Significance of p53 and presence of differentiated vulvar intra-epithelial neoplasia (dVIN) at resection margin in early stage human papillomavirus-independent vulvar squamous cell carcinoma

Emily F Thompson ,[1] Kathryn Shum,[1] Richard W C Wong,[2] Giorgia Trevisan,[3] Janine Senz,[1] Jutta Huvila,[4] Samuel Leung,[1] David G Huntsman,[1,5] C Blake Gilks ,[5] Jessica N McAlpine,[6] Lynn Hoang,[5] Amy Jamieson [6]

For numbered affiliations see end of article.

Correspondence to
Dr Amy Jamieson, Department of Gynecology and Obstetrics, Division of Gynecologic Oncology, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada; amy.jamieson@vch.ca

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ABSTRACT

Objective Vulvar squamous cell carcinoma and in situ lesions can be stratified by human papillomavirus (HPV) and *TP53* status into prognostic risk groups using p16 and p53 immunohistochemistry. We assessed the significance of vulvar squamous cell carcinoma resection margin positivity for either differentiated vulvar intra-epithelial neoplasia (dVIN) or abnormal p53 immunohistochemistry, and other pathologic variables, in a cohort of patients with HPV-independent (HPV-I) p53 abnormal (p53abn) vulvar squamous cell carcinomas.

Methods Patients with stage I–II HPV-I p53abn vulvar squamous cell carcinoma with negative invasive margins who did not receive adjuvant radiation from a single institution were included. Tumors underwent margin reassessment using p53 immunohistochemistry. Cases were segregated into (1) morphologic dVIN at margin; or (2) abnormal p53 immunohistochemistry staining at margin without morphologic dVIN (p53abn immunohistochemistry); or (3) margins negative by morphology and p53 immunohistochemistry. Clinicopathologic/outcome data were collected.

Results A total of 51 patients were evaluated: (1) 12 with dVIN on margin; (2) 12 with p53abn immunohistochemistry on margin without morphologic dVIN; and (3) 27 with margins negative for morphologic dVIN and p53abn immunohistochemistry. The recurrence rate for patients with dVIN or p53abn immunohistochemistry on the margin was equally high at 75% each, compared with 33% with margins negative for morphologic dVIN and p53abn immunohistochemistry (p=0.009). On multivariate analysis, positive in situ margins maintained an association with disease recurrence (p=0.03) whereas invasive margin distance (radial and deep), lymphovascular invasion, and tumor size did not.

Conclusions Patients with stage I–II HPV-I vulvar squamous cell carcinoma with margins positive for either dVIN or p53abn immunohistochemistry without morphologic dVIN showed increased disease recurrence, regardless of invasive margin distance. These findings show that p53 immunohistochemistry is a useful adjunct for evaluating margin status in HPV-I vulvar squamous cell carcinoma and may support repeat excision for positive in situ margins (dVIN or p53abn immunohistochemistry).

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with HPV-independent vulvar squamous cell carcinomas have been shown to experience worse outcomes compared to HPV-associated.

⇒ Current vulvar squamous cell carcinoma treatment guidelines are not stratified by HPV and TP53 status and do not address management of high risk in-situ lesions at resection margins.

WHAT THIS STUDY ADDS

⇒ p53 immunohistochemistry can detect occult in-situ lesions and is a useful adjunct for evaluating margin status in HPV-independent vulvar squamous cell carcinoma.

⇒ Disease recurrence was higher in patients with in-situ margin positive status; either differentiated vulvar intra-epithelial neoplasia (dVIN) or abnormal p53 immunohistochemistry staining.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Efforts to identify and surgically (re)-excise TP53 mutated field may reduce recurrence rates in HPV-independent p53 abnormal vulvar squamous cell carcinoma.

