The future of research on vulvar intraepithelial neoplasia: towards precision diagnostics and risk stratification

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Abstract

Vulvar intraepithelial neoplasia (VIN) represents a heterogeneous group of premalignant lesions arising through distinct human papillomavirus (HPV)-associated and HPV-independent pathways. Despite well-characterized differences in etiology, prognosis, and progression risk, current management remains largely uniform and predominantly surgical. This one-size-fits-all approach neglects opportunities for individualized care and exposes patients, particularly younger women and those with multifocal disease, to potentially avoidable psychosexual morbidity. Recent advances in molecular pathology, including immunohistochemistry, genomic profiling, DNA methylation analysis, and copy number alteration detection, offer promising avenues for refining diagnostic precision and enabling risk stratification. Integration of markers such as $p16^{INK4a}$, p53, and emerging methylation panels into diagnostic workflows may improve differentiation between lesion subtypes, guide surveillance, and identify candidates for conservative therapy. Moreover, the unique pathogenesis of vulvar high-grade squamous intraepithelial neoplasia, which diverges from squamocolumnar junction (SCJ)-driven models seen in other HPV-associated cancers, highlights the need for focused research on host-virus interactions and early oncogenic events in non-SCJ epithelium. Future directions include non-invasive sampling methods, molecularly-guided surveillance protocols, therapeutic HPV vaccines, and combined immunomodulatory treatments to reduce the burden of excisional therapy. Establishing precision-based approaches for VIN could not only preserve vulvar integrity and function but also improve oncological outcomes through targeted prevention and early intervention strategies. [J Turk Ger Gynecol Assoc.2025; 26(3): 230-4]

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Introduction

The current landscape of vulvar intraepithelial neoplasia and its limitations

Recent years have witnessed a growing emphasis on precision medicine approaches in gynaecologic oncology, including vulvar intraepithelial neoplasia (VIN). VIN encompasses a spectrum of preinvasive lesions of the vulvar epithelium, with human papillomavirus (HPV)-associated and HPV-independent lesions reflecting two distinct oncogenic pathways (1). HPV-related vulvar high-grade squamous intraepithelial neoplasia (vHSIL) is commonly diagnosed in younger women and has

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a more favorable prognosis, with a relatively low 10-year cumulative risk of invasive cancer, estimated at approximately 10% (2). In contrast, HPV-independent VIN, which often arises in the context of chronic dermatoses, such as lichen sclerosus (LS), is associated with a higher neoplastic progression rate of up to 50% and tends to affect older women (3).

Despite this evidence of difference, the clinical management of VIN is largely uniform, with excisional surgery being the mainstay of treatment regardless of individualized cancer risk (4). This approach, while effective in removing dysplastic tissue, carries significant psychosexual and anatomical consequences, especially for younger patients or those with multifocal disease (5). Moreover, the diagnosis of HPV-independent VIN remains challenging, given its subtle histological presentation and overlap with benign inflammatory dermatoses (6). Thus, a critical gap persists in our ability to identify patients at high risk for progression and those who could benefit from conservative or surveillance strategies.

The future of VIN research lies in refining diagnosis precision, integrating histopathological features with molecular biomarkers, with the aim of improving diagnostic accuracy, stratifying risk, and guiding individualized surveillance and treatment strategies. This would ultimately facilitate personalized treatment paradigms.

Biomarker-driven diagnostics and prognostics

A giant step towards addressing this gap would be the integration of objective molecular biomarkers into both diagnostic and prognostic algorithms. Advances in molecular pathology have opened new frontiers in the diagnosis and classification of VIN, offering tools that enhance histological interpretation and provide insight into the biological behavior of preinvasive lesions. The integration of immunohistochemical (IHC) and genomic markers into routine diagnostic workflows promises to improve the accuracy and reproducibility of VIN classification and identify the lesions with a higher risk of malignant transformation (7).

