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PD-1 blockade in recurrent or metastatic cervical cancer: Data from cemiplimab phase I expansion cohorts and characterization of PD-L1 expression in cervical cancer

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HIGHLIGHTS

- Cemiplimab provided clinical benefit among patients with advanced cervical cancer in a phase I study.
- Responding patients had squamous histology.
- PD-L1 protein expression in commercially available samples and PD-L1 mRNA expression in The Cancer Genome Atlas were analyzed.
- Analyses of PD-L1 protein and mRNA expression showed greater PD-L1 expression in squamous versus non-squamous cervical cancer.
- Results support the rationale and design of a global randomized phase III cemiplimab study in metastatic cervical cancer.

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ABSTRACT

Objectives. To characterize the safety, tolerability, and anti-tumor activity of cemiplimab as monotherapy or in combination with hypofractionated radiation therapy (hfRT) in patients with recurrent or metastatic cervical cancer. To determine the association between histology and programmed death-ligand 1 (PD-L1) expression.

Methods. In non-randomized phase I expansion cohorts, patients (squamous or non-squamous histology) received cemiplimab 3 mg/kg intravenously every 2 weeks for 48 weeks, either alone (monotherapy cohort) or with hfRT during week 2 (combination cohort). Due to insufficient tissue material, PD-L1 protein expression was evaluated in commercially purchased samples and mRNA expression levels were analyzed from The Cancer Genome Atlas (TCGA).

Results. Twenty patients enrolled in both cohorts in total; 10 had squamous histology. The most common adverse events of any grade were diarrhea, fatigue, and hypokalemia, occurring in 35%, 25%, and 25%, respectively. Objective response rate was 10% in each cohort; responders had squamous histology. Duration of response was 11.2 months and 6.4 months for the responder in the monotherapy and combination cohort, respectively.

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Irradiated lesions were not included in the response assessments. In separate archived specimens ($N = 155$), PD-L1 protein expression in tumor and immune cells was negative (<1%) more commonly in adenocarcinoma than in squamous tumors. PD-L1 mRNA levels were lower in adenocarcinoma than squamous cell tumors (1.2 vs 5.0 mean transcripts per million, respectively) in TCGA.

Conclusions. Cemiplimab has activity in cervical squamous cell carcinoma. The phase I results, combined with results from other anti–PD-1 trials in cervical cancer and our biomarker analyses have informed the design of the ongoing phase III trial, with the primary overall survival hierarchical analyses being done first in patients with squamous histology.

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1. Introduction

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of death in women worldwide, with approximately 570,000 cases per year and approximately 311,000 related deaths in 2018 [1]. Approximately 95% of cervical cancers stem from chronic infection with human papillomavirus [2].

Patients with recurrent or metastatic disease are managed with chemotherapy in combination with bevacizumab when indicated [3]. First-line treatment of these patients with the combination of cisplatin, paclitaxel, and bevacizumab is associated with increased overall survival (OS, 17.0 vs 13.3 months) and higher response rates (48% vs 36%) than the combination of cisplatin and paclitaxel alone [4]. However, treatment options are limited for patients once their tumors progress on these regimens and median survival is only approximately 7 months in the second-line or greater setting [3,5,6]. Pembrolizumab received accelerated approval from the Food and Drug Administration for the treatment of patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy (whose tumors express programmed death-ligand 1 [PD-L1]) based on objective response rate and durability of responses [5]. There is an unmet need to develop treatment options for patients with recurrent or metastatic cervical carcinoma after progression on standard first-line platinum-taxane based chemotherapy with or without bevacizumab. No agent has been shown to improve OS after first-line chemotherapy for metastatic cervical cancer.

Expression of the immune checkpoint PD-L1 is an immune-evasion strategy observed in both tumor cells and virally infected cells. PD-L1 expression has been detected in the majority of cervical squamous cell cancers using immunohistochemical analysis of tumor cells and the surrounding stroma [7], suggesting that anti–programmed cell death (PD)-1 therapies may be effective in cervical cancer. Anti–PD-1/PD-L1 antibodies have been shown to enhance radiation-induced tumor regression (in tumors receiving local radiation and in distant tumors via abscopal effect) and survival in mouse models [8].

