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# Potential biomarkers for predicting the efficacy of a pembrolizumab-containing regimen in advanced cervical cancer: A real-world analysis

## *İleri evre serviks kanserinde pembrolizumab içeren bir rejimin etkinliğini tahmin etmek için olası biyobelirteçler: Gerçek dünya analizi*

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**Abstract**

**Objective:** Prognostic biomarkers in patients with advanced cervical cancer treated with immune checkpoint inhibitors remain unclear. An evaluation of combined positive score (CPS) and tumor proportion score (TPS), and a comparison of their usefulness with inflammatory biomarkers in real-world data could be informative.

**Materials and Methods:** We analyzed 28 patients who were treated with the KEYNOTE-826 regimen between November 2022 and June 2024. The complete cohort (group 1), patients with no prior chemotherapy (group 2), and treatment-naïve (group 3) were evaluated as follows: 1) CPS, TPS, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and hemoglobin, albumin, lymphocyte, and platelets (HALP score) in peripheral blood samples were obtained prior to initial treatment and KEYNOTE-826 regimen, and receiver operating curve analysis was used to compare them. The optimal cut-off values that showed the highest level of discrimination for progression-free survival were identified.

**Results:** The areas under the curve (AUC) for progression-free survival in group 2 were measured for CPS, TPS, NLR, PLR, and HALP scores before the KEYNOTE-826 regimen. The AUC values for these scores were 0.644, 0.662, 0.852, 0.667, and 0.700, respectively. The lower NLR (≤5.52) group had a significantly longer median survival than the higher NLR (>5.52) group ($p<0.001$), with median survivals of 14.0 vs. 7.6 months, respectively. In group 3, CPS and TPS were highest at 0.700 for predicting progression-free survival, compared to NLR, PLR, and HALP score. CPS and TPS appear positively correlated with progression-free survival.

**Conclusion:** CPS and TPS showed a modest correlation with progression-free survival and NLR prior to immunotherapy demonstrated the best treatment efficacy for advanced cervical cancer.

**Keywords:** Cervical cancer, biomarker, combined positive score, tumor proportion score, neutrophil-to-lymphocyte ratio, immunotherapy, pembrolizumab

**PRECIS:** Combined positive score showed a modest correlation with survival, and neutrophil-to-lymphocyte ratio was shown to be the most predictive biomarker for advanced cervical cancer chemotherapy, including immunotherapy.

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## Öz

**Amaç:** İmmün kontrol noktası inhibitörleri ile tedavi edilen ileri evre serviks kanseri olan hastalarda prognostik biyobelirteçler hala belirsizliğini korumaktadır. Kombine pozitif skor (CPS) ve tümör oranı skoru (TPS) değerlendirmesi ve bunların gerçek dünya verilerindeki enflamatuvar biyobelirteçlerle yararlılıklarının karşılaştırılması bilgilendirici olabilir.

**Gereç ve Yöntemler:** Kasım 2022 ile Haziran 2024 arasında KEYNOTE-826 rejimi ile tedavi edilen 28 hastayı analiz ettik. Tam kohort (grup 1), daha önce kemoterapi almamış hastalar (grup 2) ve tedavi görmemiş hastalar (grup 3) aşağıdaki şekilde değerlendirildi: 1) Başlangıç tedavisi ve KEYNOTE-826 rejiminden önce periferik kan örneklerinde CPS, TPS, nötrofil-lenfosit oranı (NLR), trombosit-lenfosit oranı (PLR) ve hemoglobin, albümin, lenfosit ve trombosit değerleri (HALP skoru) elde edildi ve bunları karşılaştırmak için alıcı çalışma karakteristiği eğrisi analizi kullanıldı. Progresyon içermeyen sağkalım için en yüksek ayrım seviyesini gösteren optimum kesme değerleri belirlendi.

**Bulgular:** Grup 2'de progresyon içermeyen sağkalım için eğri altında kalan alanlar (AUC), KEYNOTE-826 rejiminden önce CPS, TPS, NLR, PLR ve HALP skorları için ölçüldü. Bu puanlar için AUC değerleri sırasıyla 0,644, 0,662, 0,852, 0,667 ve 0,700 idi. Düşük NLR (≤5,52) grubu, yüksek NLR (>5,52) grubundan önemli ölçüde daha uzun bir medyan sağkalıma sahipti (p<0,001), medyan sağkalımlar sırasıyla 14,0'a karşı 7,6 ay idi. Grup 3'te, CPS ve TPS, 0,700 değerinde NLR, PLR ve HALP puanına kıyasla progresyonsuz sağkalımı tahmin etmede en yüksek güce sahipti. CPS ve TPS, progresyonsuz sağkalımla pozitif olarak ilişkili görünmektedir.

**Sonuç:** CPS ve TPS, progresyonsuz sağkalımla ılımlı bir korelasyon gösterdi ve immünoterapiden önceki NLR, ileri servikal kanser için en iyi tedavi etkinliğini gösterdi.


**Anahtar Kelimeler:** Servikal kanser, biyobelirteç, kombine pozitif skor, tümör oranı skoru, nötrofil-lenfosit oranı, immünoterapi, pembrolizumab

## Introduction

Despite the widespread implementation of screening programs and introduction of the human papillomavirus vaccine, cervical cancer remains the fourth most commonly diagnosed cancer and the fourth leading cause of mortality in women[1,2]. Patients with advanced cervical cancer may benefit from the monoclonal antibody pembrolizumab (Pem), which targets the programmed death 1 (PD-1) pathway[3,4]. The PD-1 to programmed death-ligand 1 (PD-L1) signaling pathway is essential for maintaining immune homeostasis[5]. The binding of PD-L1 to PD-1 inhibits T-cell proliferation and cytokine production via the T-cell receptor, preventing excessive immune responses[6]. PD-L1 is not only expressed on tumor cells but also on tumor-infiltrating immune cells. Anti-PD-1/L1 therapy is mainly used to target the negative signals mediated by PD-L1; thus, PD-L1 expression in the tumor microenvironment is the most studied biomarker[7]. The combined positive score (CPS), tumor proportion score (TPS), and PD-L1 immunohistochemistry (IHC) assays are important methods for evaluating PD-L1 expression in patients with cancer.

