

# The Prognostic Values of HPV Genotypes and Tumor PD-L1 Expression in Patients With HPV-associated Endocervical Adenocarcinoma

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**Abstract:** Despite the well-established pathogenic effect of high-risk human papillomavirus (hrHPV) genotypes on endocervical adenocarcinomas (ECAs), the prognostic values of hrHPV genotypes and their association with other prognostic variables have not been established. We categorized 120 usual-type human papillomavirus-associated (HPVA) ECA cases into 3 species groups (HPV16+, HPV18/45+, and other genotypes+) based on the hrHPV status. The clinical-stage, invasion patterns (Silva), and programmed death ligand-1 (PD-L1) expression were compared among genotype groups. In addition, log-rank test and Kaplan-Meier survival curves were used to compare progression-free survival (PFS) among different patient groups. A total of 120 ECA cases with positive hrHPV tests were included in this study. Among them, 51 (42.5%) were positive for HPV16, 50 (41.7%) were positive for HPV18 or 18/45, 9 (7.5%) were positive for other hrHPV genotypes (not including HPV16/18/45). Our data showed patients had no significant difference in clinical stages ( $P=0.51$ ), invasion patterns ( $P=0.55$ ), and PFS ( $P=0.59$ ) across genotype groups. Overall, a relatively high prevalence of PD-L1 expression was observed in HPVA ECAs (25% by tumor proportion score [TPS] and 55% by a combined positive score [CPS]). Using TPS, 19.6% (10/51) HPV16+ cases, 32.0% (16/50) cases of HPV18 or 18/45+ cases, and 22.2% (2/9) cases of other genotypes+ cases demonstrated PD-L1 positivity. No significant difference in PD-L1 expression was seen across genotype groups

( $P=0.35$ ). PD-L1 expression in tumors with patterns B and C was significantly higher than in those with pattern A ( $P=0.00002$ ). Patients with PD-L1-positive tumors by either CPS or TPS showed significantly poorer PFS than those with PD-L1-negative tumors (CPS,  $P=0.025$ ; TPS,  $P=0.001$ ). Our data support that HPV genotypes have no prognostic value in HPVA ECAs, while PD-L1 expression serves as a negative prognostic marker in HPVA ECAs and implies an unfavorable outcome.

**Key Words:** HPV genotypes, PD-L1, HPV-associated, endocervical adenocarcinoma, immune therapy

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In the past few decades, the incidence of endocervical adenocarcinomas (ECAs) has been on the rise both in absolute numbers and overall proportion in cervical cancers.<sup>1</sup> ECAs remain a significant public health problem despite advances in treatment options.<sup>2,3</sup> Patients with ECA have a poorer survival rate than patients with squamous cell carcinoma (SCC), especially in patients with metastatic tumors.<sup>1,4–6</sup> Unlike cervical SCC, where nearly all cases are caused by human papillomavirus (HPV) infection, 10% to 25% of ECAs are not associated with HPV.<sup>7–11</sup> In the newly published 2020 World Health Organization (WHO) Classification of Female Genital Tumors,<sup>12</sup> ECAs are subclassified into human papillomavirus-associated (HPVA) and human papillomavirus-independent (HPVI) groups. Besides the different etiologies, tumors from these 2 groups also demonstrate distinct clinical behaviors.<sup>7,11</sup> The prognosis of HPVA ECAs typically depends on multiple factors. The clinical-stage is the most important and well-established prognostic variable.<sup>13,14</sup> Recently, the pattern-based classification (Silva) system (PBCS) has been shown to have a strong association with tumor prognosis.<sup>15,16</sup>

Meanwhile, the programmed death ligand-1 (PD-L1)/programmed cell death protein 1 pathway produces inhibitory effects on the activation of immune effector cells and their cytotoxic response against tumor cells,<sup>17,18</sup> which in turn leads to the escape of tumor cells from immune surveillance. In cervical cancer, the anti-PD-L1 antibody pembrolizumab has shown promising antitumor activity in PD-L1-positive tumors.<sup>19,20</sup> Most recently,

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PD-L1 expression has been shown to be a negative prognostic marker associated with a potentially unfavorable outcome for ECAs.<sup>21</sup>

Despite the well-established pathogenic effect of high-risk human papillomavirus (hrHPV) genotypes for ECAs,<sup>22</sup> the prognostic values of hrHPV genotypes in patients with HPV ECA have not been established. HPV genotypes 16, 18, and 45 accounted for ~95% of HPV ECA.<sup>23,24</sup> Moreover, ~90% of HPV ECA were the usual type.<sup>10</sup> As most hrHPV genotypes are phylogenetically clustered within either the HPV16 (alpha-9) or HPV18/45 (alpha-7) clades,<sup>25</sup> in this study, we categorized usual-type HPV ECA cases into 3 groups (HPV16+, HPV18/45+, and other genotypes+) based on hrHPV status. We also investigated the prognostic values of hrHPV genotypes and their association with other prognostic variables. In addition, we investigated the prevalence of PD-L1 expression and its prognostic value in HPV ECA.