INTRODUCTION

Vulvar cancer accounts for approximately 5% of all gynecological cancers, with squamous cell carcinoma accounting for >90% at this anatomical site.[1] Disease incidence in high income countries is increasing, and although traditionally thought to affect predominantly elderly women, the incidence in younger women has also been increasing.[1]

Vulvar squamous cell carcinoma is known to develop through precursor lesions via different etiologic pathways related to the presence of human papillomavirus

(HPV) and *TP53* mutations.[2] Immunostaining for p16 has been validated as an accurate surrogate marker for determination of HPV status.[3 4] Pattern-based p53 immunohistochemistry has also been shown to accurately reflect *TP53* mutations in vulvar squamous cell carcinoma.[5 6] Thus, with the use of p16 and p53 immunohistochemistry, vulvar squamous cell carcinoma can be sub-classified into etiologic sub-types in routine clinical practice. The prognostic significance of HPV status in vulvar squamous cell carcinoma has been shown in recent studies.[7–11] Patients with HPV-independent (HPV-I) vulvar squamous cell carcinoma have consistently been shown to experience worse outcomes than those with HPV-associated (HPV-A) disease, with 5-year overall survival rates of 22–47% and 62–81%, respectively.[9] Recent evidence has also shown that HPV-I vulvar squamous cell carcinoma can be further stratified by *TP53* status, with *TP53* mutated vulvar squamous cell carcinoma having worse survival outcomes than *TP53* wild type.[11 12]

The extent of surgical resection appears to be especially important in HPV-I vulvar squamous cell carcinoma, with two studies showing improved outcomes in HPV-I vulvar squamous cell carcinoma treated with radical surgical excision compared with more conservative surgery.[7 13] Less radical surgery resulting in worse outcomes in HPV-I p53 abnormal (p53abn) vulvar squamous cell carcinoma is consistent with the theory of 'field cancerization'; it is thought that somatic *TP53* mutations in squamous cells colonize large areas of the vulvar epithelium and develop into differentiated vulvar intra-epithelial neoplasia (dVIN) or vulvar squamous cell carcinoma. Thus, our focus on removing the primary tumor aiming for the internationally recommended microscopic margins of 8 mm may be undertreating these HPV-I *TP53* mutated cancers, where the bounds of the dVIN/*TP53* mutated epithelium on the vulva often extend beyond what can be appreciated clinically.

p53 immunohistochemistry has been shown to be a useful tool for detecting histologically under-recognized dVIN,[14] and can change dVIN margin status from negative to positive in 31% of cases.[15] In addition, we have demonstrated that p53 immunohistochemistry staining can detect morphologically occult p53abn in situ lesions at vulvar resection margins and were associated with an increased risk of local recurrence.[16]

In this study we expand on our previous findings and determine the clinical significance of vulvar resection margins that are positive for high-risk in situ lesions. We focused on patients with surgically staged I–II disease as we thought this was the cohort where residual high-risk in situ disease may have the most measurable impact on outcomes. The aim of this study was to compare outcomes in patients with either (1) margins positive for morphologic dVIN or (2) margins positive for p53abn immunohistochemistry staining (without morphologic dVIN), to (3) patients with margins negative for morphologic dVIN and p53abn immunohistochemistry.

METHODS

Case Selection and Inclusion

This study was approved by the institutional research ethics board (H21-03716). Patients with primary invasive vulvar squamous cell carcinoma were selected from the Vancouver General Hospital institutional archive. Those with surgically excised International Federation of Gynecology and Obstetrics (FIGO 2009) stage I–II (node negative) vulvar squamous cell carcinoma with margins negative for invasive carcinoma and who did not receive adjuvant radiation were included.

Pathology and Immunohistochemistry

Hematoxylin and eosin (H&E) and immunohistochemistry slides were reviewed and interpreted by three specialist gynecologic pathologists (RWCW, GT, LH) and a research fellow (ET). To confirm HPV-independent status, p16 immunohistochemistry was performed as a surrogate marker for HPV.[3 4] To confirm *TP53*-mutant status, p53 immunohistochemistry was performed on the tumor and evaluated using criteria previously described.[5 6] p53 and p16 immunohistochemistry were performed on formalin fixed paraffin embedded 4 µm tissue sections and were stained using the Dako Omnis and Dako EnVision FLEX+ detection system as per manufacturer recommendations. Sections were mounted onto Dako FLEX microscope slides, air dried for 20 min, and baked at 60°C for 20 min. The following antibodies were used: p16 (Roche CINtec, E6H4, mouse monoclonal, 1:5 dilution) and p53 (Dako, DO-7, mouse monoclonal, 1:500 dilution). Only cases of vulvar squamous cell carcinoma that were p16 negative and exhibited a mutational p53 (p53abn) immunohistochemistry pattern were included for further study.

