Gynecologic Oncology 194 (2025) 41–47

Contents lists available at ScienceDirect

Gynecologic Oncology

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

A clinical study of tremelimumab, alone or in combination with olaparib, for recurrent epithelial ovarian cancer

Stéphanie Gaillard [a,b,*,1], Neha Verma [a,1], Maureen Berg [a], Jeanne Harrison [a], Peng Huang [c], James M. Leatherman [a], Michele Doucet [a], Rupashree Sen [a], Aditya Suru [a], Hongyan Cai [a], Jennifer Durham [a], Danijela Jelovac [a], Ashley Cimino-Mathews [a,d], Christopher Cherry [a], Sudipto Ganguly [a,1], Leisha A. Emens [a,1]

[a] Department of Oncology Johns Hopkins School of Medicine, Baltimore, MD, United States of America
[b] Department of Gynecology and Obstetrics Division of Gynecologic Oncology, Johns Hopkins School of Medicine, Baltimore, MD, United States of America
[c] Department of Oncology Division of Biostatistics and Bioinformatics, Johns Hopkins School of Medicine, Baltimore, MD, United States of America
[d] Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD, United States of America

HIGHLIGHTS

- Tremelimumab, alone and with olaparib, was well-tolerated in patients with recurrent epithelial ovarian cancer.
- Tremelimumab IV 10 mg/kg/dose with olaparib orally 150 mg twice daily was a safe and feasible dose.
- Overall clinical benefit rate among patients receiving tremelimumab, alone and with olaparib, was 46 %.
- Tremelimumab 10 mg/kg/dose (but not 3 mg/kg/dose) resulted in immune activation (increased $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells).
- Immune activation as defined above did not translate into clinical responses.

ARTICLE INFO

Article history:
Received 17 December 2024
Received in revised form 30 January 2025
Accepted 31 January 2025
Available online xxxx

Keywords:
Epithelial ovarian cancer
Tremelimumab
Immune activation
Olaparib
PARP inhibitor

ABSTRACT

Objective. PARP inhibitors may work synergistically to improve the efficacy of immunotherapy in patients with epithelial ovarian cancer (EOC). We performed a parallel-arm study of tremelimumab, alone or with olaparib, in patients with recurrent EOC.

Methods. Eligibility criteria included measurable disease and progression <12 months from last platinum. Participants were randomized to Arm A (tremelimumab monotherapy, 10 mg/kg/dose intravenously [IV]) or Arm B (dose level 1 [DL1] olaparib orally 150 mg twice daily with tremelimumab IV 3 mg/kg/dose and DL2 olaparib orally 150 mg twice daily with tremelimumab IV 10 mg/kg/dose). Primary objectives were safety, change in peripheral $ICOS^+$ T cells, and identification of optimal dose combination.

Results. Among 24 total patients (12 on Arm A, 6 on Arm B-DL1, 6 on Arm B-DL2), the most common grade 3 toxicities were rash (13 %), immune-mediated hepatitis (8 %), and colitis (8 %). No grade ≥ 4 toxicities were identified. No dose-limiting toxicities were identified. One patient (Arm B-DL2) experienced a partial response; no complete responses were observed. Ten patients (7 on Arm A, 2 on Arm B-DL2, and 1 on Arm B-DL1) had a best response of stable disease. There was a significant increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells at both C1D15 and C1D22 in groups treated with tremelimumab IV 10 mg/kg/dose, but not in those treated with tremelimumab 3 mg/kg/dose.

Conclusions. Tremelimumab IV 10 mg/kg/dose with olaparib 150 mg orally twice daily was safe and feasible. Tremelimumab 10 mg/kg/dose (as opposed to 3 mg/kg/dose) was required for immune activation, although this did not translate into clinical responses.

1. Background

Epithelial ovarian cancer (EOC) is a leading cause of cancer mortality among women in the United States [1]. Recurrent EOC carries a poor prognosis, with only modest response rates to subsequent lines of

* Corresponding author at: 201 North Broadway 10-260, Baltimore, MD 21287, United States of America.
E-mail address: stephanie.gaillard@jhmi.edu (S. Gaillard).
[1] Each author contributed equally to the work presented.

https://doi.org/10.1016/j.ygyno.2025.01.015
0090-8258/

standard chemotherapy [2]. There is a critical need for development of novel therapeutic approaches for recurrent EOC.

Despite exciting results in other tumor types, response rates to immunotherapy in patients with recurrent EOC are low (≤15 %) [3–5]. One strategy to increase response rates to immune checkpoint inhibitors in patients with EOC involves combining these agents with potentially synergistic drugs such as PARP [poly (ADP-ribose) polymerase] inhibitors [3,6–9]. Approximately half of all patients with EOC have BRCA mutations or defects in homologous recombination (HR) repair. In the BRCA+/HR deficient setting, PARP inhibition causes DNA damage resulting in synthetic lethality [10]. Emerging data suggests that the DNA damage caused by PARP inhibitors may also result in a more immunogenic tumor microenvironment (TME) [7]. Indeed, PARP inhibitors have been shown to improve the efficacy of anti-PD-1/PD-L1 therapy in patients with EOC, regardless of BRCA mutation or homologous recombination deficiency (HRD) biomarker status [6,7]. PARP inhibitors may therefore also improve the efficacy of other types of immunotherapy, including anti-CTLA-4 therapy.

There is little published data regarding the safety and efficacy of anti-CTLA-4 monotherapy and/or anti-CTLA-4 therapy in combination with a PARP inhibitor in patients with recurrent EOC, particularly in those with platinum-resistant disease. Interestingly, in murine BRCA-deficient ovarian tumors treated with the PARP inhibitor veliparib in combination with anti-CTLA-4 or anti-PD-1/PD-L1, inhibition of CTLA-4 but not PD-1/PD-L1 promoted recruitment of activated T-cells and improved long-term survival [11]. However, further evaluation of the safety and bioactivity of anti-CTLA-4 therapy, alone and in combination with a PARP inhibitor, in patients with recurrent EOC is needed.

