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Original Article

Serum USP1 and PD-L1 levels independently predict treatment response and prognosis in cervical cancer: a retrospective cohort study

Yingge Zhao, Hairong Zhou

Department of Gynecology, Boshan District Hospital of Traditional Chinese Medicine, Zibo 255200, Shandong, China

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Abstract: Cervical cancer remains a common malignant tumor with highly variable treatment outcomes, and the combined predictive value of serum ubiquitin-specific protease 1 (USP1) and programmed death ligand 1 (PD-L1) has been rarely investigated. This retrospective study included 102 cervical cancer patients treated between July 2022 and July 2024, who were classified into treatment-sensitive and treatment-resistant groups according to RECIST 1.1 criteria. After propensity score matching (1:1, caliper 0.02) for age, FIGO (International Federation of Gynecology and Obstetrics) stage, tumor diameter, lymph node status, and serum biomarkers, 84 patients (42 per group) were analyzed. Serum USP1 and soluble PD-L1 levels were measured by ELISA, and logistic regression, Kaplan-Meier curves, and Cox models were used for analysis. The resistant group showed significantly higher USP1 (38.62±9.43 vs. 29.86±8.24 pg/mL, $P < 0.001$) and PD-L1 levels (156.48±42.78 vs. 124.58±38.92 pg/mL, $P < 0.001$), with a positive correlation between the two ($r = 0.51$, $P < 0.001$), which remained significant after adjustment (partial $r = 0.42$, $P = 0.002$). Multivariable analysis identified high USP1 (OR = 2.56, 95% CI: 1.34-4.89) and high PD-L1 (OR = 2.18, 95% CI: 1.15-4.13) as independent predictors of poor treatment response, and both were independent risk factors for overall survival (USP1: HR = 2.18, 95% CI: 1.24-3.83; PD-L1: HR = 1.96, 95% CI: 1.12-3.43). No significant interaction was found between the two markers ($P > 0.05$), indicating additive rather than synergistic effects. Combining both biomarkers improved predictive accuracy with a C-index of 0.74 and an NRI of 31%. In conclusion, pre-treatment serum USP1 and soluble PD-L1 levels are effective indicators for predicting treatment response and prognosis in cervical cancer, and their combined assessment helps identify high-risk patients and supports personalized treatment planning.

Keywords: Cervical cancer, ubiquitin-specific protease 1, programmed death ligand 1, prognosis, treatment response, biological markers

Introduction

Cervical cancer remains one of the most common malignant tumors affecting the female reproductive system worldwide, representing a serious global health burden. According to global cancer statistics, there were approximately 600,000 new cases of cervical cancer and over 340,000 deaths from the disease in 2020 alone, with the majority occurring in low- and middle-income countries where medical resources are scarce [1, 2]. Although the widespread implementation of human papillomavirus (HPV) vaccination and improved screening programs have led to a decline in cervical cancer incidence in some developed countries, the five-year survival rate for patients diagnosed with locally advanced or recurrent metastatic disease has not significantly improved over recent decades [3, 4]. This clinical dilemma has driven researchers to continuously explore more accurate prognostic assessment methods and efficacy predictors to better stratify patient risk beyond traditional clinical and pathological factors.

Tumor development and progression depend not only on the proliferative capacity of tumor cells themselves but also on the host's immune surveillance mechanisms and DNA repair

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capabilities [5, 6]. Programmed death ligand 1 (PD-L1) serves as a key immune checkpoint molecule. Its expression on tumor or immune cells enables binding to PD-1 on T cells, which in turn promotes T-cell dysfunction and apoptosis, allowing tumors to escape immune surveillance [7, 8]. PD-1/PD-L1 monoclonal antibodies have been widely used in the treatment of cervical cancer; however, the expression of PD-L1 is regulated by multiple factors, and its predictive value as a single biomarker has certain limitations [9, 10]. Therefore, identifying indicators that can complement PD-L1 or reflect other aspects of tumor biology is an important research direction.

Ubiquitin-specific protease 1 (USP1) belongs to the deubiquitinating enzyme family and has recently been identified as a critical regulator in DNA cross-link damage repair and the pathogenesis of Fanconi anemia [11, 12]. Under conditions of replicative stress or DNA damage, USP1 stabilizes key proteins by removing their ubiquitin markers, thereby maintaining genomic stability [13, 14]. Notably, this mechanism is activated in malignant cells under genotoxic stress induced by radiotherapy or chemotherapy, likely contributing to treatment resistance [15, 16]. Studies have shown that abnormal USP1 expression is closely associated with poor prognosis in various solid tumors, including ovarian and lung cancers [17, 18]. However, in the field of cervical cancer, clinical data on USP1 are still relatively scarce, and whether its serum levels can reflect tumor burden and biological characteristics remains unclear.

In clinical practice, patients with the same FIGO (International Federation of Gynecology and Obstetrics) stage and receiving the same treatment regimen often exhibit markedly different treatment responses and long-term prognoses [19, 20]. This variability suggests that traditional anatomical indicators alone are insufficient to meet the growing demand for precision medicine in cervical cancer [21, 22]. PD-L1 and USP1 represent two key aspects of tumor biology-immune escape and DNA damage repair, respectively. Theoretically, simultaneous detection of these two indicators could provide a more comprehensive understanding of tumor behavior. However, previous studies have mostly focused on single indicators, and the combined predictive value of serum USP1 and PD-L1 in cervical cancer has not been systematically investigated.

In clinical practice, we often observe the phenomenon that those patients with cervical cancer at the same stage and receiving the same treatment regimen may have great differences in treatment response and long-term prognosis [23, 24]. This difference means that we cannot rely solely on traditional anatomical indicators to make judgments. These indicators include FIGO stage and lymph node status, among others. Doing so cannot meet the growing demand for precision medicine in the field of cervical cancer treatment [25, 26]. PD-L1 and USP1 represent two key aspects in the mechanism of tumor immune escape and DNA damage repair, respectively. Theoretically, simultaneous detection of these two indicators can contribute to a more comprehensive understanding of the biological properties of the tumor [27, 28]. PD-L1 and USP1 represent two key aspects in the mechanism of tumor immune escape and DNA damage repair, respectively. Theoretically, simultaneous detection of these two indicators can contribute to a more comprehensive understanding of the biological properties of the tumor [28, 29]. Although advancements in surgery, chemotherapy, radiotherapy, and immunotherapy have significantly improved patient outcomes, treatment resistance and recurrence remain major challenges in clinical practice. Therefore, identifying biomarkers that can predict treatment response and prognosis is of great clinical significance for achieving personalized treatment.

Innovation and clinical significance of this study

The present study has several innovative aspects. First, while most existing studies have assessed USP1 or PD-L1 expression in tissue samples, we measured their levels in serum, offering a non-invasive, repeatable, and real-time monitoring approach that is more feasible for clinical practice. Second, this is the first study to simultaneously evaluate the predictive value of serum USP1 and soluble PD-L1 for both treatment response and long-term prognosis in cervical cancer, and to explore the potential interaction between these two biomarkers. Third, by employing propensity score matching to control for confounding factors

and conducting sensitivity analyses (including partial correlation and subgroup analyses), we aimed to validate the robustness of the association between USP1 and PD-L1. Fourth, we quantified the incremental predictive value of combining these two markers using the C-index and net reclassification improvement (NRI), providing statistical evidence for their combined use.

From a clinical perspective, identifying patients at high risk of treatment resistance and poor prognosis is crucial for personalized treatment. If serum USP1 and PD-L1 levels can independently predict outcomes, their combined detection may help stratify patients into different risk categories, guiding more intensive or alternative therapeutic strategies - such as combining PARP inhibitors with immune checkpoint inhibitors. This study therefore aims to address the following questions: (1) Are serum USP1 and PD-L1 levels associated with treatment response and survival in cervical cancer patients? (2) Do these two markers provide independent and additive predictive information? (3) Can their combined use improve risk stratification? By answering these questions, we hope to provide a practical serological tool for precision management of cervical cancer.

Research subjects and methods

Patient selection and eligibility criteria

We conducted this study using a retrospective cohort analysis. The study was conducted at an academic medical center from Boshan District Hospital of Traditional Chinese Medicine. In turn, we recruited eligible participants, all of whom were cervical cancer patients. These patients were treated in Gynecological Oncology between July 2022 and July 2024. From these patients, we selected 102 subjects who met all inclusion criteria. All cases were confirmed as primary cervical cancer by histopathological examination. According to the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) [30] patients were divided into a treatment-sensitive group and a treatment-resistant group. The treatment-sensitive group was defined as those who achieved complete or partial remission 4 weeks after completing first-line treatment. The treatment-resistant group consisted of those whose best response to treatment was disease stabilization or progression. Grouping was based solely on the initial treatment response, with no consideration given to late-onset events that occurred during follow-up. Events such as disease progression, recurrence, or death during follow-up were used only for survival analysis (PFS, OS) and were not considered for reclassification into either group. To eliminate baseline differences between the two groups of patients, we used the PSM approach to achieve a 1:1 match. Matched variables included age, FIGO stage, tumor diameter, lymph node metastasis, degree of vascular invasion, degree of tumor differentiation, pathological type, and serum levels of Squamous Cell Carcinoma Antigen (SCC-Ag), cancer antigen 125 (CA125), Cancer Antigen 19-9 (CA19-9), lactate dehydrogenase (LDH), and C-reactive protein (CRP). A total of 84 patients were finally included in the analysis, with 42 patients in each group, as shown in **Figure 1**.

Inclusion criteria are as follows [31, 32]: (1) Primary cervical cancer confirmed by histopathological examination, with FIGO (2018) stage as IB-IVA; (2) Age ≥ 18 years old; (3) No previous anti-tumor treatment for cervical cancer (including but not limited to chemotherapy, radiotherapy, targeted therapy, immunotherapy and radical surgery) before enrollment; (4) ECOG performance status score is 0-1; (5) Expected survival period ≥ 6 months; (6) Venous blood was collected on an empty stomach before treatment, and the serum samples were well preserved for testing; (7) Complete clinical and pathological data, and follow-up time ≥ 24 months.

