STUDY

Imiquimod Treatment of Anal Intraepithelial Neoplasia in HIV-Positive Men

Ulrike Wieland, MD; Norbert H. Brockmeyer, MD; Soenke J. Weissenborn, PhD; Bettina Hochdorfer, MD; Markus Stücker, MD; Jochen Swoboda, MD; Peter Altmeyer, MD; Herbert Pfister, PhD; Alexander Kreuter, MD; for the Competence Network HIV/AIDS

Objective: To evaluate the treatment of anal intraepithelial neoplasia (AIN) with the local immune response modifier imiquimod in human immunodeficiency virus (HIV)-positive men who have sex with men (MSM).

Design: Prospective, nonrandomized, open-label pilot study, with a mean follow-up time of 9½ months.

Setting: Dermatology department of a university hospital.

Patients: Twenty-eight consecutive HIV-positive MSM with histologically confirmed perianal (n=23) or intraanal (n=5) AIN.

Intervention: Overnight treatment with self-applied imiquimod cream (perianal AIN) or suppositories (intraanal AIN) 3 times a week for 16 weeks.

Main Outcome Measures: Response to treatment was documented using clinical, cytologic, and histologic criteria. Human papillomavirus (HPV) typing and HPV DNA load determination for the high-risk HPV types 16, 18, 31, and 33 were performed.

Results: Seventeen (61%) of all 28 patients included in the study and 17 (77%) of the 22 patients with AIN, who applied imiquimod as instructed, showed clinical and histologic clearance at the end of therapy. Four patients had residual AIN and 1 patient did not improve. Clinical response was accompanied by a sharp decline in HPV DNA loads and by a reduction in the number of HPV types, but long-term HPV clearance was rarely achieved. In the follow-up period, AIN cleared in 3 patients with residual AIN. Fourteen (78%) of 18 imiquimod responders with at least 5 five months of follow-up had a normal cytologic and clinical picture at the end of the follow-up period. Three primary responders developed a recurrence. In 6 noncompliant patients, there was no clinical or morphological improvement and the HPV DNA loads remained high.

Conclusions: Imiquimod appears to be a safe and effective treatment option for AIN in HIV-positive MSM. Clinical response is accompanied by a significant decrease in high-risk HPV DNA load. These results should encourage controlled randomized studies of imiquimod treatment of AIN.

Trial Registration: clinicaltrials.gov Identifier: NCT00365729

Arch Dermatol. 2006;142:1438-1444

Author Affiliations: Institute of Virology, University of Cologne, Cologne (Drs Wieland, Weissenborn, and Pfister), Department of Dermatology and Allergology, Ruhr University Bochum, Bochum (Drs Brockmeyer, Hochdorfer, Stücker, Altmeyer, and Kreuter), and Institute of Cytology, Godesberg (Dr Swoboda), Germany.
Group Information: A list of the Competence Network HIV/AIDS members appears at www.kompetenznetz-hiv.de.

LIKE CERVICAL CANCER, ANAL cancer is causally linked to persistent infection with high-risk human papillomavirus (HPV) types such as HPV-16 or HPV-18.[1-3] In human immunodeficiency virus (HIV)-positive men who have sex with men (MSM), anal HPV prevalence is approximately 90%, and infections with multiple HPV types are common.[4-6] Consequently, HIV-positive MSM have a strongly increased risk for anal cancer and its precursor, anal intraepithelial neoplasia (AIN). Despite highly active antiretroviral therapy (HAART), the incidence and prevalence of AIN and anal cancer have not decreased in HIV-positive MSM.[6-8] In a recent study, 52% of HIV-positive men presented with advanced AIN.[6]

Imiquimod (1-[2-methylpropyl]-1*H*-imidazo-[4,5-*c*]-quinolin-4-amine) is a topical immune response modifier that was recently approved for the management of anogenital warts, superficial basal cell carcinoma, and actinic keratoses (in the United States). It has been used off-label for the treatment of recalcitrant warts, nodular basal cell carcinoma, Bowen disease, cutaneous squamous cell carcinoma, vulvar intraepithelial neoplasia, and molluscum contagiosum.[9-12] Binding of imiquimod to the Toll-like receptor 7 on monocytes, macrophages, and dendritic cells activates both the innate and the cell-

Table 1. Characteristics of 22 Compliant Patients With Anal Intraepithelial Neoplasia (AIN) Before Imiquimod Therapy (Tx), at the End of Tx, and at the End of Follow-up (Fu)

