Topical cidofovir to treat high-grade anal intraepithelial neoplasia in HIV-infected patients: a pilot clinical trial

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Objective : To evaluate the efficacy of 1% topical cidofovir cream for the treatment of anal high-grade squamous intraepithelial lesions (HSILs) in HIV-infected individuals.

Design : Single-arm, open-label, pilot clinical trial.

Methods : The study medication was applied intraanally three times per week for 4 weeks. Lesions were assessed with high-resolution anoscopy and biopsy at weeks 12 and 24. The primary endpoint was complete response (CR) at week 12, defined as clinical and histological remission. We also evaluated partial response defined as regression to low-grade squamous intraepithelial lesion.

Results : We included 17 HIV-infected patients with intraanal HSIL. Median (interquartile range) age was 36 years (28–41), median (interquartile range) $CD4^+$ cell count was 545 cells/μl (358–630), and viral load was less than 50 copies/ml in 93.75%. Two patients were lost to follow-up, one of them did not apply treatment. At 12 weeks, in the intention-to-treat population, 10 out of 16 patients [62.5%; 95% confidence interval (CI), 38.22–85.78%] had achieved CR. At 24 weeks, seven of the 10 patients (70%; 95% CI, 47–93%) remained in CR, but two out of 10 patients (20%; 95% CI, 0–40%) presented HSIL. One patient did not attend the visit at 24 weeks. Three patients with persistent HSIL at 12 weeks improved at 24 weeks (partial response in one and CR in two). The mean number of human papillomavirus genotypes decreased from 5.2 to 2.73 at 12 weeks ($P = 0.002$). Local adverse effects were frequent (81%), although there were no discontinuations because of adverse events.

Conclusion : One percent topical cidofovir could be an appropriate alternative therapy in HIV-infected patients with anal HSIL.

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Introduction

Anal squamous cell carcinoma is a growing cause of morbidity and mortality in at-risk patients, such as MSM and HIV infection. The incidence in this high-risk group has been reported to be 65–109 per 100 000 person-years [1–3].

The precursor lesion of anal cancer, high-grade squamous intraepithelial lesion (HSIL), is especially prevalent in HIV-infected MSM (25–52%) [1,4]. The natural history of anal HSIL and its progression to infiltrating carcinoma is well documented in the literature, although the rate of progression seems to be lower than the progression of cervical HSIL [1,5–7].

Similarities in the natural history of anal and cervical HSIL indicate that treatment of anal HSIL could lead to a considerable decrease in the incidence of invasive anal cancer. Optimal treatment of anal HSIL has not been clearly defined, since few controlled studies have been published and no therapy guidelines have been agreed upon to date [8]. The preferred approach is ablation of localized lesions, mainly using infrared coagulation, although electrocoagulation, CO_2 laser, cryotherapy, and trichloroacetic acid have also been used [9–11]. However, in patients with extensive involvement, particularly circumferential disease, ablation is associated with considerable morbidity; therefore, the study of efficacious, alternative, topical approaches for treatment of HSIL is of particular interest [12,13].

Cidofovir is a nucleotide analog with activity against a wide range of DNA viruses, including human papillomavirus (HPV) [14]. Although the mechanism of action of cidofovir for the treatment of HPV-associated tumors is not fully elucidated, activity may result from the induction of apoptosis in HPV-infected cells [15]. Cidofovir reduces expression of E6 and E7 and enables accumulation of the tumor suppressor proteins p53 and pRb *in vitro* [16]. In addition, its antitumor activity could be due in part to its antiangiogenic effect [17].

Topical and intralesional cidofovir have been widely used in the treatment of recurrent laryngeal papillomatosis and in benign and premalignant HPV-associated cutaneous lesions, especially in immunocompromised patients [14,18,19].

We hypothesized that given its efficacy in HPV-related lesions, topical cidofovir could be efficacious for the treatment of intraanal HSIL in HIV-infected MSM. We conducted a proof of concept pilot clinical trial to determine the effect of 1% topical cidofovir in HIV-infected patients diagnosed with intraanal HSIL.

