Title: A case of usual (basaloid)-type vulvar intraepithelial neoplasia that failed to respond to imiquimod cream: clinical implications

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Abstract The author reports a case of usual-type (basaloid-type) vulvar intraepithelial neoplasia (VIN) 3 that failed to respond to imiquimod cream. A 51-year-old Japanese woman, visited her local gynecologist complaining of vulvar itching. Atypical cells were noted in cytology smears, but nine vulvar biopsy specimens showed benign proliferation of epithelial tissue. The patient was placed under watchful observation for 8 months, until the vulvar smears once again showed atypical cells and biopsy specimens revealed VIN3. The patient was then referred to our hospital. We yielded a diagnosis of VIN3, basaloid type in usual type. The biopsy specimens were positive for p16, and the lesions were confirmed to be HPV related. We recommended simple vulvectomy, but the patient requested conservative treatment with imiquimod cream. With her written informed consent, we prescribed imiquimod cream to be self-administered 3 times a week. Colposcopy and pap smear test were performed every 2 weeks. Four weeks after the start of treatment, a fingertip-sized papule was detected at the patient's vaginal introitus. By 6 weeks, the lesion had enlarged, and biopsy specimens revealed invasive squamous cell carcinoma. At 7 weeks, we performed simple vulvectomy. The surgical specimen showed stage pT1b keratinizing-type squamous cell carcinoma. HPV-16 DNA was detected in the specimen.

Keywords vulvar intraepithelial neoplasia・imiquimod・human papilloma virus

Introduction Vulvar intraepithelial neoplasia (VIN) is a premalignant lesion involving the skin of the external female genitalia. The incidence of VIN 3 is rare (approximately 2.8 new cases per 100,000 women-year) but increasing among younger women < 50 years of age [1], because most cases of VIN are caused by human papilloma virus (HPV) infection, which itself is on the rise due to the increase in sexual activity among young women. The predominant symptom is pruritus. The diagnosis of VIN is careful inspection of the vulva and adequate repetitive biopsy. The mainstay of the therapy is surgical excision of the lesion. It is performed by simple vulvectomy or skinning vulvectomy and skin graft. Jones RW et al reported that, without treatment, VIN 3 will progress to invasive cancer within 1-7 years (mean 3.9 years) [2].

Imiquimod (imidazoquinoline) is an immune response-modifying drug with antiviral and antitumor activity. It is used to treat the HPV-related genital warts. In 2008, imiquimod cream was shown to be effective for VIN 3 [3]. Young patients with the disease tend to desire conservative treatment; thus, use of imiquimod cream will become more and more common. However, the indications for treatment with imiquimod cream have not been clarifed.

We describe a case of VIN 3 of the usual type (basaloid type) that did not respond to imiquimod cream but rather progressed to invasive cancer within 6 weeks after the start of treatment.

Case report A 51-year-old Japanese woman, generally healthy and non-smoking, visited her local gynecologist complaining of vulvar itching. Upon examination, her vulva and vaginal introitus appeared slightly reddish, and atypical cells were noted in cytology smears. Nine vulvar biopsy specimens obtained subsequently showed benign proliferation of epithelial tissue. The patient was placed under watchful observation by the same gynecologist for 8 months, i.e. until the vulvar smears once again showed atypical cells and biopsy specimens (Fig. 1) revealed VIN 3. The patient was then referred to our hospital.

We reviewed the biopsy specimens and performed a colposcopic examination (Fig. 2), which together yielded a diagnosis of VIN 3, basaloid type in usual type. The biopsy specimens were positive for p16 (Fig. 3), and the lesions were confirmed to be HPV related. We recommended simple vulvectomy, but the patient requested conservative treatment with imiquimod cream. With her written informed consent, we prescribed imiquimod cream (Beselna Cream 5%, Mochida Pharmaceutical) to be self-administered 3 times a week. Colposcopy and pap smear test were performed every 2 weeks.