Patient No.	CDC Stage	Localization of AIN	Clinical Findings Before Tx	Histologic Findings Before Tx	Cytologic Findings Before Tx	Clinical Findings at End of Tx	Histologic Findings at End of Tx	Cytologic Findings at End of Tx	Cytologic (Histologic) Findings at End of Fu	Last Fu After End of Tx, mo
27	C3	P	L MoF	AIN-1	LSIL	Normal	Normal	Normal	Normal	12
6	C3	P	L MoF	AIN-1	LSIL	Normal	Normal	Normal	Normal	14
14	A2	P	L MoF	AIN-1	LSIL	Normal	Normal	Normal	Normal	17
15	A2	I	L MoF	AIN-1	LSIL	Normal	Normal	US	Normal	1
25	C3	P	L MoF	AIN-1	LSIL	Normal	Normal	Normal	Normal	6
13	A1	P	L MoF	AIN-1	LSIL	Normal	Normal	ASCUS	Normal	7
4	A2	P	L MoF	AIN-2	HSIL	Normal	Normal	Normal	Normal	2
3	A3	I	L MoF	AIN-2	HSIL	Normal	Normal	Normal	LSIL	5
16	C3	P	L MoF	AIN-2	HSIL	Normal	Normal	Normal	HSIL (AIN-2)	12
12	C3	P	B MuF	AIN-2	HSIL	Normal	Normal	Normal	Normal	18
17	B3	P	L MoF	AIN-3	HSIL	Normal	Normal	Normal	Normal	4
18	B1	P	L MoF	AIN-3	HSIL	Normal	Normal	Normal	Normal	5
26	B3	I	V MoF	AIN-3	US	Normal	Normal	LSIL	Normal	5
7	C3	P	E MoF	AIN-3	HSIL	Normal	Normal	LSIL	LSIL	6
10	C3	P	L MuF	AIN-3	HSIL	Normal	Normal	Normal	Normal	11
8	C3	P	L MuF	AIN-3	HSIL	Normal	Normal	Normal	Normal	13
9	B2	P	L MuF	AIN-3	HSIL	Normal	Normal	Normal	Normal	20
23	C3	I	L MuF	AIN-3	HSIL	Residual L	Normal	LSIL	LSIL	6
20	C3	P	L MuF	AIN-1	LSIL	Normal	Residual AIN-1	Normal	Normal	5
19	C3	P	L MuF	AIN-3	HSIL	Normal	Residual AIN-1	Normal	Normal	13
1	C3	P	V MuF	AIN-3	HSIL	Residual V	Residual AIN-1	Normal	Normal (Normal)	18
21	C3	P	L MoF	AIN-1	HSIL	L MoF	AIN-2	HSIL	HSIL	6

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; B, bowenoid; CDC, Centers for Disease Control and Prevention; E, erythroplakic; HSIL, high-grade squamous intraepithelial lesions; I, intra-anal; L, leukoplakic; LSIL, low-grade squamous intraepithelial lesions; MoF, monofocal AIN; MuF, multifocal AIN; P, perianal; US, unsatisfactory sample (scant cellularity); V, verrucous.

mediated (T_H1) immune response by the induction of several proinflammatory cytokines and chemokines, such as interferon alfa, tumor necrosis factor α, and interleukin 1, 6, 8, and 12. Furthermore, imiquimod induces apoptosis and activates B lymphocytes.[9,10,12-14] At present, its safety and efficacy in HPV-associated anogenital warts has been reported in about 40 clinical studies, including a randomized trial in HIV-infected patients.[9,10,12]

To date, there is still no universally accepted standard of treatment of AIN. Surgical excision or ablation is the primary form of treatment, although extensive anal surgery may lead to considerable morbidity, such as wound-healing problems, anal stenosis, or incontinence, especially in immunosuppressed patients.[2,15] Therefore, topical therapies, including podophyllotoxin, 80% trichloroacetic acid or liquid nitrogen, carbon dioxide laser ablation, electrocautery, and infrared coagulation, have been considered for smaller lesions.[2,3,15,16] Most ablative therapies, however, have considerable recurrence rates and frequently require retreatment.[17,18] Reasons for recurrences could be the presence of multifocal AIN, HPV persistence in clinically healthy surrounding tissue, and HIV-associated immunosuppression leading to limited control of HPV. We recently reported on the treatment of AIN with imiquimod cream in 10 HIV-infected MSM and observed complete clearance or at least histologic regression in all patients.[19] These results encouraged us to further evaluate the effects of imiquimod in an open-label prospective pilot study in HIV-positive MSM with histologically confirmed AIN.

METHODS

PATIENTS

Between November 2003 and December 2004, 124 HIV-positive MSM were screened for AIN as previously described.[20] After giving informed consent, 28 consecutive patients with newly diagnosed perianal or intra-anal AIN were included in the study. The last follow-up sample was obtained in September 2005. Patients younger than 18 years and patients with concomitant malignancy or opportunistic infections were excluded. The patients ranged in age from 31 to 69 years (mean age, 43 years). The CD4 cell counts and HIV-1 RNA loads were between 39/μL and 1015/μL (mean, 422/μL) and less than 40 to 86 300 copies/mL (mean, 7369 copies/mL), respectively. The Centers for Disease Control and Prevention stages are listed in **Table 1** and **Table 2**. Twenty-six patients received HAART. One patient (No. 11) did not receive HAART, and 1 patient (No. 13) started HAART during the follow-up period. The study, which was a substudy of the German Competence Network HIV/AIDS, was approved by the ethics review board of Ruhr University Bochum, Bochum, Germany. It was conducted according to Declaration of Helsinki principles.