Design, patients, and methods

We performed an exploratory, pilot, single-arm, open-label clinical trial to estimate a treatment effect.

The study population comprised adult patients ($\geq$18 years) with confirmed HIV infection and a history of biopsy-proven HSIL of the anal canal who had not received treatment for HSIL during the previous 12 weeks.

We excluded patients with skin disease in the anogenital region, a history of HPV-associated infiltrating neoplasm, and a history of neoplasm during the previous 5 years. We also excluded patients with a history of hematologic, renal, or hepatic disease and pregnant or breastfeeding women.

Seventeen patients were recruited from a cohort of 1850 (40% MSM) HIV-infected patients followed at the HIV Unit of the Internal Medicine Department of Hospital Universitario La Paz, Madrid, Spain. Another three patients were recruited from the cohort of 2200 (37% MSM) HIV-infected patients followed at the HIV Unit of the Infectious Diseases Service of Hospital Universitario Ramón y Cajal.

In these two centers, patients at risk for anal cancer (MSM, MSW/women with antecedent of anogenital dysplasia, anal condyloma or high-risk sexual behavior) are followed in a screening program that includes periodic anal cytology and HRA. From these two cohorts, we recruited for our trial those who met inclusion criteria and signed informed consent between September 2013 and April 2014.

All the visits were held and all the study procedures performed at Hospital Universitario La Paz. Three patients did not meet the inclusion criteria and were excluded. Two patients were lost to follow-up, and one of them did not apply treatment. Participants who received at least one application of intraanal cidofovir constituted the intention-to-treat (ITT) population. Participants who received the complete treatment course and were evaluable for the primary endpoint of the study at week 12 constituted the per-protocol population. Consequently, the ITT population comprised 16 patients and the per-protocol population comprised 15 patients (Fig. 1).

Protocol procedures

A detailed clinical history was taken to record personal data (smoking, substance abuse, sexual behavior, and parameters relative to the HIV infection and its treatment)

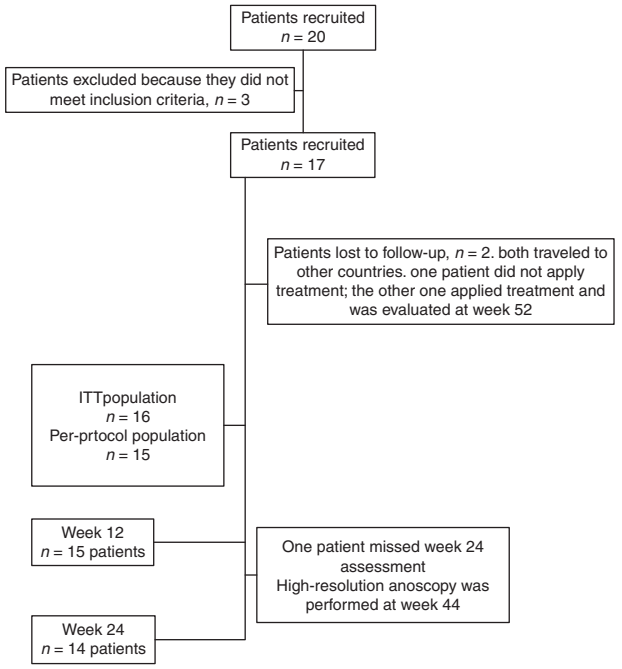

Fig. 1. Flow chart showing progress from the screened population to the intention to treat and per-protocol population. ITT, intention to treat.

and the results of HPV testing and high-resolution anoscopy. Each patient underwent a full physical examination. A blood sample was taken, and anal brush cytology was performed to obtain samples for HPV testing.

High-resolution anoscopy was performed to record anal involvement by quadrants, and a biopsy specimen was taken from the area that was clinically suggestive of HSIL.

The baseline visit (visit 0) was held from 7 to 30 days after the selection visit. Patients with histologically confirmed HSIL were given the complete treatment course in a container. Adherence was recorded using a diary. Adverse effects were recorded at each visit.

Patients attended the scheduled visits for high-resolution anoscopy at weeks 12 and 24. Involvement of the anal mucosa was recorded by quadrants, and an additional biopsy specimen was taken from the original biopsy site, even if no clinically evident lesions were found. Additional biopsy specimens were taken from sites where new lesions suggestive of HSIL appeared.

