

## ORIGINAL ARTICLE

# Treatment of Vulvar Intraepithelial Neoplasia with Topical Imiquimod

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## ABSTRACT

**BACKGROUND**

Alternatives to surgery are needed for the treatment of vulvar intraepithelial neoplasia. We investigated the effectiveness of imiquimod 5% cream, a topical immune-response modulator, for the treatment of this condition.

**METHODS**

Fifty-two patients with grade 2 or 3 vulvar intraepithelial neoplasia were randomly assigned to receive either imiquimod or placebo, applied twice weekly for 16 weeks. The primary outcome was a reduction of more than 25% in lesion size at 20 weeks. Secondary outcomes were histologic regression, clearance of human papillomavirus (HPV) from the lesion, changes in immune cells in the epidermis and dermis of the vulva, relief of symptoms, improvement of quality of life, and durability of response. Reduction in lesion size was classified as complete response (elimination), strong partial response (76 to 99% reduction), weak partial response (26 to 75% reduction), or no response ( $\leq 25\%$  reduction). The follow-up period was 12 months.

**RESULTS**

Lesion size was reduced by more than 25% at 20 weeks in 21 of the 26 patients (81%) treated with imiquimod and in none of those treated with placebo ( $P < 0.001$ ). Histologic regression was significantly greater in the imiquimod group than in the placebo group ( $P < 0.001$ ). At baseline, 50 patients (96%) tested positive for HPV DNA. HPV cleared from the lesion in 15 patients in the imiquimod group (58%), as compared with 2 in the placebo group (8%) ( $P < 0.001$ ). The number of immune epidermal cells increased significantly and the number of immune dermal cells decreased significantly with imiquimod as compared with placebo. Imiquimod reduced pruritus and pain at 20 weeks ( $P = 0.008$  and  $P = 0.004$ , respectively) and at 12 months ( $P = 0.04$  and  $P = 0.02$ , respectively). The lesion progressed to invasion (to a depth of  $< 1$  mm) in 3 of 49 patients (6%) followed for 12 months (2 in the placebo group and 1 in the imiquimod group). Nine patients, all treated with imiquimod, had a complete response at 20 weeks and remained free from disease at 12 months.

**CONCLUSIONS**

Imiquimod is effective in the treatment of vulvar intraepithelial neoplasia. (Current Controlled Trials number, ISRCTN11290871.)

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**S**URGERY, THE TREATMENT OF CHOICE FOR vulvar intraepithelial neoplasia, removes all visible lesions, with the aim of relieving symptoms and preventing vulvar cancer.<sup>1</sup> However, there are limitations to surgery. The percentage of lesions with positive surgical margins ranges from 24 to 68%.<sup>2,3</sup> Recurrences are common, because surgery does not eliminate human papillomavirus (HPV), the cause of most vulvar intraepithelial neoplasia.<sup>4,5</sup> Progression is not influenced by radical excision,<sup>4,6</sup> and surgery can mutilate the vulva, thereby causing psychosexual distress.<sup>7-10</sup> Thus, alternative treatments are needed.

Vulvar intraepithelial neoplasia is caused by HPV,<sup>11</sup> which has prompted the use of imiquimod 5% cream (Aldara, 3M Pharmaceuticals), a topical immune-response modifier,<sup>12</sup> for treatment of the disease. Efficacy has been reported, although only in small, uncontrolled studies.<sup>13-16</sup> The aim of this study was to assess the effectiveness of imiquimod 5% cream in patients with multifocal grade 2 or 3 vulvar intraepithelial neoplasia in a placebo-controlled, double-blind, randomized clinical trial.

## METHODS

### PATIENTS

All patients 18 years of age or older with grade 2 or 3 vulvar intraepithelial neoplasia who were seen at the Academic Medical Center of the University of Amsterdam or the Erasmus University Medical Center of Rotterdam between April 2001 and July 2003 were asked to participate. The inclusion criteria were histologically proven, multifocal grade 2 or 3, vulvar intraepithelial neoplasia without microinvasion and contraceptive use for sexually active, premenopausal women (to avoid any possible teratogenic effects of imiquimod). The exclusion criteria were a history of cancer or inflammatory dermatosis of the vulva, pregnancy, immunodeficiency, any treatment for vulvar intraepithelial neoplasia or warts within the previous month, hypersensitivity to the cream, or an inability to understand Dutch or English. A Consolidated Standards for the Reporting of Trials (CONSORT) diagram appears in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).

