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Putative precancerous lesions of vulvar squamous cell carcinoma

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ABSTRACT

Precursor lesions of vulvar squamous cell carcinoma (VSCC) can be divided into two major biologic and prognostic groups: HPV-associated and HPV-independent VSCC. These two pathways are categorized as usual vulvar intraepithelial neoplasia (uVIN) with progression to basaloid or warty VSCC and differentiated vulvar intraepithelial neoplasia (dVIN) with progression to the more common keratinizing VSCC. While the HPV-dependent pathway to squamous cell carcinoma is well-understood, the development of squamous cell carcinoma from HPV-independent lesions is less clear. The majority of HPV-independent lesions fall into the dVIN category, and mutations in *TP53* have been implicated as the driver behind their development. Other less common HPV-independent precursor lesions, termed differentiated exophytic vulvar intraepithelial lesion (DEVIL) and vulvar acanthosis with altered differentiation (VAAD), have also been characterized as precursors to keratinizing and verrucous VSCC. Inflammatory conditions of the vulva such as lichen sclerosus and lichen simplex chronicus also put patients at risk for developing VSCC. We herein evaluate the available evidence and biologic basis for these VSCC precursor lesions, among other speculated entities, and discuss their clinical, diagnostic, and prognostic features.

Introduction

Vulvar squamous cell carcinoma (VSCC) is an uncommon gynecologic malignancy with an annual incidence of 2.5% affecting women at the median age of 69.[1] While rare overall, squamous cell carcinoma is the most common malignancy of the vulva with over 6,000 new cases per year.[1] It is widely accepted that there are two predominant precursor lesions that give rise to VSCC via distinct oncogenic pathways.[2–5] The two major precursor lesions that progress to VSCC are usual vulvar intraepithelial neoplasia (uVIN) and differentiated vulvar intraepithelial neoplasia (dVIN). High-risk human papillomavirus (HPV) is the infectious agent that drives uVIN, whereas dVIN is HPV-independent.

VSCC can be subdivided into various histologic subtypes, most commonly keratinizing, basaloid, and warty variants. Usual vulvar intraepithelial neoplasia is the most common type of vulvar dysplasia and tends to give rise to the basaloid and warty variants of VSCC. Differentiated vulvar intraepithelial neoplasia gives rise to keratinizing VSCC which is the predominant subtype of VSCC, representing approximately 60% of cases.[6] Occasionally the morphologic features of HPV-dependent and HPV-independent VSCC overlap and ancillary studies are needed to determine the etiology.

Additional potential precursors to VSCC–all HPV-independent and seemingly more closely related to dVIN—have also been described. Furthermore, some inflammatory conditions of the vulva have overlapping morphologic features with these entities, creating diagnostic challenges. These inflammatory conditions have also been disputed as potentially premalignant. Herein we evaluate and discuss the available evidence for these putative precursor lesions to vulvar squamous cell carcinoma.

Usual vulvar intraepithelial neoplasia (uVIN)

Clinical features

Usual VIN makes up the majority (90%) of vulvar dysplasia.[2, 7] This HPV-associated lesion has the same viral-induced pathogenesis as cervical intraepithelial neoplasia (CIN) and more commonly affects younger women. The annual incidence of uVIN has been increasing in recent years; however, with the advent of the HPV vaccine, the rate is expected to decline based on cervical intraepithelial neoplasia data.[8] Clinically, uVIN can be asymptomatic or can present as a pruritic plaque, raised nodule, or warty growth. Multifocality is common, particularly in immunosuppressed individuals. Treatment for high-grade uVIN includes excision, cryoablation, and topical biologics (e.g. imiquimod).[2]

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Pathogenesis

Infection with one or more of the high-risk subtypes of HPV leads to the development of uVIN. HPV infection is very common among premenopausal women; however, only small subset of infections progress to high-grade lesions. HPV 16 is the most common high-risk subtype to cause progression to high-grade uVIN and VSCC.[9] Immunocompromised states, particularly HIV infection, can predispose to persistent HPV infection leading to multifocal uVIN and subsequent progression to VSCC.[10] Smoking is also associated with an increased recurrence risk.[11] HPV-associated VSCC have been reported to further acquire mutations in *PIK3CA, FGFR3*, and *PTEN*.[12]

Microscopic features

Histologically, uVIN can be divided into low-grade and high-grade squamous intraepithelial neoplasia. Low-grade VIN (VIN1) has koilocytes within the upper third to upper half of the epithelium and may have increased mitotic activity and nuclear-to-cytoplasmic (N:C) ratios restricted to the lower third of the epithelium, with maturation evident in the two-thirds. High-grade intraepithelial neoplasia demonstrates increased N:C ratios and mitotic activity in the lower half of the squamous epithelium with partial maturation in the upper third (VIN2), or full thickness cytologic atypia with mitotic figures reaching the upper third (VIN3) (Fig. 1A-B). Hyperkeratosis and parakeratosis may be present. Extension of dysplasia into adnexal structures is common and may mimic invasion, particularly if tangentially sectioned. As most uVIN and their associated VSCC have a basaloid appearance, basal cell carcinoma (BCC) may enter the differential (Fig. 1C). However, BCC of the vulva is much less common and is not HPV associated, thus, they can be distinguished with p16 immunohistochemistry.[13] Occasionally a multifocal nested pattern of VIN can occur, with marked cytologic atypia restricted to circumscribed foci with intervening normal squamous epithelium (Fig. 1D). This pattern is unusual, and immunohistochemistry can be applied to exclude mimics that more commonly exhibit this growth pattern such as extramammary Paget disease and melanoma. Currently, the World Health Organization (WHO) only requires distinction between low- and high-grade VIN—differentiation between VIN2 and VIN3 is not necessary as these lesions are treated the same as all high-grade uVIN has a low but significant risk of progression to VSCC.[14] Most progress to basaloid or warty subtypes, but they can also give rise to the more common keratinizing VSCC.[2, 6]