Cemiplimab is a high-affinity, human, hinge-stabilized IgG4 monoclonal antibody to the PD-1 receptor that potently blocks the interactions of PD-1 with PD-L1 and PD-L2. In the dose escalation portion of the first-in-human study of cemiplimab, objective radiographic responses were observed in two of three patients with metastatic squamous cervical cancer [9]. The dose escalation portion of the study included cemiplimab monotherapy cohorts and cohorts in which patients received cemiplimab monotherapy plus hypofractionated radiation therapy (hfRT) (to a non-target lesion) in week 2. Both of these patients who experienced objective responses were in cemiplimab + hfRT cohorts. It was unclear if these responses were due solely to cemiplimab, or if hfRT was supporting abscopal anti-tumor immune responses in non-irradiated lesions. Expansion cohorts were designed to gain further experience with cemiplimab monotherapy and cemiplimab + hfRT in patients with metastatic cervical cancer.

In this manuscript we aim to provide the clinical and biomarker data that support the design of the phase III trial in recurrent/metastatic cervical cancer. We present results from the phase I expansion cohorts of patients with recurrent or metastatic cervical cancer, regardless of histology or PD-L1 expression, who received cemiplimab as monotherapy or in combination with hfRT (NCT02383212). We also present additional analyses of gene expression profiling and PD-L1 expression in cervical cancer specimens from other independent sources to gain a deeper understanding of differences between squamous and adenocarcinoma of the cervix which may contribute to differential sensitivity to immune checkpoint inhibitors.

2. Methods

2.1. Patients

We report data from expansion cohorts 23 and 24 which included patients aged ≥18 years with histologically or cytologically confirmed recurrent or metastatic cervical cancer who were resistant to or intolerant of platinum and taxane doublet chemotherapy. Patients in cohort 23 (monotherapy cohort) received cemiplimab 3 mg/kg intravenously (IV) every 2 weeks (Q2W) for 48 weeks. Patients in cohort 24 (combination therapy cohort) received cemiplimab 3 mg/kg IV Q2W for up to 48 weeks plus hfRT (9 Gy × 3 times over 1 week to a single lesion, starting 1 week after Day 1 of cemiplimab from Day 8 to Day 12 of a 14-day cycle; Supplementary Fig. 1). The combination therapy cohort enrolled patients for whom palliative hfRT was planned to a lesion that was causing some signs or symptoms. The radiated lesion was not followed as a target lesion for response assessments. For both cohorts, the primary objective was to characterize safety and tolerability; description of anti-tumor activity in each expansion cohort was a secondary objective.

Additional eligibility criteria included an Eastern Cooperative Oncology Group performance status score of 0 or 1 and adequate organ function. Patients were required to have at least one measurable lesion per Response Evaluation Criteria in Solid Tumors 1.1 (RECIST 1.1). In the combination therapy cohort, the measurable lesion(s) was (were) in addition to the irradiated lesion.

Key exclusion criteria included ongoing or recent (within 5 years) autoimmune disease requiring systemic immunosuppression, prior treatment with an agent blocking the PD-1/PD-L1 pathway, a history of solid organ transplantation, concurrent cancer (unless indolent or non-life-threatening), or hematologic cancer.

The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The study protocol and all amendments were approved by the institutional review board (or ethics committee) at each participating study site.

2.2. Study design

This was a phase I, open-label, multicenter dose escalation and dose expansion study of cemiplimab in patients with advanced solid tumors. The primary objective in cervical cancer expansion cohorts was to characterize the safety and tolerability of cemiplimab as monotherapy or in combination with hfRT in patients with recurrent or metastatic cervical cancer. A key secondary objective was to determine the anti-tumor activity of cemiplimab as monotherapy or in combination with hfRT in these patients.

2.3. Assessments

Tumor histology was assessed per local pathology reports of tumor samples obtained at any time prior to study enrollment. Severity of treatment-emergent adverse events (TEAEs) was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03). Extensive safety evaluations were performed during screening and on Day 1 of each subsequent treatment cycle throughout the study. Routine safety evaluations were performed at each cemiplimab dosing visit. Tumor response assessments were performed by investigators, per RECIST 1.1, at the end of each 8-week treatment cycle. In combination therapy cohorts, the lesions selected for hfRT were not included in RECIST 1.1 assessments.

2.4. Statistical analysis of clinical data

There was no formal hypothesis for the expansion phase of the study presented here. The safety summaries and analyses were performed on the safety analysis set. Data were summarized using descriptive statistics, along with two-sided 95% confidence interval (CI), by dose cohort. Continuous variables were summarized with mean, median, standard deviation, minimum, and maximum. Categorical variables were summarized with frequency and percentage. Kaplan–Meier analysis was performed for progression-free survival (PFS) and OS. The data cut-off date for clinical analysis (clinical activity and safety) was April 30, 2019, which is the final data cut for this study.

