Journal of Clinical and Translational Research 2023; 9(4): 272-281

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

ORIGINAL ARTICLE

PD-L1 expression and prognosis in definitive radiotherapy patients with neuroendocrine cervical carcinoma

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ARTICLE INFO

Article history:
Received: May 15, 2023
Revised: June 10, 2023
Accepted: July 11, 2023
Published online: July 28, 2023

Keywords:
Cervical carcinoma
Neuroendocrine carcinoma
Small-cell neuroendocrine carcinoma
Chemotherapy
Chemoradiotherapy

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ABSTRACT

Background: Neuroendocrine carcinoma of the cervix (NECC) is more prone to lymphatic infiltration, lymph node involvement, local recurrence, and distant metastasis. Using concurrent chemoradiotherapy (CCRT) with or without adjuvant chemotherapy as the standard treatment for locally advanced NECCs and CCRT for patients with early lesions confined to the cervix. However, the prognosis of NECC patients treated with definitive radiotherapy (RT) is unknown. Immune checkpoint inhibitors are a promising therapeutic strategy for locally advanced cervical cancer. Some reports suggest that the expression of PD-L1 in solid tumors correlates with prognosis.
Aim: This study investigates prognostic factors for survival in patients with neuroendocrine cervical carcinoma (NECC) treated with definitive RT and the relationship between PD-L1 expression and prognosis in these patients.
Methods: This retrospective study included 66 patients with histologically confirmed NECC who received RT with or without chemotherapy. From January 2015 to December 2020, patients received routine extended-field irradiation (EFI), and PD-L1 expression was assessed by immunohistochemistry. The most commonly used chemotherapy agents were etoposide-platinum and paclitaxel-platinum.
Results: PD-L1 expression was positive in 17 of 45 (37.8%) patients. There were 52 cases of pure NECC and 14 cases of mixed carcinoma. Sixty stage IB-III patients received definitive RT. The 3- and 5-year progression-free survival (PFS) was 39.8% and 34.1%, and 3- and 5-year overall survival (OS) was 48.0% and 40.2%, respectively. There was no significant difference in 3 and 5-year PFS and 3 and 5-year OS between patients with pure and mixed carcinoma. Positive PD-L1 expression was associated with higher 3-year PFS in patients with mixed histology. Univariate analysis showed that lymph node metastasis (LNM) and the International Federation of Gynecology and Obstetrics stages predicted 3- and 5-year PFS in patients who received definitive RT. The median OS in patients receiving less than four cycles and at least four cycles of chemotherapy (CT) was 26.0 and 44.0 months, respectively ($P = 0.038$); moreover, 3- and 5-year PFS was 34.1% and 25.7% in the former and 46.4% and 40.4% in the latter. There were no significant differences in OS and PFS between pelvic irradiation and prophylactic EFI in patients treated with definitive RT. There were no significant differences in para-aortic failure rate after concurrent chemoradiotherapy between patients who underwent pelvic irradiation or prophylactic EFI ($P = 0.147$).
Conclusion: In patients with mixed NECC, positive PD-L1 expression is correlated with higher 3-year PFS. Chemoradiotherapy was effective for NECCs. The LNM and stage predicted PFS. Four or more cycles of chemotherapy improve prognosis. Prophylactic EFI did not significantly improve PFS and OS.
Relevance for Patients: This study is relevant to patients as it confirms that chemoradiotherapy is effective for both early and locally advanced NECC and that four or more cycles of chemotherapy improved prognosis. The regimen should be carefully evaluated to ensure that patients receive the most effective radiation therapy for the prophylactic of para-aortic LNM. Potential risk factors for the recurrence of radical radiotherapy should be fully understood to minimize these risks. This study observed that PD-L1 expression positive in patients with mixed NECC types is correlated with higher 3-year PFS.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

1. Introduction

Neuroendocrine carcinoma of the cervix (NECC) is a rare histologic type of cervical cancer, accounting for 0.9 – 1.5% of cervical cancer cases [1-3]. Unlike squamous cell carcinoma and adenocarcinoma, NECC is more prone to lymphatic infiltration, lymph node involvement, local recurrence, and distant metastasis (DM) [4].

Small-cell NECC (SCNEC) is the most common type of NECC, accounting for approximately 80% of NECC cases. Large-cell NECC (LCNEC) and other histological types represent approximately 12% and 8% of NECC cases, respectively. Common markers of NECC include chromogranin A (CgA), synaptophysin (Syn), and CD56.

Adjuvant chemoradiotherapy after radical hysterectomy is feasible for early-stage cervical cancer, and concurrent chemoradiotherapy (CCRT) or chemotherapy alone is feasible for locally advanced and metastatic disease [5-7]. The first-line chemotherapy for NECC is etoposide or paclitaxel combined with a platinum agent (cisplatin or carboplatin). The National Comprehensive Cancer Network (2022) recommends using CCRT with or without adjuvant chemotherapy as the standard treatment for stage IB3-IVA NECC and CCRT for patients with early lesions confined to the cervix. Prognostic factors for cervical cancer include race, age, tumor stage and grade, histological type, tumor volume, lymph node involvement and location, performance status, and type of treatment [8]. However, the prognosis of NECC patients treated with radical radiotherapy (RT) is unknown.

Immune checkpoint inhibitors are a promising therapeutic strategy for locally advanced cervical cancer (LACC) [9]. A clinical trial found that ipilimumab combined with nivolumab achieved satisfactory results in three patients with recurrent NECC, including two with positive PD-L1 expression [10]. PD-L1 expression in solid tumors correlates with prognosis. For instance, PD-L1 expression is a good prognostic biomarker in human papillomavirus (HPV)-associated head and neck cancer. Conversely, PD-L1 expression is associated with poor prognosis in patients with renal cancer [11-13].

This study assessed the efficacy of radiation therapy for NECC, prognostic factors for NECC, and the relationship between PD-L1 expression and patient survival.