Patients who were shown to have HSIL at the end of the study were offered ablative treatment of residual lesions with infrared coagulation.

All the patients signed the informed consent document before undergoing any study procedure. The protocol was approved by the local ethics committee.

Treatment

All patients received treatment with 1% cidofovir cream. Patients were given oral and written instructions on intraanal self-application (2–3 cm depth) of 2 g of the cream. Treatment was administered three times weekly for 4 weeks (12 applications).

Cidofovir was extracted from vials of cidofovir solution (Cidofovir injection, Mylan Institutional: 75 mg/ml, 5-ml vials) and formulated to 1% using Beeler base as an excipient until a homogeneous composition was achieved. The preparation was stored refrigerated (2–8°C) until it was dispensed to the patient, who also stored the product refrigerated at home. Patients were recommended to abstain from receptive anal intercourse during treatment.

Techniques

Human papillomavirus testing

Anal brush cytology (ThinPrep) was used to take samples at the selection visit, week 12, and week 24. The samples were analyzed using PCR (CLART HPV2 assay GENOMICA S.A.U.).

High-resolution anoscopy and biopsy

At the selection visit and at weeks 12 and 24, high-resolution anoscopy was performed by visualizing the anal canal through a videocolposcope (Zeiss, OPMI pico) as previously described [20]. Biopsy samples were collected under local anesthesia with Baby-Tischler forceps. Visually atypical areas (acetowhite plaques, areas with an anomalous vascular pattern, and Lugol-negative areas) were chosen for biopsy based on criteria that were predefined in the study.

At the selection visit, anal involvement was recorded by quadrants using templates and photographs. The degree of involvement in each quadrant was classified as 25% or less, 26–50%, 51–75%, or 76–100%. A biopsy specimen was taken from the area that was suggestive of the most severe HSIL. The exact location of the biopsy was recorded on the template. At weeks 12 and 24, involvement by quadrants was recorded, and a biopsy specimen was taken from the site of the biopsy performed at the selection visit.

All study specimens were blindly assessed by two pathologists (M.J.B. and E.R.B.). The results were categorized as normal, low-grade squamous intraepithelial lesion (LSIL), HSIL [anal intraepithelial neoplasia (AIN) grade 2 or 3], or invasive cancer according to the Bethesda classification [21]. Discrepancies between the findings of the pathologists were resolved by reexamining the specimens with a multiheaded microscope to reach a

consensus. In some cases, the grade of dysplasia was established based on overexpression of p16 [22,23].

Testing at the local laboratory

The analytical parameters recorded at the selection visit, week 2, and week 4 were complete blood count, $CD4^+$ cell count, HIV-1 viral load, and biochemistry (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, total bilirubin, creatinine, and glucose).

Criteria for response

Complete remission (CR) was defined as the absence of clinical and histologic squamous intraepithelial lesion in high-resolution anoscopy and biopsy.

Partial response (PR) was defined as a regression from HSIL to LSIL. Progression was defined as an increase in the grade of dysplasia or development of an infiltrating carcinoma. Stable disease was defined as disease not fulfilling any of the above criteria. Recurrence was defined as the appearance of LSIL or HSIL at week 24 at a site where the biopsy at week 12 was normal. A metachronous lesion was defined as the appearance of a new HSIL lesion that had not been reported in the previous anoscopies.

The primary endpoint of the study was the percentage of patients achieving CR at week 12. Secondary endpoints were the percentage of patients achieving PR, with recurrences/metachronous lesion at week 24, and presenting adverse effects.

Adverse events

Adverse effects were classified by the site investigator according to Common Terminology Criteria for Adverse Events v3.0 grading of toxicity. Data on adverse effects were collected from the time of the first dose of study medication until 30 days after the last dose. Data on serious adverse effects were collected throughout the study.