### STUDY DESIGN

A formalin-fixed biopsy specimen was obtained for histologic analysis within 3 months before en-

rollment. A second specimen from the same lesion was frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for HPV DNA testing and immunohistochemical analysis. Patients with extensive vulvar intraepithelial neoplasia underwent surgical mapping before enrollment to establish the extent and grade of neoplasia and to rule out invasive disease. If a lesion was suspicious for invasion (i.e., was raised, erosive, ulcerative, or indurated), a wide local excision was performed.

During the first visit, a medical history was taken and a physical examination was performed. Blood samples were drawn for pregnancy testing and for hematologic and serum chemical analysis at the first visit and at 4 weeks after treatment (20 weeks after the first visit). A cervical smear was taken at the first and last study visits.

Eligible patients were randomly assigned to receive 250 mg of imiquimod 5% or placebo cream, a complete vehicle control. Neither the patients nor the examining physicians were aware of the treatment assignments. Randomization was carried out by 3M Pharmaceuticals in blocks of four (with a two-by-two design) without stratification. The patients applied a thin layer of study medication to the lesions and let it remain overnight without a cover twice a week for a period of 16 weeks. In case of severe side effects, application could be reduced to once a week, or a treatment-free period of 1 week was permitted. The patients were advised to use sulfur precipitate 5% in zinc oxide ointment the day after application of the cream to avoid superinfection.

The patients used a diary to report concomitant medication and side effects. Every 4 weeks, the patients were monitored for the efficacy of treatment, symptoms, and side effects. At 20 weeks, a post-treatment biopsy specimen was obtained for histologic analysis, and a sample was stored at  $-80^{\circ}\text{C}$  for detection of HPV DNA and immunohistochemical analysis. Photographs were used to ensure that the post-treatment biopsy specimen was taken from the same site.

To investigate long-term effects and to evaluate possible recurrences of vulvar intraepithelial neoplasia, we performed post-treatment assessments at 7 months and at 12 months. If a recurrence was suspected at 12 months, a biopsy specimen was obtained. In cases of persistent or residual disease after 1 year, treatment with imiquimod or surgery was recommended. If lesions suspicious for invasion developed during the study, wide local excision was performed. Except for cases of serious

side effects, the randomization code was not broken until all women had been seen at 12 months. The ethics committees of the Academic Medical Center of the University of Amsterdam and the Erasmus University Medical Center of Rotterdam approved the study protocol. All women voluntarily provided written informed consent; they were informed that surgery was the treatment of choice for vulvar intraepithelial neoplasia.

All vulvar intraepithelial neoplasia lesions were measured with calipers and photographed at baseline, every 4 weeks during treatment, and at follow-up visits. A computer program (ImageJ) was used to calculate the total lesion size in square centimeters by adding the measurements for each separate lesion together. To avoid bias caused by side effects, one of the investigators and an independent gynecologist with expertise in vulvar pathology evaluated the clinical response with the use of photographs taken at the first study visit and at 20 weeks. Clinical response was defined as a reduction in total lesion size and was classified as a complete response, a strong partial response (76 to 99% reduction in lesion size), a weak partial response (26 to 75% reduction in lesion size), or no response (reduction in lesion size of 25% or less). Skin reactions during treatment were recorded. To evaluate the long-term response, photographs taken at 12 months were compared with those taken at baseline.

All biopsy evaluations were reviewed independently by two experienced gynecologic pathologists who were unaware of the clinical data. Biopsy specimens were classified as grade 1, 2, or 3 vulvar intraepithelial neoplasia.<sup>17</sup> A consensus meeting was arranged when the pathologists did not agree. Histologic regression was defined as regression from grade 2 or 3 vulvar intraepithelial neoplasia to a lower grade. If infiltration was present, the depth of infiltration was measured by Wilkinson's method.<sup>18</sup>

Frozen biopsy specimens were analyzed for the presence of HPV DNA with the use of a standard GP5+/6+ polymerase-chain-reaction (PCR) enzyme immunoassay, followed by reverse line-blot analysis.<sup>19,20</sup> High-risk and low-risk probe cocktails were used to identify the 14 most prevalent high-risk and the 22 most prevalent low-risk types of HPV. In addition, PCR amplification products were analyzed to identify individual HPV types by reverse line-blot analysis. A PCR assay for the  $\beta$ -globin gene was performed to ascertain the presence and quality of target DNA. The histochemical

analysis of immune cells and the statistical analysis of immunologic data are described in the Supplementary Appendix.