Cases of vulvar squamous cell carcinoma that had dVIN present/positive at the surgical radial margin, recognized and reported on the original pathology report and scheduled for observation only, were placed in Group 1 (positive for 'morphologic dVIN'). Cases where the vulva was re-excised due to the margin positivity for dVIN were excluded. In the remaining cases, margins were re-examined using p53 immunohistochemistry. Blocks showing the closest approach of vulvar squamous cell carcinoma to a radial surgical margin were stained with p53 immunohistochemistry.[5 6] Those with the presence of a p53abn immunohistochemistry pattern at the surgical radial margin were placed in Group 2 ('p53abn immunohistochemistry without morphologic dVIN'). Cases with margins negative for morphologic dVIN and p53abn immunohistochemistry were placed in Group 3 ('negative for both morphologic dVIN and p53abn immunohistochemistry').

Clinicopathologic Parameters

Tumor size, lymphovascular invasion, invasive margin distances (radial and deep), lichen sclerosus, age at diagnosis, FIGO 2009 stage, disease recurrence (local (in situ and invasive), nodal and distant), time to recurrence, death from vulvar squamous cell carcinoma or death from any cause were recorded. Treatment and complications of all subsequent recurrences were also recorded.

Outcomes and Statistical Analysis

We considered univariable associations between binary and categorical variables using a χ^2 test or Fisher's exact and Kruskal–Wallis test for continuous variables. Kaplan–Meier curves and log-rank test were used to illustrate and test the association between margin status and progression-free, disease-specific, and overall survival. A multivariate analysis incorporating dVIN/p53abn immunohistochemistry margin status, tumor size, invasive margin distances,

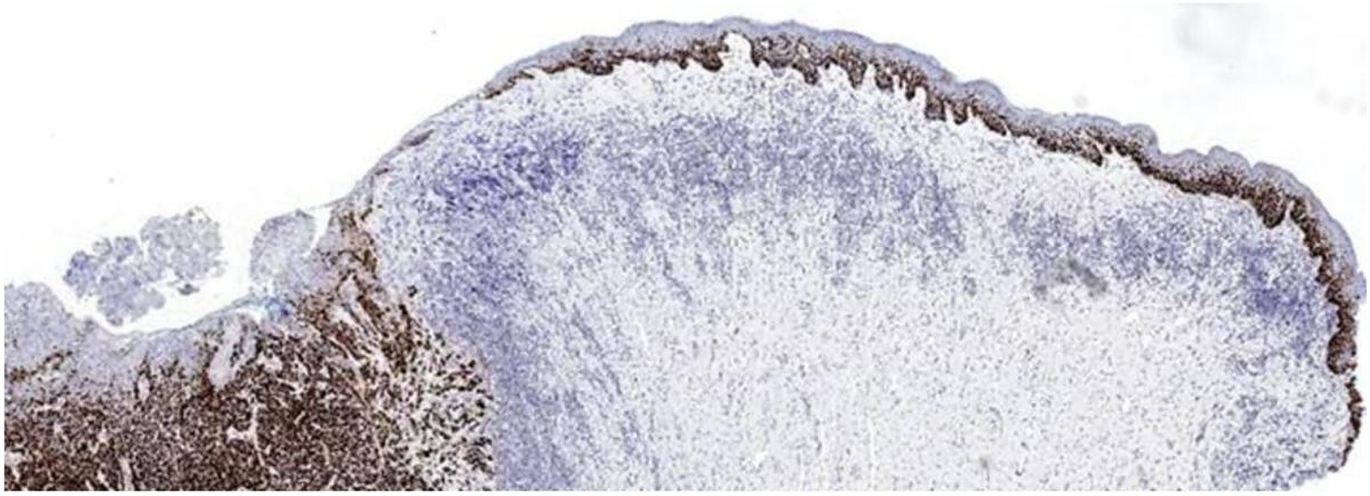

Figure 1 Immunohistochemical stain for p53 showing an abnormal (mutational) pattern in the vulvar squamous cell carcinoma (diffuse/overexpression pattern) as well as the adjacent in situ lesion which did not fulfill the traditional morphologic criteria for differentiated vulvar intra-epithelial neoplasia (dVIN). The abnormal p53 histochemistry pattern unexpectedly extended to the resection margin (magnification 30×).

and lymphovascular invasion was performed. All statistical analyses were done using R project for statistical computing with significance set at α=0.05.