Four weeks after the start of treatment, a fingertip-sized papule was detected at the patient's vaginal introitus. By 6 weeks, the lesion had enlarged, and there was no clear margin (Fig. 4). Biopsy specimens revealed invasive squamous cell carcinoma of the vulva. At 7 weeks, we performed simple vulvectomy. The surgical specimen showed stage pT1b keratinizing-type squamous cell carcinoma of the vulva (Fig. 5). The specimen was positive for p16 immunohistostaining. Furthermore, HPV-16 DNA was detected in the specimen. Adjuvant radiotherapy was undertaken (whole pelvis: 45Gy, small pelvis: 54.4Gy, local:

65.2Gy), because she had been undergone simple vulvectomy but not radical vulvectomy. The patient remains free of disease 2 years after treatment.

Discussion

Imiquimod cream has gained acceptance as an effective therapy for usual-type VIN. We questioned why it was not effective in our case and arrived at two possible explanations. The first is that there might have been invasion before therapy was begun; that is, we might have missed scant invasion. Husseinzadhe et al reported that invasion was found upon surgery in approximately 20% of cases in which VIN 3 was diagnosed by prior biopsy [4]. In our case, early colposcopy revealed a non-invasive lesion; it was during the treatment period that colposcopy revealed an invasive lesion.

The second explanation is that treatment with imiquimod cream is not suitable for the type of VIN 3 we were faced with. An International Society for the Study of Vulvar Disease subcommittee has classified VIN into two types: usual and differentiated [5]. The usual type accounts for more than 90% of cases of VIN 3, which tends to occur in relatively young patients. Furthermore, the usual type is generally caused by HPV infection and does not have high malignant potential. The usual type is further classified as warty, basaloid, or mixed [5]. The differentiated type occurs less frequently and is not related to HPV infection. Differentiated-type VIN 3 has high malignant potential and often progresses to keratinizing-type squamous cell carcinoma.

It is the relation between HPV and usual-type VIN that makes it a good candidate for imiquimod cream therapy. The overexpression of p16 is strongly correlate with HPV 16 or 18 E6/E7 oncogene expression. Sabine R et al reported that the positive predictive value of moderate to strong diffuse p16

immunostaining and HPV positivity for the diagnosis of VIN 3 was 97% [6]. Annelinde T et al recently reports that imiquimod-induced clearance of HPV results in normalization of counts for certain immune cells and is strongly correlated with histologic regression of the disease [7]. However, it is unclear whether all three subtypes of usual VIN 3 are indications for treatment with imiquimod cream. The usual-type basaloid VIN 3 in our case failed to respond to imiquimod cream. We speculate that basaloid VIN 3, but not warty or mixed VIN 3, has high malignant potential, making it unsuitable for imiquimod treatment. Unfortunately, there are no reports regarding differences in the curative effect of imiquimod cream according to histologic subtypes of VIN 3. Histologic studies are needed. Two things are certain: 1) VIN which has high malignant potential is included in basaloid type VIN. 2) HPV-related VIN can progress to invasive cancer only in 6 weeks, so when you prescribe imiquimod cream to VIN, you should check the lesion at least every 4 weeks.

Conflict of interest statement The authors have no conflicts of interest to report.