This study was designed to evaluate the safety of the anti-CTLA-4 monoclonal antibody tremelimumab, alone and in combination with olaparib, in patients with recurrent or persistent EOC where disease is relatively platinum resistant (progression <12 months from last platinum). The goal of this study was to understand the single-agent activity of tremelimumab in this patient population as well as to define a safe and bioactive dose of tremelimumab and olaparib for further clinical evaluation. Given limited data regarding the safety and bioactivity of tremelimumab in patients with advanced EOC, this study was designed as a parallel-arm study to test the activity of tremelimumab, alone or in combination with olaparib, without direct comparison of the two arms.

2. Methods

2.1. Study design and procedures

This study was designed as an investigator-initiated, non-comparative, parallel-arm study assessing the safety and bioactivity of tremelimumab, alone or in combination with olaparib, in patients with recurrent or persistent EOC. Because the optimal dose of olaparib with tremelimumab was not known, two dose levels were included in the combination arm. A third dose level and expansion at the optimal dose aiming to identify the maximum tolerated dose (MTD) of tremelimumab with olaparib were planned but were not opened due to sponsor's change in development priorities. Clinical outcomes for each dose cohort (tremelimumab monotherapy, olaparib with tremelimumab dose level 1, and olaparib with tremelimumab dose level 2) were determined separately with no direct comparison between arms. The primary objectives were to (1) determine the safety of tremelimumab alone and in combination with olaparib, (2) measure the baseline and change in peripheral $CD4^+ICOS^+$ T cells, $CD8^+ICOS^+$ T cells, and $CD4^+CD25^+FOXP3^+$ T cells by intracellular cytokine staining, and (3) identify the optimal dose combination of olaparib and tremelimumab. Secondary objectives were to measure the 6-month progression-free survival (PFS6) and determine the objective response rate by RECIST and irRECIST. Exploratory objectives included evaluating candidate biomarkers of response, PFS, and OS.

Eligible participants were randomized to either Arm A (tremelimumab) or Arm B (tremelimumab and olaparib). All participants on Arm A received tremelimumab 10 mg/kg/dose intravenously (IV), initially every 4 weeks for 7 doses and subsequently every 12 weeks. Participants on Arm B were enrolled onto 2 dose levels: dose level 1 (DL1) consisted of olaparib 150 mg orally twice daily and tremelimumab 3 mg/kg/dose IV and DL2 consisted of olaparib 150 mg orally twice daily and tremelimumab 10 mg/kg/dose IV. As above, a third dose level (olaparib 300 mg twice daily and tremelimumab 10 mg/kg/dose) and expansion at the optimal dose (based on safety and bioactivity, described below) were planned, but were not opened due to sponsor's change in development priorities. As in Arm A, tremelimumab was administered initially every 4 weeks for 7 doses and subsequently every 12 weeks. Six patients were planned at each dose level for dose limiting toxicity (DLT) assessment before enrollment at the next dose level was initiated. Participants were randomized using an A/B randomization table. During enrollment holds to Arm B DL1 for safety, accrual to Arm A was continued so as not to delay accrual to the study. When Arm B DL1 or Arm B DL2 was active, patients were randomized between Arms A and Arms B DL1 or B DL2. Treatment in each cohort continued until disease progression or unacceptable toxicity.

The clinical trial was approved by the Johns Hopkins Institutional Review Board (ClinicalTrials.gov identifier: NCT02485990). Written informed consent was obtained from all patients prior to performing study-related procedures in accordance with federal and institutional guidelines. AstraZeneca provided tremelimumab, olaparib, and study funding.

2.2. Eligibility

Participants with recurrent or persistent EOC were eligible to enroll. Key inclusion criteria included measurable disease by RECISTv1.1, one or more prior taxane-platinum-based chemotherapeutic regimen, treatment-free interval following last line platinum-based therapy of less than 12 months, Eastern Cooperative Oncology Group performance status ≤1, availability of archival tissue for review and testing, and normal organ and marrow function. Prior treatment with immune checkpoint blockade (except anti-CTLA-4) and PARP inhibitor was allowed. Key exclusion criteria included prior therapy with anti-CTLA-4, chronic inflammatory or autoimmune condition, or systemic oral corticosteroids within 28 days prior to initiating study therapy.

2.3. Safety

Grading of adverse events (AEs) was performed according to the NCI CTCAEv4.03. A DLT, as assessed in Arm B, was defined as Grade 3 or higher treatment-related toxicity that occurred during the DLT evaluation period (Cycle 1 of therapy). Any Grade 3 immune-related adverse event (irAE) that improved to Grade ≤ 2 within 3 days after onset of the event with maximal supportive care including systemic corticosteroids or improved to Grade ≤ 1 or baseline within 14 days was not considered a DLT. The following were not considered DLTs: Grade 3 endocrinopathy that was managed with or without systemic corticosteroid therapy and/or hormone replacement therapy, provided the participant was asymptomatic, and Grade 3 inflammatory reaction attributed to a local antitumor response (e.g., inflammatory reaction at sites of metastatic disease, lymph nodes).