In recurrent or metastatic cervical cancer, Pem plus chemotherapy in a previous phase 3 KEYNOTE-826 study (with or without bevacizumab; Bev) was shown to prolong progression-free survival (PFS) and overall survival (OS) compared to chemotherapy alone[3,4]. Regarding the role of PD-L1 therapy for CPS, the hazard ratio (HR) for PFS compared with the chemotherapy group was 0.62 [95% confidence interval (CI), 0.50-0.77, p<0.001] for patients with PD-L1 CPS ≥1; a conclusion was drawn that it was effective for all patients with CPS ≥1. On the other hand, immune-related events occurred in 34.5% of the Pem group, and grade 3–5 adverse events occurred in 12.1% of the group; including two patients (0.7%) who died from immune-mediated encephalitis and pancreatitis[4]. Biomarkers that can reliably guide the decision-making process for treatment strategies and be highly predictive of responses to immune checkpoint inhibitors (ICI) therapy are required to improve the treatment outcomes of these patients.

Biomarkers in cancer treatment are essential for enabling individualized treatment and predicting patient treatment responses. Important criteria include predictive ability, reliability, clinical usefulness, diversity of data sources, and non-invasive measurement methods[8-11]. Biomarkers that fulfill these criteria will enable the development of more effective treatment strategies. Many studies have evaluated PD-L1 expression in tumor cells as a predictive biomarker of ICI[7]. The PD-L1 expression rate is determined by TPS for non-small cell lung cancer and by CPS for patients with head and neck cancer, esophageal cancer, breast cancer, and cervical cancer[3,12-15].

Recent studies addressing gynecological cancers have reported that inflammatory biomarkers, including the neutrophil-to-lymphocyte ratio (NLR), are significantly associated with clinical prognosis[16-18]. Peripheral neutrophil counts assessed by NLR are directly related to intratumoral neutrophil infiltration and have been shown to impair antitumor immune responses[19,20]. In theory, neutrophilia indicates a response to systemic inflammation, and lymphocytopenia reflects a decrease in cell-mediated immunity[21]. Our recent publications report that the peripheral blood NLR score, sampled prior to Pem inclusion in the regimen, is a significant predictor for the prognosis of regimens containing Pem for endometrial cancer[17,18].

In the present study, we aimed to evaluate the possibility of using CPS and TPS as prognostic biomarkers in data from real-world settings, and to compare their usefulness with inflammatory biomarkers in advanced cervical cancer.

## Materials and Methods

### Patient Population

We performed a retrospective review of a clinical database to identify cases of advanced or recurrent cervical cancer in patients who received the KEYNOTE-826 treatment protocol, consisting of Pem, chemotherapy, and Pem ± Bev, followed by Pem ± Bev as maintenance therapy, between November 2022 and June 2024. The Institutional Review Board at Kagoshima University Graduate School of Medical Sciences granted approval for the study protocol (approval number: 230081, date: 19.09.2023). The 2018 FIGO staging system was used to classify the disease, and clinical data were collected by reviewing inpatient medical records. Pathological information was obtained from biopsies performed at our outpatient clinic on patients who did not undergo surgery or from uterine specimens that were surgically removed from patients receiving primary surgical treatment. PD-L1 expression in formalin-fixed tumor samples was evaluated at a central laboratory in our institute using a commercially available PD-L1 IHC 22C3 pharm Dx assay (Dako, Carpinteria, California, U.S.A.).

A study flowchart is shown in Figure 1. The study included 32 patients with endometrial cancer who had progressed to stages III-IV or recurred and who received ICI. Finally, after excluding four cases due to insufficient data, 28 cases formed group 1, the complete cohort of patients; patients with no prior chemotherapy formed group 2; and treatment-naïve patients formed group 3. Patients were evaluated as follows. Group 2 had the most similar inclusion criteria to the previous KEYNOTE-826 study and included patients who had undergone concurrent chemoradiotherapy.

First, the predictive prognostic biomarkers, including CPS, TPS, and inflammatory biomarkers, along with NLR, PLR, and hemoglobin, albumin, lymphocyte, and platelets (HALP) scores in peripheral blood samples, were compared using receiver operating curve (ROC) analysis. Pre-treatment values of neutrophil, hemoglobin, platelet, and albumin counts were obtained immediately prior to undergoing the KEYNOTE-826 regimen. CPS was calculated as (number of PD-L1 positive tumor cells + number of PD-L1 positive immune cells)/total number of viable tumor cells × 100. TPS was calculated as (number of PD-L1 positive tumor cells/total number of viable