Exclusion criteria include [33, 34]: (1) Include other active malignant tumors (excluding nonmelanoma skin cancer or previous cervical carcinoma in situ); (2) Have a history of other invasive malignant tumors within the past 3 years; (3) Have an active infection, autoimmune disease or other systemic disease that may affect the levels of serum markers; (4) Pregnant or lactating women; (5) Have received pelvic radiotherapy in the past (regardless of the reason); (6) The laboratory test indicators before enrollment do not meet the following standards: hemoglobin ≥ 90 g/L, absolute neutrophil count ≥ 1.5×10^9/L, platelet count ≥ 100×10^9/L, alanine aminotransferase and aspartate aminotransferase ≤ 2.5 times the upper

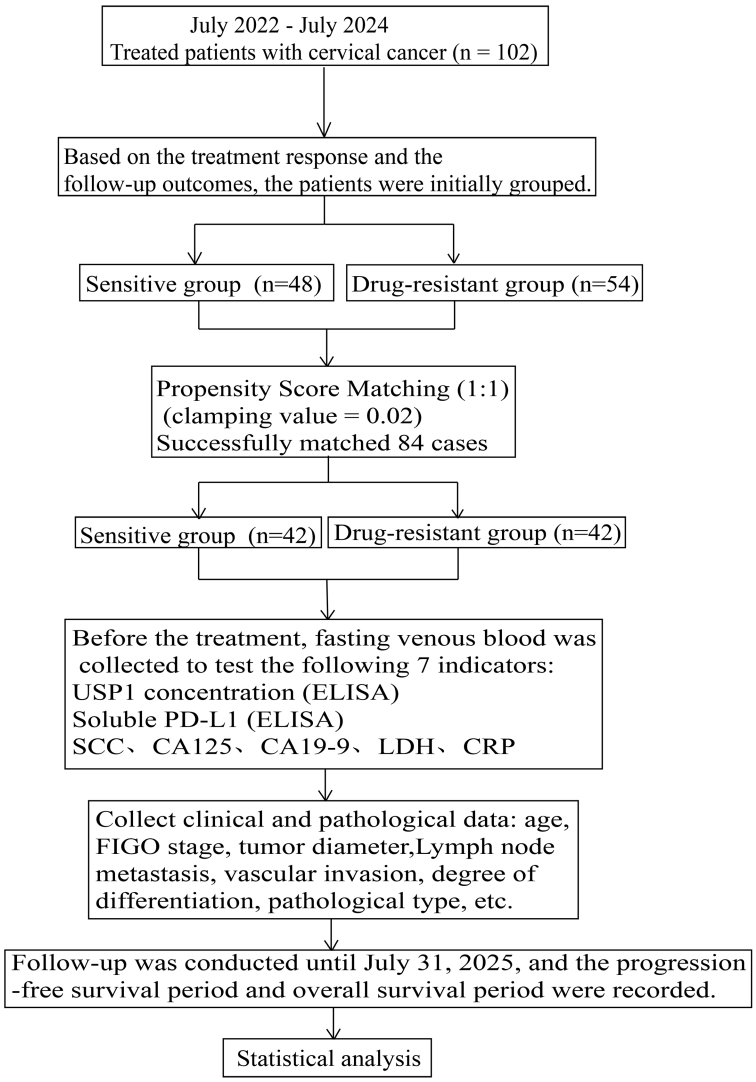

Figure 1. Flow chart. FIGO is International Federation of Gynecology and Obstetrics. USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. SCC: squamous cell carcinoma antigen; LDH: lactate dehydrogenase; SCC: squamous cell carcinoma antigen; CA125: cancer antigen 125; CA19-9: cancer antigen 19-9; LDH: lactate dehydrogenase; CRP: C-reactive protein.

limit of normal value, serum albumin ≥ 30 g/L; (7) Have uncontrollable severe infection, mental illness or cognitive impairment, which makes it impossible to cooperate with the study; (8) Are concurrently participating in other interventional clinical studies.

Data extraction

Clinical and pathological data were extracted from the hospital's electronic medical record system by two independent investigators (Y.Z. and H.R.Z.) using a standardized data collection form. The following data were extracted: (1) Demographic characteristics: age, body mass index; (2) Tumor-related characteristics: FIGO stage (I, II, III, IV), tumor diameter (≤ 4 cm or > 4 cm), histological type (squamous cell carcinoma vs. adenocarcinoma), tumor differentiation grade (well, moderate, poor); (3) Metastasis-related indicators: lymph node metastasis (present or absent), vascular invasion (present or absent); (4) Serum biomarkers: SCC-Ag, CA125, CA19-9, LDH, CRP; (5) Study-specific biomarkers: serum USP1 and soluble PD-L1 levels measured by ELISA; (6) Treatment information: first-line treatment regimens; and (7) Follow-up data: progression status, recurrence, death, and survival time.

Data extraction was performed independently by two investigators. Discrepancies were resolved by consensus or by consulting a third investigator. Data quality was assured by randomly selecting 10% of the sample for double-checking by a senior researcher.

Outcome measures

The primary outcomes were treatment response and long-term prognosis. Treatment response was assessed 4 weeks after completion of first-line treatment according to the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) [30]. Patients were classified into the treatment-sensitive group if they achieved complete or partial response, and into the treatment-resistant group if their best response was stable disease or progressive disease. This classification was based solely on initial treatment response, with no consideration of late-onset events.

Prognosis was evaluated using progression-free survival (PFS) and overall survival (OS).

PFS was defined as the time from treatment initiation to first documented disease progression (per RECIST v1.1) or death from any cause, whichever occurred first. OS was defined as the time from treatment initiation to death from any cause. Patients who were alive or lost to follow-up were censored at the last known alive date. Follow-up was conducted every 3 months for the first 2 years and every 6 months thereafter via outpatient clinic visits or telephone interviews. The median follow-up duration was 24 months (range: 6-36 months).

Secondary outcomes included the correlation between serum USP1/PD-L1 levels and clinicopathological parameters, as well as the incremental predictive value of combined USP1 and PD-L1 detection (assessed by C-index and net reclassification improvement).

Laboratory methods: Blood collection and storage: Fasting venous Blood (5 ml) was collected from each patient within 24 hours before treatment initiation using vacuum tubes containing separator gel and coagulant (Becton Dickinson, Franklin Lakes, NJ). After standing at room temperature for 30 minutes, serum was separated by centrifugation at 3000 rpm for 10 minutes using a TDZ5-WS centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Changsha, China). Serum aliquots were stored in 1.5 mL sterile cryotubes (Eppendorf, Hamburg, Germany) at -80°C in a DW-86L626 ultra-low temperature freezer (Haier Biomedical, Qingdao, China) until batch testing.

Elisa for USP1 and PD-L1: Serum USP1 concentration was measured using a double-antibody sandwich Elisa Kit (Wuhan Huamei Bioengineering Co., Ltd., Wuhan, China; catalog No.csb-el024855hu; Range Detection: 15.6-1000 pg/ml; intra-assay CV < 8%, inter-assay CV < 10%). Soluble PD-L1 level was measured using an Elisa Kit (R & D Systems, Minneapolis, MN; Catalog No. DB7H10; detection range: 31.2-2000 pg/mL; sensitivity: 4.2 pg/mL). All samples were tested in duplicate, and absorbance was read at 450 nm (with 570 nm reference for PD-L1) using a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA). Results were calculated from standard curves.

Conventional serum biomarkers: Serum SCC-Ag, CA125, and cancer antigen 19-9 (CA19-9) were measured by electrochemiluminescence immunoassays using a Cobas E 601 analyzer (Roche Diagnostics, Mannheim, Germany) with original kit (Roche). LDH was measured using the Lactate substrate method, and CRP using immunoturbidimetry, both on an AU680 automated biochemical analyzer (Beckman Coulter, Brea, CA) with complementary reagents (Beckman Coulter).

Quality control: All assays were performed in a single batch to minimize inter-batch variability. Each batch included quality control samples. Standard operating procedures were strictly followed.

Outcome measures and timing of assessments

Timing of blood collection: Fasting venous blood samples were collected from all patients within 24 hours before the initiation of first-line anti-cancer therapy (including surgery, chemotherapy, radiotherapy, or immunotherapy). Serum was separated and stored at -80°C until batch testing.

Primary outcomes: The primary outcomes were treatment response and long-term prognosis. Treatment response was assessed 4 weeks after completion of first-line treatment using RECIST v1.1 criteria [30]. Patients were classified into the treatment-sensitive group (complete or partial response) and the treatment-resistant group (stable or progressive disease). Prognosis was evaluated using progression-free survival (PFS) and overall survival (OS). PFS was defined as the time from treatment initiation to first documented disease progression or death from any cause. OS was defined as the time from treatment initiation to death from any cause. Patients alive at last follow-up were censored.

Secondary outcomes: Secondary outcomes included: (1) The correlation between serum USP1 and soluble PD-L1 levels; (2) The correlation of each biomarker with clinicopathological parameters (FIGO stage, tumor diameter, lymph node metastasis, etc.); and (3) The incremental predictive value of combined USP1 and PD-L1 detection compared with each marker alone, quantified by the C-index and net reclassification improvement (NRI).

Laboratory methods

This study was a single-center retrospective cohort study. All enrolled patients had 5 mL of fasting elbow venous blood collected before treatment. The blood was placed in vacuum tubes containing separator gel and coagulant agents. After being allowed to stand at room temperature for 30 minutes, the serum was separated by centrifugation at 3000 revolutions per minute for 10 minutes. The serum was then aliquoted and stored at -80°C for subsequent testing.

The detection of serum USP1 and soluble PD-L1 concentrations was performed using a double-antibody sandwich ELISA method. The reagents were purchased from Wuhan Huamei Bioengineering Co., Ltd. (product code: CSB-EL024855HU; detection range: 15.6-1000 pg/mL; intra-assay coefficient of variation < 8%, inter-assay coefficient of variation < 10%). Another reagent was obtained from R&D Systems, USA (product code: DB7H10; detection range: 31.2-2000 pg/mL; detection sensitivity: 4.2 pg/mL). All samples were tested in duplicate, and the average values were used for analysis.

Serum squamous cell carcinoma antigen, carcinoembryonic antigen 125, and carcinoembryonic antigen 19-9 were detected using electrochemiluminescence assays (Instrument: Cobas e 601, Roche Diagnostics). Serum LDH and CRP were measured using the lactate substrate method and immunoturbidimetry, respectively (Instrument: AU680, Beckman Coulter). The testing procedures strictly followed the instrument's standard operating procedures, and quality control samples were included in each batch.

All serological tests were conducted in a single batch after sample collection, while clinical and pathological data were collected through the hospital's electronic medical record system.

Sample size calculation

The sample size was estimated based on the differences in serum USP1 expression between resistant and sensitive groups of cervical cancer, as reported in preliminary experiments and previous literature. With a two-sided alpha level of 0.05 and a power of 1-β = 0.80, the sample size calculation formula for comparing means of two independent groups was used. Considering a 10%-15% dropout rate and data missing, it was estimated that at least 95 participants were needed. In reality, 102 participants were included in this study. After propensity score matching, 84 patients (42 in each group) were included in the final analysis. Post-hoc power calculations were performed using the actual observed USP1 levels in the matched groups: 38.6±9.4 pg/mL in the resistant group versus 29.8±8.2 pg/mL in the sensitive group. The actual test power was 0.86. Using PD-L1 levels as a measure, the actual test power was 0.82. These results indicate that the sample size after matching was sufficient to support hypothesis testing for the primary outcome indicators.