2.4. Assessment of bioactivity

Whole blood was collected from enrolled patients at baseline and up to 5 time points post-treatment. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Histopaque density-gradient centrifugation. Multi-color flow cytometry was used to evaluate dynamic changes in expression of ICOS, PD-1 and TIGIT in peripheral $CD4^+$and $CD8^+$ T cells along with frequencies of $CD4^+CD25^+FOXP3^+$ T

regulatory cells (Tregs). All antibodies (**Supplemental Table 1**) were used at manufacturer-recommended concentrations. 10 [6] PBMC were dispensed into a 96-well U-bottomed plate and stained with Aqua Live Dead viability dye (Thermofisher). Following a wash step, Human Fc Block (a cocktail of anti-CD16 and anti-CD32) was added to saturate Fc receptors and block non-specific binding. Cells were washed twice with FACS buffer (PBS + 0.1 % BSA + 2 mM EDTA) and stained with a cocktail comprising target antibodies. All staining was done for 20 min at room temperature. Surface-stained samples were fixed and permeabilized (FOXP3 staining buffer; ThermoFisher) prior to staining with anti-FOXP3. Following a final wash in permeabilization-wash buffer (1×; ThermoFisher), cells were resuspended in 0.4 ml FACS buffer and acquired on the BD Celesta flow cytometer. Data was analyzed on FlowJo software (Becton Dickinson; version 10.2).

The planned primary outcome was change from baseline in the log of the ratio: $CD4^+ICOS^+$/T reg. If there was no change in the log-ratio, it was pre-planned to use the greatest change in the ratio of $CD4^+ICOS^+$ T cells to determine greatest bioactivity. To further evaluate the phenotype of activated T cells, the change in percentage of cells co-expressing ICOS with either TIGIT or PD-1 was also assessed.

Antitumor activity was determined through radiologic tumor assessments conducted prior to starting therapy and every 8 weeks thereafter.

2.5. RNA sequencing

$CD4^+$ T cells were purified from PBMC using the EasySep human $CD4^+$ T cell isolation kit (StemCell Technologies). Total RNA was extracted from purified $CD4^+$ T cells using RNeasy Micro kit (Qiagen) and quantified. Transcript abundance within the $CD4^+$ T cell compartment was quantified using bulk RNA-seq and expressed as FPKM (Fragments per kilobase of transcript per million mapped reads) values. Counts data were analyzed for differential expression using a negative binomial model implemented with DESeq2 v1.34.0. For all response metadata, time point was included in the design matrix unless samples were subset to a specific time point. The resulting differential expression results were analyzed with gene set enrichment analysis using fgsea v1.20.0 with a selection of relevant gene sets. The complete list of gene sets can be found in the supplemental figure of gsea results (**Supplemental Fig. 5**).

2.6. Statistical considerations

Baseline patient and disease characteristics were summarized using descriptive statistics. Continuous variables were presented as median and range, categorical variables were presented using tables. Safety data were also summarized using tables. Mean percentages of ICOS-expressing $CD4^+$ or $CD8^+$ T cells were summarized across treatment arms and dates. Both fold changes and percentage of cell changes from C1D1 to C1D15 as well as from C1D1 to C1D22 within each arm were evaluated using signed rank test. The Kaplan-Meier estimate was employed to assess both the progression-free survival rate and overall survival rate. The corresponding 95 % confidence intervals were constructed using the Greenwood formula for calculating the standard deviation of the survival rate.

Initially, a sample size of 25 patients per arm was planned, which would provide approximately 80 % power to reject the null hypothesis of a 6-month PFS rate of <12 % (the undesirable 6-month PFS based on historical data) as compared to the desirable 6-month PFS rate of >30 % at a 2-sided level of significance of 0.2. This planned sample size would also provide approximately 80 % power to reject the null hypothesis of a response rate (CR + PR) of 10 % (the undesirable response rate based on historical data) as compared to the desirable response rate of 25 % at a 2-sided level of significance of 0.2.

RNA-seq data quality control was conducted prior to analysis. Duplicated genes were removed to ensure data accuracy. Changes in gene expression levels in $CD4^+$ T cells from baseline (C1D1) to follow-up (C1D15) were calculated. Next, we identified the top genes that differentiated patients who benefited from their assigned treatment versus those who did not based on this change measure. Finally, we normalized this change and constructed a heatmap to visualize the data (**Supplemental Fig. 5**). Spearman correlation rho was used to measure correlation between flow cytometry and RNA-seq.

3. Results

3.1. Baseline patient demographics and disease characteristics

Although a sample size of 25 per arm was initially planned, the study was stopped early due to sponsor's change in development priorities. As a result, a total of twenty-four patients (12 in Arm A, 12 in Arm B) were enrolled in the study between 1/8/2016 and 4/16/2019 (Table 1). Patients in Arm A received tremelimumab 10 mg/kg/dose IV. Patients in Arm B received treatment with tremelimumab and olaparib and were enrolled into two dose levels ($n = 6$ in each level). Both dose levels received olaparib 150 mg orally twice daily; patients on DL1 received tremelimumab 3 mg/kg/dose IV, while those on DL 2 received tremelimumab 10 mg/kg/dose IV.

Table 1
Baseline patient and disease characteristics.

	All	Arm A	Arm B	
			Dose Level 1	Dose Level 2
Tremelimumab Dose		10 mg/kg	3 mg/kg	10 mg/kg
Olaparib Dose		N/A	150 mg BID	150 mg BID
	***N* = 24**	***N* = 12**	**N = 6**	**N = 6**
Age, median (range)	59.5 (44–81)	59.5 (44–81)	64.5 (54–71)	53.0 (48–74)
Race, N (%)				
White	21 (88)	11	6	4
Black/African-American	1 (4)	1	0	0
Asian	1 (4)	0	0	1
Other	1 (4)	0	0	1
Ethnicity, N (%)				
Hispanic	2 (9)	0	0	2
Non-Hispanic	22 (91)	12	6	4
ECOG PS				
0	20 (83)	12	5	5
1	4 (17)	0	1	3
Primary site, N (%)				
Ovary	18 (75)	9	5	4
Fallopian tube	5 (21)	2	1	2
Peritoneal	1 (4)	1	0	0
Histology, N (%)				
Serous	20 (83)	10	5	5
Clear Cell	3 (4)	1	1	1
Adenocarcinoma, NOS	1 (4)	1	0	0
Grade				
G2	1 (4)	1	0	0
G3/high-grade	23 (96)	11	6	6
BRCA Mutation Present, N (%)				
No mutation	21 (88)	12	5	4
BRCA1	2 (8)	0	0	2
BRCA2	1 (4)	0	1	0
Prior Regimens, N (%)	3.5 (1, 9)	3.5 (2, 9)	2.5 (1, 6)	3.5 (1, 5)
Platinum-Sensitivity, N (%)				
Sensitive	7 (29)	1	3	3
Resistant	16 (67)	10	3	3
Refractory	1 (4)	1	0	0
Prior Therapy				
PARP Inhibitor	3 (13)	1	0	2
Anti-PD-1/PD-L1	2 (8)	0	1	1

NOS, not otherwise specified.

