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Clinical Investigation

The Prognostic Significance of p16 Status in Patients With Vulvar Cancer Treated With Vulvectomy and Adjuvant Radiation

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Summary

This is a retrospective analysis from a single institution examining 39 women with vulvar squamous cell carcinoma treated with adjuvant radiation therapy. This study notes how outcomes differed according to p16 status, which was measured through immunohistochemistry. We observed fewer in-field relapses and improved progression-free survival in women whose tumors were

Purpose: Vulvar squamous cell carcinoma (VSCC) is a relatively rare malignancy. Human papillomavirus has been implicated as a causative factor for a subset of these patients. The purpose of this study was to evaluate whether p16-positivity (a human papillomavirus surrogate) predicts for better response rates in women who undergo surgery followed by adjuvant radiation therapy (RT).

Methods and Materials: We retrospectively analyzed data from women with VSCC who were treated with adjuvant RT. p16-Positivity was defined as diffuse strong immunoreactivity within the tumor. Time to event outcomes was performed with Kaplan-Meier and cumulative incidence methodologies.

Results: Thirty-nine women were identified. Ten had positive results for p16 (p16+), and 29 had negative results (p16−). The median follow-up was 25.7 months. The median age at diagnosis was 59 years for women with p16+ tumors and 74 years for women with p16− tumors ($P = .022$). The distribution of stage did not differ by p16 status. The indications for adjuvant RT were close/positive margins in 19 women, positive nodes in 9 women, and both in 11 women. There were 21 recurrences: 15 vulvar, 3 isolated nodal, 2 synchronous vulvar/nodal, and 1 distant metastasis. In-

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p16 positive. No difference in overall survival was found between groups. This is the largest cohort of patients with known p16 status treated with adjuvant radiation therapy.

field relapse rates at 3 years were lower in p16+ patients (32.5%) than in p16− patients (59.1%, $P = .072$). This trend was also observed in progression-free survival ($P = .062$). A p16+ status and a lower International Federation of Gynecology and Obstetrics stage were associated with fewer in-field relapses and improved progression-free survival in multivariable analyses. The p16 status was not a predictor of overall survival.

Conclusions: p16-Positivity appears to be a prognostic factor for in-field relapse rates in patients with VSCC appropriately treated with adjuvant RT.

Introduction

Vulvar cancer is a relatively rare disease, accounting for approximately 6% of gynecologic malignancies in the United States—an incidence of 6020 in 2017.[1] Risk factors for developing vulvar squamous cell carcinoma (VSCC) typically include older age, lichen sclerosus, squamous hyperplasia (hyperplastic dystrophy), smoking, and the human papillomavirus (HPV) infection. Most patients are in their seventh decade of life, although it has been observed that younger patients more commonly have HPV-associated VSCC than their older counterparts, whose disease is associated more with inflammation and lichen sclerosus.[2,3] A growing body of evidence suggests that HPV positivity might be linked to improved outcomes; unfortunately, this relationship is not as well established as it is in HPV-related oropharyngeal cancers.[4-6]

Currently, treatment recommendations largely depend on the stage of the disease at presentation. Women with International Federation of Gynecology and Obstetrics (FIGO) 2009 stage I to II disease are initially managed surgically with either wide local excision (for stage IA) or radical local resection/modified radical vulvectomy with inguinal sentinel lymph node (LN) biopsy (for stage IB-II). The decision to include adjuvant radiation therapy (RT) depends on several factors, including surgical margins and LN status.[3,7-9] Unlike in oropharyngeal cancers, HPV status has yet to be incorporated to guide treatment paradigms.[10]

The purpose of this retrospective, single-institution study is to assess how HPV status, measured using p16 expression as a surrogate marker, affects outcomes of women with vulvar cancer treated with adjuvant RT for close/positive margins or involved LNs.

Methods and Materials

Patient population

A clinical database of 451 women with VSCC treated with radical vulvectomy alone or neoadjuvant/definitive/adjuvant RT at our institution from 1991 to 2016 was created. Tissue availability was queried with the goal of evaluation of p16 status. For patients with available tissue specimens, clinical and treatment data were collected. Thirty-nine women were identified who had undergone radical vulvectomy followed by adjuvant external beam RT with available tissue specimens and sufficient clinical data (with treatments done between 2004 and 2016).