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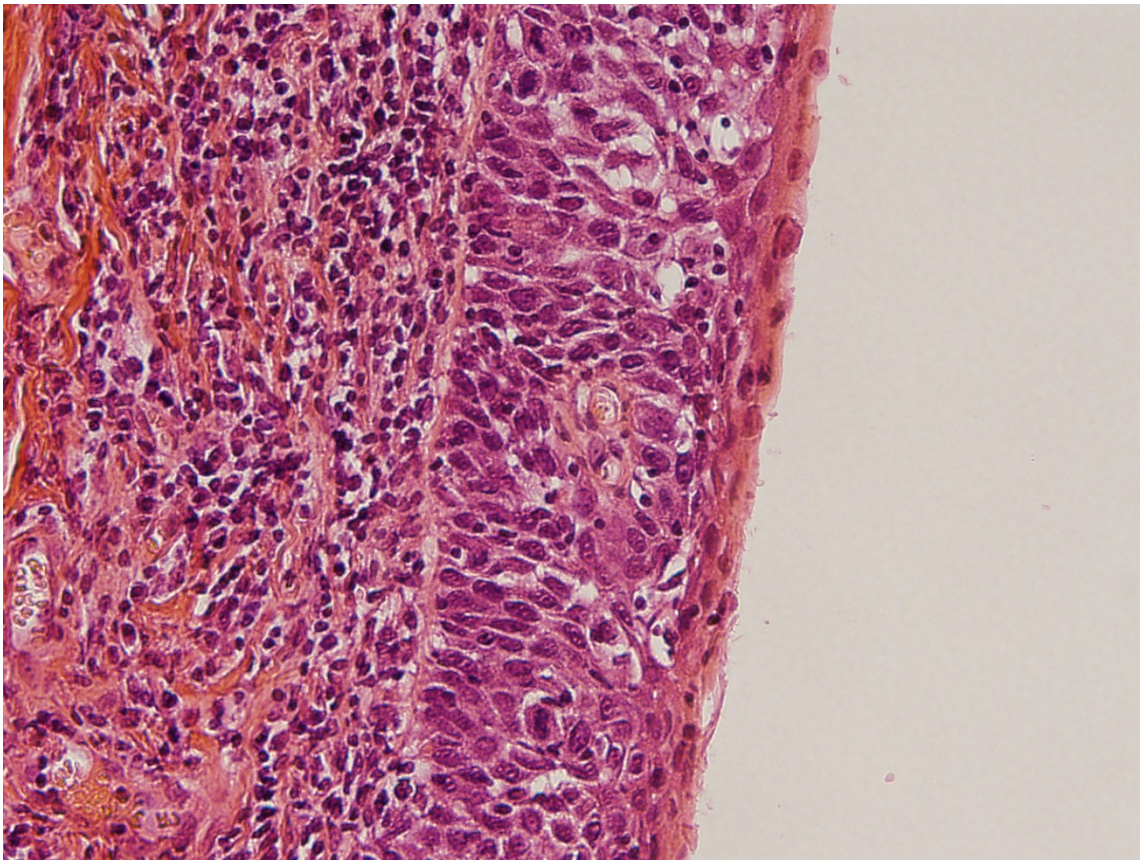

Fig. 1

Dysplastic cells extended into the upper third of the epithelium (×200)