The median age of enrolled patients was 59.5 years (range 44–81). Three patients (12 %) had germline BRCA1 or BRCA2 mutations. HRD test status was unknown for the majority ($n = 18$; 75 %) of patients; five patients (21 %) were known to be HRD test negative while one patient (4 %) was known to be HRD test positive. The most frequent primary disease site was ovary (n = 18; 75 %) and the most common histology was serous carcinoma ($n = 20$, 83 %). The median number of prior regimens received was 3.5 (range 1–9); all patients had received prior platinum and taxane therapy, 3 patients (12 %) had received prior PARP inhibitor therapy and 2 patients (8 %) had received prior anti-PD-1/PD-L1 therapy. Eight patients (33 %) had platinum sensitive disease (defined for the purposes of this study as progression 6–12 months after last platinum exposure), 15 patients (63 %) had platinum-resistant disease, and 1 patient (4 %) had platinum-refractory disease.

3.2. Safety

All patients who received at least one dose of therapy were evaluable for toxicity. The median number of cycles received by the entire study population was 2 (range 1–10). Of the 24 patients who initiated protocol treatment, 21 patients (88 %) had a treatment-related AE of any grade. The majority (68 %) of AEs were attributed solely to tremelimumab, consistent with prior studies of this agent, while 24 % were attributed to olaparib, 9 % to both study drugs, and 3 % were undetermined. There were 18 treatment-related grade 3 toxicities and no grade 4 or 5 toxicities. The most common grade 3 AEs included rash ($n = 3$, 13 %), immune-mediated hepatitis, and colitis ($n = 2$, 8 % for each) (Table 2). No DLTs were observed on either dose level of Arm B. No new safety signals were identified during the study. Six patients (25 %) discontinued treatment due to AEs; three on Arm A and three on Arm B (two on DL1 and one on DL2).

Table 2
Treatment-related adverse events.

	All Arms (N = 24)	Arm A (n = 12)		Arm B, DL1 (n = 6)		Arm B, DL2 (n = 6)	
	Any Grade	G ≤ 2	G3	G ≤ 2	G 3	G ≤ 2	G 3
Toxicity*	**No. (%)**	**No.**					
Rash	10 (42)	1	2	4	1	2	0
Diarrhea	9 (38)	4	1	3	0	1	0
Nausea	8 (33)	2	0	3	0	3	0
Fatigue	7 (29)	2	0	2	0	3	0
Pruritis	6 (25)	5	0	0	0	1	0
Abdominal Pain	5 (21)	2	0	1	0	1	1
Myalgia	5 (21)	3	0	1	0	1	0
Vomiting	4 (17)	1	0	1	0	2	0
Anorexia	3 (13)	2	0	1	0	0	0
Arthralgia	3 (13)	1	0	1	0	1	0
Dyspepsia	3 (13)	1	0	2	0	0	0
Headache	3 (13)	1	0	1	0	1	0
Hypothyroidism	3 (13)	2	0	0	0	1	0
Anemia	2 (8)	0	0	1	0	0	1
Autoimmune Hepatitis	2 (8)	0	1	0	0	0	1
Chills	2 (8)	0	0	1	0	1	0
Colitis	2 (8)	0	2	0	0	0	0
Dry Mouth	2 (8)	1	0	0	0	1	0
Dysguesia	2 (8)	1	0	0	0	1	0
Edema, facial	2 (8)	1	0	1	0	0	0
Mucositis	2 (8)	1	0	0	1	0	0
Sore Throat	2 (8)	1	0	1	0	0	0
Urticaria	2 (8)	1	0	0	0	1	0

Note: The denominator to calculate percentages is 24, the number of patients who received at least one dose of study drug.
There was 1 instance of the following (listed by study arm): Arm A (G2 constipation, G2 dry eyes, G2 sinusitis, G2 watery eyes, G3 fever, G3 sepsis); Arm B DL1 (G2 increased amylase, G2 muscle weakness, G2 peripheral sensory neuropathy, G2 urinary tract infection, G3 acute kidney injury, G3 hyponatremia, G3 increased lipase, G3 lung infection), Arm B DL2 (G2 hypophysitis).
* Toxicity is reported as the maximum grade toxicity experienced per event per patient during study treatment.

3.3. ICOS expression in peripheral T cells

ICOS expression was evaluated as a surrogate of T cell activation. The dose level with the highest activity and acceptable toxicity was to be selected as recommended dose for expansion. Of the 24 patients who initiated treatment, 23 patients had matched samples at C1D1 and C1D15, 23 patients had matched samples at C1D1 and C1D22, and 22 patients had matched samples at all three time points. The bioactivity of tremelimumab was measured by first assessing the change from baseline in the log of the ratio: $CD4^+ICOS^+$/T reg. There was no significant change in this log-ratio at any time point (**Supplemental Table 2**). In a pre-planned analysis, the fold change from baseline in $CD4^+ICOS^+$ T cells, as well as $CD8^+ICOS^+$ T cells, was then used to define a response at the individual patient level and determine whether the tremelimumab dose affects T cell activation. There was a significant increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells at both C1D15 and C1D22 in patients treated in Arm A and Arm B DL2, both groups treated with 10 mg/kg/dose IV tremelimumab (Fig. 1 and Table 3). In patients who received 3 mg/kg/dose IV tremelimumab, only the $CD4^+ICOS^+$ T cells at C1D15 were increased ($p < 0.05$). Given the safety profile was similar between Arm B-DL1 and Arm B-DL2 and the increase in $ICOS^+CD4^+$ and $CD8^+$ T cells seen in Arm B-DL2, DL2 (tremelimumab IV 10 mg/kg/dose and olaparib 150 mg orally twice daily) was considered a safe and feasible dose for further study.