Pathologic staining

Available tissue blocks were stained with p16 antibody (clone E6H4, predilute, Ventana Medical Systems, Inc, Tucson, AZ) on whole tissue sections and evaluated by 2 pathologists. Only diffuse strong p16 nuclear and cytoplasmic expression was scored as a positive result (p16+). A negative p16 result (p16−) included no reactivity or weak to moderate patchy reactivity.

The p16 nuclear expression and cytoplasmic expression were scored using a histochemical or H-score—like method in which the percentage of cell staining was recorded for each intensity level (0, no staining; 1, weak staining intensity; 2, moderate staining intensity; or 3, strong intensity staining). The percentage of cells staining for each intensity level was then multiplied by the intensity level. The resulting values for each intensity level were added together to range from 0 (no staining) to 300 (diffuse strong staining) (Figs. E1A, E1B, and E1C; available online at https://dx.doi.org/10.1016/j.ijrobp.2018.08.014.) An H-score of 200 (indicative of strong staining in at least two thirds of the tumor) or higher was considered a positive result. The H-score was used because it applies a quantitative measure that allows for more objectivity in the assessment of the specimen.

Statistical methods

SPSS version 24 (IBM, Armonk, NY) and R, version 3.4.4 (including packages "survival" [version 2.41-3] for Kaplan-Meier [KM] and Cox proportional hazard analyses) were used for analyses. X^2 analysis/Fisher exact test and independent *t* test/Mann-Whitney tests were used as appropriate to assess correlations between staining results and pathologic, clinical, and treatment characteristics. Disease endpoints—including progression-free survival (PFS) and overall survival (OS)—were defined as the time from diagnosis to failure or death and calculated using the KM method, with comparisons made using log-rank tests only for p16 status. A cumulative incidence model was also

Table 1 Baseline characteristics of women within cohort

	Total (n = 39)	p16+ (n = 10, 26%)	p16− (n = 29, 74%)	*P* value
Median age at diagnosis (y)	71	59	74	.022
History of prior vulvar cancer	7	2 (20%)	5 (17%)	.644
History of lichen sclerosis	12 (31%)	2 (20%)	10 (34%)	1
FIGO clinical stage 2009				.731
Stage I	27 (69%)	6 (60%)	21 (72%)	
Stage II	7 (18%)	2 (20%)	5 (17%)	
Stage III	5 (13%)	2 (20%)	3 (10%)	
Grade				.603
1	16 (41%)	3 (30%)	13 (45%)	
2	18 (46%)	5 (50%)	13 (45%)	
3	5 (13%)	2 (20%)	3 (10%)	
Median clinical tumor size (cm)	3	3.25	3	.582
Pathologic stage				.488
I	14 (36%)	3 (30%)	11 (38%)	
II	3 (8%)	1 (10%)	2 (7%)	
III	21 (54%)	5 (50%)	16 (55%)	
IVA	1 (3%)	1 (10%)	0 (0%)	
Pathologic T stage	29 (74%)	8 (80%)	21 (72%)	.41
I	8 (21%)	1 (10%)	7 (24%)	
II	2 (6%)	1 (10%)	1 (3%)	
III				
Pathologic N stage				.870
0	8 (21%)	3 (30%)	5 (17%)	
I	9 (23%)	2 (20%)	7 (24%)	
II	11 (28%)	3 (30%)	8 (28%)	
Unknown	11 (28%)	2 (20%)	9 (31%)	
Median pathologic tumor size (cm)	3	1.7	3.2	.031
Median depth of invasion (mm)	7.5	7.5	7.5	.895
Median margin (mm)	2	0.6	2	.322
Margin				
Positive	10 (26%)	3 (30%)	7 (24%)	.696
Close (<8 mm)	20 (51%)	5 (50%)	14 (48%)	.302
Node positive	20	5 (50%)	15 (52%)	1
Median number LN dissected	4.5	2.5	5	.453
Median size largest LN (cm)	0.6	1.1	0.5	.696
Extracapsular extension				1
Present	6 (15%)	1 (10%)	5 (17%)	
Absent	30 (77%)	8 (80%)	22 (76%)	
Unknown	3 (8%)	1 (10%)	2 (7%)	
LVSI				1
Absent	27 (69%)	6 (60%)	21 (72%)	
Present	7 (18%)	2 (20%)	5 (17%)	
Unknown	5 (13%)	2 (20%)	3 (11%)	
Median vulva dose (Gy, IQR)	54.9	54.9	54.9	.861
Margin				
Positive	55.0 (46.4-59.4)	54.0 (48.6-58.2)	56.0 (47.7-59.4)	.945
Close (<8 mm)	55.9 (54.0-59.0)	56.4 (55.8-57.6)	55.8 (52.5-59.5)	.882
Negative (5 patients, 0 vs 5)	50.4 (50.0-55.8)	NA	50.4 (50.0-55.8)	NA
LVSI				
Absent	55.9 (54.0-59.5)	56.1 (54.5-59.4)	55.9 (53.1-59.4)	.890
Present	54.0 (47.7-58.5)	55.8 (54.9-56.7)	50.4 (45.0-59.4)	.335
Median groin/nodes dose (Gy, IQR)	50.4	50.4	51.9	.066
Nodal status				
0 nodes (2 patients, 1 vs 1)	56.8 (56.4-57.2)	57.6 (57.6-57.6)	56.0 (56.0-56.0)	NA
1 node (10 patients, 2 vs 8)	50.4 (50.1-55.9)	47.7 (46.4-49.1)	52.9 (50.3-56.9)	.198
>1 nodes (8 patients, 1 vs 7)	51.2 (50.4-55.8)	43.2 (43.2-43.2)	51.9 (50.4-55.8)	NA