2. Methods

2.1. Patients

The study included patients with histologically confirmed NECC who received RT with or without chemotherapy at our cancer center between January 2009 and December 2020. Patients gave written informed consent before therapy. The diagnosis was based on the morphological and immunohistochemical characteristics of tumors. The inclusion criteria were patients with no history of previous treatment or malignancies, patients who completed a treatment course, patients with a follow-up of at least 3 months, and patients whose imaging data allowed tumor staging based on the 2018 International Federation of Gynecology and Obstetrics (FIGO) cervical cancer staging system.

2.2. Immunohistochemistry

Immunohistochemistry was performed on 3-5-μm-thick sections. The sections were incubated with antibodies against CgA, Syn, CD56, Ki-67, and PD-L1. PD-L1 immunostaining was performed using clone 28-8 as an anti-PD-L1 antibody (Dako, Carpentaria, CA, USA). PD-L1 expression was scored by counting the total number of PD-L1-positive cells, including tumor cells, lymphocytes, and macrophages, and dividing by the total number of living tumor cells ×100 [14]. PD-L1 expression in tissues (or assays) with a score of ≥1 was considered positive.

2.3. Treatment

2.3.1. RT

The standard protocol included external beam RT (EBRT) and high-dose-rate brachytherapy (HDR-BT). From January 2009 to December 2014, EFI was performed in the pelvis and para-aortic lymph nodes (PALNs) if PALN metastasis was detected at the initial diagnosis. From January 2015 to December 2020, patients received EFI routinely. The patients were planned using 3D conformal RT or intensity-modulated RT. EBRT was performed using either 40.0 – 46.0 Gy in 20 – 23 fractions or 45.0 – 50.4 Gy in 1.8 Gy fractions. HDR-BT was performed during or after EBRT at a dose of 6.0 – 7.0 Gy for each fraction once or twice a week, with a median total dose of 28.0 Gy (range, 21.0 – 35.0 Gy). Palliative RT included EBRT with or without brachytherapy.

2.3.2. Chemotherapy

Patients with no contraindications to platinum received chemotherapy. Therapies included CCRT with etoposide-platinum (EP) or paclitaxel-platinum (TP), followed by adjuvant chemotherapy with EP or TP. In addition, a few patients were treated with chemoradiotherapy involving a single platinum agent concurrent radiotherapy (CRT) followed by adjuvant chemotherapy with EP or TP.

2.3.3. Observation and follow-up

The patients were followed up every 3 months for the first 2 years, every 6 months for the next 3 years, and every 12 months after the 5th year. A physical examination, Papanicolaou smear, and routine blood tests were performed during the follow-up. Radiographic examinations were performed if disease recurrence was suspected. Patient survival with or without recurrence or metastasis was measured.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL). Median overall survival (OS) and median progression-free survival (PFS) were estimated using the Kaplan–Meier method and compared between groups using the log-rank test. Prognostic factors were analyzed by Cox regression analysis. $P < 0.05$ were considered statistically significant.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

2.5. Ethnical statements

2.5.1. Ethical approval

This study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Clinical Oncology School of Fujian Medical University, Fujian Cancer Hospital (Review Number K2022-208-01).

2.5.2. Consent to participate

Informed consent was obtained from all individual participants included in the study.

2.5.3. Consent to publish

The authors affirm that human research participants provided informed consent for the publication of the images in Figures 1 and 2, Tables 1-6.

3. Results

3.1. Patients and tumor characteristics

A total of 188 patients with newly diagnosed NECCs were treated at our center. Of these, 66 patients treated with RT were included in the study (age: 31 – 86 years; median: 50 years). Fifty-two (78.8%) patients presented pure NECCs, including 50 with SCNEC, one with LCNEC, and one with SCNEC + LCNEC. Fourteen cases (21.2%) of NECCs were associated with other malignancies, including adenocarcinoma (11 cases), squamous cell carcinoma (two cases), and adenosquamous carcinoma (one case). Sixty patients with stage IB-III received definitive RT, and six patients with stage IVB received palliative RT. Treatments included RT alone (four patients), CRT (four patients), and CCRT (58 patients).

Disease stages and the respective number of cases were as follows: IB (1), IIA (7), IIB (14), IIIA (2), IIIB (5), IIIC1 (22), IIIC2 (9), and IVB (6). The clinicopathologic features and treatment modalities are summarized in Table 1.

3.2. Immunohistochemistry

Ki-67 protein expression levels were measured in 59 patients. The number of Ki-67-positive cells in each patient ranged from 20% to 100%, with a median of 75%. Immunohistochemistry showed that 92.2% (59/64), 41.9% (26/62), and 67.2% (41/61) patients were positive for Syn, CgA, and CD56, respectively.PD-L1 expression was positive in 17 (37.8%) patients.

3.3. OS

The follow-up period ranged from 13 to 156 months, with a median of 33 months. The 3- and 5-year OS was 41.7% and 35.2%, and 3- and 5-year PFS was 35.6% and 30.6%, respectively. The 5-year OS and PFS were 60.0% and 56.3% in patients with stage I-IIA and 42.3% and 32.7% in patients with stage IIB-IIIc.

The 3-year OS in patients with true and mixed carcinoma was 50.7% and 37.3%, respectively ($P = 0.633$). Five-year OS in these groups was 40.0% and 24.9%, respectively ($P = 0.400$); 3-year PFS was 42.8% and 27.7% ($P = 0.248$), and 5-year PFS was 35.3% and 13.8% ($P = 0.178$).

3.4. PD-L1 expression and patient survival

For patients with mixed histology, positive PD-L1 expression was associated with higher 3-year PFS compared with negative PD-L1 expression (66.7% vs. 16.7%, $P = 0.042$). There were no significant differences in survival between the two pathological types (Table 2).