2.5. Analysis of PD-L1 protein and mRNA expression in independent cervical cancer samples

A total of 155 commercially available tumor blocks were evaluated for tumor-infiltrating immune cell presence and PD-L1 protein expression in both tumor and immune cells; 43 blocks were adenocarcinoma and 112 blocks were squamous cell carcinoma tumors. These tissue blocks were obtained from 12 different vendors. The number of blocks from the top five vendors are as follows: 58, 37, 36, 17, and 8. No one vendor provided more than 35% of the blocks. There was insufficient evaluable tumor material available to support PD-L1 biomarker correlative analysis in patients enrolled in the cervical cancer expansion cohorts of the phase I study. Among 20 patients in the cervical cancer expansion cohorts, no pre-treatment sample was submitted for six patients and the pre-treatment sample had insufficient tumor cells for one patient. Supplementary Table 1 lists the results of PD-L1 IHC analysis and best overall response status for the remaining 13 patients.

PD-L1 immunohistochemistry staining was conducted with the Ventana PD-L1 (SP263) rabbit monoclonal primary antibody (Roche Diagnostics; AZ) according to the manufacturer instructions for use. PD-L1 scoring was reported as percentage of tumor cells with any membrane staining above background (TC %) or the percentage of tumor-associated immune cells with staining at any intensity above background (IC %). Any tumor-associated immune cell in a mixed inflammatory setting and not part of necrosis was included in calculating the percent of tumor area occupied by tumor-associated immune cells (Immune Cells Present, ICP). In order to facilitate analysis and interpretation, cut-off values to group PD-L1 expression and immune cell presence were determined by organizing the distribution into discrete units.

PD-L1 mRNA expression was plotted for squamous and non-squamous cervical cancer tumors in The Cancer Genome Atlas (TCGA). Transcripts per million (TPM) of cervical tumors were plotted using OmicSoft ArrayStudio software, version 10.0.1.50. PD-L1 mRNA expression results are in whole based upon data generated by the TCGA Research Network (https://www.cancer.gov/tcga).

3. Results

3.1. Patients

Ten patients were enrolled in each expansion cohort. The median age was 55.0 years (range, 31.0–76.0) and 51.5 years (range, 29.0–65.0) in the monotherapy and combination therapy cohorts, respectively. Baseline characteristics were similar across the two cohorts and are summarized in Table 1. Ten patients had squamous histology, eight patients had adenocarcinoma, one patient had adenosquamous histology, and one patient had mucinous carcinoma histology. At the time of data cut-off, among patients receiving cemiplimab monotherapy, one patient had completed planned treatment (48 weeks) and nine patients had discontinued treatment, mainly due to disease progression or recurrence ($n = 8$). In patients receiving cemiplimab + hfRT, all 10 patients had discontinued treatment, mainly due to disease progression or recurrence ($n = 8$).

For patients receiving cemiplimab monotherapy, the median number of administered doses of cemiplimab was 4 (range, 2.0–23.0), with median duration of exposure of 8.1 weeks (range, 4.0–48.4) and median duration of follow-up of 5.6 months (range, 0.8–16.2). For patients receiving cemiplimab + hfRT, the median number of administered doses of cemiplimab was 8 (range, 1.0–17.0), median duration of exposure 16.0 weeks (range, 2.0–34.1) with a median follow-up of 3.76 months (range, 0.7–8.1).

3.2. Clinical activity

One patient in each cohort (10%) experienced a partial response, with a duration of 11.2 months for the monotherapy cohort responder and 6.4 months for the combination therapy cohort responder. Both responders had squamous histology. Eight patients achieved best response of stable disease (SD): three patients (30%) in the monotherapy cohort and five patients (50%) in the combination therapy cohort

Table 1
Patient demographics and baseline characteristics.