RESULTS

Cohort Description

Fifty-one patients with HPV-I p53abn FIGO stage I–II vulvar squamous cell carcinoma were suitable for inclusion: (1) 12 with morphologic dVIN on the margin; (2) 12 with p53abn immunohistochemistry (without morphologic dVIN) on the margin (Figure 1); and (3) 27 with margins negative for both morphologic dVIN and p53abn immunohistochemistry. Clinicopathologic characteristics of the study cohort by margin status are shown in Table 1. The mean age was 75.8 years (range 40.7–97). Fifty (98%) had FIGO stage I disease, 8% had lymphovascular invasion, and 75% of patients had associated lichen sclerosus. The majority of patients (82%) underwent groin lymph node assessment and none had nodal metastases.

There was no significant difference between the three groups in age, tumor size, radial and deep invasive margin distances, and lymphovascular invasion. There was a significant association with margin status (positive for morphologic dVIN or positive for p53abn immunohistochemistry without morphologic dVIN) and the presence of lichen sclerosus (p=0.0009) (Table 1).

Outcomes

Mean follow-up time for the cohort was 5.4 years (range 0.6–20.9). There was a total of 27 (52.9%) disease recurrences/progression events, 36 (70.6%) overall survival events, and 16 (31.4%) deaths specific to vulvar cancer in the full cohort. Positive in situ margins were significantly associated with an increased risk of disease recurrence, where 75% of patients with morphologic dVIN on the margin and equally 75% of patients with p53abn immunohistochemistry (without morphologic dVIN) on the margin experienced recurrent disease compared with 33% in the group where margins were negative for both morphologic dVIN and p53abn immunohistochemistry (p=0.009). The mean recurrence times also differed (13.2 months for dVIN at the margin, 30.7 months for p53abn immunohistochemistry at the margin, and 47.3 months for margins negative for dVIN and p53abn immunohistochemistry), although this did not reach statistical significance. Figure 2 shows Kaplan–Meier survival analyses comparing outcomes for the three margin groups. Positive morphologic dVIN at the margin (Group 1) was significantly associated with worse progression-free survival (p=0.004, panel A). Similarly, positive p53abn immunohistochemistry at the margin without morphologic dVIN (Group 2) was associated with worse progression-free survival (p=0.01, panel C). The progression-free survival curves for both Group 1 (morphologic dVIN at margin) and Group 2 (p53abn immunohistochemistry without morphologic dVIN) showed substantial overlapping and were not statistically different (panel E). Positive margins for morphologic dVIN and p53abn immunohistochemistry showed trends towards worse disease-specific survival but were not significant (p=0.05 and p=0.15). We also performed a multivariate analysis to assess the association of tumor size, invasive margin distance (radial and deep), lymphovascular invasion, and margin status (morphologic dVIN or p53abn immunohistochemistry without morphologic dVIN) with outcomes. Lesion present at the margins (morphologic dVIN or p53abn immunohistochemistry without morphologic dVIN) was the only parameter which maintained a statistically significantly association with increased risk of disease recurrence (p=0.03) in the multivariate model, whereas invasive margin distances (radial and deep), lymphovascular invasion, and tumor size did not (Table 2).