3.4. Expression of inhibitory receptors on peripheral T cells

To further evaluate the phenotype of activated T cells, we assessed the change in percentage of cells co-expressing ICOS with either TIGIT or PD-1, two inhibitory immune receptors (**Supplemental Fig. 1** and **Supplemental Tables 3 and 4**). Percentages of $CD4^+$ T cells and $CD8^+$ T cells expressing either ICOS and TIGIT or ICOS and PD-1 were increased at both C1D15 and C1D22 in patients who received 10 mg/kg/dose IV tremelimumab. For those receiving 3 mg/kg/dose IV tremelimumab, a significant increase in $ICOS^+TIGIT^+CD4^+$ T cells was observed on C1D22 but not C1D15. No other evaluated T cell subset was significantly changed in the patients treated with 3 mg/kg/dose IV tremelimumab.

3.5. Clinical response

Of the 24 patients who initiated treatment, 2 patients (both in Arm A) discontinued prior to first response evaluation for progression of disease ($n = 1$) or AE (n = 1). Twenty-two patients were evaluable for antitumor response by RECISTv1.1 (Table 4). Two patients on Arm B (one in each dose level) had a PFS > 6 months (Table 4 and **Supplemental Fig. 2**). Only one patient (Arm B-DL2) experienced a partial response by RECIST or irRECIST criteria; no complete responses were observed (Fig. 2 and Supplemental Fig. 3). Ten patients (42 %; 7 on Arm A, 2 on Arm B-DL2, and 1 on Arm B-DL1) had a best response of stable disease. Overall clinical benefit rate (defined as CR + PR + SD) was 46 %. Eight patients (33 %) opted to continue treatment beyond progression. Of note, the one patient who experienced a partial response was HRD test positive (BRCA 1/2 negative) and had not previously received treatment with a PARP inhibitor; this patient had platinum-resistant disease with a platinum-free interval of four months. Kaplan Meier curves showing PFS and OS data are presented in **Supplemental Fig. 4**.

3.6. Exploratory analyses

There was no significant difference between the fold change in $CD4^+ICOS^+$ T cells on Day 15 or Day 22 between patients with clinical benefit (defined as CR, PR, or SD) versus best response of progression

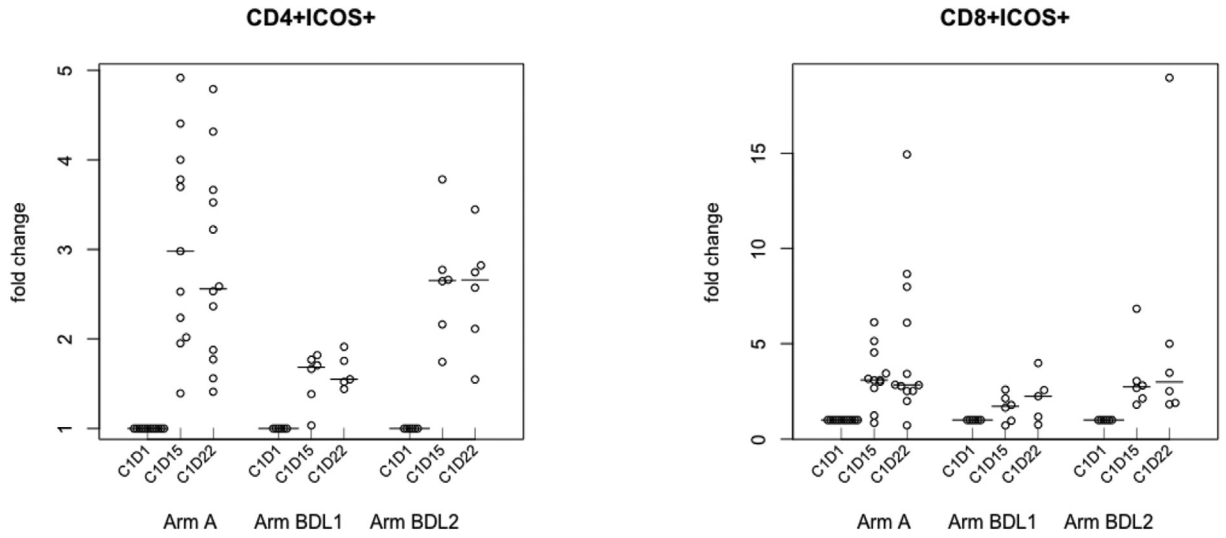

Fig. 1. Fold change in mean percentage of ICOS expressing (A) $CD4^+$ and (B) $CD8^+$ T cells at C1D1, C1D15, and C1D22 by dose level. Each dot represents fold change in mean percentage of cells for an individual patient and each bar represents the median.

(**Supplemental Table 5**). RNA sequencing on $CD4^+$ T cells was performed in a subset of patients selected by best response [benefiters (PR or SD): $n = 3$ from Arm B-DL2 and $n = 6$ from Arm A versus non-benefiters (PD): n = 3 from Arm B-DL2 and n = 3 from Arm A]. A heatmap of change in expression from C1D1 (baseline) to C1D15 shows segregation of expression by best response (**Supplemental Fig. 5**). Flow cytometry and RNA-seq expression of ICOS, TIGIT, and PD-1 were tightly correlated (**Supplemental Fig. 6**). Gene set enrichment analysis was performed, but results were not significant.