	Cemiplimab monotherapy ($n = 10$)	**Cemiplimab + hfRT ($n = 10$)**
Median age, years (range)	55.0 (31.0–76.0)	51.5 (29.0–65.0)
Race, *n* (%)		
White	9 (90.0)	8 (80.0)
Black or African American	0	1 (10.0)
Asian	0	1 (10.0)
Not reported	1 (10.0)	0
ECOG performance status, *n* (%)		
0	4 (40.0)	2 (20.0)
1	6 (60.0)	8 (80.0)
Tumor histology, *n* (%)		
Squamous	4 (40.0)	6 (60.0)
Adenocarcinoma	6 (60.0)	2 (20.0)
Other	0	2[a] (20.0)
Prior cancer-related systemic therapy, *n* (%)[b]	10 (100.0)	10 (100.0)
Platinum compounds	10 (100.0)	10 (100.0)
Taxanes	10 (100.0)	10 (100.0)
Bevacizumab	7 (70.0)	7 (70.0)
Other[c]	10 (100.0)	3 (30.0)
Prior cancer-related radiotherapy, *n* (%)	10 (100.0)	8 (80.0)

ECOG, Eastern Cooperative Oncology Group; hfRT, hypofractionated radiation therapy.
[a] Other histology was adenosquamous cell carcinoma in one patient and mucinous carcinoma in one patient.
[b] Chemical/pharmacological/therapeutic subgroup code was not available for one patient receiving cemiplimab + hfRT.
[c] Other includes other antineoplastic agents, pyrimidine analogues, anthracyclines and related substances, folic acid analogues, and investigational drugs.

Table 2
Tumor response per investigator assessment.

	Cemiplimab monotherapy ($n = 10$)	Cemiplimab + hfRT ($n = 10$)
ORR, % (95% CI)	10.0 (0.3–44.5)	10.0 (0.3–44.5)
Partial response, *n* (%)	1 (10.0)	1 (10.0)
Stable disease, *n* (%)	3 (30.0)	5 (50.0)
Progressive disease, *n* (%)	5 (50.0)	4 (40.0)
NE[a], *n* (%)	1 (10.0)	0
Disease control rate, % (95% CI)[b]	40.0 (12.2–73.8)	60.0 (26.2–87.8)
Durable disease control rate, % (95% CI)[c]	20.0 (2.5–55.6)	30.0 (6.7–65.2)
Observed time to response, months[d]	1.8	1.8
DOR, months[b]	11.2	6.4

CI, confidence interval; DOR, duration of response; hfRT, hypofractionated radiation therapy; IQR, interquartile range; NE, not evaluable; ORR, objective response rate.
[a] NE response includes missing and unknown tumor response.
[b] Defined as proportion of patients with objective respose or stable disease.
[c] Defined as the proportion of patients with objective response or stable disease without progression for at least 16 weeks, measured at least 105 days to account for scheduling windows in the protocol.
[d] Based on one patient in each group who experienced objective tumor response.

(Table 2). Of these eight patients with best response of SD, four had squamous histology. Durable disease control, defined as the proportion of patients without progressive disease for ≥105 days, was 20.0% (95% CI: 2.5–55.6) in patients receiving cemiplimab monotherapy and 30.0% (95% CI: 6.7–65.2) in patients receiving cemiplimab + hfRT. Other clinical activity results regarding tumor response status are summarized in Table 2. The time course of percent changes in target lesion for each evaluable patient is provided in Supplementary Fig. 2.

Kaplan–Meier estimation of median PFS was 1.9 (95% CI: 1.0–9.0) months in patients receiving cemiplimab monotherapy and 3.6 (95% CI: 0.6–5.7) months in patients receiving cemiplimab + hfRT. Kaplan–Meier estimation of median OS was 10.3 (95% CI: 2.1–not evaluable [NE]) months in patients receiving cemiplimab monotherapy and 8.0 (95% CI: 1.7–NE) months in patients receiving cemiplimab + hfRT.

3.3. Safety

TEAEs of any grade were reported in nine and 10 patients in the monotherapy and combination therapy cohorts, respectively, regardless of treatment attribution (Table 3). The most common TEAEs were diarrhea in 35% (7/20), fatigue in 25% (5/20), and hypokalemia in 25% (5/20) of patients enrolled in both cohorts combined (Table 3). Of patients receiving cemiplimab monotherapy, four (40.0%) patients experienced Grade ≥ 3 TEAEs. Of the patients receiving cemiplimab + hfRT, four (40.0%) patients experienced Grade ≥ 3 TEAEs. No patients in either cohort discontinued treatment due to TEAEs.

