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Correlation of histopathologic findings with clinical predictors of disease recurrence and progression to vulvar carcinoma in patients with differentiated vulvar intraepithelial neoplasia (dVIN)

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ABSTRACT

Objective: To evaluate predictors of recurrence and the risk of progression to carcinoma in patients with dVIN.
Methods: 36 self-identified White patients with dVIN from 2011 to 2022 were identified. Demographics, treatment and clinical course were abstracted. Histopathologic features and IHC stains were reviewed by 2 subspecialty pathologists. Standard statistical analyses were applied.
Results: Median cohort age was 70 years (range 39–91). Median follow-up was 29.5 months (range 1–123). All patients were Caucasian. 67% had lichen sclerosus (LS) adjacent to dVIN. 56% of patients had recurrent dVIN a median of 11 months from diagnosis. 14 patients had invasive squamous cell carcinoma of the vulva (SCCV) during the study period: 9 (25%) with synchronous dVIN, 5 (14%) developed SCCV after a median of 21.5 months (range 8–57). Patients treated with surgery were more likely to have recurrent/persistent dVIN ($p = 0.04$) and synchronous or progression to SCCV ($p = 0.02$) than patients treated with topical therapy. Excluding 9 women with synchronous SCCV, no initial treatment (observation, topical therapy, surgery) was superior at preventing recurrent/ progressive disease in isolated dVIN. Mutation-type p53 expression was identified in 18 (64%) and aberrant GATA3 staining/expression in 20 (56%) of cases. Aberrant GATA3 expression was associated with a higher frequency of synchronous/progression to SCCV ($p < 0.05$).
Conclusion: dVIN has an aggressive clinical course in white patients with a high risk of recurrence/persistence and synchronous/progression to SCCV despite treatment. Close surveillance with a low threshold for additional biopsies is warranted. P53 and GATA3 IHC stains may be useful markers of disease outcome.

1. Introduction

Differentiated vulvar intraepithelial neoplasia (dVIN) was originally described in 1960 but has only recently been classified as a distinct pathologic entity through molecular subtyping (Jin and Liang, 2019). It represents one of two significant etiologic pathways for the development of squamous cell carcinoma of the vulva (SCCV) (Jin and Liang, 2019; Hacker et al., 2020; Hinten et al., 2018; Del Pino et al., 2013; van der Avoort et al., 2006; Kaufman, 1995; Singh and Gilks, 2020). In the first pathway, HPV-associated high grade squamous intraepithelial lesion (HSIL), formerly known as usual-type vulvar intraepithelial neoplasia (uVIN) (Sideri et al., 2005), progresses to SCCV (Hinten et al., 2018; Del Pino et al., 2013; van der Avoort et al., 2006; Kaufman, 1995; Singh and Gilks, 2020; Madsen et al., 2008; Hoang et al., 2016; Preti et al., 2005). The second and more common pathway is HPV independent SCCV with a predilection for postmenopausal women who suffer from chronic inflammatory dermatoses of the vulva (Hacker et al., 2020; Hinten et al., 2018; Del Pino et al., 2013; van der Avoort et al., 2006). Studies have reported that 50–80% of all SCCV are HPV independent, although the study populations have consisted of predominantly White women potentially limiting these findings in other populations (Hacker et al., 2020; Singh and Gilks, 2020). Molecular analysis supports de novo p53

Abbreviations: dVIN, differentiated vulvar intraepithelial neoplasia; HSIL, high grade squamous intraepithelial lesion; HPV, human papillomavirus; IHC, immunohistochemical; LS, lichen sclerosus; LSC, lichen simplex chronicus; SCCV, squamous cell carcinoma of the vulva; uVIN, usual-type vulvar intraepithelial neoplasia; WLE, wide local excision.

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mutations as the etiologic basis of dVIN (Santos et al., 2004; Liu et al., 2021).

Recent data suggests the progression to SCCV occurs more commonly and more rapidly in patients with dVIN compared to those with HSIL (McAlpine et al., 2017; Bigby et al., 2016). According to the current literature, one out of three patients (33%) with untreated dVIN will develop SCCV at a median of 1.1 years from the time of diagnosis. In contrast, approximately one out of eighteen patients (5–6%) with HSIL will progress to SCCV which, like analogous cervical precursor lesions, usually takes years to develop (Hinten et al., 2018; McAlpine et al., 2017; Bigby et al., 2016; van Seters et al., 2005; Schuurman et al., 2013; Day et al., 2020; Van de Nieuwenhof et al., 2011; Trietsch et al., 2015; Eva et al., 2008). Despite the significant clinical implications, dVIN continues to pose a major diagnostic challenge, as it is sometimes difficult for even experienced gynecologic clinicians and pathologists to distinguish it from chronic vulvar dermatoses including lichen sclerosus (LS), lichen simplex chronicus (LSC) (Van de Nieuwenhof et al., 2011; Friedrich et al., 1980) and pseudoepitheliomatous hyperplasia (McMullen-Tabry et al., 2021).