Patients with Recurrent Disease

Twenty-seven patients (53%) in this cohort had disease recurrence and, of those, 50% had more than one recurrence event. There were 53 disease recurrence events in total, of which 44 were treated with surgical excision and 11 with radiation (with or without surgery). Twenty of the 27 patients (74%) had post-operative wound complications (breakdown and/or infection) following surgery for recurrent disease and 19 patients (70%) required home care or wound assistance in the community for wound complications. Sixteen patients

Table 1 Clinicopathologic characteristics of patient cohort with p53abn HPV-independent vulvar squamous cell carcinoma stratified by margin status

		In situ margin positive			
	All	dVIN	p53abn	Margins negative	P value
Total	51	12	12	27	
Follow-up, years, mean (range)	5.4 (0.1–20.9)	5.3	5.3	5.4	0.99
Age, years, mean (range)	75.8 (40.7–97)	73.4	72.8	78.2	0.376
Stage					0.99
Stage I	50 (98%)	12 (100%)	12 (100%)	26 (96%)	
Stage II	1 (2%)	0	0	1 (4%)	
Associated LS					**0.0009**
Yes	38 (75%)	11 (92%)	12 (100%)	15 (56%)	
No	12 (24%)	0	0	12 (44%)	
Unknown	1 (2%)	1			
Tumor size, cm (mean)	2.8	1.9	2.7	3.2	0.248
Invasive margin distance, cm (mean)					
Radial	0.5	0.5	0.3	0.5	0.0585
Deep	0.6	0.6	0.5	0.7	0.325
Groin nodes evaluated	42 (82%)	10 (83%)	8 (67%)	24 (88%)	0.363
LVI present	4 (8%)	0	0	4	0.99
Disease-specific outcomes					
Recurrence	27 (53%)	9 (75.0%)	9 (75.0%)	9 (33%)	**0 .009**
Died of disease	16 (31%)	6 (50.0%)	4 (33.3%)	6 (22%)	0.195
Time to recurrence, months (mean)	30.4	13.2	30.7	47.3	0.587

dVIN, differentiated vulvar intra-epithelial neoplasia; LS, lichen sclerosus; LVI, lymphovascular invasion; p53abn, abnormal p53 IHC staining without morphologic dVIN.

(59%) died from disease recurrence; 13 patients required hospital admission in the context of end-of-life care, and seven patients required palliative care input for pain/symptom control.

DISCUSSION

Summary of Main Results

In this assessment of stage I–II HPV-I p53abn vulvar squamous cell carcinomas, we observed significantly higher rates of disease recurrence in patients with in situ margin positive status; 75% of patients with morphologic dVIN and 75% of patients with p53abn immunohistochemistry without morphologic dVIN on the margin experienced recurrent disease, compared with 33% recurrence in the group where margins were negative for both morphologic dVIN and p53abn immunohistochemistry. In addition, the in situ margin status (either morphologic dVIN or p53abn immunohistochemistry) was the only parameter to remain statistically associated with progression-free survival in the multivariate analysis incorporating tumor size, invasive radial margin distance, invasive deep margin distance, and lymphovascular invasion (p=0.03). These results emphasize the clinical importance of residual morphologic dVIN at a resection margin, suggesting that re-excision of dVIN on margins could have reduced the recurrence rate by half. This study also provides further evidence of the aggressive clinical course of HPV-I vulvar squamous cell carcinoma,[7–11] with a high recurrence rate overall (53%) in this cohort restricted to HPV-I p53abn disease.

Results in the Context of Published Literature

Our findings align with those of Te Grootenhuis et al, where higher rates of recurrence were seen in patients with dVIN at the margin, independent of tumor-free margin distance.[17] Currently, treatment guidelines put heavy emphasis on invasive carcinoma margin distance, where most guidelines today recommend tumour-free pathological margins of 8 mm or more to adequately treat the primary vulvar tumour.[1 18 19] However, the recommendations for dVIN, particularly residual dVIN, remain variable. The European Society of Gynecological Oncology (ESGO) recommends "to consider additional, more superficial resection of dVIN in addition to radical local excision of invasive tumors",[19] while the International Federation of Gynecology and Obstetrics (FIGO)[1] and National Comprehensive Cancer Network (NCCN)[18] do not make a specific recommendation for residual dVIN. In a survey by the International Society for the Study of Vulvovaginal Disease (ISSVD), the majority of gynecologists reported they would surgically excise primary dVIN,[20] but this disposition does not translate to the setting of residual dVIN at a margin for the treatment of vulvar squamous cell carcinoma. In a recent survey of 27 gynecologic oncologists in Canada (GOC), only three (11%) said they would take a patient back to the operating room for re-excision of residual dVIN present at the margin after surgery for vulvar squamous cell carcinoma.[21] This study raises another important and evolving issue which is the presence of p53abn immunohistochemistry at the margin without morphologic dVIN. 'Field cancerization' is a long-standing theory

