Seminars in Diagnostic Pathology 38 (2021) 37–49

Contents lists available at ScienceDirect

Seminars in Diagnostic Pathology

journal homepage: www.elsevier.com/locate/semdp

Prognostic factors in vulvar squamous cell carcinoma

# A review of prognostic factors in squamous cell carcinoma of the vulva: Evidence from the last decade

Chen J Julia[a], LN Hoang[b,c,*]

[a] Medical Undergraduate Program, Faculty of Medicine, University of British Columbia, BC, Canada
[b] Department of Pathology, Vancouver General Hospital and University of British Columbia, 1215 – 910 West 10th Avenue, Vancouver BC, Canada
[c] Genetic Pathology Evaluation Center (GPEC), BC, Canada

ARTICLE INFO

*Keywords:*
Vulva
Squamous cell carcinoma
HPV
Prognosis
Survival

ABSTRACT

Squamous cell carcinoma of the vulva is a rare gynecologic cancer that is associated with significant patient morbidity and mortality, particularly for recurrent disease. This review summarizes the evidence and continued challenges, regarding the traditional clinicopathologic factors used to prognosticate vulvar squamous cell carcinoma. Articles published within the last 10 years (2010–2020) were identified. Relevant articles concerning the following fifteen prognostic factors were reviewed: HPV/p16 status, vulvar intraepithelial neoplasia, patient age, tumor stage, tumor grade, tumor size, depth of invasion, stromal changes, histologic patterns of invasion, lymphovascular space invasion (LVSI), perineural invasion, lymph node metastases, tumour focality, margin status and lichen sclerosus (LS). The relationship between each prognostic factor and progression-free survival (PFS) and overall survival (OS), including hazard ratios, 95% confidence intervals and *p*-values, were extracted.

## Introduction

The vulva comprises of the anatomical region external to the vagina, encompassing the labia majora, labia minora, clitoris, bulb of the vaginal vestibule, Bartholin (greater) glands and Skene (lesser) glands.[1] Cancers of the vulvar are rare, constituting less than 5% of all gynecologic malignancies. It has an incidence of 2.6 per 100,000 women per year and cumulative lifetime risk of 0.09%.[2,3] Surgical resection, ranging from simple excision to radical vulvectomy, is the mainstay of treatment, with limited roles for adjuvant radiation and chemotherapy. Physical and psychosocial morbidity is a significant challenge in vulvar cancer, particularly with treatment escalation, and one-third of patients will die from the disease with a 5 year overall survival of 70.4%.[3]

Squamous cell carcinomas (SCC) account for over 90% of malignancies occurring in the vulva. These tumors can be driven by human papillomavirus (HPV) infection (HPV-associated) or occur independently of HPV (HPV-independent). In this review, we discuss the various pathologic factors used to prognosticate vulvar squamous cell carcinoma (VSCC) and summarize the evidence behind these considerations.

## Methods

Pubmed (pubmed.ncbi.nlm.nih.gov) was searched using the following terms, “vulva OR vulvar”, “squamous cell carcinoma” and “prognosis”. Articles within the last 10 years (2010–2020) were included and articles written in English were reviewed. A total of 327 articles were identified. The articles were reviewed to determine relevance. Case reports, case series, and review articles were excluded. Original articles focusing on 15 prognostic factors of interest were included. Those prognostic factors were HPV/p16 status, vulvar intraepithelial neoplasia, patient age, tumor stage, tumor grade, tumor size, depth of invasion, stromal changes, histologic patterns of invasion, lymphovascular space invasion (LVSI), perineural invasion, lymph node metastases, tumour focality, margin status and lichen sclerosus (LS). Ten papers were found through a search of references of the relevant papers. A total of 63 articles were included. We recorded the country, years of study, number of patients, follow-up times and survival for each study (Supplemental Table 1). For each prognostic factor, we recorded the relationship with progression free survival (PFS) (local recurrence rate, disease free survival) and overall survival (OS), including the types of statistical analyses, hazards ratios, 95% confidence intervals and *p*-values. A *p*-value $\leq 0.05$ was considered statistically significant. Disease-specific survival (DSS) was recorded when reported.

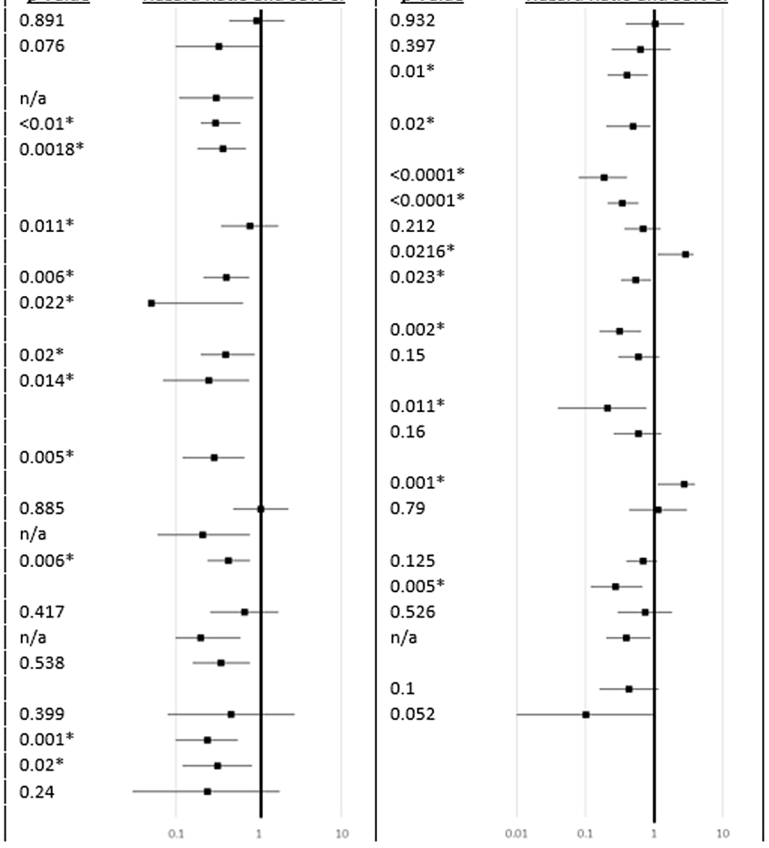
* Corresponding author at: Department of Pathology, Vancouver General Hospital and University of British Columbia, 1215 – 910 West 10th Avenue, Vancouver BC, Canada
*E-mail address:* Lien.Hoang@vch.ca (L. Hoang).

https://doi.org/10.1053/j.semdp.2020.09.004

Available online 06 September 2020

**Table 1**
Presence of HPV and relationship with PFS and OS.

| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) | | Overall Survival (OS) | |
|---|---|---|---|---|---|---|
| | | | p Value | Hazard Ratio and 95% CI | p Value | Hazard Ratio and 95% CI |
| Alonso et al. 2011 | p16 | univariate | 0.891 | | 0.932 | |
| Dohopolski et al. 2019 | p16 | univariate | 0.076 | | 0.397 | |
| Dong et al. 2015 | p16 | univariate | | | 0.01* | |
| Hay et al. 2016 | p16 | univariate | n/a | | | |
| Lee et al. 2016 | p16 | univariate | <0.01* | | 0.02* | |
| McAlpine et al. 2017 | p16 | univariate PFS | 0.0018* | | | |
| McAlpine et al. 2017 | p16 | univariate DSS | | | <0.0001* | |
| McAlpine et al. 2017 | p16 | univariate OS | | | <0.0001* | |
| Stefansson et al. 2019 | p16 | univariate | 0.011* | | 0.212 | |
| Sznurkowski et al. 2016 | p16 | univariate | | | 0.0216* | |
| Czogalla et al. 2020 | p16 | multivariate | 0.006* | | 0.023* | |
| Dohopolski et al. 2019 | p16 | multivariate | 0.022* | | | |
| Dong et al. 2015 | p16 | multivariate | | | 0.002* | |
| Lee et al. 2016 | p16 | multivariate | 0.02* | | 0.15 | |
| McAlpine et al. 2017 | p16 | multivariate PFS | 0.014* | | | |
| McAlpine et al. 2017 | p16 | multivariate DSS | | | 0.011* | |
| McAlpine et al. 2017 | p16 | multivariate OS | | | 0.16 | |
| Stefansson et al. 2019 | p16 | multivariate | 0.005* | | | |
| Sznurkowski et al. 2016 | p16 | multivariate | | | 0.001* | |
| Alonso et al. 2011 | PCR | univariate | 0.885 | | 0.79 | |
| Hay et al. 2016 | PCR | univariate | n/a | | | |
| Stefansson et al. 2019 | PCR | univariate | 0.006* | | 0.125 | |
| Wakeham et al. 2017 | PCR | univariate | | | 0.005* | |
| Weberpals et al. 2017 | PCR | univariate | 0.417 | | 0.526 | |
| Lee et al. 2016 | PCR | multivariate | n/a | | n/a | |
| Stefansson et al. 2019 | PCR | multivariate | 0.538 | | | |
| Wakeham et al. 2017 | PCR | multivariate | | | 0.1 | |
| Weberpals et al. 2017 | PCR | multivariate | 0.399 | | 0.052 | |
| Wakeham et al. 2017 | p16, PCR | univariate | 0.001* | | | |
| Wakeham et al. 2017 | p16, PCR | multivariate | 0.02* | | | |
| Nooij et al. 2016 | n/a | univariate | 0.24 | | | |




*Abbreviations: HPV = human papillomavirus; OS = overall survival; CI = confidence interval; PCR = polymerase chain reaction; PFS = progression-free survival; DFS = disease-free survival; DSS = disease-specific survival; * significant p-value <0.05*

All analyses listing a *p*-value are summarized in the text and only analyses providing a hazards ratio ( ± 95% confidence interval) are summarized in Tables.

## Results

### *HPV status*

Currently, HPV status is not used to formally prognosticate patients with VSCC, nor has it been incorporated into tailored treatment algorithms. However, there has been growing attention to HPV status, largely driven by observations made in SCC of the head and neck region. The National Cancer Comprehensive Network (NCCN) guidelines for oropharyngeal SCC require HPV testing and/or p16 immunohistochemical (IHC) staining, and provide separate treatment algorithms depending on the HPV/p16 status.[4]

Upon our review, 21 studies evaluated the prognostic significance of HPV status on survival, using either p16 IHC, HPV DNA polymerase chain reaction (PCR) or DNA in-situ hybridization (ISH) (Table 1). The vast majority found that HPV-associated VSCC had better prognosis than HPV-independent tumors, but not all reached statistical significance. Sixteen studies[5–20] looked at the prognostic significance of HPV on PFS: 23 analyses (14 univariate, 9 multivariate)[5,7–9,11,13–15,17,18,20] found that HPV was prognostic for PFS, while 15 analyses (11 univariate, 4 multivariate)[5–7,9,12,16,17,19,20] did not. Three analyses[10,13] did not report the significance. Nineteen 19 studies[5–9,11–15,17–25] assessed the prognostic significance of HPV on OS, with a total of 46 different analyses: 18 analyses (13 univariate, 5 multivariate)[5,8,11,13–15,18,21,23,24] found that HPV was prognostic for OS, and 28 analyses (17 univariate, 10 multivariate)[5–7,9,12,13,15,17–20,22,24,25] did not. One analysis[13] did not report the significance and 1 analysis[22] reported that HPV was not prognostic, but not the method of analysis.

To date, only one study has used HPV RNA in-situ hybridization. In Allo et al,[26] HPV RNA ISH was prognostic for PFS (in univariate and multivariate analyses) but not for OS. RNA ISH is a relatively new methodology for HPV assessment, which was been officially endorsed by the College of American Pathologists this year in 2020.[26] HPV RNA ISH circumvents the need for PCR equipment and reagents, and tests for HPV E6/E7 mRNA by direct visualization on glass slides, thereby avoiding confusing with environmental contamination and transient HPV infections. It has been shown to have higher sensitivity and specificity compared to both HPV DNA ISH and PCR.[26] It is possible that use of HPV RNA ISH for future prognostication studies may provide enhanced clarity, with more consistent results, in assessing the prognostic role of HPV on patient survival.

The clinicopathologic parameters that may explain the prognostic differences between HPV-associated and HPV-independent VSCC varies between studies. Some studies have found that HPV-associated SCC present at younger ages,[9,11,14,15,17] lower FIGO stages,[7,16] smaller tumor sizes[9,11,14] lower depth of invasion,[19] less margin positivity[15,19] and less nodal metastases.[16] In addition, it is very plausible that the

higher association with basaloid and warty morphology in HPV-driven lesions,[6,21] has allowed for earlier recognition and treatment of HPV-associated vulvar SCC and its precursor lesions, which contrasts the notorious under-recognition and delayed treatment of HPV-independent squamous precursor lesions.[27] In Bigsby et al 24 of 97 women had biopsies 6 months before the presentation of VSCC. In the 47 biopsies available for review, 18 harboured dVIN and of those, 14 were previously unrecognized.[27]

*Vulvar Intraepithelial Neoplasia (VIN)*

The squamous precursor lesion for the HPV-associated pathway is termed usual-type vulvar intraepithelial neoplasia (uVIN) or high-grade squamous intraepithelial lesion (HSIL), as per The Lower Anogenital Squamous Terminology (LAST) Project.[28] As alluded to above, uVIN/HSIL has conspicuous cytologic atypia, characterized by nuclear pleomorphism, hyperchromasia, suprabasilar mitoses, high nuclear:cytoplasmic ratios and dysmaturation. Due to its relative commonality, uVIN/HSIL can also be seen incidentally in cases of HPV-independent SCC.[6] In contrast, the features of dVIN, historically described as "intraepithelial carcinoma of simplex type" by Abell et al in the 1960s,[29,30] is much more difficult to recognize. dVIN shows epidermal hyperplasia with elongated rete ridges, basal atypia, dyskeratotic cells and hypereosinophilic cytoplasmic tincture.[31] Pathologists should be wary, because the features of dVIN can often be more subtle than described in textbooks, presenting as non-acanthotic lesions or lesions where the atypia is obscured by superimposed inflammation.[32] p53 IHC can be helpful in distinguishing dVIN from reactive lesions, if a null, cytoplasmic or parabasal/diffuse pattern of staining is seen.[33,34] A basal-overexpression pattern will not reliably distinguish dVIN from reactive lesions.[35]

Three studies have shown that the risk and rate of progression to invasive SCC is higher and faster in dVIN compared to uVIN/HSIL. The risk of progression to VSCC for uVIN/HSIL versus dVIN were reported to be 0% vs 86%,[36] 15% vs 80% [18] and 5.7% vs 32.8%.[37] In the most sizeable series to date ($n=1148$), the 10-year cumulative risk of VSCC was 9.7% for uVIN/HSIL and 50% for dVIN.[38] The time-frame for progression for uVIN/HSIL versus dVIN has been 41.4 months vs 22.8 months ($p=0.005$)[37] and 4.1 years vs 1.4 years (although not significant, $p=0.449$).[38] OS, DSS and PFS have been reported to be worse in dVIN than uVIN/HSIL.[6,36] In a study by McAlpine *et al*, 23/25 women with dVIN only or dVIN/SCC had progressed to or recurred with SCC, with a median progression time of 1.9 years. None of the uVIN/HSIL progressed in the study time-period (median follow up was 5 years). The vast majority (22/25) of patients died of disease, with a median overall survival of 3.4 years.[36]

*Patient Age*

Thirty-eight studies evaluated the prognostic value of age on survival, using different methods of analyses (categorical and continuous variables) (Table 2). Twenty-eight studies[6,8–10,13,15–19,39–56] yielding 50 different analyses found that age was prognostic for PFS (9 univariate,[6,9,13,18,19,39,46,48,50] 9 multivariate[13,18,19,48,53]), though more studies found that age was not prognostic (20 univariate,[15,18,40,42–45,47,49,51,52,55,56] 11 multivariate,[8,9,15,18,41,42,44,47,50,54] 1[10] method of analysis not reported). Similarly, 32 studies (70 different analyses) found mixed results for age and overall survival [prognostic in 43 (22 univariate,[6,9,13,15,17,18,21,24,39,43,48,50,57–61] 21 multivariate[8,13,15,17–19,48,50,54,58,60–63]), not prognostic in 35 (27 univariate,[9,10,15,18,19,21,25,40–42,45,46,48,52,55,56,60] 8 multivariate[9,15,18,21,25,60])].