## MATERIALS AND METHODS

### Case Selection

The study was conducted with approval from the institutional review board at Zhejiang University School of Medicine Women's Hospital and Shaoxing Maternity and Child Health Care Hospital, China. A total of 120 cases of usual-type HPV ECA resections accessioned between 2014 and 2020 were selected from 2 centers (Zhejiang University School of Medicine Women's Hospital and Shaoxing Maternity and Child Health Care Hospital) for further analysis. Patient age, tumor stage, treatment history, and clinical outcome data were extracted from the clinical information system database. hrHPV status (tested by Aptima, HC2, or Cobas assay) and p16 performed as part of the standard of clinical care were recorded. The hrHPV test was performed on either the concurrent or most recent Pap specimen (within 3 mo of the procedures). p16 immunohistochemistry (IHC) was performed on either the index case or the preceding diagnostic biopsy. The block-positive staining pattern was considered p16 positive.

### Histologic Analysis

Hematoxylin and eosin-stained slides were reviewed by 3 gynecologic pathologists (F.Z., M.L., and X.Z.) in a blinded fashion. The pathologic diagnosis of each case was confirmed and classified according to the 2020 WHO Classification of Female Genital Tumors.<sup>12</sup> In all specimens, tumors were classified as invasive patterns A, B, or C according to the PBCS initially described by Parra-Herran and colleagues.<sup>12,15,16</sup> Briefly, pattern A is characterized by well-formed glands with rounded contours; pattern B is defined by focal destructive stromal invasion arising from pattern A glands; and pattern C is defined by diffuse destructive stromal invasion. In addition, PBCS requires negative margins for a reliable assessment of invasion patterns.

### HPV Testing

Detection of hrHPV was performed by 1 of the 3 polymerase chain reaction-based tests (Aptima, HC2, or

Cobas assay) on liquid-based cytology samples according to the manufacturer's specifications. Aptima human papillomavirus (AHPV) assay (Hologic, San Diego, CA) detects the E6/E7 mRNA of 14 hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). AHPV+ samples were then reflex-tested with the AHPV-GT, which can detect the HPV E6/E7 mRNA in hrHPV genotypes 16 or 18/45. AHPV-GT-negative means the other 11 hrHPV genotypes were positive; Cobas testing (Roche, Pleasanton, CA) was performed according to the manufacturer's specifications. Under this system, HPV16 and 18 are detected separately and other 12 hrHPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are detected as a pool by a cocktail of probes with 3 different fluorochromes; Hybrid Capture (HC2) (Detong, Hangzhou, CH) is a nucleic acid hybridization assay for the semiquantitative detection of 14 hrHPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in bulk with HPV16 and 18 tested in 1 group, and the other 12 hrHPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) detected in the other group.

### PD-L1 IHC

IHC was performed according to the previously published protocol.<sup>26</sup> Briefly, positively charged slides with formalin-fixed, paraffin-embedded tissue sections cut at 4  $\mu$ m thickness were dried in an oven for 1 hour at 56 to 60°C. Deparaffinization, rehydration, and target retrieval were performed according to the manufacturer by fully submerging the slides in preheated (65°C) EnVision FLEX Target Retrieval Solution and incubating at 97°C for 20 minutes. Then the slides were immediately submerged in a wash buffer for 5 minutes at room temperature. With antigen retrieval, the slides were placed in the Autostainer Link 48 platform (Dako/Agilent), where they were incubated with the monoclonal mouse anti-human PD-L1 antibody, clone 22C3 pharmDx (Dako/Agilent, Santa Clara, CA), then the anti-mouse linker antibody and finally a DAB+ substrate-chromogen solution. After rinsing with wash buffer for 5 minutes, slides were incubated with the EnVision FLEX/HRP visualization reagent for 30 minutes at room temperature. In the end, the enzymatic conversion by 3, 3'-diaminobenzidine (DAB) tetrahydrochloride chromogen was performed for 10 minutes at room temperature, followed by DAB enhancer for 5 minutes at room temperature. The instrument monitored the incubation time and rinsing of slides between reagents. The reagent times were preprogrammed in the Dako Link software. Slides were subsequently counterstained for 5 minutes with hematoxylin. Mounting was performed using nonaqueous, permanent mounting media. The quality of testing was monitored by the performance of positive and negative tissue controls.