4. Discussion

In this parallel-arm study, we found that tremelimumab, alone and in combination with olaparib, was tolerable with AEs as expected in patients with recurrent EOC. Among the two combination dose levels evaluated, DL2 (olaparib orally 150 mg twice daily and tremelimumab IV 10 mg/kg/dose) was tolerable without DLT, and no new safety signals were identified. There was a significant increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells at both C1D15 and C1D22 in both groups treated with 10 mg/kg/dose tremelimumab (Arm A and Arm B-DL2). Given the safety profile was similar between Arm B-DL1 (olaparib orally 150 mg twice daily and tremelimumab IV 3 mg/kg/dose) and Arm B-DL2 (olaparib orally 150 mg twice daily and tremelimumab IV 10 mg/kg/dose) and the increase in $ICOS^+CD4^+$ and $CD8^+$ T cells seen in Arm B-DL2, tremelimumab IV 10 mg/kg/dose with olaparib 150 mg orally twice daily was considered a safe and feasible dose for further study. A third dose level (olaparib orally 300 mg twice daily and tremelimumab IV 10 mg/kg/dose) and expansion at the optimal dose (based on safety and bioactivity) were initially planned, but the study was stopped early due to sponsor's change in development priorities. However, this dose combination was found to be safe based on preliminary results of a recent phase I/II study [12].

The significant increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells at both C1D15 and C1D22 in both groups treated with 10 mg/kg/dose tremelimumab (Arm A and Arm B-DL2) suggests that this dose results in activation of an immune response in this population, as ICOS (inducible T cell co-stimulator) has previously been established as an indicator of T cell mediated response to cancer immunotherapy [13]. However, in this patient population, immune activation as defined by an increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells did not translate into clinical responses. One patient (Arm B-DL2) in this study experienced a partial response and ten patients total (7 on Arm A, 2 on Arm B-DL2, and 1 on Arm B-DL1) had a best response of stable disease, with a clinical benefit rate of 46 %. The one patient who experienced a partial response was HRD test positive (BRCA 1/2 negative) and had not previously received treatment with a PARP inhibitor; this patient had platinum-resistant disease with a platinum-free interval of four months. There was no significant difference between the fold change in $CD4^+ICOS^+$ T cells between patients with clinical benefit (defined as CR + PR + SD) versus best response of progression.

Promising pre-clinical data has demonstrated the potential therapeutic efficacy of a PARP inhibitor with anti-CTLA-4 therapy in patients with EOC [11]. Additionally, PARP inhibitors have been shown in recent clinical studies to improve the efficacy of anti-PD-1/PD-L1 therapy in patients with EOC, regardless of BRCA mutation or HRD biomarker status; this is thought to be due to a synergistic interaction resulting in a more immunogenic TME [6,7,19,20]. We would therefore expect greater anti-tumor effect with the combination of a PARP inhibitor and anti-CTLA-4 therapy in our study, particularly given evidence of immune activation (defined as an increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells) in patients who received tremelimumab 10 mg/kg/dose.

Table 3
Mean percentage of ICOS expressing T cells by dose level.

		$CD4^+ICOS^+$			$CD8^+ICOS^+$		
	n	**C1D1**	**C1D15**	**C1D22**	**C1D1**	**C1D15**	**C1D22**
Arm A	12	17.18 ± 10.56	46.57 ± 10.08***	40.99 ± 12.05***	5.41 ± 7.91	12.25 ± 6.37**	17.99 ± 15.41***
Arm B DL1	6	18.25 ± 5.38	27.74 ± 6.74*	32.07 ± 11.99	6.37 ± 4.67	11.44 ± 11.18	9.30 ± 5.59
Arm B DL2	6	16.69 ± 7.73	40.60 ± 11.05*	39.22 ± 10.84*	3.52 ± 1.63	10.24 ± 4.45*	21.69 ± 30.41*

Mean percentage ± standard deviation presented.
*$p < 0.05$, **$p < 0.002$, ***$p < 0.001$ when compared to Day 1 (signed rank test).

Table 4
Duration of treatment, six-month progression free survival (PFS6), and best objective response (OR) by treatment group.

	No. of Patients		
	Arm A	Arm B	
		DL1	DL2
	(n = 12)	(n = 6)	(n = 6)
Duration of Treatment			
N° of cycles, median (range)	2 (1–7)	2.5 (1–8)	5 (1−10)
PFS6			
Yes	0	1	1
No	12	5	5
PFS6, % (95 % CI)	0 (0, 26.5)	16.7 (0.8, 51.7)	16.7 (0.8, 51.7)
OR			
CR	0	0	0
uPR	0	0	1
SD			
confirmed	1	1	2
unconfirmed	6	0	0
PD	3	5	3
Not evaluable	2	0	0
ORR, % (95 % CI)	0 (0, 26.5)	0 (0, 45.9)	16.7 (0, 46.5)