*Tumor stage*

In 30 studies, the association between stage and survival, was stronger for OS than for PFS. Varied staging methods were used, although the FIGO (The International Federation of Gynecology and Obstetrics) staging system was the most common. In Table 3, 59% of analyses (18 univariate[6,13,16–18,42,45,46,50,57,64–66] and 15 multivariate[9,13,15,17,50,54,66]) found stage to be prognostic for PFS, while 41% of analyses (11 univariate[9,15,19,20,43,44,47,51,52,55,56,67] and 12 multivariate[8,9,16,18,42,44,53,65]) did not. In contrast, for overall survival, 55% of analyses were prognostic (27 univariate[6,13,18,21,24,42,45,46,48,50,51,59–61,65,66,68] and 12 analyses[8,9,13,15,18,21,50,54,61,62,66]) while 45% of analyses (24 univariate[9,15,17,19,43,52,55,56] and 7 multivariate[9,15,18,42,61,65]) were not. The vast majority of analyses (87%) found stage to be an adverse prognosticator, but not all met the threshold for statistical significance.

*Tumor grade*

Pathologic grade is divided into well-differentiated (G1), moderately differentiated (G2), poorly differentiated (G3) and undifferentiated (G4) by FIGO,[1] and G1, G2, G3 by the American Joint Committee on Cancer (AJCC).[69] Like many squamous cell carcinomas in the body, there are no specific criteria and grade is based essentially on the pathologists' gestalt, evaluating features such as degree of keratinization, intercellular bridges, nuclear pleomorphism, mitotic activity and pattern of invasion.[70] The Gynecology Oncology Group (GOG) assigns grade by the proportion of the tumor that is undifferentiated (UC) (seen as small cells with scant cytoplasm, infiltrating as small nests or cords): G1: no UC; G2: 1–30% UC; G3: 31–50% UC; G4: >50% UC.[71]

Due to such inconsistencies in grading, it is not surprising that only a minority of studies found tumor grade to be prognostic for PFS (7 analyses[39,42,46,48,66] were significant; 35 analyses[8,19,40,42–45,49,50,52,54–56,65–67,72] were not significant) or OS (19 analyses[39,46,48,57,59,63,66] were significant; 33[8,19,39–43,45,50,52,54–56,59,61,65,66,72] were not significant) (Table 4).

Other grading schemes such as tumor budding, has been trialed in cervical SCC,[73] but not yet in vulvar SCC.

*Tumor size and depth of invasion*

The 2018 FIGO guidelines and AJCC 8th edition use a tumor size of 2 cm and depth of invasion of 1 mm as cut-off values to distinguish between stage IA and IB disease.[1,69]

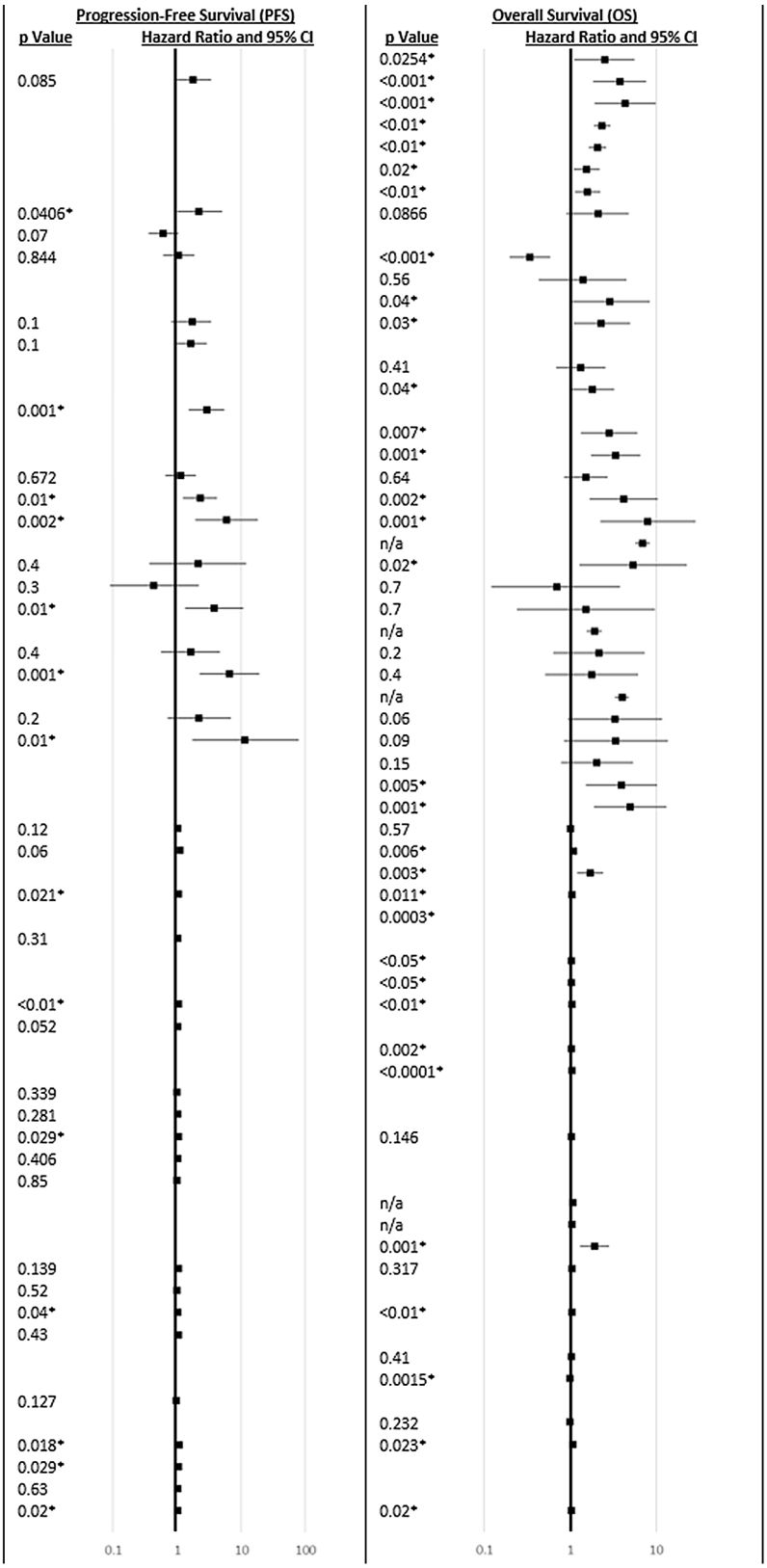
Twenty-eight studies assessed the prognostic value of tumor size on survival (Table 5). Using a tumor size cut-off of 2 cm in 4 studies, 2 studies found that tumor size was prognostic for OS and none for PFS.[6,49,61,72] Even though 2 cm is the cut-off in formal staging systems, a greater number of studies (12 studies) used 4 cm as a cut-off value. In these studies, tumor size was prognostic for PFS (6 of 7 univariate analyses, 2 of 6 multivariate analyses) and OS (4 of 9 univariate analyses, 1 multivariate analyses).[10,40–42,45,47,52,57,60,64,65,74] In 3 studies assessing tumor size as a continuous variable, only 1 study found tumor size was prognostic for both PFS and OS in the multivariate analysis.[41,56,67]

Depth of invasion (DOI) is measured from the epithelial-stromal junction of the adjacent most superficial dermal papilla to the deepest point of the invasive tumor. Tumors with a DOI <1 mm, also known as microinvasive carcinoma,[28] have a negligible risk of lymph node metastases (<1 %) and low local recurrence rates (~5%)[75–78]. For these tumors, radical wide local excision should be sufficient, without the need for groin node dissection.[1] Tumors invading 1–3 mm had 6% risk and 3–5 mm have 20% risk of lymph node spread.[79] However, as in many sites in the gynecologic tract, depth of invasion can be difficult to measure and quantify uniformly amongst pathologists.[80] Yoonessi *et al* proposed using Clark levels instead of DOI, but this has not been widely studied or adopted in practice.[81] It is important to acknowledge that tumor thickness is measured from the bottom of the granular layer to the deepest point of invasion and is not used as an element in staging.[82]

The studies evaluating DOI and the impact on recurrence and

**Table 2**
Patient age and relationship with PFS and OS.

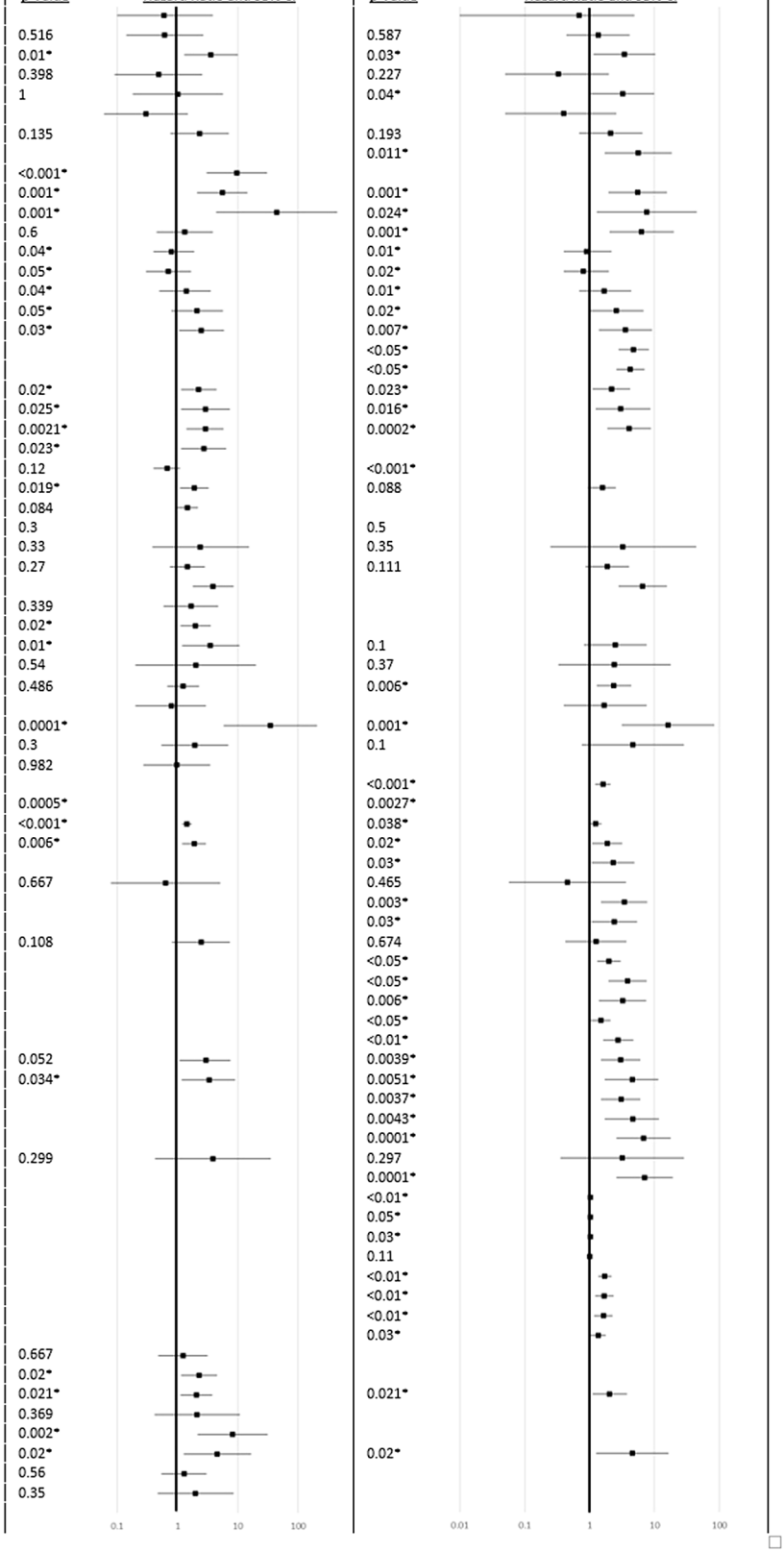
| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) p Value | Overall Survival (OS) p Value |
|---|---|---|---|---|
| Sznurkowski et al. 2016 | >60yo | univariate | | 0.0254* |
| Stefansson et al. 2019 | ≥60yo | univariate | 0.085 | <0.001* |
| Stefansson et al. 2019 | ≥60yo | multivariate | | <0.001* |
| Xanthopoulos et al. 2018 | ≥68yo | univariate | | <0.01* |
| Xanthopoulos et al. 2018 | ≥68yo | multivariate unadj | | <0.01* |
| Xanthopoulos et al. 2018 | ≥68yo | multivariate adj | | 0.02* |
| Xanthopoulos et al. 2018 | ≥68yo | multivariate imp | | <0.01* |
| Nicoletto et al. 2010 | >70yo | univariate | 0.0406* | 0.0866 |
| Stankevica et al. 2012 | >70yo | univariate | 0.07 | |
| Czogalla et al. 2020 | ≥70yo | multivariate | 0.844 | <0.001* |
| Imoto et al. 2016 | >70yo | multivariate DSS | | 0.56 |
| Imoto et al. 2016 | >70yo | multivariate OS | | 0.04* |
| Seebacher et al. 2012 | >70yo | multivariate | 0.1 | 0.03* |
| Panici et al. 2015 | ≥75yo | univariate PFS | 0.1 | |
| Panici et al. 2015 | ≥75yo | univariate DSS | | 0.41 |
| Panici et al. 2015 | ≥75yo | univariate OS | | 0.04* |
| Panici et al. 2015 | ≥75yo | multivariate PFS | 0.001* | |
| Panici et al. 2015 | ≥75yo | multivariate DSS | | 0.007* |
| Panici et al. 2015 | ≥75yo | multivariate OS | | 0.001* |
| Laliscia et al. 2019 | >76yo | univariate | 0.672 | 0.64 |
| Alonso et al. 2011 | ≥78yo | univariate | 0.01* | 0.002* |
| Wakeham et al. 2017 | >80yo | univariate | 0.002* | 0.001* |
| Rauh-Hain et al. 2014 | >80yo | multivariate | | n/a |
| Wakeham et al. 2017 | >80yo | multivariate | 0.4 | 0.02* |
| Wakeham et al. 2017 | 50-59yo | univariate | 0.3 | 0.7 |
| Wakeham et al. 2017 | 50-59yo | multivariate | 0.01* | 0.7 |
| Rauh-Hain et al. 2014 | 50-64yo | multivariate | | n/a |
| Wakeham et al. 2017 | 60-69yo | univariate | 0.4 | 0.2 |
| Wakeham et al. 2017 | 60-69yo | multivariate | 0.001* | 0.4 |
| Rauh-Hain et al. 2014 | 65-79yo | multivariate | | n/a |
| Wakeham et al. 2017 | 70-79yo | univariate | 0.2 | 0.06 |
| Wakeham et al. 2017 | 70-79yo | multivariate | 0.01* | 0.09 |
| Dong et al. 2015 | 2nd quartile | univariate | | 0.15 |
| Dong et al. 2015 | 3rd quartile | univariate | | 0.005* |
| Dong et al. 2015 | 4th quartile | univariate | | 0.001* |
| Bogani et al. 2017 | continuous | univariate | 0.12 | 0.57 |
| Collarino et al. 2019 | continuous | univariate | 0.06 | 0.006* |
| Dhakal et al. 2013 | continuous | univariate | | 0.003* |
| Dohopolski et al. 2019 | continuous | univariate | 0.021* | 0.011* |
| Dong et al. 2015 | continuous | univariate | | 0.0003* |
| Gasimli et al. 2018 | continuous | univariate | 0.31 | |
| Hinten et al. 2015 | continuous | univariate 5-yr | | <0.05* |
| Hinten et al. 2015 | continuous | univariate 10-yr | | <0.05* |
| Lee et al. 2016 | continuous | univariate | <0.01* | <0.01* |
| McAlpine et al. 2017 | continuous | univariate PFS | 0.052 | |
| McAlpine et al. 2017 | continuous | univariate DSS | | 0.002* |
| McAlpine et al. 2017 | continuous | univariate OS | | <0.0001* |
| Nooij et al. 2015 | continuous | univariate | 0.339 | |
| Nooij et al. 2016 | continuous | univariate | 0.281 | |
| Weberpals et al. 2017 | continuous | univariate | 0.029* | 0.146 |
| Baiocchi et al. 2015 | continuous | multivariate | 0.406 | |
| Bogani et al. 2017 | continuous | multivariate | 0.85 | |
| Courtney-Brooks et al. 2010 | continuous | multivariate DSS | | n/a |
| Courtney-Brooks et al. 2010 | continuous | multivariate OS | | n/a |
| Dhakal et al. 2013 | continuous | multivariate | | 0.001* |
| Dohopolski et al. 2019 | continuous | multivariate | 0.139 | 0.317 |
| Gasimli et al. 2018 | continuous | multivariate | 0.52 | |
| Lee et al. 2016 | continuous | multivariate | 0.04* | <0.01* |
| McAlpine et al. 2017 | continuous | multivariate PFS | 0.43 | |
| McAlpine et al. 2017 | continuous | multivariate DSS | | 0.41 |
| McAlpine et al. 2017 | continuous | multivariate OS | | 0.0015* |
| Nooij et al. 2015 | continuous | multivariate | 0.127 | |
| Trietsch et al. 2014 | continuous | multivariate | | 0.232 |
| Weberpals et al. 2017 | continuous | multivariate | 0.018* | 0.023* |
| Woelber et al. 2011 | continuous | multivariate | 0.029* | |
| Woelber et al. 2016† | continuous | multivariate PFS | 0.63 | |
| Woelber et al. 2016† | continuous | multivariate DFS | 0.02* | 0.02* |