(*continued on next page*)

Table 1 (*continued*)

	Total (n = 39)	p16+ (n = 10, 26%)	p16− (n = 29, 74%)	*P* value
ECE				
Present	51.2 (50.4-54.5)	50.4 (50.4-50.4)	51.9 (50.4-55.4)	NA
Absence	50.4 (48.8-56.0)	45.0 (44.1-51.3)	50.4 (50.4-56.0)	.474
Use of chemotherapy				1
2 cycles	5 (13%)	2 (20%)	3 (10%)	
4 cycles	1 (3%)	0 (0%)	1 (3%)	
6 cycles	1 (3%)	0 (0%)	1 (3%)	
Unspecified*	5 (13%)	2 (20%)	3 (10%)	
RT alone	27 (69%)	6 (60%)	20 (70%)	

Abbreviations: ECE = extracapsular extension; FIGO = International Federation of Gynecology and Obstetrics; IQR = interquartile range; LN = lymph node; LVSI = lymphovascular space invasion; NA = not applicable; RT = radiation therapy.

* Unspecified chemotherapy regimens were regimens administered to treat the patient's vulvar cancer, but the type of chemotherapy was not found on record.

constructed (using the R package cmprsk 2.2-7) to estimate in-field relapse (IFR) rates.

Patients alive but without relapse were censored at the date of last follow-up. Patients who relapsed outside the treatment field were censored at the time of relapse. Predictors of outcomes were obtained using Cox proportional hazards methods for univariable analyses. Factors with $P < .15$ were included in a multivariable Cox proportional hazards model. Models were then adjusted for FIGO stage and age.

Results

Patient and treatment characteristics

Ten women (26%) had p16+ results, and 29 women (74%) had p16− results. The median age at diagnosis of the entire cohort of 39 patients was 71 years (interquartile range, 57-79): 59 years for p16+ patients and 74 years for p16− patients ($P = .022$). The median follow-up time was 25.7 months (interquartile range, 14.0-46.3 months), with no difference between the 2 groups. Baseline characteristics—history of lichen sclerosus, FIGO stage, grade, pathologic data including staging—are summarized in Table 1. The p16− patients tended to be older and to have larger pathologically measured tumors (3.4 cm vs 2.1 cm; $P = .031$).

Thirty women received both vulvar and groin radiation. Nine women were treated with vulvar-only radiation—all had pathologically node-negative disease. The median dose to the vulva was approximately 55 Gy in both groups; the median dose to the groin LNs was 50 Gy for p16+ patients and 52 Gy for p16− patients. If patients received chemotherapy, they most commonly received cisplatin (n = 11), although 1 patient received cisplatin and 5-fluorouracil. Two women received an unspecified regimen of chemotherapy.