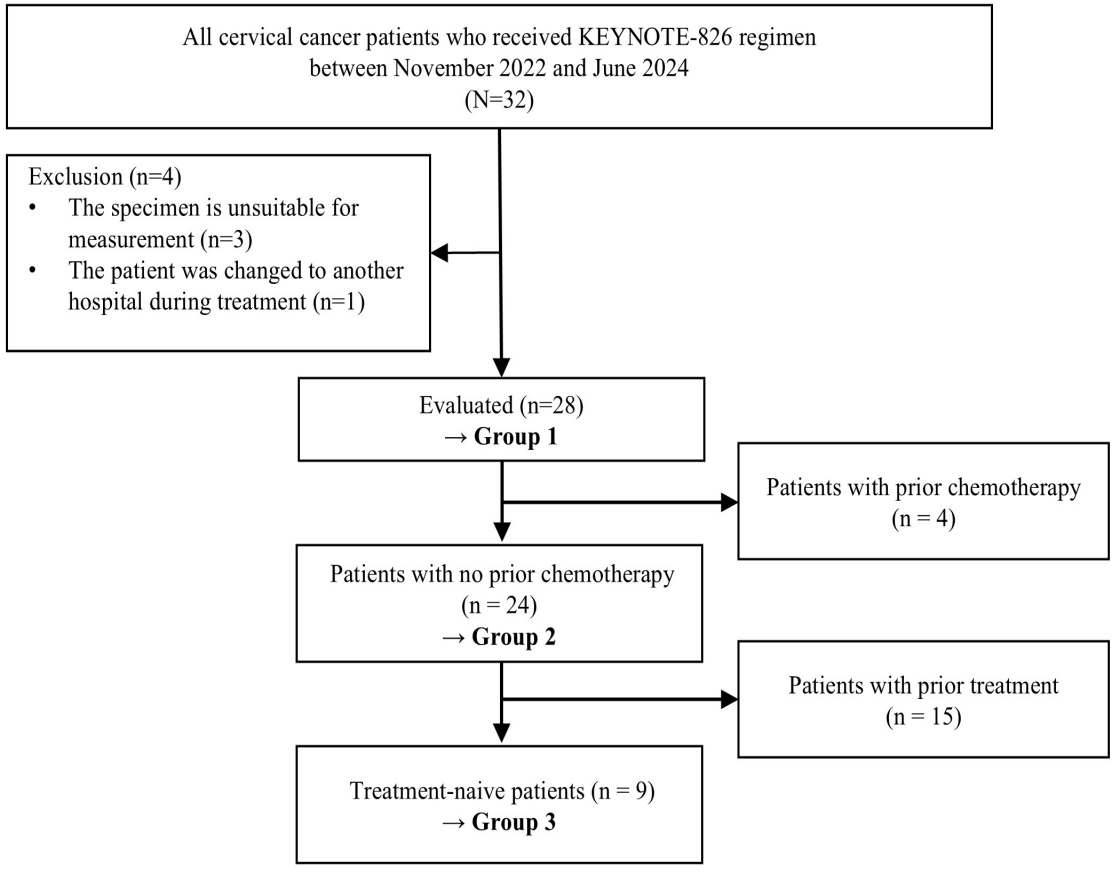


**Figure 1.** Flowchart summarizing the study. The KEYNOTE-826 regimen was defined as Pem plus chemotherapy ± Bev followed by maintenance therapy with Pem ± Bev

*Pem: Pembrolizumab, Bev: Bevacizumab*

tumor cells) × 100. NLR, PLR, HALP scores were defined as follows: neutrophil [L]/lymphocytes [L], platelets [L]/lymphocytes [L], and hemoglobin [g/L] × albumin [g/L] × lymphocytes [L]/platelets [L]. The usefulness of each prediction parameter in identifying overall response (OR), disease control (DC), and a progression-free (PF) period of 8 months or more was evaluated, and their potential as surrogates for clinical benefit was assessed. Second, the Youden index was employed to identify the optimal cut-off values for the predictor that demonstrated the highest level of discrimination for PF.

All participants were admitted to and provided care at the Kagoshima University Hospital. All patients who had previously undergone chemotherapy had fully recovered from any bone marrow suppression caused by the treatment, and none of them were administered immunosuppressive drugs, including steroids, that might influence the complete blood count. PFS was described as the time span from the initiation of the treatment plan to the confirmation of tumor advancement. The proportion of patients who achieve either a partial response (PR) or a complete response (CR) is typically defined as OR. DC was achieved with PR, CR, and stable disease. The KEYNOTE-826 regimen was defined as at least one course of chemotherapy plus Pem ± Bev, followed by Pem ± Bev, as maintenance therapy.

### Statistical Analysis

The threshold for statistical significance was established at $p<0.05$. The Kaplan-Meier method was employed to generate survival curves, and the log-rank test was used to compare PFS across the groups. All statistical analyses were performed on a personal computer using a statistical software package (SPSS for Windows, v.29; SPSS Inc., Chicago, IL, USA).

## Results

Table 1 shows the characteristics of group 1 patients enrolled in the KEYNOTE-826 regimen receiving Pem for advanced or recurrent cervical cancer. The median age of participants was 51.5 years, with a median follow-up time of 9.5 months (range, 1-25 months); and 19 (67.9%) cases were recurrent. All patients were initially treated with chemotherapy with paclitaxel plus carboplatin (TC) along with bevacizumab (Bev), and continued with the KEYNOTE-826 regimen for at least six courses. However, two patients were excluded from completing the regimen due to disease progression, and one patient did not receive the sixth course because of complications from COVID-19.

Of the four patients with prior chemotherapy regimens, two had undergone only one regimen, one had undergone two regimens, and one had undergone four regimens; all patients underwent the TC ± Bev regimen. The two cases with CPS of only 1.0 progressed within 0 and 1 month, respectively, and both died of the disease. Three out of the four cases with zero TPS had recurrence at 0, 8, and 11 months, and disease death at 1, 12, and 22 months. The remaining patient survived 11 months.

### Potential as a Biomarker of CPS, TPS, and Exploratory Research for NLR, PLR, and HALP Score for the KEYNOTE-826 Regimen

The median values for the predictive biomarkers are shown in Table 2. In group 3, the CPS and the NLRs prior to initial treatment and prior to the KEYNOTE-826 regimen appear to be higher than those for groups 1 and 2.

The areas under the ROC curve for predictive biomarkers for the KEYNOTE-826 regimen in all groups are shown in Table 3. Among the inflammatory biomarkers (NLR, PLR, and HALP score) measured prior to the KEYNOTE-826 regimen NLR was the highest predictor of OR, DC, and PF. The CPS and TPS had only modest OR, DC, and PF prediction accuracy in groups 1 and 2, but they were higher for predicting PF than for NLR, PLR, and the HALP score in group 3. In all groups, CPS and TPS were consistently higher in the order of PF, DC, and OR levels.

Major analysis results for groups 1-3 in advanced cervical cancer treated with the KEYNOTE-826 regimen are shown in Figures 2-4. In group 1, the area under the curve (AUC) and scatter diagrams between CPS, TPS, and NLR for PF and PFS of NLR using the Kaplan-Meier method is shown in Figure 2. The AUC in group 1 of PF included CPS, TPS NLR, PLR, and HALP scores measured prior to the KEYNOTE-826 regimen; results were 0.636, 0.636, 0.826, 0.674, and 0.686, respectively. Scatter diagrams of CPS and TPS for PFS revealed a mild positive correlation. The lower NLR (NLR ≤5.525) group had a significantly longer PFS than the higher NLR (5.525 <NLR) group ($p<0.001$, median survival: 13.6 months vs. 7.8 months; Figure 2E); furthermore, NLR and PFS were each negatively correlated (Figure 2F).