AIN SCREENING

All participants underwent a standardized anal screening program as previously described.[16,20] Diagnosis of AIN was estab-

Table 2. Characteristics of 6 Noncompliant Patients With Anal Intraepithelial Neoplasia (AIN) Before Imiquimod Therapy (Tx), at the End of Tx, and at the End of Follow-up (Fu)

Patient No.	CDC Stage*	Localization of AIN	Clinical Findings Before Tx	Histologic Findings Before Tx	Cytologic Findings Before Tx	Clinical Findings at End of Tx	Histologic Findings at End of Tx	Cytologic Findings at End of Tx	Cytologic Findings at End of Fu	Last Fu After End of Tx, mo
28	A2	P	B MuF	AIN-1	LSIL	B MuF	AIN-1	ASCUS	No Fu	0
5	C3	P	V MuF	AIN-1	LSIL	V MuF	AIN-3	LSIL	No Fu	0
22	A3	P	V MuF	AIN-2	HSIL	V MuF	AIN-3	HSIL	HSIL	2
24	B3	P	V MuF	AIN-3	HSIL	V MuF	AIN-3	HSIL	No Fu	0
2	C3	I	V MuF	AIN-3	HSIL	V MuF	AIN-3	HSIL	No Fu	0
11	C3	P	V MuF	AIN-3	HSIL	V MuF	AIN-3	HSIL	HSIL	0.5

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; B, bowenoid; CDC, Centers for Disease Control and Prevention; E, erythroplakic; HSIL, high-grade squamous intraepithelial lesions; I, intra-anal; L, leukoplakic; LSIL, low-grade squamous intraepithelial lesions; MuF, multifocal AIN; P, perianal; V, verrucous.

*An explanation of the CDC staging system appears at www.aids-ed.org/aetc/aetc?page=cm-105_disease.

lished according to clinical and histopathologic criteria (Table 1 and Table 2).[20,21] Posttreatment biopsy specimens were obtained next to the site of the first specimen. Smears for cytology and HPV detection were collected from both the perianal area and the anal canal at each visit.[20,22] Pathologists were blinded to the patients' diagnoses.

TREATMENT PROTOCOL

Patients with histologically confirmed AIN were treated overnight with self-applied imiquimod cream or suppositories 3 times a week for 16 weeks. Patients with perianal AIN received 5% imiquimod cream (Aldara; 3M Medica, Borken, Germany). In cases involving intra-anal AIN, suppositories containing 5% imiquimod cream were prepared as described.[23] Imiquimod application was increased to 5 times a week in 1 patient (No. 1) because no signs of treatment-induced inflammation (erythema or mild erosion) were seen. In cases involving strong local adverse effects (eg, burning, marked erythema, or erosions), interruption of treatment for up to 7 days was allowed. All patients were instructed to refrain from receptive anal intercourse during imiquimod therapy. Follow-up visits were scheduled every 4 to 6 weeks.

HPV TYPING AND HPV DNA LOAD DETERMINATION

Screening for HPV DNA was performed with nested polymerase chain reaction for anogenital HPV types.[24] For HPV typing, polymerase chain reaction products were hybridized with oligonucleotide probes in an enzyme immunoassay.[20,25,26] Determinations of HPV DNA loads were performed by real-time polymerase chain reaction with type-specific primers and probes for HPV-16, -18, -31, and -33.[24,27] The HPV DNA load was expressed as HPV DNA copies per β-globin gene copy.[27] If more than 1 of the 4 quantified HPV types were present in a sample, cumulative HPV DNA loads were calculated. The HPV-16, -18, -31, or -33 loads were determined for all samples from a patient if the respective HPV type had been found in any of the patient's samples (**Table 3**).

STATISTICAL ANALYSIS

Statistical analyses (Wilcoxon test for paired samples) were performed with SPSS software (Version 11.0.1; SPSS Inc, Chicago, Ill). $P<.05$ was considered statistically significant.

RESULTS

CLINICAL AND HISTOLOGIC RESPONSE TO IMIQUIMOD

Imiquimod was applied according to instructions by 22 (79%) of the 28 patients with AIN. All 22 patients developed erythema at the application site (**Figure 1**A). At the end of therapy, a complete clinical and histologic response was observed in 17 (61%) of all 28 study patients and in 17 (77%) of the 22 compliant patients (Table 1 and Figures 1, **2**, and **3**). Residual AIN, either clinical and/or histologic, was seen in 4 patients (Nos. 1, 19, 20, and 23 [Table 1]). One compliant patient (No. 21) with monofocal AIN-1 did not respond to imiquimod therapy and progressed to AIN-2 within 16 weeks. This patient's disease was Centers for Disease Control and Prevention stage C3, but his CD4 cell counts were stable at approximately 1000/uL and his HIV replication was well controlled by HAART.

Neither the grade nor the localization of AIN seemed to influence the success of therapy. Six (75%) of the 8 compliant patients with AIN-1, 4 (100%) of 4 patients with AIN-2, and 7 (70%) of 10 patients with AIN-3 showed complete clinical and histologic clearance of AIN at the end of therapy. Three (75%) of the 4 patients with intra-anal disease and 14 (78%) of the 18 patients with perianal disease had a complete response.

Adverse effects of imiquimod therapy, in addition to the erythema that was seen in all patients at the application site, occurred in 11 of the 22 compliant patients. Seven patients (32%) (Nos. 1, 4, 6, 16, 18, 25, and 27) developed mild erosions that led to the interruption of therapy for a maximum of 7 days. One patient (No. 9) had severe erosions as a result of overdosage (imiquimod application twice daily for 2 weeks). Four patients (18%) (Nos. 6, 15, 20, and 21) reported influenzalike symptoms within the first 2 weeks of therapy.