Among the 60 patients who received definitive EBRT, whole pelvis irradiation, EFI, and prophylactic EFI were performed in 27, 9, and 24 patients, respectively. Thirty-six (54.55%) patients experienced tumor persistence, recurrence, metastasis, or progression. Distal metastases were more common in supraclavicular, mediastinum, and hilum lymph nodes. The most common hematogenous metastasis was pulmonary in 16 cases (16/30), hepatic in 10 cases (10/30), bone in 12 cases (12/30), and pancreatic in four cases (4/30). Brain metastasis occurred in one case. The survival status of patients is shown in Table 3. The

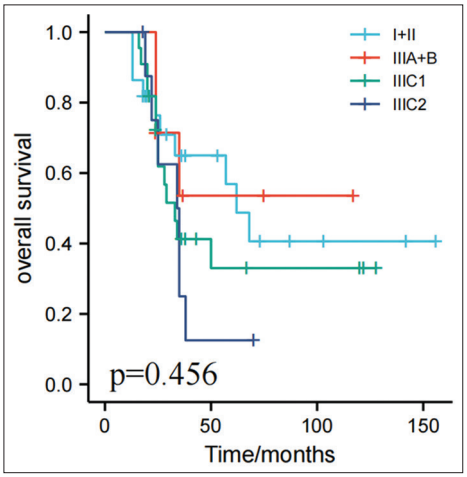

Figure 1. Overall survival by International Federation of Gynecology and Obstetrics stage.

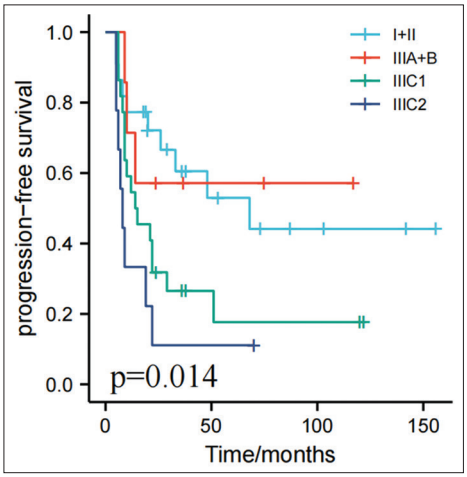

Figure 2. Progression-free survival by International Federation of Gynecology and Obstetrics stage.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 1. Patients, tumor characteristics, and treatment modalities.

Characteristics	Number of patients	Percentage
Age (years)		
<60	50	75.8 (50/66)
≥60	16	24.2 (16/66)
Histology		
Pure	52	
Small-cell neuroendocrine carcinoma	50	75.8 (50/66)
Large-cell neuroendocrine adenocarcinoma	1	1.5 (1/66)
Small-cell+large-cell	1	1.5 (1/66)
Mixed	14	
Small-cell neuroendocrine carcinoma and adenocarcinoma	11	16.7 (11/66)
Small-cell neuroendocrine carcinoma and squamous cell carcinoma	1	3.0 (1/66)
Small-cell neuroendocrine carcinoma and adenosquamous carcinoma	2	3.0 (2/66)
FIGO stage		
IB	1	1.5 (1/66)
IIA	7	10.6 (7/66)
IIB	14	21.2 (14/66)
IIIA	2	3.0 (2/66)
IIIB	5	7.6 (5/66)
IIIC1	22	33.3 (22/66)
IIIC2	9	13.6 (9/66)
IVB	6	9.1 (6/66)
Immunohistochemistry		
Syn-positive	59	92.2 (59/64)
CgA-positive	26	41.9 (26/62)
CD56-positive	41	67.2 (41/61)
Tumor size (cm)		
<4	10	15.2 (10/66)
≥4	56	84.9 (56/66)
Lymph node involvement		
Pelvic	22	33.3 (22/66)
Pelvic and para-aortic	9	13.6 (9/66)
Radiotherapy		
3DCRT	21	31.8 (21/66)
IMRT	45	68.2 (45/66)
Definitive	60	90.9 (60/66)
Palliative	6	16.7% (6/66)
Chemotherapy		
TP	28	42.4 (28/66)
EP	31	46.9 (31/66)
Treatment		
CCRT	58	45.5 (58/66)
Radiotherapy	4	6.1 (4/66)
Platinum + radiotherapy	4	6.1 (4/66)
Number of chemotherapy cycles (EP or TP)		
1 – 3	23	34.9 (23/66)
4 – 7	36	57.6 (36/66)
Definitive external beam radiotherapy		
Pelvic irradiation	27	45.0 (27/60)
Extended-field irradiation	33	55.0 (33/60)

(*Contd...*)

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 1. (*Continued*)

Characteristics	Number of patients	Percentage
PD-L1-positive		
≥1	17	37.8 (17/45)
<1	20	44.4 (20/45)
0	8	17.8 (8/45)
Ki-67-positive		
<75	14	23.7 (14/59)
≥75	45	76.3 (45/59)

3DCRT: 3D conformal radiotherapy; IMRT: Intensity-modulated radiotherapy; CCRT: Concurrent chemo radiotherapy; EP: Etoposide-platinum; TP: Paclitaxel-platinum; CgA: Chromogranin A, Syn: Synaptophysin

Table 2. Progression-free survival and overall survival based on PD-L1 expression in 45 patients with neuroendocrine cervical carcinoma.

Factors	Cases	3-year PFS			5-year PFS			3-year OS			5-year OS		
		PD-L1 Positive	PD-L1 Negative	*P*	PD-L1 Positive	PD-L1 Negative	*P*	PD-L1 Positive	PD-L1 Negative	*P*	PD-L1 Positive	PD-L1 Negative	*P*
Total	45	45.4	34	0.559	15.1	34	0.897	39.8	41.3	0.685	39.8	26.6	0.733
Age (years)													
≤50	22	37.5	36.9	0.933	0	36.9	0.594	33.8	42.3	0.402	33.8	31.7	0.386
>50	23	57.1	31.5	0.428	57.1	31.5	0.428	51.4	40.4	0.721	51.4	20.2	0.721
Histology													
Pure	36	41.7	38.4	0.897	20.8	38.4	0.689	37.3	40.9	0.553	37.7	30.7	0.407
Mixed	9	66.7	16.7	0.042	0	16.7	0.194	50	41.7	0.735	50	0	0.441
FIGO stage													
I+II	15	83.3	48.6	0.349	41.7	48.6	0.663	0	51.9	0.405	83.3	34.6	0.708
III	26	33.3	26.5	0.705	0	26.5	0.902	33.3	40.1	0.395	33.3	30.1	0.527
IV	4	0	0	0.808	0	0	0.808	0	0	0.695	0	0	0.695
Lymph node involvement													
No	17	71.4	52.5	0.624	35.7	52.5	0.927	64.3	52.1	0.867	64.3	34.7	0.875
Pelvic or PALN	28	37.5	19	0.421	0	19	0.601	37.5	38.1	0.72	37.5	28.6	0.881
Number of chemotherapy cycles													
≤3	18	33.3	34.1	0.892	0	34.1	0.515	33.3	35.1	0.703	33.3	0	0.368
4–6	27	51.3	36.3	0.741	25.6	36.3	0.890	45.7	62.9	0.332	45.7	25.2	0.636