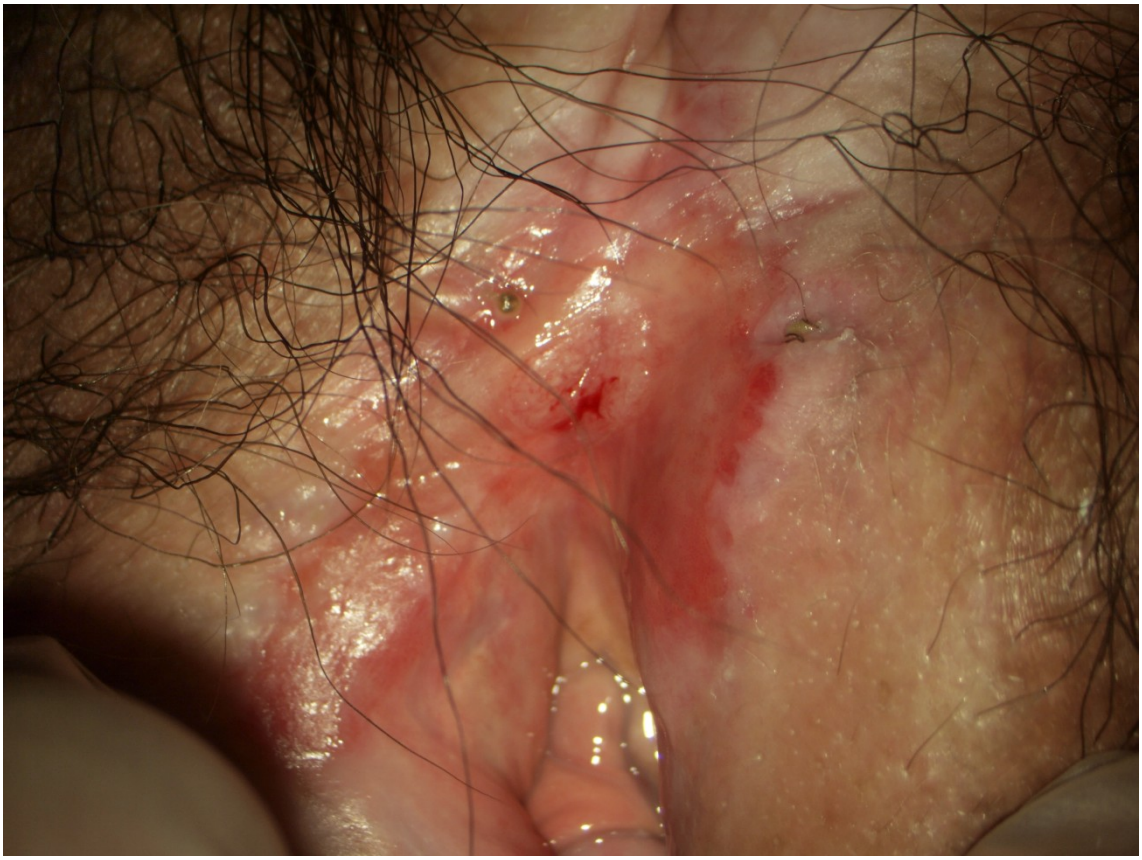

Fig. 2

White-appearing epithelium with irregular contour appeared after the application of 5% acetic acid