However, this study had a relatively small sample size, and given that it was stopped early, it was not powered to assess for efficacy. In addition to the increase in $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells, there was also a statistically significant increase in $CD4^+$ T cells and $CD8^+$ T cells co-expressing either ICOS and TIGIT or ICOS and PD-1 at both C1D15 and C1D22 in patients who received tremelimumab 10 mg/kg/dose. TIGIT and PD-1 are inhibitory immune receptors, each with a distinct mechanism of action from CTLA4 [18,21]. It is possible that targeting the immune response at multiple levels (i.e., anti-CTLA-4 therapy with anti-PD-1 therapy or anti-TIGIT therapy, along with a PARP inhibitor), is needed for an anti-tumor response in this patient population. It is also notable that a majority (67 %) of participants in our study had platinum-resistant or platinum-refractory disease, which has been demonstrated to have a lower response to immune checkpoint blockade than platinum-sensitive disease [22]. Additionally, it is possible that while anti-CTLA-4 therapy in combination with a PARP inhibitor does result in activation of an immune response in this patient population at the doses studied, a higher olaparib dose of 300 mg is needed for synergistic anti-tumor effect. A recent preliminary report of a phase I/II study evaluating tremelimumab with olaparib in 49 patients with BRCA-deficient recurrent ovarian cancer (including platinum-sensitive or platinum-resistant disease) showed that olaparib 300 mg twice daily could be safely administered with tremelimumab 10 mg/kg. The frequency of dosing of tremelimumab was changed in this study due to immune-related adverse events from every four weeks continuously to every four weeks for 4 doses followed by maintenance dosing every 12 weeks, similar to our strategy of every four weeks for 7 doses followed by maintenance dosing every 12 weeks [12]. Among 44 evaluable patients, ORR was 39 %, with a clinical benefit rate of 48 % and median PFS of 3.4 months. While the clinical benefit rate in this study was similar to our cohort, the higher response rate seen in this study may be attributable to the underlying tumor BRCA-deficiency or the higher olaparib dose used. The phase II NRG-GY021 trial evaluating olaparib 300 mg twice daily alone or in combination with tremelimumab 10 mg/kg (every 4 weeks × 4 followed by every 12 weeks for up to 2 years) in patients with recurrent platinum-sensitive ovarian cancer, including both BRCA-mutated and BRCA-wild type patients, was stopped early due to immune-related toxicities. Ongoing correlative studies will help clarify factors that may predict the efficacy of this treatment combination [23].

Strengths of this study included: focus on patients with platinum-resistant or platinum-refractory disease, a population in great need of more effective therapies; and assessment of immune cell activation as a measure of bioactivity. Weaknesses of the study included: stopped early due to changes in sponsor development priorities, which precluded evaluation at a third dose level and expansion at the optimal dose. Additionally, biopsies were not performed prior to or during treatment, limiting a more comprehensive assessment of the immune TME.

Collectively, our results suggest that tremelimumab can be administered, alone and in combination with olaparib, with a manageable safety profile in this population. Tremelimumab 10 mg/kg/dose (as opposed to 3 mg/kg/dose) resulted in evidence of immune activation, but this did not translate into clinical responses. Further studies designed to evaluate factors that promote an anti-tumor response to tremelimumab 10 mg/kg/dose in patients with recurrent EOC are needed.

Funding sources

AstraZeneca provided tremelimumab, olaparib, and funding in support of this study. SG and NV are supported in part by NIH/NCI P30 CA006973.

CRediT authorship contribution statement

Stéphanie Gaillard: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Neha Verma:** Writing – review & editing, Writing – original draft, Investigation. **Maureen Berg:** Writing – review & editing, Data curation. **Jeanne Harrison:** Writing – review & editing, Project administration, Data curation. **Peng Huang:** Writing – review & editing, Writing – original draft, Formal analysis. **James M. Leatherman:** Writing – review & editing, Project administration. **Michele Doucet:** Writing – review & editing, Investigation. **Rupashree Sen:** Writing – review & editing, Investigation. **Aditya Suru:** Writing – review & editing, Investigation. **Hongyan Cai:** Writing – review & editing, Project administration. **Jennifer Durham:** Writing – review & editing, Project administration. **Danijela Jelovac:** Writing – review & editing, Investigation. **Ashley Cimino-Mathews:** Writing – review &

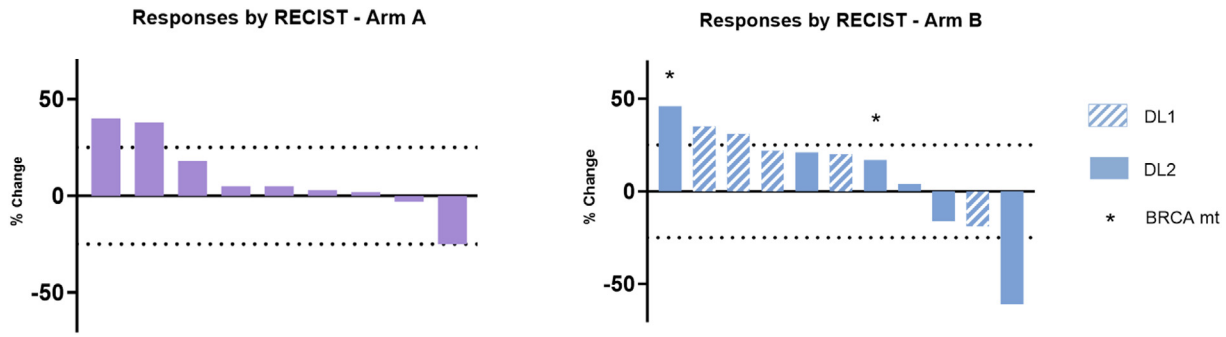

Fig. 2. Waterfall plots showing treatment responses by RECIST according to treatment arm.

editing, Investigation. **Christopher Cherry:** Writing – review & editing, Investigation. **Sudipto Ganguly:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Leisha A. Emens:** Writing – review & editing, Investigation, Conceptualization.

Data availability

Deidentified data will be made available by the authors on request.

Declaration of competing interest

As above, funding was provided by AstraZeneca to the institution in support of this study.

Unrelated to this manuscript:

Stephanie Gaillard has received research funding to institution from Compugen, Genentech/Roche, Clovis/Pharma&, Iovance, Tempest, Tesaro/GSK, Blueprint, Immunogen, Volastra, Beigene, GOG-Foundation, served as a consultant for Immunogen, Novartis, Verastem, and Compugen, participated on an Advisory Board for SignPath Pharma, and serves as a Phase I subcommittee chair for NRG Oncology.

Ashley Cimino-Mathews has received research funding to institution from Bristol-Myers Squibb.

Christopher Cherry is the owner of CMCC Consulting LLC.