*Abbreviations: CI = confidence interval; PFS = progression-free survival; DSS = disease-specific survival; DFS = disease-free survival; OS = overall survival; unadj = unadjusted, adj = adjusted, imp = imputed; * significant p value <0.05; † analysis was done with margins <8mm. analyses done with continuous margins had very similar values*

**Table 3**
Tumor stage and relationship with PFS and OS.

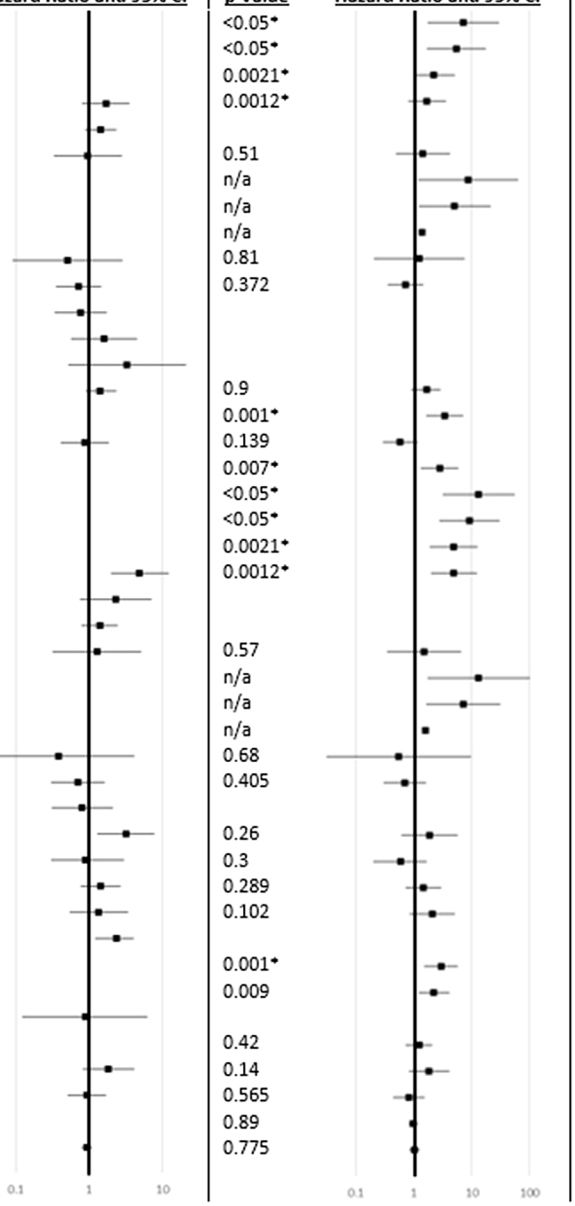
| Study | Staging | Uni-/Multi-Variate | Progression Free Survival (PFS) p Value | Overall Survival (OS) p Value |
|---|---|---|---|---|
| Collarino et al. 2019 | FIGO (unspecified) | univariate | | |
| Dohopolski et al. 2019 | FIGO 2009 | univariate | 0.516 | 0.587 |
| Wakeham et al. 2017 | FIGO 2009 | univariate | 0.01* | 0.03* |
| Dohopolski et al. 2019 | FIGO 2009 | multivariate | 0.398 | 0.227 |
| Wakeham et al. 2017 | FIGO 2009 | multivariate | 1 | 0.04* |
| Collarino et al. 2019 | FIGO (unspecified) | univariate | | |
| Dohopolski et al. 2019 | FIGO 2009 | univariate | 0.135 | 0.193 |
| Goes et al. 2012† | FIGO 2009 | univariate | | 0.011* |
| Minar et al. 2018 | FIGO (unspecified) | univariate | <0.001* | |
| Wakeham et al. 2017 | FIGO 2009 | univariate | 0.001* | 0.001* |
| Dohopolski et al. 2019 | FIGO 2009 | multivariate | 0.001* | 0.024* |
| Wakeham et al. 2017 | FIGO 2009 | multivariate | 0.6 | 0.001* |
| Lee et al. 2016 | FIGO 2009 | univariate | 0.04* | 0.01* |
| Lee et al. 2016 | FIGO 2009 | multivariate | 0.05* | 0.02* |
| Lee et al. 2016 | FIGO 2009 | univariate | 0.04* | 0.01* |
| Lee et al. 2016 | FIGO 2009 | multivariate | 0.05* | 0.02* |
| Bogani et al. 2017 | FIGO 2009 | univariate | 0.03* | 0.007* |
| Hinten et al. 2015 | FIGO 1988/2009 | univariate 5-yr | | <0.05* |
| Hinten et al. 2015 | FIGO 1988/2009 | univariate 10-yr | | <0.05* |
| Laliscia et al. 2019 | FIGO (unspecified) | univariate | 0.02* | 0.023* |
| Long et al. 2019 | FIGO (unspecified) | univariate | 0.025* | 0.016* |
| Nicoletto et al. 2010 | FIGO (unspecified) | univariate | 0.0021* | 0.0002* |
| Nooij et al. 2016 | FIGO 2009 | univariate | 0.023* | |
| Stankevica et al. 2012 | FIGO (unspecified) | univariate | 0.12 | <0.001* |
| Stefansson et al. 2019 | FIGO (unspecified) | univariate | 0.019* | 0.088 |
| te Grootenhuis et al. 2019 | FIGO 2009 | univariate | 0.084 | |
| Zannoni et al. 2011 | FIGO 2009 | univariate | 0.3 | 0.5 |
| Bogani et al. 2017 | FIGO 2009 | multivariate | 0.33 | 0.35 |
| Long et al. 2019 | FIGO (unspecified) | multivariate | 0.27 | 0.111 |
| Nicoletto et al. 2010 | FIGO (unspecified) | multivariate | | |
| Nooij et al. 2016 | FIGO 2009 | multivariate | 0.339 | |
| Stefansson et al. 2019 | FIGO (unspecified) | multivariate | 0.02* | |
| Bogani et al. 2017 | FIGO 2009 | univariate | 0.01* | 0.1 |
| Bogani et al. 2017 | FIGO 2009 | multivariate | 0.54 | 0.37 |
| Czogalla et al. 2020† | FIGO (unspecified) | multivariate | 0.486 | 0.006* |
| Collarino et al. 2019 | FIGO (unspecified) | univariate | | |
| Wakeham et al. 2017 | FIGO 2009 | univariate | 0.0001* | 0.001* |
| Wakeham et al. 2017 | FIGO 2009 | multivariate | 0.3 | 0.1 |
| Nooij et al. 2015 | FIGO 2009 | univariate | 0.982 | |
| Sznurkowski et al. 2016 | FIGO 2009 | univariate | | <0.001* |
| Wakeham et al. 2017 | FIGO 2009 | univariate | 0.0005* | 0.0027* |
| Polterauer et al. 2019 | FIGO (unspecified) | multivariate | <0.001* | 0.038* |
| Seebacher et al. 2012 | FIGO 2009 | multivariate | 0.006* | 0.02* |
| Dong et al. 2015 | Stage | univariate | | 0.03* |
| Weberpals et al. 2017 | Stage | univariate | 0.667 | 0.465 |
| Dong et al. 2015 | Stage | multivariate | | 0.003* |
| Dong et al. 2015 | Stage | univariate | | 0.03* |
| Weberpals et al. 2017 | Stage | univariate | 0.108 | 0.674 |
| Courtney-B et al. 2010† | Stage | multivariate OS | | <0.05* |
| Courtney-B et al. 2010† | Stage | multivariate DSS | | <0.05* |
| Dong et al. 2015 | Stage | multivariate | | 0.006* |
| Courtney-B et al. 2010† | Stage | multivariate OS | | <0.05* |
| Courtney-B et al. 2010† | Stage | multivariate DSS | | <0.01* |
| McAlpine et al. 2017 | Stage | univariate | 0.052 | 0.0039* |
| McAlpine et al. 2017 | Stage | multivariate | 0.034* | 0.0051* |
| McAlpine et al. 2017 | Stage | multivariate | | 0.0037* |
| McAlpine et al. 2017 | Stage | multivariate | | 0.0043* |
| Dong et al. 2015 | Stage | univariate | | 0.0001* |
| Weberpals et al. 2017 | Stage | univariate | 0.299 | 0.297 |
| Dong et al. 2015 | Stage | multivariate | | 0.0001* |
| Xanthopoulos et al. 2018 | AJCC 6th | univariate | | <0.01* |
| Xanthopoulos et al. 2018 | AJCC 6th | multivariate | | 0.05* |
| Xanthopoulos et al. 2018 | AJCC 6th | multivariate | | 0.03* |
| Xanthopoulos et al. 2018 | AJCC 6th | multivariate | | 0.11 |
| Xanthopoulos et al. 2018 | AJCC 6th | univariate | | <0.01* |
| Xanthopoulos et al. 2018 | AJCC 6th | multivariate | | <0.01* |
| Xanthopoulos et al. 2018 | AJCC 6th | multivariate | | <0.01* |
| Xanthopoulos et al. 2018 | AJCC 6th | multivariate | | 0.03* |
| Woelber et al. 2011 | TNM 6th | multivariate | 0.667 | |
| Woelber et al. 2016†† | TNM 6th | multivariate PFS | 0.02* | |
| Woelber et al. 2016†† | TNM 6th | multivariate OS | 0.021* | 0.021* |
| Woelber et al. 2011 | TNM 6th | multivariate | 0.369 | |
| Woelber et al. 2016†† | TNM 6th | multivariate PFS | 0.002* | |
| Woelber et al. 2016†† | TNM 6th | multivariate O | 0.02* | 0.02* |
| Gasimli et al. 2018 | TNM 7th | univariate | 0.56 | |
| Gasimli et al. 2018 | TNM 7th | multivariate | 0.35 | |




*Abbreviations: CI = confidence interval; PFS = progression-free survival; OS = overall survival; DFS = disease-free survival; DSS = disease-specific survival; * significant p-value <0.05; † hazard ratios were flipped; †† analyzed with margins <8mm. analysis with continuous margins had similar values; Δ referenced to change in stage*