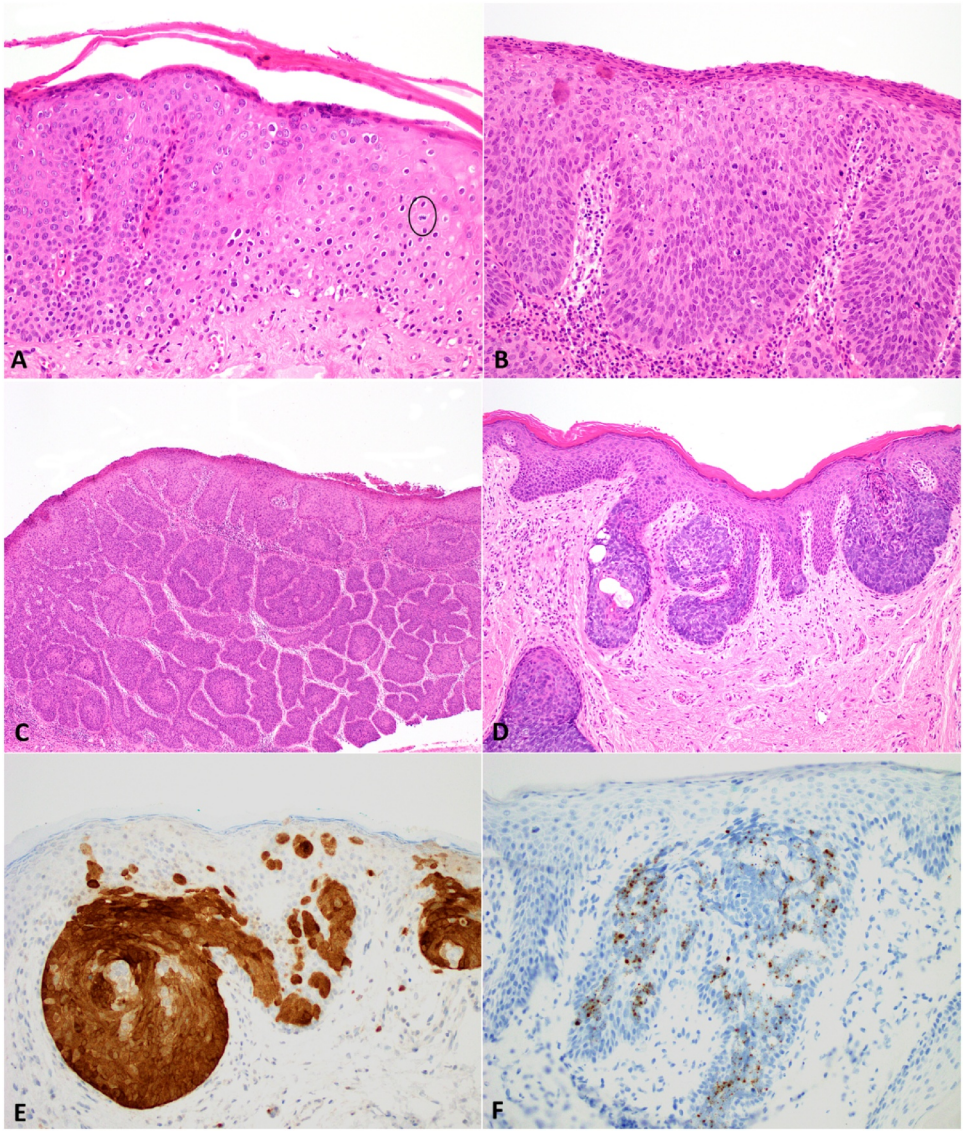

Fig. 1. Distinction between VIN1 and VIN2 can be challenging and this case highlights koilocytic changes in the surface epithelium, but close examination reveals a mitotic figure mid-epithelium (circle), consistent with VIN2 (1A). Classic VIN3 demonstrates diffuse cytologic atypia with high N:C ratio cells and numerous mitotic figures throughout the epithelium (1B). This example of VIN3 is notable for a hyperplastic jigsaw-like growth pattern mimicking basal cell carcinoma (1C). Note that all of the nests are well-circumscribed and do not have jagged borders or paradoxical maturation to suggest invasion. Rarely, VIN3 can present with a patchy, nested appearance with expansion of the rete ridges by neoplastic cells (1D). This may mimic melanoma and extramammary Paget disease; thus, confirmatory p16 and high-risk HPV in-situ hybridization studies can be helpful. The p16 is diffusely positive in the neoplastic cells (1E) and the HPV in-situ hybridization demonstrates dark, punctate positivity (1F).

Differentiated vulvar intraepithelial neoplasia (dVIN)

Clinical features

Differentiated VIN is less common than uVIN, comprising approximately 2-10% of total VIN cases and typically affecting post-menopausal women.[2] Unlike uVIN which is separated into low- and high-grade lesions, dVIN is by definition high-grade. While dVIN is overall much less common than uVIN, the rate of progression to VSCC is much higher.[11] Clinically these lesions frequently present in a background of lichen sclerosus or lichen simplex chronicus.[5] They may appear as an erythematous, raised lesion in a background of thin, pale, papery plaques.

Pathogenesis

Differentiated VIN is an HPV-independent precursor to VSSC. The pathogenesis of dVIN is less clear than the HPV-driven uVIN, although underlying chronic inflammation and damage to the epithelium as seen in lichen sclerosus (LS) and other inflammatory conditions is believed to be a predisposing factor. Clonal relationships between dVIN and VSCC have been established, although there is minimal available data to suggest a clonal relationship with LS.[15] Acquisition of a mutation in the tumor suppressor *TP53* gene is seen in approximately 70-90% of dVIN cases, and is likely the driver contributing to progression from an underlying chronic inflammatory condition.[2,16–20] Similarly, the majority of keratinizing VSCC also have mutations in *TP53*.[19, 21] Other mutations, including *NOTCH1, HRAS, PIK3CA, PTEN, BRAF, MET,* and *CDKN2A,* have also been described in both HPV-independent premalignant and malignant lesions.[12, 19, 20] Tessier-Cloutier et al. recently described a series of HPV-independent precursor lesions and VSCC and found that cases with *TP53* and *PIK3CA* co-mutations had significantly worse clinical outcomes.[20]

Microscopic features

Differentiated VIN can be quite challenging to diagnose, as the cytologic features are often very subtle. A retrospective study evaluating premalignant lesions in women with VSCC found that dVIN is frequently under-recognized on biopsies preceding malignant diagnoses.[22] Architecturally, dVIN lesions are usually acanthotic with sharp, elongated rete ridges with anastomoses (Fig. 2A). They characteristically have basal cytologic atypia with hyperchromasia and increased mitotic activity along the basal and parabasal layers (Fig. 2A-C). They are often hyperkeratotic and parakeratotic (Fig. 2A).[2, 11] Abnormal keratinocytes with increased keratinization or keratin pearls can also be helpful clues. A background of lichen sclerosus is often present (Fig. 3A). The juxtaposition of the atrophic, banal epithelium seen in lichen sclerosus with an acanthotic proliferation should prompt close evaluation of the lower epithelial layers for atypia and increased proliferation (Fig. 3).

Impact of HPV status on prognosis and treatment

Distinction between HPV-associated and HPV-independent precursor lesions and VSCC is important. Studies have shown that HPV-associated VSCC has a better prognosis and lower recurrence rate than HPV-independent VSCC.[23, 24] In the head and neck, HPV-associated squamous cell carcinomas have been categorized as a separate entity from non-HPV-associated squamous cell carcinoma in the WHO due to their marked difference in prognosis. While these patients typically present with lymph node involvement, they fare far better than their non-HPV-associated counterparts.[25, 26] In the vulva, patients with HPV-associated SCC treated with radiation have a significantly higher five-year progression free survival, overall survival, and fewer relapses as compared to HPV-independent SCC.[27] HPV-associated and HPV-independent VSCC have distinctly different pathogeneses, and if additional studies continue to demonstrate different prognoses and outcomes, separation into two separation of these lesions into two separate categories—similar to head and neck SCC—may be warranted in order to provide more accurate prognostic information and reduce overtreatment of HPV-associated cancers.

Other putative precancerous lesions

More recently, other HPV-independent precursor lesions to VSCC have also been described. These lesions include differentiated exophytic vulvar intraepithelial lesion (DEVIL) and vulvar acanthosis with altered differentiation (VAAD), which are verrucous-like lesions occurring in post-menopausal women that have been identified in association with VSCC. Both DEVIL and VAAD are biologically more closely related to dVIN, although their pathogeneses may be different.

Differentiated exophytic vulvar intraepithelial lesion (DEVIL)

DEVIL is a recently identified HPV-independent vulvar lesion, described by Watkins, et al. in 2017.[28] These lesions have an atypical verruciform morphology and are seen in association with keratinizing VSCC. In contrast to dVIN, these lesions do not have *TP53* abnormalities, but were identified by Watkins, et al. to commonly harbor *PIK3CA* and *ARID2* mutations.[28] *HRAS* mutations, and less commonly *BRAF* mutations, have also been reported.[20, 29] While VSCC, including those in association with DEVIL, often do not have *PIK3CA* mutations, the reproducibility of identifying these mutations in the precursor lesion suggests a specific pathway to their development.[28] Morphologically, DEVIL is exophytic with either acanthosis or verrucous architecture, shows keratinocyte maturation, and does not have significant basal cytologic atypia to suggest dVIN or morphologic changes of uVIN such as koilocyte formation or full-thickness basaloid atypia (Fig. 4).[28] Underlying chronic inflammation can be seen. Prominent hyperkeratosis and parakeratosis is often present (Fig. 4A). Areas of hypogranulosis—evidence of keratinocyte dysmaturation—is a common feature (Fig. 4B and C). DEVIL was initially identified as an atypical verrucous lesion adjacent to well-differentiated keratinizing squamous cell carcinoma.[28] Subsequently, DEVIL lesions have also been identified in association with dVIN and VAAD.[4]

Vulvar acanthosis with altered differentiation (VAAD)

Vulvar acanthosis with altered differentiation (VAAD) was initially described in 2004 by Nascimento et al. as a precursor lesion to verrucous squamous cell carcinoma of the vulva.[30] This lesion is described as having prominent verrucous architecture, hyperkeratosis, hypogranulosis, and conspicuous abundant pale eosinophilic cytoplasm with a glassy appearance. VAAD is most commonly associated with progression to verrucous squamous cell carcinoma. Fig. 5 demonstrates a case of recurrent VAAD presenting in a patient with a history of a partial vulvectomy for verrucous carcinoma a few years prior. One case of progression from VAAD to verrucous carcinoma and then to an aggressive poorly differentiated carcinoma with anaplastic features within a five-year timeframe has been reported.[31] These examples demonstrate that these atypical verrucous lesions can recur or can progress to aggressive malignancies, highlighting the importance of continued clinical follow-up, as well as recognition of these lesions by pathologists.