Pruritus and pain were rated by the patients every 4 weeks during treatment and at follow-up visits on a visual analogue scale from 0 (no symptoms) to 10 (severe symptoms). The mental health scale of the Medical Outcomes Study 36-Item Short-Form General Health Survey (ranging from 0 to 100, with higher numbers indicating a better health-related quality of life) and the overall quality-of-life scale of the European Organization for Research and Treatment of Cancer (EORTC) quality-of-life questionnaire (QLQ-C30) were used to assess generic and cancer-specific health-related quality of life, respectively.<sup>20-23</sup> Body image and sexuality were assessed with the EORTC QLQ-BR23.<sup>24</sup> These questionnaires were administered at baseline, at 20 weeks, and at 12 months.

#### PRIMARY AND SECONDARY END POINTS

The primary outcome was a reduction in lesion size of more than 25% 4 weeks after the end of treatment (20 weeks after the beginning of treatment). The secondary outcomes were histologic regression from grade 2 or 3 vulvar intraepithelial neoplasia to a lower grade, clearance of HPV, and changes in immune cells in the epidermis and dermis at 20 weeks; relief of clinical symptoms and improvement of quality of life at 20 weeks and at 12 months; and durability of the clinical response at 12 months.

#### STATISTICAL ANALYSIS

In the placebo group, we expected no reduction in lesion size in 95% of the patients. For the purpose of calculating sample size, we considered treatment with imiquimod adequate if 50% of the patients showed at least a weak partial response. To detect such a difference with a power of 80% ( $\alpha=0.05$  and  $\beta=0.20$ ), a sample size of 36 patients would be needed. Taking into account the possibility of withdrawal by some patients, we chose to include 52 patients.

Analyses were performed according to the intention-to-treat principle. For a comparison of responses between the two groups, Fisher's exact test was used. The Pearson chi-square test was used to compare clinical, histologic, and viral outcomes between the groups. To assess the correlation between post-treatment histologic findings and viral clearance, a test for trend was performed. Repeated-measures analysis of variance was used

<b>Table 1. Baseline Characteristics of the Patients.*</b>			
<b>Characteristic</b>	<b>Imiquimod (N = 26)</b>	<b>Placebo (N = 26)</b>	<b>P Value†</b>
Age (yr)			0.08
Median	39	44	
Range	22–56	31–71	
Previous surgical treatment (no. of patients)			1.00
Any treatment	18	19	
1 treatment	6	7	
2 or 3 treatments	7	7	
4 or 5 treatments	2	4	
>5 treatments	3	1	
No treatment	8	7	
Symptoms (no. of patients)			0.50
Any symptom	22	19	
Itchiness	2	8	
Pain	3	1	
Itchiness and pain	17	10	
No symptoms	4	7	
Smoking status			1.00
Smoking (no. of patients)	23	23	
1–9 cigarettes/day	3	5	
10–20 cigarettes/day	12	7	
>20 cigarettes/day	8	11	
No smoking (no. of patients)	3	3	
Previous cervical neoplasia (no. of patients)			0.78
Yes	15	17	
No	11	9	
Vulvar intraepithelial neoplasia grade (no. of patients)‡			0.60
1	1	0	
2	2	2	
3	23	24	
HPV DNA (no. of patients)			1.00
Positive	25	25	
Negative	1	1	
HPV type (no. of patients)			0.47
16	20	21	
18	0	1	
33	5	3	
Lesion size (cm <sup>2</sup> )§			0.37
Median	4.7	5.4	
Range	0.0–20.8	0.7–45.0	

\* HPV denotes human papillomavirus.

† P values were calculated by Fisher's exact test, except for age and lesion size, for which the Mann–Whitney U test was used, and vulvar intraepithelial neoplasia grade and HPV type, for which the Pearson chi-square test was used.

‡ The grades were reviewed by two independent gynecologic pathologists.