Six (21%) of the 28 patients with AIN did not apply imiquimod at all or only occasionally (Table 2). None of these 6 patients had any erythema at follow-up visits or any adverse effects that were typical of imiquimod therapy. Also, they did not show any clinical response. The histo-

Table 3. Human Papillomavirus (HPV) Types and HPV DNA Loads in 22 Compliant and 6 Noncompliant Patients With Anal Intraepithelial Neoplasia (AIN) Before Imiquimod Therapy (Tx), at the End of Tx, and at the End of Follow-up (Fu)

Patient No.	Compliance	HPV Types* Before Tx	HPV Types* at End of Tx	HPV Types* at End of Fu†	HPV DNA Load‡ Before Tx	HPV DNA Load‡ at End of Tx	HPV DNA Load‡ at End of Fu†
27	Yes	16	16, 61	16, 51, 61, 73	205	12	21
6	Yes	33, 52, 58	HPV neg	16, 83, 89	17634	0	3
14	Yes	16, 18, 33, 58, 59, 89	HPV neg	HPV neg	0.2	0	0
15	Yes	11, 16	11, 16, 52	11, 16, 52	0.03	0.01	3
25	Yes	33, 52, 58, 59, 89	33, 35, 44, 58, 68, 83	33, 44, 58	0.1	0.3	1
13	Yes	61, 66, 73	33, 35, 45, 61	45, 61, 73	No QTs	0	No QTs
4	Yes	16, 54	16	31, 35	365	3	21
3	Yes	16, 18, 31, 33, 35, 42, 45, 52, 59, 66, 70, 83, 89	16, 18, 42, 44, 52	18, 42, 45, 52	17	1	0
16	Yes	16, 18, 52, 83	16, 18	16, 18	16073	22	1340
12	Yes	11, 16, 18, 42	16, 52	11, 42	1092	111	0
17	Yes	16	16	16, 82	1859	31	81
18	Yes	16	16	HPV neg	139	15	0
26	Yes	11, 16, 18, 31, 58	11, 16, 18, 45, 51, 58, 68, 82, 83	11, 18, 33, 45, 51, 58, 68, 82	41	0.2	0.7
7	Yes	33, 52, 56, 58, 59, 70, 73, 81, 83, 84, 89	81	33, 52, 55, 56, 58, 59, 68, 70, 82, 83	5	0	8
10	Yes	16	54	16, 52, 54, 66, 73, 81	2	0	0.3
8	Yes	16, 34, 73	89	16	193	0	3
9	Yes	53, 56	HPV neg	53	No QTs	HPV neg	No QTs
23	Yes	6, 16, 18, 44, 83, 89	6, 16, 18	6, 16, 18, 31, 83	35	2	12
20	Yes	16, 35, 40, 42, 52, 59, 66, 70, 72, 81, 84	35, 42, 52, 54, 59, 66, 70, 72, 81, 84, 89	35, 42, 52, 59, 66, 70, 72, 81, 84, 89	NE	No QTs	No QTs
19	Yes	16, 18, 33, 35, 52, 58, 68, 83, 89	HPV neg	33, 84, 89	772	0	0
1	Yes	16, 42, 45, 59	42	16, 42, 45, 84, 89	NE	No QTs	45
21	Yes	6, 16	6, 16, 34, 42	6, 16, 56	177	42	322
28	No	16, 33, 52, 58, 59, 83	16, 42, 61, 70, 83	No Fu	25	180	No Fu
5	No	18, 26, 31, 61, 83	18, 26, 31, 35, 61	No Fu	21	438	No Fu
22	No	16, 58, 73, 81	16, 34	16, 61	92	106	99
24	No	16, 18, 31, 83	16, 18, 31, 35, 61	No Fu	128	91	No Fu
2	No	16, 18, 33, 52, 58, 66, 83	16, 18, 33, 52, 66, 83	No Fu	500	3721	No Fu
11	No	11, 16, 18, 52, 72, 73, 82	16, 72, 83	16, 18, 52, 73	283	259	142

Abbreviations: HPV neg, HPV DNA negative; NE, not evaluable because cellular input was too low (<40 β-globin gene copies/5 μL input DNA); no QTs, no quantified types (HPV types other than HPV-16, -18, -31 or -33 were present).

*HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -73, and -82 were considered high-risk types and HPV-6, -11, -34, -40, -42, -43, -44, -54, -55, -61, -70, -72, -81, -83, -84, and -89 low-risk types.[28]

†The end of the Fu period (months after the end of therapy) for the respective patients can be seen in the last column of Table 1 and Table 2.

‡HPV DNA load is the cumulative load of HPV-16, -18, -31, and -33, if the respective types were present.

logic AIN grade did not change in 4 of the noncompliant patients, but 2 patients (Nos. 5 and 22) progressed from AIN-1/2 to AIN-3 within 16 weeks. Unwillingness to refrain from sexual intercourse (patients 2, 22, 24, and 28), pain during and after imiquimod application (patients 2, 22, 24, and 28), or depression (patient 5) was given as the reason for noncompliance. One patient (No. 11) turned out to have an HIV encephalopathy and therefore was not able to apply imiquimod. Surgical therapy was offered to the noncompliant patients and was accepted by 2 (patients 5 and 28) of them. One patient (No. 22) asked for a second course of imiquimod; the other 3 patients were unavailable for follow-up.