Similar to DEVIL, *TP53* mutations are not seen in VAAD lesions. Instead, they most frequently have mutations in *HRAS* (71.4%) and *NOTCH1* (28.6%).[32] A recent study that performed mutational analysis in a variety of HPV-independent premalignant and malignant lesions identified two of two cases of VAAD with *PIK3CA* mutations.[20] In this same study, one of three cases of DEVIL was found to have a *PIK3CA* mutation and one had an *HRAS* mutation. As expected, none of the DEVIL nor VAAD cases had *TP53* mtuations.[20]

While the data is relatively recent and limited, there appears to be

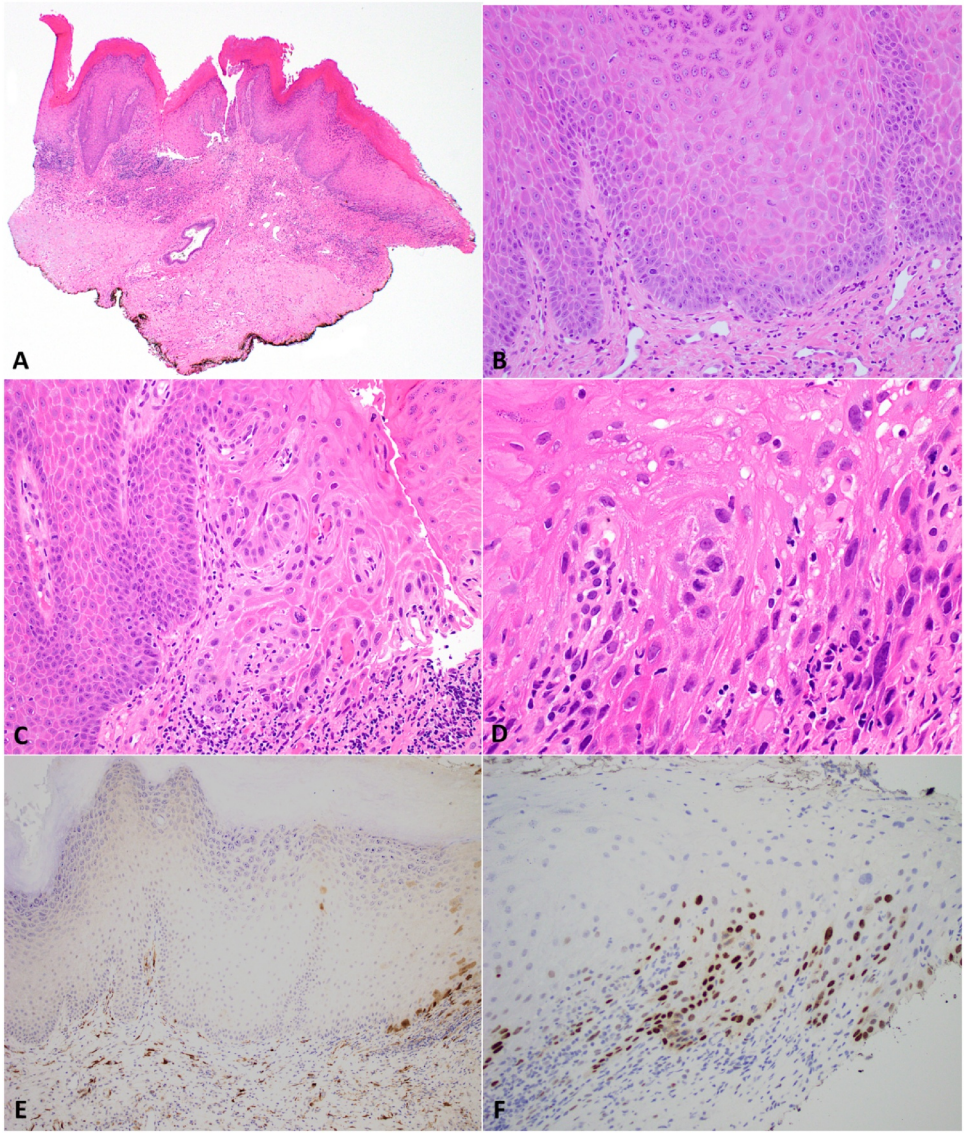

Fig. 2. Differentiated VIN is often acanthotic with jagged rete and hyperkeratosis (2A). Variable degrees of granulosis can be seen (2B) and the hallmark feature is prominent cytologic atypia and mitotic activity restricted to the basal layers (2C-2D). P16 is negative or focally patchy positive (2E) and there is confluent strong p53 expression in the areas with cytologic atypia in the corresponding H&E (2F).

sufficient evidence supporting that DEVIL and VAAD are indeed precancerous lesions. In reality, even when these lesions are identified, there is significant morphologic overlap between DEVIL and VAAD and distinction can be very difficult. The histologic features separating DEVIL and VAAD are subjective, and immunohistochemical stains provide similar results. Additionally, both have been described in association with each other and with dVIN.[4] What is more important, however, is recognition of an atypical verrucous lesion, as both DEVIL and VAAD have been shown to precede HPV-independent VSCC—well-differentiated VSCC and verrucous carcinoma, respectively. A recently clinicopathologic and molecular review of verrucous lesions by Akbari et al. reported multiple cases of DEVIL, rather than VAAD, seen in association with verrucous carcinoma.[29] These authors prefer the term DEVIL over VAAD for HPV-negative verruciform lesions, given the morphologic and mutational overlap.[29] While this study, along with others, found similarity in mutational profiles in DEVIL and VAAD, there is some molecular evidence suggesting that these are distinct entities; further molecular studies are needed to confirm this.[29, 33]

Singh and Gilks suggest that DEVIL, VAAD, and dVIN are likely related entities on a spectrum of well-differentiated atypical squamous lesions.[4] Perhaps these three lesions should be considered as similar entities under the umbrella of HPV-independent VIN.

Inflammatory dermatoses of the vulva: are they pre-neoplastic?

Other inflammatory lesions of the vulva—particularly lichenoid dermatoses—have been described as potential precursors to VSSC; however, the overall evidence is lacking. The reported prevalence of lichen sclerosus (LS) is variable and is estimated at approximately 1.7% percent.[34, 35] LS has a bimodal age distribution affecting both prepubertal and postmenopausal patients and is thought to be associated with autoimmune conditions and trauma or repeated irritation.[34, 35] In prepubertal patients, LS often regresses after menarche although in some cases patients relapse and have continued disease for years. In contrast, in postmenopausal patients LS usually does not regress and poses a risk for neoplastic transformation. LS is well-known to be associated with dVIN (Fig. 3) and keratinizing VSCC. VSCC is identified in 3.5-7% of patients with vulvar LS.[35] While the risk of neoplasia each year is approximately 1%, after 25-years it can be as high as 37%; thus, lifelong clinical follow up is necessary in patients with LS.[35] In a study

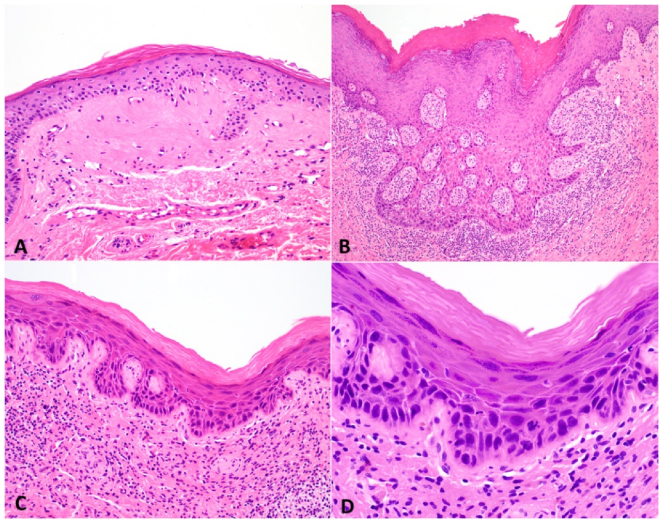

Fig. 3. This is an example of dVIN arising in the setting of LS. LS has thin, atrophic epithelium with loss of rete ridges, no significant hyperkeratosis, and abundant dense collagen deposition in the superficial papillary dermis (3A). No cytologic atypia should be seen. In contrast, dVIN can either be hypertrophic (3B) or flat, similar to LS (3C). However, in the flat dVIN, the cytologic atypia should be prominent. There is hyperchromasia, nuclear membrane irregularities, and increased mitotic activity in the basal and parabasal layers in this case (3C-D).

