Safety, tolerability and efficacy of agonist anti-CD27 antibody (varlilumab) administered in combination with anti-PD-1 (nivolumab) in advanced solid tumors

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ABSTRACT
Background Phase 1/2 dose-escalation and expansion study evaluating varlilumab, a fully human agonist anti-CD27 mAb, with nivolumab in anti-PD-1/L1 naive, refractory solid tumors. Methods Phase 1 evaluated the safety of varlilumab (0.1–10 mg/kg) with nivolumab (3 mg/kg) administered once every 2 weeks. Phase 2 evaluated varlilumab regimens (3 mg/kg once every 2 weeks, 3 mg/kg once every 12 weeks, and 3 mg/kg once every 4 weeks) with nivolumab 240 mg once every 2 weeks in tumor-specific cohorts. Primary objective was safety; key clinical endpoints included objective response rate (ORR) and overall survival rate at 12 months (OS12). Exploratory objectives included determination of effects on peripheral blood and intratumoral immune signatures. Results 175 patients were enrolled (36 in phase I and 139 in phase 2). Phase 1 dose-escalation proceeded to the highest varlilumab dose level without determining a maximum tolerated dose. In phase 2, ORR were ovarian cancer 12.5%, squamous cell carcinoma of the head and neck, colorectal cancer 5%, and renal cell carcinoma 0%; GBM OS12 was 40.9%. Increased tumor PD-L1 expression was associated with improved clinical outcomes in a subset of patients, particularly ovarian cancer. Conclusion Varlilumab and nivolumab were well tolerated, without significant toxicity beyond that expected for each agent alone. Clinical activity was observed in patients that are typically refractory to anti-PD-1 therapy, however, overall was not greater than expected for nivolumab monotherapy. Treatment was associated with proinflammatory changes in the tumor microenvironment, particularly in ovarian cancer where the changes were associated with better clinical outcomes.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ Strategies to enhance the clinical activity of anti-PD-1 therapy include combinations with T cell costimulatory pathway agonists. CD27 is a key T cell costimulatory molecule that mediates T cell proliferation, activation, and effector function. This phase 1/2 clinical study combined nivolumab with the anti-CD27 agonist mAb varlilumab in patients with checkpoint-naive advanced solid tumors.

WHAT THIS STUDY ADDS
⇒ Treatment was well tolerated without unexpected toxicities. In phase 2, overall clinical activity in ovarian cancer, squamous cell carcinoma of the head and neck, colorectal cancer, renal cell carcinoma, and glioblastoma cohorts was not greater than expected for nivolumab monotherapy. However, interrogation of the tumor microenvironment demonstrated treatment-induced increases in CD8+ T cells and tumor PD-L1 expression associated with improved clinical outcomes in a subset of patients, particularly ovarian cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ This study provides evidence that varlilumab in combination with PD-1 blockade is safe and biologically active. Future investigation of combining CD27 costimulation with checkpoint blockade should aim to identify biomarkers of response.

INTRODUCTION
The costimulatory molecule CD27 is a member of the tumor necrosis factor receptor superfamily (TNFRSF7) and is constitutively


→ Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2022-005147).

This manuscript contains original material. Interim results of the study have been previously presented as follows: American Associate for Cancer Research (AACR) Annual Meeting (April 2016); Abstract #CT023/Poster. American Society of Clinical Oncology (ASCO) Annual Meeting (June 2017); Presentation. ASCO Annual Meeting (June 2018); Presentation. Society for NeuroOncology (SNO) Annual Meeting (Nov 2018); Abstract ID# ATIM-23/Poster.

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expressed on the majority of mature T cells. In the appropriate context of T cell receptor engagement, the interaction of CD27 with its ligand CD70 promotes T cell activation, proliferation, survival, maturation of effector capacity, and T cell memory. CD27 is also expressed on subsets of B cells and NK cells, where it plays roles in mediating B cell proliferation, germinal center formation and immunoglobulin production and NK cell cytolytic activity, respectively. The critical role of CD27 in regulating immune responses is highlighted by the observation that loss of function mutations in the molecule or its ligand are associated with persistent EBV viremia and a combined immunodeficiency syndrome.

Varililumab (CDX-1127) is a fully human agonist anti-CD27 monoclonal antibody (mAb) that activates CD27 expressing T cells. In vitro studies demonstrate that varililumab mediated costimulation is only effective in enhancing T cell responses in the context of CD3/TCR engagement. In human CD27 transgenic mice, varililumab demonstrated antitumor activity in several models. The antitumor activity of varililumab was enhanced by blocking the PD-1/PD-L1 pathway with an anti-PD-L1 mAb. The combination therapy synergized to increase CD8+ T cell expansion and effector function. The synergy was shown to be dependent on increases in cytotoxicity and proliferation gene expression programs mediated by PD-1/L1 blockade and CD27 agonism, respectively.

In a phase 1 clinical study, varililumab was shown to be well tolerated in patients with advanced solid and hematologic malignancies, without evidence of significant immune-related adverse events characteristic of immune checkpoint blockade. Biological activity was demonstrated, with acute and transient increases in serum cytokines and chemokines, increased effector memory T cells, and a marked and persistent decrease in Treg cells. Modest monotherapy clinical activity was observed, with a durable complete response (CR) of over 2 years in a patient with non-Hodgkin’s lymphoma and durable partial responses (PR) in two patients with renal cell carcinoma (RCC).

PD-1-based and PD-L1-based immunotherapy regimens have become a cornerstone therapy in oncology, with clinical benefit demonstrated in multiple tumor types. However, only a subset of patients responds to such therapy and responding patients may eventually relapse. Strategies to combine anti-PD-1 or anti-PD-L1 therapy with other modalities targeting non-redundant pathways may enhance antitumor immune responses and have the potential to increase the durability of responses and increase the percentage of responders.