The utility of immunohistochemical (IHC) staining to aid in the diagnosis of dVIN can be unreliable without proper individualized correlation to the clinicopathologic features. Given the occasional overlap in histology of HSIL and dVIN, IHC markers p16 and p53 are used together to delineate HPV-mediated versus HPV-independent lesions (Santos et al., 2004; Cheng et al., 2016). High-risk HPV related squamous lesions will show diffuse, block-like expression of p16. P53-mutated squamous lesions should express a mutational pattern; either contiguous nuclear basal overexpression or a complete loss of nuclear basal expression in a "null" pattern (Liu et al., 2021). However, mutational-type expression patterns can also be seen when assessing non-neoplastic vulvar conditions with IHC (McMullen-Tabry et al., 2021). Furthermore, not all cases of dVIN show mutational staining patterns resulting from the different pathways that can abrogate the integrity of p53 (Carreras-Dieguez et al., 2023). As such, the diagnosis relies heavily on correlation with clinical history and exam findings coupled with a high suspicion of the histomorphologic findings (Yang and Hart, 2000). Recent studies have suggested GATA-binding protein 3 (GATA3) which is expressed in other solid organ malignancies, as a potential new IHC marker for dVIN. GATA3 is strongly expressed in breast and urothelial carcinoma; however, the literature suggests a loss of GATA3 expression may correlate with the tumorigenesis of dVIN lesions (Goyal et al., 2018; Clark et al., 2014).

In this study, we report the natural history of dVIN and evaluate potential clinical predictors of persistence or recurrence and the risk of progression to squamous cell carcinoma in a cohort of patients with histologically confirmed dVIN. Additionally, we show the potential diagnostic utility of GATA3 immunohistochemical staining in the identification of dVIN to improve early detection, and p53 and GATA3 characterization as potential prognostic markers of the clinical outcome.

2. Materials and methods

A retrospective chart review was performed on all patients with histologically documented dVIN at Dartmouth-Hitchcock Medical Center in Lebanon, NH between 2011 and 2022. Institutional Review Board approval was obtained with a waiver of informed consent. Clinical data was abstracted from medical records. Patients self-identified race from 4 categories: American Indian/Indigenous American, Asian, White, Black/African American. Ethnicity was not recorded during the study period. Demographic and clinical variables included age, race, BMI, evidence of immunosuppression (recent or current systemic steroid use, use of immune modulating agents, HIV infection or other immunocompromised state, autoimmune condition and/or concurrent neoplastic disease), current or former tobacco use, history of abnormal Pap smear, history of lower genital tract dysplasia, and presenting symptoms. Histopathologic features, treatment and clinical course included: anatomic location, focality of lesions, presence of LS on resection specimen, initial treatment (observation, topical therapy (e.g., clobetasol, imiquimod), or surgical excision), and persistence or recurrence and/or progression to or synchronous SCCV were described. No patients received more than one initial treatment concurrently. No patients were treated with an ablative procedure (e.g., laser). Initial treatment modality was at the discretion of the provider depending on the anatomic location, extent of the abnormal vulvar tissue and synchronous pathology such as LS or SCCV. Providers consisted of four gynecologic oncologists and/or one general gynecology attending with a specialized vulvar pathology practice. Patients were followed with semi-annual examinations and biopsies at the discretion of their gynecologic provider.

The slides of all patients with a diagnosis of dVIN were reviewed retrospectively for diagnostic confirmation by two pathologists with relevant subspecialty training in gynecologic pathology and dermatopathology. Patients met inclusion criteria if there was a consensus diagnosis of dVIN by both pathologists and there was sufficient tissue available for ancillary immunohistochemical staining. Immunohistochemical staining for p53, p16, GATA3, ER and PR was performed on all cases and independently reviewed and scored by each pathologist. Patients without consensus agreement on the diagnosis of dVIN were excluded from the study (N = 3) as were those with insufficient biopsy material to accommodate IHC staining (N = 8). p53 immunohistochemical stains were scored as "p53 mutant-type" expression if the stain showed contiguous nuclear basal expression or complete loss of nuclear basal expression, in contrast with wild-type non-contiguous nuclear basal expression. GATA3 was scored as "positive" (intact nuclear basal expression), or "aberrant" with partial or complete loss of nuclear basal expression. Patients were followed until death, completion of the study timeframe, or lost to follow-up.

The primary outcome measures were the prevalence of persistence or recurrence of dVIN and the prevalence of progression to or presence of synchronous SCCV. Patients were categorized as having persistent disease if repeat vulvar biopsy performed at a subsequent visit confirmed dVIN. Patients were considered to have recurrent disease when there was resolution of dVIN by examination or vulvar biopsy following initial treatment with a subsequent biopsy confirming dVIN. Patients who developed SCCV on a subsequent biopsy after dVIN diagnosis were considered to have progressive disease and those who had resection specimens showing concurrent SCCV at the time of dVIN diagnosis were considered to have synchronous disease. Chi-square analysis and Fisher's exact test were used to compare categorical differences between patients. Continuous variables were examined with the Mann-Whitney *U* test. A two-tailed p-value of 0.05 was statistically significant. Statistical analysis was performed using GraphPad Prism (version 5.0 for Windows; GraphPad Software, San Diego, CA).