In group 2 of PF, the areas under the curve included CPS, TPS, and NLR; PLR, and HALP scores measured prior to the KEYNOTE-826 regimen; results were 0.644, 0.662, 0.852, 0.667, and 0.700, respectively. The group with a lower NLR (NLR ≤5.525) exhibited a notably longer survival compared to the group with a higher NLR (5.525< NLR) ($p<0.001$, median survival: 14 months vs. 7.6 months; Figure 3E). Scatter diagrams of the CPS and TPS for PFS revealed a mild positive correlation; and the NLR for PFS was negatively correlated (Figure 3F).

In group 3, CPS and TPS were the most predictive biomarkers for PF, with an area under the ROC curve of 0.700 for both. The higher CPS (20.0≤ CPS) group tended to have longer PFS than the lower CPS (CPS <20) group ($p=0.210$, median survival: 3 months vs. not statistically reached; Figure 4B). Similarly, the higher TPS (25.0≤ TPS) group tended to have longer PFS than the lower TPS (TPS <25) group ($p=0.210$, median survival: 3.5 months compared to a median survival that was not statistically reached). The scatter diagrams of CPS and TPS for PFS seem to be positively correlated (Figure 4C, D).

**Table 1**. Baseline characteristic of 28 patients in advanced cervical cancer

| Case | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 45 | 52 | 41 | 56 | 38 | 48 | 41 | 51 | 50 | 46 | 57 | 65 | 64 | 57 |
| Histological type | Gastric | SCC | SCC | SCC | SCC | SCC | SCC | Gastric | SCC | Adeno | SCC | Others | SCC | Others |
| FIGO stage (FIGO 2013) | IVB | IB3 | IIB | IIIC2 | IIIC1 | IVA | IVB | IIIC1 | IIA2 | IB3 | IIIC1 | IVB | IB2 | IVB |
| Timing of TC plus Pem ± Bev administration | Initial | Rec | Rec | Rec | Rec | Rec | Initial | Rec | Rec | Rec | Rec | Initial | Rec | Initial |
| Sites of recurrence | Pelvic+Distant | Distant | Distant | Distant | Pelvic+Distant | Distant | Pelvic+Distant | Pelvic+Distant | Pelvic | Pelvic+Distant | Pelvic | Pelvic+Distant | Distant | Pelvic+Distant |
| TC plus Pem + Bev | 6 | 5 | 8 | 6 | 6 | 6 | 6 | 6 | 4 | 9 | 6 | 6 | 6 | 1 |
| TC plus Pem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Pem + Bev | 4 | 7 | 10 | 14 | 10 | 5 | 9 | 19 | 5 | 0 | 16 | 17 | 5 | 0 |
| Pem | 4 | 3 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Numeber of prior chemotherapy | 0 | 0 | 2 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prior RT or CCRT | Nil | CCRT | CCRT | CCRT | Nil | CCRT | Nil | Nil | Nil | CCRT | Nil | Nil | Nil | Nil |
| Treatment delay | Nil | Once | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Dose reduction of TC | 1 level | Nil | 1 level | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Best overall response | PR | CR | SD | CR | PR | PD | PR | CR | CR | PR | PR | CR | SD | PD |
| Prognosis | DOD | AWD | DOD | NED | DOD | DOD | DOD | NED | AWD | DOD | NED | NED | DOD | DOD |
| Specimen of CPS and TPS | Bx | Resected uterus | Resected uterus | Bx | Bx | Bx | Bx | Resected uterus | Resected uterus | Resected uterus | Bx | Bx | Resected uterus | Bx |
| Timinig of examination | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial |
| CPS value | 10 | 10 | 20 | 10 | 10 | 80 | 30 | 30 | 30 | 1 | 100 | 40 | 15 | 1 |
| TPS value | 10 | 0 | 10 | 5 | 5 | 70 | 30 | 1 | 20 | 0 | 100 | 40 | 10 | 0 |
| Pre-initial treatment | | | | | | | | | | | | | | |
| NLR | 2.11 | 1.74 | 2.91 | 4.02 | 5.02 | 7.43 | 11.01 | 1.26 | 6.75 | 2.30 | 2.31 | 4.42 | 3.53 | 8.78 |
| PLR | 206.6 | 134.1 | 21.5 | 203.9 | 321.6 | 34.3 | 546.6 | 133.1 | 216.2 | 164.0 | 220.3 | 298.2 | 267.6 | 366.1 |
| HALP | 0.24 | 0.24 | 1.91 | 0.22 | 0.13 | 1.25 | 0.07 | 0.40 | 0.04 | 0.31 | 0.14 | 0.15 | 0.17 | 0.06 |
| Pre-TC plus Pem ± Bev | | | | | | | | | | | | | | |
| NLR | 1.13 | 3.42 | 2.02 | 1.56 | 5.09 | 7.43 | 11.01 | 1.78 | 10.38 | 5.69 | 2.99 | 2.90 | 5.36 | 10.37 |
| PLR | 121.2 | 330.3 | 213.9 | 248.4 | 199.3 | 343.3 | 546.6 | 170.6 | 521.8 | 313.9 | 205.8 | 160.1 | 560.0 | 342.4 |
| HALP | 0.41 | 0.15 | 0.25 | 0.24 | 0.22 | 0.11 | 0.06 | 0.29 | 0.08 | 0.12 | 0.20 | 0.28 | 0.08 | 0.05 |