Statistical methods

A multilevel analytical approach was employed. Continuous variables were tested for normality using the Shapiro-Wilk test. Normally distributed data were presented as mean ± standard deviation and compared using independent sample t-tests; non-normally distributed data were presented as median (interquartile range) and compared using the Mann-Whitney U test. Categorical variables were presented as frequencies and percentages and compared using the chi-square test or Fisher's exact test.

Correlations were assessed using Pearson or Spearman correlation coefficients depending on data distribution. To control for confounding factors, propensity score matching (PSM) was performed using a 1:1 nearest neighbor algorithm with a caliper of 0.02. There were 48 cases in the sensitive group before matching and 54 cases in the resistant group. Matching variables included age, FIGO stage, tumor diameter, lymph node metastasis, vascular invasion, differentiation grade, pathological type, and serum levels of SCC-Ag, CA125, CA19-9, LDH, and CRP.

Binary logistic regression was used to identify independent predictors of treatment response (sensitive = 0, resistant = 1). Variables with $P < 0.10$ in univariate analysis were entered into the multivariate model using the Enter method. USP1 and PD-L1 were dichotomized at their median values (34.2 pg/mL and 140.5 pg/mL, respectively). FIGO stage was dichotomized as

early (I-II = 0) vs. advanced (III-IV = 1). Lymph node metastasis was coded as absent = 0 vs. present = 1. Model fit was assessed using the Hosmer-Lemeshow test.

Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify independent prognostic factors for PFS and OS. Variables with P < 0.10 in univariate analysis were entered into the multivariate model. Results were presented as hazard ratios (HRs) with 95% confidence intervals (CIs).

To evaluate potential interaction between USP1 and PD-L1, a product term (USP1 × PD-L1) was included in the logistic and Cox regression models. A *P*-value < 0.10 was considered significant for interaction.

All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and R software (version 4.1.0, R Foundation for Statistical Computing). A two-sided *P*-value < 0.05 was considered statistically significant unless otherwise specified. No adjustment was made for multiple comparisons, as this was an exploratory hypothesis-generating study.

Variable selection for multivariate logistic regression: Candidate variables for multivariate analysis were selected based on two criteria: (1) Clinical relevance established in previous literature (including age, FIGO stage, tumor diameter, lymph node metastasis, vascular invasion, differentiation grade, pathological type, SCC, CA125, CA19-9, LDH, and CRP); and (2) Statistical significance in univariate analysis. Univariate logistic regression was first performed for each candidate variable, with treatment response (sensitive = 0, resistant = 1) as the dependent variable. Variables with a univariate *P*-value < 0.10 were then entered into the multivariate logistic regression model using the Enter method (forced entry). This threshold (*P* < 0.10) was chosen to avoid omitting potentially important variables while maintaining model parsimony. The Hosmer-Lemeshow goodness-of-fit test was used to assess model calibration.

Variable assignment for multivariate logistic regression: The following variable assignments were used in the multivariate logistic regression model. Treatment response (dependent variable) was coded as 0 for sensitive (complete or partial response) and 1 for resistant (stable or progressive disease). Independent variables were coded as: USP1 - low expression (≤ 34.2 pg/mL, median) = 0, high expression (> 34.2 pg/mL) = 1; PD-L1 - low expression (≤ 140.5 pg/mL, median) = 0, high expression (> 140.5 pg/mL) = 1; FIGO stage - early stage (I-II) = 0, advanced stage (III-IV) = 1; Lymph node metastasis - absent = 0, present = 1; Tumor diameter - ≤ 4 cm = 0, > 4 cm = 1; Vascular invasion - absent = 0, present = 1; Differentiation grade - well/moderate = 0, poor = 1; SCC, CA125, LDH, CRP - entered as continuous variables (per 1 ng/mL, 1 U/mL, 1 U/L, 1 mg/L increase, respectively). All assignments were determined before model fitting and were not altered during analysis.

Multivariate logistic regression: To identify independent predictors of poor treatment response, candidate variables were first screened by univariate logistic regression (see Table S1). Variables with a univariate *p* value < 0.10 were entered into the multivariate model. The Enter method (forced entry) was used, meaning all selected variables were entered simultaneously into the model without stepwise selection. This approach was chosen to avoid overfitting and to preserve the ability to test prespecified hypotheses based on clinical relevance.

Cutoff values and variable assignment: Serum USP1 and PD-L1 levels were dichotomized at their respective medians (34.2 pg/mL for USP1, 140.5 pg/mL for PD-L1), as no clinically established cutoffs exist. Low expression (≤ median) was coded as 0, high expression (> median) as 1. Other variables were coded as follows: FIGO stage (I-II = 0, III-IV = 1); lymph node metastasis (absent = 0, present = 1); tumor diameter (≤ 4 cm = 0, > 4 cm = 1); vascular invasion (absent = 0, present = 1); differentiation grade (well/moderate = 0, poor = 1); conventional serum markers (SCC, CA125, LDH, CRP) were entered as continuous variables (per 1 ng/mL, 1 U/mL, 1 U/L, 1 mg/L increase, respectively). All assignments were pre-specified and not altered during analysis.

Ethical statement

The study protocol has been approved by the Medical Ethics Committee of Boshan District

Table 1. Comparison of baseline clinical and pathological characteristics of the first two groups of patients

Indicators	Sensitive group (n = 48)	Drug-resistant group (n = 54)	t/χ^2	*P*	95% CI	SMD
Age (years)	52.36±9.24	53.18±8.97	0.45	0.65	-2.42 to 3.78	0.09
BMI (kg/m^2)	23.45±3.12	23.67±3.34	0.34	0.73	-1.04 to 1.48	0.07
FIGO staging [n (%)]			4.82	0.19	-	0.31
Phase I	18 (35.29)	12 (23.53)				
Phase II	20 (39.22)	18 (35.29)				
Phase III	10 (19.61)	15 (29.41)				
Stage IV	3 (5.88)	6 (11.76)				
Tumor diameter > 4 cm [n (%)]	22 (43.14)	31 (60.78)	3.22	0.07	-	0.36
Lymph node metastasis [n (%)]	16 (31.37)	27 (52.94)	4.90	0.03	-	0.45
Vascular infiltration [n (%)]	14 (27.45)	22 (43.14)	2.78	0.10	-	0.33
Degree of differentiation [n (%)]			2.95	0.23	-	0.28
Well-differentiated	10 (19.61)	7 (13.73)				
Moderate differentiation	25 (49.02)	20 (39.22)				
Poorly differentiated	16 (31.37)	24 (47.06)				
Pathological type [n (%)]			0.45	0.50	-	0.13
Squamous cell carcinoma	39 (76.47)	36 (70.59)				
Adenocarcinoma	12 (23.53)	15 (29.41)				
SCC (ng/mL)	3.45±2.12	4.89±3.01	2.78	0.01	0.39 to 2.49	0.55
CA125 (U/mL)	18.45±8.12	24.67±12.34	3.01	0.003	2.10 to 10.34	0.60
CA19-9 (U/mL)	16.32±7.45	20.18±9.67	2.28	0.02	0.53 to 7.19	0.45
LDH (U/L)	185.36±42.12	210.45±56.78	2.52	0.01	5.42 to 44.76	0.50
CRP (mg/L)	5.12±3.45	8.67±5.89	3.67	< 0.001	1.62 to 5.48	0.73

Note: BMI: Body Mass Index; FIGO: International Federation of Gynecology and Obstetrics; SCC: Squamous Cell Carcinoma Antigen; CA125: Cancer Antigen 125; CA19-9: Cancer Antigen 19-9; LDH: Lactate Dehydrogenase; CRP: C-reactive Protein; SMD: Standardized Mean Difference. The 95% CI for continuous variables represents the confidence interval of the mean difference.

Hospital of Traditional Chinese Medicine (Approval No.: BZ20220608001), and the study process fully complies with the ethical standards stipulated in the Declaration of Helsinki. Given that this was a retrospective observational study, only the remaining clinical test samples and the patient's medical records were used during the study and no interventions were conducted. Additionally, all study data has been anonymised and the patient's personal information has been strictly protected. The Ethics Committee agreed to exempt patients from the procedure of signing informed consent.

Results

Baseline information

The median follow-up duration was 24 months (range: 6-36 months). All serum biomarkers were measured at baseline before treatment initiation. In this pilot study, a total of 102 cervical cancer patients were included in the study. These patients were divided into "Sensitive group" (n = 48) and "Drug-resistant group" (n = 54). We compared the baseline data for each group in **Table 1**. For each key clinicopathological index, the standardized mean difference was between 0.45 and 0.73. This result indicates that there are significant confounding factors and that a direct comparison of data from different groups may lead to bias. Therefore, we adopt the method of propensity score matching to eliminate this bias. We used the 1:1 nearest neighbor algorithm for matching and set the matching criterion to 0.02. We also carefully selected corresponding matching variables based on clinical significance, which included demographic characteristics such as age. In addition, we considered factors related to the tumor, such as FIGO stage, tumor diameter, degree of differentia-

Table 2. Comparison of baseline clinicopathological characteristics between the two matched patient groups

Indicators	Sensitive group (n = 42)	Drug-resistant group (n = 42)	t/χ^2	*p*	95% CI	SMD
Age (years)	52.48±9.12	52.76±8.85	0.14	0.89	-3.56 to 4.12	0.03
BMI (kg/m^2)	23.51±3.08	23.62±3.21	0.16	0.87	-1.23 to 1.45	0.03
FIGO staging [n (%)]			0.62	0.89	-	0.12
Phase I	14 (33.33)	12 (28.57)				
Phase II	17 (40.48)	16 (38.10)				
Phase III	8 (19.05)	10 (23.81)				
Stage IV	3 (7.14)	4 (9.52)				
Tumor diameter > 4 cm [n (%)]	20 (47.62)	23 (54.76)	0.43	0.51	-	0.14
Lymph node metastasis [n (%)]	16 (38.10)	18 (42.86)	0.20	0.66	-	0.10
Vascular infiltration [n (%)]	13 (30.95)	15 (35.71)	0.22	0.64	-	0.10
Degree of differentiation [n (%)]			0.92	0.63	-	0.15
Well-differentiated	8 (19.05)	6 (14.29)				
Moderate differentiation	20 (47.62)	18 (42.86)				
Poorly differentiated	14 (33.33)	18 (42.86)				
Pathological type [n (%)]			0.24	0.62	-	0.08
Squamous cell carcinoma	32 (76.19)	30 (71.43)				
Adenocarcinoma	10 (23.81)	12 (28.57)				
SCC (ng/mL)	3.89±2.34	4.12±2.56	0.43	0.67	-0.82 to 1.28	0.09
CA125 (U/mL)	19.51±9.08	21.62±10.21	1.00	0.32	-2.10 to 6.32	0.22
CA19-9 (U/mL)	17.21±8.12	18.34±8.56	0.62	0.54	-2.45 to 4.71	0.14
LDH (U/L)	190.45±45.23	198.67±48.92	0.80	0.43	-12.34 to 28.78	0.17
CRP (mg/L)	5.89±4.12	6.45±4.56	0.59	0.56	-1.34 to 2.46	0.13

Note: BMI: Body Mass Index; SMD: Standardized Mean Difference; FIGO: International Federation of Gynecology and Obstetrics; SCC: Squamous Cell Carcinoma Antigen; CA125: Cancer Antigen 125; CA19-9: Cancer Antigen 19-9; LDH: Lactate Dehydrogenase; CRP: C-reactive Protein; After matching, all *p*-values were > 0.05, and the SMD values were < 0.2, indicating good inter-group balance. The 95% CI of continuous variables is the confidence interval of the mean difference.

tion, as well as pathological type. Metastasis-related indicators were also taken into consideration, such as lymph node involvement and vascular invasion. Serum biomarkers were also matched, including SCC-Ag, CA125, CA19-9, LDH, and CRP. After matching, there were 84 patients in the analysis group. The sample size of each group was comparable, with 42 patients in each group. As shown in **Table 2**, the covariate distribution of each group was more balanced, with all standardized mean differences less than 0.2 (range: 0.03-0.22), and no statistically significant differences between the groups (*p*-values for all variables greater than 0.05), indicating that confounding factors have been effectively controlled.