Statistical analysis

Quantitative data were expressed as mean (standard deviation), median (interquartile range), and minimum and maximum. Qualitative data were expressed as absolute frequencies and percentages. Qualitative variables were compared using the Pearson χ^2 test or Fisher exact test, and 95% confidence intervals (CIs) were calculated. The association between quantitative and qualitative variables was determined using the Mann–Whitney test, *t* test, or analysis of variance, depending on the normality of the distribution and the sample size (Kolmogorov–Smirnov test or Shapiro–Wilk test).

Given that the objective was to perform a preliminary exploration of the effect of the drug on anal HSIL, the sample size was not formally estimated. We arbitrarily decided to include 20 patients.

Table 1. Baseline demographic characteristics.

Demographic and baseline characteristics	
Sex: *n* (%)	
Male	17 (100%)
Age (years)	
Median	36
Interquartile range	28–41
Ethnicity: *n* (%)	
White/Non-Hispanic	2 (12%)
American/Hispanic	7 (41%)
White/Hispanic	8 (50%)
CDC risk group: *n* (%)	
MSM	17 (100%)
$CD4^+$ cell count at baseline (cells/μl)	
Median	545
Interquartile range	358–630
Nadir $CD4^+$ (cells/μl)	
Median	225
Interquartile range	127–225
Percentage of patients with viral load <50 copies/ml	93.7%
Years since first diagnosis of HIV	
Median	10
Interquartile range	3–6

Results

The baseline characteristics of the study patients are shown in Table 1. Of note, all the patients included were MSM. There were 17 patients with HSIL, two of whom had AIN3 (12%) and the remainder AIN2 (88%). Fifteen patients (94%) had multifocal involvement. A total of 47 biopsies were performed under high-resolution anoscopy during the study. The p16 technique was used in 20 biopsies (42%).

Three patients (20%) had already received treatment for HSIL (5-fluorouracil, two patients; infrared coagulation, two patients; both, one patient), although not during the previous 12 weeks.

Clinical response

The analysis of the ITT group ($n = 16$) at week 12 revealed that 10 of the 16 patients had reached CR (62.5%; 95% CI, 38.2–85.7%). One patient achieved PR at week 12 (patient 10) (6.2%; 95% CI, 0–17%). The disease remained stable in four patients (patients 5, 6, 8, and 9) although in two of them (patients 6 and 9), the area affected decreased by more than 50% (Table 2).

At week 24, 10 of the 16 patients (62.5%; 95% CI, 38–85%) had a normal biopsy result. Seven of the 10 patients with a CR at week 12 remained in CR (70%; 95% CI, 47–93%). Of the three patients without a CR at week 12, two with HSIL (patients 6 and 5) and one with LSIL (patient 10) had a normal biopsy specimen at week 24.

Table 2. Clinical and histological characteristics at baseline, week 12, and week 24 (8 and 20 weeks after completing treatment).

	Week 0		Week 12		Week 24	
Patient	Histology	Number of affected quadrants	Histology	Number of affected quadrants	Histology	Number of affected quadrants
1	HSIL (AIN2)	4	Normal	0	UK W44:LSIL	UK W44:1
2	HSIL (AIN2)	4	Normal	0	HSIL (AIN2)	1
3	HSIL (AIN3)	1	Normal	0	Normal	0
4	HSIL (AIN2)	2	Normal	1	Normal	1
5	HSIL (AIN2)	4	HSIL (AIN2)	4	Normal	4
6	HSIL (AIN2)	2	HSIL (AIN2)	1	Normal	1
7	HSIL (AIN2)	4	Normal	1	Normal	0
8	HSIL (AIN2)	4	HSIL (AIN2)	3	LSIL (AIN1)	3
9	HSIL (AIN2)	4	HSIL (AIN2)	1	HSIL (AIN 2)	1
10	HSIL (AIN2)	3	LSIL	0	Normal	0
11	HSIL (AIN2)	2	Normal	0	Normal	0
12	HSIL (AIN3)	2	Normal	1	Normal	1
13	HSIL (AIN2)	3	Normal	0	Normal	0
14	HSIL (AIN2)	4	Normal	1	HSIL (AIN2)	1
15	HSIL (AIN2)	4	Normal	0	Normal	0
16	HSIL (AIN2)	4	UK	UK	UK W52:LSIL	UK W52:1

AIN, anal intraepithelial neoplasia; HSIL, high-grade intraepithelial neoplasia; LSIL, low-grade intraepithelial neoplasia.