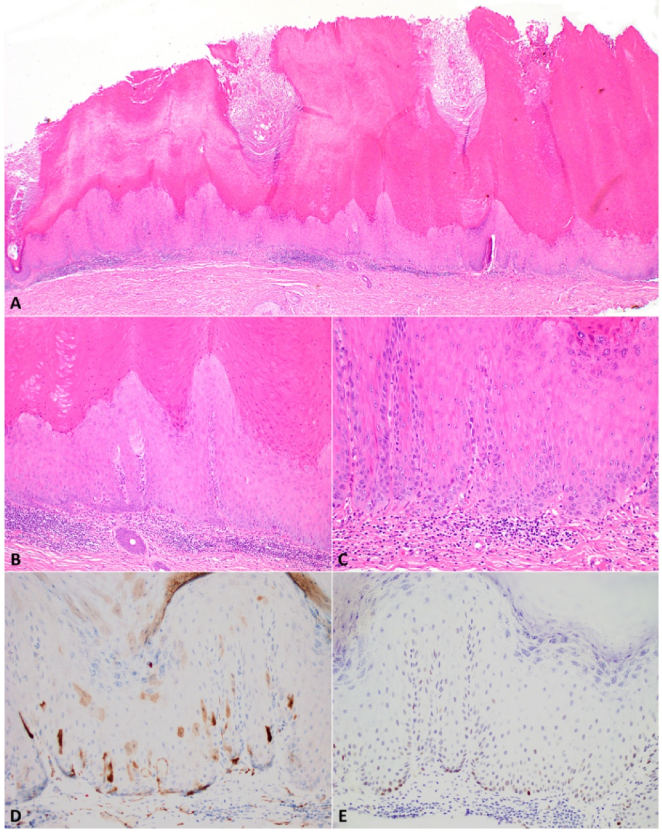

Fig. 4. Morphologic features of DEVIL include verruciform acanthosis and marked hyperkeratosis (4A), keratinocyte maturation with only focal dysmaturation in the form of hypogranulosis (4B-C), and no significant basal atypia to suggest dVIN (4C). P16 demonstrates a negative or patchy positive normal expression pattern (4D) and p53 has wild-type expression (4E).

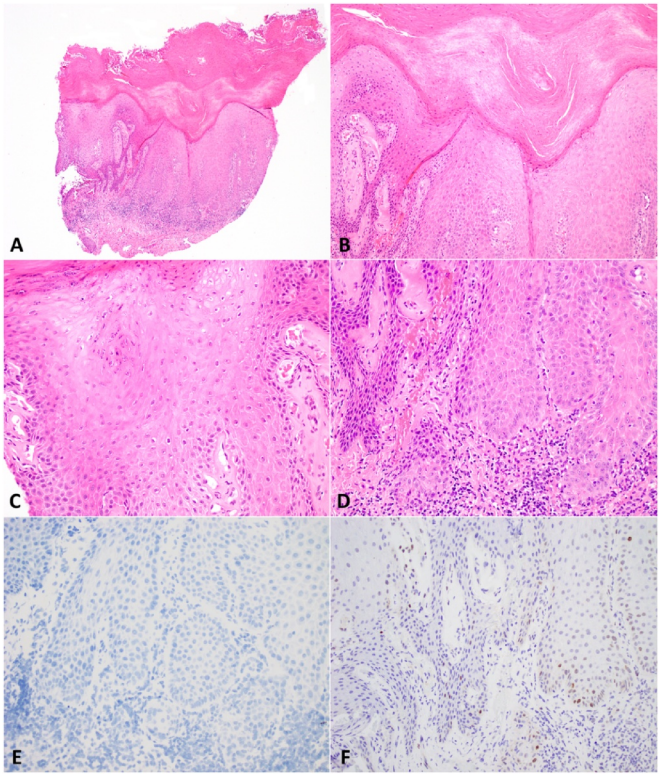

Fig. 5. VAAD is typically distinctly acanthotic or verrucous with marked hyperkeratosis and parakeratosis and hypogranulosis (5A-B). The epithelium has a distinct almost glassy eosinophilic pallor (5C). The basal layers should only have reactive changes with no significant cytologic atypia as seen in dVIN (5D). P16 is negative (5E) and p53 has a wild-type pattern of expression (5F).

of 3038 women with vulvar LS, the 20-year incidence of VSCC was found to be 6.7%.[36] Out of those patients, over 50% had diagnoses of VIN prior to a diagnosis of VSCC, although the authors postulate that this number is probably higher. The overall 10-year incidence of progression to VSCC was 3% in patients with LS along and 18% in patients with LS and VIN.[36] Another retrospective study of 976 cases of LS found a cumulative risk of progression of 1.2% at 24 months and 36.8% at 300 months.[37] Notably, the risk of progression was significantly higher in women over 70 years. Allelic imbalance in the same chromosomal loci as VSCC, suggesting monoclonality, has been reported in approximately 40% of LS in one study.[38] Mutations in *TP53* and *CDKN2A*—both common in VSCC—are not seen in solitary LS and LS associated with VSCC, however.[39] Overall, LS has a nonnegligible risk (~5%) to progression to VSCC—usually through the dVIN pathway—thus, it should be considered a precursor lesion to VSCC and patients with LS should be regularly monitored.[32]

Similarly, lichen simplex chronicus (LSC) is another common inflammatory condition of the vulva and has significant morphologic overlap with early lichen sclerosus. Both of these lesions show a band-like or perivascular infiltrate of chronic lymphocytic inflammation within the superficial dermis and an overlying reactive epithelium. Acanthosis, spongiosis, hypergranulosis, and hyperkeratosis are common features. Of note, keratinocyte maturation is maintained. LSC is thought to be an atopic dermatitis and is triggered by irritation and further progresses due to repeated pruritus and scratching.[34] These lesions typically present as lichenoid plaques with variable pigmentation and scale. Superimposed infections can also occur and create diagnostic confusion. LSC of the vulva is difficult to treat due to the repeated itch-scratch cycle and prolonged therapy with steroids is often needed. While LSC is a risk factor for VSCC, the only direct association that has been reported is in cases of “verruciform” LSC, which, as Watkins

suggests, are probably cases that would be better classified as DEVIL or VAAD today.[32] There is not sufficient evidence to classify LSC as a precursor lesion to VSCC and these patients do not need the same rigorous follow-up as patients with protracted LS.

Lichen planus (LP) in the vulvovaginal region is less frequently encountered than LS and LSC. Nonetheless, it is an important diagnostic consideration, particularly because it can mimic dVIN. Histologically, LP has a lichenoid lymphocytic infiltrate in the superficial dermis, wedge-shaped hypergranulosis, basal colloid bodies, and sharp rete ridges. In the erosive subtype of LP, the epidermis is lost. This particular variant is more challenging to treat than non-erosive LP and can cause scarring. While LP is associated with SCC in the oral cavity, in the vulva this has not been well-established and the evidence for progression from vulvar LP to VSCC is mixed. A study of 43 HPV-independent VSCC excisions revealed that 42% of these women had preoperative diagnoses of LS, but none had a diagnosis of LP.[40] After review, 95% of cases had coexisting LS, one had a non-specific lichenoid reaction, and no cases of LP were seen.[40] Simpson et al. reviewed four case series of erosive LP and demonstrated limited evidence for progression to VSCC.[41] Additionally, none of the series reviewed commented on HPV infection in any of the patients that developed SCC. The only prospective series included in this review identified two of 114 women with erosive anogenital LP that developed VSCC over the course of 72 months; however, in one patient VIN3 preceded the diagnosis of VSCC, confounding this data as this is almost certainly the precursor lesion.[41, 42] Thus, the overall rate of progression from vulvar LP to VSCC was <1%. At this time there is not sufficient evidence to conclude that LP is a precursor lesion to VSCC.