3. Results

Forty-seven patients had a diagnosis of dVIN during the study period.. Three patients were excluded due to lack of consensus on the dVIN diagnosis upon re-review by the two pathologists and eight patients had insufficient biopsy material to accommodate IHC staining resulting in a final study cohort of 36 patients who met inclusion criteria. [Fig. 1]. Median age of the cohort was 70 years (range 39–91) and all patients were Caucasian. Median follow-up time was 29.5 months (range 1–123). Fourteen patients (39%) had a history of current or former tobacco use and 26 patients (72%) had a BMI $\geq$ 26. Twelve patients (33%) had evidence of immunosuppression including one patient with concurrent diffuse large B-cell lymphoma treated with an experimental monoclonal antibody following multiple unsuccessful chemotherapeutic regimens. Seventeen (47%) patients had a history of lower genital tract dysplasia including ten patients (28%) with a history of an abnormal Pap smear: five of these patients had been previously treated for cervical intraepithelial neoplasia (CIN), one of which subsequently developed anal intraepithelial neoplasia (AIN) 3. One patient had a

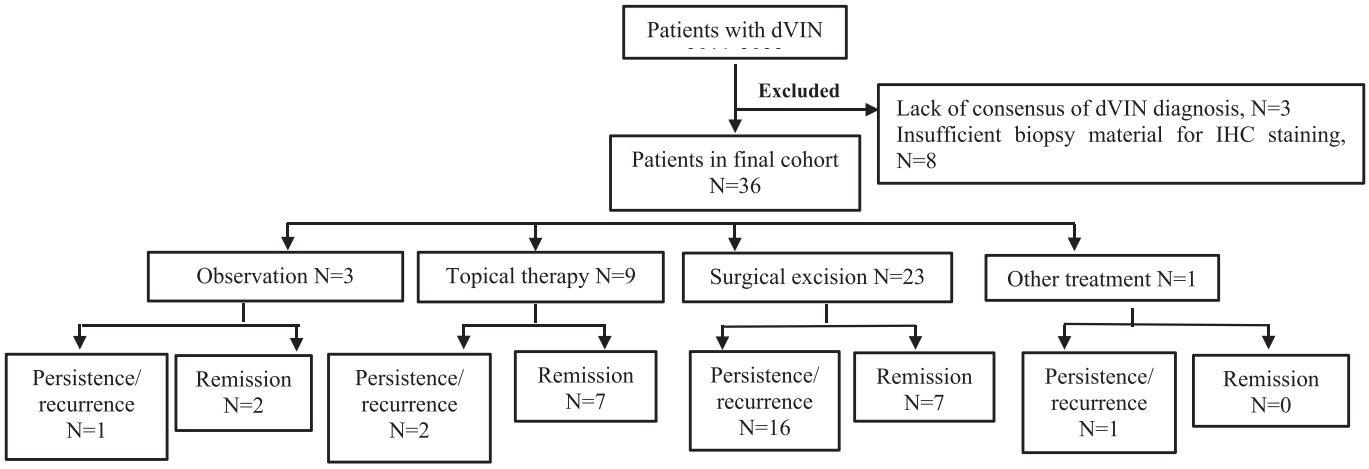

Fig. 1. Patient cohort, initial treatment and clinical course of patients with differentiated vulvar intraepithelial neoplasia (dVIN).

remote history of stage IIB cervical adenocarcinoma treated with radiation.

All patients had visibly abnormal vulvar tissue that was biopsied at diagnosis. The most common presenting symptoms of dVIN were vulvar itching (56%) and/or pain (37%). Nine patients with had dVIN associated SCCV diagnosed by biopsy. In one case, the patient presented with no vulvar symptoms but was noted to have a white perineal plaque on routine examination with biopsy revealing dVIN. The most common location of dVIN in this cohort was the labium majus (70%) followed by the perineum (19%). Remaining lesions were found on the labium minus and clitoris.

Initial treatment for patients with dVIN is outlined in Fig. 1 and consisted of topical therapy in 9 (25%) and surgical excision in 23 (63%). Three patients were observed due to complete resolution of any lesion following the vulvar biopsy that established the diagnosis of dVIN. Topical treatment with clobatesol proprionate was used in 8 patients with adjacent LS and imiquimod in 1 patient following vulvar biopsy with no visible residual abnormalities of the vulva. Surgery was undertaken for patients with visible discrete lesions and all women with histologically confirmed, resectable SCCV. Sixteen patients had simple partial vulvectomy and 7 had a primary radical vulvectomy with or without lymph node assessment and reconstruction due to synchronous SCCV. One of these patients had a vulvar biopsy highly suspicious for cancer followed by a right simple partial vulvectomy with a frozen section that failed to demonstrate definitive invasive carcinoma. The final pathology showed an invasive squamous cell carcinoma with a depth of invasion of 1.37 mm and the patient was counseled to have a right radical vulvectomy with right inguinal lymphadenectomy. The patient sought a second opinion and ultimately underwent a right partial radical vulvectomy with right inguinal lymphadenectomy. The final pathology revealed no residual squamous cell carcinoma and negative lymph nodes. One patient with dVIN-associated SCCV underwent primary radiation with sensitizing cisplatin prior to undergoing radical vulvectomy with plastic surgery reconstructive flap. This was preferred due to the location of the lesion deeply involving the urethra for which a primary surgical resection would necessitate a urinary diversion procedure.

Twenty of 36 study patients (56%) had persistent or recurrent dVIN (Table 1). Recurrent disease occurred at a median of 11 months (range 3–32) from the time of initial diagnosis. There was no statistically significant difference in the age ($p = 0.3$) or length of follow-up ($p = 0.4$) between those who had persistent or recurrent dVIN compared to those

Table 1
Demographics and Histopathologic characteristics of patients with persistent/recurrent dVIN and progression to or synchronous squamous cell carcinoma.

