doses of steroids, mycophenolate mofetil and tacrolimus guided by a multidisciplinary team. While undergoing treatment for these toxicities, her pulmonary nodules continued to decrease in size (Fig. 1, Panel C). Unfortunately, despite escalating treatment for her steroid-refractory immunotherapy-related toxicities, she ultimately died from sequelae of grade 5 autoimmune hepatitis. Her time on treatment was 3 months and her documented duration of response was 6 months.

3.2. Case 2

Patient 2 was diagnosed with Stage IIIC OCCC at age 61, received neoadjuvant carboplatin and paclitaxel and had an interval suboptimal cytoreduction. Postoperatively, she received adjuvant carboplatin, paclitaxel and bevacizumab with her best response being stable disease. After 19 months on bevacizumab maintenance, she had disease progression and subsequently progressed through second line platinum doublet chemotherapy prior to initiating immunotherapy. Her intended regimen was pembrolizumab, bevacizumab and cyclophosphamide, but bevacizumab was held due to metastatic involvement of the colon. She had progression of disease on her first CT scan 2 months after initiating immunotherapy. Genomic testing of the colon tumor demonstrated low TMB and MSS (Table 2).

3.3. Case 3

Patient 3 underwent optimal cytoreduction of Stage IIIB OCCC at age 46. She received 1 cycle of carboplatin and paclitaxel but declined further treatment after a hypersensitivity reaction to paclitaxel. She was diagnosed with recurrent disease 10 months later but declined all cytotoxic chemotherapy. She had somatic genomic testing demonstrating mutations in ARID1A and PPP2R1A (Table 2). An ICI-based regimen was recommended. She ultimately declined cyclophosphamide, but initiated pembrolizumab and bevacizumab. After 1 cycle, she presented with exertional chest pain. A comprehensive workup revealed elevated troponin, ST changes on electrocardiogram, echocardiogram with nodular thickening of mitral valve concerning for endocarditis, though no evidence of myocarditis on cardiac magnetic resonance imaging. She underwent an endomyocardial biopsy, but pathology was not consistent with an immune-mediated process. She was ultimately diagnosed with non-bacterial thrombotic endocarditis treated with antibiotics, steroids and therapeutic anticoagulation with normalization of troponin levels and resolution of symptoms. In discussion with a multidisciplinary team of immunotherapy, cardiology, and oncology experts, the decision was made to restart immunotherapy. Despite her negative biopsy, she continued to have asymptomatic troponin elevations that occurred consistently with pembrolizumab re-dosing and always responded well to steroids, clinically consistent with an underlying immune-mediated grade 2 myocarditis. She ultimately received 7 doses

Table 1
Patient immunotherapy regimen and response.

Patient	Number of prior lines of therapy	Intended regimen	Regimen Given	Time on treatment (months)	Duration of response (months)	Best response	Time to progression (months)	Adverse events	Discontinuation reason
1	6	pembrolizumab, cyclophosphamide, bevacizumab	pembrolizumab, cyclophosphamide, bevacizumab	3	6	partial response	N/A	Grade 5 hepatitis Grade 2 myocarditis	toxicity
2	3	pembrolizumab, cyclophosphamide, bevacizumab	pembrolizumab, cyclophosphamide*	2	0	progression	2	None	progression
3	1	pembrolizumab, cyclophosphamide, bevacizumab	pembrolizumab, bevacizumab+	6	7	partial response	7	Grade 2 myocarditis	progression
4	1	pembrolizumab, cyclophosphamide, bevacizumab	pembrolizumab, cyclophosphamide, bevacizumab	15	15	partial response	15	None	progression

* Bevacizumab was held for Patient 2 in the setting of metastatic involvement of the colon
+ Cyclophosphamide was not given to Patient 3 due to patient preference.

Table 2
Somatic genomic testing results.

Patient	Site of testing	Tumor proportion score	Tumor mutational burden (muts/megabase)	Microsatellite stability	Germline BRCA status	ARID1A Mutations		Other somatic gene mutations
						Genetic Alteration	Amino Acid Alteration	
1*	primary	0	4	stable	negative	c.31_56del	S11fs*91	FANCC, SMARCA4
2*	recurrence	not available	5	stable	negative	not applicable	not applicable	PIK3CA, TP53
3*	primary	50	5	stable	negative	c.48GCC	D1998fs*32	PIK3R1, PPP2R1A
4+	recurrence	not available	3	stable	negative	c.5299G > T c.5407G > C	E1767* E1803Q	ATM, FBXW7, FGFR2, KMT2C, LRP1B, PIK3CA

* Results from FoundationOne CDx.
+ Results from MD Anderson Mutation Analysis Precision Panel.