**Table 4**
Tumor grade and relationship with PFS and OS.

| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) p Value | Progression-Free Survival (PFS) Hazard Ratio and 95% CI | Overall Survival (OS) p Value | Overall Survival (OS) Hazard Ratio and 95% CI |
|---|---|---|---|---|---|---|
| Hinten et al. 2015 | G1 vs G2 | univariate 5-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | G1 vs G2 | univariate 10-yr DSS | | | <0.05* | |
| Nicoletto et al. 2010 | G1 vs G2 | univariate OS | | | 0.0021* | |
| Nicoletto et al. 2010 | G1 vs G2 | univariate PFS | 0.0012* | | 0.0012* | |
| te Grootenhuis et al. 2019 | G1 vs G2 | univariate | 0.148 | | | |
| Weberpals et al. 2017 | G1 vs G2 | univariate | 0.946 | | 0.51 | |
| Hinten et al. 2015 | G1 vs G2 | multivariate 5-yr DSS | | | n/a | |
| Hinten et al. 2015 | G1 vs G2 | multivariate 10-yr DSS | | | n/a | |
| Rauh-Hain et al. 2014 | G1 vs G2 | multivariate | | | n/a | |
| Weberpals et al. 2017 | G1 vs G2 | multivariate | 0.446 | | 0.81 | |
| Woelber et al. 2016 | G1 vs G2 | multivariate OS/DFS† | 0.372 | | 0.372 | |
| Woelber et al. 2016 | G1 vs G2 | multivariate RR† | 0.535 | | | |
| Gasimli et al. 2018 | G1 vs G2 vs G3 | univariate | 0.37 | | | |
| Gasimli et al. 2018 | G1 vs G2 vs G3 | multivariate | 0.206 | | | |
| Seebacher et al. 2012 | G1 vs G2 vs G3 | multivariate | 0.2 | | 0.9 | |
| Sznurkowski et al. 2016 | G1 vs G2/3 | univariate | | | 0.001* | |
| Czogalla et al. 2020 | G1 vs G2/3 | multivariate | 0.728 | | 0.139 | |
| Sznurkowski et al. 2016 | G1 vs G2/3 | multivariate | | | 0.007* | |
| Hinten et al. 2015 | G1 vs G3 | univariate 5-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | G1 vs G3 | univariate 10-yr DSS | | | <0.05* | |
| Nicoletto et al. 2010 | G1 vs G3 | univariate OS | | | 0.0021* | |
| Nicoletto et al. 2010 | G1 vs G3 | univariate PFS | 0.0012* | | 0.0012* | |
| Pleunis et al. 2018 | G1 vs G3 | univariate | 0.145 | | | |
| te Grootenhuis et al. 2019 | G1 vs G3 | univariate | 0.242 | | | |
| Weberpals et al. 2017 | G1 vs G3 | univariate | 0.725 | | 0.57 | |
| Hinten et al. 2015 | G1 vs G3 | multivariate 5-yr DSS | | | n/a | |
| Hinten et al. 2015 | G1 vs G3 | multivariate 10-yr DSS | | | n/a | |
| Rauh-Hain et al. 2014 | G1 vs G3 | multivariate | | | n/a | |
| Weberpals et al. 2017 | G1 vs G3 | multivariate | 0.426 | | 0.68 | |
| Woelber et al. 2016 | G1 vs G3 | multivariate OS/DFS† | 0.405 | | 0.405 | |
| Woelber et al. 2016 | G1 vs G3 | multivariate RR† | 0.667 | | | |
| Bogani et al. 2017 | G1/2 vs G3 | univariate | 0.01* | | 0.26 | |
| Collarino et al. 2019 | G1/2 vs G3 | univariate | 0.8 | | 0.3 | |
| Laliscia et al. 2019 | G1/2 vs G3 | univariate | 0.264 | | 0.289 | |
| Long et al. 2019 | G1/2 vs G3 | univariate | 0.521 | | 0.102 | |
| Panici et al. 2015 | G1/2 vs G3 | univariate DFS | 0.005* | | | |
| Panici et al. 2015 | G1/2 vs G3 | univariate DSS | | | 0.001* | |
| Panici et al. 2015 | G1/2 vs G3 | univariate OS | | | 0.009 | |
| Bogani et al. 2017 | G1/2 vs G3 | multivariate | 0.92 | | | |
| Le et al. 2018 | G1/2 vs G3 | multivariate OD | | | 0.42 | |
| Le et al. 2018 | G1/2 vs G3 | multivariate DM/DSD | 0.14 | | 0.14 | |
| Long et al. 2019 | G1/2 vs G3 | multivariate | 0.813 | | 0.565 | |
| Xanthopoulos et al. 2018 | I vs II vs III vs IV | univariate | | | 0.89 | |
| Polterauer et al. 2019 | n/a | multivariate | 0.43 | | 0.775 | |




*Abbreviations: CI = confidence interval; G = grade; DSS = disease-specific survival; OS = overall survival; RR = recurrence rate; DM = distant metastasis; DSD = disease-specific death; OD = overall death; RFS = recurrence-free survival; * significant p value <0.05; † analyzed with margins <8mm analysis with continuous margins had similar values.*

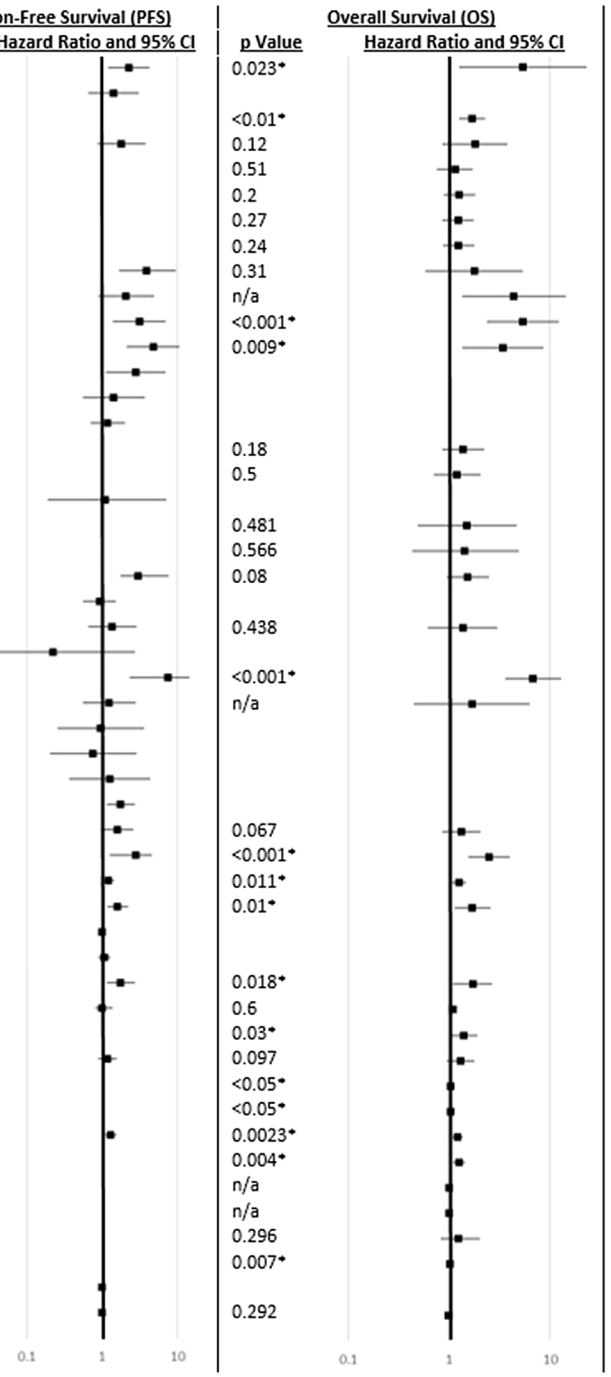
survival have used highly variable cut-off values (Table 6). In 22 studies, DOI was prognostic for PFS (5 univariate analyses[40,42,46,64,65] and 6 multivariate analyses[42,46,54,65,72]) but more often it was not significant (14 univariate analyses[6,16,19,20,43,47,49,52,55,56,67,83] and 7 multivariate analyses[19,40,47,54,56,83]). The levels of significance did not change drastically using increasing DOI cut-offs and often nodal recurrences were not assessed separately from local recurrences. Similar results were observed for overall survival (prognostic in 7 univariate analyses[6,40,46,56,59,65] and 3 multivariate analyses[40,65,72]; not prognostic in 14 univariate analyses[19,24,41–43,52,55] and 7 multivariate analyses[6,19,54,56,59]).

*Patterns of Invasion*

In 1996, Ambros *et al.* studied 51 VSCC and found that a prominent fibromyxoid stromal response (>25%) was associated with a significantly older age group, clitoral involvement, extensive lymph node metastases and poorer overall survival.[84] The fibromyxoid response was seen as extracellular matrix made of immature collagen and fibroblasts surrounding the edge of the tumor cells, which often have a blue stromal hue.[84] This feature was also analysed in a cohort of 184 Brazilian women by Pinto *et al*, who found this to be an adverse prognosticator by univariate analysis but not by multivariate analysis.[85]

**Table 5**
Tumor size and relationship with PFS and OS.

| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) p Value | Progression-Free Survival (PFS) Hazard Ratio and 95% CI | Overall Survival (OS) p Value | Overall Survival (OS) Hazard Ratio and 95% CI |
|---|---|---|---|---|---|---|
| Alonso et al. 2011 | ≥2cm | univariate | 0.217 | | 0.023* | |
| Pleunis et al. 2018 | ≥2cm | univariate | 0.372 | | | |
| Xanthopoulos et al. 2018 | >2cm | univariate | | | <0.01* | |
| Le et al. 2018 | ≥2cm | multivariate DSS | 0.12 | | 0.12 | |
| Le et al. 2018 | ≥2cm | multivariate OD | | | 0.51 | |
| Xanthopoulos et al. 2018 | >2cm | multivariate unadj | | | 0.2 | |
| Xanthopoulos et al. 2018 | >2cm | multivariate adj | | | 0.27 | |
| Xanthopoulos et al. 2018 | >2cm | multivariate imp | | | 0.24 | |
| Bogani et al. 2017 | >4cm | univariate | 0.002* | | 0.31 | |
| Hay et al. 2016 | >4cm | univariate | n/a | | n/a | |
| Laliscia et al. 2019 | >4cm | univariate | 0.005* | | <0.001* | |
| Long et al. 2019 | >4cm | univariate | <0.001* | | 0.009* | |
| Minar et al. 2018 | ≥4cm | univariate | 0.027* | | | |
| Nooij et al. 2016 | >4cm | univariate | 0.456 | | | |
| Baiocchi et al. 2013 | >4cm | multivariate PFS | 0.49 | | | |
| Baiocchi et al. 2013 | >4cm | multivariate OS | | | 0.18 | |
| Baiocchi et al. 2013 | >4cm | multivariate DSS | | | 0.5 | |
| Bogani et al. 2017 | >4cm | multivariate | 0.86 | | | |
| Imoto et al. 2016 | >4cm | multivariate OS | | | 0.481 | |
| Imoto et al. 2016 | >4cm | multivariate DSS | | | 0.566 | |
| Long et al. 2019 | >4cm | multivariate | 0.022* | | 0.08 | |
| te Grootenhuis et al. 2019 | ≥4cm | multivariate | 0.763 | | | |
| Tu et al. 2017 | ≥4cm | multivariate | 0.41 | | 0.438 | |
| Pleunis et al. 2018 | ≥5cm | univariate | 0.307 | | | |
| Aragona et al. 2014 | ≥8cm | multivariate | <0.001* | | <0.001* | |
| Hay et al. 2016 | >2-4cm | univariate | n/a | | n/a | |
| Nooij et al. 2015 | <2 vs >4cm | univariate | 0.947 | | | |
| Nooij et al. 2015 | <2 vs 2-4cm | univariate | 0.684 | | | |
| Nooij et al. 2015 | 2-4 vs >4cm | univariate | 0.717 | | | |
| Zannoni et al. 2011 | >2-<5 vs ≥ 5cm | univariate | 0.05* | | | |
| Aragona et al. 2014 | 4-<6cm | multivariate | 0.197 | | 0.067 | |
| Aragona et al. 2014 | 6-<8cm | multivariate | <0.001* | | <0.001* | |
| Weberpals et al. 2017 | Δ1cm | univariate | 0.03* | | 0.011* | |
| Weberpals et al. 2017 | Δ1cm | multivariate | 0.004* | | 0.01* | |
| te Grootenhuis et al. 2019 | continuous | univariate | 0.666 | | | |
| Baiocchi et al. 2015 | continuous | multivariate | 0.41 | | | |
| Zannoni et al. 2011 | continuous | multivariate | 0.009* | | 0.018* | |
| Collarino et al. 2019 | n/a | univariate | 0.9 | | 0.6 | |
| Dhakal et al. 2013 | n/a | univariate | | | 0.03* | |
| Dohopolski et al. 2019 | n/a | univariate | 0.291 | | 0.097 | |
| Hinten et al. 2015 | n/a | univariate 5-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | n/a | univariate 10-yr DSS | | | <0.05* | |
| McAlpine et al. 2017 | n/a | univariate OS | 0.0064* | | 0.0023* | |
| McAlpine et al. 2017 | n/a | univariate DSS | | | 0.004* | |
| Courtney-B et al. 2010 | n/a | multivariate OS | | | n/a | |
| Courtney-B et al. 2010 | n/a | multivariate DSS | | | n/a | |
| Dohopolski et al. 2019 | n/a | multivariate | | | 0.296 | |
| Trietsch et al. 2014 | n/a | multivariate | | | 0.007* | |
| Woelber et al. 2016 | n/a | multivariate | 0.373 | | | |
| Woelber et al. 2016 | n/a | multivariate OS/DFS | 0.292 | | 0.292 | |



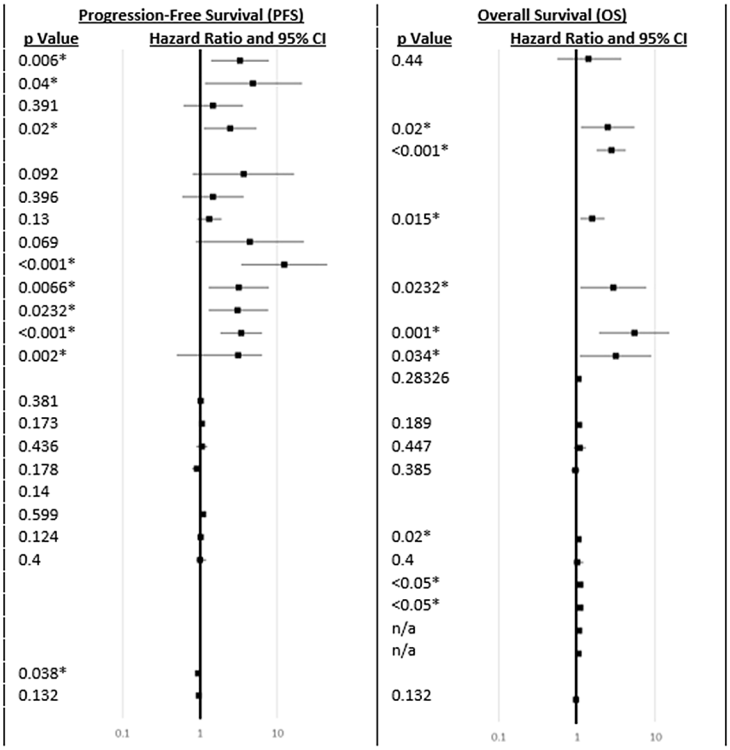

*Abbreviations: CI = confidence interval; DSS = disease-specific survival; OS = overall survival; OD = overall death; RR = recurrence rate; DM = distant metastasis; unadj = unadjusted; adj = adjusted; imp = imputed; RFS = recurrence-free survival; * significant p value <0.05*

More recently, Jeffus *et al* found that the fibromyxoid stromal response was associated with greater depth of invasion, lymph node involvement, extranodal extension and perineural invasion.[86] This was observed irrespective of the pattern of invasion and in the presence of any fibromyxoid stromal response, without the minimum 25% cut-off as originally reported by Ambros *et al*[86]

As mentioned briefly above, pattern of tumor invasion has also been sparsely studied. Jeffus *et al* categorized the pattern of tumors as solid/pushing, nested or infiltrative. The infiltrative pattern had cells invading as strands, cords or single cells in a spray-like pattern. The infiltrative pattern was strongly associated with a fibromyxoid stromal response and greater DOI, but not with tumor size or lymph node status.[86] Hay *et al* and te Grootenhius *et al* found no statistical differences in different invasion patterns (pushing versus infiltrative; spray, invasive versus confluent).[10,67] Again, other patterns and stromal changes, such as tumor-budding and tumor-infiltrating lymphocytes (raised here due to a recent reports of potential immune checkpoint inhibitor therapy[8,87]), has not been studied in the vulva.

