

Prognostic significance of PD-L1 protein expression and copy number gains in locally advanced cervical cancer

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Abstract

Background: Although immune checkpoint inhibitors against programmed death-1 (PD-1) and its ligand (PD-L1) have demonstrated promising results in several solid malignancies, including cervical cancer, there are some limitations to using PD-L1 immunohistochemical expression as a predictive biomarker for selecting patients who may benefit from such therapy.

Objective: To examine the protein expression and genetic status of PD-L1 with clinical outcomes in locally advanced cervical cancer.

Methods: We investigated the PD-L1 gene copy number gains assessed by fluorescence in situ hybridization (FISH) and PD-L1 expression using immunohistochemistry in 123 patients with locally advanced cervical cancers between December 2008 and December 2016.

Results: The prevalence of PD-L1 immunohistochemical expression was detected in 103/123(83%) cases. PD-L1 gene amplification and polysomy were detected in 7% and 40% of cases, respectively. PD-L1 gene amplification and polysomy were associated with positive PD-L1 immunostaining (score 1+ to 3+) in 88% and 68% of cases, respectively. Clinically, PD-L1 immunopositivity was associated with parametrial invasion at diagnosis. In contrast, PD-L1 polysomy was associated with parametrial invasion and FIGO stages III-IV, whereas PD-L1 amplification was associated with nodal metastasis. In multivariate analysis, PD-L1 amplification was predictive of worse RFS (HR, 5.68; 95%CI, 1.98-16.28; $p = 0.001$), whereas PD-L1 polysomy was predictive of worse LRR (HR, 4.13; 95%CI, 1.63-10.49; $p = 0.003$). PD-L1 immunohistochemical expression was not associated with worse outcomes in Cox models.

Conclusions: Our results showed that an increase in PD-L1 gene copy number could be a novel prognostic and possible predictive biomarker for anti-PD-1/PD-L1 therapy in locally advanced cervical cancer.

Key words: Prognosis, programmed cell death-ligand 1, protein expression, copy number status, cervical cancer

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Introduction

Cervical cancer is the second most common cancer in women worldwide, particularly in developing countries.¹ Most patients present with locally advanced stages² defined as stages IB2-IVA by the International Federation of Gynaecology and Obstetrics (FIGO), and concurrent chemoradiation remains the standard of treatment for these patients. However, the majority of recurrences occur within two years after treatment.²⁻⁴

Most patients who experience recurrence still have a poor prognosis. In addition, a molecular predictor of tumour recurrence after concurrent chemoradiotherapy is lacking. If patients who are at risk for recurrence could be effectively classified, then more specific treatments might have great benefit to these patients. Potentially, identification of cervical cancer with effective molecular biomarkers could be helpful for determining prognosis.

Theoretically, tumours can evade immune surveillance by upregulating programmed cell death-ligand 1 (PD-L1) expression. PD-L1 is known to play a key role in the inhibition of the T cell-mediated immune response, leading to the progression of tumours. PD-L1 can interact with programmed cell death 1 (PD-1), an inhibitory immune checkpoint receptor that plays a major role in immune evasion during tumour progression,⁵ leading to the inactivation of T cells via the inhibition of T-cell receptor signalling and co-stimulatory signals.⁶⁻⁹

PD-L1 overexpression has been identified in many solid cancers,¹⁰ such as malignant melanoma,¹¹ pulmonary cancer¹² and colorectal cancer.¹³ However, the relationship between PD-L1 expression levels and overall disease prognosis is still inconclusive. Wu et al.¹⁰ demonstrated that PD-L1 overexpression is related to worse overall survival in gastric carcinoma, hepatocellular carcinoma, oesophageal carcinoma, and transitional cell carcinoma, whereas this relationship was not observed in pulmonary cancer and malignant melanoma.

In addition, PD-L1 immunohistochemical expression on the surfaces of tumour cells could show some limitations for identifying patients who may benefit from immune checkpoint inhibitors, such as heterogeneous expression and its dynamic expression.

Interestingly, in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma, 9p24.1 gene amplification has recently been demonstrated to be a key mechanism for PD-L1 protein overexpression.¹⁴ Accordingly, in a subset of carcinomas in the stomach as well as the colon, triple-negative breast cancers and glioblastomas, the 9p24.1 gene mechanism has been discovered.^{15,16} More recently, cervical and vulvar squamous cell carcinomas showed the genetic origin of amplified PD-L1 expression as well. In 67% of cervical and 43% of vulvar squamous cell carcinomas assessed using FISH, the genes encoding PD-L1 and PD-L2, *CD274* and *PDCD1LG2*, were co-amplified or could be found on multiple copies of the same chromosome.¹⁷ As a result, 9p24.1 gene copy number changes are an essential mechanism of increased PD-L1 expression in cervical squamous cell carcinomas, as proposed by the data. However, this study did not investigate the correlation of genetic changes with clinical outcomes.

Hence, the present study aimed to assess the pretreatment of PD-L1 protein and gene expression in patients with locally advanced cervical carcinoma. We investigated the correlation between the frequency of copy number gains of the PD-L1 gene and the upregulation of the corresponding proteins and determined its prognostic impact using a clinically well-characterized cohort comprising 123 patients with cervical cancer.

Materials and Methods

The retrospective cohort consisted of cervical cancer patients with FIGO stage IB2-IVA who underwent tissue biopsies of squamous cell carcinoma and adenocarcinoma of the cervix between December 2008 and December 2016 at the Faculty of Medicine, Navamindradhiraj University. The study was approved by the Institutional Review Board of the Faculty of Medicine, Navamindradhiraj University. H&E-stained sections were reviewed by two pathologists (KL, CS). Complete clinicopathologic data were available for all patients.