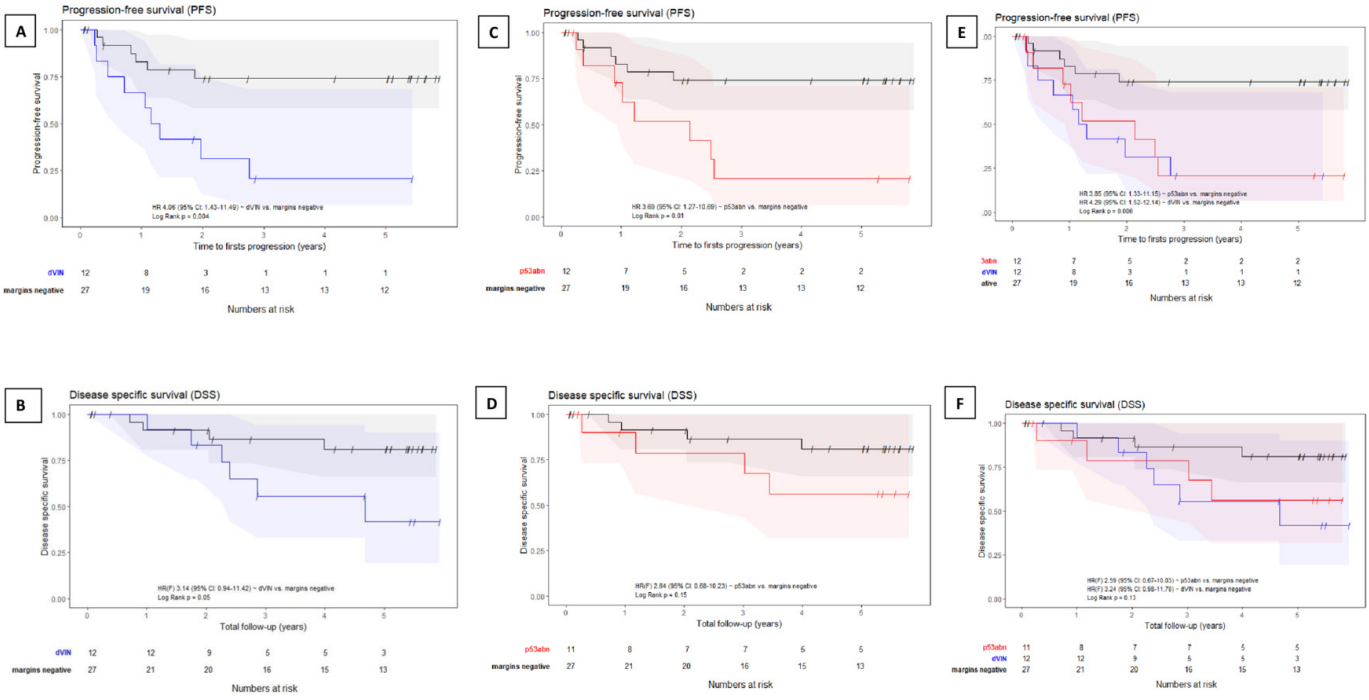

Figure 2 Kaplan–Meier survival analyses comparing outcomes for the three margin groups. Positive morphologic differentiated vulvar intra-epithelial neoplasia (dVIN) at the margin (Group 1) was significantly associated with worse progression-free survival (p=0.004, panel A) with a trend towards worse disease-specific survival (p=0.05, panel B). Similarly, positive p53 abnormal (p53abn) immunohistochemistry at the margin without morphologic dVIN (Group 2) was associated with significantly worse progression-free survival (p=0.01, panel C), with a trend towards worse disease-specific survival (panel D). The progression-free survival and disease-specific survival curves for both Group 1 (morphologic dVIN at margin) and Group 2 (p53abn immunohistochemistry without morphologic dVIN) showed substantial overlapping and were not statistically different (panels E and F).