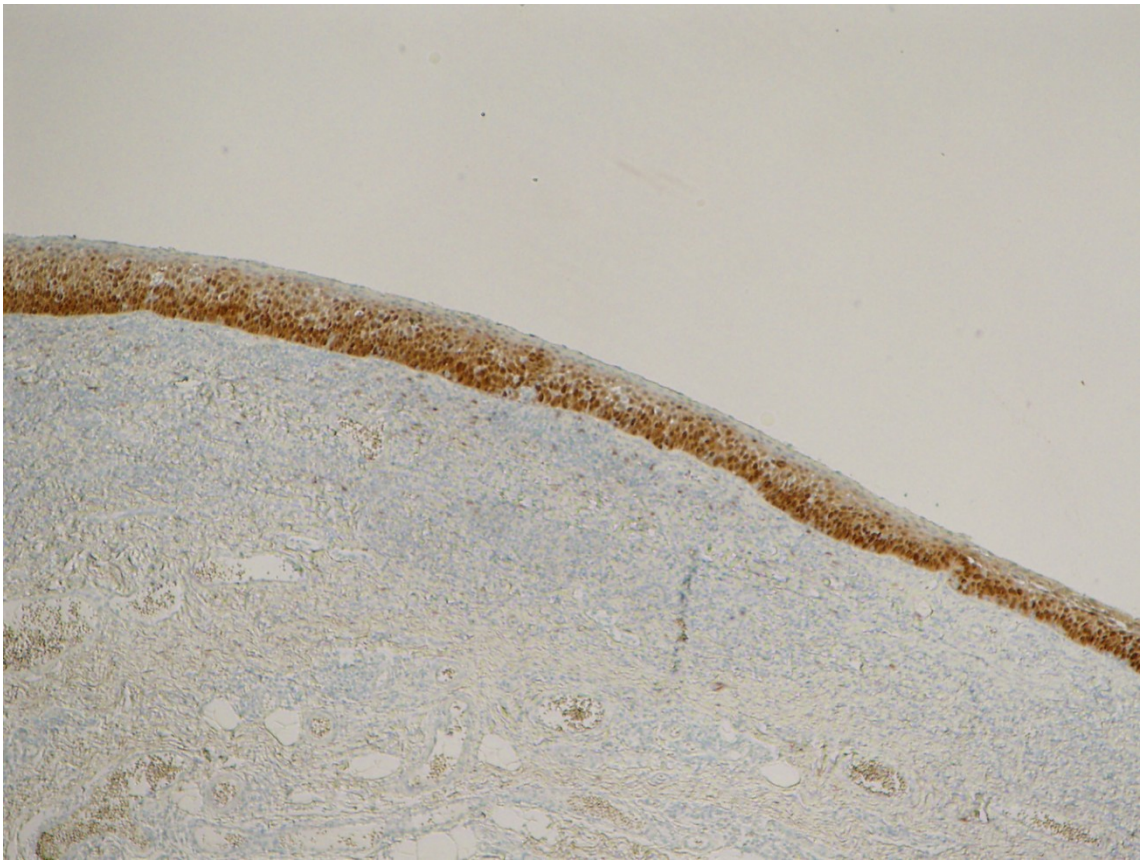

Fig. 3

Overexpression of p16 was detected in her biopsied specimen (×40)

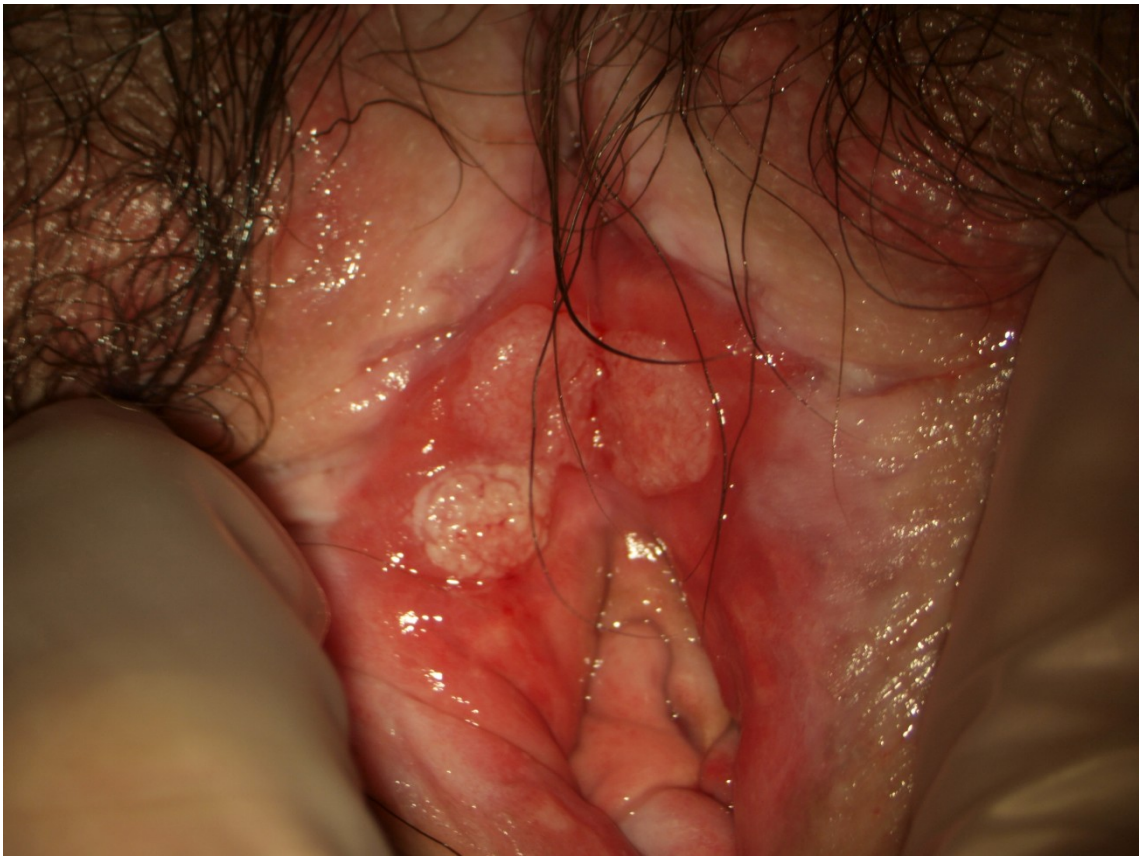

Fig. 4

The enlargement of the size and irregularity of the contour became more prominent