§ Vulvar intraepithelial neoplasia lesions spontaneously disappeared after the initial biopsy in one patient in the imiquimod group. No lesions could be measured at the time of initiation of treatment.

to test for between-group differences over time in self-reported symptoms, health-related quality of life, body image, and sexuality. Analysis of covariance was used to compare group scores on these outcomes at 20 weeks and at 12 months, with adjustment for baseline scores. All reported P values are two-sided and are not adjusted for multiple testing. No interim analyses of efficacy were performed. 3M Pharmaceuticals was not involved in the study except to provide study medication and to perform randomization.

## RESULTS

### STUDY POPULATION

Table 1 shows the baseline characteristics of the 52 patients assigned to study groups. The two groups were well balanced. The patients had received a diagnosis of vulvar intraepithelial neoplasia at a mean of 5.4 years before enrollment (range, 1 month to 20 years). The most recent surgery was performed more than 3 months before enrollment in all patients. Mapping (which involved more than three biopsies) was performed in 12 patients (6 in the imiquimod group and 6 in the placebo group). To rule out invasion, wide local excision was performed in three patients (all in the placebo group). One patient in the imiquimod group with a positive test result for HPV DNA had coexisting lichen sclerosus. Two patients were using local corticosteroids at enrollment and discontinued corticosteroid use before starting the study.

One patient in the imiquimod group discontinued study medication at 4 weeks because her lesions had spontaneously disappeared after the initial biopsy. One patient in the placebo group stopped at 14 weeks because of a lack of response. Two other patients, one in each group, needed a treatment-free period of more than 1 week for personal reasons. The median number of sachets of cream used was 32 (range, 27 to 33) in the placebo group and 30 (range, 6 to 32) in the imiquimod group. The frequency of imiquimod application was reduced to once a week in five patients because of severe local inflammation. Other side effects were itching or burning immediately after application of imiquimod or on the next day, flu-like symptoms, headache, apathy, weariness, and muscular ache (Table 2). Skin reactions noted by the investigator included erythema, erosion, vesiculation, and edema. Hematologic and serum bio-

**Table 2. Side Effects.**

Side Effect	Imiquimod (N=26)	Placebo (N=26)	P Value*
	no. of patients		
<b>Reported by the patient<sup>†</sup></b>			
Vulvar pain or pruritus	24	7	<0.001
Headache	7	5	0.52
Apathy	5	0	0.03
Weariness	8	4	0.20
Muscular ache	3	1	0.35
Flulike symptoms	5	3	0.47
Other side effects <sup>‡</sup>	4	4	1.00
No side effects	1	13	<0.001
<b>Reported by the investigator</b>			
Erythema			
Mild-to-moderate	14	2	<0.001
Severe	6	0	0.02
Erosion			
Mild-to-moderate	17	5	0.001
Severe	0	0	1.00
Vesiculation	4	0	0.06
Edema	11	0	<0.001
Ulceration	0	0	1.00

\* P values were calculated by the Pearson chi-square test.