One death, due to pneumonitis, was reported in a patient receiving cemiplimab + hfRT and was considered treatment-related. However, other co-morbidities with potential influence on the patient's outcome included possible diffuse malignant pulmonary involvement, pericardial tumor potentially impacting diastolic filling pressures, and decreased general condition, along with medical history elements such as tobacco use and chronic obstructive pulmonary disease. The lesion selected for palliative radiation therapy (RT) in this patient was on the pericardium. At time of death from pneumonitis, the patient's overall tumor response was partial response. Another patient also experienced Grade 3 pneumonitis, and this resolved with treatment that included steroids and empiric antibiotics. One patient with baseline fatigue experienced Grade 3 immune-related myalgia and Grade 2 immune-related hypothyroidism that was associated with intermittent Grade 2 and 3 fatigue. Myalgia resolved with steroid treatment. Treatment-related TEAEs (≥Grade 3) occurred in 10% (1/10) patients in the monotherapy cohort and 30% (3/10) patients in the combination therapy cohort (Supplementary Table 2).

Table 3
Summary of TEAEs by treatment cohort.

	Cemiplimab monotherapy ($n = 10$)		Cemiplimab + hfRT ($n = 10$)	
TEAE, *n* (%)	**Any grade**	**Grade ≥ 3**	**Any grade**	**Grade ≥ 3**
Any	9 (90.0)	4 (40.0)	10 (100.0)	4 (40.0)
Led to discontinuation	0	0	0	0
Death	0	0	1 (10.0)	1 (10.0)
Most common[a]				
Diarrhea	4 (40.0)	1 (10)	3 (30.0)	0
Fatigue	3 (30.0)	1 (10)	2 (20.0)	0
Hypokalemia	3 (30.0)	1 (10)	2 (20.0)	0
Pain in extremity	3 (30.0)	0	0	0
Anemia	2 (20.0)	1 (10)	1 (10.0)	1 (10.0)
Constipation	2 (20.0)	0	2 (20.0)	0
Dyspnea	2 (20.0)	0	0	0
Headache	2 (20.0)	0	1 (10.0)	0
Hypothyroidism	2 (20.0)	0	1 (10.0)	0
Nasal congestion	2 (20.0)	0	0	0
Nausea	1 (10.0)	0	2 (20.0)	0
Pneumonitis	1 (10.0)	0	2 (20.0)	2 (20.0)
Hematuria	1 (10.0)	1 (10.0)	1 (10.0)	0
Hyponatremia	1 (10.0)	0	1 (10.0)	1 (10.0)
Localized edema	1 (10.0)	1 (10.0)	1 (10.0)	0
Activated partial thromboplastin time prolonged	1 (10.0)	1 (10.0)	0	0
Hypoalbuminemia	1 (10.0)	1 (10.0)	0	0
Myalgia	1 (10.0)	1 (10.0)	0	0
Pneumonia	1 (10.0)	1 (10.0)	0	0
Macular rash	1 (10.0)	1 (10.0)	0	0
Cervical vertebral fracture	0	0	1 (10.0)	1 (10.0)
Urinary tract infection	0	0	3 (30.0)	0
Peripheral edema	0	0	2 (20.0)	0
Pyrexia	0	0	2 (20.0)	0
Stomatitis	0	0	2 (20.0)	0
Increased alanine aminotransferase	0	0	2 (20.0)	0
Wheezing	0	0	2 (20.0)	0

hfRT, hypofractionated radiation therapy; TEAE, treatment-emergent adverse event; TRAE, treatment-related adverse event.
In the cemiplimab monotherapy cohort, there were nine patients who experienced TRAEs. For the cemiplimab + hfRT cohort, there were ten patients who experienced TRAEs. In the combination therapy cohort, there were two cases of Grade ≥ 3 pneumonitis, considered serious and related to treatment. The treatment-related death occurred in one of these patients with pneumonitis and is described in the text.
[a] Occurred in two or more patients in either cohort of any grade, or ≥ Grade 3 in any patient; ordered by overall frequency in cemiplimab monotherapy cohort.

3.4. Protein and PD-L1 mRNA expression in cervical cancer

The observation that responses occurred in patients with squamous histology prompted exploration of potential associations between PD-L1 expression and histology in cervical cancer. There were insufficient samples available from study patients, and therefore, archived cervical cancer specimens from other sources were interrogated. Among 155 tumor samples analyzed by immunohistochemistry, tumor PD-L1 expression was undetectable (<1%) in 69.8% (30/43) of adenocarcinomas and 40% (45/112) of squamous cell carcinoma samples. PD-L1 expression in immune cells was undetectable (<1%) in 30.2% (13/43) of adenocarcinomas in contrast to 4.5% (5/112) in squamous cell carcinomas (Table 4). Combined enrichment analysis demonstrated that both immune cell presence and expression of PD-L1 in tumor cells were more

common in squamous cell carcinomas than in adenocarcinoma tumors (Supplementary Fig. 3).