As preclinical data support the concept of combining PD-1 blockade with agonist anti-CD27 mAb therapy as a complementary and effective combination to augment antitumor immune responses, this phase 1/2, open-label study was conducted to assess the safety, pharmacokinetics, pharmacodynamics, and activity of varililumab when administered in combination with nivolumab, an anti-PD-1 mAb, to patients with advanced refractory solid tumors.

**METHODS**

**Patients**

The study was open to patients with one of the following unresectable and/or metastatic histologically diagnosed tumors: in phase 1, non-small cell lung cancer (NSCLC), melanoma, colorectal cancer (CRC), squamous cell carcinoma of the head and neck (SCCHN) or ovarian cancer, and in Phase 2, CRC, SCCHN, glioblastoma (GBM), clear cell RCC or ovarian cancer (including fallopian tube or primary peritoneal carcinoma). Patients could have no more than five prior anticancer regimens for advanced (recurrent, locally advanced or metastatic) disease and no prior therapy with an anti-CD27, anti-PD-1, anti-PD-L1, or anti-PD-L2 antibody. Anti-CTLA-4 antibody or any other antibody targeting T cell check point or co-stimulation pathways must have been discontinued at least 3 months prior to the planned start of study treatment. Additional eligibility requirements included males or females ≥18 years of age; documented progressive disease; measurable (target) disease by RECIST 1.1 criteria or Immunotherapy Radiologic Assessment in Neuro-Oncology (iRANO) criteria for GBM; life expectancy ≥12 weeks; Eastern Cooperative Oncology Group performance status of 0 or 1; adequate organ function; and no history of autoimmune disease. Patients with GBM must have received radiotherapy and temozolomide and could not be receiving dexamethasone doses ≥2 mg daily. Protocol eligibility requirements closely followed those for prior nivolumab monotherapy studies at the time the study was conducted, in order to provide a basis to compare safety and activity in the selected patient populations.

An independent data review committee was charged with approving dose-escalation in phase 1 and reviewing safety data throughout the course of the study.

**Study design and treatment**

This was a phase 1/2 study which consisted of a dose-escalation phase 1, followed by tumor-specific phase 2 cohorts. The tumor types and patient populations selected were intended to represent varying degrees of responsiveness to nivolumab monotherapy known at the time the study was designed. In both phase 1 and phase 2, combination treatment was administered for four cycles, followed by maintenance nivolumab.

In phase 1, varililumab was administered on a once every 2 weeks schedule at three dose levels (0.1, 1.0, and 10 mg/kg) in combination with nivolumab (3 mg/kg once every 2 weeks). Initially, six patients were enrolled into a cohort and if three evaluable patients completed 12 weeks; ≥6 months; on April 16, 2024 by guest. Protected by copyright. http://jitc.bmj.com/
240 mg administered once every 2 weeks dosing regimen. To study the hypothetical potential for immune exhaustion associated with a once every 2 weeks dosing regimen and tonic CD27 signaling, alternative regimens were explored in ovarian and SCCHN patients. The ‘A’ cohorts explored varilumab 3 mg/kg once every 12 weeks, and the ‘B’ cohorts explored varilumab 0.3 mg/kg once every 4 weeks; nivolumab 240 mg was administered once every 2 weeks. Because of the hypothetical possibility that the combination therapy could promote or exacerbate cerebral edema, the phase 2 GBM cohort first enrolled 6 patients to determine the safety of the treatment before enrolling the remaining patients in the cohort. Enrollment into the SCCHN ‘A’ and RCC cohorts were not completed due to slow recruitment.

Varilumab was administered as a 90 min intravenous infusion without prophylactic medication (unless clinically indicated). After a break of at least 30 min, nivolumab was given as a 60 min infusion for patients in phase 1 and as a 30 min infusion for patients in phase 2. Treatment was continued until disease progression or intolerance to the therapy was documented.

Restaging assessments were performed once every 8 weeks, and tumor responses were determined by the investigator in accordance with RECIST 1.1, except for patients with GBM who were assessed using iRANO criteria. The primary objective for phase 1 was to determine the safety and tolerability of varilumab in combination with nivolumab. The primary end point of phase 2 was to determine the objective response rate (ORR) of the combinations as determined by RECIST for CRC, RCC, SCCHN, and ovarian cancer and determine the overall survival at 12 months (OS12) for GBM. Exploratory end points included analyzing the association of measured biomarkers with antitumor activity.

**Pharmacokinetic and biomarker assessments**

Pharmacokinetics: Quantitative results were determined by immunoassay in which recombinant CD27 was immobilized to capture varilumab in human serum samples, which was detected by subsequent addition of goat anti-human antibody-HRP conjugate, and visualization at 450 nm with a lower limit of sensitivity at 1 µg/mL.

Immunohistochemistry: Formalin-fixed, paraffin embedded samples were processed and stained at a commercial laboratory (Mosaic Laboratories) and read by a certified pathologist. PD-L1 staining was performed using the 28-8 pharmDX assay. Positive staining in tumor was defined as linear staining over the plasma membrane that could be partial or complete on greater than or equal to 1% of tumor cells in a minimum of 100 viable tumor cells. Staining for other T cell markers (CD3, CD8, CD4, FOXP3) was performed using qualified assays and reported as % positive tumor cells.

Flow cytometry: Immunophenotyping of circulating lymphocytes (in whole blood samples) was performed using validated flow cytometry panels at a commercial laboratory (Covance Central Laboratory Services).

Whole-exome sequencing: Tumor biopsies were provided to Tempus Labs for whole-exome sequencing and analysis. The tumor mutational burden (TMB) was calculated as somatic variants at allele fractions above 10% and reported as total number of non-synonymous mutations per Mb.