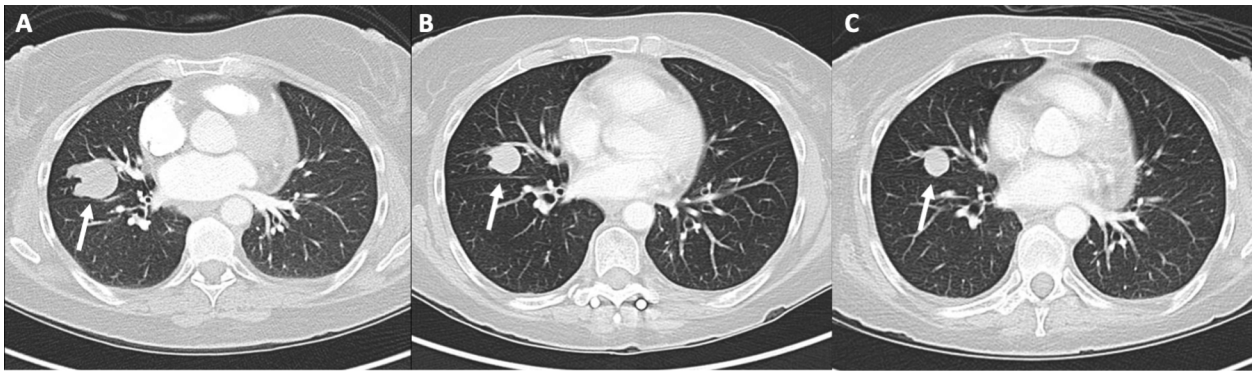

Fig. 1. Patient 1 Radiographic Treatment Response. (A) Baseline imaging prior to initiating pembrolizumab, lesion measures 2.7x2.6 cm; (B) Initial response after 3 cycles of pembrolizumab, lesion measures 1.9x2.0 cm; (C) Continued response even after holding cycle 6 of pembrolizumab, lesion measures 1.5x1.6 cm.

of pembrolizumab over 6 months (3 doses with concurrent bevacizumab). Notably, bevacizumab was held for two cycles after diagnosis of thrombogenic endocarditis and then resumed after sufficient time on anticoagulation. Progression was diagnosed 6 months after initiation of ICI.

3.4. Case 4

Patient 4 had complete gross resection of Stage IC disease at age 62 followed by adjuvant carboplatin and paclitaxel. She was diagnosed with an isolated recurrence in the right acetabulum 10 months later which prompted initiation of pembrolizumab, cyclophosphamide and bevacizumab as second line treatment. She received 1 cycle of this regimen prior to undergoing a hemipelvectomy and reconstruction to resect the acetabular lesion, then continued immunotherapy. Somatic genomic testing of this recurrence demonstrated mutations in ARID1A with MSS and low TMB (Table 2). Approximately 10 months later she had imaging findings of isolated recurrence near her prior resection bed. Given oligometastatic disease, and that the patient was otherwise tolerating the immunotherapy regimen, this solitary site was targeted with stereotactic radiation therapy while she continued this regimen. She remained on immunotherapy for 15 months without any toxicity, but ultimately CT imaging demonstrated distant progression prompting discontinuation.

4. Discussion

We present a case series of 4 patients with recurrent OCCC who were treated with pembrolizumab in combination with either bevacizumab and/or cyclophosphamide. We found that 3 of 4 patients had a sustained (≥6 months) partial response with this regimen. Somatic genomic testing showed 3 of 4 patients had mutations in ARID1A and, despite low TMB and MSS, all responded to immunotherapy. These cases highlight that patients with OCCC lacking the traditional biomarkers predictive of immunotherapy response may still benefit and indicate that the subset of patients who will benefit most has not yet been elucidated.

Our series adds to the growing body of literature supporting a potential role for immunotherapy for OCCC. In subgroup analysis of the KEYNOTE-100 trial, which investigated single-agent pembrolizumab for treatment of recurrent OC, 15.8 % of patients with OCCC responded to this treatment as compared to 8.5 % of patients with high-grade serous histology (Matulonis et al., 2019). This finding has been reinforced by case reports and case series, the largest including 16 patients, showing response to ICI treatment among OCCC patients (Calo et al., 2023; Sia et al., 2022; Zhao and Jiang, 2022). However, to date, only one clinical trial has investigated ICI treatment compared to standard chemotherapy in the recurrent OCCC patient population. This phase II trial enrolled 47 patients randomly assigned to receive durvalumab or physician's choice chemotherapy and results demonstrated no significant difference in outcomes between these treatments (Tan et al., 2022). It is clear that more data are needed to better guide use of ICI in this patient population.