	Recurrence/ persistence N = 20	No recurrence/ persistence N = 16	P value	Cancer N = 14	No Cancer N = 22	P value
Age median, range (years)	67 (39–82)	71 (48–91)	0.3	69 (45–82)	70 (39–91)	0.8
Follow-up median, range (years)	31 (1–123)	29 (3–53)	0.4	35 (1–123)	29 (3–53)	0.5
BMI ≥ 26	15 (75%)	11 (69%)	0.7	11 (79%)	15 (68%)	0.7
Immunosuppression	5 (25%)	7 (44%)	0.3	2 (14%)	10 (45%)	0.1
Current or former tobacco use	9 (45%)	5 (31%)	0.5	6 (43%)	8 (36%)	0.7
Hx abnormal Pap	6 (30%)	4 (25%)	1	3 (21%)	7 (32%)	0.7
Hx lower genital tract dysplasia	12 (60%)	5 (31%)	0.1	8 (57%)	9 (41%)	0.5
Initial Treatment All dVIN			0.04			0.02
Observation	1 (5%)	2 (13%)		0	3 (13%)	
Topical therapy	2 (10%)	7 (44%)		1 (7%)	8 (36%)	
Surgical excision	16 (80%)	7 (44%)		12 (86%)	11 (50%)	
Other	1 (5%)	0		1 (7%)	0	
Initial Treatment Isolated dVIN	Recurrence/ Persistence N = 11	No Recurrence/ Persistence N = 16	p	Cancer N = 5	No Cancer N = 22	p
Observation	1 (9%)	2 (13%)	0.3	0	3 (13%)	0.4
Topical	2 (18%)	7 (44%)		1 (20%)	8 (36%)	
Surgical Excision	8 (40%)	7 (44%)		4 (80%)	11 (50%)	

who did not. No demographic characteristics or histologic features were associated with persistent or recurrent dVIN. Patients treated with topical therapy were less likely to have recurrent/persistent dVIN than those treated with surgical excision 10% versus 80% ($p = 0.04$). Patients treated with surgery included the 9 women with dVIN associated synchronous SCCV. Excluding the 9 patients with dVIN associated SCCV, 27 of 36 study patients (75%) had isolated dVIN lesions and there was no statistically significant difference in the frequency of persistent/recurrent dVIN between treatment modalities. All isolated dVIN lesions were <1.5 cm.

Fourteen of 36 study patients (39%) had synchronous SCCV ($n = 9$) or progressed to SCCV ($n = 5$) during the study period. (Table 1). The median time to progression to SCCV was 21.5 months (range 8–57). There was no difference in median age at the time of initial dVIN diagnosis between those who progressed or had synchronous cancer compared to those who did not ($p = 0.8$). Additionally, there was no difference in the length of follow up between those who progressed to cancer or had synchronous cancer versus those who did not ($p = 0.5$). No demographic characteristics were associated with progression to or synchronous SCCV. Patients treated with topical therapy were less likely to have synchronous or progression to SCCV than those treated with surgical excision 7% versus 86% ($p = 0.02$). Excluding the 9 patients with dVIN associated SCCV, there was no statistically significant difference in the frequency of progression to SCCV among the 27 women with isolated dVIN between topical versus surgical treatment. Of the 5 patients who progressed to SCCV after initial treatment for dVIN, one had topical treatment and the remaining patients were treated with surgery (Table 2). These patients all had dVIN in the surgical resection margins. Five of these patients had recurrent SCCV at a median of 24 months from primary SCCV diagnosis and four remain without evidence of disease and one patient had recurrent dVIN.

Fig. 2 panels A, C and E highlight the hematoxylin and eosin (H&E) images of lichen sclerosus, dVIN and invasive SCCV, respectively from a select patient within the study cohort. Panels B, D and F demonstrate the progressive loss of GATA3 staining from lichen sclerosus to dVIN to SCCV, respectively. The pathologic and immunohistochemical

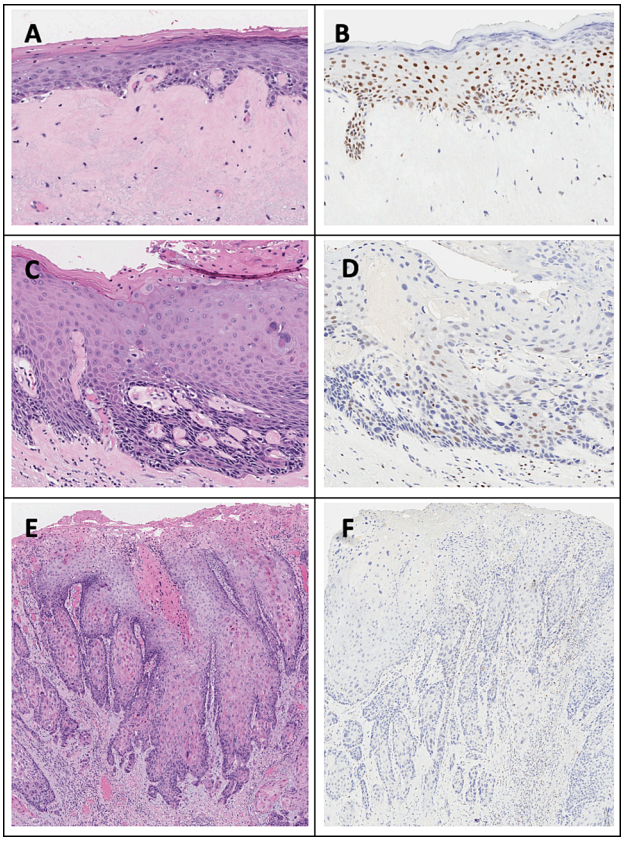

Fig. 2. Aberrant GATA3 staining/expression correlates with pre-invasive and invasive vulvar lesions. Footnote: Panels A), C) and E) demonstrate H&E appearance of LS, dVIN and SCCV respectively. Panel B) demonstrates positive GATA3 staining in LS. Panels D) and F) demonstrate aberrant GATA3 staining in dVIN and SCCV, respectively.