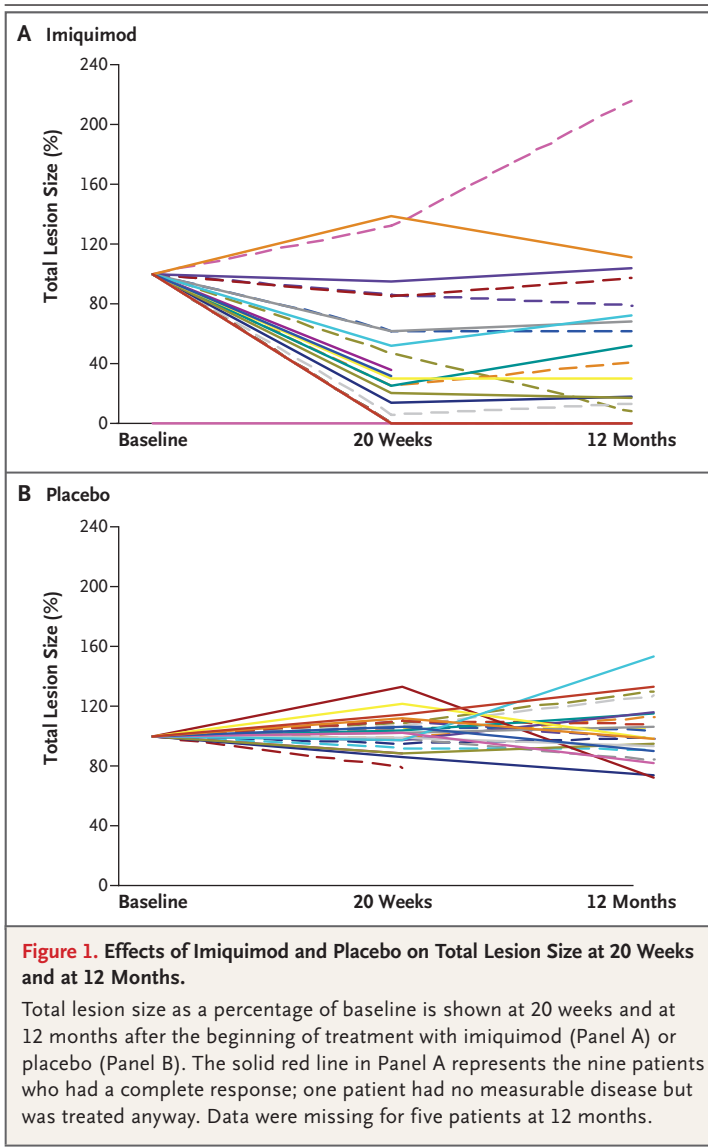
<sup>†</sup> In the imiquimod group, one patient reported no side effects during treatment, nine patients reported one side effect, five patients reported two side effects, eight patients reported three side effects, and three patients reported four side effects. In the placebo group, 13 patients reported no side effects, 7 patients reported one side effect, 2 patients reported two side effects, 3 patients reported three side effects, and 1 patient reported four side effects. The number of patients reporting no side effects differed significantly between the two groups (P<0.001).

<sup>‡</sup> The other side effects were loss of hair, excessive vulvar perspiration, loss of blood from treated skin, watery eyes, and reactive lymph nodes.

chemical test results remained within the normal range in both study groups.

### CLINICAL OUTCOME

Figure 1 summarizes the change in total lesion size after treatment with imiquimod or placebo at 20 weeks and at 12 months. At 20 weeks, lesion size was reduced by more than 25% in 21 of 26 patients treated with imiquimod (81%) and in no patients in the placebo group (P<0.001). The lesions had completely disappeared in nine imiquimod-treated patients and were reduced by more than 75% in five (Table 3, and Fig. 1 of the Supplementary Appendix). There were no significant differ-



ences between the findings of the two investigators who evaluated the clinical response.

#### HISTOLOGIC, VIRAL, AND IMMUNOLOGIC OUTCOMES

Pretreatment biopsies showed that 47 patients had grade 3 vulvar intraepithelial neoplasia, 4 had grade 2, and 1 had grade 1. Table 4 summarizes the histologic results after treatment. There was disagreement between the observers in the grading of 19 of 104 biopsies (18%), mainly concerning grades 2 and 3 (13 of 19), and consensus was reached in all 19 cases. Histologic regression from grade 2 or 3 to a lower grade was seen in 18 patients treated with imiquimod (69%) and in 1 pa-

tient treated with placebo (4%) ( $P < 0.001$ ). Eight patients no longer had vulvar intraepithelial neoplasia; before treatment, six of these patients had grade 3 vulvar intraepithelial neoplasia, one had grade 2, and one had grade 1. One patient with grade 2 vulvar intraepithelial neoplasia at baseline had grade 3 after treatment with imiquimod.

At baseline, 25 patients in each group had lesions positive for HPV DNA (Table 1). At 20 weeks, HPV was cleared in 17 lesions: 15 after treatment with imiquimod and 2 after treatment with placebo ( $P < 0.001$ ) (Table 4). Fifteen lesions had HPV type 16, and two lesions (one treated with imiquimod and one treated with placebo) had HPV type 33. There was no significant association between HPV type and viral outcome. There was a strong association between viral clearance and histologic regression ( $P < 0.001$ ). Of 14 lesions that regressed to grade 1 vulvar intraepithelial neoplasia or to no neoplasia, 13 were cleared of HPV after treatment with imiquimod and the other lesion was HPV-negative at baseline.