Immunohistochemistry

Immunohistochemistry was carried out in all cases using a monoclonal antibody recognizing PD-L1 (clone SP263, Ventana Medical Systems, Inc., Tucson, AZ, USA) and performed on a systematized staining platform (Benchmark ULTRA; Ventana Medical Systems, Inc., Tucson, AZ, USA). Four-micron whole tissue sections were cut and stained. An OptiView DAB IHC Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA) was used according to the manufacturer's guidelines for the visualization of the primary anti-PD-L1 antibody. For all immunohistochemical staining reactions, human placental tissue was used as a positive control. The staining of PD-L1 in tissues was quantified using two scoring systems as follows: (1) intensity scores: IHC 0 specified no appreciable staining in cancer cells or staining in under 10% of cancer cells; IHC 1+ specified slightly noticeable partial membrane staining in > 10% of cancer cells; IHC 2+ specified moderate staining of the whole membrane in > 10% of cancer cells; and IHC 3+ specified intense staining of the whole membrane in > 10% of cancer cells; (2) modified histochemical scores (H-scores): the modified H-score was calculated by multiplying the average membranous intensity score (0, absent; 1, weak; 2, moderate; 3, strong) by the percentage of stained cells (from 0% to 100%). Consequently, the H-score could range from 0 to 300. These scoring systems were also used in previous studies.^{17,18}

Tumours with 10% or more cells exhibiting the PD-L1 immunohistochemical reaction, irrespective of intensity, were defined as PD-L1 positive because a previous study had established this cut-off value using the same antibody clone.¹⁹

PD-L1 fluorescence in situ hybridization

Tissue microarrays (TMAs) with 3 mm core diameter were obtained from representative cervical cancer tissues. A minimum of 2 and up to 4 tumour cores from the tumour invasion front and/or the tumour centre were taken from the tumours in areas previously marked by two pathologists (KL, CS). All TMA cores were validated to contain a sufficient number of tumour cells by reviewing haematoxylin and eosin (HE)-stained sections. Dual-colour FISH analysis was performed on 4 µm FFPE TMA sections. A SPEC CD274, *PDCD1LG2/CEN9* Dual Colour Probe (Zytovision, Bremerhaven, Germany) was used according to the manufacturer's guidelines. After screening the entire area of individual cores, the probe signals from a monolayer of at least 50 tumour cell nuclei were counted at × 100 magnification in at least five representative images per case. As previously described,²⁰ PD-L1 amplification was identified if PD-L1/CEP9 ratio ≥ 2.0, while polysomy was identified if the median copy number of the PD-L1 gene ≥ 3.0 and the ratio of this value to the CEP9 signals was < 2.0. All other tumours were deemed to display disomy.

Statistical analysis

Statistical analysis was performed using Stata Statistical Software (College Station, TX: StataCorp LP; <http://www.stata.com>). The distribution of qualitative data was compared between groups using a χ^2 -test or Fisher's exact test, depending on the cell counts of the corresponding contingency tables.

For survival analysis, the Kaplan-Meier method was used to compute recurrence-free survival (RFS), cancer-specific survival (CSS), and locoregional-recurrence-free survival (LRR). Univariate and multivariate analyses were performed using the Cox proportional hazards model, and the differences between groups were analysed by a log-rank test. For all statistical analyses, $p < 0.05$ was considered statistically significant.

Results

Clinicopathological significance of PD-L1 expression and PD-L1 copy number status in locally advanced cervical cancer

The clinicopathological characteristics of the cervical cancer patients in relation to PD-L1 immunohistochemical expression and PD-L1 copy number status are shown in **Tables 1 and 2**, respectively.

Table 1. Clinicopathological data according to PD-L1 immunohistochemical expression.

| Clinicopathologic Parameters | PD-L1 positive | PD-L1 negative | <i>p</i> -value |
|------------------------------|----------------|----------------|-----------------|
| n (%) | 103 (83) | 20 (17) | |
| Age (years) | 55.39 ± 13.18 | 53.90 ± 9.82 | 0.346 |
| < 60 | 66 (64) | 15 (75) | |
| ≥ 60 | 37 (36) | 5 (25) | |
| Histology | | | < 0.001* |
| SCC | 97 (94) | 13 (65) | |
| Adeno | 6 (6) | 7 (35) | |
| Tumor Size (cm) | | | 0.076 |
| < 4 | 22 (21) | 8 (40) | |
| ≥ 4 | 81 (79) | 12 (60) | |

| Clinicopathologic Parameters | PD-L1 positive | PD-L1 negative | <i>p</i> -value |
|------------------------------|----------------|----------------|-----------------|
| Staging | | | 0.079 |
| Stage IB2-IIIB | 61 (59) | 16 (80) | |
| Stage IIIA-IVA | 42 (41) | 4 (20) | |
| Parametrial invasion | | | < 0.001* |
| No | 15 (15) | 10 (50) | |
| Yes | 88 (85) | 10 (50) | |
| Lymph node positive | | | 0.617 |
| No | 77 (75) | 16 (80) | |
| Yes | 26 (25) | 4 (20) | |
| Treatment | | | 0.370 |
| CTRT | 99 (96) | 20 (100) | |
| Radical RT | 4 (4) | 0 (0) | |

Values are presented as number (%), mean ± standard deviation.

Abbreviations: SCC, squamous cell carcinoma; Adeno, adenocarcinoma; CTRT, concurrent chemoradiotherapy; RT, radiation therapy.