Serum USP1 and soluble PD-L1 levels

We studied the patient population after paired comparison, and the relevant results are shown in **Table 3**. Serum levels of USP1 were much higher in those patients who developed resistance to treatment or worsened their condition. Its mean value was 38.62±9.43 pg/ml. In those patients who were sensitive to treatment, the mean level of USP1 was 29.86±8.24 pg/ml. This difference was highly significant: the t-value was 4.53 and the *p*-value was less than 0.001. Similar results were obtained for soluble PD-L1. The level of soluble PD-L1 was 156.48±42.78 pg/mL in those patients who developed resistance to treatment or worsened the condition, which was significantly higher Ithan that in those patients who were sensitive to treatment, whose mean level was 124.58± 38.92 pg/mL. The t-value at this time is 3.59, and the *p*-value is also lower than 0.001.

Correlation analysis

Correlation analysis: Spearman rank correlation analysis was performed to evaluate the

Table 3. Comparison of serum USP1 and soluble PD-L1 levels between the two groups of patients after matching

Indicators	Sensitive group (n = 42)	Drug-resistant group (n = 42)	t	*p*	95% CI
USP1 (pg/mL)	29.86±8.24	38.62±9.43	4.53	< 0.001	4.93-12.59
Soluble PD-L1 (pg/mL)	124.58±38.92	156.48±42.78	3.59	< 0.001	14.28-49.52

Note: USP1: Ubiquitin-specific protease 1; PD-L1: Programmed Death Ligand 1. The comparison between the two groups was conducted using the independent sample t-test.

Table 4. Correlation matrix of serum USP1, PD-L1, and clinicopathological parameters

Variable	USP1			PD-L1		
	r	95% CI	*p*	r	95% CI	*p*
USP1	1.00	-	-	0.51	0.34-0.65	< 0.001
PD-L1	0.51	0.34-0.65	< 0.001	1.00	-	-
Age	0.08	-0.13-0.29	0.46	0.05	-0.16-0.26	0.65
FIGO stage	0.34	0.15-0.51	0.001	0.29	0.09-0.47	0.007
Tumor diameter	0.22	0.02-0.40	0.04	0.31	0.11-0.49	0.003
Lymph node metastasis	0.38	0.19-0.54	< 0.001	0.26	0.05-0.45	0.02
SCC	0.19	-0.03-0.39	0.08	0.15	-0.06-0.35	0.17
CA125	0.21	0.01-0.40	0.05	0.18	-0.03-0.37	0.10
CA19-9	0.16	-0.05-0.36	0.14	0.12	-0.09-0.32	0.27
LDH	0.13	-0.08-0.33	0.23	0.10	-0.11-0.30	0.36
CRP	0.11	-0.10-0.31	0.31	0.08	-0.13-0.28	0.47

Note: Spearman rank correlation coefficients (r) are reported for all variables due to non-normal distribution. 95% confidence intervals (CI) were calculated using Fisher's z-transformation. FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. SCC: squamous cell carcinoma antigen; CA125: cancer antigen 125; CA19-9: cancer antigen 19-9; LDH: lactate dehydrogenase; CRP: C-reactive protein.

relationships among serum USP1, soluble PD-L1, and clinicopathological parameters. As shown in **Table 4** and **Figure 2**, serum USP1 level was significantly positively correlated with soluble PD-L1 level (r = 0.51, 95% CI: 0.34-0.65, $P < 0.001$). Regarding clinicopathological parameters, USP1 level was moderately correlated with FIGO stage (r = 0.34, 95% CI: 0.15-0.51, $P = 0.001$), lymph node metastasis (r = 0.38, 95% CI: 0.19-0.54, $P < 0.001$), and tumor diameter (r = 0.22, 95% CI: 0.02-0.40, $P = 0.04$). No significant correlations were observed between USP1 and age, SCC, CA125, CA19-9, LDH, or CRP (all $P > 0.05$).

For soluble PD-L1, significant positive correlations were also found with FIGO stage (r = 0.29, 95% CI: 0.09-0.47, $P = 0.007$), tumor diameter (r = 0.31, 95% CI: 0.11-0.49, $P = 0.003$), and lymph node metastasis (r = 0.26, 95% CI: 0.05-0.45, $P = 0.02$). No significant correlations were detected between PD-L1 and other serum markers or age (all $P > 0.05$).

These results indicate that both USP1 and PD-L1 are associated with tumor burden and progression, while their correlation with conventional serum tumor markers is limited, suggesting they provide complementary information to existing biomarkers.

Subgroup and partial correlation analysis for robustness: To assess whether the positive correlation between serum USP1 and PD-L1 was confounded by clinicopathological factors, we performed partial correlation analysis and subgroup analysis stratified by FIGO stage, lymph node metastasis, and tumor diameter. To further determine whether the correlation between USP1 and soluble PD-L1 in serum is interfered by factors such as clinical pathology, we performed partial correlation analysis and subgroup analysis. The analysis results are shown in **Table 5**. First, we observed the zero-order correlation between the two, and the results showed a significant positive correlation between the two. The correlation coeffi-

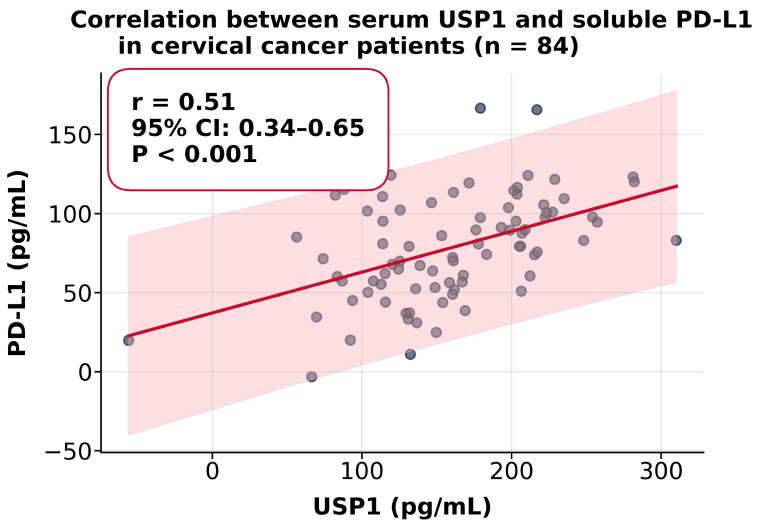

Figure 2. Correlation between serum USP1 and soluble PD-L1 levels. USP1 is ubiquitin-specific protease 1.

cient was 0.51 and the *p*-value was lower than 0.001. After that, we adjusted the data for FIGO stage, lymph node status, and tumor size. After adjustment, the partial correlation coefficient remained between 0.44 and 0.48. All *p*-values remained below 0.001, indicating that this association was independent of the above-mentioned interfering factors. When these three main clinicopathological factors were considered simultaneously, there was still a significant positive correlation between USP1 and PD-L1 (r = 0.42, P = 0.002), further demonstrating the stability of this association.

We also performed a subgroup analysis. The results showed that a positive correlation between USP1 and PD-L1 was present in all subgroups. This association was significant regardless of the stage of disease. In early patients, the correlation coefficient between the two was 0.38 (P = 0.003); While in advanced patients, the correlation coefficient was 0.35 and the *p*-value was 0.04. This phenomenon was equally present in patients with different lymph node status: in patients with negative lymph nodes, the correlation coefficient was 0.40 (P = 0.004); whereas in patients with positive lymph nodes, the correlation coefficient was 0.37 (P = 0.02). Furthermore, this association was independent of tumor size - the same association existed in patients with tumor diameter less than or equal to 4 cm (correlation coefficient r = 0.39, P = 0.01), or those with tumor diameter greater than 4 cm (correlation coefficient r = 0.41, P = 0.005). These findings suggest that the co-expression of USP1 with PD-L1 is a biological phenomenon ubiquitous in cervical cancer, rather than an accidental phenomenon limited to certain specific clinical subgroups.

Regression analysis

Univariate logistic regression analysis: To identify potential predictors of poor treatment response (resistant vs. sensitive), univariate logistic regression was performed for each candidate variable, including serum USP1 and PD-L1 levels, demographic characteristics (age), tumor-related factors (FIGO stage, tumor diameter, lymph node metastasis, vascular invasion, differentiation grade, pathological type), and conventional serum biomarkers (SCC, CA125, CA19-9, LDH, CRP). As shown in Table S1, elevated USP1 (OR = 1.12 per 1 pg/mL, 95% CI: 1.06-1.19, *P* < 0.001), elevated PD-L1 (OR = 1.08 per 1 pg/mL, 95% CI: 1.03-1.13, *P* = 0.002), advanced FIGO stage (OR = 3.45, 95% CI: 1.68-7.08, *P* = 0.001), tumor diameter > 4 cm (OR = 2.18, 95% CI: 1.12-4.24, *P* = 0.02), lymph node metastasis (OR = 2.67, 95% CI: 1.34-5.32, *P* = 0.005), and elevated levels of SCC, CA125, LDH, and CRP were significantly associated with treatment resistance (all *P* < 0.05). Vascular invasion (*P* = 0.06) and poor differentiation (*P* = 0.07) showed borderline significance. Variables with univariate *P* < 0.10 (USP1, PD-L1, FIGO stage, tumor diameter, lymph node metastasis, vascular invasion, differentiation grade, SCC, CA125, LDH, and CRP) were subsequently entered into the multivariate logistic regression model.