Potential associations between PD-L1 expression and tumor histology in cervical cancer were also explored at the mRNA level in TCGA samples. PD-L1 mRNA expression was greater in squamous versus non-squamous cervical cancer samples. The median and mean TPM of PD-L1 mRNA for squamous cervical tumors ($n = 253$) were 4.5 and 5.0, respectively. The median and mean for non-squamous cervical tumors ($n = 53$) were 1.3 and 1.2, respectively (Fig. 1). Additional TCGA analyses for expression of selected genes (PD-1, PD-L1, CD8A) showed that cervical cancer clusters with other solid tumor types for which anti–PD-1 therapy improves OS, such as melanoma, non-small cell lung cancer, renal clear cell carcinoma, and head and neck squamous cell carcinoma (Supplementary Fig. 4).

4. Discussion

Clinical activity results of these expansion cohorts demonstrate that treatment with cemiplimab induces responses and clinical benefit among recurrent or metastatic cervical cancer patients. This supports the clinical activity signal observed in the dose escalation cohort of the cemiplimab first-in-human study, in which two of three cervical cancer patients had durable responses [9]. The sum total equates to a 17% objective response rate among cervical cancer patients enrolled in the phase I study (4/23 combined; 2/3 responding patients in dose escalation plus 2/20 in expansion cohorts). All responders had squamous histology. The safety results observed here are consistent with what has been observed in other studies of cemiplimab and other inhibitors of the PD-1/PD-L1 axis. There was no apparent improvement to objective response rate from adding hfRT after the initiation of cemiplimab treatment (in fact, one of the cervical squamous patients who had objective response to cemiplimab + hfRT in dose escalation subsequently had disease recurrence after completion of planned treatment, and experienced complete response to retreatment with cemiplimab monotherapy).

In our analysis of cervical cancer specimens independent of the phase I trial, the combination of both high PD-L1 protein expression and immune cell presence was enriched in squamous relative to adenocarcinoma histology. These results confirm and extend a prior report which used a different anti–PD-L1 antibody and found higher expression in squamous than in adenocarcinoma cervical cancer samples [7]. The current study supports these observations by reporting that in TCGA cervical cancer samples, PD-L1 mRNA expression is greater in squamous than in non-squamous samples. Increased PD-L1 expression among squamous tumors at both the protein and mRNA level indicates that mechanisms of immune-evasion may differ between squamous and non-squamous cervical cancers and may impact clinical response to immunotherapy. PD-L1 gene amplification was previously described in TCGA analyses of cervical cancer specimens, but expression levels of PD-L1 mRNA in squamous versus non-squamous histologies was not described [2]. Observations regarding PD-L1 expression and histology in cervical cancer should not be extrapolated to other tumor types in the absence of additional data.

The importance of the PD-1/PD-L1 axis in squamous cervical cancer is also supported by studies with other PD-1 inhibitors. In the multi-cohort study KEYNOTE-158, objective response rate in the cervical cancer cohort was 12.2% (12/98) with pembrolizumab, and 11 of the 12 observed responses were in patients with squamous histology [9]. In CheckMate 358, a phase I/II study in patients with squamous histology only, the objective response rate was 26.3% (5/19) for cervical cancer with nivolumab [10]. Another study, with a patient population of 60% squamous histology, also reported a 4% response rate to nivolumab [11]. Although these observations are directionally consistent with the hypothesis that the clinical activity of PD-1 blockade in cervical cancer may partition with squamous histology, it is not possible to draw conclusions from small cross-study comparisons. The present

Table 4
Presence of immune cells and frequencies of PD-L1 expression in tumor and immune cells in cervical SCC and adenocarcinoma.

	Tumor staining, %	Distribution, *n* (%)	Immune cell staining, %	Distribution, *n* (%)	Immune cell present, %	Distribution, *n* (%)
Adenocarcinoma ($n = 43$)	<1	30 (69.8)	<1	13 (30.2)	<1	29 (67.4)
	<25	11 (25.6)	<20	18 (41.9)	<10	7 (16.3)
	<50	0 (0.0)	<40	10 (23.3)	<25	7 (16.3)
	≥50	2 (4.7)	≥41	2 (4.7)	≥25	0 (0.0)
SCC ($n = 112$)	<1	45 (40.2)	<1	5 (4.5)	<1	29 (25.9)
	<25	36 (32.1)	<20	55 (49.1)	<10	45 (40.2)
	<50	16 (14.3)	<40	42 (37.5)	<25	34 (30.4)
	≥50	15 (13.4)	≥41	10 (8.9)	≥25	4 (3.6)

PD-L1, programmed death-ligand 1; SCC, squamous cell carcinoma.