* p -value < 0.05, Statistically significant.

Table 2. Clinicopathological data according to PD-L1 copy number status.

| Clinicopathologic Parameters | PD-L amplification | PD-L1 polysomy | PD-L1 disomy | <i>p</i> -value ^a | <i>p</i> -value ^b |
|------------------------------|--------------------|----------------|---------------|------------------------------|------------------------------|
| n (%) | 8 (7) | 50 (40) | 65 (53) | | |
| Age (years) | 56.5 ± 14.51 | 55.02 ± 13.12 | 55.07 ± 12.29 | 0.912 | 0.879 |
| < 60 | 5 (63) | 33 (66) | 42 (64) | | |
| ≥ 60 | 3 (37) | 17 (34) | 23 (36) | | |
| Histology | | | | 0.370 | 0.423 |
| SCC | 8 (100) | 43 (86) | 59 (91) | | |
| Adeno | 0 (0) | 7 (14) | 6 (9) | | |
| Tumor Size (cm) | | | | 0.057 | 0.083 |
| < 4 | 0 (0) | 9 (18) | 21 (32) | | |
| ≥ 4 | 8 (100) | 41 (82) | 44 (68) | | |
| Staging | | | | 0.314 | < 0.001* |
| Stage IB2-IIIB | 5 (63) | 21 (42) | 51 (78) | | |
| Stage IIIA-IVA | 3 (37) | 29 (58) | 14 (22) | | |

Table 2. (Continued)

| Clinicopathologic Parameters | PD-L amplification | PD-L1 polysomy | PD-L1 disomy | <i>p</i> -value ^a | <i>p</i> -value ^b |
|------------------------------|--------------------|----------------|--------------|------------------------------|------------------------------|
| Parametrial invasion | | | | 0.066 | 0.007* |
| No | 0 (0) | 5 (10) | 20 (31) | | |
| Yes | 8 (100) | 45 (90) | 45 (69) | | |
| Lymph node positive | | | | 0.003* | 0.153 |
| No | 3 (37) | 36 (72) | 54 (83) | | |
| Yes | 5 (63) | 14 (28) | 11 (17) | | |
| Treatment | | | | 0.615 | 0.789 |
| CTRT | 8 (100) | 48 (96) | 63 (97) | | |
| Radical RT | 0 (0) | 2 (4) | 2 (3) | | |

Values are presented as number (%), mean ± standard deviation.

^a comparison between PD-L1 amplification and PD-L1 disomy.

^b comparison between PD-L1 polysomy and PD-L1 disomy.

Abbreviations: SCC, squamous cell carcinoma; Adeno, adenocarcinoma; CTRT, concurrent chemoradiotherapy; RT, radiation therapy. **p*-value < 0.05, Statistically significant.

Patients with PD-L1 immunopositive tumours were associated with squamous cell carcinoma ($p < 0.001$) and had a significantly higher risk for parametrial invasion at diagnosis ($p < 0.001$), whereas PD-L1 immunohistochemical expression was not relevantly correlated with age, FIGO stage, tumour size or radio (chemo) therapy. In contrast, PD-L1 polysomy was associated with the presence of parametrial invasion ($p = 0.007$) and FIGO stage III-IV ($p < 0.001$), whereas PD-L1 amplification was found to be relevantly correlated with nodal metastasis ($p = 0.003$). An increase in PD-L1 gene copy number was not correlated with age, histologic subtype, tumour size or radio (chemo) therapy.

Status of PD-L1 protein expression

PD-L1 expression was evaluated in tumour cells of cervical carcinoma by immunohistochemistry. PD-L1 expression in at least 10% of tumour cells was identified in 103/123 (83%) cervical carcinomas. The mean percentage of positive tumour cells (any strength of expression) was 25% (range: 1%-100%). Strong, membranous staining (3+) was identified in 18/123 (15%) cases, moderate staining (2+) in 27/123 (22%) cases, and weak staining (1+) in 20/123 (16%) cases (Figure 1a-d).

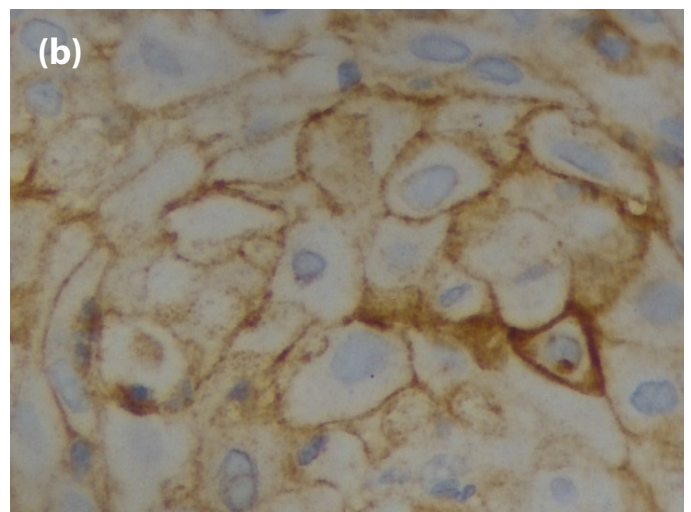
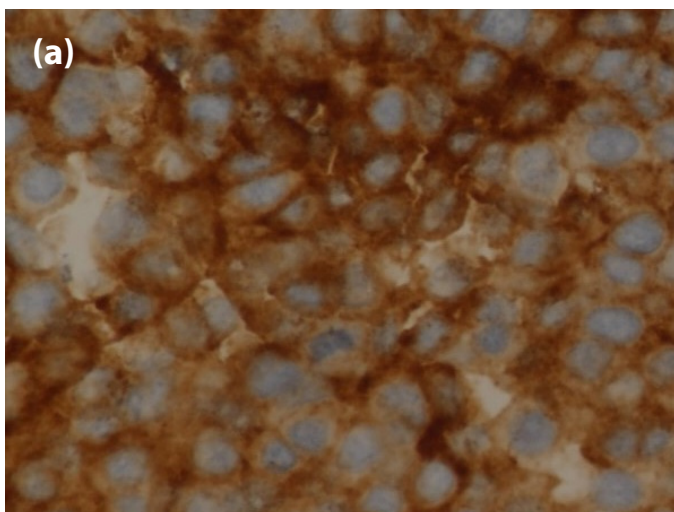