**Statistics**

At the time of this study initiation, with the exception of RCC, minimal data on the expected clinical activity of anti-PD-1 or anti-PD-L1 monotherapy was available for several of the tumor types chosen for study. Therefore, the estimate of the ORR was calculated for each phase 2 cohort (except GBM) based on the maximum likelihood estimator (ie, crude proportion of patients whose best overall response is CR or PR) and was accompanied by 2-sided 95% exact binomial CIs. The OS12 rate and its two-sided 95% CI was estimated for the GBM cohort, where the OS12 rate was based on the Kaplan-Meier (KM) method and the CI was estimated by log-log transformation. The duration of objective response was summarized descriptively using the KM method. The progression-free survival (PFS) was summarized descriptively using the KM method and OS was assessed by KM plots. Biomarker data was analyzed using two-tailed paired t-test.

For CRC, SCCHN, and ovarian phase 2 cohorts, eighteen evaluable patients were to be enrolled per cohort to have the maximum width of the 95% CI of the estimated ORR to be no greater than 48%. For the RCC phase II cohort, 25 evaluable patients were to be enrolled to have the maximum width of the 95% CI of the estimated ORR to be no greater than 41%. If 10 responses were observed (ie, the estimated ORR is 40%) among the 25 enrolled patients, then the lower limit of the two-sided 95% CI of the estimated ORR was 21%. For the GBM phase 2 cohort, 20 patients were to be enrolled to have the maximum width of the 95% CI of the estimated OS12 rate to be no greater than 44%. If 10 patients survived at least 1 year (ie, the estimated OS12 rate is 50%) among the 20 enrolled patients, then the lower limit of the two-sided 95% CI of the estimated OS12 rate would be 28%. The CI calculation for OS12 rate was based on the Greenwood formula. The OS12 rate was estimated as 28.8%. These calculations were based on the Clopper-Pearson method for exact CIs.

**RESULTS**

**Patient characteristics and disposition**

One hundred and seventy-five patients with advanced refractory solid tumors were enrolled at 15 centers from February 11, 2015 to December 12, 2018.

In the phase 1 portion of the study, 36 patients (0.1 mg/kg, n=6; 1.0 mg/kg, n=13; 10 mg/kg, n=13), were enrolled, received study treatment and were evaluable for response (online supplemental table S1). The most frequent tumor type in phase 1 was CRC (n=21), followed by ovarian (n=8), melanoma (n=4), and SCCHN (n=3).
The majority (97%) of patients had stage IV disease. Most patients (89%) previously received at least one anticancer treatment regimen with 75% receiving ≥3 prior regimens. None had received prior anti-PD-1 or anti-PD-L1 therapy. A total of nine (25%) patients completed all four cycles of combination therapy per protocol. Twenty-seven (75%) did not complete all 4 cycles of combination therapy, due to progressive disease in 17 (47%), adverse events in 4 (11%) and other reasons in 6 (17%).

In the phase 2 portion of the study, 139 patients were enrolled (table 1). All received study treatment and 133 (96%) patients were evaluable for response. Of the response evaluable patients in phase 2 the most frequent tumor type was ovarian cancer (n=56), followed by SCCHN (n=24), GBM (n=22), CRC (n=21), and RCC (n=14). Ninety-five per cent of patients had stage IV disease, excluding the GBM patients. All patients (100%) received at least one anticancer treatment regimen previously, with 49% receiving ≥3 prior regimens. A majority (90%) received prior chemotherapy and 6% had prior immunotherapy (cytokines or Toll-like receptor agonist); none had received prior anti-CTLA-4, anti-PD-1 or anti-PD-L1 therapy. Previous therapy by tumor type was consistent with standard-of-care at the time the study was conducted and included chemotherapy (100%) in CRC; platinum-based chemotherapy in ovarian cancer (100%); chemotherapy (100%) and immunotherapy (8%) in SCCHN; protein kinase inhibitor (100%) and immunotherapy (7%) in RCC, and surgery and chemoradiation (100%) in GBM. Out of the 139 enrolled patients, 26 (18.7%) patients completed all 4 cycles of combination therapy per protocol and 113 (81.3%) did not. Reasons for treatment discontinuation are as follows: progressive disease 78 (56.1%), symptomatic deterioration 15 (10.8%), adverse events 11 (7.9%) and other reasons 9 (6.4%).

Safety
All 175 patients in the study reported a treatment emergent adverse event (TEAE), with the most common being fatigue (42%), nausea (37%), vomiting (27%), pruritus (25%), and constipation, cough, decreased appetite, diarrhea, and dyspnea (21% each). Overall, most TEAEs (84%) were grade 1 or 2. Grade 3 or 4 TEAEs that occurred in ≥10% of all patients in phase 1 were lymphopenia (22.2%), blood alkaline phosphatase increased (13.9%), blood bilirubin increased (13.9%), and fatigue.