**Table 1.** continued

| Case | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 46 | 43 | 50 | 55 | 54 | 44 | 51 | 60 | 64 | 60 | 65 | 68 | 56 |
| Histological type | SCC | Adeno | SCC | SCC | SCC | SCC | Others | Adeno | SCC | SCC | Adeno | Others | Others |
| FIGO stage (FIGO 2013) | IVB | IVB | IIIC1 | IIIC1 | IVB | IB | IVB | IIIB | IIIB | IIIC1p | IVB | IVB | IVB |
| Timing of TC plus Pem ± Bev administration | Initial | Initial | Rec | Rec | Initial | Rec | Rec | Rec | Rec | Rec | Initial | Rec | Initial |
| Sites of recurrence | Distant | Pelvic+Distant | Pelvic+Distant | Distant | Pelvic+Distant | Pelvic | Pelvic | Distant | Distant | Distant | Distant | Pelvic+Distant | Distant |
| TC plus Pem + Bev | 6 | 4 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 1 | 6 | 6 |
| TC plus Pem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| Pem + Bev | 14 | 0 | 1 | 7 | 6 | 2 | 0 | 6 | 7 | 0 | 0 | 4 | 7 |
| Pem | 0 | 0 | 11 | 0 | 0 | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Numeber of prior chemotherapy | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prior RT or CCRT | Nil | Nil | Nil | Nil | Nil | RT | Nil | CCRT | CCRT | CCRT | Nil | CCRT | Nil |
| Treatment delay | Once | Nil | Nil | Nil | Nil | Nil | Once | Nil | Nil | Once | Once | Nil | Nil |
| Dose reduction of TC | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | 1 level | 1 level | Nil | Nil |
| Best overall response | PR | PD | SD | SD | PR | CR | CR | CR | PR | PR | SD | PR | CR |
| Prognosis | NED | DOD | NED | NED | NED | NED | NED | NED | NED | NED | AWD | NED | NED |
| Specimen of CPS and TPS | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx |
| Timinig of examination | Initial | Initial | Initial | Initial | Initial | Initial | Rec | Rec | Initial | Initial | Initial | Rec | Initial |
| CPS value | 5 | 10 | 5 | 60 | 40 | 100 | 10 | 5 | 30 | 10 | 10 | 5 | 20 |
| TPS value | 1 | 1 | 0 | 60 | 20 | 100 | 20 | 20 | 40 | 20 | 20 | 10 | 30 |
| Pre-initial treatment | | | | | | | | | | | | | |
| NLR | 4.89 | 3.49 | 4.33 | 6.48 | 21.27 | 3.81 | 3.40 | 3.02 | 6.48 | 1.81 | 5.03 | 2.69 | 6.08 |
| PLR | 249.1 | 254.9 | 358.1 | 388.0 | 788.9 | 159.0 | 199.2 | 121.3 | 208.5 | 100.5 | 234.0 | 174.8 | 309.4 |
| HALP | 0.20 | 0.21 | 0.13 | 0.13 | 0.06 | 0.36 | 0.22 | 0.47 | 0.25 | 0.55 | 0.24 | 0.28 | 0.10 |
| Pre-TC plus Pem ± Bev | | | | | | | | | | | | | |
| NLR | 4.83 | 6.07 | 2.71 | 5.32 | 26.60 | 3.81 | 11.33 | 3.56 | 2.25 | 4.05 | 4.19 | 4.62 | 1.02 |
| PLR | 296.6 | 223.3 | 290.0 | 335.6 | 695.6 | 159.0 | 384.6 | 206.0 | 193.2 | 169.8 | 175.2 | 201.4 | 174.8 |
| HALP | 0.15 | 0.23 | 0.13 | 0.16 | 0.07 | 0.35 | 0.11 | 0.25 | 0.40 | 0.30 | 0.28 | 0.09 | 0.21 |

SCC: Squamous cell carcinoma, Adeno: Adenocarcinoma, FIGO: International Federation of Gynecology and Obstetrics, TC: Paclitaxel plus carboplatin, Pem: Pembrolizumab, Bev: Bevacizumab, RT: Radiothearapy, CCRT: Concurrent chemoradiotherapy, CR: Complete response, PR: Partial response, SD: Stable disease, PD: Progressive disease, DOD: Dead of disease, AWD: Alive with disease, NED: No evidence of disease, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HALP score: hemoglobin, albumin, lymphocyte, and platelet score, Rec: Recurrence

**Table 2.** Median values for each predictive biomarkers

| | | | Measurement periods | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Prior to initial treatment | | | Prior to KEYNOTE-826 regimen | | |
| | CPS | TPS | NLR | PLR | HALP score | NLR | PLR | HALP score |
| Group 1 (range) | 12.5 (1.0-100) | 20 (0-100) | 3.91(1.26-21.27) | 214.9 (21.5-788.9) | 0.220 (0.04-1.91) | 4.12 (1.02-26.60) | 235.9 (121.2-695.6) | 0.185 (0.050-0.041) |
| Group 2 (range) | 12.5 (1.0-100) | 20.0 (0-100) | 4.18 (1.74-21.30) | 218.3 (34.3-788.9) | 0.215 (0.04-1.25) | 4.12 (1.02-26.60) | 251.4 (121.23-695.6) | 0.165 (0.05-0.41) |
| Group 3 (range) | 30.0 (1.0-100) | 20 (0-100) | 5.03 (2.31-21.27) | 254.9 (34.3-788.9) | 0.150 (0.06-1.25) | 6.07 (2.90-26.60) | 296.6 (160.1-695.6) | 0.150 (34.33-788.89) |

NLR, PLR, and HALP scores were sampled in peripheral blood. CPS: Combined positive score, TPS: Tumor proportion score, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HALP score: Hemoglobin, albumin, lymphocyte, and platelet score

**Table 3.** Area under the ROC curve and cut-off value in predictive biomarkers

| | | Area under the ROC curve | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Measurement periods | | | | | |
| | | | | Prior to initial treatment | | | Prior to KEYNOTE-826 regimen | | |
| | | CPS | TPS | NLR | PLR | HALP score | NLR | PLR | HALP score |
| Group 1 | | | | | | | | | |
| | Overall response | 0.583 | 0.602 | 0.534 | 0.526 | 0.508 | 0.620 | 0.703 | 0.638 |
| | Disease control | 0.580 | 0.633 | 0.760 | 0.533 | 0.513 | 0.804 | 0.707 | 0.727 |
| | Progression-free (*estimated cut-off value) | 0.636 | 0.636 | 0.689 | 0.492 | 0.534 | 0.826 (5.52) | 0.674 | 0.686 |
| Group 2 | Overall response | 0.579 | 0.632 | 0.539 | 0.593 | 0.554 | 0.664 | 0.614 | 0.621 |
| | Disease control | 0.579 | 0.635 | 0.730 | 0.508 | 0.516 | 0.857 | 0.698 | 0.714 |
| | Progression-free (*estimated cut-off value) | 0.644 | 0.662 | 0.657 | 0.546 | 0.505 | 0.852 (5.52) | 0.667 | 0.700 (0.12) |
| Group 3 | Overall response | 0.500 | 0.500 | 0.700 | 0.850 | 0.725 | 0.600 | 0.650 | 0.575 |
| | Disease control | 0.639 | 0.694 | 0.500 | 0.611 | 0.639 | 0.667 | 0.611 | 0.667 |
| | Progression-free (*estimated cut-off value) | 0.700 (20.0) | 0.700 (25.0) | 0.500 | 0.700 | 0.775 | 0.550 | 0.550 | 0.525 |