Figure 1. Representative pictures of PD-L1 IHC and FISH, showing (a) strong staining (3+), (b) moderate staining (2+), (c) weak staining (1+), (d) negative staining (0), (e) PD-L1 amplification, (f) polysomy, and (g) disomy. The PD-L1 gene is labelled in green and centromere 9 in red. (a-d: original magnification × 60, e-g: original magnification × 100).

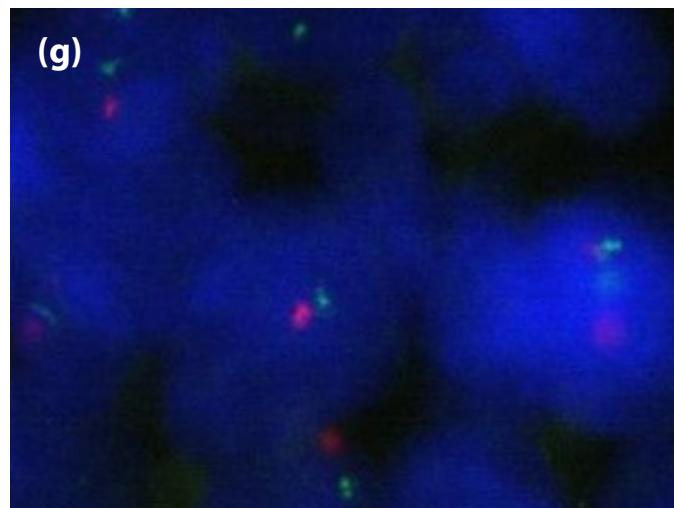
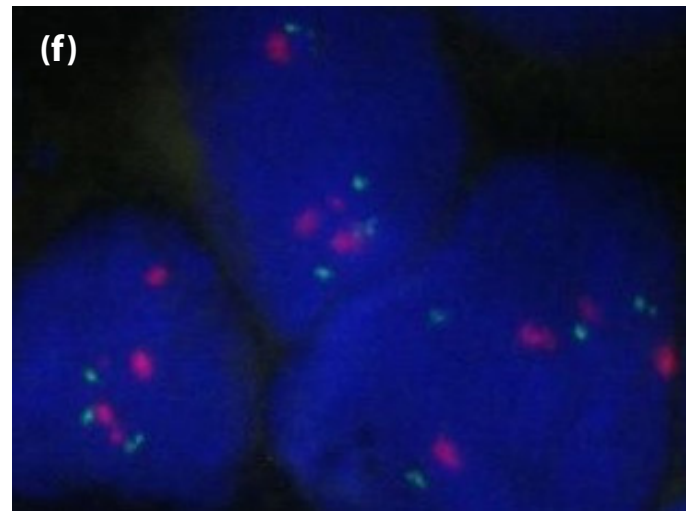
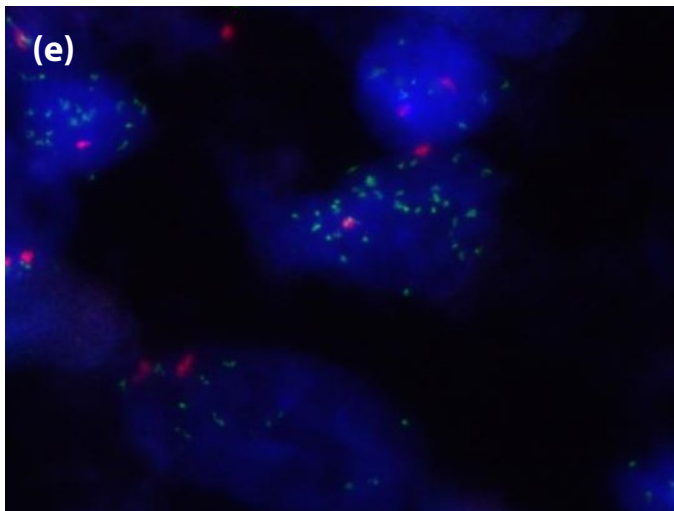
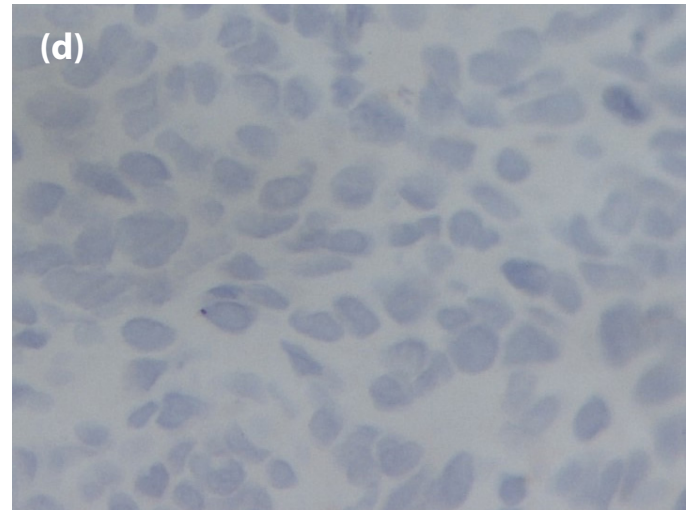
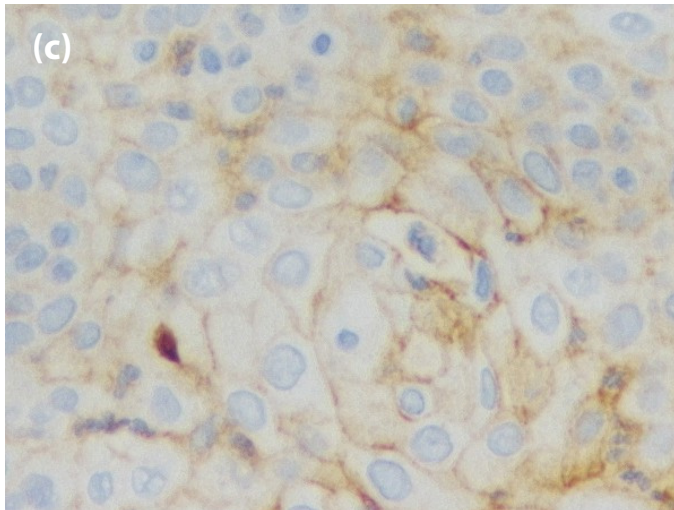