**Table 6**
Tumor depth of invasion and relationship with PFS and OS.

| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) | | Overall Survival (OS) | |
|---|---|---|---|---|---|---|
| | | | p Value | Hazard Ratio and 95% CI | p Value | Hazard Ratio and 95% CI |
| Bogani et al. 2017 | >2mm | univariate | 0.006* | | 0.44 | |
| Bogani et al. 2017 | >2mm | multivariate | 0.04* | | | |
| Pleunis et al. 2018 | ≥2.5mm | univariate | 0.391 | | | |
| Le et al. 2018 | ≥3mm | multivariate DSS | 0.02* | | 0.02* | |
| Le et al. 2018 | ≥3mm | multivariate OS | | | <0.001* | |
| Nooij et al. 2015 | >4mm | univariate | 0.092 | | | |
| Nooij et al. 2016 | ≥4mm | univariate | 0.396 | | | |
| Aragona et al. 2014 | >4mm | multivariate | 0.13 | | 0.015* | |
| Nooij et al. 2015 | >4mm | multivariate | 0.069 | | | |
| Minar et al. 2018 | >5mm | univariate | <0.001* | | | |
| Nicoletto et al. 2010 | >9mm | univariate | 0.0066* | | 0.0232* | |
| Nicoletto et al. 2010 | >9mm | multivariate | 0.0232* | | | |
| Long et al. 2019 | >20mm | univariate | <0.001* | | 0.001* | |
| Long et al. 2019 | >20mm | multivariate | 0.002* | | 0.034* | |
| Sznurkowski et al. 2016 | continuous | univariate | | | 0.28326 | |
| te Grootenhuis et al. 2019 | continuous | univariate | 0.381 | | | |
| Weberpals et al. 2017 | continuous | univariate | 0.173 | | 0.189 | |
| Weberpals et al. 2017 | continuous | multivariate | 0.436 | | 0.447 | |
| Zannoni et al. 2011 | continuous | multivariate | 0.178 | | 0.385 | |
| Zannoni et al. 2011 | ≤4 vs >4-<11 vs ≥ 11cm | univariate | 0.14 | | | |
| Holthoff et al. 2015 | doubled DOI | multivariate | 0.599 | | | |
| Alonso et al. 2011 | n/a | univariate | 0.124 | | 0.02* | |
| Collarino et al. 2019 | n/a | univariate | 0.4 | | 0.4 | |
| Hinten et al. 2015 | n/a | univariate 5-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | n/a | univariate 10-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | n/a | multivariate 5-yr DSS | | | n/a | |
| Hinten et al. 2015 | n/a | multivariate 10-yr DSS | | | n/a | |
| Woelber et al. 2016 | n/a | multivariate RR | 0.038* | | | |
| Woelber et al. 2016 | n/a | multivariate OS/DFS | 0.132 | | 0.132 | |



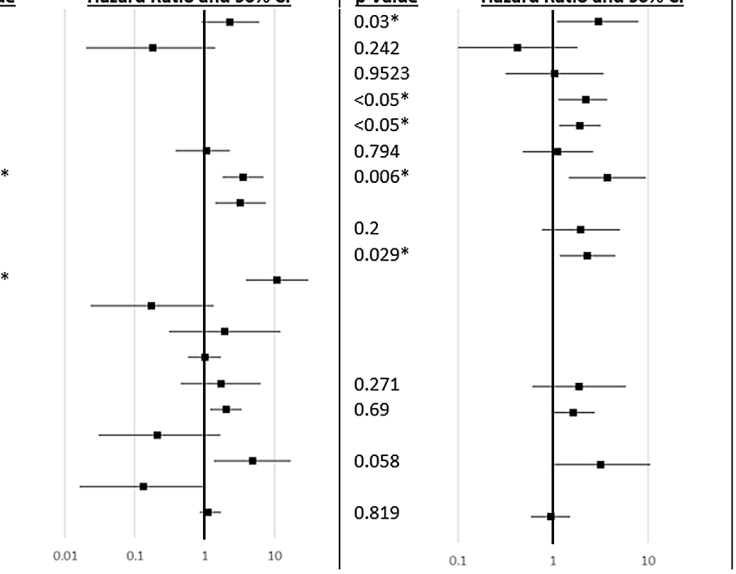

*Abbreviations: CI = confidence interval; DOI = doubling of infiltration; DM = distant metastasis; DSD = disease-specific death; DSS = disease-specific survival; OS = overall survival; PFS = progression-free survival; RR = recurrence rate; * significant p value <0.05*

*Lymphovascular Invasion*

Eighteen studies assessed the relationship between lymphovascular space invasion on recurrence and overall survival (Table 7). The relationship between LVSI and recurrence was equivocal. Amongst 14 studies, 9 analyses (6 univariate and 3 multivariate)[15,40,64–66] found that LVSI was prognostic for progression-free survival, while 12 analyses (8 univariate,[9,19,39,43,45,47,49,67] 3 multivariate[9,19,39,43,45,47,49,66,67]

**Table 7**
Lymphovascular space invasion and relationship with PFS and OS.

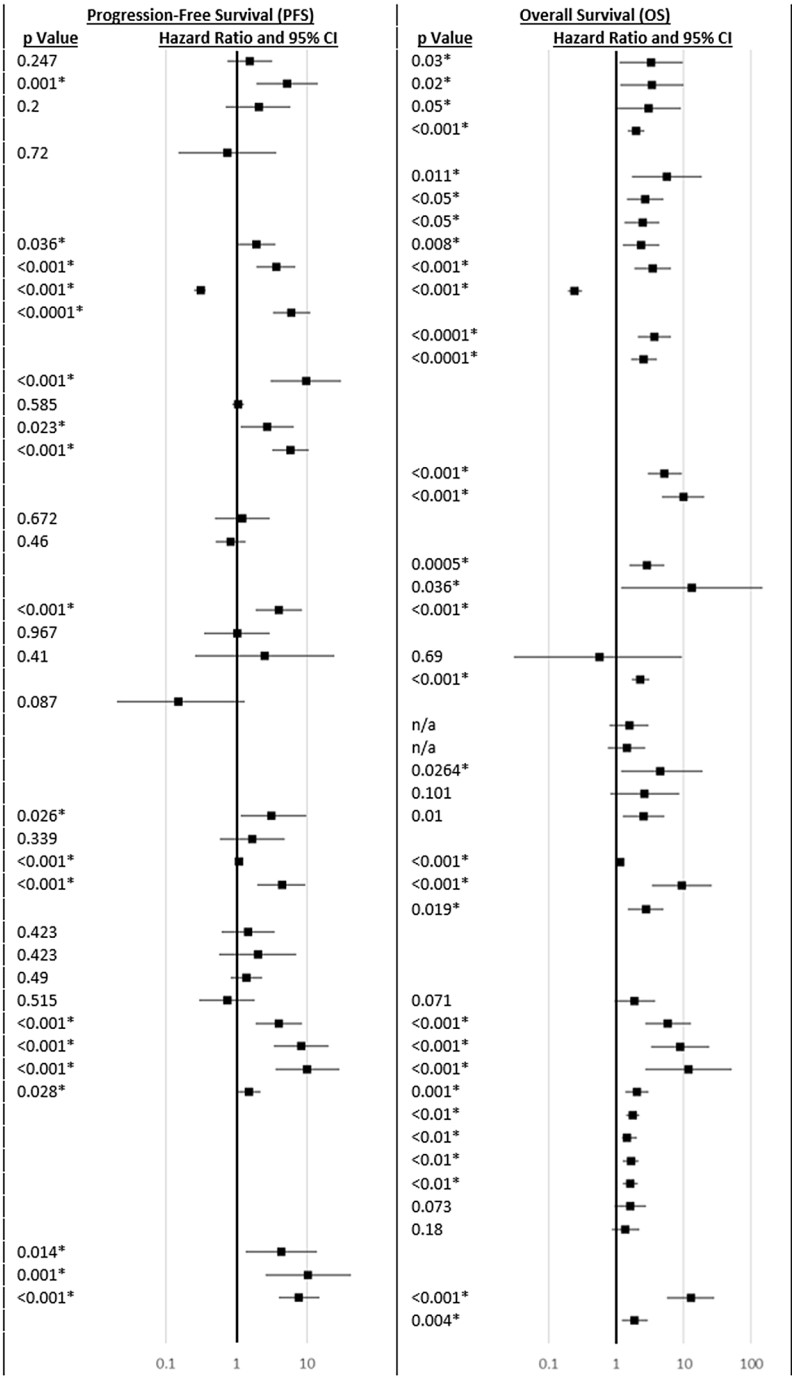
| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) | | Overall Survival (OS) | |
|---|---|---|---|---|---|---|
| | | | p Value | Hazard Ratio and 95% CI | p Value | Hazard Ratio and 95% CI |
| Collarino et al. 2019 | LVSI | univariate | 0.08 | | 0.03* | |
| Dohopolski et al. 2019 | LVSI | univariate | 0.102 | | 0.242 | |
| Goes et al. 2012 | LVSI | univariate | | | 0.9523 | |
| Hinten et al. 2015 | LVSI | univariate 5-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | LVSI | univariate 10-yr DSS | | | <0.05* | |
| Laliscia et al. 2019 | LVSI | univariate | 0.894 | | 0.794 | |
| Long et al. 2019 | LVSI | univariate | <0.001* | | 0.006* | |
| McAlpine et al. 2017 | LVSI | univariate PFS | 0.015* | | | |
| McAlpine et al. 2017 | LVSI | univariate DSS | | | 0.2 | |
| McAlpine et al. 2017 | LVSI | univariate OS | | | 0.029* | |
| Minar et al. 2018 | LVSI | univariate | <0.001* | | | |
| Nooij et al. 2015 | LVSI | univariate | 0.093 | | | |
| Pleunis et al. 2018 | LVSI | univariate | 0.478 | | | |
| te Grootenhuis et al. 2019 | LVSI | univariate | 0.99 | | | |
| Weberpals et al. 2017 | LVSI | univariate | 0.422 | | 0.271 | |
| Aragona et al. 2014 | LVSI | multivariate | 0.007* | | 0.69 | |
| Dohopolski et al. 2019 | LVSI | multivariate | 0.141 | | | |
| Long et al. 2019 | LVSI | multivariate | 0.015* | | 0.058 | |
| Nooij et al. 2015 | LVSI | multivariate | 0.054 | | | |
| Polterauer et al. 2019 | LVSI | multivariate | 0.29 | | 0.819 | |




*Abbreviations: LVSI = lymphovascular space invasion; DFS = disease-free survival; DSS = disease-specific survival; PFS = progression-free survival; OS = overall survival; CI = confidence interval; * significant p value <0.05*

**Table 8**
Lymph node metastases and relationship with PFS and OS.

| Study | Criteria | Uni-/Multi-Variate | Progression-Free Survival (PFS) p Value | Progression-Free Survival (PFS) Hazard Ratio and 95% CI | Overall Survival (OS) p Value | Overall Survival (OS) Hazard Ratio and 95% CI |
|---|---|---|---|---|---|---|
| Alonso et al. 2011 | ≥1 positive | univariate | 0.247 | | 0.03* | |
| Bogani et al. 2017 | ≥1 positive | univariate | 0.001* | | 0.02* | |
| Collarino et al. 2019 | ≥1 positive | univariate | 0.2 | | 0.05* | |
| Dhakal et al. 2013 | ≥1 positive | Univariate | | | <0.001* | |
| Gasimli et al. 2018 | ≥1 positive | Univariate | 0.72 | | | |
| Goes et al. 2012 | ≥1 positive | Univariate | | | 0.011* | |
| Hinten et al. 2015 | ≥1 positive | univariate 5-yr DSS | | | <0.05* | |
| Hinten et al. 2015 | ≥1 positive | univariate 10-yr DSS | | | <0.05* | |
| Laliscia et al. 2019 | ≥1 positive | univariate | 0.036* | | 0.008* | |
| Long et al. 2019 | ≥1 positive | univariate | <0.001* | | <0.001* | |
| Mahner et al. 2015 | ≥1 positive | univariate | <0.001* | | <0.001* | |
| McAlpine et al. 2017 | ≥1 positive | univariate PFS | <0.0001* | | | |
| McAlpine et al. 2017 | ≥1 positive | univariate DSS | | | <0.0001* | |
| McAlpine et al. 2017 | ≥1 positive | univariate OS | | | <0.0001* | |
| Minar et al. 2018 | ≥1 positive | univariate | <0.001* | | | |
| Nooij et al. 2015 | ≥1 positive | univariate | 0.585 | | | |
| Nooij et al. 2016 | ≥1 positive | univariate | 0.023* | | | |
| Panici et al. 2015 | ≥1 positive | univariate DFS | <0.001* | | | |
| Panici et al. 2015 | ≥1 positive | univariate OS | | | <0.001* | |
| Panici et al. 2015 | ≥1 positive | univariate DSS | | | <0.001* | |
| Pleunis et al. 2018 | ≥1 positive | univariate | 0.672 | | | |
| Stankevica et al. 2012 | ≥1 positive | univariate | 0.46 | | | |
| Sznurkowski et al. 2016 | ≥1 positive | univariate | | | 0.0005* | |
| Alonso et al. 2011 | ≥1 positive | multivariate | | | 0.036* | |
| Aragona et al. 2014 | ≥1 positive | multivariate | <0.001* | | <0.001* | |
| Baiocchi et al. 2015 | ≥1 positive | multivariate | 0.967 | | | |
| Bogani et al. 2017 | ≥1 positive | multivariate | 0.41 | | 0.69 | |
| Dhakal et al. 2013 | ≥1 positive | multivariate | | | <0.001* | |
| Gasimli et al. 2018 | ≥1 positive | multivariate | 0.087 | | | |
| Hinten et al. 2015 | ≥1 positive | multivariate 5-yr | | | n/a | |
| Hinten et al. 2015 | ≥1 positive | multivariate 10-yr | | | n/a | |
| Imoto et al. 2016 | ≥1 positive | multivariate DSS | | | 0.0264* | |
| Imoto et al. 2016 | ≥1 positive | multivariate OS | | | 0.101 | |
| Long et al. 2019 | ≥1 positive | multivariate | 0.026* | | 0.01 | |
| Nooij et al. 2016 | ≥1 positive | multivariate | 0.339 | | | |
| Polterauer et al. 2019 | ≥1 positive | multivariate | <0.001* | | <0.001* | |
| Seebacher et al. 2012 | ≥1 positive | multivariate | <0.001* | | <0.001* | |
| Sznurkowski et al. 2016 | ≥1 positive | multivariate | | | 0.019* | |
| Tu et al. 2017 | ≥1 positive | multivariate | 0.423 | | | |
| Tu et al. 2017 | >2 positive | multivariate | 0.423 | | | |
| Baiocchi et al. 2013 | >3 positive | multivariate | 0.49 | | | |
| Aragona et al. 2014 | 0 vs 1 positive | multivariate | 0.515 | | 0.071 | |
| Aragona et al. 2014 | 0 vs 2 positive | multivariate | <0.001* | | <0.001* | |
| Aragona et al. 2014 | 3-5 positive | multivariate | <0.001* | | <0.001* | |
| Aragona et al. 2014 | >5 positive | multivariate | <0.001* | | <0.001* | |
| Laliscia et al. 2019 | 0 vs 1 vs >1 | univariate | 0.028* | | 0.001* | |
| Xanthopoulos et al. 2018 | 0 vs 1 vs >2 | univariate | | | <0.01* | |
| Xanthopoulos et al. 2018 | 0 vs 1 vs >2 | multivariate unadj | | | <0.01* | |
| Xanthopoulos et al. 2018 | 0 vs 1 vs >2 | multivariate adj | | | <0.01* | |
| Xanthopoulos et al. 2018 | 0 vs 1 vs >2 | multivariate imp | | | <0.01* | |
| Baiocchi et al. 2013 | 1-2 vs >3 | multivariate DSS | | | 0.073 | |
| Baiocchi et al. 2013 | 1-2 vs >3 | multivariate OS | | | 0.18 | |
| Woelber et al. 2011 | 0 vs 1 | multivariate | 0.014* | | | |
| Woelber et al. 2011 | 0 vs 2 | multivariate | 0.001* | | | |
| Polterauer et al. 2019 | lymph node ratio | multivariate | <0.001* | | <0.001* | |
| Lérias et al. 2019 | met ≥ 5mm size | multivariate | | | 0.004* | |




*Abbreviations: DSS = disease-specific survival; OS = overall survival; PFS = progression-free survival; CI = confidence interval; unadj = unadjusted; adj = adjusted; imp = imputed*
** significant p value <0.05*

1 method not specified) did not. The results for LVSI and OS was similar. Amongst 13 studies, 8 univariate analyses[15,40,43,59,65,66] found LVSI to be prognostic for OS, but this was not supported by any multivariate analyses. 13 analyses (8 univariate,[9,15,19,39,41,45,68] 4 multivariate[40,65,66] and 1 method not specified[10]) did not find a statistically significant relationship between LVSI and OS. The studies did not distinguish between focal or extensive LVSI.