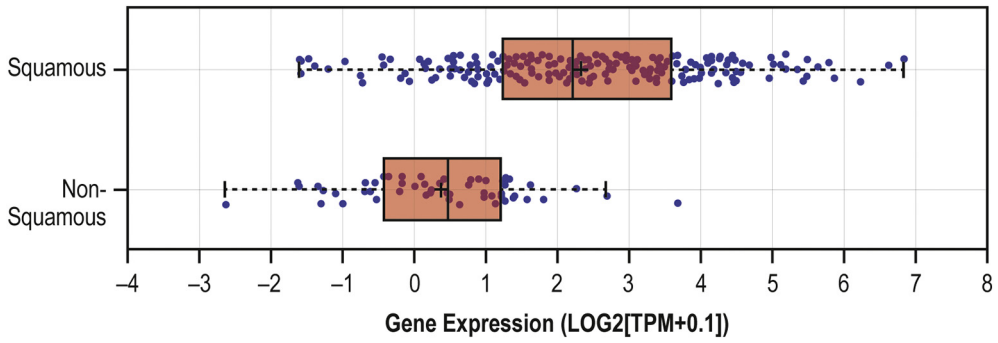

Fig. 1. Box plot of PD-L1 mRNA expression in TCGA cervical cancers by histology. mRNA, messenger RNA; PD-L1, programmed death-ligand 1; TCGA, The Cancer Genome Atlas; TPM; transcript per million.

data, and other studies reviewed here, do not exclude the possibility that some patients with non-squamous cervical cancers may also benefit from immunotherapy.

Although most patients with recurrent or metastatic cervical cancer do not experience objective responses with PD-1 blockade, the potential for durable responses (or durable stable disease) could lead to meaningful survival benefits. The current study is too small to provide robust estimates of OS.

Analysis of gene expression data for selected genes (PD-1, PD-L1, CD8A) in TCGA demonstrates that cervical cancer clusters with other solid tumor types for which anti–PD-1 therapy improves OS, such as melanoma, non-small cell lung cancer, renal clear cell carcinoma, and head and neck squamous cell carcinoma [12–17]. In a publication by Lee and Ruppin, TCGA analysis of 36 variables in 21 tumor types found that CD8+ T cell abundance, PD-1 gene expression, and tumor mutational burden were the three most predictive variables of objective response to anti–PD-1/PD-L1 therapy [18]. Two of these variables (CD8+ T cell abundance, PD-1 gene expression) are consistent with the TCGA analysis in the current report that clusters cervical cancer with other immunotherapy-responsive tumors. The third variable in the Lee and Ruppin report, tumor mutation burden, may also contribute to the clinical activity of PD-1 blockade on some cervical cancer patients. In exploratory analyses of expansion cohorts in a phase II study of pembrolizumab (KEYNOTE-158), cervical squamous cell cancer was among the tumor types in which high tissue tumor mutation burden (defined as >10 mutations/megabase) was associated with increased clinical activity [19]. Therefore, both the TCGA analyses (as presented here, and in the report of Lee and Ruppin), provide indirect evidence that PD-1 blockade warrants further study in cervical cancer.

A randomized phase III trial is ongoing in second-line or greater metastatic cervical cancer patients, comparing cemiplimab versus investigator's choice of chemotherapy (NCT03257267). The primary analysis for OS will be hierarchical, first for patients with squamous histology and then for all patients (squamous, adenocarcinoma, or adenosquamous histology). Associations between PD-L1 expression, clinical activity, and histology will be explored.

There are several limitations of PD-L1 expression as a tool to identify patient groups more likely to benefit from PD-1 blockade, including spatial and temporal heterogeneity in PD-L1 expression in tumor cells and lack of standard positive threshold [20]. Nevertheless, it has been used successfully in other tumor types, e.g. head and neck cancer, resulting in approvals based on PD-L1 expression. In the ongoing phase III trial, tumor histology is being explored as an alternative strategy to PD-L1 immunohistochemistry for enrichment of cervical cancer patients more likely to benefit from anti–PD-1 antibody therapy.