Ancillary studies

uVIN versus dVIN

Usual VIN and differentiated VIN are not always easy to differentiate. Application of various ancillary studies can help distinguish vulvar precancerous lesions from one another. The two most important immunohistochemical stains are p16 and p53. Ki-67, while often used in cervical neoplasia, is not helpful in distinguishing uVIN from dVIN due to similar patterns of expression.[43] In high-grade uVIN, p16 demonstrates strong and diffuse block positivity in at least the lower two-thirds of the epithelium (Fig. 1).[44, 45] In contrast, dVIN is typically weakly, patchy positive, or negative for p16. In dVIN, there is an increase in p53 nuclear positivity with confluence of staining within the basal and parabasal epithelial cells (Fig. 2). This increase in p53 expression is a reflection of the *TP53* mutations seen in the majority of dVIN.[16, 17] Similar to other tumors with *TP53* mutations, while increased p53 expression is the most common immunohistochemical staining pattern, complete absence of staining (null expression) can also be seen in approximately 25-30% of cases (Fig. 6).[46] Lack of p53 expression is often more difficult to recognize, and in cases with suspected null p53, careful examination of the surrounding stromal and inflammatory cells for positive internal control is necessary. Recently, Thompson et al. described a null-like pattern of p53 expression in HPV-associated lesions of the female genital tract, including VSCC.[47] In this study, they evaluated p53 expression in combination with p16 and HPV ISH in cases with difficult to interpret p53 IHC. They found that HPV-associated VSCC with a markedly reduced/null-like pattern of p53 expression had an inverse relationship with HPV ISH.[47] These VSCC also had strong and diffuse p16 expression, consistent with HPV viral integration. Another study also identified absent basal p53 expression in uVIN cases with superimposed lichen simplex chronicus (LSC).[21] While there was absent basal p53 expression, raising the possibility of a null phenotype, the parabasal and mid-epithelial layers had retained wild-type p53 expression, which argues against a *TP53* mutation. These studies emphasize the need for using multiple ancillary markers to determine the etiology of VSCC.

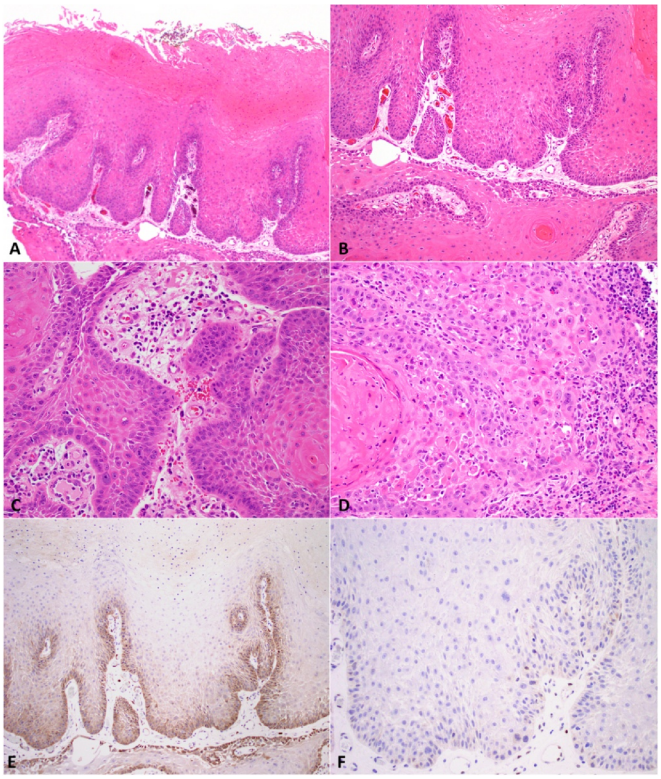

Fig. 6. This atypical squamous proliferation is acanthotic with thickened, anastomosing rete ridges, hyperkeratosis, and parakeratosis with an absent granular cell layer (6A-B). On higher power examination, there is prominent basal cytologic atypia and deep keratinization, concerning for neoplasia (6C-D). A p16 is negative (6E) and p53 appears to be completely negative with some positive stromal cells serving as the positive control (6F), raising the possibility of a null phenotype. Overall, this case was signed out as an atypical squamous proliferation, favor differentiated VIN with null p53 expression.

While application of p16 and p53 immunohistochemistry frequently resolves the question of uVIN versus dVIN, occasionally these stains can be difficult to interpret. In these cases, supplementation with high-risk HPV chromogenic RNA in-situ hybridization (CISH) is extremely useful punctate nuclear and cytoplasmic reactivity with HPV CISH confirms HPV infection. In contrast to the cervix, in the vulva care must be taken to avoid misinterpretation of melanin pigment in the epithelium as a positive HPV CISH result.[48]

Strong parabasal and mid-epithelial p53 positivity with notable sparing of the basal layer has been reported as a unique finding in HPV-associated uVIN and VSCC.[21]

dVIN versus LS

Morphologic distinction between dVIN and LS is often challenging. Strong and diffuse contiguous staining of a clonal population along the basal/parabasal layers is helpful in making a diagnosis of dVIN. In contrast, lichen sclerosus should have patchy staining of variable intensity. The utility of cytokeratin CK13 and CK17 expression in distinguishing dVIN and LS has been evaluated.[49, 50] In one study, increased CK17 expression was seen in 74% of dVIN cases in contrast to just 15% of LS cases. CK13 did not prove to be useful as the expression was similar in both entities (30-40%).[49] Podoll et al. suggest that CK17, particularly when diffuse suprabasal or full thickness expression is identified, can be used in combination with morphology, p53, and Ki-67 expression to help arrive at a diagnosis of dVIN.[50] Another study identified that loss of basal GATA3 expression in 88% of dVIN cases can help distinguish this lesion from LS and uVIN, which both retain moderate to strong GATA3 expression throughout the epithelium.[51]

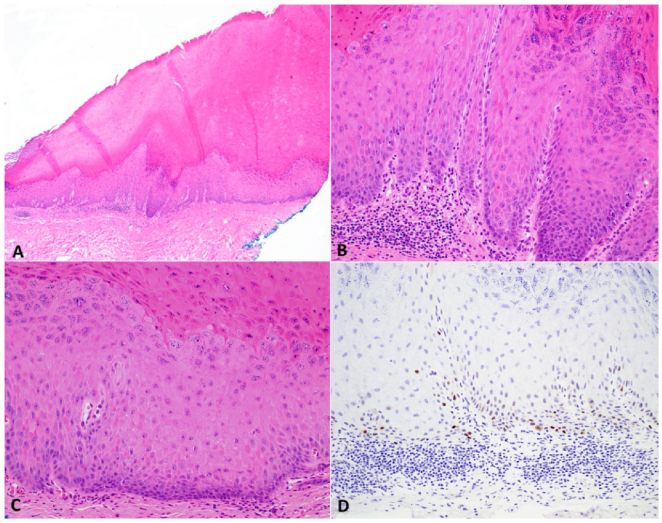

Fig. 7. This example of an atypical squamous proliferation demonstrates acanthosis with thickened rete and marked hyperkeratosis with alternating parakeratosis (7A). Some areas have a retained granular layer (7B) while others have hypogranulosis (7C). There is minimal cytologic atypia and no significant mitotic activity (7B-C). Despite the lack of atypia, the atypical architecture and evidence of dysmaturation prompted evaluation with a p53 stain, which shows a wild-type expression pattern (7D). This case was signed out as an atypical squamous proliferation with a comment explaining that the p53 pattern argues against a diagnosis of dVIN but the atypical architecture warrants close clinical follow-up with repeat biopsy if the lesion persists or enlarges. On follow up, another biopsy was taken and was diagnosed as DEVIL (Figure 4).

DEVIL and VAAD

DEVIL and VAAD are similar to dVIN in that they are negative or patchy positive for p16 and negative for HPV CISH. In contrast, however, they do not have aberrant p53 expression. Instead, they have patchy positive (wild-type) p53 expression along the basal layer (Fig. 4E). Thus, in addition to morphology, application of p53 IHC can be useful in distinguishing DEVIL or VAAD from dVIN.

Diagnostic challenges in practice

Conceptually, these different precursor lesions are relatively easy to distinguish; however, in practice there is often morphologic overlap, conflicting clinical history, and sampling issues that can impede accurate diagnosis. Sometimes lesions have some, but not all of the diagnostic features of HPV-independent neoplasia and a diagnosis of atypical squamous proliferation with recommendation for close follow-up can be made (Fig. 7). In addition, various non-neoplastic inflammatory and hyperplastic dermatologic lesions can be mimickers, particularly of dVIN, DEVIL, and VAAD (Fig. 8).