Three patients presented HSIL at week 24, one with previous HSIL (patient 9) and two who had achieved a CR at week 12 (patients 2 and 14). Patient 2 presented a recurrence, as HSIL was observed at the original site. In patient 14, the biopsy result was normal in the area of the previous HSIL, although a metachronous HSIL lesion was observed in a different quadrant. One patient (patient 8) with stable disease at week 12 presented PR (LSIL) at week 24. Finally, one patient with CR at week 12 did not attend the visit at week 24 (patient 1); therefore, the corresponding result was not included in the analysis. However, at week 44, this patient's biopsy specimen revealed LSIL. Patient 16, who was lost to follow-up, applied the full course of treatment and returned to the clinic at week 52, and although the data for this patient are not included in the analysis, the biopsy result revealed LSIL.

The analysis of the per-protocol group ($n = 15$) at week 12 revealed that 10 of the 15 patients had reached CR (66.6%; 95% CI, 42–89%) and one patient achieved a PR at (patient 10) (6.5%; 95% CI, 0–17%). At week 24, 10 of the 15 patients (66.6%; 95% CI, 42–89%) had a normal biopsy result.

Human papillomavirus analysis

Anal brush cytology revealed HPV in 100% of patients at the selection visit. HPV16 was detected in 50% of cases. The most common genotypes were HPV52, 16, 58, 51, and 6. The mean (standard deviation) number of genotypes at the baseline visit was 5.2 (3.2), which decreased to 2.73 (3.3) at week 12 and 3.87 (2.2) at week 24. The reduction in the mean number of genotypes between the baseline visit and week 12 was statistically significant ($P = 0.002$).

No significant differences were found between the mean number of HPV genotypes in patients whose SIL lesions persisted and those whose biopsy result was normal.

Adherence and adverse events

Adherence to treatment was good, and it was not necessary to modify the regimen or suspend treatment in any case. No systemic adverse effects or serious local adverse effects were detected. One patient presented acute hepatitis C virus infection at week 2, although this was not considered related to treatment. Local adverse effects were reported in 13 of the 16 patients (81%; 95% CI, 62–100%). The most common was local discomfort (itching/stinging/pain), which affected 13 patients (30% grade 1, 54% grade 2, 15% grade 3). Six of 16 patients (37%) reported bleeding (100% grade 1). Four patients reported flatulence (6% grade 1, 18% grade 2) and three patients diarrhea (two patients grade 1 and one patient grade 2). Mean time to a local adverse effect was 16.4 (9.3) days, and median time was 15 (11–19) days. Symptoms resolved before 3 weeks in all cases.

With the exception of the patient who developed hepatitis C virus infection, no clinically significant abnormalities were seen in the blood count or biochemistry parameters at 2 and 4 weeks (during and at the end of treatment). We observed no significant variations in creatinine levels or glomerular filtration estimated by means of the Modification of Diet in Renal Disease formula, $CD4^+$ cell count, or HIV viral load (data not shown).

Discussion

To our knowledge, this is the first study to evaluate topical cidofovir for the treatment of intraanal HSIL in

HIV-infected patients. We found that two out of three participants who completed treatment achieved CR and that all the study participants completed the treatment course despite frequent local adverse effects. This response was associated with a significant decrease in the number of HPV types at week 12.

Previous studies on topical cidofovir have shown the drug to be effective even in severely immunosuppressed patients [14]. Most of the previous studies on topical cidofovir have focused on the treatment of anogenital warts or dysplasia in HIV-infected patients and vulvar or cervical HSIL in immunocompetent women [19,24,25].