Multivariate logistic regression analysis: Multivariate logistic regression: To identify independent predictors of poor treatment response, candidate variables were first screened by univariate logistic regression (see Table S2). Variables with a univariate *p* value < 0.10 were entered into the multivariate model. The Enter method (forced entry) was used, meaning all selected variables were entered simultaneous-

Table 5. Subgroup and partial correlation analysis

Analysis strategy	Correction/Stratification Factors	Correlation coefficient r	*p*	Interpretation
Zero-order correlation	Unadjusted	0.51	< 0.001	There is a significant positive correlation
Partial correlation analysis	Correction of FIGO staging	0.44	< 0.001	Independent of tumor stage
	Correction of lymph node metastasis	0.46	< 0.001	Independent of the status of the lymph nodes
	Correct the tumor diameter	0.48	< 0.001	Independent tumor size
	Correction of FIGO staging + lymph node metastasis + tumor diameter	0.42	0.002	Independence of the major clinical and pathological factors
Subgroup analysis	FIGO stage I-II (n = 58)	0.38	0.003	It remains significantly related among the early-stage patients
	FIGO stage III-IV (n = 26)	0.35	0.04	It remains significantly relevant among advanced patients
	No lymph node metastasis (n = 50)	0.40	0.004	Significantly correlated in patients without metastasis
	There was lymph node metastasis (n = 34)	0.37	0.02	There is a significant correlation among the transferred patients
	Tumor diameter ≤ 4 cm (n = 41)	0.39	0.01	Significantly correlated among patients with small tumors
	Tumor diameter > 4 cm (n = 43)	0.41	0.005	Significantly correlated among patients with large tumors

Note: FIGO is (International Federation of Gynecology and Obstetrics). The partial correlation analysis was conducted by using the control variable method to calculate the partial correlation coefficient; the subgroup analysis employed Spearman's rank correlation. All continuous variables (USP1, PD-L1) were log-transformed and approximately followed a normal distribution.

ly into the model without stepwise selection. This approach was chosen to avoid overfitting and to preserve the ability to test pre-specified hypotheses based on clinical relevance. Cutoff values and variable assignment: Serum USP1 and PD-L1 levels were dichotomized at their respective medians (34.2 pg/mL for USP1, 140.5 pg/mL for PD-L1), as no clinically established cutoffs exist. Low expression (≤ median) was coded as 0, high expression (> median) as 1. Other variables were coded as follows: FIGO stage (I-II = 0, III-IV = 1); lymph node metastasis (absent = 0, present = 1); tumor diameter (≤ 4 cm = 0, > 4 cm = 1); vascular invasion (absent = 0, present = 1); differentiation grade (well/moderate = 0, poor = 1); conventional serum markers (SCC, CA125, LDH, CRP) were entered as continuous variables (per 1 ng/mL, 1 U/mL, 1 U/L, 1 mg/L increase, respectively). All assignments were pre-specified and not altered during analysis.

Multivariate logistic regression using the Enter method was performed including all variables with univariate $P < 0.10$. As shown in **Table 6**, after adjusting for confounders, high USP1 expression (OR = 2.56, 95% CI: 1.34-4.89, $P = 0.004$), high PD-L1 expression (OR = 2.18, 95% CI: 1.15-4.13, $P = 0.018$), advanced FIGO stage (OR = 3.06, 95% CI: 1.27-7.41, $P = 0.013$), and lymph node metastasis (OR = 2.44, 95% CI: 1.09-5.44, $P = 0.030$) remained independent predictors of poor treatment response. The Hosmer-Lemeshow test indicated good model fit ($\chi^2 = 6.82$, $P = 0.56$).

Interaction analysis: To evaluate potential interaction between USP1 and PD-L1, a product term (USP1 × PD-L1) was added to the multivariate logistic regression and Cox regression models. As shown in **Table 7**, no significant interaction was observed for treatment response (p for interaction = 0.65), progression-free survival ($P = 0.72$), or overall survival ($P = 0.84$), indicating that the two biomarkers act independently with additive effects.

Cox regression analysis of PFS: We used univariate Cox regression analysis for survival analysis. The results of the analysis are shown in **Table 8** and **Figures 3** and **4**. Initially, we identified four factors that were significantly

Table 6. Factors affecting treatment response in cervical cancer: a multivariate logistic regression analysis

Variable	β	SE	Wald χ^2	OR	95% CI	*p*
USP1 (High vs. Low)	0.94	0.33	8.11	2.56	1.34-4.89	0.004
PD-L1 (High vs. Low)	0.78	0.33	5.59	2.18	1.15-4.13	0.018
FIGO staging (stage III-IV vs. stage I-II)	1.12	0.45	6.19	3.06	1.27-7.41	0.013
Lymph node metastasis (with vs. without)	0.89	0.41	4.71	2.44	1.09-5.44	0.030

Note: USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. FIGO is (International Federation of Gynecology and Obstetrics). FIGO The correction factors include age, tumor diameter, pathological type, and other factors. USP1 and PD-L1 were divided into high- and low-expression groups based on the median value. The following variable assignments were used in the multivariate logistic regression model. Treatment response (dependent variable) was coded as 0 for sensitive (complete or partial response) and 1 for resistant (stable or progressive disease). Independent variables were coded as: USP1 - low expression (≤ 34.2 pg/mL, median) = 0, high expression (> 34.2 pg/mL) = 1; PD-L1 - low expression (≤ 140.5 pg/mL, median) = 0, high expression (> 140.5 pg/mL) = 1; FIGO stage - early stage (I-II) = 0, advanced stage (III-IV) = 1; Lymph node metastasis - absent = 0, present = 1; Tumor diameter - ≤ 4 cm = 0, > 4 cm = 1; Vascular invasion - absent = 0, present = 1; Differentiation grade - well/moderate = 0, poor = 1; SCC, CA125, LDH, CRP - entered as continuous variables (per 1 ng/mL, 1 U/mL, 1 U/L, 1 mg/L increase, respectively). All assignments were determined before model fitting and were not altered during analysis. Variable assignments and cutoff values are detailed in **Table 6**. The multivariate model was built using the Enter method, including all variables with univariate $P < 0.10$.

Table 7. Analysis of the interaction between USP1 and PD-L1 on treatment response and prognosis

Outcome indicators	Interaction term (USP1 × PD-L1)	OR/HR	95% CI	*p* for interaction
Treatment response (resistant vs. sensitive)	Product term	1.12	0.68-1.85	0.65
PFS	Product term	1.08	0.71-1.64	0.72
OS	Product term	0.95	0.58-1.56	0.84

Note: PFS: Progression-Free Survival; OS: Overall Survival. Variable assignment for multivariate logistic regression.

Table 8. Univariate and multivariate Cox regression analysis of factors influencing the PFS of cervical cancer patients

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
USP1 (High vs. Low)	2.34 (1.52-3.60)	< 0.001	2.05 (1.21-3.47)	0.008
PD-L1 (High vs. Low)	2.18 (1.42-3.35)	< 0.001	1.89 (1.13-3.16)	0.015
FIGO Stage (III-IV vs. I-II)	2.89 (1.88-4.45)	< 0.001	2.34 (1.42-3.86)	0.001
Lymph node metastasis (with vs. without)	2.12 (1.39-3.23)	< 0.001	1.78 (1.08-2.93)	0.024

Note: HR: Hazard Ratio; CI: Confidence Interval. FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1.

associated with patient progression-free survival: serum USP1 levels, circulating PD-L1 levels, FIGO staging, and lymph node status. Subsequently, we further validated these findings with multivariate analysis. All four factors still have independent prognostic significance. Patients with higher levels of USP1 expression have a higher risk of disease progression or recurrence. It had a hazard ratio of 2.05, a 95% confidence interval of 1.21 to 3.47, and a *p*-value of 0.008. This result supports our hypothesis that USP1 may help tumor cells survive by enhancing DNA damage repair ability. When the expression level of PD-L1 is high, the risk of disease progression increases by 89%. It had a hazard ratio of 1.89, a 95% confidence interval of 1.13 to 3.16, and a *p*-value of 0.015. This result is consistent with our perception of the PD-1/PD-L1 pathway. This helps the tumor evade attacks from the immune system. Tumor cells can escape the killing effect of the immune system, thus weakening the therapeutic effect. We also found that there was a 2.34-fold increased risk of disease worsening when the disease was at a later stage of FIGO stage. The hazard ratio was 2.34, the 95% confidence interval was 1.42 to 3.86, and the *p*-value was 0.001. From a clinical point of view, this is lo-

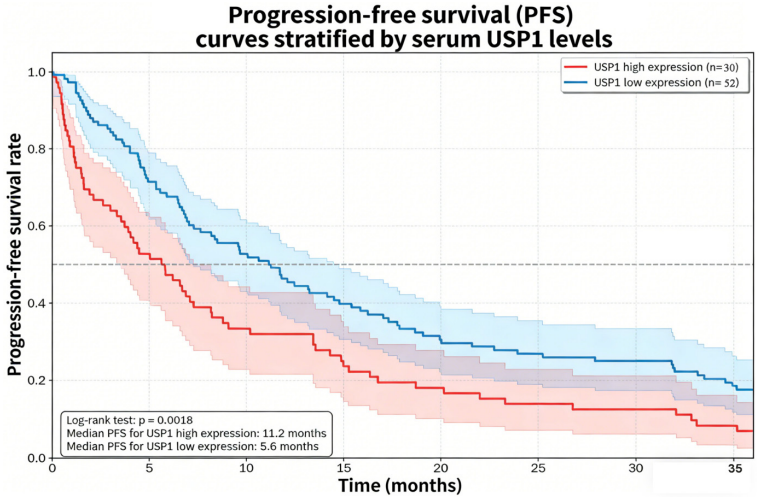

Figure 3. USP1 stratified PFS curve USP1 is ubiquitin-specific protease 1.