### Analysis of PD-L1 Expression

PD-L1 expression was evaluated by 2 pathologists (F.Z. and X.Z.). Currently, 2 scoring systems, the combined positive score (CPS) and the tumor proportion score (TPS), are used for the evaluation of PD-L1 expression of

solid tumors.<sup>27</sup> Recently, the Food and Drug Administration (FDA) approved a companion diagnostic IHC test for PD-L1 expression in advanced cervical cancers where the PD-L1 positivity was defined as CPS  $\geq 1$ .<sup>28</sup> Notably, the FDA-approved test did not discriminate among the histologic types of cervical cancers. The published study focused mainly on the efficacy of SCC.<sup>20</sup> With this in mind, both CPS and TPS were calculated in this study. CPS was calculated as the number of PD-L1-staining cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells multiplied by 100. In addition, only intratumoral and peritumoral (within one  $\times 20$  field of the tumor nest or gland edge) immune cells (lymphocytes and histocytes) were counted. Stromal immune cells distant from the tumor were excluded. TPS was calculated as the percentage of tumor cells with membranous PD-L1 expression. Both scores ranged from 0 to 100. A cutoff score  $\geq 1$  for CPS and  $\geq 1\%$  for TPS was used to define PD-L1 positivity.

**Statistical Analysis**

To correlate clinical stages, invasion patterns, PD-L1 expression among different hrHPV genotype groups were compared using either a 2-tailed  $\chi^2$  or Fisher exact test. A *P*-value  $< 0.05$  was deemed statistically significant. The analysis was performed using JMP 11.2.0 (SAS Institute Inc., Cary, NC). The progression interval was censored for patients for whom there was no recorded date of progression in the data field “months to new tumor event after initial diagnosis.” Log-rank test and Kaplan-Meier survival curves were used to compare progression-free survival (PFS) among different patient groups. A significance level of 0.05 was used. GraphPad Prism 7.04 software (San Diego, CA) was used for survival analysis.

**RESULTS**

**Clinicopathologic Characteristics of Patients**

Patients’ demographics, hrHPV status, clinical stages, and follow-up information are summarized in Table 1. A total of 120 cases of usual-type HPVA ECAs were included in the current study. The specimens included 94 hysterectomies, 4 cold knife cone excisions, and 22 loop electrosurgical excision procedures. Among them, 107/120 patients were clinically staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2018 system,<sup>29</sup> and 119/120 patients had complete follow-up information available.

Since commonly used HPV DNA tests (such as Hybrid Capture 2 assay [HC2] and Cobas 4800) and the HPV mRNA test (Aptima) have shown similar sensitivity in detecting hrHPV in liquid-based cytology specimens,<sup>30,31</sup> 120 ECA cases with positive hrHPV test by 1 of the 3 tests were included in this study. Then, the cases were further categorized into 3 genotype groups based on the hrHPV test results: group 16 (HPV16+), group 18 (HPV18 or HPV18/45+), and group O (other genotype+). Among them, 51 (42.5%) were positive for HPV16, 50 (41.7%) were positive for HPV18 or 18/45, 9 (7.5%) were positive for other hrHPV genotypes (not including HPV16/18/45). In addition, 10 were positive for HPV16 and/or HPV18 (HC2 test), which were excluded from statistical analysis among genotype groups because both HPV16 and 18 were detected simultaneously in HC2 test. All cases were p16 block positive.

**Correlation of HPV Genotypes With Clinical Stages, Invasion Patterns, and PFS of Patients With HPVA ECA**

As illustrated in Table 1, among 107 patients with clinical staging information, 45 (42.1%) were HPV16+, 43 (40.2%) were HPV18 or 18/45+, 9 (8.4%) were other genotypes+, and 10 (9.3%) were positive for either HPV16 or 18 (HC2 test). Overall, 8.9% (4/45) of HPV16+ patients had advanced clinical stages (FIGO II to IV). In all, 11.6% (5/43) of HPV18 or 18/45+ patients had advanced clinical stages. In all, 22.2% (2/9) of other genotypes+ patients had advanced clinical stages. No significant correlation of clinical-stage was found across 3 genotype groups (*P* = 0.51). Ten HPV16 and/or 18+ (HC2 test) patients were not included in statistical analysis, all of which had stage I tumors.