Watkins, et al. described a series of challenging uVIN cases with superimposed lichen simplex chronicus (LSC) that mimicked dVIN.[21] They found overlapping morphologic features including acanthosis, hyperkeratosis, hypergranulosis, abnormal maturation, nuclear hyperchromasia, and basal atypia between cases of dVIN and uVIN with LSC. The uVIN cases with LSC demonstrated diffuse p16 expression in the lower two-thirds of the epithelium, consistent with HPV-association.[43]

Interestingly, Ordi et al. reported a small series of four cases of dVIN with morphologic features that pathologists typically associate with uVIN.[52] They noted diffuse replacement of the entire squamous epithelium by an atypical, monomorphic population of basaloid keratinocytes with only minimal maturation.[52] The lesions were grossly more similar to uVIN as well with elevated red plaques. The p53 expression was strong and diffuse in the basal layers with suprabasal extension while p16 was negative in all cases. Confirmatory HPV testing was also

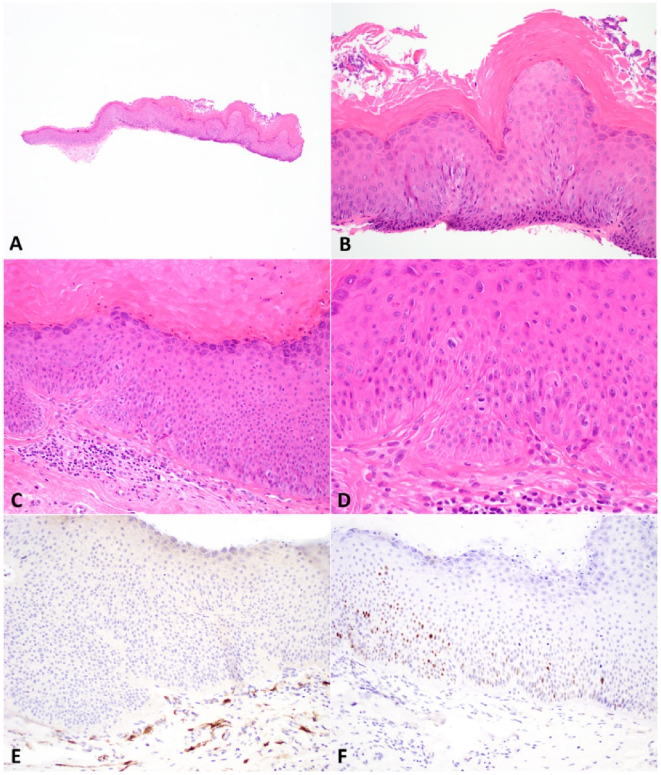

Fig. 8. This squamous proliferation that has a fairly normal epidermal thickness with mild hyperkeratosis and keratinocyte maturation (8A-B). The granular layer is retained and there is some loss of rete architecture and mild chronic inflammation in the superficial papillary dermis (8C). On higher power, the nuclei are regular with occasional prominent nucleoli; scattered parabasal mitotic figures are prominent (8D). Given the somewhat atypical morphology, p16 and p53 were performed to evaluate for uVIN and dVIN. Both stains are reassuring as p16 is negative (8E) and p53 demonstrates a wild-type expression pattern (8F). Special stains for microorganisms were also performed and were negative. Overall, this case has some atypical features and warrants follow-up, but is favored to be reactive.

negative in all cases. While this basaloid pattern of dVIN has a similar appearance to typical uVIN, there were a few clinicopathologic features of dVIN as well. The average age of the patients was 61, which is more typical of dVIN. One case had associated lichen sclerosus, two had associated squamous cell hyperplasia, and in three cases areas of more typical dVIN were identified. The majority (75%) were associated with conventional keratinizing VSCC—more commonly seen with dVIN—-while one case had a basaloid VSCC.[52] This case series demonstrates the existence of basaloid dVIN and highlights the importance of applying, at a minimum, p16 and p53 immunohistochemical stains in order to accurately subclassify vulvar neoplasia into HPV-associated versus HPV-independent precursor lesions and VSCC.

HPV-associated VSCC arising out of uVIN can also have areas with LS and dVIN-like morphology (Fig. 9). Rakislova et al. described a large series of HPV-associated VSCC with a small proportion demonstrating histologically ambiguous precursor lesions.[53] They identified a subset of 14 VSCC with adjacent unusual intraepithelial lesions with dVIN, LS, or dVIN and LS-like features. Definitive HPV-association was identified in 43% of these intraepithelial lesions with HPV16 DNA detection, HPV mRNA detection, and strong p16 expression. The remaining 57% of cases were positive for HPV DNA, but negative for p16 in both the tumor and precursor. HPV mRNA was negative in half and the other half was not tested.[53] While only a small subset in this series showed these ambiguous features, it is important to note that almost all of the associated VSCC were keratinizing, which is more commonly seen in association with dVIN. Thus, it is important to perform ancillary

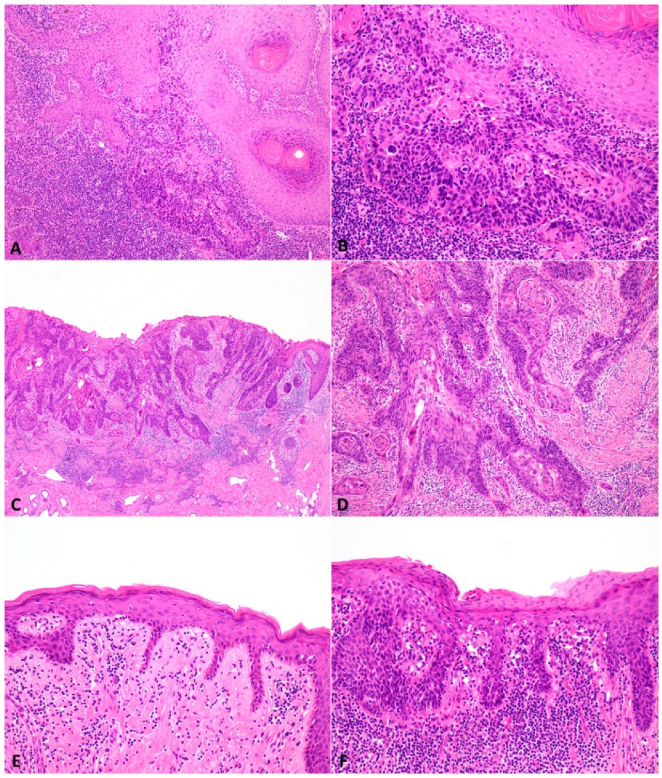

Fig. 9. Biopsies from a vulvar lesion in a postmenopausal woman reveal a tangentially sectioned atypical squamous proliferation with acanthosis, hyperkeratosis, and marked basal and parabasal cytologic atypia with areas concerning for, but not diagnostic of superficial invasion (9A-B). Partial vulvectomy revealed an invasive keratinizing squamous cell carcinoma (9C-D) with adjacent areas showing LS-like (9E) and dVIN-like (9F) change. P16 (not pictured) was strongly and diffusely positive, supporting an HPV-associated etiology.

studies, namely p16 and p53, in keratinizing VSCC given the prognostic implications.

Another common challenge in practice when reviewing VSCC precursor lesions is assessment for early invasion. Features such as paradoxical maturation appearing at the base of a basaloid uVIN or small, irregular nests dripping off of the elongated rete of dVIN can be a clues to superficial invasion and should prompt close examination for additional small nests or single cells in the dermis. Differentiated VIN can be particularly problematic because the lesion is already mature-appearing and the rete are jagged and irregular, making distinction from in-situ to early carcinoma challenging. Additionally, marked lichenoid inflammatory infiltrates can obscure small foci of invasion. Performing levels and p53 IHC can often bring out these foci; however, separation between VIN and microinvasive SCC is not always possible in a small biopsy (Fig. 9A and B). Pseudoepitheliomatous hyperplasia is another possibility to keep in mind when considering invasion. Pseudoepitheliomatous hyperplasia has been seen in association with LS and can mimic both dVIN and invasive SCC.[54] Careful search for basal atypia and desmoplasia is necessary to exclude this possibility. Additionally, a wild-type p53 expression pattern can be reassuring. Perhaps more challenging than assessing for superficial microinvasion is evaluating verrucous lesions for signs of pushing invasion. In these cases, the only reliable method is direct comparison of the thickest portion of the lesion to the adjacent in-situ foci in the excision specimen.