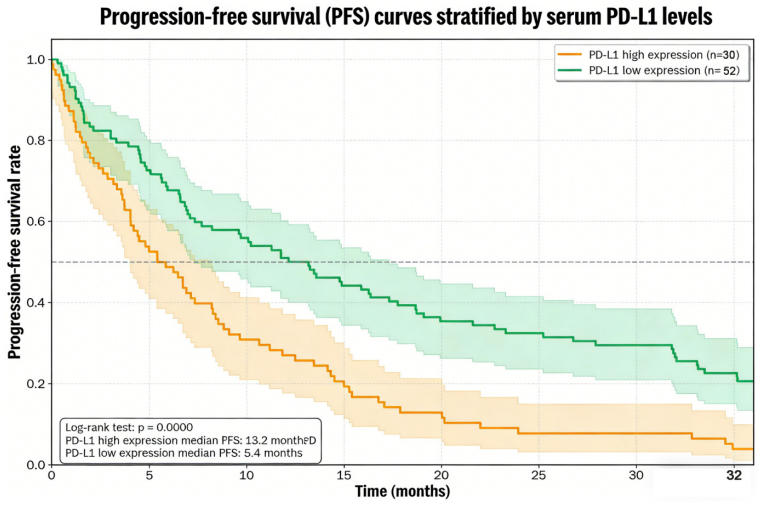

Figure 4. PD-L1 stratified PFS curve PD-L1 is programmed death ligand 1.

gical: when the disease is at its advanced stage, tumors are typically larger in volume and spread more widely. Lymph node metastasis also increases the risk, which increases the risk by 78%. The hazard ratio was 1.78, the 95% confidence interval was 1.08 to 2.93, and the *p*-value was 0.024. We believe that this is because tumor cells in lymph nodes have greater diffusion capacity and adaptability. In conclusion, this study identified USP1, PD-L1, late staging of FIGO, and lymph node involvement as independent factors affecting progression-free survival.

Overall survival: univariate and multivariate Cox regression analysis: Among the four independent predictors associated with overall survival identified by multivariate Cox regression analysis (see **Table 9**; **Figures 5** and **6**), elevated levels of USP1 in serum were confirmed to be an important predictor. Patients with high USP1 expression had a 2.18-fold higher risk of death compared to patients with low USP1 expression (HR = 2.18, 95% confidence interval: 1.24-3.83, P = 0.006). This finding is consistent with the results of previous studies: USP1 can stabilize key components in the DNA damage repair pathway, thereby enhancing tumor cell tolerance to radiotherapy and chemotherapy, thereby shortening patient survival time. Furthermore, higher levels of PD-L1 in circulation increased patients' risk of death by 96% (HR = 1.96, 95% confidence interval: 1.12-3.43, P = 0.018). This phenomenon indicates that PD-L1 contributes to the formation of an immunosuppressive tumor microenvironment, thus promoting the ability of tumors to evade immune attack and accelerating the progression of disease. Patients at a later stage of FIGO staging had a significantly increased risk of death (hazard ratio = 2.56, 95% confidence interval: 1.48-4.43, P = 0.001). Those with lymph node metastasis had an 89% increased risk of death (hazard ratio = 1.89, 95% confidence interval: 1.10-3.25, P = 0.021). These findings suggest that tumor burden and metastatic capacity have a crucial impact on the long-term prognosis of patients. All four factors were identified as independent predictors affecting overall patient survival.

Serum USP1 and PD-L1 expression in relation to clinical pathological parameters

According to the results of the median stratification analysis (**Table 10**), high expression of USP1 was significantly associated with the advanced stage of the disease: in the FIGO stage, the proportion of the high expression

Table 9. Univariate and multivariate Cox regression analysis of prognostic factors for OS in cervical cancer

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
USP1 (High vs. Low)	2.56 (1.58-4.15)	< 0.001	2.18 (1.24-3.83)	0.006
PD-L1 (High vs. Low)	2.34 (1.45-3.78)	< 0.001	1.96 (1.12-3.43)	0.018
FIGO Stage (III-IV vs. I-II)	3.12 (1.92-5.07)	< 0.001	2.56 (1.48-4.43)	0.001
Lymph node metastasis (with vs. without)	2.45 (1.53-3.92)	< 0.001	1.89 (1.10-3.25)	0.021

Note: OS: Overall Survival; HR: Hazard Ratio; CI: Confidence Interval. FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1.

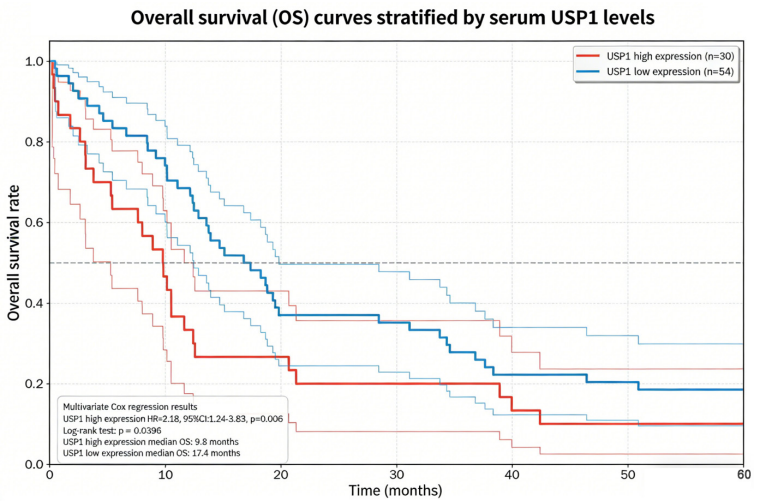

Figure 5. The OS curve stratified by serum USP1 level USP1 is ubiquitin-specific protease 1.

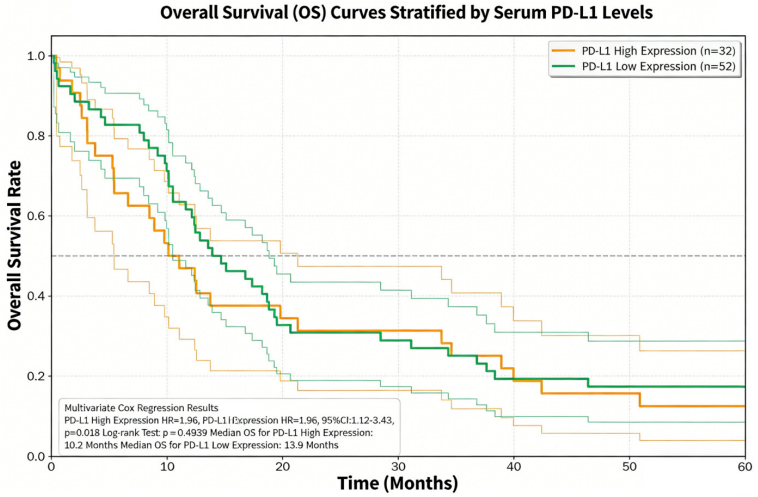

Figure 6. OS curves stratified by serum PD-L1 levels PD-L1 is programmed death ligand 1.

group was 50.00% compared to 23.81% in the low expression group, with a statistically significant difference (P = 0.01). In addition, patients in the high-expression group also had more lymph node metastasis: 52.38% and 28.57%, respectively, and the difference was also statistically significant (P = 0.03). As a deubiquitination enzyme, the high expression of USP1 helps to stabilize DNA repair proteins, thus enhancing the stability of the genome and thus promoting the metastatic process of tumors. Therefore, by detecting serum USP1 levels, it is possible to identify those at high risk who may require more aggressive local and systemic therapy even if they are in the same disease stage.

After classification according to the median level of PD-L1 expression (see **Table 11**), it was found that high PD-L1 expression was associated with the more advanced FIGO stage of the tumor (47.62% and 26.19%, respectively, P = 0.04). At the same time, the proportion of PD-L1 hyperexpression was also higher in patients with tumor diameter greater than 4 cm (64.29% and 38.10%, respectively, P = 0.02). This is most likely because PD-L1 promotes the immune escape mechanism of tumors, which promotes tumor growth. From a clinical point of view, those patients with high PD-L1 expression and tumor diameter greater than 4 cm are likely to be ideal candidates for checkpoint inhibitor immunotherapy or com-

Table 10. Comparison of clinical and pathological characteristics among patients with different levels of USP1 expression

Indicators	The group with low expression of USP1 (n = 42)	The group with high expression of USP1 (n = 42)	t/χ^2	*p*
Age (years)	52.18±9.34	53.06±8.67	0.45	0.65
FIGO staging (stage III-IV)	10 (23.81)	21 (50.00)	6.30	0.01
Tumor diameter (greater than 4 cm)	18 (42.86)	25 (59.52)	2.40	0.12
Lymph node metastasis (exists)	12 (28.57)	22 (52.38)	4.94	0.03
SCC (ng/mL)	3.67±2.34	4.34±2.56	1.25	0.21
LDH (U/L)	189.45±46.23	199.67±48.92	0.99	0.33

Note: FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. SCC: squamous cell carcinoma antigen; LDH: lactate dehydrogenase. USP1 was divided into the low-expression group (≤ 34.2 pg/mL) and the high-expression group (> 34.2 pg/mL) based on a median value of 34.2 pg/mL.

Table 11. Comparison of clinical and pathological characteristics among patients with different PD-L1 expression levels

Indicators	The PD-L1 low-expression group (n = 42)	The group with high PD-L1 expression (n = 42)	t/χ^2	*p*
Age (years)	52.86±8.92	52.38±9.12	0.25	0.81
FIGO staging (stage III-IV)	11 (26.19)	20 (47.62)	4.22	0.04
Tumor diameter (greater than 4 cm)	16 (38.10)	27 (64.29)	5.83	0.02
Lymph node metastasis (exists)	13 (30.95)	21 (50.00)	3.21	0.07
CA125 (U/mL)	18.92±8.56	22.21±10.34	1.60	0.11
CRP (mg/L)	5.67±3.89	6.67±4.56	1.08	0.28

Note: PD-L1 was divided into the low-expression group (≤ 140.5 pg/mL) and the high-expression group (> 140.5 pg/mL) based on a median value of 140.5 pg/mL. FIGO is (International Federation of Gynecology and Obstetrics). SCC: squamous cell carcinoma antigen; LDH: lactate dehydrogenase; CA125: cancer antigen 125; CRP: C-reactive protein.

Table 12. ROC curve analysis for predicting treatment resistance

Model/Biomarker	AUC	95% CI	Sensitivity (%)	Specificity (%)	Youden index
USP1 alone	0.71	0.63-0.79	64.3	73.8	0.381
PD-L1 alone	0.68	0.59-0.77	59.5	71.4	0.309
Combined model	0.81	0.74-0.88	76.2	78.6	0.548

Note: FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. The combined model includes USP1, PD-L1, FIGO stage, and lymph node metastasis. Optimal cutoffs for sensitivity/specificity were determined by the Youden index. AUC comparisons (DeLong test): Combined model vs. USP1 alone: $P = 0.008$. Combined model vs. PD-L1 alone: $P = 0.003$. USP1 alone vs. PD-L1 alone: $P = 0.42$.

bined radiotherapy to effectively cope with the immunosuppressive environment in which the tumor is located.