The PBCS classification was successfully applied to all 120 cases. Among 51 HPV16+ cases, 20 (39.2%) were classified as pattern A, 18 (35.3%) were classified as pattern B, and 13 (25.5%) were classified as pattern C. Among 50 HPV18 or 18/45+ cases, 16 (32.0%) were classified as pattern A, 16 (32.0%) were classified as pattern B, and 18 (36.0%) were classified as pattern C. Among 9 other genotypes+ cases, 3 (33.3%) were classified as pattern A, 2 (22.2%) were classified as pattern B, and 4 (44.4%) were classified as pattern C. No significant correlation of invasion patterns was found between HPV16+ and HPV18 or 18/45+ groups (*P* = 0.51). Overall, no significant correlation of invasion patterns was found across 3 genotype groups (*P* = 0.55). Ten HPV16 and/or 18+

**TABLE 1.** Clinicopathologic Information of Patients With HPVA ECA

Genotype Groups	Age, Median (Range)	Test Modules, n (%)			FIGO Stages, n (%)				Follow-up, n (%)			Total
		AHPV	Cobas	HC2	I	II-IV	NA	P	R	M	D	
16	47 (28-67)	37 (73)	14 (27)	0	41 (80)	4 (8)	6 (12)	0.51	2 (4)	2 (4)	0	51
18	45 (27-62)	34 (68)	16 (32)	0	38 (76)	5 (10)	7 (14)		3 (6)	0	2 (4)	50
O	58 (45-73)	9 (100)	0	0	7 (78)	2 (22)	0		0	0	0	9
16 or 18+	47 (37-57)	0	0	10 (100)	10	0	0	NA	0	0	0	10
Total												120

16 indicates HPV16+; 18, HPV18 or 18/45+; D, death; M, metastasis; NA, not available; O, other HPV genotypes+; R, recurrence.

**TABLE 2.** PD-L1 Expression in HPV A ECA in Various HPV Genotype Groups

HPV Genotype Groups	Invasion Patterns, n (%)			PD-L1 Expression, n (%)				Total
	A	B	C	TPS		CPS		
				+	-	+	-	
16	20 (39)	18 (35)	13 (26)	10 (20)	41 (80)	24 (47)	27 (53)	51
18	16 (32)	16 (32)	18 (36)	16 (32)	34 (68)	32 (64)	18 (36)	50
O	4 (40)	4 (40)	2 (20)	2 (22)	7 (78)	7 (78)	2 (22)	9
16 or 18+ (HC2 test)	3 (33)	2 (22)	4 (44)	2 (20)	8 (80)	3 (30)	7 (70)	10
Total								120
<i>P</i>	0.51 (16 vs. 18); 0.55 (across groups)			0.15 (16 vs. 18); 0.35 (across groups)		0.11 (16 vs. 18); 0.10 (across groups)		

16 indicates HPV16+; 18, HPV18 or 18/45+; O, other HPV genotypes+.

(HC2 test) cases were not included in statistical analysis. Among them, 4 (40.0%) were classified as pattern A, 4 (40.0%) were classified as pattern B, and 2 (20.0%) were classified as pattern C. Detailed analysis was summarized in Table 2.

Complete follow-up information was available for 119 patients. Median follow-up was 22 months (range, 3 to 72 mo). In total, 5.9% (7/119) of the patients were diagnosed with recurrent/late metastatic tumor, and 1.7% (2/119) had died of the disease (Table 1). No significant correlation of PFS were found between HPV16+ and HPV18 or 18/45+ patient groups ( $P=0.59$ , log-rank test), with median progression-free interval undefined in both groups. The adjusted hazard ratio (HR) for PFS was 0.62 (95% confidence interval [CI]: 0.11-3.62) for the HPV16+ group and 1.63 (95% CI: 0.28-9.55) for the HPV18/45+ group. Overall, no significant correlation of PFS was found across 3 genotype groups ( $P=0.74$ ; log-rank test) (Fig. 1A). Ten HPV16 and/or 18+ (HC2 test) patients were not included in survival analysis, none of which showed progression during the follow-up period. When survival analysis was performed separately for each invasion pattern group, patients with pattern C tumors showed significantly poorer PFS ( $P=0.03$ ) than patients with patterns A or B tumors (Fig. 1B).

### PD-L1 Expression in HPV A ECAs and its Correlation With hrHPV Genotypes

The PD-L1 expression in HPV A ECAs tends to be heterogenous and could be focal and patchy (Fig. 2). Overall, a relatively high prevalence of PD-L1 expression was observed in HPV A ECAs (25% by TPS and 55% by CPS). Using TPS, 19.6% (10/51) HPV16+ cases, 32.0% (16/50) cases of HPV18 or 18/45+ cases, and 22.2% (2/9) cases of other genotypes+ cases demonstrated PD-L1 positivity. No significant difference in PD-L1 expression was seen across genotype groups ( $P=0.35$ ). Using CPS, 47.1% (24/51) of HPV16+ cases, 64.0% (32/50) of HPV18 or 18/45+ cases, and 77.8% (7/9) of other genotypes+ cases demonstrated PD-L1 positivity. Similarly, no significant difference in PD-L1 expression was seen across genotype groups ( $P=0.10$ ). Ten HPV16 and/or 18+ (HC2 test) cases were not included in statistical analysis. Detailed analysis is summarized in Table 2.