Indications for adjuvant RT were associated with node and margin status. Eleven of 39 women (28%) had node-positive disease with either close margins (<8 mm; 7 patients) or positive margins (in 4 patients). Thirteen women (13%) had only close margins (<8 mm), and 6 (15%) had positive margins. Six women (15%) had positive nodes but negative margins. Three women (8%) had positive nodes but unknown margin status. The margin status was similar between the 2 groups ($P = .352$), as was nodal status (Table 1).

Recurrences and prognostic factors

Total recurrences occurred in 21 of 39 women (54%). Fifteen recurrences were vulvar-only (1 p16+ and 14 p16−). Three were isolated nodal (2 groin LN and 1 lower pelvic LN: 2 p16− and 1 p16+, respectively), 2 were synchronous vulvar/LN (1 p16+ and 1 p16−), and 1 was a distant metastasis (skin metastasis, p16−).

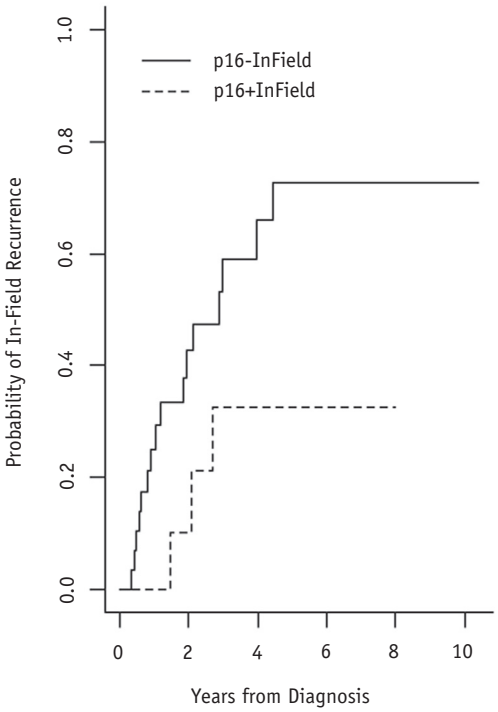

Fig. 1. In-field relapse rates differentiated by p16 status.

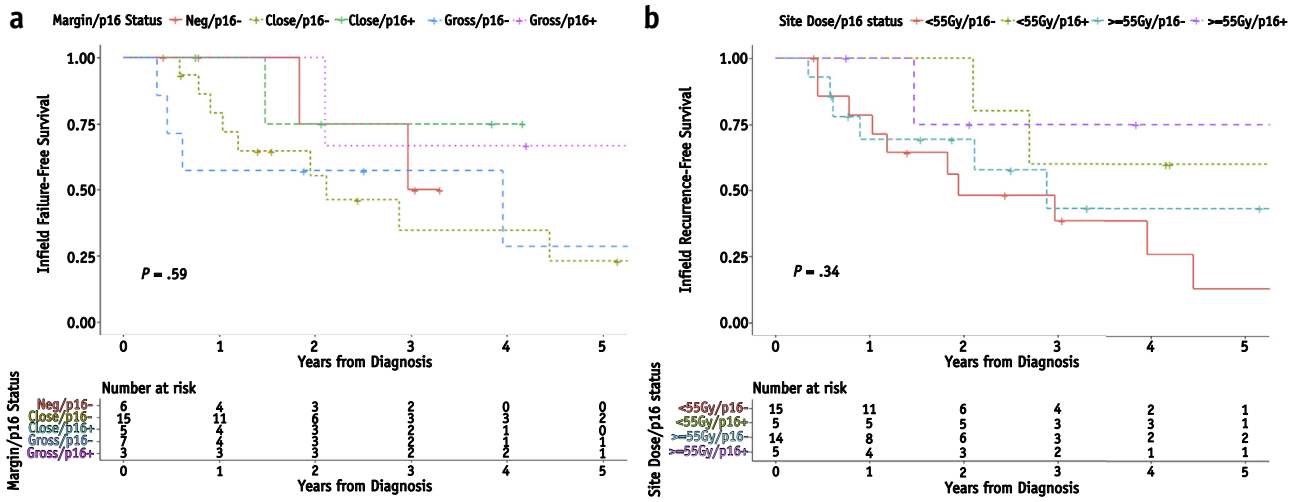

Fig. 2. (a) In-field recurrence-free survival stratified by margin/p16 status. (b) In-field recurrence-free survival stratified by prescribed radiation dose/p16 status.