There is biological rationale that antiangiogenic therapy may enhance response to ICI therapy. Vascular endothelial growth factor (VEGF) is highly expressed in OCCC and downregulates adhesion molecules that traditionally attract immune cells (Mabuchi et al., 2010). Thus, inhibiting this factor is thought to increase immunogenicity and improve response to ICI (Fukumura et al., 2018). Our series supports the theory that combining an ICI with anti-VEGF therapy may be advantageous in recurrent OCCC as all 3 patients who had partial response received bevacizumab with pembrolizumab. Two other cases in the literature demonstrate the success of combining these agents, one with complete response and another with sustained partial response over 12 months (Zhao and Jiang, 2022; Lin et al., 2020). More recently, the combination of pembrolizumab and lenvatinib, a kinase inhibitor with antiangiogenic properties, was reported in a series of 3 OCCC patients, all with partial response (Calo et al., 2023). In fact, a phase II trial investigating the efficacy and safety of combining pembrolizumab and

lenvatinib in patients with OCCC is currently recruiting participants (NCT05296512). While this combination shows promise, lenvatinib has a significant side effect profile and high rates of adverse events have been reported in the endometrial cancer patient population (Makker et al., 2020). Bevacizumab, however, is generally well tolerated and we demonstrate that combining this therapy with metronomic chemotherapy and ICI may elicit a similar response to that reported by Calo et al (Calo et al., 2023). Prospective studies are needed to further evaluate bevacizumab, metronomic cyclophosphamide and ICI for recurrent OCCC.

It is possible that differences in response to ICI treatment among OCCC patients could be explained by tumor molecular profiles within this histologic subtype. ARID1A is a tumor suppressor gene and loss of expression has emerged as a potential predictor of immunotherapy response (Kuroda et al., 2021). In mouse models, ARID1A-mutated OC demonstrates sensitivity to ICI, with longer overall survival (OS) of mice with ARID1A-mutated tumors treated with ICI compared to those without ARID1A alterations (Shen et al., 2018). Clinically, case reports and one series have shown ICI as an effective treatment in ARID1A-mutated OCCC (Calo et al., 2023; Zhao and Jiang, 2022). In our case series, all patients with partial response had ARID1A mutations, though interestingly had low TMB and MSS. This molecular profile was also seen in the collective 3 cases reported by Zhao et al and Calo et al. Of note, treatment of OCCC with ICI in the absence of high TMB or MSI is not FDA approved or included in NCCN recommendations. For the patients in our series, specific insurance authorization or pharmaceutical compassionate use approval was obtained for their ICI regimen. Our findings indicate that further studies are needed to determine the underlying mechanism of ICI response in ARID1A-mutated OCCC that is otherwise MSS with low TMB, and establish the utility of ARID1A mutations as a predictor of response to immunotherapy.

Patient 3 additionally had a mutation in PPP2R1A, which encodes protein phosphatase 2 (PP2A). A recent abstract highlighted that, among OCCC patients treated with ICI, 7 patients with mutations in PPP2R1A had significantly longer OS compared to the 21 patients without a PPP2R1A mutation (Hinchcliff et al., 2022). Interestingly, one patient reported in the series by Calo et al also had a tumor with mutations in both PPP2R1A and ARID1A, and this patient had a durable response to ICI treatment lasting over 25 months (Calo et al., 2023). More data are needed to understand the relationship between immunotherapy response, PPP2R1A-mutated tumors, and OS among OCCC patients.

Importantly, our case series highlights the significant adverse events that can occur with ICI, underscoring the need to identify the most appropriate patient cohort for this class of agents. Immune-related adverse events have been reported in up to 22.6 % of OC patients treated with pembrolizumab (Matulonis et al., 2019). When OC patients were treated with a combination of pembrolizumab, bevacizumab and cyclophosphamide specifically, 10.5 % of reported adverse events were immune-related, though all were less than grade 3 (Zsiros et al., 2021). In our series, two patients experienced autoimmune side effects attributed to their ICI therapy and for one patient this ultimately proved lethal. Careful risk assessment and counseling of patients as well as prompt recognition and intervention of immunotherapy-related toxicities are important for maximizing safety and efficacy. Determining who will benefit most will better inform our best clinical practice.

5. Conclusion

In summary, we present 4 cases of recurrent OCCC that suggest that pembrolizumab, in combination with bevacizumab and cyclophosphamide, may be an effective treatment option for this treatment-resistant subtype of OC. However, these findings must be considered in the context of potentially serious toxicities. These cases underscore the need for randomized controlled trials in OCCC that examine outcomes by tumor mutation profile, to identify the best molecular markers to predict response to treatment.

6. Consent

Written informed consent was obtained from the patients (or, where applicable, the patient's guardian or next of kin) for publication of this case report and accompanying images.

CRediT authorship contribution statement

Shannon M. Glynn: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Stephanie Gaillard:** Writing – review & editing. **Rebecca L. Stone:** Writing – review & editing. **Amanda N. Fader:** Writing – review & editing. **Anna L. Beavis:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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