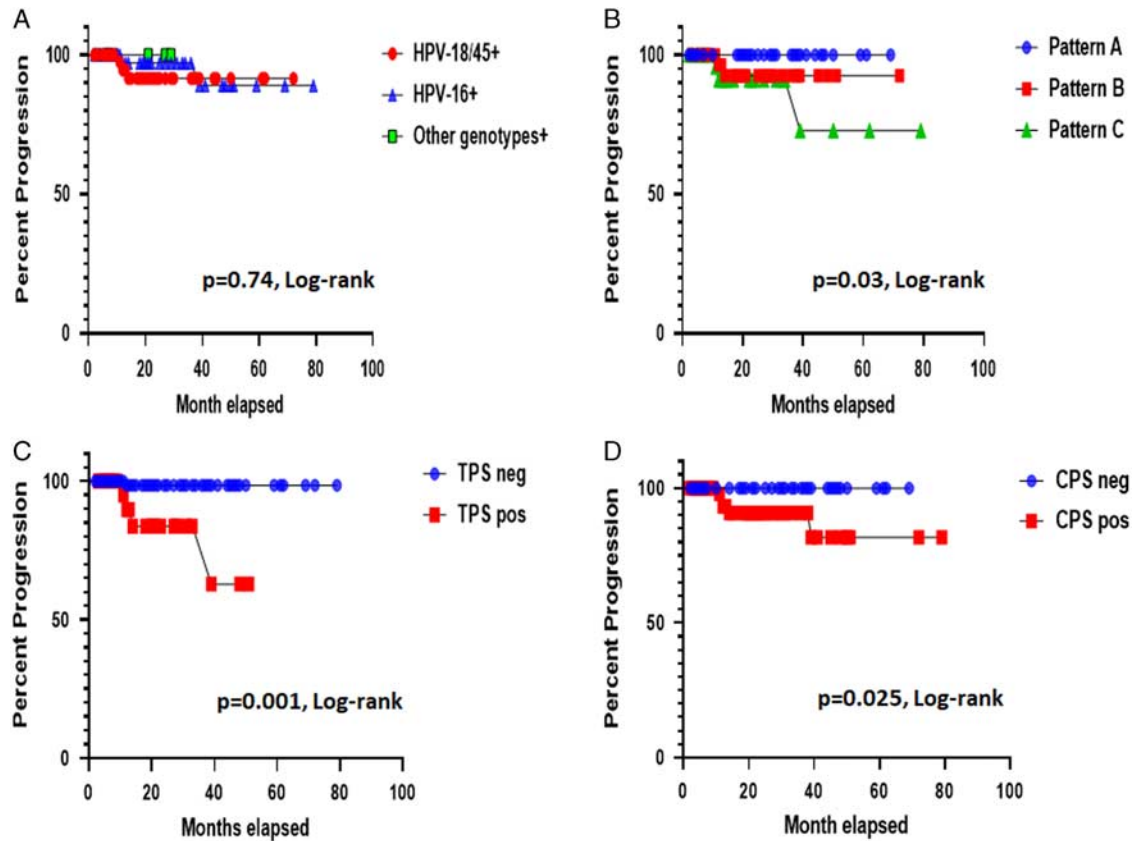
### Correlation of PD-L1 Expression With Invasion Patterns and PFS of Patients With HPV A ECA

The PD-L1 expression was analyzed based on invasion patterns. Using TPS, 2.3% (1/43) of tumors with pattern A, 32.5% (13/40) with pattern B, and 43.2% (16/37) with pattern C were PD-L1-positive. PD-L1 expression in tumors with patterns B and C was significantly higher than in those with pattern A ( $P=0.0002$  [A vs. B];  $<0.0001$  [A vs. C]; 0.00002 [A vs. B+C]). There was no significant difference in PD-L1 expression between tumors with patterns B and C ( $P=0.33$ ). Using CPS, 18.6% (8/43) of cases with pattern A, 77.5% (31/40) with pattern B, and 75.7% (28/37) with pattern C demonstrated PD-L1 positivity. Similarly, PD-L1 expression in tumors with patterns B and C was significantly higher than in those with pattern A ( $P<0.00001$  [A vs. B];  $<0.0001$  [A vs. C];  $<0.00001$  [A vs. B+C]). There was no significant difference in PD-L1 expression between tumors with patterns B and C ( $P=0.85$ ). Detailed PD-L1 analysis is illustrated in Table 3.