CHANGES IN HPV STATUS DURING IMIQUIMOD THERAPY

Before therapy, all 28 patients were HPV DNA positive and carried high-risk HPV types (Table 3). Twenty-four patients (86%) were infected with more than 1 HPV type, and the mean number of types was 4.7. The most frequent high-risk types were HPV-16 (n=22 [79%]), HPV-18 (n=11 [39%]), HPV-52 (n=10 [36%]), HPV-58 (n=9 [32%]), and HPV-33 (n=8 [29%]). Also, 22 patients carried low-risk HPV types (Table 3). Imiquimod therapy generally led to a rapid decrease in high-risk HPV DNA loads within the first 4 to 8 weeks of therapy. The mean and median HPV DNA loads in compliant patients with analyzable loads and normal or improved histologic findings (n=17) were 2261 and 139 (range, 0.03-17 634) HPV DNA copies per β-globin gene copy before therapy compared with 12 and 0.3 (range, 0-111) HPV DNA copies per β-globin gene copy at the end of therapy ($P<.001$). During the follow-up period, HPV DNA loads again slightly increased in most patients (Table 3). The mean and median HPV DNA loads in the patients' last follow-up samples were 88 and 3 (range, 0-1340) HPV DNA copies per β-globin gene copy. In 1 compliant patient (No.

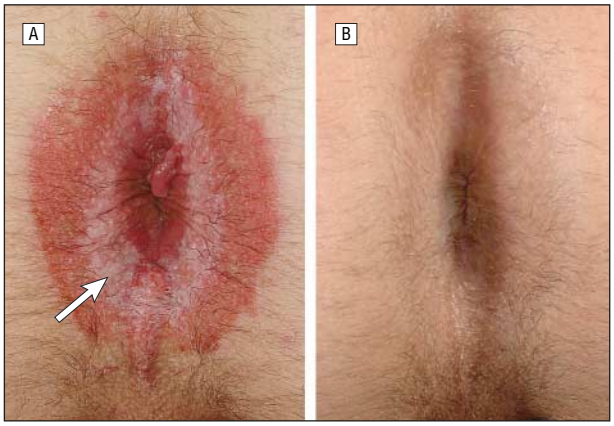

Figure 1. Imiquimod treatment of anal intraepithelial neoplasia (patient 4). A, Complete circumferential demarcation of anal intraepithelial neoplasia (AIN) after 2 weeks of imiquimod therapy. Clinically, leukoplakic AIN was visible only at the lower part of the anus (arrow) before treatment. B, Complete clearance of AIN with no residual signs of imiquimod-related erythema 16 weeks later.

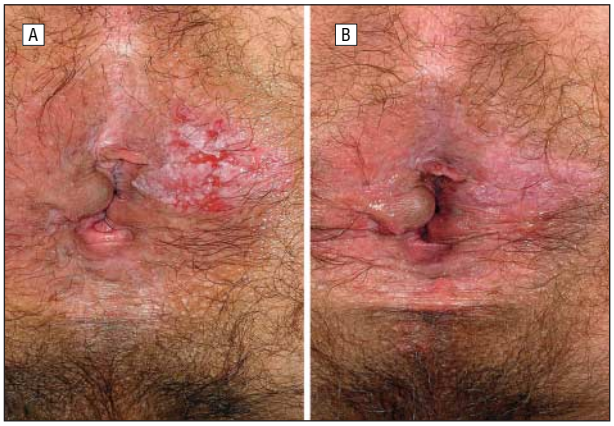

Figure 2. Imiquimod treatment of anal intraepithelial neoplasia (patient 7). A, Perianal indurated, erythematous plaque (2 cm in diameter) with superficial erosions (erythroplakic grade 3 anal intraepithelial neoplasia) before therapy. B, Clinical appearance after 16 weeks of imiquimod therapy.

21) who did not respond to imiquimod therapy, the HPV-16 load first decreased from 177 to 2 HPV DNA copies per β-globin gene copy within the first 4 weeks of therapy, then increased gradually to 42 HPV DNA copies per β-globin gene copy at the end of therapy, and then further increased to 322 HPV DNA copies per β-globin gene copy during follow-up. In the 6 noncompliant patients, the HPV DNA loads persisted at a high level or increased within the observation period (Table 3). The mean and median high-risk HPV DNA loads in the noncompliant patients were 175 and 110 (range, 21-500) HPV DNA copies per β-globin gene copy before therapy and 799 and 229 (range, 91-3721) HPV DNA copies per β-globin gene copy 16 weeks later.

In most compliant patients with complete or partial histologic response (n=21), not only was there a decrease in HPV DNA loads, but there was also a reduction in the number of infecting HPV types. The mean and median numbers of HPV types were 4.6 and 4.0 (range, 1-13) before therapy compared with 2.6 and 1.0 (range, 0-11) at the end of therapy (P=.01). In the last follow-up samples, the mean and median numbers of HPV types were 3.7 and 3.0 (range, 0-10). At the end of therapy, only 4 patients (Nos. 6, 9, 14, and 19) were HPV DNA negative, and only 1 (patient 14) of them remained HPV DNA negative during the follow-up period (Table 3). Patient 14 and another patient (No. 18) who became HPV DNA negative during the follow-up period were in the earlier stages of HIV infection (Table 1).