Treatment with imiquimod increased the numbers of CD1a+ dendritic cells, CD8+ T cells, and CD94+ natural killer cells in the epidermis 4 weeks after the end of therapy. The increase was significant only for patients whose lesions regressed by more than 75%. In the dermis of imiquimod-treated patients whose lesions regressed by more than 75%, the numbers of CD207+ dendritic cells, CD208+ dendritic cells, and regulatory T cells were reduced; the reduction was significant for CD207+ dendritic cells and regulatory T cells (see the Supplementary Appendix for details).

#### SELF-REPORTED SYMPTOMS AND QUALITY OF LIFE

As compared with placebo, treatment with imiquimod reduced pruritus and pain at 20 weeks ( $P = 0.008$  and  $P = 0.004$ , respectively) and 12 months ( $P = 0.04$  and  $0.02$ , respectively), according to analysis of covariance with adjustment for baseline scores. In a repeated-measures analysis, no significant differences at baseline, at 20 weeks, or at 12 months were observed between the imiquimod group and the placebo group in self-reported health-related quality of life, body image, or sexuality.

#### FOLLOW-UP

All but three patients were followed for 12 months (Table 3). One patient did not have a response to the study medication and withdrew at 20 weeks.

**Table 3. Clinical Response at 20 Weeks and at 12 Months after the Beginning of Treatment.\***

Response	20 Weeks†		12 Months	
	Imiquimod (N=26)	Placebo (N=26)	Imiquimod (N=26)	Placebo (N=26)
None	5	26‡	5	21
Weak partial	7§	0	6	2
Strong partial	5	0	4	0
Complete	9¶	0	9	0
No data	0	0	2	3

\* Clinical response was classified as no response (reduction in lesion size of 25% or less), weak partial response (26 to 75% reduction), strong partial response (76 to 99% reduction), or complete response (100% reduction).

† The difference between the treatment groups at 20 weeks was significant ( $P < 0.001$  by Pearson chi-square test for trend).

‡ Progression to invasive disease occurred in two of these patients during the study. One of the patients underwent surgery before the end of the study, so the total lesion size was not measured at 12 months. A third patient did not have a response to the study medication and withdrew from the study at 20 weeks. A fourth patient, who had severe pain, withdrew from the study at 8 months to undergo surgery.

§ Progression to invasive disease occurred in one of these patients during the study. She underwent surgery before the end of the study, so the total lesion size was not measured at 12 months. Another patient was lost to follow-up at 20 weeks because of unrelated medical problems.

¶ In one patient, vulvar intraepithelial neoplasia lesions spontaneously disappeared after the initial biopsy. Another patient, with an original diagnosis of grade 2 vulvar intraepithelial neoplasia, received a diagnosis of grade 1 disease after independent review.

Another patient was lost to follow-up at 20 weeks because of an unrelated medical problem. A third patient with grade 3 vulvar intraepithelial neoplasia stopped treatment at 8 months when she underwent surgery (skinning vulvectomy) for severe pain. Complete follow-up information was available for 49 patients. All patients with a complete response after treatment with imiquimod at 20 weeks remained free of disease at 12 months. Two of 12 patients (17%) with a partial response after imiquimod treatment had enlargement of their lesions.

In one patient treated with imiquimod, newly developed vulvar intraepithelial neoplasia progressed to invasion at 7 months. A radical local excision confirmed the presence of invasion to a depth of less than 1 mm. Invasion also occurred in two other patients treated with placebo. Invasion to a depth of less than 1 mm developed at 12 months in one patient with preexisting vulvar intraepithelial neoplasia. A subsequent radical local excision showed no further invasion. In the other patient, progression of newly developed vulvar intraepithelial neoplasia was found at 20 weeks. A radical local excision confirmed the presence of invasion to a depth of less than 1 mm.

neoplasia during an observation period of 1 year. Complete response was achieved in 9 (35%) and partial response in 12 (46%) of 26 patients treated with the cream. Regression from grade 2 or 3 vulvar intraepithelial neoplasia to a lower grade was seen in 18 of 26 lesions (69%), 15 of which tested negative for HPV DNA after treatment. None of the nine patients with a complete response showed any evidence of vulvar intraepithelial neoplasia at 12 months. Four of the nine patients who had a complete response had undergone surgery two or three times before receiving imiquimod. Three lesions (6%) progressed to invasion to a depth of less than 1 mm, two after treatment with placebo and one after treatment with imiquimod. These results are similar to those of other studies showing progression of vulvar intraepithelial neoplasia in 9% of untreated patients and 3% of surgically treated patients.<sup>4,5</sup> In our study, two of three patients with progression to invasive disease (one treated with imiquimod and two with placebo) had newly developed vulvar intraepithelial neoplasia.