*Lymph node metastases*

Both the FIGO 2018 and AJCC 8th Edition use a cut-off of 5 mm as well as extracapsular extension, as factors for staging.[1,69] The 2 mm cut-off used to separate micro- and macrometastases is not used. The AJCC in addition, includes a category of isolated tumor cells (pN0(i+), <0.2 mm) and fixed/ulcerated regional lymph nodes (pN3).

Thirty-four studies evaluated the prognostic significance of lymph node metastases (LNM) (Table 8). The majority of studies used any number of lymph nodes positive (≥1) as the cut-off. The majority of studies found that LNM was prognostic but not always statistically significant. For PFS, 26/50 (52%) univariate and 10/18 (56%) multivariate analyses were statistically significant.[6,15,16,20,40–45,47–53,56,64–66,74,88–91] However, the relationship between LNM and OS much stronger, 50/67 (75%) univariate analyses and 34/44 (77%) multivariate analyses were statistically significant.[6,15,22,24,40–43,45,48,50,52,56,58–61,65,66,68,74,88,89,91–93]

Sentinel lymph node procedure can be performed to reduce surgical morbidity. Based off the GROINSS-V study, it is performed in unifocal tumors confined to the vulva, size <4 cm, stromal invasion >1 mm and clinically negative groin nodes.[94] There is no consensus on the need for pathologic ultrastaging. Unlike in the gastrointestinal tract, there is no recommended minimum for number of lymph nodes, although some authors recommend a minimum of 10 for a unilateral groin dissection.[48,62] Polterauer *et al* has been the only study to suggest that assessment of lymph node ratio is superior to lymph node count in predicting PFS and OS.[66] This has not been reiterated in the literature.

### *Perineural invasion*

Few studies assessed perineural invasion and prognosis, with mixed results. PNI was prognostic for PFS in 3/5 analyses.[49,51,59] and OS in 2/3 analyses[49,51,59]

### *Tumor focality*

Tumor location is important for guiding surgical management. Tumors <4 cm in size and ≥2 cm from the midline can have ipsilateral groin dissection (the risk of contralateral groin involvement is <1%), and tumors close to the midline (<2 cm or crossing the midline) should have bilateral groin dissection.[1] In the assessment of tumor location, midline/medial versus lateral, there were mixed findings. Three studies found that lateral tumors had worse prognosis, 1 was statistically significant [51,55,74] The authors believe this is likely due to the more aggressive treatment given for midline tumors. In contrast, Minar *et al* found that involvement of midline had worse local recurrence.[64]

In the few studies that evaluated the significance of unifocal versus multifocal tumors, multifocal tumors had a tendency towards worse PFS and DSS/OS, but did not reach statistical significance.[49,51,59]

### *Margin status*

To provide some guidance on margin assessment, Kortekaas *et al* surveyed 57 pathologists and provided practical guidelines on how to measure margins. They proposed that the margin should be assessed form the peripheral edge of the invasive tumor nests to the inked peripheral margin. This can be the distance between the invasive nests and the epithelial–stromal edge or the stromal edge, depending on which is shorter. The margin should measured in a straight lined, instead of a jagged line that would follow tissue folds, and reported in millimeters.[95]

The NCCN[96] and FIGO[1] recommend a clinical gross margin of 1–2 cm, which yields a histologic margin clearance of >8 mm, after tissue shrinkage from formalin fixation. Similarly, the British Royal College of Obstetricians & Gynaecologists,[97] the Society of Obstetricians and Gynecologists of Canada[98] and the European Society of Gynecological Oncology,[99] recommend 1 cm margins, again with a 8 mm pathologic clearance. More conservative margins are allowable to preserve function of the midline structures (clitoris, urethra, anus).[99] The German guidelines [Arbeitsgemeinschaft Gynäkologische Onkologie (AGO), Study Group for Gynecologic Oncology] recommend a 3 mm margin clearance.[100] Close margins, defined as <5 mm by FIGO, may benefit from post-operative radiotherapy if re-excision is not possible.[1]

Studies reviewing margin status have used varied cut-off ranges (Table 9). In studies comparing positive vs negative margins, margin status was predictive of PFS in only 2/9 (22%) analyses [6,15,39,42,43,46] and OS in 3/10 (30%).[6,15,19,21,42,43,46] In 1990, Heaps *et al* ($n=135$), found that a surgical tumor-free margin ≥8 mm resulted in no recurrences, versus <8 mm resulted in 49% local recurrence rate ($p<0.0001$).[101] This was reiterated by Hullu *et al* ($n=253$)[102] and Chan *et al* ($n=90$),[103] using the same 8 mm cut-off, finding local recurrence rates of 0% versus 22% and 0% versus 23%, respectively. In our review using studies with a cut-off of 8 mm, published within the last decade, 4/14 analyses (29%) and 2/4 (50%) was significant for PFS and OS respectively.[16,49,53,64,65,67,89,104] Micheletti *et al* analysed 8 different margin cut-offs between 3 and 10 mm, and found that 5 mm was the best cut-off, as differences in OS/DSS were not apparent using the 8 mm cut-off.[104]

The issue of minimum margin distance remains contested, and one of the biggest deficiencies in the literature is that margin status has not been separately analyzed for HPV-associated and HPV-independent tumors. McAlpine *et al* published extremely compelling data, where patients who had surgery after 1995, when there was a shift from radical to more conservative surgeries, led to worse outcomes for the HPV-independent tumors, that was not apparent prior to 1995.[33] This raises the longstanding issue of 'field cancerization', where the bounds of dysplasia, particularly in HPV-independent disease which is largely driven by *TP53* mutations, are not visible clinically and thus not always amendable to surgical resection.[105,106] Recent small studies of recurrent and multifocal VSCC demonstrated molecular clonality in 35–50% of cases.[107,108] This finding can be explained by the phenomenon of the cancerization field, or alternatively, by under-recognized dysplasia at margins. Singh *et al* found that by using p53 IHC for mapping, focusing on null pattern staining, margin status changed from negative to positive in 4/13 specimens and from focally to more extensive in 3 other specimens.[109]

For in-situ neoplasia, The American College of Obstetricians and Gynecologists (ACOG) and FIGO recommend a gross margin clearance 0.5–1 cm, while the German AGO recognized there was insufficient evidence to recommend a specific number.[1,100,110] Very few studies have looked at the prognostic differences between the presence of uVIN/HSIL or dVIN at a margin. Te Grootenhius *et al* found that dVIN or LS/dVIN at a margin correlated with a higher risk of local recurrence, whereas uVIN/HSIL at a margin did not.[67]

### *Lichen sclerosus*

Lichen sclerosus is more frequently identified in HPV-independent VSCC.[21] In 5 of 6 studies studies,[17,41,42,49,67,72] LS was associated with worse PFS (statistically significant in 2 studies), but not with OS.

## Summary

This review summarizes the most recent evidence, published within the last 10 years, supporting the various prognostic factors used to assess squamous cell carcinoma of the vulva. Not surprisingly, tumor stage and lymph node metastases had the strongest associations with survival.

## Declaration of Competing Interest

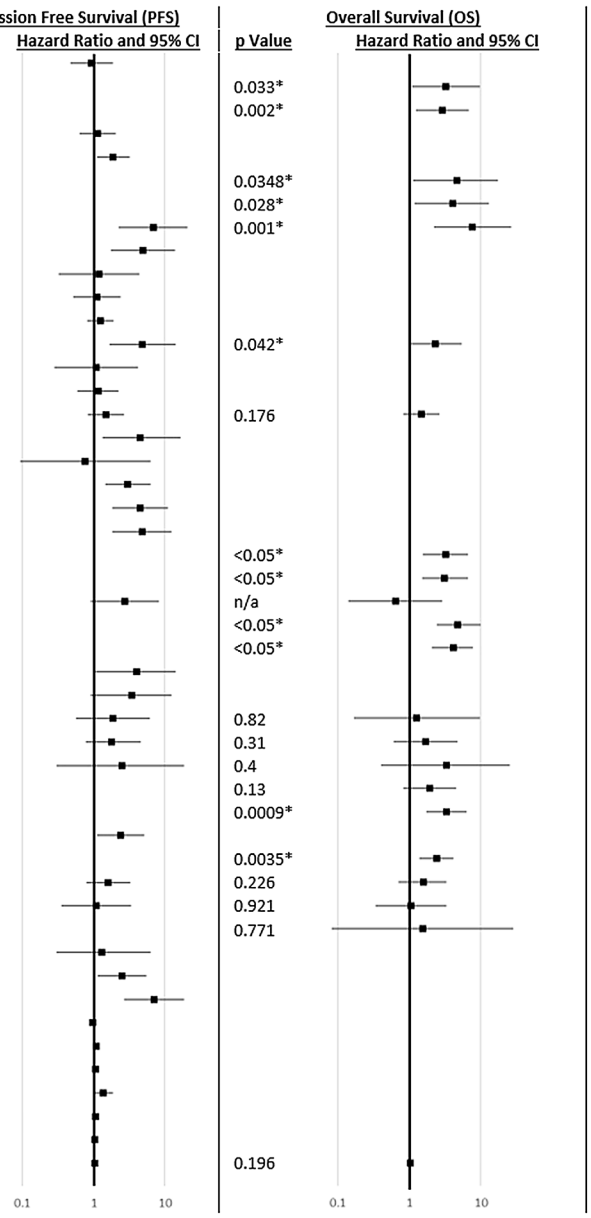
None

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1053/j.semdp.2020.09.004.

**Table 9**
Margin status/distance and relationship with PFS and OS.

| Study | Criteria | Reference | Uni-/Multi-Variate | Progression Free Survival (PFS) p Value | Overall Survival (OS) p Value |
|---|---|---|---|---|---|
| te Grootenhuis et al. 2019 | <3mm | ≥3mm | univariate | 0.831 | |
| Micheletti et al. 2018 | <5mm | ≥5mm | univariate DSS | | 0.033* |
| Micheletti et al. 2018 | <5mm | ≥5mm | univariate OS | | 0.002* |
| te Grootenhuis et al. 2019 | <5mm | ≥5mm | univariate | 0.678 | |
| Viswanathan et al. 2013† | <5mm | ≥5mm | n/a | 0.002* | |
| Imoto et al. 2016 | <6mm | ≥6mm | multivariate DSS | | 0.0348* |
| Imoto et al. 2016 | <6mm | ≥6mm | multivariate OS | | 0.028* |
| Long et al. 2019 | <8mm | ≥8mm | univariate | <0.001* | 0.001* |
| Minar et al. 2018 | <8mm | ≥8mm | univariate | 0.003* | |
| Nooij et al. 2016 | <8mm | ≥8mm | univariate | 0.808 | |
| Pleunis et al. 2018 | <8mm | ≥8mm | univariate | 0.787 | |
| te Grootenhuis et al. 2019 | <8mm | ≥8mm | univariate | 0.308 | |
| Long et al. 2019 | <8mm | ≥8mm | multivariate | 0.004* | 0.042* |
| Nooij et al. 2016 | <8mm | ≥8mm | multivariate | 0.903 | |
| Woelber et al. 2016† | <8mm | ≥8mm | multivariate RR | 0.675 | |
| Woelber et al. 2016† | <8mm | ≥8mm | multivariate OS/DFS | 0.176 | 0.176 |
| Gasimli et al. 2018† | <9mm | ≥9mm | univariate | 0.017* | |
| Gasimli et al. 2018† | <9mm | ≥9mm | multivariate | 0.8 | |
| Viswanathan et al. 2013 | <10mm | ≥10mm | multivariate | n/a | |
| Nooij et al. 2016† | positive | <8mm | univariate | 0.001* | |
| Nooij et al. 2016† | positive | <8mm | multivariate | 0.001* | |
| Hinten et al. 2015† | positive | <8mm | univariate 5-yr | | <0.05* |
| Hinten et al. 2015† | positive | <8mm | univariate 10-yr | | <0.05* |
| Hay et al. 2016 | positive | ≥8mm | univariate | n/a | n/a |
| Hinten et al. 2015† | positive | ≥8mm | univariate 5-yr | | <0.05* |
| Hinten et al. 2015† | positive | ≥8mm | univariate 10-yr | | <0.05* |
| Nooij et al. 2016† | positive | ≥8mm | univariate | 0.41 | |
| Nooij et al. 2016† | positive | ≥8mm | multivariate | 0.07 | |
| Alonso et al. 2011 | positive | negative | univariate | 0.307 | 0.82 |
| Bogani et al. 2017 | positive | negative | univariate | 0.16 | 0.31 |
| Collarino et al. 2019 | positive | negative | univariate | 0.6 | 0.4 |
| Dong et al. 2015 | positive | negative | univariate | | 0.13 |
| McAlpine et al. 2017 | positive | negative | univariate DSS | | 0.0009* |
| McAlpine et al. 2017 | positive | negative | univariate PFS | 0.045* | |
| McAlpine et al. 2017 | positive | negative | univariate OS | | 0.0035* |
| Nicoletto et al. 2010 | positive | negative | univariate | 0.171 | 0.226 |
| Weberpals et al. 2017 | positive | negative | univariate | 0.887 | 0.921 |
| Weberpals et al. 2017 | positive | negative | multivariate | | 0.771 |
| Bogani et al. 2017 | positive | negative | multivariate | 0.67 | |
| Viswanathan et al. 2013 | positive | negative | multivariate RFS | n/a | |
| Viswanathan et al. 2013 | positive | negative | multivariate RR | n/a | |
| te Grootenhuis et al. 2019† | continuous | - | univariate | 0.153 | |
| Woelber et al. 2016† | continuous | - | univariate LR | 0.125 | |
| Woelber et al. 2016† | continuous | - | univariate DFS | 0.09 | |
| Viswanathan et al. 2013† | continuous | - | n/a | n/a | |
| Woelber et al. 2011† | continuous | - | multivariate | 0.218 | |
| Woelber et al. 2016† | continuous | - | multivariate RR | 0.462 | |
| Woelber et al. 2016† | continuous | - | multivariate OS/DFS | 0.196 | 0.196 |