Using TPS, 25% (30/120) patients had PD-L1-positive tumors, while 55% (66/120) patients had PD-L1-positive tumors by CPS. Patients with PD-L1-positive tumors by either CPS or TPS showed significantly poorer PFS than those with PD-L1-negative tumors (CPS,  $P=0.025$ ; TPS,  $P=0.001$ ; log-rank test, with an undefined median progression-free interval in both PD-L1-positive and negative groups (Fig. 1C). Using TPS, the adjusted HR for PFS was 36.89 (95% CI: 4.23-321.4) for the PD-L1-positive group and 0.03 (95% CI: 0.003-0.24) for the PD-L1-negative group. Using CPS, the adjusted HR for PFS was 7.48 (95% CI: 1.29-43.48) for the PD-L1-positive group and 0.13 (95% CI: 0.02-0.78) for the PD-L1-negative group (Fig. 1D).

### DISCUSSION

The prevalence of hrHPV genotypes varies among different histologic subtypes of cervical cancers. HPV16 is the most frequent genotype in SCCs,<sup>32</sup> while HPV18 is often but not always the most frequent genotype in ECAs.<sup>22,32</sup> Moreover, HPV A and HPV I ECAs demonstrate different clinical behaviors.<sup>7,11</sup> Despite the well-established role of hrHPV genotypes in the carcinogenesis of cervical cancers,<sup>22</sup> the prognostic values of various hrHPV genotypes (especially HPV16, 18, and 45) have not



**FIGURE 1.** Kaplan-Meier curves of PFS in patients with HPVA ECA. A, PFS in patients with HPVA ECA from different HPV genotype groups (red circle: group 18; blue triangle: group 16; green square: group O). B, PFS in patients with HPVA ECA with 3 different invasion patterns (blue circle: pattern A tumors; red square: pattern B tumors; green triangle: pattern C tumors). C, PFS in PD-L1-positive group (red) and PD-L1-negative group (blue) using TPS (cutoff 1%). D, PFS in PD-L1-positive group (red) and PD-L1-negative group (blue) using CPS (cutoff 1).