A subset of 19 patients had IFRs. Fifteen were vulvar only (1 was p16+ and 14 were p16−), 2 were isolated nodal (1 groin LN was p16− and 1 lower pelvic LN was p16+), and 2 were synchronous vulvar/LN (1 p16+ and 1 p16−).

IFR rates at 3 years were the same (32.5%) for p16+ and p16− patients; 5-year rates were 59.1% for p16+ patients and 72.7% for p16− patients (hazard ratio [HR], 0.34; 95% confidence interval [CI], 0.11-1.10; Gray's $P = .072$, Fig. 1). Older age at diagnosis was the only predictor for more frequent IFRs on univariable analysis (HR, 1.04; 95% CI, 1.00-1.09; $P = .031$). On multivariable analysis, p16+ status and FIGO stage I disease were predictors of less frequent IFRs (HR, 0.05; 95% CI, 0.00-0.70; $P = .026$; stage III HR, 45.8; 95% CI, 4.48-468; $P = .001$). IFR-free survival was further investigated by stratifying groups by margin status/p16 status (Fig. 2a, $P = .59$) and dose at site of failure/p16 status (Fig. 2b, $P = .34$).

Three-year PFS rates were 63.5% (95% CI, 37.7%-100%) for p16+ patients and 36.8% (95% CI, 21.5%-63.0%) for p16− patients (log-rank $P = .062$, Fig. 3). Five-year rates were 63.5% (95% CI, 37.7%-100%) for p16+ patients and 22.1% (95% CI, 9.03%-54.0%) for p16− patients. On univariable analysis, older age at diagnosis was the only predictor for worse PFS (HR, 1.05; 95% CI, 1.01-1.09; $P = .021$). A p16+ status and a lower FIGO stage were predictors of improved PFS on multivariable analysis (HR, 0.05; 95% CI, 0.00-0.65; $P = .022$; stage III HR, 44.7; 95% CI, 4.39-455; $P = .001$). These results can be seen in Tables 2 and 3.

OS and prognostic factors

The median OS was 4.39 years. The 3- and 5-year OS rates were 61.2% (95% CI, 47.1%-79.7%) and 42.0% (95% CI, 27.4%-64.5%) for all patients, respectively. OS was not different when segregated by p16 status (Fig. 4). Three-year OS rates for p16+ and p16− patients were 77.8% (95% CI, 54.9%-100%) and 55.5% (95% CI, 39.3%-78.4%), respectively. The p16+ and p16− associated 5-year OS rates were 33.3% (95% CI, 11.3%-100%) and 45.2% (95% CI, 28.9%-70.9%), respectively.

On univariable analysis (Table 2), older age at diagnosis predicted for worse OS (HR, 1.05; 95% CI, 1.01-1.10; $P = .011$). On multivariable analysis, FIGO stage (stage III HR, 7.64; 95% CI, 1.30-44.9; $P = .024$) and extracapsular extension (HR, 5.72; 95% CI, 1.00-32.6; $P = .049$) were the only statistically significant factors predicting worse OS (Table 3).

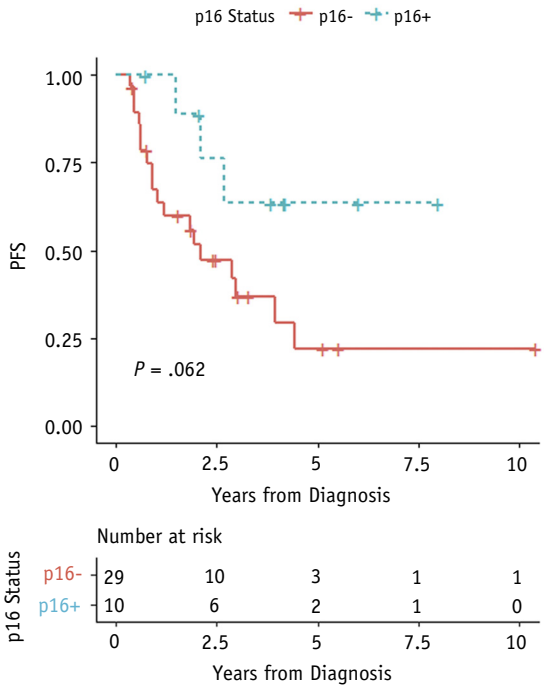

Fig. 3. Progression-free survival (PFS) differentiated by p16 status.