The IHC markers $p16^{INK4a}$ and p53 are central to the distinction between HPV-related and HPV-independent pathways:

- p16 overexpression is a surrogate marker of high-risk HPV oncogenic activity. It is consistently positive in HSIL and facilitates its recognition, especially in morphologically ambiguous cases (8).
- p53 expression patterns, on the other hand, are particularly valuable in identifying HPV-independent VIN. Aberrant p53 staining is frequently associated with mutations in the *TP53* gene and correlate with HPV-negative carcinogenic pathways (7). In LS lesions, mutant p53 staining has been significantly associated with progression to vulvar squamous cell carcinoma (VSCC), with odds ratios exceeding 30, suggesting a pivotal role for *TP53* dysfunction in HPV-independent carcinogenesis (9).

Despite their usefulness, both markers have limitations in sensitivity and specificity, and interpretation can vary among pathologists. Beyond p16 and p53, several additional biomarkers are being explored:

- Ki-67, a proliferation marker, is typically elevated in VIN, but lacks the discriminatory power needed for subtype differentiation (10).
- CK17, SOX2 and GATA3 can be useful in the diagnosis of HPV-independent VIN, p53 wild-type lesion and its distinction from hyperplastic non-neoplastic vulvar lesions (11).

Emerging next-generation sequencing studies have begun to elucidate the genomic alterations that differentiate HSIL, HPV-independent VIN, and invasive VSCC (12). These include mutations in *NOTCH1*, *TP53*, *CDKN2A*, and others, with HPV-independent VIN lesions showing higher molecular instability (13).

Recent studies have identified DNA methylation signatures as promising tools for both diagnostic and prognostic applications in VIN. A panel including *ZNF582, SST,* and *miR124-2* has shown robust performance in detecting VIN, including the HPV-independent subtype, with area under the curve values approaching 0.90 in large validation cohorts (7). These markers not only differentiate VIN from benign dermatoses, like LS, but also correlate with an increased risk of malignant transformation.

Emerging evidence demonstrates that copy number alterations (CNAs) co-occur with elevated DNA methylation levels and are associated with increased severity of disease. A study using modified fast aneuploidy screening sequencing (mFAST-SeqS) identified gains in chromosomal arms (e.g., 1pq, 3q, 9q) and losses (e.g., 2q, 4q) as potential markers of progression risk in HPV-associated VIN (14). A significant positive correlation between aneuploidy scores and methylation burden suggests a synergistic model of genetic and epigenetic dysregulation in VIN pathogenesis (14).

Pathogenesis of HPV-associated vulvar high-grade squamous intraepithelial lesion

In the cervix, anus and oropharynx, high-risk HPV (hr-HPV) preferentially infects multipotent reserve cells that lie immediately beneath the columnar epithelium of the squamocolumnar junction (SCJ). These reserve cells, which retain an embryonic immunophenotype (CK7-positive/p63-negative), appear exquisitely vulnerable to viral oncoprotein-driven deregulation and give rise to most high-grade squamous intraepithelial lesions and carcinomas in those organs (15).

By contrast, the vulva lacks a discrete SCJ, indicating that hr-HPV-driven carcinogenesis in the vulva must originate in non-SCJ basal keratinocytes, possibly in the hair-bearing labial skin or its associated appendages and, at the level of the vulvar vestibule, micro abrasions or epithelial disruptions may enable HPV to access basal cells in an otherwise intact stratified squamous epithelium (16). The absence of a privileged SCJ-derived stem-cell niche may explain the substantially lower incidence of vulvar neoplasm compared to cervical cancer, despite comparable HPV exposure. Research should explore putative different host-virus interactions, immune surveillance pressures and micro-environmental cues that govern malignant progression in the vulva.

Current molecular work is directed at identifying the earliest driver events in vHSIL (e.g., E6/E7-mediated $p16^{INK4a}$ up-regulation, APOBEC mutational signatures, and field changes in the vestibular epithelium). Clarifying this pathway is expected to uncover targetable vulnerabilities, such as epigenetic modifiers or immune checkpoints, and to refine risk stratification algorithms for lesions that may otherwise remain indolent.

Prevention and treatment strategies

Standard of care

All guideline-issuing bodies agree that every histologically confirmed VIN warrants treatment because of the possible progression to invasive carcinoma if left untreated. Wide local excision with free macroscopic margins remains the reference therapy and is recommended whenever invasion cannot be excluded clinically or histologically (17). Excision provides a specimen for margin assessment but is intrinsically disfiguring and may compromise sexual function.