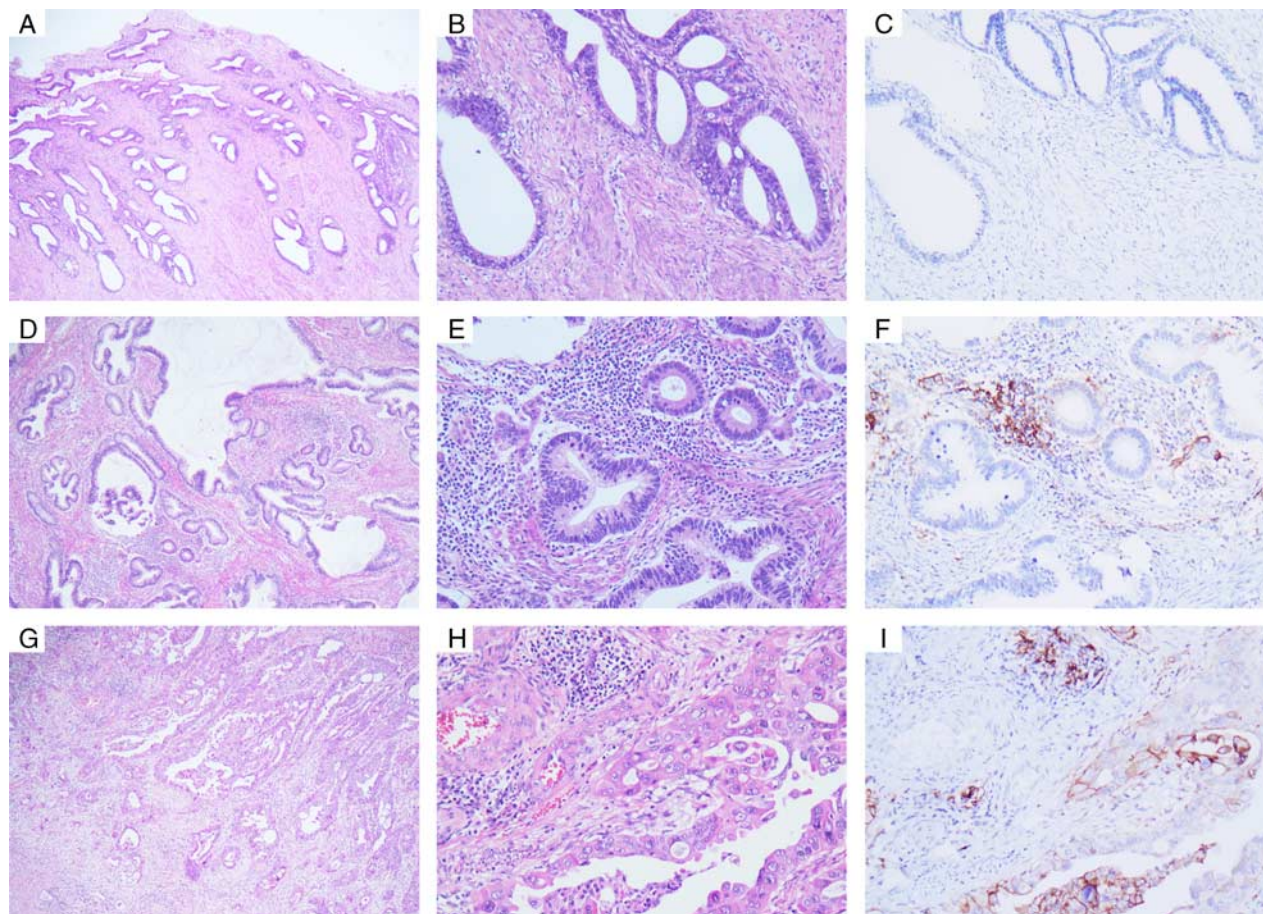
been well-established. Previously published data have not yielded definitive clarity on the topic. Some studies reported favorable outcomes for cervical cancers with HPV16 and/or 18 positivity.<sup>33–38</sup> In contrast, some studies suggested unfavorable outcomes for HPV16/18 positive cervical cancers.<sup>39–50</sup> Moreover, some studies reported no prognostic value for hrHPV genotypes.<sup>51–54</sup> While many factors may contribute to those contradicting results, not separating SCCs and ECAs in those studies may play an important role. Only one study focused mainly on ECAs<sup>36</sup>; however, it did not separate HPVA and HPV16 ECAs. Considering the significant difference in clinical behaviors between SCCs and ECAs, HPVA, and HPV16 ECAs, all these studies carry an intrinsic bias. Therefore, it may be optimal to investigate the prognostic values of hrHPV genotypes based on the histologic types and etiologies of cervical cancers.

In this study, we investigated the prognostic values of hrHPV genotypes in patients with usual-type HPVA ECAs and their association with other known prognostic factors of ECAs. Our data showed that patients had no significant difference in both clinical stages and PFS across hrHPV genotype groups. In addition, no correlation was found between hrHPV genotypes and other known prognostic factors including invasion patterns and PD-L1

expression. Altogether, our data support that hrHPV genotypes have no prognostic value in HPVA ECAs.

As one of the most important immune checkpoints, several studies have investigated PD-L1 prevalence in cervical cancers.<sup>21,55–64</sup> Studies have demonstrated a significant correlation between HPV infection and increased PD-L1 expression in cervical SCC and cervical intraepithelial neoplasia.<sup>60,61,63,65</sup> Although the underlying mechanism is not fully understood, those studies suggested impaired immune surveillance by the upregulated PD-L1 expression caused by HPV infection plays a crucial role in the SCC/cervical intraepithelial neoplasia development. Most recently, a study by Rivera-Colon et al<sup>21</sup> suggested that PD-L1 may be an unfavorable prognostic marker for ECAs. Similar to that study, our data demonstrated a high prevalence of PD-L1 in usual-type HPVA ECAs (25% by TPS and 55% by CPS). We also investigated the association among HPV genotypes, the Silva PBCS, and PDL-1 expression in HPVA ECAs. No significant correlation was found between hrHPV genotypes and patterns of invasion. Similarly, no significant difference in PD-L1 expression was found across hrHPV genotype groups. On the other hand, our data showed: (1) enhanced PD-L1 expression in destructive invasion patterns (patterns B and C) compared