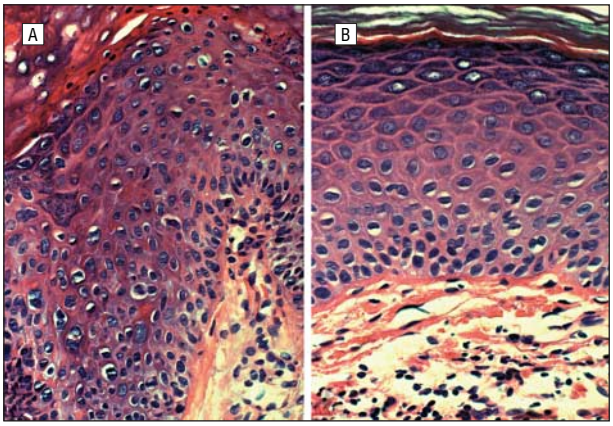

Figure 3. Histologic features of anal intraepithelial neoplasia treated with imiquimod (patient 18). A, Keratinocytic polymorphism, hyperchromatic nuclei, and numerous mitoses consistent with grade 3 anal intraepithelial neoplasia before imiquimod therapy (hematoxylin-eosin, original magnification ×40). B, Almost-normal epidermis with a moderate residual inflammatory infiltrate at the end of therapy (hematoxylin-eosin, original magnification ×40).

FOLLOW-UP PERIOD

Follow-up cytology results were available in all cases with normal histologic findings and clinical healing (n=17) or with residual AIN at the end of therapy (n=4). The duration of follow-up after the end of therapy ranged from 1 to 20 months (mean, 9.5 months; median, 7 months) (Table 1). Fourteen of the 17 patients with complete response at the end of therapy had normal cytologic results and a normal clinical picture at the last follow-up visit. Two patients (Nos. 3 and 7) had low-grade squamous intraepithelial lesions (LSIL) 5 and 6 months after the end of therapy, but the lesions appeared clinically inconspicuous. One year after the end of therapy, 1 patient with AIDS (No. 16) developed a clinical relapse and high-grade squamous intraepithelial lesions (HSIL)/AIN-2. One patient (No. 23) with residual clinical disease and normal histologic findings at the end of therapy still had LSIL on cytologic examination but had a normal clinical picture 6 months later. Interestingly, 3 patients (Nos. 1, 19, and 20) who had residual AIN-1 at the end of therapy demonstrated normal cytologic findings during follow-up, without further intervention, and had a normal clinical picture 5, 13, and 18 months after the end of therapy. In summary, of the 21 patients with normal or improved histologic findings at the end of therapy, 17 (81%) had normal cytologic findings and a normal clinical picture at their last visit. When only patients with a follow-up of at least 5 months are considered, 78% (14/18) had normal cytologic findings and a normal clinical picture at their last visit.

COMMENT

Human immunodeficiency virus–positive MSM develop AIN and anal cancer more frequently than HIV-negative MSM, and progression from normal findings or LSIL to HSIL occurs fast.[1-3,5-8,15,29,30] Therefore, regular anal screening has been recommended for MSM to detect and treat anal dysplasias as early as possible.[16,31] Compared with AIN in HIV-negative individuals, AIN in HIV-positive patients is more recalcitrant to therapeutic interventions.[3,15,17] Many different approaches, including topical therapies, electrocautery, laser ablation, infrared coagulation, and surgical excision, have been used. Most approaches, however, have not been validated in controlled randomized trials.[2,3,15,16] The successful use of imiquimod cream for the treatment of AIN has been described in an HIV-positive man with carcinoma in situ, in a nonimmunocompromised woman with Bowen disease, and, recently by us, in 10 HIV-positive MSM.[19,32,33] In the present study, we used imiquimod to treat 28 HIV-positive MSM with histologically confirmed AIN. After 16 weeks of therapy, 17 (77%) of 22 compliant patients and 17 (61%) of all study patients were free of disease by clinical and histologic criteria. Four (22%) of 18 primary imiquimod responders with a follow-up period of at least 5 months had cytologic abnormalities at the end of the observation period, but only 1 patient (6%) developed HSIL. The relapse rate during follow-up compares favorably with the relapse rates in studies in which ablative techniques were used.[17,18] Chang et al[17] found persistent or recurrent HSIL in 23 (79%) of 29 HIV-positive patients with high-grade AIN who were treated with excision or cauterization, but their duration of follow-up was longer than ours and the mean time to recurrence was 12 months. We had 9 patients with a follow-up period of 12 months or longer; 8 were clinically and cytologically normal at the end of follow-up, and only 1 had developed HSIL/AIN-2. Goldstone et al[18] used infrared coagulation to treat 165 high-grade AIN lesions in 68 HIV-positive MSM. Only 28% of the lesions persisted, which is similar to our results. However, 65% of the patients developed new or persistent HSIL within a median follow-up time (217 days) that was similar to the follow-up period in our study. One advantage of imiquimod therapy over ablative therapies could be the induction of an HPV-specific T-cell immunity, as has been shown with imiquimod treatment of condylomas and vulvar intraepithelial neoplasia.[11,34] The decrease in HPV DNA load and infecting HPV types during therapy and the persistent low HPV loads in the follow-up period that were seen in our patients could be the result of an imiquimod-induced T_H1 response. The T_H1 response has also been assumed to be the reason for the decreases in HPV DNA and messenger RNA that have been seen after imiquimod treatment of condylomas.[13,34,35] In our study, 3 patients with residual AIN-1 at the end of therapy had complete clearance of their AIN in the follow-up period, a result that further supports this assumption. It is possible that the "end-of-treatment" biopsy specimen should not be obtained in the first week after the last application of imiquimod, as in this study, but, instead, should be obtained a few weeks later to give time for final clearance of dysplasias.

In a recent review article, imiquimod was not recommended for intra-anal AIN or high-grade perianal AIN.[2] In our study, neither the localization nor the grade of AIN seemed to have an influence on the response to treatment. However, larger and controlled studies are needed to confirm this finding, especially since imiquimod is not licensed for the treatment of AIN or for intra-anal application.