Table 2
14 Patients with differentiated vulvar intraepithelial neoplasia with synchronous squamous cell carcinoma of the vulva or progression to cancer during study period.

Synchronous N = 9

Patient ID	Stage SCCV at Dx	Treatment	Disease Status	Follow-up since vulvar cancer Dx (months)
4	1A	Simple vulvectomy	NED	6
11	IB	Left partial radical vulvectomy, left inguinal lymphadenectomy	Recurrent dVIN	89
12	IB	Partial radical vulvectomy, bilateral inguinal lymphadenectomy w/rhomboid flap	NED	24
21	IB	EBRT w/sensitizing cisplatin followed by radical vulvectomy, VY flap reconstruction	AWD	15
24	II	Left partial radical vulvectomy, left inguinal lymphadenectomy	AWD	24
30	IB	Partial radical vulvectomy, bilateral inguinal lymphadenectomy followed by adjuvant radiation	AWD	14
32	IA	Left partial radical vulvectomy	NED	48
33	IB	Partial right radical vulvectomy	DOD	53
38	IB	Left partial radical vulvectomy, sentinel left inguinal lymph node biopsy	DOD	46

Dx diagnosis SCCV squamous cell carcinoma, NED, no evidence of disease; EBRT, external beam radiation therapy; AWD, alive with disease; DOD, died of disease

Progression N=5

Patient ID	Initial Treatment dVIN	Time to SCCV (months)	Stage SCCV	Treatment SCCV	Disease Status	Follow-up since vulvar cancer Dx (months)
3	WLE	57	IB	Left partial radical vulvectomy, left inguinal lymphadenectomy	NED	66
6	Simple partial vulvectomy	22	IB	Partial radical vulvectomy, bilateral inguinal lymphadenectomy	NED	84
20	Simple partial vulvectomy	21	IB	Left radical vulvectomy, right partial radical vulvectomy	AWD	31
35	Simple partial vulvectomy	8	IB	Right partial radical vulvectomy, right inguinal lymphadenectomy w/rhomboid flap	NED	32
39	Clobetasol	11	IA	WLE	NED	11

SCCV squamous cell carcinoma, NED, no evidence of disease; EBRT, external beam radiation therapy; AWD, alive with disease; DOD, died of disease WLE wide local excision

characteristics of the cohort are described in Table 3. The presence or absence of IHC staining of dVIN with p16 and ER positivity was not associated with persistence/recurrence or synchronous/progression to SCCV. No case exhibited PR positivity. In our cohort, 50% of patients had aberrant mutation-type expression of p53, 53% had patchy/negative p16 expression, and no cases showed diffuse, block-like expression of p16 as is seen in high-risk HPV-related vulvar squamous lesions. Fifty-six percent of cases had aberrant GATA3 expression/staining with partial or complete loss of GATA3 by IHC. Most patients (64%) had concurrent LS adjacent to dVIN on resection specimens. Aberrant GATA3 staining was more common in cases with p53 mutation-type expression compared to normal 53 expression, 72% versus 50%, although this finding was not significant. A statistically higher proportion of women with persistent or recurrent dVIN had aberrant staining/expression of GATA3 (85%) compared to those who did not have persistent or recurrent dVIN (19%) $p < 0.001$. No other IHC or pathologic characteristics were correlated with persistent or recurrent dVIN. Excluding the nine women with synchronous SCCV, aberrant GATA-3 expression was more common among the 27 women with isolated dVIN who had recurrence compared to those who did not recur (63% versus 30%), however the association was not statistically significant in this subgroup ($p = 0.3$). P53-mutation type staining was not associated with a higher likelihood of recurrent dVIN in the 27 women with isolated dVIN ($p = 0.9$). A statistically higher proportion of women with synchronous or progression to SCCV had p53 mutation-type staining (86%) compared to those who did not have synchronous or progressive SCCV (27%) $p < 0.001$. A statistically higher proportion of women with synchronous or progression to SCCV also had aberrant GATA 3 staining/expression (86%) compared to those who did not have synchronous or progression to SCCV (36%) $p < 0.001$). Five women progressed to SCCV among the 27 women with isolated dVIN which precludes analysis of any association of aberrant GATA-3 or mutation-type p53 staining with progression to cancer.

4. Discussion

Differentiated vulvar intraepithelial neoplasia (dVIN) carries a significant risk of recurrence and malignant transformation and requires ongoing surveillance. More than half of patients with dVIN in this study of 36 women had persistent or recurrent disease and 39% of patients had either synchronous invasive squamous cell carcinoma (SCCV) of the vulva (9 cases) or progressed to SCCV during the study (5 cases). Immunohistochemical staining (IHC) was a useful adjunct diagnostic and prognostic marker in this cohort of dVIN. Aberrant IHC staining of p53 and GATA3 was identified in greater than half of dVIN cases and was associated with worse disease outcome. Aberrant expression/staining of GATA3 may portend a higher likelihood of persistent or recurrent disease, while mutation-type p53 and aberrant GATA3 staining were associated with a higher probability of synchronous, or progression to, vulvar cancer.