Figure 1. (Continued)

Status of PD-L1 gene copy number alterations

PD-L1 amplification was identified in 8/123 (7%) cases (Figure 1e). Gene copy number gain was confined to tumour cells and was not present in the white blood cell component. PD-L1 polysomy was observed in 50/123 (40%) cases (Figure 1f). A total of 65/123 (53%) cases displayed a disomy (Figure 1g).

Correlation between PD-L1 copy number gain and PD-L1 protein expression

There was no association between PD-L1-positive tumours and the genetic category of the tumour ($p = 0.169$)

The results for both PD-L1 IHC scores and PD-L1 FISH are shown in Table 3. Overall, tumours with PD-L1 gene amplification and polysomy displayed membranous PD-L1 immunostaining (scores 1+ to 3+) by immunohistochemistry in 7/8 (88%) and 34/50 (68%) cases, respectively. A significantly higher frequency of cases with PD-L1 amplification was PD-L1 immunopositive (scores 1+ to 3+) than cases without amplification ($p = 0.042$). Likewise, the immunohistochemical expression of PD-L1 in tumours with PD-L1 polysomy was significantly higher than in tumours with disomy ($p < 0.001$). Moreover, 6/8 carcinoma cases with strong, membranous PD-L1 immunostaining (score 3+) showed PD-L1 amplification, 8/50 showed a polysomy and 4/65 cases displayed a disomy. Interestingly, one of carcinoma cases with PD-L1 amplification was PD-L1 immunonegative (score 0). To confirm the findings and to exclude false negative staining due to tumour heterogeneity in this case, FISH analysis and immunohistochemistry were repeated on whole tissue sections. Likewise, whole sections of all carcinoma cases with PD-L1 immunopositivity (score 3+) but negative results for amplification were also repeated, showing similar results.

Table 3. PD-L1 FISH and PD-L1 immunohistochemistry.

| PD-L1 FISH | Cases n = 123 | PD-L1 IHC | | | |
|---------------|------------------|-------------|-------------|-------------|------------|
| | | Score 3+ | Score 2+ | Score 1+ | Score 0 |
| Amplification | 8 (7%) | 6/8 | 1/8 | 0/8 | 1/8 |
| Polysomy | 50 (40%) | 8/50 | 16/50 | 10/50 | 16/50 |
| Disomy | 65 (53%) | 4/65 | 10/65 | 10/65 | 41/65 |

In further analyses, PD-L1 expression levels in tissues were evaluated using a modified H scoring system. The PD-L1 protein expression levels according to the copy number status of PD-L1. The mean H score in amplified tumours was significantly higher than in polysomic tumours and disomic tumours (228 ± 95 , 62 ± 53 , and 16 ± 14 , respectively; $p < 0.001$).

Survival outcomes

Figure 2 shows the Kaplan-Meier survival curves for cervical cancer patients, according to the IHC-based (Figure 2a-c) and FISH-based (Figure 2d-f) expression status of PD-L1 in tumours. The results of univariate and multivariate analyses evaluating the impact of various known prognostic factors on LRR, RFS and CSS are summarized in Table 4. Overall, FIGO stage, tumour size, number of metastatic lymph nodes and PD-L1 amplification were univariately associated with RFS and CSS. Nevertheless, on multivariate analysis, FIGO stage continued to show a significant impact on RFS (HR, 2.22; 95%CI, 1.17-4.20; $p = 0.015$) and CSS (HR, 13.25; 95%CI, 4.45-39.45; $p < 0.001$), whereas PD-L1 amplification showed a significant impact on RFS on multivariate analysis (HR, 5.68; 95%CI, 1.98-16.28; $p = 0.001$). Only PD-L1 polysomy showed a significant impact on LRR in both univariate and multivariate analyses (HR, 4.13; 95%CI, 1.63-10.49; $p = 0.003$).