Table 2 Cox proportional hazards univariable analyses

Univariable	In-Field HR (95% CI)	*P*	PFS HR (95% CI)	*P*	OS HR (95% CI)	*P*
Age at diagnosis	1.04 (1.00-1.09)	**.031**	1.05 (1.01-1.09)	**.021**	1.05 (1.01-1.10)	**.011**
FIGO stage 2009						
Stage I	Reference		Reference		Reference	
Stage II	0.72 (0.16-3.21)	.663	0.61 (0.14-2.70)	.516	1.36 (0.44-4.19)	.587
Stage III	2.63 (0.85-8.14)	.093	2.33 (0.77-7.07)	0.135	2.11 (0.69-6.52)	.193
p16 status						
Negative	Reference		Reference		Reference	
Positive	0.37 (0.11-1.27)	.114	0.33 (0.10-1.12)	0.076	0.65 (0.24-1.76)	.397
Pathologic tumor size (cm)	1.18 (0.87-1.60)	.300	1.17 (0.88-1.57)	0.291	1.31 (0.95-1.79)	.097
Size largest LN (cm)	0.51 (0.07-3.82)	.508	1.67 (0.49-5.65)	0.413	2.20 (0.86-5.58)	.098
Extracapsular extension						
Absence	Reference		Reference		Reference	
Presence	1.02 (0.21-4.87)	.985	1.46 (0.38-5.64)	0.579	2.54 (0.82-7.87)	.107
LVSI						
Absence	Reference		Reference		Reference	
Presence	0.19 (0.25-1.46)	.111	0.18 (0.02-1.40)	0.102	0.42 (0.10-1.81)	.242

Abbreviations: CI = confidence interval; FIGO = International Federation of Gynecology and Obstetrics; HR = hazard ratio; LN = lymph node; LVSI = lymphovascular space invasion; OS = overall survival; PFS = progression-free survival.
Bolded *P* values considered significant with threshold of $P < 0.05$.

Discussion

Vulvar cancer is a rare and heterogeneous disease affecting an increasing number of women in the United States. There are 2 types of vulvar cancer, squamous cell and nonsquamous cell, with VSCC accounting for more than 90% of diagnoses.[11] VSCC develops through one of 2 pathways, an HPV-associated pathway or a non−HPV-associated pathway. The HPV-independent VSCCs are normally seen in older women in their 60s and 70s and manifest from areas of chronic skin irritation, as in lichen sclerosus. HPV-dependent VSCCs have been repeatedly noted to affect younger women. The proportion of HPV-associated VSCCs has varied among studies, but a recent meta-analysis calculated that roughly 40% of all VSCCs arise from HPV—concordant with our cohort, in which 26% were p16+.[11]

Several studies have reviewed the prognostic significance of p16/HPV status in vulvar cancer (Table 4); yet, consensus establishing its importance, as is seen in oropharyngeal or anal cancers, is lacking.[10,12-22] Recent

Table 3 Cox proportional hazards multivariable analyses

Multivariable	In-Field HR (95% CI)	*P*	PFS HR (95% CI)	*P*	OS HR (95% CI)	*P*
Age at Diagnosis	1.04 (0.99-1.10)	.153	1.04 (0.99-1.10)	0.139	1.04 (0.96-1.12)	0.317
FIGO stage 2009						
Stage I	Reference		Reference		Reference	
Stage II	0.53 (0.10-2.87)	.462	0.49 (0.09-2.57)	. 398	0.33 (0.05-2.01)	.227
Stage III	45.8 (4.48-468)	**.001**	44.7 (4.39-455)	**.001**	7.64 (1.30-44.9)	**.024**
p16 status						
Negative	Reference		Reference		-	-
Positive	0.05 (0.00-0.70)	**.026**	0.05 (0.00-0.65)	**. 022**	-	-
Pathologic tumor size (cm)	-	-	-	-	1.23 (0.83-2.01)	. 296
Size largest LN (cm)	-	-	-	-	-	-
Extracapsular extension						
Absence	-	-	-	-	Reference	
Presence	-	-	-	-	5.72 (1.00-32.6)	**.049**
LVSI						
Absence	Reference		Reference		-	-
Presence	0.22 (0.03-1.74)	.151	0.21 (0.03-1.68)	.141	-	-