Table 1  Baseline characteristics phase 2

<table>
<thead>
<tr>
<th>Tumor type, n (%)</th>
<th>CRC n=21</th>
<th>Ovarian n=58</th>
<th>SCCHN n=24</th>
<th>GBM n=22</th>
<th>RCC n=14</th>
<th>All n=139</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53.0</td>
<td>64.0</td>
<td>65.5</td>
<td>58.0</td>
<td>63.5</td>
<td>62.0</td>
</tr>
<tr>
<td>Range</td>
<td>40.0, 72.0</td>
<td>40.0, 89.0</td>
<td>34.0, 77.0</td>
<td>35.0, 75.0</td>
<td>46.0, 78.0</td>
<td>34.0, 89.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (52.4)</td>
<td>0 (0.0)</td>
<td>17 (70.8)</td>
<td>15 (68.2)</td>
<td>11 (78.6)</td>
<td>60 (43.2)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (47.6)</td>
<td>58 (100)</td>
<td>7 (29.2)</td>
<td>7 (31.8)</td>
<td>3 (21.4)</td>
<td>79 (56.8)</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10 (47.6)</td>
<td>20 (34.5)</td>
<td>9 (37.5)</td>
<td>8 (36.4)</td>
<td>9 (64.3)</td>
<td>56 (40.3)</td>
</tr>
<tr>
<td>1</td>
<td>11 (52.4)</td>
<td>38 (65.6)</td>
<td>15  62.5</td>
<td>14 (63.6)</td>
<td>5 (35.7)</td>
<td>83 (59.7)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>III</td>
<td>0 (0.0)</td>
<td>5 (8.6)</td>
<td>1 (4.2)</td>
<td>N/A</td>
<td>0 (0.0)</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>IV</td>
<td>21 (100)</td>
<td>33 (56.9)</td>
<td>23 (95.8)</td>
<td>N/A</td>
<td>14 (100)</td>
<td>111 (79.9)</td>
</tr>
<tr>
<td>No of prior regimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>2 (9.6)</td>
<td>17 (29.3)</td>
<td>19 (79.2)</td>
<td>20 (90.9)</td>
<td>13 (92.9)</td>
<td>71 (51.1)</td>
</tr>
<tr>
<td>3–4</td>
<td>10 (47.6)</td>
<td>20 (34.5)</td>
<td>4 (16.7)</td>
<td>2 (9.1)</td>
<td>1 (7.1)</td>
<td>37 (26.6)</td>
</tr>
<tr>
<td>≥5</td>
<td>9 (42.9)</td>
<td>21 (36.2)</td>
<td>1 (4.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>31 (22.3)</td>
</tr>
<tr>
<td>Prior systemic treatment</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>21 (100)</td>
<td>58 (100)</td>
<td>24 (100)</td>
<td>22 (100)</td>
<td>0 (0.0)</td>
<td>125 (89.9)</td>
</tr>
<tr>
<td>Biologics</td>
<td>21 (100)</td>
<td>30 (51.7)</td>
<td>13 (54.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>64 (46.0)</td>
</tr>
<tr>
<td>Kinase inhibitor</td>
<td>6 (28.6)</td>
<td>4 (6.9)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>14 (100)</td>
<td>24 (17.3)</td>
</tr>
<tr>
<td>Immunotherapy*</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (8.3)</td>
<td>3 (13.6)</td>
<td>1 (7.1)</td>
<td>8 (5.8)</td>
</tr>
</tbody>
</table>

* Cytokines or Toll-like receptor agonist.
CRC, colorectal cancer; ECOG, Eastern Cooperative Oncology Group; GBM, glioblastoma; N/A, not available; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck.
(11.1%). In phase 2, the only grade 3 or 4 TEAEs that occurred in ≥10% of all patients was anemia (10.8%).

Dose-escalation in phase 1 proceeded to the highest varililumab dose level tested in combination with nivolumab without identifying the maximum tolerated dose (MTD). For treatment-related adverse events (TRAE), there was a trend for a higher percentage of patients experiencing lymphopenia, increased alanine aminotransferase, arthralgia, and pruritus in the 1 mg/kg and 10 mg/kg varililumab phase 1 cohorts compared with the 0.1 mg/kg cohort (online supplemental table S2). A higher percentage of infusion reactions occurred in the varililumab 0.1 mg/kg cohort (50%) compared with the 1.0 mg/kg and 10 mg/kg cohorts (13.3% and 20%, respectively). There were 2 DLTs in the phase 1 part of the study, with both events attributed to varililumab and nivolumab. One patient with ovarian cancer who was treated with varililumab at 10 mg/kg experienced grade 4 hepatitis which resolved after corticosteroid treatment and one patient with CRC who was treated with varililumab at 1.0 mg/kg experienced grade 3 pruritic rash on all extremities which resolved with topical corticosteroid treatment.

Overall, 100 (72%) patients reported adverse events considered related to varililumab and nivolumab (table 2). In phase 2, the most frequently reported TRAEs considered related to both study drugs were rash (27.3%), fatigue (15.1%), pruritus (14.4%), and nausea (7.9%). Grade ≥3 adverse events considered related to varililumab were reported in 39 (22%) patients with all except one also considered related to nivolumab. In addition, four patients reported grade 3 TRAEs that were related