One of our cases progressed to AIN-2 during imiquimod therapy. Reasons for the failure of imiquimod therapy could be (1) a reduction of dermal dendritic cells, as has been shown in cases involving imiquimod-resistant condylomas; (2) the lack of an HPV-specific T_H1 immunity, as has been seen in patients with vulvar intraepithelial neoplasia that did not respond to imiquimod, or (3) Toll-like receptor 7 polymorphisms.[11,36,37]

A considerable number of patients (21%) were noncompliant. The main reason patients gave for not applying imiquimod was incompatibility with their sexual lifestyle. This issue should be discussed with patients before they begin therapy. Ablative therapies are probably the better choice for patients who are not ready to change their sexual activities during imiquimod application. An advantage of ablative techniques is that they do not require long-term compliance or an ability to administer the therapy. Therefore, ablative therapies presumably should be the first choice for patients with mental disorders. In our study, noncompliant patients had verrucous and multifocal AIN more often than compliant patients (Table 1 and Table 2). This outcome possibly reflects a more reluctant attitude to seek and adhere to medical advice, especially since mean HIV RNA loads and CD4 cell counts were also less favorable in noncompliant patients than in compliant patients (HIV RNA, 26 642 copies/mL vs 2113 copies/mL; CD4 cell count, 359/μL vs 484/μL).

The limitations of our study are the lack of a control group receiving an ablative therapy, the unequal length of the follow-up period, and the relatively small number of patients. The high primary response rates, the low recurrence rates, the significant decrease in HPV loads, and the tolerable side effects shown in this pilot study should encourage the initiation of larger studies to compare imiquimod therapy for AIN with ablative techniques. It has been suggested that a combination of vaccination (inducing a HPV-specific T_H1 response) and local imiquimod application could increase the likelihood of a strong clinical response in patients with vulvar intraepithelial neoplasia.[11] This assumption could be incorporated in the planning of future studies of imiquimod therapy for AIN.

Accepted for Publication: April 29, 2006.
Correspondence: Alexander Kreuter, MD, Department of Dermatology and Allergology, Ruhr University Bochum, Gudrunstrasse 56, D-44791 Bochum, Germany (a.kreuter@derma.de).
Author Contributions: Dr Kreuter had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Wieland, Brockmeyer, Pfister, and Kreuter. *Acquisition of data:* Wieland, Hochdorfer,

Stücker, Swoboda, and Kreuter. *Analysis and interpretation of data:* Wieland, Brockmeyer, Weissenborn, Altmeyer, and Kreuter. *Drafting of the manuscript:* Wieland and Kreuter. *Critical revision of the manuscript for important intellectual content*: Wieland, Brockmeyer, Weissenborn, Hochdorfer, Stücker, Swoboda, Altmeyer, Pfister, and Kreuter. *Statistical analysis:* Weissenborn. *Obtained funding:* Wieland. *Administrative, technical, and material support:* Wieland, Hochdorfer, Stücker, and Swoboda. *Study supervision:* Wieland, Brockmeyer, Hochdorfer, Stücker, Altmeyer, Pfister, and Kreuter.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grant 01 KI 0211 from the Federal Ministry of Education and Research, German Network of Competence HIV/AIDS.

Role of the Sponsor: The funding organization (German Federal Ministry of Education and Research) was not involved in the design and conduct of the study; the collection, management, analysis, and interpretation of data; or in the preparation, review, or approval of the manuscript.

Acknowledgment: We thank Monika Junk, Nicole Girrulat, and Tanja Blome for excellent technical assistance.