Abbreviations: CI = confidence interval; FIGO = International Federation of Gynecology and Obstetrics; HR = hazard ratio; LN = lymph node; LVSI = lymphovascular space invasion; OS = overall survival; PFS = progression-free survival.
Bolded *P* values considered significant with threshold of $P < 0.05$.

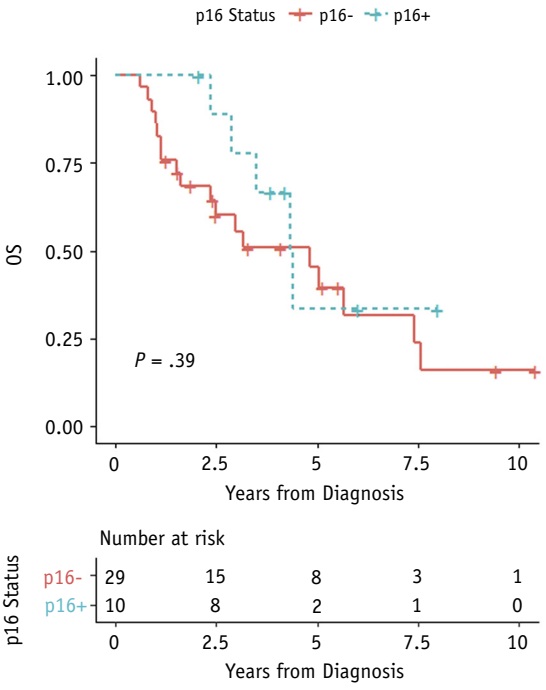

Fig. 4. Overall survival (OS) differentiated by p16 status.

articles exploring its significance for women receiving radiation have noted improved outcomes in p16+ patients.[4,5,16] Yet, of these studies, only a few published studies have cohorts that exclusively include patients receiving RT, and these studies include patients receiving a mixture of preoperative RT, postoperative RT, or definitive RT. Our retrospective, single-institution study evaluated patients receiving adjuvant RT and observed improved locoregional outcomes in women whose tumors were p16+ compared to women with p16− tumors (IFR, 32.5% vs 59.1%).

To the authors' knowledge, there are only 2 other published articles evaluating the locoregional relapse/IFR rates after radiation: the studies by Yap et al and Lee et al.[4,5] Yap et al conducted a retrospective analysis of 40 patients treated with preoperative RT, postoperative RT, or RT alone (postoperative RT, n = 26). They noted 5-year locoregional relapse—primary site or inguinal/lower pelvic LN relapse—rates of 15.4% and 81.2% for p16+ and p16− tumors, respectively (Gray's test, $P = .003$). Lee et al conducted a retrospective analysis of 57 patients treated with preoperative RT, postoperative RT, or definitive RT (postoperative RT, n = 28); they observed similar IFR—vulvar or regional LN relapse or both—with rates of 19% and 75% at 5 years, respectively ($P < .01$). We also observed similar IFR rates at 5 years for p16+ patients (32.5%) and p16− patients (70.5%), with the trend of fewer relapses for p16+ patients. Reasons for improved outcomes in p16+ vulvar cancers have not been well studied, and therefore the mechanisms are unclear. With extrapolation from data from oropharyngeal squamous cell carcinoma, it is likely that these tumors are more radiosensitive.[6,23,24]

Although our analysis does suggest improved IFR/PFS in p16+ patients, this benefit did not translate into increased OS, as appreciated in the similar and overlapping KM survival curves in Figure 4. In fact, the relationship between p16 status and OS has been difficult to establish in general because several studies have reported contradictory results.[4,5,12,13,25] Even the 2 recent studies whose entire cohort received RT differ. However, given our small sample size and patients being salvaged after local relapse, we cannot exclude any potential survival benefit for patients treated in the adjuvant setting.