*The estimated cut-off value is only shown for progression-free of ≥0.700. Bold indicates ≥0.8, and underlined indicates ≥0.7. NLR, PLR, and HALP scores were measured from peripheral blood samples. ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HALP score: hemoglobin, albumin, lymphocyte, and platelet score

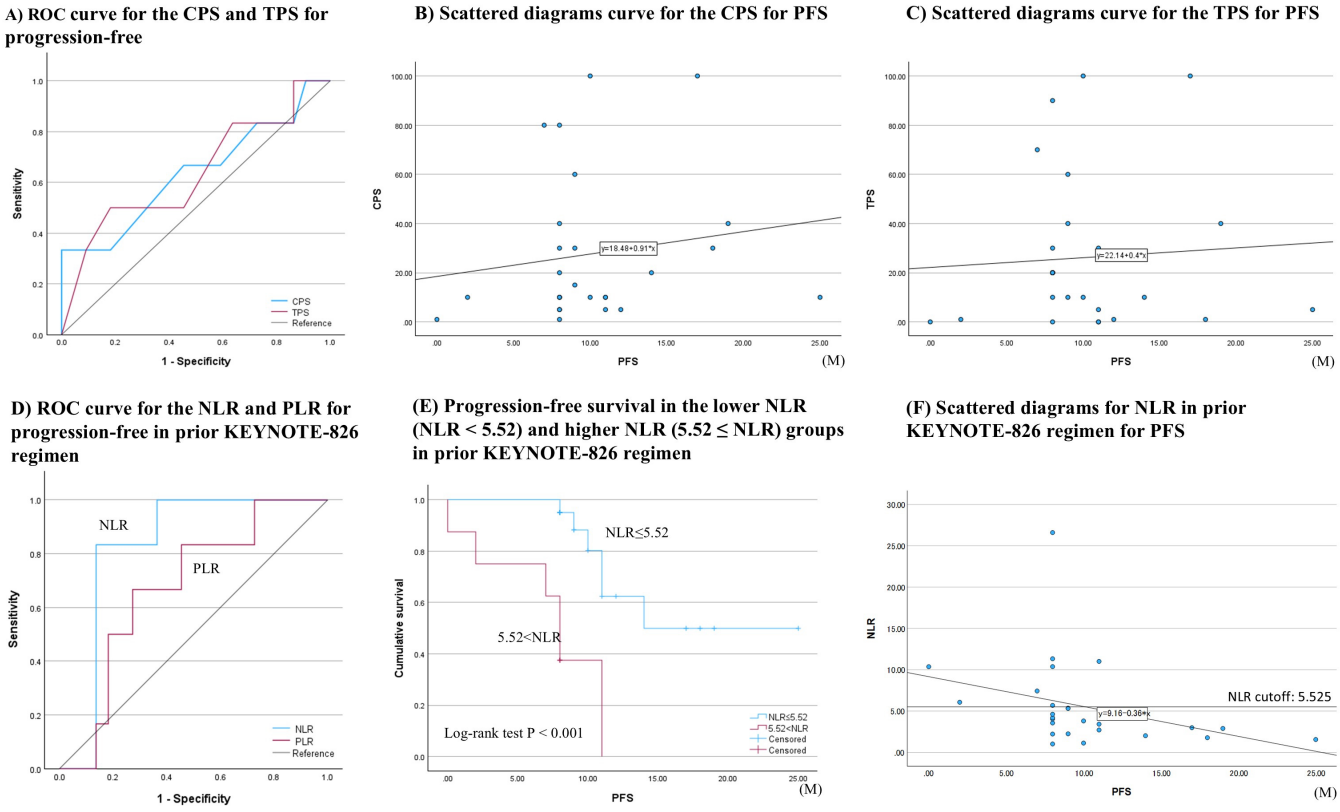


**Figure 2.** Major analysis results of group 1 in advanced cervical cancer treated with the KEYNOTE-826 regimen. A) ROC curve for CPS and TPS for progression-free. The areas under the curve were 0.636 and 0.636, respectively. Scatter diagrams for CPS and TPS for PFS are shown in (B) and (C). In both cases, a mild positive correlation seems to be observed. (D) ROC curve for NLR and PLR for progression-free in the Prior to KEYNOTE-826 regimen. The AUC was 0.826 and 0.674, and the cut-off value for NLR was 5.52. (E) PFS in the lower NLR (NLR <5.52) and higher NLR (5.52 ≤NLR) groups in the Prior KEYNOTE-826 regimen. The lower NLR (NLR ≤5.52) group had a significantly longer PFS than the higher NLR (5.52< NLR) group (p<0.001, median survival: 13.6 M vs. 7.8 months), (F) Scattered diagram for NLR in the Prior to the KEYNOTE-826 regimen for PFS. A negative correlation was observed between the NLR and PFS

*ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, PFS: Progression-free survival, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, AUC: Area under the curve*

## Discussion

In our study, CPS and TPS showed a modest positive correlation with PF, but they could not be established as absolute biomarkers in patients receiving the KEYNOTE-826 regimen. In contrast, inflammatory biomarkers, measured using the NLR in peripheral blood samples immediately prior to the KEYNOTE-826 regimen, were the most predictive of treatment efficacy for advanced cervical cancer.

The complex and individual interactions between host factors, which indicate dysfunctional immune responses, and tumor factors, which contribute to aggressive malignancies, are crucial[22,23]. There is increasing evidence that both neutrophils and lymphocytes, components of the immune system, are involved in tumor progression and prognosis[24]. The presence of neutrophils in peripheral blood indicates inflammation, and lymphocytes are important indicators of immune status. In healthy human participants, the mean value and corresponding 95% reference interval for the inflammatory biomarkers NLR and PLR were 1.76 (0.83-3.92) and 120 (61-239), respectively[25]; both values were clearly high in our study population, as shown in Table 2, especially in patients in group 3. In group 3, the median NLR was remarkably high, and in such a population with inherently poor prognosis, NLR may be less useful as a biomarker of treatment efficacy[22,24]. However, it is also known that an increase in baseline NLR does not necessarily prevent long-term survival, and such an increase alone does not seem to have prognostic significance sufficient to warrant discontinuation of ICI[22]. In patients with an extremely high NLR, it is necessary to predict treatment efficacy using multiple biomarkers such as CPS and TPS.