For cases of multifocal, HPV-associated, HSIL cosmetic outcome is paramount, laser or radio-frequency ablation that destroys the full epithelial thickness is an accepted alternative when stromal invasion is excluded with adequate biopsies. Topical 5% imiquimod three times weekly for 12-20 weeks yields complete histologic regression in 35%-60% of selected cases, with durability linked to clearance of oncogenic HPV DNA (18).

As mutilating surgery is still the price of cure for many women, translational research should prioritize:

1. Combined modality regimens (e.g., fractional ablation plus topical or intralesional immunomodulators) aimed at maximizing viral clearance while preserving anatomy;
2. Therapeutic HPV vaccines that expand lesion-specific tissue-resident memory T cells; and
3. Molecularly-guided surveillance using viral or host methylation markers to individualize follow-up intensity and trigger pre-emptive therapy only for lesions with a high progression signature.

Future research should also aim to explore non-invasive treatment approaches for HPV-independent VIN, recognizing its significantly higher propensity for rapid progression to invasive carcinoma compared to HPV-associated vHSIL. Notably, the molecular and genetic landscape of dVIN closely mirrors that of invasive squamous cell carcinoma, underscoring its biological aggressiveness and the urgency for early, effective, and potentially conservative therapeutic interventions.

Collectively, these strategies aspire to reduce treatment morbidity without sacrificing oncological safety, a goal that can only be met if the unique vulvar pathogenetic cascade is fully elucidated.

Clinical implications and future research directions

VIN lies at the crossroads of gynecology, dermatology, pathology, virology, and oncology. Strengthening interdisciplinary collaboration is therefore paramount for both research and especially in patient care pathways.

Current surveillance protocols for VIN are largely empirical and not risk-adapted (4). By integrating methylation testing, CNA profiling, and p53 IHC, clinicians could stratify patients into low-, intermediate-, and high-risk categories. This would facilitate tailored and more judicious use of surgical intervention, earlier detection of progression and different follow-up intervals.

Translating molecular diagnostics into clinical practice requires accessible and acceptable testing modalities. In analogy to cervical and anal cancer screening, methylation-based assays could be adapted for non-invasive sampling, such as vulvar swabs or self-collection devices. This would be especially valuable in the context of immunocompromised women, women with other lower genital tract HPV lesions or long-term monitoring of patients with LS.

Raising awareness of vulvar health is a key factor for improving women's cancer prevention strategies. Despite being a critical part of the lower genital tract, the vulva is often overlooked during routine gynecologic care (19). Evidence has highlighted that incorporating vulvar inspection into cervical cancer screening may facilitate earlier detection of vulvar precancerous lesions and malignancies, leading to improved patient outcomes (20).

Public health campaigns should focus on educating both healthcare providers and the general population about the importance of vulvar examination. Routine inspection of the vulva, combined with appropriate patient education on recognizing symptoms, such as persistent itching, lesions, or pain, can significantly reduce diagnostic delays.

Clear, evidence-based public health messages and multidisciplinary collaborations are needed to normalize discussions around vulvar health, reduce stigma, and empower women to seek timely medical advice. By integrating vulvar

inspection into established cancer screening programs, we could take an important step forward in comprehensive women's health care.

Conclusion

The future of research on VIN is moving towards an integrated molecular framework that transcends traditional morphology. By leveraging advances in DNA methylation profiling, genomic instability assessment, and IHC markers, we are approaching an era of precision gynecologic oncology, in which risk-adapted strategies can improve outcomes while preserving quality of life.

Elucidating the distinct, non-SCJ pathway by which high-risk HPV drives vHSIL is essential, as it will clarify early oncogenic events and identify molecular or immunologic targets for management. While wide local excision remains the oncologic standard for non-HPV-associated VIN, future combined therapeutic vaccines, topical immunomodulators and biomarker-guided surveillance should be researched in order to reduce mutilation while maintaining cure rates. Raising awareness about VIN remains a critical public health priority. Many cases are undiagnosed because of non-specific symptoms, individual patient stigma, or lack of routine vulvar examination in gynecologic care. Educational campaigns targeting both healthcare professionals and patients will be important for promoting and improving early detection.

Footnotes

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