PALN: Para-aortic lymph nodes; PFS: Progression-free survival; OS: Overall survival

median PFS and OS were 22.0 and 35.0 months, respectively. In addition, 3- and 5-year PFS was 39.8% and 34.1%, and 3- and 5-year OS was 48.0% and 40.2%, respectively.

The 3-year OS in patients with stages I+II, IIIA+B, IIIC1, and IIIC2 was 60.5%, 57.1%, 26.5%, and 11.1%,respectively; 5-year OS in these groups was 53.0%, 57.1%, 17.7%, and 10.3%. The disease stage increased as survival rates decreased (Figure 1). Advanced-stage NECC (P = 0.011), lymph node metastasis (LNM), and the number of chemotherapy cycles predicted PFS. The 3- and 5-year PFS was 60.5% and 53.0% in stage I+II, 57.1% and 57.1% in stage IIIA+B, 26.5% and 17.7% in stage IIIC1, and 11.1% and 11.1% in stage IIIC2 (Figure 2). The 3- and 5-year PFS was 39.7% and 27.6% in patients with LNM and 48.7% and 30.1% in patients without LNM, respectively (P = 0.029). The median OS in patients receiving less than four cycles and at least four cycles of CT was 26.0 and 44.0 months, respectively (P = 0.038); moreover, 3-year and 5-year PFS was 34.1% and 25.7% in the former and 46.4% and 40.4% in the latter (Table 3).

Univariate analysis showed that LNM and FIGO stages predicted 3- and 5-year PFS in patients who received definitive RT (Table 4). Multivariate Cox regression analysis demonstrated that FIGO stages were independent factors affecting PFS (Table 5).

There were no significant differences in OS and PFS between pelvic irradiation and EFI. Furthermore, there was no significant difference in the incidence of para-aortic failure after CCRT or CRT between patients treated with pelvic irradiation or prophylactic EFI (P = 0.147) (Table 6).

4. Discussion

NECC is strongly associated with HPV infections [15], providing a rationale for studying the molecular characteristics of NECC. Since the efficacy of CRT for advanced diseases is low, it is critical to identify biomarkers associated with survival, local control, and DM. PD-L1 is highly expressed in NECC [16,17] and is thus a potential therapeutic target. PD-L1 expression was positive in more than 50% of patients

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 3. Progression-free survival and overall survival based on the clinical characteristics of 60 patients with neuroendocrine cervical carcinoma treated with definitive radiotherapy.

Factors	Cases	Median PFS 95% CI	*P*-value	3-year PFS	5-year PFS	Median OS 95% CI	*P*-value	3-year OS	5-year OS
Total	60	22.0 (14.0, 68.0)		0.398 (0.288, 0.549)	0.341 (0.231, 0.502)	35.0 (33.0, NR)		0.480 (0.362, 0.637)	0.402 (0.284, 0.569)
Median age (years)			0.527				0.894		
≤50	32	19.0 (9.0, NR)		0.427 (0.283, 0.643)	0.332 (0.194, 0.569)	35.0 (33.0, NR)		0.475 (0.322, 0.701)	0.435 (0.284, 0.666)
>50	28	22.0 (14.0, NR)		0.367 (0.220, 0.611)	----	35.0 (28.0, NR)		0.489 (0.326,0.733)	0.335 (0.172, 0.655)
Histology			0.178				0.393		
Pure	47	26.0 (14.0, NR)		0.428 (0.304, 0.602)	0.392 (0.267, 0.575)	50.0 (33.0, NR)		0.508 (0.377, 0.685)	0.438 (0.304, 0.630)
Mixed	13	15.0 (6.0, NR)		0.277 (0.110, 0.699)	0.138 (0.026, 0.733)	33.0 (24.0, NR)		0.373 (0.173, 0.806)	0.249 (0.082, 0.755)
FIGO stage			0.011				0.456		
I+II	22	68.0 (26.0, NR)		0.605 (0.422, 0.867)	0.530 (0.339, 0.826)	62.0 (33.0, NR)		0.650 (0.468, 0.902)	0.569 (0.374, 0.865)
IIIA+B	7	NR (10.0, NR)		0.571 (0.301, 1.000)	0.571 (0.301, 1000)	NR (24.0, NR)		0.536 (0.257, 1.000)	0.536 (0.257, 1.000)
IIIC1	22	14.5 (9.0, NR)		0.265 (0.131, 0.539)	0.177 (0.061, 0.515)	33.0 (25.0, NR)		0.413 (0.244, 0.697)	0.330 (0.167, 0.654)
IIIC2	9	8.0 (6.0, NR)		0.111 (0.017, 0.705)	0.111 (0.017, 0.705)	34.5 (25.0, NR)		0.250 (0.075, 0.830)	0.125 (0.020, 0.782)
Tumor size (cm)			0.113				0.333		
≤4	18	NR (10.0, NR)		0.530 (0.335, 0.839)	0.530 (0.335, 0.839)	57.0 (33.0, NR)		0.635 (0.437, 0.921)	0.463 (0.259, 0.826)
>4	42	20.0 (10.0, 48.0)		0.343 (0.153, 0.464)	0.267 (0.153, 0.464)	35.0 (26.0, NR)		0.419 (0.287, 0.613)	0.381 (0.250, 0.582)
Lymph node involvement			0.029				0.191		
No	29	48.0 (26.0, NR)		0.487 (0.436, 0.818)	0.397 (0.389, 0.683)	40.0 (33.0, NR)		0.581 (0.452, 0.838)	0.524 (0.382, 0.803)
Pelvic or PALN	31	18.0 (9.0, 22.0)		0.301 (0.113, 0.432)	0.276 (0.369, 0.398)	32.9 (25.0, NR)		0.365 (0.244, 0.596)	0.321 (0.141, 0.518)
Number of chemotherapy cycles			0.023				0.038		
1 – 3	21	15.0 (9.0, 22.0)		0.341 (0.064, 0.459)	0.257 (0.209, 0.474)	26.0 (24.0,50.0)		0.316 (0.091, 0.513)	0.252 (0.058, 0.455)
4 – 6	32	33.0 (29.0, NR)		0.464 (0.450, 0.809)	0.404 (0.450, 0.809)	44.0 (27.0, NR)		0.576 (0.599, 0.930)	0.486 (0.464, 0.872)
EBRT			0.434				0.073		
Pelvic irradiation	27	26.0 (11.0, 41.0)		0.310 (0.189, 0.509)	0.186 (0.078, 0.445)	53.0 (39.0,75.0)		0.329 (0.197, 0.549)	0.211 (0.093, 0.481)
Extended-field irradiation	33	14.0 (7.0, 28.0)		0.310 (0.189, 0.509)	0.186 (0.078, 0.445)	33.0 (28.0,42.0)		0.414 (0.312, 0.660)	0.338 (0.241, 0.595)
PD-L1 expression			0.687				0.723		
Positive	15	48.0 (10.0, NR)		0.333 (0.189, 0.589)	0.333 (0.189, 0.589)	33.0 (25.0, NR)		0.463 (0.254, 0.845)	0.463 (0.254, 0.845)
Negative	27	20.0 (9.0, NR)		0.389 (0.218, 0.694)	0.389 (0.218, 0.694)	35.0 (33.0, NR)		0.473 (0.304, 0.736)	0.331 (0.169, 0.651)