This report has several limitations. Comparison of clinical outcomes between cohorts is limited by the small sample sizes and by potential differences in baseline characteristics between the cohorts. Specifically, each patient in the combination therapy cohort was required to have a symptomatic lesion for which palliative RT was clinically indicated. Additionally, there was insufficient tumor material to perform correlative analyses of PD-L1 expression from study patients. Due to the limited clinical annotation of the immunohistochemistry and TCGA analyses, it was difficult to ascertain whether patients in the expansion cohorts had similar clinical characteristics to those represented by the independent tumor samples used in those analyses.

5. Conclusion

Among patients with recurrent or metastatic cervical cancer who were resistant to or intolerant of platinum and taxane doublet chemotherapy, cemiplimab demonstrated clinical benefit and a safety profile similar to those observed with other PD-1 inhibitors. The results from this phase I cemiplimab trial in conjunction with data with other anti–PD-1 agents suggest that clinical activity may be associated with histology in cervical cancer. Furthermore, the potential association between histology and clinical activity of PD-1 inhibition is supported indirectly by analyses of cervical cancer specimens from other sources in which PD-L1 protein and mRNA expression is greater in squamous than in non-squamous histologies. A phase III randomized trial of cemiplimab versus investigator's choice of chemotherapy is ongoing, and the primary OS hierarchical analysis will be first in patients with squamous histology (NCT03257267).

Author contributions

Conceptualization: DR, KPP, IL, MM, MGF.
Data curation: DR, MGM, AMG, IB, JYH, DC, GF, SF, SJ, KM, AN, KPP, JB.
Formal analysis: JL, NAY-D, S-YY.
Investigation: DR, MGM, AMG, IB, JYH, DC, GF, SF, SJ, KM, AN, KPP, JB, WF, MF, ES, JL, ANY-D, S-YY, IL, MM, MGF.
Methodology: DR, KPP, IL, MM, MGF.
Writing: MGF.

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Data sharing

Qualified researchers may request access to study documents (including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan) that support the methods and findings reported in this manuscript. Individual anonymized participant data will be considered for sharing once the indication has been approved by a regulatory body, if there is legal authority to share the data and there is not a reasonable likelihood of participant re-identification. Submit requests to https://vivli.org/.

Declaration of Competing Interest

DR has received institutional clinical trial funding from Genentech and Kura Oncology; institutional clinical trial funding and uncompensated scientific committee and advisory board support from Regeneron Pharmaceuticals, Inc., Bristol Myers-Squibb, and GSK; and institutional clinical trial funding, uncompensated scientific committee, advisory board, and travel support from Merck.

AGM has received consulting, advisory, speakers' bureau, and travel accommodation expenses from AstraZeneca, GSK-Tesaro, Clovis, Roche, Pharmamar, Genmab, MSD, Oncoinvent, Pfizer-Merck, Amgen, and Immunogen; and personal fees from Sotio.

IB reports research funding and personal fees from AstraZeneca, Bristol-Myers Squibb, Merck Serono, MSD, Orion Pharma, Rakuten Aspyrian, Roche; research funding from Celgene, GlaxoSmithKline, Incyte, Janssen, Kura, Novartis, Pfizer, Shattuck Lab, VCN Biosciences.

JYH is a consultant and receives fees from Foundation Medicine, Massive Bio, Inc., and Atheneum Consulting.

DC has received consulting fees from Pfizer, Nectar, Torque, and Puretech.

GF reports advisory roles to Fujifilm, EMD Serono and has received travel expenses from Bristol-Myers Squibb, EMD Serono, Fujifilm, Millennium, and Sarah Cannon Research Institute; speakers bureau expenses from Total Health Conferencing, royalties from Wolters Kluwer; and research funding to his institution from 3-V Biosciences, Abbisko, Abbvie, ADC Therapeutics, Aileron, American Society of Clinical Oncology, Amgen, ARMO, AstraZeneca, BeiGene, Bioatla, Biothera, Celldex, Celgene, Ciclomed, Curegenix, Curis, Cyteir, Daiichi, DelMar,

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MGM and SF declare no conflict of interest.

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JB is a stockholder in Loxo Oncology, Forty-Seven, MorphoSys AG, and Zymeworks.

MF, ES, JL, and S-YY are employees and shareholders of Regeneron Pharmaceuticals, Inc.

MGF, IL, MM, NAY-D, and WF are employees of, patent holders (pending approval), and shareholders in Regeneron Pharmaceuticals, Inc.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2020.08.026.

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