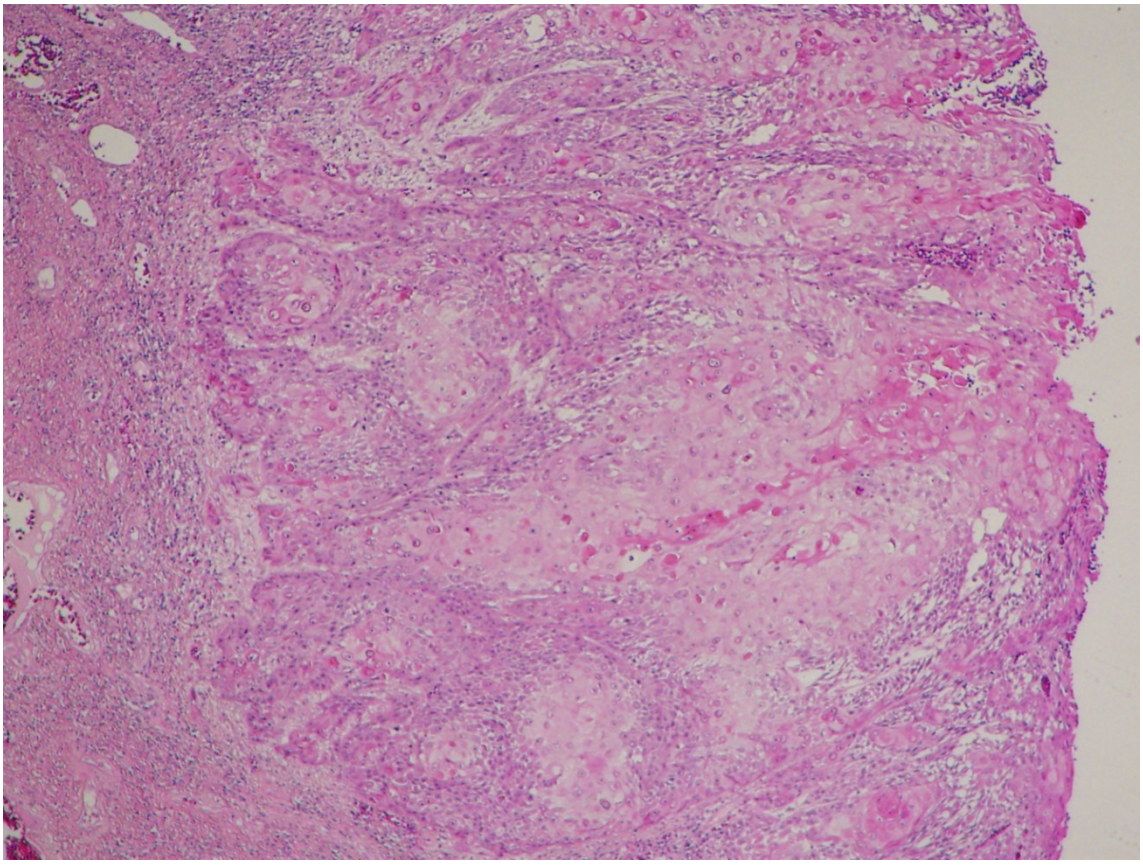

Fig. 5

The invasive neoplastic cells were mature with ample eosinophilic cytoplasm and show prominent keratinization with keratin pearl formation (×40)