PFS: Progression-free survival; OS: Overall survival; NR: Not reached

Table 4. Univariate analysis of progression-free survival and overall survival.

Factors	3-year PFS		5-year PFS		3-year OS		5-year OS	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Median age (years)								
≤50	1		1		1		1	
>50	0.860 (0.438 – 1.689)	0.662	0.797 (0.412 – 1.542)	0.501	1.024 (0.487 – 2.153)	0.950	1.047 (0.526 – 2.083)	0.896
Histology								
Pure	1		1		1		1	
Mixed	1.567 (0.730 – 3.363)	0.249	1.699 (0.817 – 3.530)	0.156	1.361 (0.578 – 3.206)	0.480	1.406 (0.632 – 3.128)	0.404
FIGO stage								
I+II	1		1		1		1	
IIIA+B	1.530 (0.395 – 5.982)	0.538	1.401 (0.371 – 5.295)	0.619	1.340 (0.346 – 5.195)	0.672	1.077 (0.294 – 3.940)	0.911
IIIC1	2.263 (0.929 – 5.511)	0.072	2.191 (0.942 – 5.094)	0.069	1.419 (0.558 – 3.607)	0.462	1.180 (0.515 – 2.702)	0.696
IIIC2	4.769 (1.714 – 13.275)	0.003	4.330 (1.609 – 11.649)	0.004	1.915 (0.643 – 5.702)	0.243	1.832 (0.691 – 4.858)	0.224
Lymph node metastasis								
No	1		1		1		1	
Pelvic or PALN	2.447 (1.168 – 5.129)	0.018	2.389 (1.172 – 4.871)	0.017	1.435 (0.662 – 3.110)	0.360	1.323 (0.656 – 2.669)	0.434
Tumor size (cm)								
≤4	1		1		1		1	
>4	1.740 (0.877 – 3.450)	0.113	1.873 (0.956 – 3.668)	0.067	1.421 (0.672 – 3.007)	0.358	1.227 (0.618 – 2.439)	0.559
Number of chemotherapy cycles								
1 – 3	1		1		1		1	
4 – 6	0.688 (0.331 – 1.434)	0.391	0.694 (0.341 – 1.412)	0.314	0.616 (0.267 – 1.422)	0.256	0.687 (0.322 – 1.463)	0.330
PD-L1 expression								
Positive	1		1		1		1	
Negative	1.519 (0.624 – 3.696)	0.357	1.201 (0.530 – 2.722)	0.661	0.895 (0.351 – 2.281)	0.816	0.926 (0.387 – 2.215)	0.863
Ki-67								
<75%	1		1		1		1	
≥75%	0.985 (0.453 – 2.144)	0.971	0.931 (0.442 – 1.960)	0.851	1.173 (0.492 – 2.794)	0.718	1.082 (0.497 – 2.353)	0.843

PALN: Para-aortic lymph nodes

with SCNEC [18,19]. In turn, Carroll *et al.* [20] examined 40 specimens from patients with NECC, including SCNEC (23 cases), LCNEC (five cases), undifferentiated NECC (three cases), and mixed NECC (nine cases), and showed that only two (8%) of 25 patients with pure NECC and three (50%) of six patients with mixed NECC were PD-L1-positive, and all 28 (100%) samples were microsatellite stable. Another study found that PD-L1 expression was positive in 10% of patients with NECC [21]. In our cohort, PD-L1 expression was positive in 37.8% (17/45) of the patients.

The prognostic value of PD-L1 for cervical cancer is debatable [22,23]. Kim *et al*. [24] observed that PD-L1 positivity was associated with lower OS in patients with gastroenteropancreatic neuroendocrine tumors. Chen *et al.* [18] evaluated 46 patients with SCNEC and found that recurrence and mortality in PD-L1-positive patients were lower than in PD-L1-negative patients (P = 0.048 and 0.033, respectively). Another study involving 48 cases of SCNEC showed that PD-L1 positivity was correlated with high survival in SCNEC (P = 0.039) [19]. In patients with mixed histology, we found that positive PD-L1 expression was associated with higher 3-year PFS compared with negative PD-L1 expression (66.7% vs. 16.7% , P = 0.042).