which proposes that there is a dysplastic field in the vulva that develops due to chronic inflammatory injury and oxidative stress, harbors most commonly somatic *TP53* mutations, and is the substrate from which vulvar squamous cell carcinoma develops.[2 22] The morphology of lesions within this *TP53*-mutant field can vary widely, encompassing lesions acceptable as dVIN and lesions which show much more subtle morphologic findings (where the morphologic changes do not meet the diagnostic threshold for dVIN).[23] We have referred to these lesions in this study as 'p53abn immunohistochemistry without morphologic dVIN' and have described them elsewhere as 'occult p53abn (dysplasia)' or 'skin showing mutant pattern p53 immunohistochemistry staining that is in continuity with HPV-I p53abn VIN (dVIN) showing subtle morphological abnormalities that do not reach the diagnostic threshold for traditional dVIN'.[23] Without going into the deep abyss of challenges surrounding dVIN inter-observer variation and diagnostic

Table 2 Multivariate analysis to assess the association of tumor size, lymphovascular invasion, invasive margin distance (radial and deep), and in situ margin status (either dVIN or p53abn immunohistochemistry) with overall survival, disease-specific survival, and progression-free survival

	Overall survival			Disease-specific survival			Progression-free survival		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Invasive deep margin (cm)	0.84	(0.18 to 3.97)	0.824	0.5	(0.07 to 3.5)	0.446	0.96	(0.18 to 5.15)	0.966
Invasive radial margin (cm)	0.91	(0.11 to 7.49)	0.930	0.4	(0.01 to 7.82)	0.459	0.51	(0.05 to 5.32)	0.567
Tumor size (cm)	1.27	(0.95 to 1.7)	0.098	1.11	(0.73 to 1.69)	0.629	0.95	(0.69 to 1.3)	0.748
LVI	1.22	(0.13 to 11.02)	0.864	4.31	(0.36 to 38.48)	0.319	4.61	(0.74 to 28.82)	0.132
In situ positive margin									
dVIN	3.58	(0.97 to 13.15)	0.148	5.09	(1.02 to 32.62)	0.124	4.21	(1.04 to 17.03)	**0.03**
p53abn	1.99	(0.56 to 7.12)	0.148	2.41	(0.37 to 15.46)	0.124	5.36	(1.38 to 20.83)	**0.03**

dVIN, differentiated vulvar intra-epithelial neoplasia; LVI, lymphovascular invasion; p53abn, anormal p53 IHC staining without morphologic dVIN.

thresholds, our study shows that, regardless of the morphologic impression, the presence of p53abn immunohistochemistry at a margin is equally as important as morphologic dVIN at a margin. Our findings suggest that excising the *TP53* mutated field should be given equal weight to dVIN and support repeat excision for positive in situ margins (either dVIN or p53abn immunohistochemistry) in HPV-I vulvar squamous cell carcinoma.

Implications for Practice and Future Research

The management of patients with vulvar squamous cell carcinoma remains challenging to gynecologic oncologists. Surgery to remove the primary tumor and assess the groin lymph node status is the treatment of choice in early stage vulvar squamous cell carcinoma, but is associated with a significant risk of long-term physical and psychosexual morbidity.[1] We also know that approximately one-third of women with vulvar squamous cell carcinoma will die from their disease, and many of the women that do survive will suffer from local disease recurrence. As such, the balance of adequate treatment and reducing treatment morbidity is an important consideration in the management of all patients with vulvar squamous cell carcinoma. HPV-I vulvar squamous cell carcinomas are known to arise in older women compared with HPV-A.[10] Some clinicians may be reluctant to offer patients with HPV-I vulvar squamous cell carcinoma with positive in situ margins repeat surgical excision or a wider resection margin at initial surgery because these patients are often elderly with co-morbidities and often have atrophic anatomy with background lichen sclerosus. In this study, we found patients who had disease recurrence(s) experienced substantial post-treatment wound complications from subsequent surgery and radiotherapy, which precipitated acute hospital admissions and required community-based wound care nursing. In addition, disease-associated mortality was high (59%) in patients with recurrent disease.