None of the demographic characteristics in our study were associated with persistent or recurrent dVIN or progression to or synchronous SCCV. In two population-based studies, the peak incidence of dVIN occurred between ages 75 and 85 which is comparable to the reported median age of 70 years at diagnosis in this study cohort (Van de Nieuwenhof et al., 2009; Heller et al., 2021). Compared to high-risk HPV-related vulvar lesions, which are typically found in younger sexually active females, dVIN almost always occurs in older, postmenopausal females with chronic inflammatory dermatoses (Hinten et al., 2018).

The diagnostic challenge of discriminating dVIN, especially solitary dVIN, from benign dermatoses including pseudo epithelial hyperplasia and lichen sclerosus further contributes to the lack of clarity regarding the clinical course of dVIN.30,32 In a study by Van De Neiuwenhof et al., 42% of vulvar biopsies initially diagnosed as lichen sclerosus taken from patients who had progression to SCCV were reclassified as dVIN after re-review by two experienced gynecologic pathologists. Similar to other reports, we found a high proportion of LS adjacent to dVIN in two-thirds of cases (Bigby et al., 2016; Van de Nieuwenhof et al., 2011). It can be difficult to differentiate dVIN from non-neoplastic vulvar dermatoses using H&E and immunohistochemical analyses which could contribute to diagnostic delay and inconsistent patient surveillance (McMullen-Tabry et al., 2021). Differentiated VIN is found adjacent or contiguous to SCCV in 46%-95% of cases depending on the methodology and time period of the study (Bigby et al., 2016; Heller et al., 2021). There is much less data about the natural history and progression to primary SCCV of isolated dVIN lesions without synchronous SCCV (McAlpine et al., 2017; Heller et al., 2021; Voss et al., 2021). Unlike most previously published studies, the majority of our study cohort had isolated dVIN ($n = 27$) rather than dVIN in the setting of synchronous SCCV ($n = 9$). Several studies have reported the risk of primary SCCV in women with solitary dVIN as summarized in a systematic review by Voss et al. (2021) The authors highlight that most of these small studies consist of fewer than 20 cases with heterogenous populations subject to selection bias and incomplete data regarding treatment and follow up. The authors described an absolute risk of dVIN progressing to primary SCCV between 33 and 86%, with a median time to progression to SCCV of 9–23 months. Among women with dVIN-associated SCCV they reported an absolute risk of SCCV recurrence of 32–94% occurring within 3 years of diagnosis. Our study outcomes are comparable with 19% of women with solitary dVIN progressing to primary SCCV and a 56% risk of recurrence in women with SCCV and adjacent dVIN. Our findings support the observation that dVIN carries a high risk and rapid progression of developing into squamous cell carcinoma of the vulva in White women (McAlpine et al., 2017; Van de Nieuwenhof et al., 2009).

The optimal treatment for dVIN and risk factors for recurrent disease remain uncertain. The recommendation for surgical excision with negative margins of dVIN is based upon case series and expert opinion. Surgery is the mainstay of treatment for dVIN with synchronous resectable SCCV (Heller et al., 2021; Voss et al., 2021; Pretti et al., 2022). Surgery was the primary treatment for most of the study cohort including those with synchronous SCCV, which may biasany comparisons of outcomes with the higher risk patients being treated with surgery. Yang et al described three patients who developed SCCV following a diagnostic biopsy while the none of the other five patients treated with surgical excision developed SCCV (Yang and Hart, 2000). Among women with isolated dVIN, none of the treatment modalities was

Table 3
Immunohistochemical characteristics of differentiated vulvar intraepithelial neoplasia specimens in patients with persistence or recurrence and/or progression to or synchronous squamous cell carcinoma of the vulva.

	Recurrence/ Persistence N = 20	No recurrence/ persistence N = 16	P value	Cancer N = 14	No cancer N = 22	P value
p 53 Mutation	13 (65%)	5 (31%)	0.4	12 (86%)	6 (27%)	<0.001
p 16 positive	12 (60%)	7 (44%)	1	7 (50%)	12 (55%)	0.4
ER positive	3 (15%)	2 (13%)	1	2 (14%)	3 (14%)	1
GATA 3 loss/heterogenous	17 (85%)	3 (19%)	<0.001	12 (86%)	8 (36%)	<0.05
Adjacent LS	14 (70%)	10 (63%)	1	13 (93%)	11 (50%)	1

LS = lichen sclerosus ER Estrogen receptor.

associated with a higher probability of recurrence or progression to invasive squamous cell carcinoma. No one has described the use of topical treatment among women with solitary dVIN. In the current cohort, nine women with solitary dVIN were treated with topical therapy. Eight patients with associated lichen sclerosus were treated with clobatesol and 1 patient with a history of usual type VIN was treated with imiquimod. Seven women treated with clobaetsol remain without recurrence during the study follow up. The small number of these women and variable regimens and schedules of treatment precludes any comment on factors that might predict who would be a candidate for topical treatment. Our data do suggest that topical treatment may be an option for appropriately counseled women with isolated dVIN and coexisting lichen sclerosus, who can be closely followed.