Although NECC patients treated with chemoradiotherapy had satisfactory outcomes, few studies assessed the efficacy of this type of therapy in NECC patients. NECC has a worse prognosis than other types of cervical cancer because of the high rates of early LNM and DM [25,26]. Moreover, prognostic factors of definitive RT and chemotherapy in locally advanced NECC patients with stage IB3, IIA2, or IIB-IIIC have not been identified.

There is controversy regarding the effectiveness of radiation therapy in early-stage NECC [7,27]. Chen *et al.* [28] reported that the curative effect of radical surgery was slightly better than that of RT for stage I-II patients. However, Ruiz *et al.* [29] and Hou *et al*. [26] observed that RT was as effective as surgery for patients with early-stage NECC. Patients with late-stage NECC are successfully treated with RT and chemotherapy [30,31]. A study based on the SEER database showed that 5-year OS for AJCC stage III was 28% [25]. In our cohort, 5-year OS was 35.2%, higher than previously reported (30%) [32]. In addition, 5-year OS in patients with stage I-IIA and stage IIB-IIIc2 (LACC) was 56.3% and 42.3%.

LNM is a prognostic factor for carcinoma of the uterine cervix. Chen *et al.* have reported that initial LNM is a poor prognostic factor for LACC [33]. Yamashita *et al.* [34] found that PLN and

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067

Table 5. Multivariate analysis of progression-free survival and overall survival.

Factors	3-year PFS		5-year PFS		3-year OS		5-year OS	
	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value	HR (95%CI)	*P*-value
Median age (years)								
≤50	1		1		1		1	
>50	1.479 (0.497 – 4.405)	0.482	1.389 (0.490 – 3.938)	0.537	1.173 (0.360 – 3.822)	0.792	1.368 (0.438 – 4.275)	0.590
Histology								
Pure	1		1		1		1	
Mixed	1.342 (0.400 – 4.501)	0.633	2.250 (0.733 – 6.907)	0.156	0.495 (0.102 – 2.405)	0.383	0.612 (0.163 – 2.295)	0.467
FIGO stage								
I+II	1		1		1		1	
IIIA+B	1.386 (0.227 – 8.462)	0.724	1.212 (0.211 – 6.948)	0.829	1.826 (0.252 – 13.240)	0.551	1.713 (0.259 – 11.340)	0.577
IIIC1	3.948 (1.103 – 14.130)	0.035	3.412 (1.044 – 11.152)	0.042	3.446 (0.814 – 14.589)	0.093	2.265 (0.635 – 8.079)	0.208
IIIC2	6.427 (1.116 – 36.997)	0.037	5.231 (1.044 – 26.218)	0.044	2.832 (0.323 – 24.837)	0.347	2.400 (0.384 – 14.989)	0.349
Tumor size (cm)								
≤4	1		1		1		1	
>4	0.927 (0.287 – 2.997)	0.899	1.162 (0.382 – 3.532)	0.792	0.739 (0.206 – 2.653)	0.643	0.573 (0.173 – 1.901)	0.363
Number of chemotherapy cycles								
1–3	1		1		1		1	
4–6	0.639 (0.205 – 1.994)	0.441	0.614 (0.198 – 1.902)	0.398	0.258 (0.057 – 1.172)	0.079	0.376 (0.093 – 1.526)	0.171
PD-L1 expression								
Positive	1		1		1		1	
Negative	1.061 (0.344 – 3.268)	0.918	0.878 (0.299 – 2.578)	0.813	0.430 (0.121 – 1.532)	0.193	0.453 (0.136 – 1.501)	0.195
Ki-67								
<75%	1		1		1		1	
≥75%	1.131 (0.323 – 3.968)	0.847	0.819 (0.253 – 2.653)	0.740	0.897 (0.208 – 3.876)	0.938	1.330 (0.241 – 3.653)	0.926

HR: Hazard ratio; CI: Confidence interval

Table 6. Para-aortic failure after pelvic irradiation and prophylactic extended-field irradiation.

Pelvic lymph node	Pelvic irradiation			Prophylactic extended-field irradiation			*P*-value
	Cases	Para-aortic failure	Failure rate	Cases	Para-aortic failure	Failure rate	
Yes	14	6	22.2% (6/27)	10	1	4.2% (1/24)	0.172
No	13	1	3.7% (1/27)	14	1	4.2% (1/24)	1.000
Total	27	7	25.9% (7/27)	24	2	8.3% (2/24)	0.147

PALN status significantly affected survival, and PALN metastasis was the most important prognostic factor for LACC. Similarly, for neuroendocrine tumors of the uterine cervix, PALN metastasis was associated with poor survival [35]. In our cohort, univariate analysis showed that LNM and FIGO stages predicted 3-and 5-year PFS, and multivariate Cox regression analysis demonstrated that FIGO stages predicted 3-and 5-year PFS in patients treated with definitive RT.

Pelvic RT combined with prophylactic EFI can reduce the incidence of para-aortic failure in patients without positive PALN on imaging. However, whether prophylactic EFI can reduce para-aortic failure in patients with cervical cancer is unknown [36]. Hoskins *et al.* [30] analyzed 31 cases of SCNEC, including 17 patients treated with CCRT and EBRT (PLN plus or minus PALN) and 14 treated with CCRT combined with the routine irradiation of PALNs. The outcomes of the two irradiation methods were similar: 3-year OS and failure-free survival were 60% and 57%, respectively. In our cohort, metastasis to PALNs alone after treatment occurred in one case, and metastasis to PALNs associated with LN metastasis in other sites or hematogenous metastasis occurred in nine cases. Prophylactic EFI did not significantly improve PFS and OS, irrespective of PLN metastasis. Nonetheless, larger clinical trials are needed to assess the efficacy of prophylactic EFI in NECC.