Conclusions

In summary, while HPV drives the majority of vulvar neoplasia, HPV-independent precursor lesions of VSCC are becoming increasingly

Table 1
Comparison of histologic features of precancerous vulvar lesions and inflammatory dermatoses.

	uVIN	dVIN	DEVIL	VAAD	LS	LSC	LP
Architecture	Acanthotic, usually without HK/PK	Thin or acanthotic, often with HK/PK	Exophytic, acanthotic, and/or verrucous; significant HK/PK	Acanthotic and verrucous; significant HK/PK	Thin and atrophic, usually without HK/PK	Acanthotic with marked HK/PK	Acanthotic with HK/PK
Rete ridges	Thickened rete	Elongated, jagged, anastomosing rete	Thickened, blunted rete	Thickened and blunted or elongated rete	Atrophic or absent rete	Elongated and thickened rete	Usually retained rete; colloid bodies in basal epithelium
Evidence of maturation	Hypogranulosis, absent or minimal maturation	Granular layer intact, retained maturation	Hypogranulosis, retained maturation	Hypogranulosis, retained maturation	Atrophic but intact granular layer, retained maturation	Hypergranulosis, retained maturation	Granular layer and maturation retained
Cytologic atypia	Koilocytes (if VIN1-2); full thickness atypia with high N:C ratios (VIN2-3)	Cytologic atypia with hyperchromasia in the basal/parabasal layers	No significant cytologic atypia	No significant cytologic atypia	No significant cytologic atypia	Reactive changes including prominent nucleoli	Reactive changes including prominent nucleoli
Mitotic activity	Abundant mitotic figures throughout epithelium	Increased mitotic activity basal/parabasal layers	Can be increased along basal/parabasal layers	Can be increased along basal/parabasal layers	Minimal	Minimal	Minimal
Inflammatory component	Mild/moderate superficial dermal lymphocytic infiltrate	Mild/moderate superficial dermal lymphocytic infiltrate	Mild superficial dermal lymphocytic infiltrate	Mild superficial dermal lymphocytic infiltrate	Superficial dermal lymphocytic infiltrate*	Mild to moderate perivascular lymphocytic and histiocytic infiltrate with occasional eos.	Moderate/severe band-like lymphocytic infiltrate, can extend into lower epithelium
VSCC subtype	Basaloid/warty SCC	Keratinizing SCC	WD keratinizing SCC	Verrucous SCC	Keratinizing SCC	N/A	N/A

Notes: HK hyperkeratosis; PK parakeratosis; WD well-differentiated; eos eosinophils; *early LS (in late LS inflammation is minimal/absent).

Table 2
Summary of ancillary studies used to aid in diagnosing precancerous vulvar lesions.

	uVIN	dVIN	DEVIL	VAAD	LS	LSC	LP
p16	Strong and diffuse	Patchy or negative	Patchy or negative	Patchy or negative	Patchy or negative	Patchy or negative	Patchy or negative
p53	Wild-type*	Confluent basal staining or null expression	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type
HR-HPV ISH	Punctate nuclear and/or cytoplasmic positivity	Negative	Negative	Negative	Negative	Negative	Negative
Ki-67	Increased in ≥lower 2/3 of epithelium	Increased basal/parabasal expression	Restricted to basal layer	Restricted to basal layer	Not increased	Not increased	Not increased
CK17	N/A	Diffuse suprabasal or full-thickness expression**	N/A	N/A	Patchy expression	N/A	N/A
GATA3	Moderate to strong full thickness expression	Loss of expression in basal cells**	N/A	N/A	Moderate to strong full thickness expression	N/A	N/A

Notes: *Occasionally, HPV-associated VSCC arising from uVIN have absent basal p53 with wild-type p53 expression in the mid-epithelial layers. **Diffuse CK17 expression and/or loss of GATA3 expression are not required for a diagnosis of dVIN but can be helpful to distinguish from LS when there are overlapping features.

recognized. HPV-independent neoplasia—which encompasses dVIN, DEVIL, and VAAD—is much more likely to progress to invasive VSCC than HPV-associated VIN. Furthermore, HPV-independent VSCC tends to have worse outcomes than HPV-associated VSCC; thus, recognition of these often-subtle precursor lesions is critical. Differentiated VIN can have inconspicuous cytologic atypia, and DEVIL and VAAD typically only show dysmaturation without atypia; thus, particular attention to the atypical architecture is essential for recognition of these entities. While non-neoplastic inflammatory conditions of the vulva are often seen in association with dVIN, DEVIL, VAAD, VSCC, and occasionally uVIN, only lichen sclerosus has sufficient evidence for progression to VSCC. It is important to appropriately characterize both premalignant and malignant vulvar squamous lesions as HPV-associated or HPV-independent, as this information can provide important prognostic information to the patient and clinician.

Table 1, Table 2

References

1. Vulvar Cancer — Cancer stat facts. Accessed July 5, 2020. https://seer.cancer.gov/statfacts/html/vulva.html.
2. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. *WHO Classification of Tumours of Female Reproductive Organs.* Fourth; 2014.
3. 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.
4. Singh N, Gilks CB. Vulval squamous cell carcinoma and its precursors. *Histopathology.* 2020;76(1):128–138. https://doi.org/10.1111/his.13989.
5. Jin C, Liang S. Differentiated vulvar intraepithelial neoplasia: a brief review of clinicopathologic features. *Arch Pathol Lab Med.* 2019;143(6):768–771. https://doi.org/10.5858/arpa.2018-0019-RS.
6. Reyes MC, Cooper K. An update on vulvar intraepithelial neoplasia: terminology and a practical approach to diagnosis. *J Clin Pathol.* 2014;67(4):290–294. https://doi.org/10.1136/jclinpath-2013-202117.
7. Terlou A, Blok LJ, Helmerhorst TJM, Van Beurden M. Premalignant epithelial disorders of the vulva: Squamous vulvar intraepithelial neoplasia, vulvar Paget's disease and melanoma in situ. *Acta Obstet Gynecol Scand.* 2010;89(6):741–748. https://doi.org/10.3109/00016341003739575.
8. Hariri S, Bennett NM, Niccolai LM, et al. Reduction in HPV 16/18-associated high grade cervical lesions following HPV vaccine introduction in the United States - 2008-2012. *Vaccine.* 2015;33(13):1608–1613. https://doi.org/10.1016/j.vaccine.2015.01.084.
9. Riethdorf S, Neffen EF, Cviko A, Löning T, Crum CP, Riethdorf L. p16INK4A expression as biomarker for HPV 16-related vulvar neoplasias. *Hum Pathol.* 2004;35(12):1477–1483. https://doi.org/10.1016/j.humpath.2004.09.004.
10. Adams TS, Mbatani NH. Clinical management of women presenting with field effect of HPV and intraepithelial disease. *Best Pract Res Clin Obstet Gynaecol.* 2018;47:86–94. https://doi.org/10.1016/j.bpobgyn.2017.08.013.
11. Quick C.M.Squamous neoplasms of the vulva. In: Nucci MR, Parra-Herran C, Goldblum JR, eds. *Gynecologic Pathology*. 2nd ed.Elsevier; 2021:47-70.
12. 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.
13. Elwood H, Kim J, Yemelyanova A, Ronnett BM, Taube JM. Basal cell carcinomas of the vulva: high-risk human papillomavirus DNA detection, p16 and BerEP4 expression. *Am J Surg Pathol.* 2014;38:542–547. https://doi.org/10.1097/PAS.0000000000000143.
14. Management of Vulvar Intraepithelial Neoplasia | ACOG. Accessed August 11, 2020. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2016/10/management-of-vulvar-intraepithelial-neoplasia.
15. Pouwer AFW, van Den Einden LCG, van der Linden M, et al. Clonal relationship between lichen sclerosus, differentiated vulvar intra-epithelial neoplasia and non HPV-related vulvar squamous cell carcinoma. *Cancer Genom Proteom.* 2020;17(2):151–160. https://doi.org/10.21873/cgp.20175.
16. Saglam O, Samayoa E, Somasekar S, Naccache S, Iwasaki A, Chiu CY. No viral association found in a set of differentiated vulvar intraepithelial neoplasia cases by human papillomavirus and pan-viral microarray testing. *PLoS One.* 2015;10(4) https://doi.org/10.1371/journal.pone.0125292.
17. Trietsch MD, Nooij LS, Gaarenstroom KN, Van Poelgeest MIE. Genetic and epigenetic changes in vulvar squamous cell carcinoma and its precursor lesions: a review of the current literature. *Gynecol Oncol.* 2015;136(1):143–157. https://doi.org/10.1016/j.ygyno.2014.11.002.
18. 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.
19. Nooij LS, Ter Haar NT, Ruano D, et al. Genomic characterization of vulvar (pre) cancers identifies distinct molecular subtypes with prognostic significance. *Clin Cancer Res.* 2017;23(22):6781–6789. https://doi.org/10.1158/1078-0432.CCR-17-