Mutation-type immunohistochemical expression of p53 is an established marker used by pathologists to assist with diagnosing dVIN as this staining pattern is a frequent finding in HPV-negative SCCV (McMullen-Tabry et al., 2021). In our cohort, p53 mutation-type expression was found to be associated with synchronous SCCV or progression of dVIN to carcinoma (p=<0.001). This association of mutation-type staining of p53 and recurrent dVIN was not found in the subgroup of women with isolated dVIN which may temper the utility of p53 IHC to predict disease course. Given the challenges with clinical and pathologic diagnosis of dVIN, additional diagnostic markers are being investigated to distinguish dVIN from benign mimickers or potentially stratify the risk of progression to SCCV. A recent study by Goyal et al. described GATA3 as a potential new biomarker for dVIN. Specifically, moderate to strong GATA3 immunostaining was reported in uVIN (similar to non-neoplastic epidermis), but (88%) of dVIN cases exhibited aberrant GATA3 staining (partial or complete loss) (Goyal et al., 2018). Our results support that aberrant GATA3 staining occurs in the majority (56%) of dVIN cases. In our study, aberrant GATA3 staining was a poor prognostic finding associated with both a higher risk of recurrence of dVIN and synchronous or progression to SCCV, although not as useful a predictor of recurrence in women with isolated dVIN. These results corroborate the findings of Goyal et al, suggesting there is utility of GATA3 as a potential mnyarker for improving the histologic diagnosis and management of dVIN (Goyal et al., 2018).

The strengths of our study include retrospective confirmation of dVIN by 2 pathologists with subspecialty training in gynecologic and dermatopathology. Our health system has a comprehensive regional electronic database, thereby decreasing the number of patients lost to follow-up. Our rural academic medical center provides specialty care from a large region with highly centralized, experienced providers with aligned practice patterns including the cadence of surveillance and treatment. Notable limitations of our study include the small cohort size, retrospective design and a racially and ethnically homogeneous cohort of all White patients. The exclusion of eight patients due to lack of consensus diagnosis or available tissue for ancillary immunohistochemical staining underscores the limitations of our findings on clinical outcomes in a relatively uncommon condition like dVIN. These results may potentially limit the ability of our results to generalize to other patient populations.

In summary, differentiated vulvar intraepithelial neoplasia has an aggressive clinical course and merits careful and regular surveillance with a low threshold to perform additional biopsies by experienced providers to exclude progression to SCCV. Larger multi-institutional studies with a greater variety of dVIN mimickers are needed to confirm our results since p53 and GATA3 are not entirely sensitive or specific for dVIN. However, our results provide evidence showing a decrease or loss of GATA3 expression in dVIN and associated squamous cell carcinoma which strongly questions the utility of GATA3 IHC as an ancillary marker in the diagnosis of this challenging entity. Further characterization of the molecular drivers of dVIN progression may also translate into better outcome data and the opportunity for novel treatment modalities.

CRediT authorship contribution statement

Jill N.T. Roberts: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jessica L. Bentz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Robert E. LeBlanc:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ilana Cass:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bigby, S.M., Eva, L.J., Fong, K.L., Jones, R.W., 2016. The natural history of vulvar intraepithelial neoplasia, differentiated type: evidence for progression and diagnostic challenges. Int. J. Gynecol. Pathol. 35 (6), 574–584.
Carreras-Dieguez, N., Saco, A., Del Pino, M., Marimon, L., Del Campo, R.L., Manziotti, C., Fuste, et al., 2023. HPV and p53 status define three types of vulvar squamous cell carcinoma with distinct clinical, pathological and prognostic features. Histopathology 83 (1), 17–30.
Cheng, A.S., Karnezis, A.N., Jordan, S., Singh, N., McAlpine, J.N., Gilks, C.B., 2016. p16 immunostaining allows for accurate subclassification of vulvar squamous cell carcinoma into HPV-associated and HPV-Independent cases. Int. J. Gyn. Pathol. 35 (4), 385–393.
Clark, B.Z., Beriwal, S., Dabbs, D.J., Bhargava, R., 2014. Semiquantitative GATA-3 immunoreactivity in breast, bladder, gynecologic tract, and other cytokeratin 7-positive carcinomas. Am. J. Clin. Pathol. 142 (1), 64–71.
Day, T., Marzol, A., Pagano, R., Jaaback, K., Scurry, J., 2020. Clinicopathologic diagnosis of differentiated vulvar intraepithelial neoplasia and vulvar aberrant maturation. J. Low. Genit. Tract Dis. 24 (4), 392–398.
Del Pino, M., Rodriguez-Carunchio, L., Ordi, J., 2013. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. Histopathol. 62 (1), 161–175.
Eva, L., Ganesan, R., Chan, K., Honest, H., Malik, S., Luesley, D., 2008. Vulval squamous cell carcinoma occurring on a background of differentiated vulval intraepithelial neoplasia is more likely to recur: a review of 154 cases. J. Reprod. Med. 53 (6), 397–401.
Friedrich Jr, E.G., Wilkinson, E.J., Fu, Y.S., 1980. Carcinoma in situ of the vulva: a continuing challenge. Am. J. Obstet. Gynecol. 136 (7), 830–838.
Goyal, A., Zhang, G., Yang, B., 2018. Differential expression patterns of GATA3 in usual and differentiated types of vulvar intraepithelial neoplasia: potential diagnostic implications. Mod. Pathol. 31 (7), 1131–1140.
Hacker, N.F., Eifel, P.J., 2020. Vulvar cancer. In: Berek, J.S., Hacker, N.F. (Eds.), Berek & Hacker's Gynecologic Oncology, seventh ed. Lippincott Williams & Wilkins, Philadelphia, pp. 503–547.
Heller, D., Day, T., Allbritton, J.I., Scurry, J., Radici, F., Welch, K., Preti for the ISSVD Difficult Pathologic Diagnoses Committee, 2021. Diagnostic criteria for differentiated vulvar intraepithelial neoplasia and vulvar aberrant maturation. J. Lower Genit. Tract. Disease 45, 57–70.
Hinten, F., Molijn, A., Eckhardt, L., Massuger, L.F., Quint, W., Bult, P., et al., 2018. Vulvar cancer: two pathways with different localization and prognosis. Gynecol. Oncol. 149 (2), 310–317.
Hoang, L.N., Park, K.J., Soslow, R.A., Murali, R., 2016. Squamous precursor lesions of the vulva: current classification and diagnostic challenges. Pathology 48 (4), 291–302.
Jin, C., Liang, S., 2019. Differentiated vulvar intraepithelial neoplasia: a brief review of clinicopathologic features. Arch. Pathol. Lab. Med. 143 (6), 768–771.
Kaufman, R., 1995. Intraepithelial neoplasia of the vulva. Gynecol. Oncol. 56 (1), 8–21.
Liu, Y.A., Ji, J.X., Almadani, N., Crawford, R.I., Gilks, C.B., Kinloch, M., Hoang, L., 2021. Comparison of p53 immunohistochemical staining in differentiated vulvar intraepithelial neoplasia (dVIN) with that in inflammatory dermatoses and benign squamous lesions in the vulva. Histopathology 78 (3), 424–433.
Madsen, B.S., Jensen, H.L., van den Brule, A., Wholfahrt, J., Frisch, M., 2008. Risk factors for invasive squamous cell carcinoma of the vulva and vagina—population-based case-control study in Denmark. Int. J. Cancer 122 (12), 2827–2834.
McAlpine, J.N., Kim, S.Y., Akbari, A., Eshragh, S., Reuschenbach, M., von Knebel, D.M., Prigge, E.S., et al., 2017. HPV-independent differentiated vulvar intraepithelial neoplasia is associated with an aggressive clinical course. Int. J. Gynecol. Pathol. 36 (6), 507–516.
McMullen-Tabry, E.R., Schechter, S.A., Wang, G.Y., Sciallis, A.P., Hrycaj, S.M., Chan, M. P., Skala, S.L., 2021. p53/ck17 dual stain improves accuracy of distinction between