Leisha A. Emens is a former employee of Ankyra Therapeutics, has received research funding to institution from AbbVie, AstraZeneca, Bolt Therapeutics, Bristol Meyers Squibb, Compugen, Corvus, CytomX, EMD Serono, Genentech, F Hoffman La Roche, Immune Onc, Merck, Next Cure, Silverback, Takeda, and Tempest, served as a consultant/advisor/speaker for AstraZeneca, BioLineRx, DNAMx, Genentech, F Hoffman La Roche, GPCR, Gilead, Immune Onc, Immunitas, Immutep, Lilly, Macrogenics, Mersana, and Shionogi, is a royalty and patent beneficiary with potential for royalties in the future from Molecuvax, and has publicly traded stocks/stock options from Ankyra Therapeutics.

The authors otherwise declare no conflicts of interest.

Acknowledgements

The authors would like to thank Richard D. Gross and Cathy Neuman for their philanthropic donation to the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, funds from which in part supported this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2025.01.015.

References

[1] R.L. Siegel, A.N. Giaquinto, A. Jemal, Cancer statistics, 2024, CA Cancer J. Clin. 74 (2024) 12–49.
[2] E. Pujade-Lauraine, S. Banerjee, S. Pignata, Management of platinum-resistant, relapsed epithelial ovarian cancer and new drug perspectives, J. Clin. Oncol. 37 (2019) 2437–2448.
[3] E. Pujade-Lauraine, K. Fujiwara, J.A. Ledermann, et al., Avelumab alone or in combination with chemotherapy versus chemotherapy alone in platinum-resistant or platinum-refractory ovarian cancer (JAVELIN ovarian 200): an open-label, three-arm, randomised, phase 3 study, Lancet Oncol. 22 (2021) 1034–1046.
[4] U.A. Matulonis, R. Shapira-Frommer, A.D. Santin, et al., Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study, Ann. Oncol. 30 (2019) 1080–1087.
[5] J. Hamanishi, M. Mandai, T. Ikeda, et al., Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer, J. Clin. Oncol. 33 (2015) 4015–4022.
[6] P.A. Konstantinopoulos, S. Waggoner, G.A. Vidal, et al., Single-arm phases 1 and 2 trial of niraparib in combination with pembrolizumab in patients with recurrent platinum-resistant ovarian carcinoma, JAMA Oncol. 5 (2019) 1141–1149.
[7] E.J. Lampert, A. Zimmer, M. Padget, et al., Combination of PARP inhibitor olaparib, and PD-L1 inhibitor durvalumab, in recurrent ovarian cancer: a proof-of-concept phase II study, Clin. Cancer Res. 26 (2020) 4268–4279.
[8] J.Y. Lee, B.G. Kim, J.W. Kim, et al., Biomarker-guided targeted therapy in platinum-resistant ovarian cancer (AMBITION; KGOG 3045): a multicentre, open-label, five-arm, uncontrolled, umbrella trial, J. Gynecol. Oncol. 33 (2022), e45.
[9] A.S. Zimmer, E. Nichols, A. Cimino-Mathews, et al., A phase I study of the PD-L1 inhibitor, durvalumab, in combination with a PARP inhibitor, olaparib, and a VEGFR1-3 inhibitor, cediranib, in recurrent women's cancers with biomarker analyses, J. Immunother. Cancer 7 (2019) 197.
[10] C.C. O'Sullivan, D.H. Moon, E.C. Kohn, et al., Beyond breast and ovarian cancers: PARP inhibitors for BRCA mutation-associated and BRCA-like solid tumors, Front. Oncol. 4 (2014) 42.
[11] T. Higuchi, D.B. Flies, N.A. Marjon, et al., CTLA-4 blockade synergizes therapeutically with PARP inhibition in BRCA1-deficient ovarian cancer, Cancer Immunol. Res. 3 (2015) 1257–1268.
[12] S. Adams, C. Muller, D. O'Malley, et al., RNAseq correlative biomarkers IFIT1B and VSTM5 predict progression free survival and clinical benefit in a multi-site phase I/II trial of Olaparib and tremelimumab for gBRCAm recurrent ovarian cancer (LBA 11), Gynecol. Oncol. 166 (2022) S60–S61.
[13] Z. Xiao, A.T. Mayer, T.W. Nobashi, et al., ICOS is an indicator of T-cell–mediated response to cancer immunotherapy, Cancer Res. 80 (2020) 3023–3032.
[18] J.D. Wolchok, H. Kluger, M.K. Callahan, et al., Nivolumab plus ipilimumab in advanced melanoma, N. Engl. J. Med. 369 (2013) 122–133.
[19] J. Liu, S. Gaillard, A.W. Hendrickson, et al., An open-label phase II study of dostarlimab (TSR-042), bevacizumab (bev), and niraparib combination in patients (pts) with platinum-resistant ovarian cancer (PROC): cohort a of the OPAL trial, Gynecol. Oncol. 162 (2021) S17–S18.
[20] G. Freyer, A. Floquet, O. Tredan, et al., 733P bevacizumab (Bev), olaparib (Ola) and durvalumab (Durva) in patients with recurrent advanced ovarian cancer (AOC): the GINECO BOLD study, Ann. Oncol. 32 (2021) S734–S735.
[21] N.A. Manieri, E.Y. Chiang, J.L. Grogan, TIGIT: a key inhibitor of the cancer immunity cycle, Trends Immunol. 38 (2017) 20–28.
[22] J.F. Liu, C. Herold, K.P. Gray, et al., Assessment of combined nivolumab and bevacizumab in relapsed ovarian cancer: a phase 2 clinical trial, JAMA Oncol. 5 (2019) 1731–1738.
[23] ClinicalTrials.gov: Testing the Addition of an Immunotherapy Drug, Tremelimumab, to the PARP Inhibition Drug, Olaparib, for Recurrent Ovarian, Fallopian Tube or Peritoneal Cancer (NCT04034927). Bethesda (MD): National Library of Medicine (US).