1302.
20. 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.* 2020:1–11. https://doi.org/10.1038/s41379-020-00651-3 Published online August 13.
21. Watkins JC, Yang E, Crum CP, et al. Classic vulvar intraepithelial neoplasia with superimposed lichen simplex chronicus: a unique variant mimicking differentiated vulvar intraepithelial neoplasia. *Int J Gynecol Pathol.* 2019;38(2):175–182. https://doi.org/10.1097/PGP.0000000000000509.
22. 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.
23. 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. https://doi.org/10.1038/s41379-020-0524-1 Published online.
24. Nooij LS, Ter Haar NT, Ruano D, et al. Genomic characterization of vulvar (pre) cancers identifies distinct molecular subtypes with prognostic significance. *Clin Cancer Res.* 2017;23(22):6781–6789. https://doi.org/10.1158/1078-0432.CCR-17-1302.
25. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med.* 2010;363(1):24–35. https://doi.org/10.1056/NEJMoa0912217.
26. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst.* 2008;100(4):261–269. https://doi.org/10.1093/jnci/djn011.
27. 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.
28. Watkins JC, Howitt BE, Horowitz NS, et al. Differentiated exophytic vulvar intraepithelial lesions are genetically distinct from keratinizing squamous cell carcinomas and contain mutations in PIK3CA. *Mod Pathol.* 2017;30:448–458. https://doi.org/10.1038/modpathol.2016.187.
29. Akbari A, Pinto A, Amemiya Y, Seth A, Mirkovic J, Parra-Herran C. Differentiated exophytic vulvar intraepithelial lesion: clinic opathologic and molecular analysis documenting its relationship with verrucous carcinoma of the vulva. *Mod Pathol.* 2020. https://doi.org/10.1038/s41379-020-0573-5 Published online.
30. Nascimento AF, Granter SR, Cviko A, Yuan L, Hecht JL, Crum CP. Vulvar acanthosis with altered differentiation: a precursor to verrucous carcinoma. *Am J Surg Pathol.* 2004;28(5):638–643. https://doi.org/10.1097/00000478-200405000-00012.
31. Al-Bannai R, Miller D, Sadownik L, Gilks CB. Vulvar acanthosis with altered differentiation (VAAD): report of a case with progression to poorly differentiated carcinoma over a 5-yr period. *Int J Gynecol Pathol.* 2015;34(4):385–389. https://doi.org/10.1097/PGP.0000000000000182.
32. Watkins JC. Human papillomavirus–independent squamous lesions of the vulva. *Surg Pathol Clin.* 2019;12(2):249–261. https://doi.org/10.1016/j.path.2019.01.001.
33. Watkins JC. Human papillomavirus–independent squamous lesions of the vulva. *Surg Pathol Clin.* 2019;12(2):249–261. https://doi.org/10.1016/j.path.2019.01.001.
34. Fruchter R, Melnick L, Pomeranz MK. Lichenoid vulvar disease: a review. *Int J Women's Dermatology.* 2017;3(1):58–64. https://doi.org/10.1016/j.ijwd.2017.02.017.
35. Singh N, Etiology Ghatage P. Clinical features, and diagnosis of vulvar lichen sclerosus: a scoping review. *Obstet Gynecol Int.* 2020;2020. https://doi.org/10.1155/2020/7480754.
36. Bleeker MCG, Visser PJ, Overbeek LIH, Van Beurden M, Berkhof J. Lichen sclerosus: incidence and risk of vulvar squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2016;25(8):1224–1230. https://doi.org/10.1158/1055-9965.EPI-16-0019.
37. Micheletti L, Preti M, Radici G, et al. Vulvar lichen sclerosus and neoplastic transformation: a retrospective study of 976 cases. *J Low Genit Tract Dis.* 2016;20(2):180–183. https://doi.org/10.1097/LGT.0000000000000186.
38. Pinto AP, Lin MC, Sheets EE, Muto MG, Sun D, Crum CP. Allelic imbalance in lichen sclerosus, hyperplasia, and intraepithelial neoplasia of the vulva. *Gynecol Oncol.* 2000;77(1):171–176. https://doi.org/10.1006/gyno.2000.5739.
39. Zięba S, Pouwer AFW, Kowalik A, et al. Somatic mutation profiling in premalignant lesions of vulvar squamous cell carcinoma. *Int J Mol Sci.* 2020;21(14):1–13. https://doi.org/10.3390/ijms21144880.
40. Day T, Otton G, Jaaback K, Weigner J, Scurry J. Is vulvovaginal lichen planus associated with squamous cell carcinoma? *J Low Genit Tract Dis.* 2018;22(2):159–165. https://doi.org/10.1097/LGT.0000000000000384.
41. Simpson RC, Murphy R. Is vulval erosive lichen planus a premalignant condition. *Arch Dermatol.* 2012;148(11):1314–1316. https://doi.org/10.1001/2013.jamadermatol.84.
42. Cooper SM, Wojnarowska F. Influence of treatment of erosive lichen planus of the vulva on its prognosis. *Arch Dermatol.* 2006;142(3):289–294. https://doi.org/10.1001/archderm.142.3.289.
43. Watkins JC, Yang E, Crum CP, et al. Classic vulvar intraepithelial neoplasia with superimposed lichen simplex chronicus: a unique variant mimicking differentiated vulvar intraepithelial neoplasia. *Int J Gynecol Pathol.* 2019;38(2):175–182. https://doi.org/10.1097/PGP.0000000000000509.
44. Darragh TM, Colgan TJ, Thomas Cox J, 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.
45. McCluggage WG. Recent developments in vulvovaginal pathology. *Histopathology.* 2009;54(2):156–173. https://doi.org/10.1111/j.1365-2559.2008.03098.x.
46. 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.
47. Thompson EF, Chen J, Huvila J, et al. p53 Immunohistochemical patterns in HPV-related neoplasms of the female lower genital tract can be mistaken for TP53 null or missense mutational patterns. *Mod Pathol.* 2020. https://doi.org/10.1038/s41379-020-0527-y Published online.
48. Mills AM, Dirks DC, Poulter MD, Mills SE, Stoler MH. HR-HPV E6/E7 mRNA in situ hybridization: validation against PCR, DNA in situ hybridization, and p16 immunohistochemistry in 102 samples of cervical, vulvar, anal, and head and neck neoplasia. *Am J Surg Pathol.* 2017;41(5):607–615. https://doi.org/10.1097/PAS.0000000000000800.
49. Dasgupta S, Ewing-Graham PC, van Kemenade FJ, van Doorn HC, Noordhoek Hegt V, Koljenović S. Differentiated vulvar intraepithelial neoplasia (dVIN): the most helpful histological features and the utility of cytokeratins 13 and 17. *Virchows Arch.* 2018;473(6):739–747. https://doi.org/10.1007/s00428-018-2436-8.
50. Podoll MB, Singh N, Blake Gilks C, Moghadamfalahi M, Sanders MA. Assessment of CK17 as a marker for the diagnosis of differentiated vulvar intraepithelial neoplasia. *Int J Gynecol Pathol.* 2017;36(3):273–280. https://doi.org/10.1097/PGP.0000000000000317.
51. Goyal A, Zhang G, Yang B. Differential expression patterns of GATA3 in usual and differentiated types of vulvar intraepithelial neoplasia: potential diagnostic implications. *Mod Pathol.* 2018;31(7):1131–1140. https://doi.org/10.1038/s41379-018-0021-y.
52. Ordi J, Alejo M, Fusté V, et al. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: An unrecognized variant of simplex (differentiated) VIN. *Am J Surg Pathol.* 2009;33(11):1659–1665. https://doi.org/10.1097/PAS.0b013e3181b40081.
53. Rakislova N, Alemany L, Clavero O, et al. Differentiated vulvar intraepithelial neoplasia-like and lichen sclerosus-like lesions in HPV-associated squamous cell carcinomas of the vulva. *Am J Surg Pathol.* 2018;42(6):828–835. https://doi.org/10.1097/PAS.0000000000001047.
54. Lee ES, Allen D, Scurry J. Pseudoepitheliomatous hyperplasia in lichen sclerosus of the vulva. *Int J Gynecol Pathol.* 2003;22(1):57–62. https://doi.org/10.1097/00004347-200301000-00012.