*Abbreviations: CI = confidence interval; PFS = progression-free survival; RR = recurrence rate; OS = overall survival; DFS = disease-free survival; DSS = disease-specific survival; LR = local recurrence; * significant p value <0.05; †hazard ratios were flipped*

## References


1. Rogers LJ, Cuello MA. Cancer of the vulva. *Int J Gynaecol Obstet.* 2018;143:4–13. https://doi.org/10.1002/ijgo.12609.
2. Vulvar cancer — cancer stat facts. Surveillance, Epidemiology, and End Results Program. National Cancer Institute. https://seer.cancer.gov/statfacts/html/vulva.html. Accessed August 15, 2020.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68:394–424. https://doi.org/10.3322/caac.21492.
4. Head and neck cancers. NCCN logy. https://www.nccn.org/professionals/physician_gls/default.aspx. Accessed August 15, 2020.
5. Allo G, Yap ML, Cuartero J, et al. HPV-independent vulvar squamous cell carcinoma is associated with significantly worse prognosis compared with HPV-associated tumors. *Int J Gynecol Pathol.* 2020;39:391–399. https://doi.org/10.1097/PGP.0000000000000620.
6. Alonso I, Fusté V, del Pino M, et al. Does human papillomavirus infection imply a different prognosis in vulvar squamous cell carcinoma? *Gynecol Oncol.* 2011;122(3):509–514. https://doi.org/10.1016/j.ygyno.2011.05.016.
7. Arians N, Prigge E-S, Nachtigall T, et al. Overexpression of p16INK4a serves as prognostic marker in squamous cell vulvar cancer patients treated with radiotherapy irrespective of HPV-status. *Front Oncol.* 2019;9:891. https://doi.org/10.3389/fonc.2019.00891.
8. Czogalla B, Pham D, Trillsch F, et al. PD-L1 expression and survival in p16-negative and -positive squamous cell carcinomas of the vulva. *J Cancer Res Clin Oncol.* 2020;146(3):569–577. https://doi.org/10.1007/s00432-020-03126-9.
9. Dohopolski MJ, Horne ZD, Pradhan D, et al. The prognostic significance of p16 status in patients with vulvar cancer treated with vulvectomy and adjuvant radiation. *Int J Radiat Oncol Biol Phys.* 2019;103(1):152–160. https://doi.org/10.1016/j.ijrobp.2018.08.014.
10. Hay CM, Lachance JA, Lucas FL, Smith KA, Jones MA. Biomarkers p16, human papillomavirus and p53 predict recurrence and survival in early stage squamous cell carcinoma of the vulva. *J Low Genit Tract Dis.* 2016;20(3):252–256. https://doi.org/10.1097/LGT.0000000000000182.
11. Horne ZD, Dohopolski MJ, Pradhan D, et al. Human papillomavirus infection mediates response and outcome of vulvar squamous cell carcinomas treated with

radiation therapy. *Gynecol Oncol.* 2018;151(1):96–101. https://doi.org/10.1016/j.ygyno.2018.08.002.
12. Lavorato-Rocha AM, de Melo Maia B, Rodrigues ISA, et al. Prognostication of vulvar cancer based on p14ARF status: molecular assessment of transcript and protein. *Ann Surg Oncol.* 2013;20(1):31–39. https://doi.org/10.1245/s10434-012-2560-7.
13. Lee LJ, Howitt B, Catalano P, et al. Prognostic importance of human papillomavirus (HPV) and p16 positivity in squamous cell carcinoma of the vulva treated with radiotherapy. *Gynecol Oncol.* 2016;142(2):293–298. https://doi.org/10.1016/j.ygyno.2016.05.019.
14. Lindell G, Näsman A, Jonsson C, et al. Presence of human papillomavirus (HPV) in vulvar squamous cell carcinoma (VSCC) and sentinel node. *Gynecol Oncol.* 2010;117(2):312–316. https://doi.org/10.1016/j.ygyno.2009.12.031.
15. McAlpine JN, Leung SCY, Cheng A, et al. Human papillomavirus (HPV)-independent vulvar squamous cell carcinoma has a worse prognosis than HPV-associated disease: a retrospective cohort study. *Histopathology.* 2017;71(2):238–246. https://doi.org/10.1111/his.13205.
16. Nooij LS, van der Slot MA, Dekkers OM, Stijnen T, Gaarenstroom KN, Creutzberg CL, et al. Tumour-free margins in vulvar squamous cell carcinoma: does distance really matter? *Eur J Cancer.* 2016;65:139–149. https://doi.org/10.1016/j.ejca.2016.07.006.
17. Stefansson K, Oda H, Öfverman C, Lundin E, Hedman H, Lindquist D. LRIG1-2 and LMO7 immunoreactivity in vulvar squamous cell carcinoma: association with prognosis in relation to HPV-DNA and p16INK4a status. *Oncol Rep.* 2019;42(1):142–150. https://doi.org/10.3892/or.2019.7138.
18. Wakeham K, Kavanagh K, Cuschieri K, et al. HPV status and favourable outcome in vulvar squamous cancer. *Int J Cancer.* 2017;140(5):1134–1146. https://doi.org/10.1002/ijc.30523.
19. Weberpals JI, Lo B, Duciaume MM, et al. Vulvar Squamous Cell Carcinoma (VSCC) as two diseases: HPV status identifies distinct mutational profiles including oncogenic fibroblast growth factor receptor 3. *Clin Cancer Res.* 2017;23(15):4501–4510. https://doi.org/10.1158/1078-0432.CCR-16-3230.
20. Yap ML, Allo G, Cuartero J, et al. Prognostic significance of human papilloma virus and p16 expression in patients with vulvar squamous cell carcinoma who received radiotherapy. *Clin Oncol (R Coll Radiol).* 2018;30(4):254–261. https://doi.org/10.1016/j.clon.2018.01.011.
21. Dong F, Kojiro S, Borger DR, Growdon WB, Oliva E. Squamous cell carcinoma of the vulva: a subclassification of 97 cases by clinicopathologic, immunohistochemical, and molecular features (p16, p53, and EGFR). *Am J Surg Pathol.* 2015;39(8):1045–1053. https://doi.org/10.1097/PAS.0000000000000454.
22. Lérias S, Esteves S, Silva F, Cunha M, Cochicho D, Martins L, et al. CD274 (PD-L1), CDKN2A (p16), TP53, and EGFR immunohistochemical profile in primary, recurrent and metastatic vulvar cancer. *Mod Pathol.* 2020;33(5):893–904. https://doi.org/10.1038/s41379-019-0429-z.
23. Rodrigues IS, Lavorato-Rocha AM, de M, Maia B, et al. Epithelial-mesenchymal transition-like events in vulvar cancer and its relation with HPV. *Br J Cancer.* 2013;109(1):184–194. https://doi.org/10.1038/bjc.2013.273.
24. Sznurkowski JJ, Zawrocki A, Biernat W. The overexpression of p16 is not a surrogate marker for high-risk human papilloma virus genotypes and predicts clinical outcomes for vulvar cancer. *BMC Cancer.* 2016;16:465. https://doi.org/10.1186/s12885-016-2503-y.
25. Trietsch MD, Spaans VM, ter Haar NT, et al. CDKN2A(p16) and HRAS are frequently mutated in vulvar squamous cell carcinoma. *Gynecol Oncol.* 2014;135(1):149–155. https://doi.org/10.1016/j.ygyno.2014.07.094.
26. Keung ES, Souers RJ, Bridge JA, et al. Comparative performance of high-risk human papillomavirus RNA and DNA in situ hybridization on college of American pathologists proficiency tests. *Arch Pathol Lab Med.* 2020;144(3):344–349. https://doi.org/10.5858/arpa.2019-0093-CP.
27. Bigby SM, Eva LJ, Fong KL, Jones RW. The natural history of vulvar intraepithelial neoplasia, differentiated type: evidence for progression and diagnostic challenges. *Int J Gynecol Pathol.* 2016;35(6):574–584. https://doi.org/10.1097/PGP.0000000000000280.
28. Darragh TM, Colgan TJ, Cox JT, et al. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the college of American pathologists and the American society for colposcopy and cervical pathology. *Int J Gynecol Pathol.* 2013;32(1):76–115. https://doi.org/10.1097/PGP.0b013e31826916c7.
29. Abell MR. Intraepithelial carcinomas of epidermis and squamous mucosa of vulva and perineum. *Surg Clin North Am.* 1965;45(5):1179–1198. https://doi.org/10.1016/S0039-6109(16)37686-1.
30. Abell MR, Gosling JRG. Intraepithelial and infiltrative carcinoma of vulva: bowen's type. *Cancer.* 1961;14(2):318–329 10.1002/1097-0142(196103/04)14:2<318::AID-CNCR2820140212>3.0.CO;2-J.
31. Hoang LN, Park KJ, Soslow RA, Murali R. Squamous precursor lesions of the vulva: current classification and diagnostic challenges. *Pathology.* 2016;48(4):291–302. https://doi.org/10.1016/j.pathol.2016.02.015.
32. Tessier-Cloutier B, Pors J, Thompson E, et al. Molecular characterization of invasive and in situ squamous neoplasia of the vulva and implications for morphologic diagnosis and outcome. Mod Pathol Published online August 13, 2020. doi:10.1038/s41379-020-00651-3.
33. Tessier-Cloutier B, Kortekaas KE, Thompson E, et al. Major p53 immunohistochemical patterns in in situ and invasive squamous cell carcinomas of the vulva and correlation with TP53 mutation status. *Mod Pathol.* 2020;33(8):1595–1605. https://doi.org/10.1038/s41379-020-0524-1.
34. Kortekaas KE, Solleveld-Westerink N, Tessier-Cloutier B, et al. Performance of the pattern-based interpretation of p53 immunohistochemistry as a surrogate for TP53 mutations in vulvar squamous cell carcinoma. *Histopathology.* 2020;77(1):92–99. https://doi.org/10.1111/his.14109.
35. Liu Y, Ji J, Almadani N, et al. Comparison of p53 immunohistochemical staining in Differentiated Vulvar Intraepithelial Neoplasia (dVIN) to inflammatory dermatoses and benign squamous lesions in the vulva. Histopathology 2020 [In Press]. doi:10.1111/his.14238.
36. McAlpine JN, Kim SY, Akbari A, et al. HPV-independent Differentiated Vulvar Intraepithelial Neoplasia (dVIN) is associated with an aggressive clinical course. *Int J Gynecol Pathol.* 2017;36(6):507–516. https://doi.org/10.1097/PGP.0000000000000375.
37. van de Nieuwenhof HP, Massuger LFAG, van der Avoort IAM, et al. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. *Eur J Cancer.* 2009;45(5):851–856. https://doi.org/10.1016/j.ejca.2008.11.037.
38. Thuijs NB, van Beurden M, Bruggink AH, Steenbergen RDM, Berkhof J, Bleeker MCG. Vulvar intraepithelial neoplasia: Incidence and long-term risk of vulvar squamous cell carcinoma. Int J Cancer Published online July 7, 2020. doi:10.1002/ijc.33198.
39. Alanyali S, Ö Duran, Özsaran Z, et al. Treatment results and prognostic factors of patients with vulvar cancer treated with postoperative or definitive radiotherapy. *Tumori.* 2016;102(3):311–315. https://doi.org/10.5301/tj.5000509.
40. Aragona AM, Cuneo NA, Soderini AH, Alcoba EB. An analysis of reported independent prognostic factors for survival in squamous cell carcinoma of the vulva: is tumor size significance being underrated? *Gynecol Oncol.* 2014;132(3):643–648. https://doi.org/10.1016/j.ygyno.2013.12.022.
41. Baiocchi G, Mantoan H, de Brot L, et al. How important is the pathological margin distance in vulvar cancer? *Eur J Surg Oncol.* 2015;41(12):1653–1658. https://doi.org/10.1016/j.ejso.2015.09.024.
42. Bogani G, Cromi A, Serati M, et al. Predictors and patterns of local, regional, and distant failure in squamous cell carcinoma of the vulva. *Am J Clin Oncol.* 2017;40(3):235–240. https://doi.org/10.1097/COC.0000000000000138.
43. Collarino A, Garganese G, Fragomeni SM, et al. Radiomics in vulvar cancer: first clinical experience using 18 F-FDG PET/CT images. *J Nucl Med.* 2019;60(2):199–206. https://doi.org/10.2967/jnumed.118.215889.
44. Gasimli K, Straussner M, Schmeil I, et al. Impact of re-excision of residual adjacent vulvar intraepithelial neoplasia (VIN III) and histological tumour-free margin (hTFM) on survival in primary squamous cell carcinoma of vulva. *Arch Gynecol Obstet.* 2018;298(5):945–950. https://doi.org/10.1007/s00404-018-4887-1.
45. Laliscia C, Gadducci A, Fabrini MG, et al. Definitive radiotherapy for recurrent vulvar carcinoma after primary surgery: a two-institutional Italian experience. *Tumori.* 2019;105(3):225–230. https://doi.org/10.1177/0300891618811279.
46. Nicoletto MO, Parenti A, del Bianco P, et al. Vulvar cancer: prognostic factors. *Anticancer Res.* 2010;30(6):2311–2317.
47. Nooij LS, Ongkiehong PJ, van Zwet EW, et al. Groin surgery and risk of recurrence in lymph node positive patients with vulvar squamous cell carcinoma. *Gynecol Oncol.* 2015;139(3):458–464. https://doi.org/10.1016/j.ygyno.2015.09.081.
48. Panici PB, Tomao F, Domenici L, et al. Prognostic role of inguinal lymphadenectomy in vulvar squamous carcinoma: younger and older patients should be equally treated. A prospective study and literature review. *Gynecol Oncol.* 2015;137(5):373–379. https://doi.org/10.1016/j.ygyno.2015.03.013.
49. Pleunis N, Leermakers MEJ, van der Wurff AA, et al. Surgical margins in squamous cell carcinoma, different for the vulva? *Eur J Surg Oncol.* 2018;44(10):1555–1561. https://doi.org/10.1016/j.ejso.2018.05.031.
50. Seebacher V, Polterauer S, Grimm C, et al. The impact of plasma fibrinogen levels on patients with vulvar cancer. *Eur J Obstet Gynecol Reprod Biol.* 2012;161(1):88–91. https://doi.org/10.1016/j.ejogrb.2011.11.030.
51. Stankevica J, Macuks R, Baidekalna I, Donina S. Midline involvement as a risk factor for vulvar cancer recurrence. *Asian Pac J Cancer Prev.* 2012;13(10):5237–5240. https://doi.org/10.7314/apjcp.2012.13.10.5237.
52. Tu H, Sun P, Gu HF, et al. Clinical significance and prognostic value of femoral lymph node metastasis in FIGO stage III vulvar carcinoma. *Eur J Surg Oncol.* 2017;43(9):1768–1775. https://doi.org/10.1016/j.ejso.2017.05.019.
53. Woelber L, Choschzick M, Eulenburg C, et al. Prognostic value of pathological resection margin distance in squamous cell cancer of the vulva. *Ann Surg Oncol.* 2011;18(13):3811–3818. https://doi.org/10.1245/s10434-011-1778-0.
54. Woelber L, Griebel LF, Eulenburg C, et al. Role of tumour-free margin distance for loco-regional control in vulvar cancer—a subset analysis of the arbeitsgemeinschaft gynäkologische onkologie CaRE-1 multicenter study. *Eur J Cancer.* 2016;69:180–188. https://doi.org/10.1016/j.ejca.2016.09.038.
55. Xu LQ, Luo RZ, Sun XM, He JH, Zhang YN. Prognostic analysis of early-stage squamous cell carcinoma of the vulva. *World J Surg Oncol.* 2013;11:20. https://doi.org/10.1186/1477-7819-11-20.
56. Zannoni GF, Prisco MG, Vellone VG, et al. Cytoplasmic expression of oestrogen receptor beta (ERβ) as a prognostic factor in vulvar squamous cell carcinoma in elderly women. *Histopathology.* 2011;59(5):909–917. https://doi.org/10.1111/j.1365-2559.2011.04029.x.
57. Cormio G, Loizzi V, Carriero C, Cazzolla A, Putignano G, Selvaggi L. Groin recurrence in carcinoma of the vulva: management and outcome. *Eur J Cancer Care (Engl).* 2010;19(3):302–307. https://doi.org/10.1111/j.1365-2354.2008.01011.x.
58. Dhakal HP, Nesland JM, Førsund M, Trope CG, Holm R. Primary Tumor Vascularity, HIF-1α and VEGF expression in vulvar squamous cell carcinomas: their relationships with clinicopathological characteristics and prognostic impact. *BMC Cancer.* 2013;13:506. https://doi.org/10.1186/1471-2407-13-506.
59. Hinten F, van Einden LCGD, Cissen M, IntHout J, Massuger LFAG, de Hullu JA. Clitoral involvement of squamous cell carcinoma of the vulva: localization with the worst prognosis. *Eur J Surg Oncol.* 2015;41(4):592–598. https://doi.org/10.1016/j.ejso.2015.01.002.
60. Imoto S, Inamine M, Kudaka W, Nagai Y, Wakayama A, Nakamoto T, et al. Prognostic factors in patients with vulvar cancer treated with primary surgery: a