**FIGURE 2.** Examples of HPVA ECA with different patterns of invasion and corresponding PD-L1 immunostain. A–C, Pattern A tumor. A, Hematoxylin and eosin (H&E) at low magnification shows well-formed glands with smooth contours and absence of destructive-type invasion or stromal desmoplasia. B, Malignant glands with rounded contours and absence of destructive invasion at medium power (H&E). C, Corresponding negative PD-L1. D–F, Pattern B tumor with corresponding PD-L1. D, Predominantly rounded glands with a few foci of early destructive-type invasion and desmoplastic stromal response (H&E). E, A gland with a focus of early destructive invasion, stromal desmoplasia and adjacent inflammatory infiltrate from the same case (H&E). F, Corresponding PD-L1 showing expression in both tumor cells and immune cells. G–I, Pattern C tumor with corresponding PD-L1. G, Invasive tumor with diffusely destructive invasion pattern (H&E). H, Irregularly shaped, angulated glands with destructive-type invasion (H&E). I, Corresponding PD-L1 with high expression in both tumor cells and immune cells.

with tumors with nondestructive invasion (pattern A); (2) PD-L1 expression inversely correlated with PFS of patients. Our study supports that PD-L1 is an unfavorable

prognostic marker for patients with HPVA ECA. PD-L1 expression may reflect a tumor defense strategy against immune attack triggered by compensatory upregulation of PD-L1 through negative feedback or an adaptive immune resistance.<sup>66,67</sup> The overall clinical outcome often depends on the balance between tumor-induced immune response and immune inhibitory machineries such as programmed cell death protein 1/PD-L1 interaction. Since a parallel increase in CD8<sup>+</sup> tumor-infiltrative lymphocytes and PD-L1 expression has been reported in ECAs,<sup>21</sup> the above-mentioned balance is likely leaning to immune resistance which resulted in more aggressive tumor behavior.

The role of HPV in cervical cancer carcinogenesis has been well-established. The initial step appears to be the integration of HPV into the host genome, which leads to genomic instability, accumulation of somatic mutations.<sup>68–72</sup> In addition, HPV E6 and E7-oncogene proteins can respectively inhibit p53 and RB functions.<sup>73,74</sup> The Cancer

**TABLE 3.** PD-L1 Expression in ECA With Various Invasion Patterns

Diagnosis	PD-L1 Expression, n (%)				Total
	TPS		CPS		
	+	-	+	-	
Pattern A	1 (2.3)	42 (97.7)	8 (18.6)	35 (81.4)	43
Pattern B	13 (32.5)	27 (67.5)	31 (77.5)	9 (22.5)	40
Pattern C	16 (43.2)	21 (56.8)	28 (75.7)	9 (24.3)	37
Total					120
P	0.0002 (A vs. B); <0.0001 (A vs. C); 0.00002 (A vs. B+C); 0.33 (B vs. C)		<0.00001 (A vs. B); <0.0001 (A vs. C); <0.00001 (A vs. B+C); 0.85 (B vs. C)		

Genome Atlas (TCGA) study demonstrated a difference in genomic integration prevalence and rate of gene silencing between HPV16 and HPV18 positive cervical cancers.<sup>72</sup> Our data suggest that instead of pathogenic HPV genotype, the global genomic alteration of the tumor determine the clinical behavior of HPV ECA.

In comparison with previous studies, our study has several strengths. First, our study focused only on patients with HPV ECA to eliminate the possible bias arising from including various histologic and etiological entities into one study. Second, this is a comprehensive study regarding the prognostic significance of hrHPV genotypes and their association with other known prognostic factors including clinical stages, patterns of invasion, and PD-L1 expression. Third, we also investigated the prevalence of PD-L1 expression and its association with hrHPV genotypes and patterns of invasion in HPV ECA. Our study also has limitations, notably that only 9 cases in other genotypes group were included. Therefore, it is uncertain whether there is significant clinical behavior in this group in a large cohort. Second, group 18 includes HPV18 and/or HPV45 (both belong to clade alpha-7). Due to the limitation of the test, we were unable to analyze HPV18 and HPV45 separately. Third, although HPV testing on the most recent Pap specimen (within 3 mo of the procedures) may be a good indicator of the tumor HPV status, ideally, polymerase chain reaction–based testing on the tumor itself is the most accurate indicator. However, such testing is not validated and thus unavailable in our clinical laboratory. Future studies include: (1) next-generation sequencing to investigate the genomic profiles and HPV status of tumors; (2) HPV in situ hybridization on a large cohort to validate our findings.

In summary, our findings suggest that hrHPV genotypes have no prognostic value in patients with HPV ECA. Furthermore, no significant correlation is identified between hrHPV genotypes and other prognostic factors including clinical stages, the pattern of invasion, and tumor PD-L1 expression. Finally, our data demonstrate a relatively high prevalence of PD-L1 expression in HPV ECA, especially in tumors with destructive invasion patterns (patterns B and C). Positive PD-L1 expression is inversely correlated with the PFS of patients. Our data support PD-L1 as a potential therapeutic target and as a negative prognostic marker for patients with HPV ECA.

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