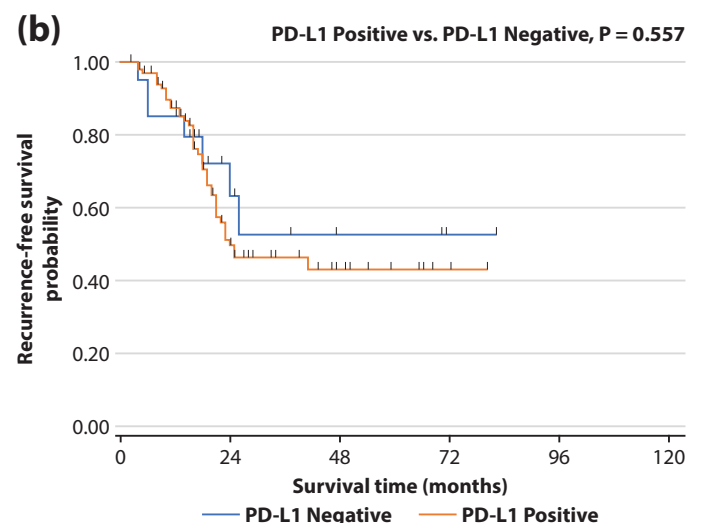
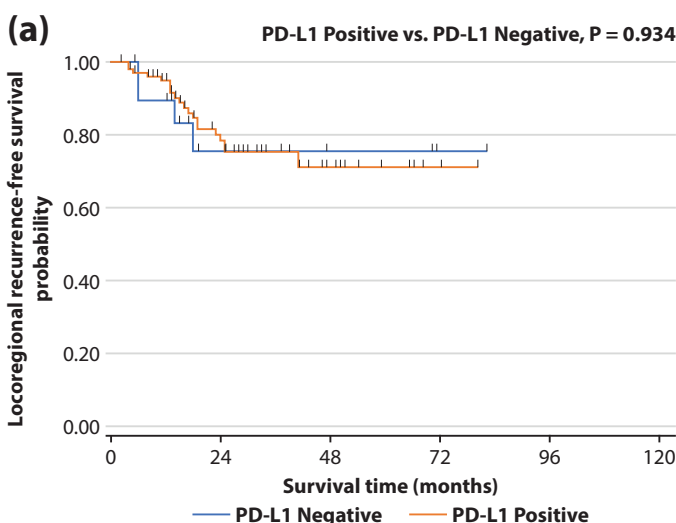


Figure 2. Kaplan-Meier survival curves for cervical cancer patients in relation to PD-L1 immunoreactivity (Figure 2a-c) and genetic category (amplification, polysomy, and disomy) (Figure 2d-f) in regard to LRR, RFS, and CSS. LRR, locoregional recurrence-free survival; RFS, recurrence-free survival; CSS, cancer-specific survival.