The role of biomarkers in the KEYNOTE-826 regimen for CPS has not been fully analyzed. Concerns exist regarding using PD-L1 IHC as a prognostic biomarker for anti-PD-1 or PD-L1

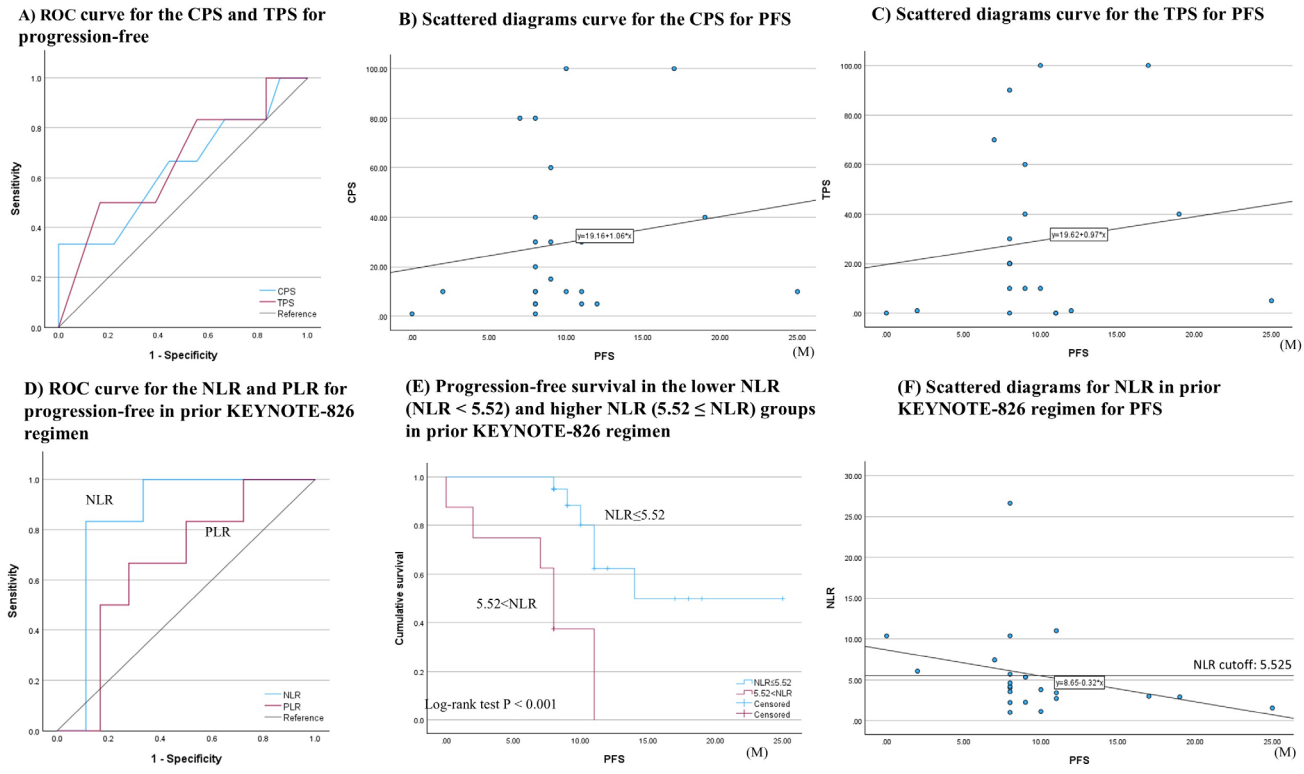


**Figure 3.** Major analysis results of group 2 in advanced cervical cancer treated with the KEYNOTE-826 regimen. A) ROC curve for CPS and TPS for progression-free. The areas under the curves are 0.644 and 0.662, respectively. Scatter diagrams for CPS and TPS for PFS are shown in (B) and (C). In both the cases, a mild positive correlation was observed. (D) ROC curve for NLR and PLR for progression-free in the Prior to the KEYNOTE-826 regimen. The AUC was 0.852 and 0.667, and the cut-off value for the NLR was 5.52. (E) PFS in the lower NLR (NLR <5.52) and higher NLR (5.52≤ NLR) groups in the Prior to the KEYNOTE-826 regimen. The lower NLR (NLR ≤5.52) group had a significantly longer PFS than the higher NLR (5.52< NLR) group (p<0.001, median survival: 14 months vs. 7.6 months). (F) Scatter diagrams of NLR in the Prior to the KEYNOTE-826 regimen for PFS. A negative correlation was observed between the NLR and PFS

*ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, PFS: Progression-free survival, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, AUC: Area under the curve*

therapy, 1) localized PD-L1 expression may be underestimated in small biopsy specimens, 2) the expression of PDL1 in multiple tumor lesions may change depending on the time course and anatomical site, and 3) there is a possibility that the expression of PDL1 may change over time due to anticancer treatment after biopsy. Considering these factors, CPS or TPS obtained from the target lesion immediately before administration may have greater potential for improving the accuracy of biomarkers; in group 3 of this study, the AUC for PFS was slightly higher than that in the other groups. However, 89% of patients had multiple lesions of stage IV; therefore, it does not necessarily reflect the tumor environment in all areas. Therefore, in the real world, patients eligible for the KEYNOTE-826 regimen may not necessarily be suitable for biomarker evaluation of the local environment using CPS or TPS.