REFERENCES

1. Palefsky JM. Human papillomavirus infection and anogenital neoplasia in human immunodeficiency virus–positive men and women. *J Natl Cancer Inst Monogr*. 1998;23:15-20.
2. Chin-Hong PV, Palefsky JM. Human papillomavirus anogenital disease in HIV-infected individuals. *Dermatol Ther*. 2005;18:67-76.
3. Panther LA, Schlecht HP, Dezube BJ. Spectrum of human papillomavirus–related dysplasia and carcinoma of the anus in HIV-infected patients. *AIDS Read*. 2005;15:79-91.
4. Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *J Infect Dis*. 1998;177:361-367.
5. Lacey HB, Wilson GE, Tilston P, et al. A study of anal intraepithelial neoplasia in HIV positive homosexual men. *Sex Transm Infect*. 1999;75:172-177.
6. Palefsky JM, Holly EA, Efirdc JT, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. *AIDS*. 2005;19:1407-1414.
7. Piketty C, Darragh TM, Heard I, et al. High prevalence of anal squamous intraepithelial lesions in HIV-positive men despite the use of highly active antiretroviral therapy. *Sex Transm Dis*. 2004;31:96-99.
8. Bower M, Powles T, Newsom-Davis T, et al. HIV-associated anal cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome? *J Acquir Immune Defic Syndr*. 2004;37:1563-1565.
9. Hengge UR, Ruzicka T. Topical immunomodulation in dermatology: potential of toll-like receptor agonists. *Dermatol Surg*. 2004;30:1101-1112.
10. Garland SM. Imiquimod. *Curr Opin Infect Dis*. 2003;16:85-89.
11. van Poelgeest MI, van Seters M, van Beurden M, et al. Detection of human papillomavirus (HPV) 16–specific CD4+ T-cell immunity in patients with persistent HPV16-induced vulvar intraepithelial neoplasia in relation to clinical impact of imiquimod treatment. *Clin Cancer Res*. 2005;11:5273-5280.
12. Hengge UR, Benninghoff B, Ruzicka T, Goos M. Topical immunomodulators—progress towards treating inflammation, infection, and cancer. *Lancet Infect Dis*. 2001;1:189-198.
13. McInturff JE, Modlin RL, Kim J. The role of toll-like receptors in the pathogenesis and treatment of dermatological disease. *J Invest Dermatol*. 2005;125:1-8.
14. Meyer T, Nindl I, Schmook T, Ulrich C, Sterry W, Stockfleth E. Induction of apoptosis by Toll-like receptor-7 agonist in tissue cultures. *Br J Dermatol*. 2003;149(suppl 66):9-14.
15. Abbasakoor F, Boulos PB. Anal intraepithelial neoplasia. *Br J Surg*. 2005;92:277-290.
16. Berry JM, Palefsky JM, Welton ML. Anal cancer and its precursors in HIV-positive patients: perspectives and management. *Surg Oncol Clin N Am*. 2004;13:355-373.
17. Chang GJ, Berry JM, Jay N, Palefsky JM, Welton ML. Surgical treatment of high-grade anal squamous intraepithelial lesions: a prospective study. *Dis Colon Rectum*. 2002;45:453-458.
18. Goldstone SE, Kawalek AZ, Huyett JW. Infrared coagulator: a useful tool for treating anal squamous intraepithelial lesions. *Dis Colon Rectum*. 2005;48:1042-1054.
19. Kreuter A, Hochdorfer B, Stücker M, et al. Treatment of anal intraepithelial neoplasia in patients with acquired HIV with imiquimod 5% cream. *J Am Acad Dermatol*. 2004;50:980-981.
20. Kreuter A, Brockmeyer N, Hochdorfer B, et al. Clinical spectrum and virologic characteristics of anal intraepithelial neoplasia in HIV infection. *J Am Acad Dermatol*. 2005;52:603-608.
21. Fenger C, Nielsen VT. Precancerous changes in the anal canal epithelium in resection specimens. *Acta Pathol Microbiol Immunol Scand A*. 1986;94:63-69.
22. Luff RD. The Bethesda System for reporting cervical/vaginal cytologic diagnoses: report of the 1991 Bethesda workshop. *Am J Clin Pathol*. 1992;98:152-154.
23. Kaspari M, Gutzmer R, Kaspari T, Kapp A, Brodersen JP. Application of imiquimod by suppositories (anal tampons) efficiently prevents recurrences after ablation of anal canal condyloma. *Br J Dermatol*. 2002;147:757-759.
24. Wieland U, Jurk S, Weissenborn S, Krieg T, Pfister H, Ritzkowsky A. Erythroplasia of queyrat: coinfection with cutaneous carcinogenic human papillomavirus type 8 and genital papillomaviruses in a carcinoma in situ. *J Invest Dermatol*. 2000;115:396-401.
25. Jacobs MV, Snijders PJF, van den Brule AJC, Helmerhorst TJM, Meijer CJLM, Walboomers JMM. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol*. 1997;35:791-795.
26. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol*. 2002;40:779-787.
27. Weissenborn SJ, Funke AM, Hellmich M, et al. Oncogenic human papillomavirus DNA loads in human immunodeficiency virus–positive women with high-grade cervical lesions are strongly elevated. *J Clin Microbiol*. 2003;41:2763-2767.
28. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348:518-527.
29. Frisch M, Biggar RJ, Goedert JJ. Human papillomavirus–associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. *J Natl Cancer Inst*. 2000;92:1500-1510.
30. Palefsky JM, Holly EA, Hogeboom CJ, et al. Virologic, immunologic, and clinical parameters in the incidence and progression of anal squamous intraepithelial lesions in HIV-positive and HIV-negative homosexual men. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;17:314-319.
31. Chin-Hong PV, Palefsky JM. Natural history and clinical management of anal human papillomavirus disease in men and women infected with human immunodeficiency virus. *Clin Infect Dis*. 2002;35:1127-1134.
32. Pehoushek J, Smith KJ. Imiquimod and 5% fluorouracil therapy for anal and perianal squamous cell carcinoma in situ in an HIV-1–positive man. *Arch Dermatol*. 2001;137:14-16.
33. Gutzmer R, Kaspari M, Vogelbruch M, et al. Successful treatment of anogenital Bowen's disease with the immunomodulator imiquimod, and monitoring of therapy by DNA image cytometry. *Br J Dermatol*. 2002;147:160-165.
34. Tyring SK, Arany I, Stanley MA, et al. A randomized, controlled, molecular study of condylomata acuminata clearance during treatment with imiquimod. *J Infect Dis*. 1998;178:551-555.
35. Arany I, Tyring SK, Stanley MA, et al. Enhancement of the innate and cellular immune response in patients with genital warts treated with topical imiquimod cream 5%. *Antiviral Res*. 1999;43:55-63.
36. Arrese J, Paquet P, Claessens N, Pierard-Franchimont C, Pierard G. Dermal dendritic cells in anogenital warty lesions unresponsive to an immune-response modifier. *J Cutan Pathol*. 2001;28:131-134.
37. Schroder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis*. 2005;5:156-164.