Zivanovic *et al.* [37] support the use of chemotherapy for distant control and radiation therapy for the local control of SCNEC. In chemoradiotherapy for patients with stage IIB-IVB SCNEC, at least five cycles of primary chemotherapy with etoposide and platinum were associated with significantly higher 5-year disease-free survival (42.9% vs. 11.8%, $P = 0.041$) and OS (45.6% vs. 17.1%, $P = 0.035$) than fewer cycles. In addition, more than five cycles of CCRT and EP therapy were associated with higher 5-year disease-free survival (62.5% vs. 13.1%, $P = 0.025$) and OS (75.0% vs. 16.9%, $P = 0.016$) [38]. Ishikawa *et al.* [35] found

that less than four cycles of chemotherapy were associated with lower OS in patients with cervical neuroendocrine tumors. In our cohort, compared with less than four cycles of chemotherapy, four or more cycles were associated with significantly higher 3-year PFS (46.4% vs. 34.1%) and 5-year PFS (40.4% vs. 25.7%) and significantly higher 3-year OS (57.6% vs. 31.6%) and 5-year OS (48.6% vs. 25.2%).

This study has limitations. First, the small number of cases with a complete follow-up, the single-center design, and changes in the treatment plan and FIGO staging during the study period (2009 – 2020) may have caused bias in selection, implementation, and measurements. Second, the retrospective design did not allow assessing the clinical effects of anti-PD-L1 therapies in NECC. Third, immunohistochemistry has a limited ability to detect PD-L1 because of the heterogeneity of PD-L1 expression in tumor specimens.

5. Conclusion

Positive PD-L1 expression was associated with higher 3-year PFS in patients with mixed histology. RT for patients with early NECC has the same effect as surgery and is effective for treating locally advanced disease. Four or more cycles of chemotherapy are more effective than a smaller number of courses. Prophylactic EFI did not significantly improve PFS and OS. Nonetheless, the effects of prophylactic EFI should be further studied.

Acknowledgments

None.

Funding

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

References

[1] Gadducci A, Carinelli S, Aletti G. Neuroendrocrine Tumors of the Uterine Cervix: A Therapeutic Challenge for Gynecologic Oncologists. Gynecol Oncol 2017;144:637-46.

[2] Burzawa J, Gonzales N, Frumovitz M. Challenges in the Diagnosis and Management of Cervical Neuroendocrine Carcinoma. Expert Rev Anticancer Ther 2015;15:805-10.

[3] Salvo G, Martin AG, Gonzales NR, Frumovitz M. Updates and Management Algorithm for Neuroendocrine Tumors of the Uterine Cervix. Int J Gynecol Cancer 2019;29:986-95.

[4] Kitajima K, Kihara T, Kawanaka Y, Kido A, Yoshida K, Mizumoto Y, *et al.* Neuroendocrine Carcinoma of Uterine Cervix Findings Shown by MRI for Staging and Survival Analysis-Japan Multicenter Study. Oncotarget 2020;11:3675-86.

[5] Atienza-Amores M, Guerini-Rocco E, Soslow RA, Park KJ, Weigelt B. Small Cell Carcinoma of the Gynecologic Tract: A Multifaceted Spectrum of Lesions. Gynecol Oncol 2014;134:410-8.

[6] Gardner GJ, Reidy-Lagunes D, Gehrig PA. Neuroendocrine Tumors of the Gynecologic Tract: A Society of Gynecologic Oncology (SGO) Clinical Document. Gynecol Oncol 2011;122:190-8.

[7] Zhang Y, Li L, Wang Z, Huang Y, Luo S, Peng Y, *et al.* Preferred Method of Therapy for Patients with Early-stage High-grade Neuroendocrine Carcinoma of the Cervix. Am J Cancer Res 2021;11:4595-606.

[8] Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical Cancer. Lancet 2019;393:169-82.

[9] De Felice F, Marchetti C, Palaia I, Ostuni R, Muzii L, Tombolini V, *et al.* Immune Check-point in Cervical Cancer. Crit Rev Oncol Hematol 2018;129:40-3.

[10] Towner M, Novak K, Chae YK, Matei D. Ipilimumab and Nivolumab for Recurrent Neuroendocrine Cervical Carcinoma. Gynecol Oncol Rep 2022;42:101039.

[11] Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, *et al.* PD-1-expressing Tumor-infiltrating T Cells are a Favorable Prognostic Biomarker in HPV-associated Head and Neck Cancer. Cancer Res 2013;73:128-38.

[12] Droeser RA, Hirt C, Viehl CT, Frey DM, Nebiker C, Huber X, *et al.* Clinical Impact of Programmed Cell Death Ligand 1 Expression in Colorectal Cancer. Eur J Cancer 2013;49:2233-42.

[13] Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, *et al.* PD-1 is Expressed by Tumor-infiltrating Immune Cells and is Associated with Poor Outcome for Patients with Renal Cell Carcinoma. Clin Cancer Res 2007;13:1757-61.

[14] Loharamtaweethong K, Puripat N, Praditphol N, Thammasiri J, Tangitgamol S. PD-L1 Protein Expression and Copy Number Gains in HIV-positive Locally Advanced Cervical Cancer. Ther Adv Med Oncol 2020;12:1-15.

[15] Alejo M, Alemany L, Clavero O, Quiros B, Vighi S, Seoud M, *et al.* Contribution of Human Papillomavirus in Neuroendocrine Tumors from a Series of 10,575 Invasive Cervical Cancer Cases. Papillomavirus Res 2018;5:134-42.

[16] Morgan S, Slodkowska E, Parra-Herran C, Mirkovic J. PD-L1, RB1 and Mismatch Repair Protein Immunohistochemical Expression in Neuroendocrine Carcinoma, Small Cell Type, of the Uterine Cervix. Histopathology 2019;74:997-1004.

[17] Ji X, Sui L, Song K, Lv T, Zhao H, Yao Q. PD-L1, PARP1, and MMRS as Potential Therapeutic Biomarkers for Neuroendocrine Cervical Cancer. Cancer Med 2021;10:4743-51.

[18] Chen L, Yang F, Feng T, Wu S, Li K, Pang J, *et al.* PD-L1, Mismatch Repair Protein, and NTRK Immunohistochemical Expression in Cervical Small Cell Neuroendocrine Carcinoma. Front Oncol 2021;11:752453.