One limitation resulting from the retrospective nature of this study was our inability to distinguish between a true relapse of the treated index lesion versus an occurrence of a new primary vulvar lesion. Distinguishing between them is important because there might be correlations between relapse type and p16 status that could help guide interventions. For example, if the index lesion recurred, then opportunities for radiation dose escalation or concurrent chemotherapy could be explored. Dose escalation (≥56 Gy) has already been suggested to be beneficial in patients with positive margins; yet, it is possible that even further dose escalation could be achieved.[26] Within our cohort, along with others, the rate of IFR is quite high—at times, higher than for patients receiving adjuvant head and neck radiation. One consideration for this phenomenon is that patients with vulvar cancer are being locally underdosed compared with patients with head and neck cancer (a median of 55 Gy in this cohort vs 60+ Gy in patients with head and neck cancer). This is not the only consideration, however. Patients with head and neck cancer at times receive concurrent chemotherapy. Although 2 National Cancer Database studies demonstrate the benefits of adjuvant chemoradiation in vulvar cancer, it was only in patients with positive LNs—possibly only those with 2+ positive LNs.[27,28] Unfortunately, there are no prospective data on these matters.

Our study has additional limitations as well, including those normally affecting retrospective analyses: selection bias and incomplete data. More specific to this study, we evaluated HPV status only through p16 immunohistochemical staining and did not evaluate for HPV DNA. Multiple studies have shown high concordance with p16 and HPV status.[4,29]

Our most significant limitation, however, stems from our small sample size. Vulvar cancer is a rare disease, which makes collecting large cohorts of patient data difficult for a single institution. Other studies evaluating this disease and the relations of p16 status are of similar size, although our study is the largest for patients receiving strictly adjuvant RT. We elected not to group all women with vulvar cancer receiving RT together in a single cohort (as other studies have done) in an attempt to better elucidate the role of p16 in each circumstance; these studies are being published separately. In future clinical trials for vulvar cancers, stratification by p16/HPV status should be established to more accurately define the role that p16/HPV plays in outcomes.

Table 4 Contemporary studies evaluating HPV/p16 status and their relationships with outcomes

Study	Patients	Radiation regimen	HPV detection	p16 Detection	Prognostic value
Yap et al[4]	40	Preop, postop, definitive	PCR	IHC	LRR: yes DFS: yes OS: no
Lee et al[5]	57	Preop, postop, definitive	PCR	IHC	IFR: yes PFS: yes OS: possible
Kim et al[17]	56	"Curative RT"	Hybrid capture 2	-	DFS: no OS: no
Alonso et al[13]	98 (9 RT)	"Radio/chemo" and adjuvant RT	PCR	IHC	DFS: no OS: no
Lindell et al[12]	75 (24 RT)	Adjuvant RT	PCR	-	RFS: yes DSS: yes OS: yes
Larsson et al[18]	130	Unknown	PCR	Yes (unknown method)	PFS: no OS: yes
McAlpine et al[19]	201 (61 RT)	Unspecified	-	IHC	PFS: yes DSS: yes
Wakeham et al[20]	62 (12 RT)	Surgery/CRT, CRT or RT alone	PCR	IHC	PFS: yes OS: possible
Weberpals et al[21]	43 (21 RT)	CRT, surgery/RT	PCR	IHC	PFS: no OS: no
Rasmussen et al[22]	Meta-analysis	-	-	-	DFS: yes OS: yes
Current study	39	Adjuvant RT	-	IHC	IFR: possible OS: no

Abbreviations: CRT = chemoradiation therapy; DFS = disease-free survival; DSS = disease-specific survival; HPV = human papillomavirus; IFR = in-field relapse; IHC = immunohistochemistry; LRR = locoregional relapse; OS = overall survival; PCR = polymerase chain reaction; PFS = progression-free survival; RFS = recurrence-free survival; RT = radiation therapy.

Conclusions

VSCC that arises as a result of p16 mutation because of HPV has a lower propensity for IFR compared with p16− VSCC after radical vulvectomy and adjuvant RT.

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