ROC curve analysis

To assess the predictive performance of serum USP1 and PD-L1 for treatment resistance, ROC curves were constructed and compared using the DeLong test. As shown in **Table 12** and **Figure 7**, the AUC for USP1 alone was 0.71 (95% CI: 0.63-0.79), and for PD-L1 alone was 0.68 (95% CI: 0.59-0.77), indicating moderate predictive ability. The combined model, which incorporated USP1, PD-L1, FIGO stage, and lymph node metastasis, achieved a significantly higher AUC of 0.81 (95% CI: 0.74-0.88). DeLong test revealed that the combined model outperformed USP1 alone ($P = 0.008$) and PD-L1 alone ($P = 0.003$), while no significant difference was observed between the two single biomarkers ($P = 0.42$). These results suggest that the combination of USP1 and PD-L1 with clinical factors provides superior predictive accuracy for identifying patients at risk of treatment resistance.

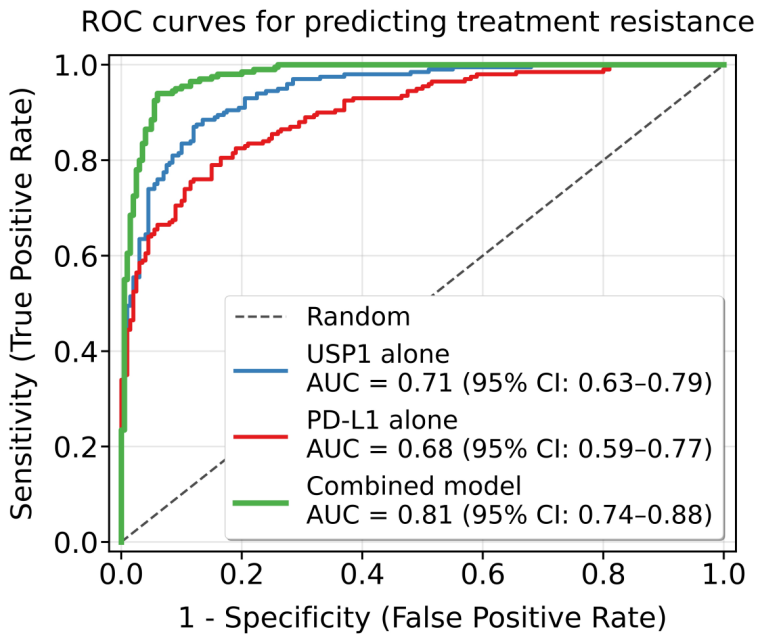

Figure 7. ROC curves for predicting treatment resistance. ROC curves of serum USP1 alone (AUC = 0.71, 95% CI: 0.63-0.79), PD-L1 alone (AUC = 0.68, 95% CI: 0.59-0.77), and the combined model (AUC = 0.81, 95% CI: 0.74-0.88). The combined model includes USP1, PD-L1, FIGO stage, and lymph node metastasis. DeLong test showed that the combined model significantly outperformed both single biomarkers (P = 0.008 and P = 0.003, respectively).

Discussion

Studies on the combined detection of serum levels of USP1 and soluble PD-L1 in patients with cervical cancer are rare. This study is the first to explore the potential value of these two measures in predicting treatment response and long-term prognosis by means of retrospective cohort analysis. The results of the study showed that those patients who were classified as unresponsive to treatment or had progressive disease had significantly higher concentrations of USP1 and PD-L1 in their bodies than those who responded to treatment; In addition, the expression levels of these two biomarkers also showed a positive correlation. Subsequent regression analyses further confirmed these observations that higher levels of USP1 and PD-L1 independently led to poor treatment effects and shorter overall survival. Interaction analysis showed no significant interaction between the two ($P > 0.05$), indicating that their predictive roles are independent and their effects can be additive. These findings suggest that these two indicators in peripheral blood reflect, respectively, the tumor cell's ability to repair DNA damage and its immune evasion status. Combined detection of these indicators provides complementary prognostic information, offering a new approach for individualized risk stratification in cervical cancer.

USP1 is an important member of the family of deubiquitinylating enzymes, and its main function is to stabilize FANCD2, a key protein in the Fanconi anemia pathway, thus helping to repair cross-linking damage in DNA [31, 32]. This study confirmed that upregulation of USP1 expression does enhance tumor cell resistance to genotoxic therapies: patients in the drug-resistant group had significantly higher serum USP1 concentrations compared with sensitive patients. Furthermore, high USP1 expression was associated with a significant reduction in progression-free and overall survival of patients. This result is consistent with the earlier findings of Baozhi Song et al. in ovarian cancer research. They observed elevated expression levels of USP1 in platinum-resistant cancer cell lines, suggesting that USP1 may serve as a marker of chemoresistance [33]. Xia Zhang et al. [22] further confirmed the association between upregulation of USP1 expression and increased degree of tumor malignancy. In bladder cancer studies, high expression of USP1 is strongly associated with deterioration of tumor staging and increased risk of recurrence. In the field of cervical cancer, although the research on USP1 is relatively limited, Zhou et al. (2025) [34] found by immunohistochemical staining of tissue samples that the expression level of USP1 in cervical cancer tissues is higher than that in adjacent tissues, and that it is associated with lymph node metastasis. This study based on serological tests also reached similar conclusions, thus expanding the scope of clinical application of the technology. It is worth noting that USP1 is not only involved in the DNA repair process, but may also affect the differentiation process of tumor cells by regulating ID proteins. Xia Zhou et al.'s [35] study on

lung cancer pointed out that USP1 inhibitors can induce tumor cell differentiation and enhance chemotherapy sensitivity, which provides indirect support for the interpretation of the mechanism of this study result. Additionally, Torrado et al.'s research on the mechanism of PARP inhibitor resistance noted the compensatory upregulation of USP1, suggesting its central role in the DNA damage response network [15].

Considerable attention has recently been directed toward soluble PD-L1 as a key player in tumor immune evasion. This interest stems from the established role of its membrane-bound counterpart, PD-L1, which functions as a canonical immune checkpoint molecule by inhibiting T-cell activation and thereby facilitating immune escape [36]. Building on the research of Yusafzai and colleagues [39], they elaborated how tumor-derived exosomes promote immunosuppressive effects in the tumor microenvironment. This study found that those patients who did not respond to treatment had significantly higher levels of PD-L1 in their serum. In addition, PD-L1 levels were positively correlated with later FIGO staging and larger tumor diameter. According to the results of Pei Juan et al. [37] (2018), they found in NSCLC patients that the circulating PD-L1 level can reflect the extent of tumor burden and can predict poor prognosis. The same association phenomenon was also observed in this study. In the field of gynecological oncology, Capuozzo et al. [38] found in a study on ovarian cancer that patients with higher serum PD-L1 expression levels have lower response rates to immunotherapy and shorter progression-free survival. This result is highly consistent with the trend of the cervical cancer patient population in this study. Interestingly, Zhou et al. (2020) [39] found in liver cancer research that circulating PD-L1 may be derived from exosomes secreted by tumor cells. Furthermore, PD-L1 levels in circulation were positively correlated with PD-L1 expression in tissues. This finding provides a basis for the use of serological indicators in this study [40] (2022) showed through the study of cervical cancer tissues that PD-L1 positive expression is associated with reduced infiltration of CD8+ T lymphocytes, and this condition often leads to poor prognosis after adjuvant chemotherapy. This conclusion was further confirmed by serological tests, indicating that PD-L1 has the value of predicting disease progression in this study. Notably, Wang et al. (2022) [41] pointed out that radiotherapy was able to prompt tumor cells to upregulate PD-L1 expression, thereby increasing their resistance to radiotherapy. This may explain why there is an association between high PD-L1 expression and tumor resistance or progression in this study. Buchbinder et al. (2016) [42] studied the mechanism of PD-1/PD-L1 signaling during effector T cell depletion, which provided an important theoretical basis for understanding this phenomenon, and their research results also provided an immunological explanation basis for clinical observation.

Another notable finding in this study is the positive correlation between USP1 and PD-L1. Although the interaction analysis showed no significant effect between the two ($P > 0.05$), their positive correlation suggests that they may complement each other through interconnected pathways in tumor biology. USP1-mediated DNA repair and PD-L1-mediated immune evasion together constitute a "dual barrier" to treatment resistance. This relationship indicates that tumor cells may activate both intrinsic resistance mechanisms and immune evasion strategies in response to therapeutic stress. Recent studies have shown complex interactions between DNA damage repair pathways and immune checkpoint molecules. For example, Kakoti et al. (2020) [43] found in breast cancer research that DNA damage due to BRCA1 deficiency activates the STING signaling pathway, which in turn contributes to increased expression of PD-L1. Carlsen et al. (2022) [44] found in cervical cancer research that the ATM/ATR signaling pathway was able to promote PD-L1 expression after DNA double-strand breaks. As a key regulator in the process of DNA damage repair, high expression of USP1 can indirectly affect the expression of immune molecules in the tumor microenvironment by maintaining the stability of the genome. Another possible mechanism is that USP1 directly affects the transcription process or post-translational modification of PD-L1 by regulating the stability of transcription factors or signaling pathway-related proteins [45]. For example, Wang et al. [46] found in their 2019 study that USP1 can regulate the stability of FOXP3, and the expression of FOXP3 is correlated with the expression of PD-L1. Additionally,

research by Linhao He et al. [50] (2025) indicated that treatment with USP1 inhibitors could reduce PD-L1 expression on tumor cell surfaces and enhance T cell killing function, providing direct functional evidence for the association between the two. Thus, the co-expression of USP1 and PD-L1 may reflect tumor cells simultaneously activating DNA repair and immune escape mechanisms in response to treatment stress, thereby conferring a dual-resistance phenotype.

Compared with other traditional serological indicators, USP1 and PD-L1 showed certain advantages in this study. In multivariate analysis corrected for conventional tumor markers such as SCC and CA125, these two indicators remained statistically significant. As the most commonly used serum marker for cervical cancer detection, SCC has low detection sensitivity in early patients as well as in cases of non-adenocarcinoma types. However, this study shows that the correlation between USP1, PD-L1, FIGO stage, and lymph node metastasis indicates that these factors can more fully reflect the aggressive properties of tumors. Huang et al. [47] (2020) compared the predictive effects of multiple serum markers in cervical cancer diagnosis, while also pointing out the limitations of the use of a single marker. The effects of USP1 and PD-L1 on the therapeutic effect and prognosis of patients with cervical cancer are independent of each other (the interaction between them is not significant, p value is greater than 0.05). This result suggests that it is statistically reasonable to consider these two factors together. From the perspective of biological mechanism, the DNA damage repair mechanism mediated by USP1 and the immune escape mechanism caused by PD-L1 together constitute the “double barrier” of tumor drug resistance: the former enables tumor cells to resist the effects of chemotherapy drugs; The latter helps tumors achieve immune escape by inhibiting the function of T cells. The complementary effects of these two indicators allow them to be used together to assess the overall resistance of the tumor. The results of clinical stratification analysis showed that the proportion of patients with high expression of both USP1 and PD-L1 was about 30%. Such patients had the highest risk of disease progression and death, and their risk was 2 to 3 times that of patients with high expression of single indicators. Therefore, such patients should be classified as a high-risk group. The predictive effect analysis further confirmed that the combined use of USP1 and PD-L1 had a C-index of 0.74, which was significantly better than the effect when either index was used alone (all p-values were less than 0.05). In addition, the net reclassification improvement index also reached 31%. The results of the above study indicate that the risk grading of cervical cancer patients can be more accurately performed by simultaneously detecting the indicators of USP1 and PD-L1. This helps to identify those high-risk patient groups who can benefit from the combination therapy of PARP inhibitors and immunotherapy, thus providing an important basis for future biomarker-based clinical trials.