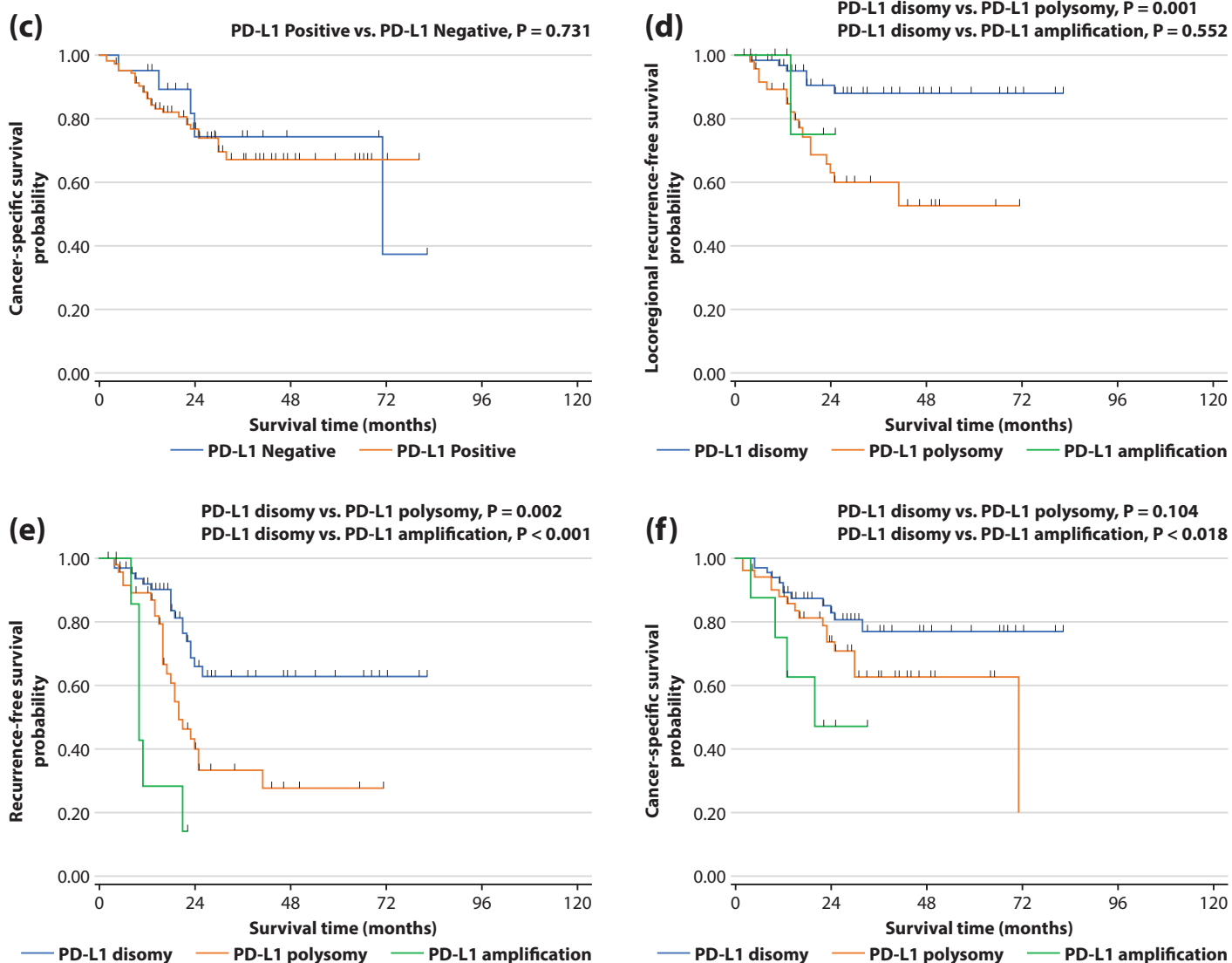


Figure 2. (Continued)

Table 4. Univariate and Multivariate survival analysis. (n = 123)

| Variables | Number (n) | Univariate analysis (p-value) | | | Multivariate analysis (p-value) | | |
|-----------------------------------|------------|-------------------------------|--------|----------|---------------------------------|--------|----------|
| | | LRR | RFS | CSS | LRR | RFS | CSS |
| Age (years) | | | | | | | |
| < 60 Vs. ≥ 60 | 81 Vs. 42 | 0.229 | 0.202 | 0.577 | | | |
| Histology | | | | | | | |
| SCC Vs. Adeno | 110 Vs. 13 | 0.115 | 0.580 | 0.690 | | | |
| Tumor size (cm) | | | | | | | |
| < 4 Vs. ≥ 4 | 30 Vs. 93 | 0.4 | 0.016* | 0.020* | | 0.453 | 0.221 |
| FIGO stage | | | | | | | |
| Stage IB2-IIIB Vs. Stage IIIA-IVA | 77 Vs. 46 | 0.163 | 0.001* | < 0.001* | | 0.015* | < 0.001* |
| Parametrial invasion | | | | | | | |
| Absent Vs. Present | 25 Vs. 98 | 0.985 | 0.198 | | | | |

Table 4. (Continued)

| Variables | Number (n) | Univariate analysis (p-value) | | | Multivariate analysis (p-value) | | |
|-------------------------------|------------|-------------------------------|----------|--------|---------------------------------|--------|-------|
| | | LRR | RFS | CSS | LRR | RFS | CSS |
| Metastatic lymph node | | | | | | | |
| Absent Vs. Present | 93 Vs. 30 | 0.122 | 0.022* | 0.009* | | 0.509 | 0.143 |
| Treatment | | | | | | | |
| CTRT Vs. Radical RT | 119 Vs. 4 | | | | | | |
| PD L1 expression | | | | | | | |
| Negative Vs. Positive | 20 Vs.103 | 0.934 | 0.563 | 0.732 | | | |
| PD L1 copy number alterations | | | | | | | |
| Polysomy Vs. Disomy | 50 Vs. 65 | 0.003* | 0.003* | 0.112 | 0.003* | 0.138 | 0.377 |
| Amplification Vs. Disomy | 8 Vs. 65 | 0.524 | < 0.001* | 0.018* | 0.524 | 0.001* | 0.221 |

Abbreviations: LRR, locoregional recurrence; RFS, recurrence-free survival; CSS, cancer-specific survival; SCC, squamous cell carcinoma; Adeno, adenocarcinoma; CTRT, concurrent chemoradiotherapy; RT, radiation therapy; *p-value < 0.05, Statistically significant.

Discussion

Cancer cells exert various methods of immune inactivation to oppose anticancer immunity. One of these methods is the modification of the PD-1/PD-L1 pathway, which is known as the immune checkpoint.²¹ The PD-1/PD-L1 pathway not only normally regulates exaggerated immune responses but also appears to be a route exploited by cancer cells to escape the immune system.²² The activation of this pathway can give rise to cancer immune evasion and facilitate cancer cell proliferation through mechanisms including T cell tolerance, T cell exhaustion, T cell apoptosis, enhancement of immunosuppressive Treg cell function and PD-1 disbalance.²³ Clinically, expression of PD-L1 in cancer cells is thought to be predictive of tumour response to immunomodulatory therapies targeting the PD-1/PD-L1 pathway.¹⁸

PD-L1 expression has been identified in many solid cancers,¹⁰ such as malignant melanoma,¹¹ pulmonary cancer¹² and colorectal cancer.¹³ However, data on PD-1 expression in cervical carcinoma regarding prevalence, prognostic impact and variation of expression in disease course is limited. In this study, our results showed that the amplification of the PD-L1 gene can be identified in a subset of cervical carcinomas and that gene amplification is correlated with expression levels of the PD-L1 protein in the majority of amplified cases.

Using immunohistochemistry and a cut-off point for positivity defined as at least 10% of malignant cells showing membranous PD-L1 staining, we identified PD-L1 expression in 83% of cervical carcinomas, a relatively higher proportion than in previous studies.²⁴⁻²⁷ To date, only a few studies have investigated PD-L1 expression in cervical carcinoma with positivity rates ranging between 30%-70%.²⁴⁻²⁷ This wide range of detection rates is likely due to several factors: (1) the use of different scoring methods and cut-off points for the definition of positivity; (2) the use of different antibody clones for immunohistochemistry—the SP263 clone used in our study might have a superior sensitivity compared to the other clones due to the number of positively stained cells and its staining intensity;²⁸⁻³⁰ (3) the condition of tissue fixation

and stability of epitopes during immunohistochemistry reactions; and (4) the heterogeneity of PD-L1 staining in different areas of the tumour.