### Table 2  Phase 2 treatment-related AEs (≥10% of patients) by preferred term

<table>
<thead>
<tr>
<th>Patients with at least 1</th>
<th>CRC n=21</th>
<th>Ovarian n=58</th>
<th>SCCHN n=24</th>
<th>GBM n=22</th>
<th>RCC n=14</th>
<th>All n=139</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAEs related to varililumab and nivolumab, n (%)</td>
<td>16 (76.2)</td>
<td>39 (67.2)</td>
<td>18 (75.0)</td>
<td>18 (81.8)</td>
<td>9 (64.3)</td>
<td>100 (71.9)</td>
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<tr>
<td>Blood and lymphatic system disorders</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>3 (14.3)</td>
<td>2 (3.4)</td>
<td>1 (4.2)</td>
<td>1 (4.5)</td>
<td>1 (7.1)</td>
<td>8 (5.8)</td>
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<tr>
<td>Endocrine disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>0</td>
<td>0</td>
<td>2 (8.3)</td>
<td>3 (13.6)</td>
<td>0</td>
<td>5 (3.6)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1 (4.8)</td>
<td>0</td>
<td>3 (12.5)</td>
<td>1 (4.5)</td>
<td>1 (7.1)</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
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<td></td>
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<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>2 (3.4)</td>
<td>1 (4.2)</td>
<td>0</td>
<td>0</td>
<td>3 (2.2)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (9.0)</td>
<td>3 (5.2)</td>
<td>1 (4.2)</td>
<td>1 (4.5)</td>
<td>1 (7.1)</td>
<td>10 (7.2)</td>
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<tr>
<td>Dry mouth</td>
<td>0</td>
<td>3 (5.2)</td>
<td>3 (12.5)</td>
<td>0</td>
<td>0</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (9.5)</td>
<td>4 (6.9)</td>
<td>2 (8.3)</td>
<td>2 (9.1)</td>
<td>1 (7.1)</td>
<td>11 (7.9)</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>2 (9.5)</td>
<td>11 (19.0)</td>
<td>3 (12.5)</td>
<td>2 (9.1)</td>
<td>3 (21.4)</td>
<td>21 (15.1)</td>
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<tr>
<td>Injury, poisoning and procedural complications</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Infusion-related reactions</td>
<td>3 (14.3)</td>
<td>4 (6.9)</td>
<td>0</td>
<td>2 (9.1)</td>
<td>0</td>
<td>9 (6.5)</td>
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<tr>
<td>Investigations</td>
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<tr>
<td>Alanine aminotransferase increased</td>
<td>0</td>
<td>4 (6.9)</td>
<td>2 (8.3)</td>
<td>2 (9.1)</td>
<td>0</td>
<td>8 (5.8)</td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>0</td>
<td>4 (6.9)</td>
<td>2 (8.3)</td>
<td>1 (4.5)</td>
<td>0</td>
<td>7 (5.0)</td>
</tr>
<tr>
<td>Lipase increased</td>
<td>0</td>
<td>4 (6.9)</td>
<td>2 (8.3)</td>
<td>1 (4.5)</td>
<td>0</td>
<td>7 (5.0)</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1 (4.8)</td>
<td>4 (6.9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (3.6)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>2 (9.5)</td>
<td>5 (8.6)</td>
<td>1 (4.2)</td>
<td>3 (13.6)</td>
<td>0</td>
<td>11 (7.9)</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pruritus</td>
<td>4 (19.0)</td>
<td>8 (13.8)</td>
<td>2 (8.3)</td>
<td>4 (18.2)</td>
<td>2 (14.3)</td>
<td>20 (14.4)</td>
</tr>
<tr>
<td>Rash*</td>
<td>8 (38.0)</td>
<td>14 (24.1)</td>
<td>3 (12.5)</td>
<td>6 (27.2)</td>
<td>7 (50.0)</td>
<td>38 (27.3)</td>
</tr>
</tbody>
</table>

*Includes preferred terms rash and rash maculopapular.

AE, adverse event; CRC, colorectal cancer; GBM, glioblastoma; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; TEAE, treatment emergent adverse event.
to nivolumab only (elevated lipase, abdominal pain, acute interstitial nephritis and lymphocyte decreased). The most common grade ≥3 TRAEs were lymphopenia, reported by 10 (6%) patients (and considered related to varlilumab and nivolumab combination in all cases) and increased lipase and lymphocyte count decrease, both reported by nine (5%) patients (with eight cases considered related to varlilumab and nivolumab, and one case related to nivolumab alone). Other drug-related grade ≥3 TRAEs that occurred in ≥1% of patients were alanine aminotransferase increase (1%), aspartate aminotransferase increase (1%), amylase increase (3%), and maculopapular rash (1%). There were two deaths in the phase 2 part of the study that were attributed to varlilumab and nivolumab. One patient with CRC died due to pneumonitis and one patient with SCCHN died due to an acute cardiac event. There were no cases of drug-related cerebral edema in the GBM cohort.

**Antitumor activity**

**Phase 1**

The ORR in the varlilumab 0.1 mg/kg, 1 mg/kg, and 10 mg/kg phase 1 dose cohorts in combination with nivolumab was 0, 6.7%, and 6.7%, respectively (online supplemental table S3). One patient with immunotherapy naive CRC refractory to two prior lines of chemotherapy based regimens was treated at the varlilumab 1 mg/kg dose level and experienced a PR with 95% decrease in target lesions that was ongoing at 40.5 months at the time of study closure. While immunohistochemical analysis performed prior to study entry suggested mismatch repair deficiency, tumor genomic analysis demonstrated a high mutational burden, potentially due to mutations observed in MLH-1 and MSH-6, together suggesting the tumor was mismatch repair deficient. One patient with SCCHN who was previously treated with three prior lines of chemotherapy was treated at the varlilumab 10 mg/kg dose level and experienced a PR for 16 weeks. The disease control rate (DCR), defined as CR, PR, or SD ≥3 months, in the varlilumab 0.1 mg/kg, 1 mg/kg, and 10 mg/kg phase 1 dose cohorts was 16.7%, 33.3%, and 33.3%, respectively.

**Phase 2**

In phase 2, 13 patients (10%) achieved an objective response, including 2 patients in the GBM cohort. Response rates by tumor type are shown in table 3. Response duration ranged from 16 to 88 weeks, with eight responses ongoing at the time of study closure. Median PFS and OS by tumor type are provided in online supplemental tables S3 and S4.

Ovarian cancer was the most extensively studied tumor type and included cohorts that examined multiple varlilumab dosing regimens. Across all varlilumab dosing regimens, the ORR in ovarian cancer was 12.5% (95% CI 5.2% to 24.1%). Of the seven patients with confirmed responses, five had serous adenocarcinoma and two had clear cell histology. By varlilumab dosing regimens, the ORR was 12% (95% CI 1.5% to 36.4%) in the 3 mg/kg once every 2 weeks regimen, 16% (85% CI 3.4% to 39.6%) in the 3.0 mg/kg once every 12 weeks regimen, and 10% (95% CI 1.2% to 31.7%) in the 0.3 mg/kg once every 4 weeks regimen. The DCR was 47% in the 3 mg/kg once every 2 weeks regimen, 32% in the 3.0 mg/kg once every 12 weeks regimen, and 20% in the 0.3 mg/kg once every 4 weeks regimen. Data from the SCCHN cohorts, which also included different varlilumab dosing cohorts, demonstrated an overall ORR of 12.5% (95% CI 2.7% to