single-center experience. *Springerplus.* 2016;5:125. https://doi.org/10.1186/s40064-016-1767-7.
61. Xanthopoulos EP, Grover S, Puri PM, et al. Survival benefit of adjuvant radiation therapy in node-positive vulvar cancer. *Am J Clin Oncol.* 2018;41(9):845–850. https://doi.org/10.1097/COC.0000000000000393.
62. Courtney-Brooks M, Sukumvanich P, Beriwal S, Zorn KK, Richard SD, Krivak TC. Does the number of nodes removed impact survival in vulvar cancer patients with node-negative disease? *Gynecol Oncol.* 2010;117:308–311. https://doi.org/10.1016/j.ygyno.2010.01.007.
63. Rauh-Hain J, Clemmer J, Clark R, et al. Management and outcomes for elderly women with vulvar cancer over time. *BJOG.* 2014;121(6):719–727. https://doi.org/10.1111/1471-0528.12580.
64. Minar L, Felsinger M, Cihalova M, Zlamal F, Bienertova-Vasku J. Vulvar cancer recurrence - An analysis of prognostic factors in tumour-free pathological margins patients group. *Ginekol Pol.* 2018;89(8):425–432. https://doi.org/10.5603/GP.a2018.0073.
65. Long Y, Yao DS, Wei YS, Wei CH, Chen XY. Prognostic significance of perineural invasion in vulvar squamous cell carcinoma. *Cancer Manag Res.* 2019;11:4461–4469. https://doi.org/10.2147/CMAR.S198047.
66. Polterauer S, Schwameis R, Grimm C, et al. Lymph node ratio in inguinal lymphadenectomy for squamous cell vulvar cancer: results from the AGO-CaRE-1 study. *Gynecol Oncol.* 2019;153(2):286–291. https://doi.org/10.1016/j.ygyno.2019.02.007.
67. te Grootenhuis NC, Pouwer AW, de Bock GH, et al. Margin status revisited in vulvar squamous cell carcinoma. *Gynecol Oncol.* 2019;154(2):266–275. https://doi.org/10.1016/j.ygyno.2019.05.010.
68. Goes RS, Carvalho JP, Almeida BGL, et al. Intratumoral lymphatic vessel density in vulvar squamous cell carcinomas: a possible association with favorable prognosis. *Int J Gynecol Pathol.* 2012;31(1):8–14. https://doi.org/10.1097/PGP.0b013e31821f4de2.
69. Amin MB, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, et al. *AJCC Cancer Staging Manual. 8th ed.* New York, NY: Springer International Publishing: American Joint Commission on Cancer; 2017.
70. Cancer protocol templates | college of american pathologists. https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates/. Accessed August 23, 2020.
71. The society of gynecologic oncology of Canada. https://www.gog.org/. Accessed August 23, 2020.
72. Le ST, Karia PS, Vollenhoven BJ, Besaw RJ, Feltmate CM, Schmults CD. Evaluation of AJCC and an alternative tumor classification system for primary vulvar squamous cell carcinoma. *J Natl Compr Canc Netw.* 2018;16(1):42–49. https://doi.org/10.6004/jnccn.2017.7022.
73. Jesinghaus M, Strehl J, Boxberg M, et al. Introducing a novel highly prognostic grading scheme based on tumour budding and cell nest size for squamous cell carcinoma of the uterine cervix. *J Pathol Clin Res.* 2018;4(2):93–102. https://doi.org/10.1002/cjp2.95.
74. te Grootenhuis NC, van der Zee AGJ, van Doorn HC, et al. Sentinel nodes in vulvar cancer: long-term follow-up of the GROningen INternational Study on Sentinel nodes in Vulvar cancer (GROINSS-V) I. *Gynecol Oncol.* 2016;140(1):8–14. https://doi.org/10.1016/j.ygyno.2015.09.077.
75. Wilkinson EJ, Rico MJ, Pierson KK. Microinvasive carcinoma of the vulva. *Int J Gynecol Pathol.* 1982;1(1):29–39. https://doi.org/10.1097/00004347-198201000-00005.
76. Berman ML, Soper JT, Creasman WT, Olt GT, DiSaia PJ. Conservative surgical management of superficially invasive stage I vulvar carcinoma. *Gynecol Oncol.* 1989;35(3):352–357. https://doi.org/10.1016/0090-8258(89)90078-4.
77. Boice CR, Seraj IM, Thrasher T, King A. Microinvasive squamous carcinoma of the vulva: present status and reassessment. *Gynecol Oncol.* 1984;18(1):71–76. https://doi.org/10.1016/0090-8258(84)90008-8.
78. Magrina JF, Gonzalez-Bosquet J, Weaver AL, Gaffey TA, Leslie KO, Webb MJ, et al. Squamous cell carcinoma of the vulva stage IA: Long-term results. *Gynecol Oncol.* 2000;76(1):24–27. https://doi.org/10.1006/gyno.1999.5638.
79. Yoder BJ, Rufforny I, Massoll NA, Wilkinson EJ. Stage IA vulvar squamous cell carcinoma: An analysis of tumor invasive characteristics and risk. *Am J Surg Pathol.* 2008;32(5):765–772. https://doi.org/10.1097/PAS.0b013e318159a2cb.
80. Abdel-Mesih A, Daya D, Onuma K, Sur M, Tang S, Akhtar-Danesh N, et al. Interobserver agreement for assessing invasion in stage 1A vulvar squamous cell carcinoma. *Am J Surg Pathol.* 2013;37(9):1336–1341. https://doi.org/10.1097/PAS.0b013e31829f306a.
81. Yoonessi M, Goodell T, Satchidanand S, Fett W, Solis F. Microinvasive squamous carcinoma of the vulva. *J Surg Oncol.* 1983;24(4):315–321. https://doi.org/10.1002/jso.2930240415.
82. Protocol for the Examination of Specimens From Patients With Primary Carcinoma of the Vulva. Cancer Protocol Templates | College of American Pathologists. https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates/. Accessed August 23, 2020.
83. Holthoff ER, Jeffus SK, Gehlot A, Stone R, Erickson SW, Kelly T, et al. Perineural invasion is an independent pathologic indicator of recurrence in vulvar squamous cell carcinoma. *Am J Surg Pathol.* 2015;39(8):1070–1074. https://doi.org/10.1097/PAS.0000000000000422.
84. Ambros RA, Malfetano JH, Mihm MC. Clinicopathologic features of vulvar squamous cell carcinomas exhibiting prominent fibromyxoid stromal response. *Int J Gynecol Pathol.* 1996;15(2):137–145. https://doi.org/10.1097/00004347-199604000-00008.
85. ÁP Pinto, LB Signorello, Crum CP, Harlow BL, Abrão F, Villa LL. Squamous cell carcinoma of the vulva in Brazil: prognostic importance of host and viral variables. *Gynecol Oncol.* 1999;74(1):61–67. https://doi.org/10.1006/gyno.1999.5458.
86. Jeffus SK, Gehlot A, Holthoff E, et al. A fibromyxoid stromal response is associated with an infiltrative tumor morphology, perineural invasion, and lymph node metastasis in squamous cell carcinoma of the vulva. *Am J Surg Pathol.* 2015;39(9):1226–1233. https://doi.org/10.1097/PAS.0000000000000486.
87. Cocks M, Chaux A, Jenson EG, et al. Immune checkpoint status and tumor microenvironment in vulvar squamous cell carcinoma. *Virchows Arch.* 2020;477(1):93–102. https://doi.org/10.1007/s00428-020-02759-y.
88. Baiocchi G, Silva Cestari FM, Rocha RM, et al. Prognostic value of the number and laterality of metastatic inguinal lymph nodes in vulvar cancer: revisiting the FIGO staging system. *Eur J Surg Oncol.* 2013;39(7):780–785. https://doi.org/10.1016/j.ejso.2013.03.004.
89. Baiocchi G, Silva Cestari FM, Rocha RM, et al. Does the count after inguinofemoral lymphadenectomy in vulvar cancer correlate with outcome? *Eur J Surg Oncol.* 2013;39(4):339–343. https://doi.org/10.1016/j.ejso.2013.02.012.
90. Diehl A, Volland R, Kirn V, et al. The number of removed lymph nodes by inguinofemoral lymphadenectomy: impact on recurrence rates in patients with vulva carcinoma. *Arch Gynecol Obstet.* 2016;294(1):131–136. https://doi.org/10.1007/s00404-015-3932-6.
91. Mahner S, Jueckstock J, Hilpert F, et al. Adjuvant therapy in lymph node-positive vulvar cancer: the AGO-CaRE-1 study. *J Natl Cancer Inst.* 2015;107(3):426. https://doi.org/10.1093/jnci/dju426.
92. van der Steen S, van de Nieuwenhof HPV, Massuger L, Bulten J, de Hullu JA. New FIGO staging system of vulvar cancer indeed provides a better reflection of prognosis. *Gynecol Oncol.* 2010;119(3):520–525. https://doi.org/10.1016/j.ygyno.2010.08.036.
93. Yap ML, Allo G, Cuartero J, et al. Prognostic significance of human papilloma virus and p16 expression in patients with vulvar squamous cell carcinoma who received radiotherapy. *Clin Oncol (R Coll Radiol).* 2018;30(4):254–261. https://doi.org/10.1016/j.clon.2018.01.011.
94. van der Zee AGJ, Oonk MH, de Hullu JA, et al. Sentinel node dissection is safe in the treatment of early-stage vulvar cancer. *J Clin Oncol.* 2008;26(6):884–889. https://doi.org/10.1200/JCO.2007.14.0566.
95. Kortekaas KE, van de Vijver KK, van Poelgeest MIE, et al. Practical guidance for measuring and reporting surgical margins in vulvar cancer. *Int J Gynecol Pathol.* 2020;39(5):420–427. https://doi.org/10.1097/PGP.0000000000000631.
96. Vulvar Cancer. NCCN clinical practice guidelines in oncology. https://www.nccn.org/professionals/physician_gls/default.aspx/. Accessed August 25, 2020.
97. Guidelines for the Diagnosis and Management of Vulval Carcinoma. British Royal College of Obstetricians & Gynaecologists. https://www.rcog.org.uk/en/guidelines-research-services/guidelines/vulval-carcinoma-guidelines-for-the-diagnosis-and-management-of/. Accessed August 25, 2020.
98. Francis JA, Eiriksson L, Dean E, Sebastianelli A, Bahoric B, Salvador S. No. 370-management of squamous cell cancer of the vulva. *J Obstet Gynaecol Can.* 2019;41(1):89–101. https://doi.org/10.1016/j.jogc.2018.07.004.
99. Oonk MHM, Planchamp F, Baldwin P, et al. European society of gynaecological oncology guidelines for the management of patients with vulvar cancer. *Int J Gynecol Cancer.* 2017;27(4):832–837. https://doi.org/10.1097/IGC.0000000000000975.
100. Schnürch HG, Ackermann S, Alt CD, et al. Diagnosis, therapy and follow-up care of vulvar cancer and its precursors guideline of the DGGG and DKG (S2k-Level, AWMF Registry Number 015/059, November 2015. *Geburtshilfe Frauenheilkd.* 2016;76(10):1035–1049. https://doi.org/10.1055/s-0042-103728.
101. Heaps JM, Fu YS, Montz FJ, Hacker NF, Berek JS. Surgical-pathologic variables predictive of local recurrence in squamous cell carcinoma of the vulva. *Gynecol Oncol.* 1990;38(3):309–314. https://doi.org/10.1016/0090-8258(90)90064-R.
102. de Hullu JA, Hollema H, Lolkema S, et al. Vulvar carcinoma: the price of less radical surgery. *Cancer.* 2002;95(11):2331–2338. https://doi.org/10.1002/cncr.10969.
103. Chan JK, Sugiyama V, Pham H, et al. Margin distance and other clinico-pathologic prognostic factors in vulvar carcinoma: a multivariate analysis. *Gynecol Oncol.* 2007;104(3):636–641. https://doi.org/10.1016/j.ygyno.2006.10.004.
104. Micheletti L, Preti M, Cintolesi V, et al. Prognostic impact of reduced tumor-free margin distance on long-term survival in FIGO stage IB/II vulvar squamous cell carcinoma. *J Gynecol Oncol.* 2018;29(5) https://doi.org/10.3802/jgo.2018.29.e61.
105. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol.* 2010;23(3):404–412. https://doi.org/10.1038/modpathol.2009.179.
106. Thompson E, Trevisan G, Wong R, et al. Use of p53 as an ancillary tool for the assessment of margin status in cases of differentiated vulvar intraepithelial neoplasia (dVIN) and HPV (human papillomavirus)-independent Squamous Cell Carcinoma (SCC) of the Vulva [abstract]. *Mod Pathol.* 2019;32:1–140.
107. Pors J, Tessier-Cloutier B, Thompson E, et al. Targeted molecular sequencing of recurrent and multifocal non HPV-associated squamous cell carcinoma of the vulva. Int J Gynecol Pathol 2020. [Accepted for Publication].
108. Regauer S, Kashofer K, Reich O. Time series analysis of TP53 gene mutations in recurrent HPV-negative vulvar squamous cell carcinoma. *Mod Pathol.* 2019;32(3):415–422. https://doi.org/10.1038/s41379-018-0141-4.
109. Singh N, Leen SL, Han G, et al. Expanding the morphologic spectrum of differentiated VIN (dVIN) through detailed mapping of cases with p53 loss. *Am J Surg Pathol.* 2015;39(1):52–60. https://doi.org/10.1097/PAS.0000000000000291.
110. Management of vulvar intraepithelial neoplasia. *Obstet Gynecol.* 2016;128(4):e178–e182. https://doi.org/10.1097/AOG.0000000000001713.