Theoretically, the expression of PD-L1 in malignant cells is regulated by different pathways. An *in vitro* study in cultured SCCHN cell lines demonstrated that PD-L1 expression is significantly upregulated in response to interferon gamma (IFN- γ), an important cytokine that triggers de novo PD-L1 induction in malignant cells as well as in normal tissues.³¹ The expression of PD-L1 in malignant cells is controlled by a variety of intracellular signalling pathways. PD-L1 expression can be stimulated by autocrine/paracrine mediators within the cancer microenvironment, especially IFN- γ . The interaction between extrinsic stimuli and the IFN- γ receptor could lead to the expression and activation of various downstream signalling pathways, including NF- κ B, MAPK, PI3K, mTOR and JAK/STAT, that promote cell cycle progression and the activation of transcription factors. Such signalling pathways further regulate the nuclear translocation of transcription factors to the PD-L1 promoter.³²

Nevertheless, the expression of PD-L1 can fluctuate at different times during the disease course. In contrast, IFN- γ -induced PD-L1 expression can be continuously activated via gene amplification events involving the gene locus on chromosome 9p24.1. The additional somatic copy number alterations resulting in an increase of the fraction of DNA regions could be associated with carcinogenesis and cancer progression.

Several important genes are known to be amplified and have been identified as prognostic markers, mechanisms of drug resistance, or treatment targets in some cancers, such as non-small-cell lung cancer.³³ The 9p24.1 chromosomal locus contains the PD-L1, PD-L2 and JAK2 genes. Selective 9p24.1 amplification has been recently recognized as a key mechanism for increased PD-L1 expression in nodular sclerosis, classical Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma¹⁴ and has also been identified

in a subset of colorectal carcinomas, triple-negative breast cancers, glioblastomas, gastric adenocarcinomas,^{15,16} and cervical and vulvar carcinomas.¹⁷

In the present study, our results demonstrated that PD-L1 copy number gain (amplification and polysomy) can be observed in a subset of cervical cancer patients using FISH analysis (47% of cervical cancer cases). Notably, gene copy number gain was confined to tumour cells and was not present in tumour-infiltrating immune cells. The results indicated that PD-L1 expression on tumour cells could be regulated by tumour-intrinsic mechanisms involving gene copy number gains, whereas PDL1 expression on immune cells was more likely associated with IFN- γ -induced adaptive regulation.

In addition, we found that PD-L1 copy number gain was superior to PD-L1 immunohistochemical expression and could act as an independent and strong predictor of survival outcomes in cervical carcinoma. The amplification-driven PD-L1 expression in a subgroup of cervical carcinomas may demonstrate a new subgroup of cervical cancer with a disease-specific genetic alteration. Further studies are required to evaluate the impact of PD-L1 amplification on pathogenesis and disease progression and on the prognosis of this newly recognized subgroup of cervical carcinomas.

In the case of PD-L polysomy, the data we gathered indicated that the survival rate for patients with polysomy lay between those for disomy and for amplification. These findings showed that it is possible to further categorize PD-L polysomy as high or low polysomy. For low polysomy, the prognosis was similar to that of disomy, while the prognosis for high polysomy more closely followed that of amplification. However, it is necessary to further validate the optimal cut-off values for these polysomy subtypes. In contrast to the previous study,¹⁷ our results showed that PD-L1 amplification can be identified in only a minority of cases (7% of cases). These conflicting data can be explained by differences in sample size, disease stage, or underlying diseases in the studied population.

In this study, it was interesting to note that one of the amplified carcinoma cases was PD-L1 immunonegative (score 0). It is not yet clear why this discrepancy arose, although it is possible that technical difficulties occurring with the antibody clone might be responsible; alternatively, the reason may be linked to posttranscriptional or posttranslational modifications.

Blocking the PD-1/PD-L1 axis could be a promising treatment option in several tumour types, such as malignant melanoma and non-small-cell lung cancer, as well as cervical cancer. These studies have shown better response rates in patients with high PD-L1 expression. However, immune checkpoint blockage has shown remarkable response rates in lymphomas, particularly Hodgkin's lymphoma, even with PD-L1 amplification as well.³⁴ The identification of PD-L1 gene copy number gain as a powerful mechanism for PD-L1 expression in the present study may provide a rationale for the treatment of cervical cancer patients, particularly in a subgroup of cervical cancer with PDL1 gene amplification. Future studies will need to investigate whether PD-L1 copy number status might be a better predictive molecular biomarker for tumour response to blockage of the PD-1/PD-L1 pathway than PD-L1 immunohistochemical status.

Conclusions

PD-L1 copy number increase was associated with PD-L1 protein expression and was demonstrated to be a strong and independent factor of poor survival outcomes in patients with cervical cancer. An increase in PD-L1 gene copy number can be an alternative biomarker for predicting the response to anti-PD-1/PD-L1 therapy in cervical cancer patients. The identification of PD-L1 gene copy number gain as a potential mechanism for PD-L1 overexpression in the present study may provide a rationale for the treatment of cervical cancer patients in this subgroup as well. Further research is needed to examine whether PD-L1 copy number alterations are clinically associated with a benefit of anti-PD-1/PD-L1 treatment in cervical cancer patients.

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Competing interests

The authors declare that they have no conflict of interest.

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