differentiated vulvar intraepithelial neoplasia and its mimics. Int. J. Gynecol. Pathol. 41 (3), 298–306.
Preti, M., Van Seters, M., Sideri, M., Van Beurden, M., 2005. Squamous vulvar intraepithelial neoplasia. Clin. Ob. Gynecol. 48 (4), 845–861.
Pretti, M., Joura, E., Vieira-Baptista, P., van Beurden, M., Bevilacqua, F., Bleeker, M.C.G., Bornsetin, J., et al., 2022. ESGO, ISSVD, ECSVD and EFC consensus statement on pre-invasive lesions. J. Lower Genit. Tract. Disease 26 (3), 229–244.
Santos, M., Montagut, C., Mellado, B., Garcia, A., Ramon y Cajal, S., Cardesa, A., et al., 2004. Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. Int. J. Gynecol. Pathol. 23 (3), 206–214.
Schuurman, M.S., van den Einden, L.C., Massuger, L.F., Kiemeney, L.A., van der Aa, M. A., de Hullu, J.A., 2013. Trends in incidence and survival of Dutch women with vulvar squamous cell carcinoma. Eur. J. Cancer 49, 3872.
Sideri, M., Jones, R., Wilkinson, E., Preti, M., Heller, D., Scurry, J., et al., 2005. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD vulvar oncology subcommittee. J. Reprod. Med. 50 (11), 807–810.
Singh, N., Gilks, B.C., 2020. Vulval squamous cell carcinoma and its precursors. Histopathol. 76 (1), 128–138.
Trietsch, M.D., Nooij, L.S., Gaarenstroom, K.N., van Poelgeest, M., 2015. Genetic and epigenetic changes in vulvar squamous cell carcinoma and its precursor lesions: a review of the current literature. Gynecol. Oncol. 136 (1), 143–157.
Van de Nieuwenhof, H.P., Massager, L.F., van der Avoort, I.A., Bekkers, R.L., Casparie, M., Aba, W., et al., 2009. Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. Eur. J. Cancer 45, 851–856.
Van de Nieuwenhof, H.P., Bulten, J., Hollema, H., Dommerholt, R.G., Massuger, L., van der Zee, A., et al., 2011. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. Mod. Pathol. 24 (2), 297–305.
van der Avoort, I., Shirango, H., Hoevenaars, B., Grefte, J., de Hullu, J., de Wilde, P., et al., 2006. Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. Int. J. Gynecol. Pathol. 25 (1), 22–29.
van Seters, M., van Beuden, M., de Craen, A., 2005. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. Gynecol. Oncol. 97 (2), 645–651.
Voss, E.O., Thuijs, N.B., Vermeulen, R.F.M., Wilthagen, E.A., van Beurden, M., Bleeker, M.C.G., 2021. The vulvar cancer risk in differentiated vulvar intraepithial neoplasia: a systematic review. Cancers 13 (6170), 1–10.
Yang, B., Hart, W.R., 2000. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. Am. J. Surg. Pathol. 24, 429–441.