### Table 3 Phase 2 objective response rates

<table>
<thead>
<tr>
<th>Cohorts (n)</th>
<th>Tumor types</th>
<th>Best overall response, n (%)</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>NE</th>
<th>ORR* n (%)</th>
<th>95% CI</th>
<th>DCR n (%)</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>3 (n=20)</td>
<td>CRC</td>
<td></td>
<td>0</td>
<td>1 (5.0)†</td>
<td>4 (20.0)</td>
<td>14 (70.0)</td>
<td>1 (5.0)</td>
<td>1 (5.0)</td>
<td>(0.1 to 24.9)</td>
<td>3 (15.0)</td>
<td>(3.2 to 37.9)</td>
</tr>
<tr>
<td>4 (n=17)</td>
<td>Ovarian</td>
<td></td>
<td>0</td>
<td>2 (11.8)</td>
<td>6 (35.3)</td>
<td>4 (23.5)</td>
<td>5 (29.4)</td>
<td>2 (11.8)</td>
<td>(1.5 to 36.4)</td>
<td>8 (47.1)</td>
<td>(23 to 72.2)</td>
</tr>
<tr>
<td>4A (n=19)</td>
<td>Ovarian</td>
<td></td>
<td>0</td>
<td>3 (15.8)</td>
<td>4 (21.1)</td>
<td>9 (47.4)</td>
<td>3 (15.8)</td>
<td>3 (15.8)</td>
<td>(3.4 to 39.6)</td>
<td>6 (31.6)</td>
<td>(12.6 to 56.6)</td>
</tr>
<tr>
<td>4B (n=20)</td>
<td>Ovarian</td>
<td></td>
<td>0</td>
<td>2 (10.0)</td>
<td>6 (30.0)</td>
<td>11 (55.0)</td>
<td>1 (5.0)</td>
<td>2 (10.0)</td>
<td>(1.2 to 31.7)</td>
<td>4 (20.0)</td>
<td>(5.7 to 43.7)</td>
</tr>
<tr>
<td>Total (n=56)</td>
<td>Ovarian</td>
<td></td>
<td>0</td>
<td>7 (12.5)</td>
<td>16 (28.6)</td>
<td>24 (42.9)</td>
<td>9 (16.1)</td>
<td>7 (12.5)</td>
<td>(5.2 to 24.1)</td>
<td>18 (32.1)</td>
<td>(20.3 to 46)</td>
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<tr>
<td>5 (n=6)</td>
<td>SCCHN</td>
<td></td>
<td>0</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
<td>(0.4 to 64.1)</td>
<td>3 (50.0)</td>
<td>(11.8 to 88.2)</td>
</tr>
<tr>
<td>5A (n=1)</td>
<td>SCCHN</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (100)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>(0 to 97.5)</td>
<td>0 (0.0)</td>
<td>(0 to 97.5)</td>
</tr>
<tr>
<td>5B (n=17)</td>
<td>SCCHN</td>
<td></td>
<td>1 (5.0)</td>
<td>1 (5.0)</td>
<td>6 (35.3)</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
<td>2 (11.8)</td>
<td>(1.5 to 36.4)</td>
<td>5 (29.4)</td>
<td>(10.3 to 56)</td>
</tr>
<tr>
<td>Total (n=24)</td>
<td>SCCHN</td>
<td></td>
<td>1 (4.2)</td>
<td>2 (8.3)</td>
<td>8 (33.3)</td>
<td>8 (33.3)</td>
<td>5 (20.8)</td>
<td>3 (12.5)</td>
<td>(2.7 to 32.4)</td>
<td>8 (33.3)</td>
<td>(15.6 to 55.3)</td>
</tr>
<tr>
<td>6 (n=20)</td>
<td>GBM</td>
<td></td>
<td>0</td>
<td>2 (10.0)</td>
<td>4 (20.0)</td>
<td>13 (65.0)</td>
<td>1 (5.0)</td>
<td>2 (10.0)</td>
<td>(1.2 to 31.7)</td>
<td>6 (30.0)</td>
<td>(11.9 to 54.3)</td>
</tr>
<tr>
<td>7 (n=13)</td>
<td>RCC</td>
<td></td>
<td>0</td>
<td>0</td>
<td>5 (38.5)</td>
<td>7 (53.8)</td>
<td>1 (7.7)</td>
<td>0 (0.0)</td>
<td>(0 to 24.7)</td>
<td>4 (30.8)</td>
<td>(9.1 to 61.4)</td>
</tr>
</tbody>
</table>

*Only confirmed responses are included.
†The patient was determined to have microsatellite instability-high tumor prior to enrolling in the study.
CR, complete response; CRC, colorectal cancer; DCR, disease control rate; GBM, glioblastoma; NE, not evaluable; ORR, objective response rate; PD, progressive disease; PR, partial response; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; SD, stable disease.
32.4%), with an ORR of 16.7% (95% CI 0.4% to 64.1%) in the 3 mg/kg once every 2 weeks regimen and 11.8% (85% CI 1.5% to 36.4%) in the 0.3 mg/kg once every 4 weeks regimen (only one patient was enrolled in the varilumab 3 mg/kg once every 12 weeks regimen). DCR was 50% in the 3 mg/kg once every 2 weeks regimen and 29.4% in the 3.0 mg/kg once every 12 weeks regimen.

The OS rate at 12 months in the GBM cohort was 40.9% (95% CI 20.9% to 60.1%). An exploratory analysis of the relationship of clinical benefit to MGMT methylation status in GBM patients revealed a trend for greater clinical benefit in those patients with unmethylated MGMT. The OS12 in patients with methylated (n=5) and unmethylated (n=17) MGMT status was 20% (95% CI 0.8% to 58.2%) and 50% (95% CI 24.5% to 71.0%), respectively, and median OS was 9.1 months (95% CI 6.6 to 14.8) and 12.5 months (95% CI 7.0 to 27.2), respectively. Median PFS was 1.9 months for methylated and unmethylated MGMT status (95% CI 1.2 to 7.4 and 95% CI 1.6 to 11.3, respectively).