In the last few years there has finally been some movement in refining the classification of vulvar squamous cell carcinoma, and the World Health Organization now endorses that vulvar squamous cell carcinoma be separated into HPV-associated and HPV-independent in the pathology report.[24] Although not formally incorporated, we anticipate that p53 will eventually be integrated. In the meantime, while classification shifts, current international guidelines remain unchanged and clinicians continue to manage vulvar squamous cell carcinoma as a single entity, despite these differences in prognostic and therapeutic factors. For example, HPV status in vulvar squamous cell carcinoma has also been shown to be predictive of response to radiation. Lee et al showed that, in 57 patients with vulvar squamous cell carcinoma treated with radiation with or without surgical resection, HPV-A had higher progression-free survival and overall survival as well as lower in-field relapse compared with HPV-I.[25] These findings were confirmed in a subsequent study of 48 patients with vulvar squamous cell carcinoma treated with primary chemoradiation.[26] Higher in-field radiation relapse in HPV-I vulvar squamous cell carcinoma has also resulted in an increase in pelvic exenterations in this patient population, and recurrent vulvar squamous cell carcinoma has become the most common indication for pelvic exenteration in many centers.[27] Prior studies have emphasized that the extent of surgical resection is of particular importance for patients with HPV-I vulvar squamous cell carcinoma (the majority of which are *TP53* mutated).[7 13] The limited effectiveness of radiation in HPV-I vulvar squamous cell carcinoma again emphasizes the need for optimizing surgical management for this particular sub-set of patients. It is not possible to determine if the patients in this series would have had more favorable outcomes with initial wider surgical resection and/or with re-excision of their positive in situ margins, and clinical outcomes were poor overall in this aggressive disease. However, given the high rates of recurrence with HPV-I vulvar squamous cell carcinoma and positive in situ margins, it does raise the question of whether surgical practice needs to change. This important question should be addressed in a prospective clinical trial to ensure that any future changes to clinical practice are informed by high-quality data as well as patient-reported outcomes to reflect the impact of this change in surgical decision making. The STRIVE study (STRatIfication of Vulvar squamous cell carcinoma by HPV and p53 status to guide Excision) was developed with international collaboration to address this important clinical challenge and may hopefully provide guidance in this understudied disease.

Strengths and Weaknesses

A strength of this study is the rigorous approach to etiologic subtyping and the inclusion of only those with high molecular risk (*TP53* mutated) vulvar squamous cell carcinomas, a sub-set of patients that might be expected to benefit from primary radical surgical treatment of *TP53* mutated 'fields of dysplasia'. Study limitations include the retrospective nature of these analyses and the small sample size.

CONCLUSION

Patients with HPV-I vulvar squamous cell carcinoma with margins positive for either morphologic dVIN or p53abn immunohistochemistry without morphologic dVIN showed increased disease recurrence, regardless of invasive margin distance. Excising these in situ lesions may reduce disease recurrence and improve outcomes in HPV-I vulvar squamous cell carcinoma, compared with the current guidelines based on invasive margin distance only that are not stratified by HPV and p53 status.

Author affiliations
[1]Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, British Columbia, Canada
[2]Department of Pathology, United Christian Hospital, Hong Kong, Hong Kong
[3]Department of Pathology, Barts Health NHS Trust, London, UK
[4]Department of Pathology, University of Turku, Turku, Finland
[5]Department of Pathology and Laboratory Medicine, The University of British Columbia, Vancouver, British Columbia, Canada
[6]Department of Gynecology and Obstetrics, Division of Gynecologic Oncology, University of British Columbia, Vancouver, British Columbia, Canada

Contributors ET: conceptualization, investigation, data curation, writing. KS: investigation, data curation, writing. RWCW: investigation, data curation. GT: investigation, data curation. JS: investigation, data curation. SL: statistical analysis, data curation. JH: statistical analysis, visualization, writing - review and editing. DGH: investigation, data curation. BG: writing - review and editing, JNM: writing - review and editing. LH: funding, conceptualization, methodology, investigation, data curation, writing. AJ: funding, conceptualization, methodology, investigation, data curation, writing, guarantor.

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ORCID iDs
Emily F Thompson http://orcid.org/0000-0002-1486-3304
C Blake Gilks http://orcid.org/0000-0001-7889-8250
Amy Jamieson http://orcid.org/0000-0002-3210-9102

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