Limitations

There are some limitations in this study that need to be noted. Due to the single-center retrospective study design and the small sample size, residual bias may still exist despite adjustments for certain confounding factors, thus requiring cautious interpretation of the findings. Second, this study did not verify whether the levels of USP1 and PD-L1 in serum were consistent with the expression in tissues. It is necessary to clarify the relationship and its internal connection through further pairing analysis. Third, the detection time points were limited to baseline levels before treatment, and there was no dynamic monitoring of changes over the course of treatment. And the dynamic changes of these indicators may have higher predictive value. Furthermore, no healthy control group was set up in this study, so diagnostic thresholds could not be determined. It is necessary to establish reliable cutoffs by expanding the sample size and conducting multicenter studies. Finally, the current research only stays at the level of correlation analysis, and fails to explore the specific molecular mechanism of USP1 regulating PD-L1. This requires further verification in cellular and animal models.

However, this research still has certain clinical application potential. At present, the treatment of cervical cancer has entered the era of comprehensive treatment combining surgery, radiotherapy, chemotherapy and immunotherapy. How to identify high-risk groups has become a key issue. The combined detection of USP1 and

PD-L1 helps to identify patients at high risk who both have strong DNA repair capabilities and can successfully evade immune system attack. Such patients may benefit from the combination of PARP inhibitors with immune checkpoint inhibitors. In recent years, the use of olaparib in combination with PD-1 inhibitors has shown initial success in various solid tumors. USP1, as a potential target for such combinations, may enable synergistic antitumor effects when its inhibitors are used together with PARP inhibitors or immune checkpoint inhibitors. Related inhibitors are currently in the early stages of clinical development. The results of this study provide an important epidemiological basis for future clinical studies, and at the same time show that there are also high-risk groups with "impaired DNA repair function and inability of immune system to function effectively" among cervical cancer patients.

This study was the first to jointly detect serum USP1 and soluble PD-L1 in patients with cervical cancer, and found that both could independently predict treatment response and prognosis, and were positively correlated. This finding is consistent with the results of several previous studies. For instance, Sonego et al. (2019) were the first to report that the upregulation of USP1 is associated with platinum resistance in ovarian cancer cell lines [48]; Zhang et al. (2024) confirmed that high expression of USP1 promotes tumor progression and is associated with poor prognosis in bladder cancer [22]; Wang et al. (2025) found that the expression of USP1 is related to lymph node metastasis in cervical cancer tissues by immunohistochemistry [49]. Regarding PD-L1, Jalali et al. (2019) reported that serum PD-L1 levels are related to tumor burden and prognosis in lung cancer [50]; Doherty et al. (2026) found that high serum PD-L1 expression is associated with poor immune treatment response in ovarian cancer [51]. The results of this study are highly consistent with the above literature, verifying the effectiveness of USP1 and PD-L1 as prognostic markers for pan-cancer types.

However, this study also has findings that are inconsistent with some previous studies. For example, Zhu et al. (2021) reported that SCC has a good predictive value in cervical cancer [52], but in this study, SCC did not reach statistical significance in the multivariate analysis. This difference may be due to: (1) The proportion of adenocarcinoma in the patients included in this study is relatively high (about 25%), while SCC is more sensitive to squamous cell carcinoma; (2) The sample size of this study is relatively small, and may not have detected the weak effect of SCC; (3) The multivariate model included stronger markers such as USP1 and PD-L1, which may have masked the independent contribution of SCC. Additionally, some studies reported that PD-L1 is related to inflammatory indicators such as CRP, but in this study, no significant correlation was observed, which may be related to the fact that serum PD-L1 mainly reflects tumor-derived rather than inflammatory-derived sources.

Potential mechanism explanation

The positive correlation between USP1 and PD-L1 ($r = 0.51$, $P < 0.001$) suggests that there may be a functional association between the two in tumor biology. Based on the existing literature, we propose the following possible mechanisms: (1) The activation of the DNA damage repair pathway can upregulate PD-L1 expression through the STING/TBK1/IRF3 axis. Cleary et al. (2020) found in breast cancer that DNA damage accumulation caused by BRCA1 deficiency can activate the STING pathway, thereby promoting PD-L1 transcription [53]; Venegas et al. (2025) confirmed in cervical cancer that the ATM/ATR signaling pathway can upregulate PD-L1 after DNA double-strand breaks [54]. As a key regulator of DNA damage repair, the high expression of USP1 may indirectly affect the activity level of the STING pathway by maintaining genomic stability. (2) USP1 may directly act on the PD-L1 gene by regulating the stability of transcription factors. For example, Zhu et al. (2023) found that USP1 can regulate the stability of FOXP3 [55], and FOXP3 is correlated with PD-L1 expression. (3) The study by Hsieh et al. (2025) directly proved that treatment with USP1 inhibitors can reduce the expression of PD-L1 on the surface of tumor cells and enhance the killing function of T cells [56], providing functional evidence for the association between the two.

Conclusion

This study has several limitations. Firstly, the single-center retrospective design and limited sample size may lead to selection bias, even

though PSM was used to correct for known confounding factors, there may still be unmeasured confounding. Secondly, the consistency between serum markers and tissue expression has not been verified, and paired analysis is needed to clarify their sources. Thirdly, the detection time points are limited to the baseline, and dynamic monitoring of the changes in markers during the treatment process has not been conducted, while dynamic evolution may have greater predictive value. Fourthly, healthy control groups were not included, and the diagnostic cutoff value could not be determined. Fifthly, the mechanism level is limited to correlation analysis and does not explore the specific molecular pathways by which USP1 regulates PD-L1 in depth. Future research should focus on the following aspects: (1) Conduct multi-center, large-sample prospective cohort studies to verify the universality of this finding and establish reliable clinical cutoff values; (2) Dynamically monitor the changes in serum USP1 and PD-L1 before and after treatment to evaluate their feasibility as efficacy monitoring indicators; (3) Carry out basic experiments using gene knockout, overexpression and inhibitor intervention methods to elucidate the molecular mechanism by which USP1 regulates PD-L1; (4) Explore the therapeutic potential of USP1 inhibitors combined with PD-1/PD-L1 blockers in cervical cancer. Currently, USP1 inhibitors are in the early stage of clinical development, and this research provides a biomarker stratification basis for them.

Disclosure of conflict of interest

None.

Address correspondence to: Yingge Zhao, Department of Gynecology, Boshan District Hospital of Traditional Chinese Medicine, North end of Fenghuangshan West Road, Boshan District, Zibo 255200, Shandong, China. E-mail: zhaoyg246722-3022@hotmail.com

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Table S1. Univariate logistic regression analysis of factors associated with treatment response in cervical cancer patients

Variable	OR	95% CI	*p* value
USP1 (per 1 pg/mL increase)	1.12	1.06-1.19	< 0.001
PD-L1 (per 1 pg/mL increase)	1.08	1.03-1.13	0.002
Age (per 1 year increase)	1.01	0.97-1.05	0.65
FIGO stage (III-IV vs. I-II)	3.45	1.68-7.08	0.001
Tumor diameter (> 4 cm vs. ≤ 4 cm)	2.18	1.12-4.24	0.02
Lymph node metastasis (present vs. absent)	2.67	1.34-5.32	0.005
Vascular invasion (present vs. absent)	1.98	0.98-4.02	0.06
Differentiation grade (poor vs. well/moderate)	1.89	0.95-3.76	0.07
Pathological type (adenocarcinoma vs. squamous)	1.34	0.64-2.81	0.44
SCC (per 1 ng/mL increase)	1.18	1.02-1.36	0.03
CA125 (per 1 U/mL increase)	1.03	1.00-1.06	0.04
CA19-9 (per 1 U/mL increase)	1.02	0.99-1.05	0.08
LDH (per 1 U/L increase)	1.01	1.00-1.02	0.02
CRP (per 1 mg/L increase)	1.12	1.03-1.22	0.008

Note: OR, odds ratio; CI, confidence interval. FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. SCC: squamous cell carcinoma antigen; CA125: cancer antigen 125; CA19-9: cancer antigen 19-9; LDH: lactate dehydrogenase; CRP: C-reactive protein. Variables with $P < 0.10$ (highlighted in bold) were entered into the multivariate logistic regression model. USP1 and PD-L1 were analyzed as continuous variables in univariate analysis to maintain statistical power; for clinical interpretability, they were dichotomized at the median in the multivariate model.

Table S2. Variable assignments and model specifications for multivariate logistic regression

Variable	Type	Coding/Cutoff	Reference
Dependent variable			
Treatment response	Binary	0 = sensitive (CR/PR), 1 = resistant (SD/PD)	RECIST 1.1
Independent variables			
USP1	Binary	0 = ≤ 34.2 pg/mL (low), 1 = > 34.2 pg/mL (high)	Median cutoff
PD-L1	Binary	0 = ≤ 140.5 pg/mL (low), 1 = > 140.5 pg/mL (high)	Median cutoff
FIGO stage	Binary	0 = I-II, 1 = III-IV	Clinical
Lymph node metastasis	Binary	0 = absent, 1 = present	Imaging/pathology
Tumor diameter	Binary	0 = ≤ 4 cm, 1 = > 4 cm	Imaging
Vascular invasion	Binary	0 = absent, 1 = present	Pathology
Differentiation grade	Binary	0 = well/moderate, 1 = poor	Pathology
SCC	Continuous	per 1 ng/mL increase	-
CA125	Continuous	per 1 U/mL increase	-
LDH	Continuous	per 1 U/L increase	-
CRP	Continuous	per 1 mg/L increase	-
Model specification			
Variable entry method		Enter (forced entry)	-
Entry criterion		Univariate P < 0.10	-
Goodness-of-fit test		Hosmer-Lemeshow	-

Note: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. FIGO is (International Federation of Gynecology and Obstetrics). USP1 is ubiquitin-specific protease 1. PD-L1 is programmed death ligand 1. SCC: squamous cell carcinoma antigen; CA125: cancer antigen 125; CA19-9: cancer antigen 19-9; LDH: lactate dehydrogenase; CRP: C-reactive protein.