The strength of our study lies in the randomized, placebo-controlled comparison. To avoid any bias caused by side effects of study medication, two independent observers using photographs obtained before and after treatment evaluated the reduction in lesion size. No significant differences between the results of the two observers were found. Histologic evidence of regression was eval-

## DISCUSSION

This trial demonstrates the effectiveness of imiquimod in the treatment of vulvar intraepithelial

**Table 4. Histologic and Virologic Results 20 Weeks after Beginning of Treatment with Imiquimod or Placebo.\***

Vulvar Intraepithelial Neoplasia Grade†	Imiquimod (N=26)		Placebo (N=26)	
	no. of patients	no. HPV-negative	no. of patients	no. HPV-negative
No disease	8‡	8	0	0
Grade 1	7	6§	1	1
Grade 2	3	2	0	0
Grade 3	8¶	0	24	2§
Invasive disease (<1 mm)	0		1	0

\* HPV denotes human papillomavirus.

† The grades were reviewed by two independent gynecologic pathologists.

‡ Two patients received a diagnosis of grade 2 vulvar intraepithelial neoplasia at baseline; the diagnosis was changed to grade 1 in one of these patients after revision.

§ One patient was already HPV-negative at baseline.

¶ One patient received a diagnosis of grade 2 vulvar intraepithelial neoplasia at baseline.

|| Two patients received a diagnosis of grade 2 vulvar intraepithelial neoplasia at baseline.

uated independently by two pathologists, and consensus was reached when they disagreed as to whether lesions should be classified as grade 2 or grade 3. Difficulty in grading vulvar intraepithelial neoplasia<sup>25,26</sup> led to a new classification in which grades 2 and 3 are combined as vulvar intraepithelial neoplasia (usual type or differentiated type).<sup>27</sup> Grade 1 vulvar intraepithelial neoplasia as an entity was abandoned, since the minimal changes associated with this grade are usually a result of transient HPV infection. According to the new classification, all patients would have been classified as having vulvar intraepithelial neoplasia (usual type), and 15 patients (58%) instead of 8 (31%) would have shown complete histologic regression after treatment with imiquimod.

It is not known why some patients had a response to imiquimod and others did not. Imiquimod binds to toll-like receptor 7, a cell-surface receptor on the immature plasmacytoid dendritic cell. Binding initiates an intracellular signaling cascade that results in innate and cell-mediated immune responses. Imiquimod promotes maturation of antigen-presenting cells and secretion of proinflammatory cytokines and initiates a shift to type 1 T-cell-mediated immunity.<sup>28</sup> Imiquimod also has direct proapoptotic activity against tumor cells.<sup>29</sup> We found that a preexisting type 1 T-cell response specific to HPV type 16 is associated with an improved outcome after imiquimod treatment.<sup>30</sup> Perhaps induction of this specific T-cell

response before imiquimod treatment would be useful.

HPV infection suppresses chemokine expression, resulting in the inhibition of infiltration and activation of T cells and natural killer cells. Moreover, the number of Langerhans' cells is significantly reduced at HPV-infected sites.<sup>31</sup> The increase in the number of immune cells in the epidermis of patients with a clinical response of more than 75% after imiquimod treatment may reflect reactivation of the resident epidermal cells. The decrease in the number of immune cells in the dermis of these patients may reflect a return to normal conditions after a successful immune response against HPV.

In conclusion, imiquimod 5% cream is a promising agent for the treatment of vulvar intraepithelial neoplasia. Regression of lesions is strongly associated with clearance of HPV. As a convenient, self-administered treatment, imiquimod is well tolerated, is less invasive than surgery, relieves itching and pain, and does not influence health-related quality of life, body image, or sexuality. Therefore, we consider imiquimod the first-choice treatment for vulvar intraepithelial neoplasia.

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No potential conflict of interest relevant to this article was reported.

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