In the previous KEYNOTE-826 study, the effect of Pem on OS and PFS increased with CPS ≥1; however, there was no further significant increase in the CPS ≥10 group. Nevertheless, the results from group 3, which was treatment-naïve, may show further therapeutic benefit with a CPS ≥10. In contrast, the HRs for OS and PFS in the subgroup with CPS <1 (11% of the study population) were approximately 1 compared to conventional chemotherapy, but the 95% CIs for estimates, were wide and overlapped with those for the entire population[26]. Non-squamous tumors are more likely to be PD-L1 negative; however, the KEYNOTE-826 study suggested that non-squamous histology may still be beneficial[26]. Therefore, we did not restrict the application of the KEYNOTE-826 regimen for CPS or TPS, and administered the regimen to patients with CPS <1. Although we did not identify any cases with CPS <1 in our study, of the four cases with TPS <1, one was disease-free.

Identification of the optimal measurement period for biomarker testing is also an important factor. In particular, CPS and TPS in group 3 patients were measured immediately prior

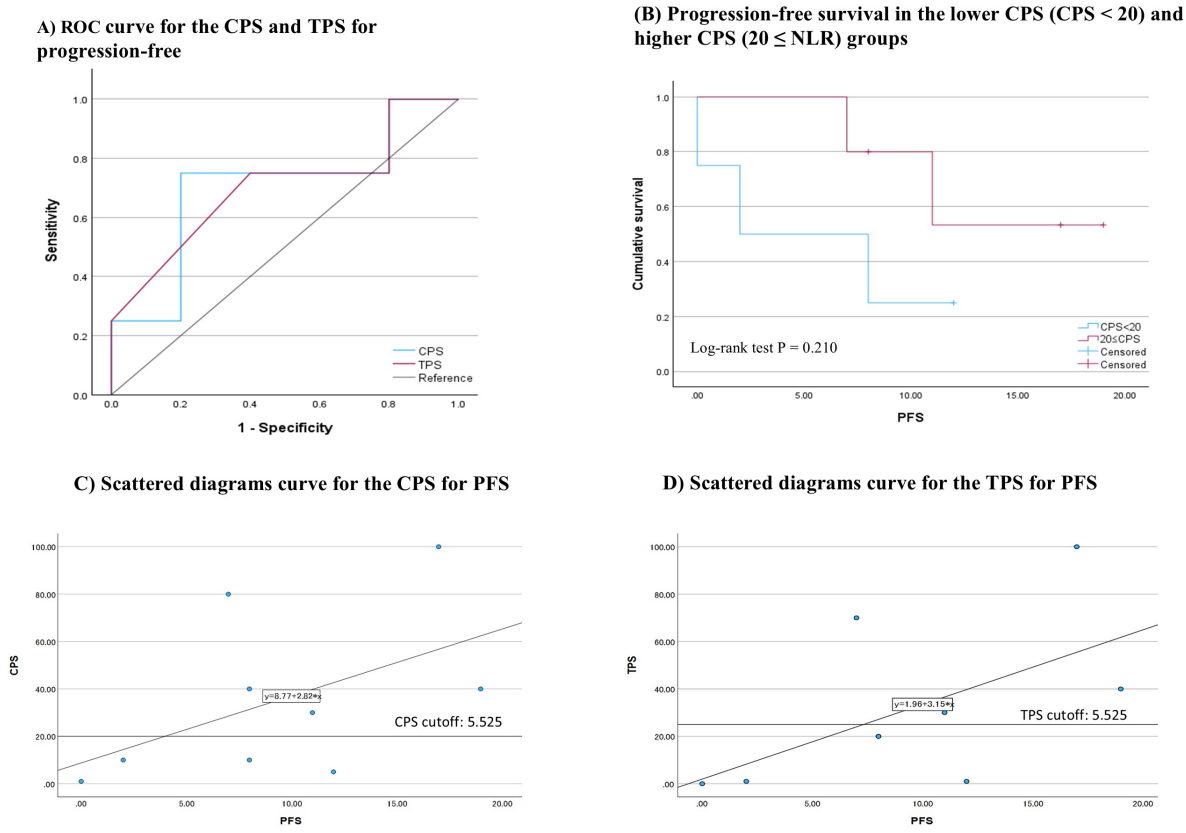


**Figure 4.** Major analysis results of group 3 in advanced cervical cancer treated with the KEYNOTE-826 regimen. A) ROC curve for CPS and TPS for progression-free. The AUC was 0.700 and 0.700, and the cut-off values for NLR were 20 and 25, respectively. The higher CPS (20≤ CPS) group tended to have longer PFS than the lower CPS (CPS <20) group (p=0.210, median survival: 3 months vs. statistically not reached). Scatter diagrams for CPS and TPS for PFS are shown in (C) and (D). A positive correlation seems to be observed in both cases

*ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, PFS: Progression-free survival, AUC: Area under the curve*

to administering the KEYNOTE-826 regimen, which may be one of the reasons why they were the most useful candidate biomarkers. when considering the measurement period after the initial treatment, we did not find any useful correlation with inflammatory biomarkers, including NLR. These results are also consistent with a similar study that we previously reported regarding regimens containing Pem for endometrial cancer[17].

### Study Limitations

This study has the following limitations: First, the sample size was relatively small; and the study was conducted with a retrospective design at a single facility. Second, the limited observation period made it difficult to evaluate long-term prognoses; therefore, an analysis of OS was not performed. We do, however, believe that there would be no significant new findings in the interpretation of biomarkers through further long-term observations when retrospectively exploring treatment outcomes.

## Conclusion

CPS and TPS from tissue samples taken directly from isolated target regions immediately prior to ICI use have the potential to become useful prognostic biomarkers. However, in the real world, patients eligible for the KEYNOTE-826 regimen already have systemic diseases, and tissue samples may not always be available. The usefulness of inflammatory biomarkers, such as NLR, which are easily measured, inexpensive, and minimally invasive, may also be significant. Our study suggests that further investigation is warranted into the utility of inflammatory indicators as prognostic biomarkers, for regimens containing ICI for cervical cancer.

### Ethics

**Ethics Committee Approval:** The Institutional Review Board at Kagoshima University Graduate School of Medical Sciences granted approval for the study protocol (approval number: 230081, date: 19.09.2023).

**Informed Consent:** Retrospective study.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.Y., I.K., S.T., A.T., H.K., Concept: S.Y., A.T., Design: S.Y., Data Collection or Processing: I.K., Analysis or Interpretation: S.Y., I.K., Literature Search: S.T., A.T., H.K., Writing: S.Y.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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