[19] Sun X, Liu L, Wan T, Huang Q, Chen J, Luo R, *et al.* The Prognostic Impact of the Immune Microenvironment in Small-cell Neuroendocrine Carcinoma of the Uterine Cervix: PD-l1 and Immune Cell Subtypes. Cancer Cell Int 2022;22:348.

[20] Carroll MR, Ramalingam P, Salvo G, Fujimoto, Soto LMS, Phoolcharoen N, *et al*. Evaluation of PARP and PDL-1 as potential therapeutic targets for women with high-grade neuroendocrine carcinomas of the cervix. Int J Gynecol Cance 2020;30:1303-7.

[21] Cimic A, Vranic S, Arguello D, Contreras E, Gatalica Z, Swensen J. Molecular Profiling Reveals Limited Targetable Biomarkers in Neuroendocrine Carcinoma of the Cervix. Appl Immunohistochem Mol Morphol 2021;29:299-304.

[22] Gu X, Dong M, Liu Z, Mi Y, Yang J, Zhang Z, *et al.* Elevated PD-L1 Expression Predicts Poor Survival Outcomes in Patients with Cervical Cancer. Cancer Cell Int 2019;19:146.

[23] Enwere EK, Kornaga EN, Dean M, Koulis TA, Phan T, Kalantarian M, *et al.* Expression of PD-L1 and Presence of CD8-positive T Cells in Pre-treatment Specimens of Locally Advanced Cervical Cancer. Mod Pathol 2017;30:577-86.

[24] Kim ST, Ha SY, Lee S, Ahn S, Lee J, Park SH, et alThe impact of PD-L1 expression in patients with metastatic GEP-NETs. J Cancer 2016;7:484–9.

[25] Pei X, Xiang L, Chen W, Jiang W, Yin L, Shen X, *et al.* The Next Generation Sequencing of Cancer-related Genes in Small Cell Neuroendocrine Carcinoma of the Cervix. Gynecol Oncol 2021;161:779-86.

[26] Hou WH, Schultheiss TE, Wong JY, Wakabayashi MT, Chen YJ. Surgery Versus Radiation Treatment for High-grade Neuroendocrine Cancer of Uterine Cervix: A Surveillance Epidemiology and End Results Database Analysis. Int J Gynecol Cancer 2018;28:188-93.

[27] Winer I, Kim C, Gehrig P. Neuroendocrine Tumors of the Gynecologic Tract Update. Gynecol Oncol 2021;162:210-9.

[28] Chen J, Sun Y, Chen L, Zang L, Lin C, Lu Y, *et al.* Prognostic Factors and Treatment of Neuroendocrine Tumors of the Uterine Cervix Based on the FIGO 2018 Staging System: A Single-institution Study of 172 Patients. PeerJ 2021;9:e11563.

[29] Ruiz MP, Dziadek OL, Algren SD. Nonsurgical Management of Neuroendocrine Cancer of the Cervix: Brief Report. Int J Gynecol Cancer 2016;26:1290-2.

[30] Hoskins PJ, Swenerton KD, Pike JA, Lim P, Aquino-Parsons C, Wong F, *et al.* Small-cell Carcinoma of the Cervix: Fourteen Years of Experience at a Single Institution Using a Combined-modality Regimen of Involved-field Irradiation and Platinum-based Combination Chemotherapy. J Clin Oncol 2003;21:3495-501.

[31] Bajaj A, Gopalakrishnan M, Harkenrider MM, Lurain JR, Small W Jr. Advanced Small Cell Carcinoma of the Cervix-successful Treatment with Concurrent Etoposide and Cisplatin Chemotherapy and Extended Field Radiation: A Case Report and Discussion. Gynecol Oncol Rep 2018;23:4-6.

[32] Cohen JG, Kapp DS, Shin JY, Urban R, Sherman AE, Chen LM, *et al.* Small Cell Carcinoma of the Cervix: Treatment and Survival Outcomes of 188 Patients. Am J Obstet Gynecol 2010;203:347.e1-6.

[33] Chen CC, Wang L, Lin JC, Jan JS. The Prognostic Factors for Locally Advanced Cervical Cancer Patients Treated by Intensity-modulated Radiation Therapy with Concurrent Chemotherapy. J Formos Med Assoc 2015;114:231-7.

[34] Yamashita H, Nakagawa K, Tago M, Shiraishi K, Nakamura N, Ohtomo K. Treatment Results and Prognostic Analysis of Radical Radiotherapy for Locally Advanced Cancer of the Uterine Cervix. Br J Radiol 2005;78:821-6.

[35] Ishikawa M, Kasamatsu T, Tsuda H, Fukunaga M, Sakamoto A, Kaku T, *et al.* A Multi-center Retrospective Study of Neuroendocrine Tumors of the Uterine Cervix: Prognosis According to the New 2018 Staging System, Comparing Outcomes for Different Chemotherapeutic Regimens and Histopathological Subtypes. Gynecol Oncol 2019;155:444-51.

[36] Wang W, Zhou Y, Wang D, Hu K, Zhang F. Prophylactic Extended-field Irradiation in Patients with Cervical Cancer: A Literature Review. Front Oncol 2020;10:579410.

[37] Zivanovic O, Leitao MM Jr., Park KJ, Zhao H, Diaz JP, Konner J, *et al.* Small Cell Neuroendocrine Carcinoma of the Cervix: Analysis of Outcome, Recurrence Pattern and the Impact of Platinum-based Combination Chemotherapy. Gynecol Oncol 2009;112:590-3.

[38] Wang KL, Chang TC, Jung SM, Chen CH, Cheng YM, Wu HH, *et al.* Primary Treatment and Prognostic Factors of Small Cell Neuroendocrine Carcinoma of the Uterine Cervix: A Taiwanese Gynecologic Oncology Group Study. Eur J Cancer 2012;48:1484-94.

DOI: http://dx.doi.org/10.18053/jctres.09.202304.23-00067