**Pharmacokinetic evaluations and association with clinical activity**

Analysis of patients in phase 1 and phase 2 demonstrated that varilumab serum levels administered once every 2 weeks were dose proportional between 0.1 and 10 mg/kg (online supplemental figure S1).

Pharmacokinetic analyses were performed in patients treated with three different varilumab regimens. The 3 mg/kg once every 2 weeks regimen demonstrated high and consistent exposure with accumulating varilumab levels after each of five doses administered. With the modified dosing regimens of 3 mg/kg once every 12 weeks and 0.3 mg/kg once every 4 weeks, varilumab serum levels completely cleared prior to subsequent dosing.

**Biomarker analysis and correlations**

**Peripheral blood analysis**

Changes in peripheral blood cells were similar across tumor types and varilumab doses. The most significant findings were rapid and sustained decreases in the number of circulating CD4+ (median: −66% at week 8) and regulatory T cells (median: −71% at week 8) (figure 1). Among the CD4+ and CD8+ T cells the greatest decrease was observed in naive and central memory subtypes, compared with effector memory or differentiated effector memory (data not shown).

Rapid, and transient increases in serum cytokines were observed following treatment, particularly for chemokines: MIP-1β (CCL4), MCP-1 (CCL2), IP-10 (CXCL10), and MIG (CXCL9) (figure 2). The magnitude of change in MIP-1β was significantly higher in patients with SD or PR relative to patients with PD.

**Tumor biopsy analysis**

One hundred and fifty patients had PD-L1 results for their pretreatment biopsy, of which 50 patients had PD-L1 positive results (1% or greater positive tumor cells). No clear correlation of baseline pretreatment PD-L1 expression and ORR was observed (figure 3 and online supplemental table S6); however, higher baseline level of CD8+ T cells and to a lesser extent CD4+ T cells were associated with better clinical outcome (figure 3).

Paired biopsies were available from patients in the ovarian cancer (n=23) and CRC (n=26) cohorts. A significant increase in tumor PD-L1 expression and T cell infiltrates (CD8+, CD4+ and FOXP3+ cells) was observed in the on-study biopsy (figure 4A) in the tumors from ovarian cancer patients. Similar changes were not observed in the paired biopsies from patients with CRC.

The changes in the tumor microenvironment in the on-study biopsies suggested a correlation with better clinical outcomes in the ovarian cancer cohort (figure 4B). Absolute increase in CD4+ T cells of 5% or greater correlated with an improved PFS (9.2 months vs 2.2 months, p=0.003), while similar increases in PD-L1 (7.4 months vs 3.5 months, p=0.07) and CD8+ T cells (7.4 months vs 3.1 months, p=0.08) also trended toward improved PFS. FOXP3+ cells, while increased over baseline, remained low with <5% increase. Analysis of other checkpoints/exhaustion markers across these patients revealed similar increases in TIM-3 and LAG-3 positive cells in both responder and non-responders (online supplemental figure S2).

Whole-exome tumor sequencing from ovarian cancer patients with PR (n=6) or PD (n=6) revealed that the mean TMB for the two groups was 2.85 mutations per Megabase (1.6–5.7) and 2.68 mutations per Megabase (1.1–4.3), respectively. Most tumor samples harbored P53 mutations; no clear pattern was associated with mutation of known significance and in one or more additional genes.

**DISCUSSION**

Combining immune checkpoint inhibitor therapy with immune costimulation therapy is a potential strategy for enhancing cancer immunotherapy efficacy. This phase 1/2 study of the agonist anti-CD27 mAb varilumab administered with the anti-PD-1 mAb nivolumab in patients with immune checkpoint therapy naïve advanced metastatic disease demonstrated that the combination was well tolerated and biologically active. Overall, the clinical activity in each of the phase 2 indications studied, ovarian cancer, CRC, RCC, SCCHN, and GBM, was not clearly distinguishable from the experience with nivolumab monotherapy. However, exploratory analyses identified specific patient subsets that may have derived additional clinical benefit from the combination therapy.

Treatment with varilumab in combination with nivolumab was without significant toxicity beyond that expected for each agent alone and the addition of varilumab did not appear to increase the incidence or severity of adverse events associated with nivolumab monotherapy. Dose-escalation of varilumab up to 10 mg/kg in combination with nivolumab did not exceed the MTD.
The primary clinical objective in the phase 2 part of the study was ORR in each of the tumor histologies except GBM, where the primary objective was OS12. For ovarian cancer, SCCHN, CRC, and RCC, ORR was 12.5%, 12.5, 5%, and 0, respectively. The OS12 and ORR in GBM was 40.9% and 10%, respectively. These results are comparable to that seen for anti-PD-1 or anti-PD-L1 monotherapy, with the exception of RCC.19–23 The absence of any objective responses in the RCC cohort was surprising given that nivolumab has a 25% ORR in the same patient population and that varilumab had demonstrated activity in this tumor type,12 but may have been due to the small number of patients enrolled into that cohort (n=14). The one PR observed in the Phase 2 CRC cohort was a patient with microsatellite instability-high tumor.

Most of the phase 2 cohorts were treated with varilumab 3 mg/kg once every 2 weeks, a dose level and regimen chosen because it was intermediate between the 1 mg/kg and 10 mg/kg Q2W regimen studied in phase 1, in which both regimens demonstrated comparable biological and clinical activity, and 3 mg/kg once every 2 weeks was expected to be saturating over the dosing interval. Additionally, patients in ovarian and SCCHN cohorts were assigned to alternative varilumab regimens in order to compare persistent exposure at the varilumab 3 mg/kg dose level with less frequent dosing regimens (varilumab 0.3 mg/kg once every 4 weeks or 3.0 mg/kg once every 12 weeks) to address the potential concern that chronic CD27 stimulation may lead to T cell exhaustion.24 ORR and DCR appeared to favor the 3 mg/kg once every 2 weeks regimen suggesting that chronic CD27 stimulation was not detrimental or inducing T cell exhaustion. This is further supported by two PRs observed in the dose-escalation at the highest varilumab dose of 10 mg/kg once every 2 weeks.

Treatment-related changes in peripheral blood cells and cytokines were consistent with varilumab monotherapy. A profound and sustained decrease in circulating T cells...
predominated by CD4+ T cells and Tregs was observed. These cell populations have high CD27 expression and the mechanism for their enhanced depletion from peripheral blood is not clear, though it could involve effector functions of varilumab, which has an unmodified IgG1 backbone. Multiplex analysis of serum showed

Figure 2 Changes in circulating chemokines during treatment and association with outcome. (A) Serum concentration of chemokines (D1 sample is approximately 2.5 hours postinfusion). (B) Magnitude of MIP-1β increase within 24 hours. (Increases at D1 and/or D2) are associated with better clinical outcome. PD, progressive disease; PR, partial response; SD, stable disease.

Figure 3 Analysis of PD-L1 and T cell infiltrates on pretreatment biopsies and association with outcome. All patients with available immunohistochemistry data included. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
a robust and transient increase of chemokines indicative of immune activation and interferon signaling. An intriguing correlation was observed in the magnitude of the MIP-1β response within 24 hours of treatment and clinical outcome, with significantly higher increases in MIP-1β in patients with PR or SD relative to PD. MIP-1β is made by a variety of activated immune cells, is chemoattractant for dendritic cells, T and NK cells, and localized

Figure 4  Changes in PD-L1 and T cell infiltrates on paired biopsies and association with outcome. (A) Tumor cell expression of PD-L1 and % CD8+, CD4+, and FOXP3+ cells on paired samples from baseline and on-study biopsy (approximately 4 weeks post first dose) was performed by IHC. (B) Kaplan-Meier curves for PFS comparing ovarian patients with or without ≥5% absolute increase in PD-L1+ tumor cells, and CD8+ and CD4+ T cells. CRC, colorectal cancer; IHC, immunohistochemistry, PFS, progression-free survival.
expression in tumor has therapeutic effects in mouse models, suggesting this relationship is relevant and not just coincidental.

Not unexpectedly, based on the indications enrolled in this study the immunohistochemical analyses on pretreatment biopsies revealed most patients had no or low PD-L1 expression on tumor cells (figure 3) and no correlation of PD-L1 expression was observed with clinical outcome. In contrast, the pretreatment levels of tumor associated CD8+ T cells were significantly associated with PR or SD relative to PD with a similar trend for CD4+ T cells. These data support that some level of T cell infiltration/tumor recognition is important for response to this immunotherapy regimen and is consistent with data from other studies with immune checkpoint blockade.

Ovarian cancer is generally considered a non-immunogenic tumor and has a poor response to PD-1 blockade with an overall response rate of 5%–10%. However, our study demonstrates that T cell infiltration can be increased and when this occurs it is correlated with improved outcome. In particular, patients with a 5% or greater absolute increase in CD4+ T cells in the on-study biopsies had a 4-fold improvement in median PFS (9.2 months) relative to patients without significant increase in CD4+ T cells (2.2 months). A similar trend was observed for CD8+ T cells (7.4 vs 3.1 months), and for increased PD-L1 expression on tumor cells (7.4 vs 3.5 months). We interpret the enhanced PD-L1 expression as evidence of functional effector T cells producing IFN-γ, a known regulator of PD-L1 expression on tumor cells. Consistent with these findings, of the patients with on-study biopsies, four had confirmed responses and each had a significant increase in CD4+ T cells, with 3 of 4 having significant increase in CD8+ T cells and PD-L1 expression (data not shown). Although the data set is too small to draw absolute conclusions, the stronger correlation with CD4+ T cells than CD8+ T cells is intriguing and adds to the growing support of the importance of CD4+ T cells in the response to immunotherapy. In the same biopsies, we also observed an increase in FOXP3+ cells, that are broadly characterized as Treg cells, though FOXP3 expression is not restricted to Treg cells. Nevertheless, the increase in FOXP3 cells was substantially lower relative to CD4+ and CD8+ T cells. For reasons that are not clear, in patients with CRC with paired biopsies the regimen was unable to result in enhanced T cell infiltration, which correlated with the poor outcome in these patients except for two patients that had high TMB and durable responses. Too few paired biopsies were collected from other indications for meaningful analysis.

To further investigate the differences in patients with ovarian cancer that responded to therapy compared with those that did not, additional analysis was performed on biopsies from selected patients. Whole-exome sequencing of pretreatment tumor samples from patients with PR or PD revealed a similar level of somatic mutations and overlapping mutations in known cancer associated genes. Similarly, expression of TIM-3 and LAG-3, which have been associated with immune escape in ovarian cancer models, did not correlate with clinical outcomes, as similar increases in the expression of these markers were observed in biopsies from patients with durable responses to those without responses.

Identification of biomarkers for selection of ovarian patients that are more likely to respond to CD27 agonism combined with PD-1 blockade merits further study as some patients had durable clinical benefit. Further efforts could better define the baseline characteristics of ovarian patients and their tumor with a goal of identifying a gene signature associated with outcome to treatment. In particular, further characterization of the impact on patients with clear cell carcinoma would be valuable as both patients with this histology had PR.
Contributors Conception or design of the study; TKe, MY, TRH, RES, DR. Patient recruitment and data acquisition: RES, MP, MC, AW, BS, OR, DCC, NR, MZ, JL, JEB, RB, AS, AJ, DR, Tka, Ff, JMB, DSS, JBA. All authors contributed to the analysis and interpretation of data, as well as drafting of the manuscript for publication. RES is the guarantor of the study.

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Patient consent for publication Not applicable.

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