Van Pachterbeke *et al.* [23] evaluated the efficacy of cidofovir gel versus placeboin 48 women diagnosed with cervical intraepithelial neoplasia (CIN) 2+ who underwent conization. The authors found that 60.8% of patients in the cidofovir group compared with 20% in the placebo group were free of CIN after three applications of 2% cidofovir gel. No systemic toxicity was recorded. Tristram *et al.* [24] recently compared the efficacy and safety profile of 1% cidofovir gel three times a week with that of imiquimod for treatment of vulvar HSIL in a randomized phase 2 trial. CR was achieved by 46% of patients receiving cidofovir and by 46% of those receiving imiquimod. Finally, Stier *et al.* [19] evaluated the efficacy of 1% topical cidofovir for 6×2-week cycles to treat high-grade perianal and vulvar intraepithelial neoplasm in HIV-positive patients. CR was recorded in 15% of patients and PR in 36%. Treatment was well tolerated, and most common adverse effects were local.

In comparison with the aforementioned studies, the CR rates of our clinical trial are similar to those of Van Pachterbeke *et al.* [23] for treatment of CIN2+ after three applications of 2% cidofovir gel. However, the percentage of patients achieving a CR in our study is higher than reported for perianal intraepithelial neoplasia and vulvar intraepithelial neoplasia in the studies of Stier *et al.* [19] and Tristram *et al.* [24], even though these authors used more prolonged cidofovir treatment regimens. We hypothesize that our better results could be because of a more pronounced effect of cidofovir on the mucosa than on the skin. As cidofovir is highly polar across the skin, its bioavailability through an intact stratum corneum could be limited [14].

Other topical drugs used to treat intraanal HSIL include imiquimod and 5-fluorouracil. Although evidence from controlled clinical trials is scarce, both treatments proved efficacious in the treatment of intraanal HSIL, with CR rates ranging from 17 to 61% [12,13,26]. Nevertheless, we consider that cidofovir may offer a series of advantages over other drugs. In contrast to the cytotoxic effect of 5-fluorouracil, cidofovir induces selective apoptosis with no effect on surrounding healthy tissue [15,24,25]. Moreover, the effect of cidofovir does not seem to depend on host immune status, whereas immune response modifiers such as imiquimod may be unable to modulate innate and acquired cellular immune responses in very severely immunosuppressed patients [27].

High recurrence rates have been documented for HIV-infected patients following all types of treatment for HSIL [26]. The 13.3% rate of recurrence/metachronous lesions among complete responders in the present study is lower than reported after other treatment options for intraanal HSIL [12,13]. However, our sample size was too small and the follow-up period in our study was too short to draw firm conclusions.

Previous studies have evaluated changes in HPV types and viral load in the course of HSIL treatment. Both imiquimod and 5-fluorouracil significantly decreased the number of HPV types detected and high-risk HPV-DNA loads recorded. Specifically, imiquimod decreased the number of HPV types in patients who responded to treatment [12]. In the case of 5-fluorouracil, this decrease was independent of the clinical/histological response [13]. Richel *et al.* [26] speculated that in contrast to the imiquimod-induced specific anti-HPV immune response, 5-fluorouracil might decrease HPV-DNA load via nonspecific mucosal cell destruction. However, infrared coagulation affected neither the number of HPV types nor the HPV-DNA loads.

In our study, treatment with cidofovir significantly decreased the number of HPV types at week 12. As with 5-fluorouracil, this decrease was independent of the clinical/histological response. Nevertheless, the small sample size of our study prevents us from drawing definitive conclusions about the effect of cidofovir on viral clearance.

The adverse effects of intravenous cidofovir include nephrotoxicity, neutropenia, and, rarely, metabolic acidosis [14]. Topical cidofovir seems to be well tolerated, and we observed no systemic toxicity in our study. However, tolerance was poor, as a high percentage of patients reported local adverse effects. This finding contrasts with the proposed mechanism of action of cidofovir, which induces apoptosis only in neoplastic cells and not in noninfected keratinocytes [15]. We consider that the high rate of local adverse effects in our study could be because of the large number of patients with very extensive HSIL recruited (93.7%).

Our study is subject to a series of limitations. First, it was an uncontrolled, open-label, pilot study of a small sample of HIV-infected patients with HSIL. Second, in the absence of a placebo arm, we were not able to discern to what extent the response could be directly attributed to cidofovir as opposed to spontaneous regression. A recent study on the natural history of anal HSIL in HIV-infected and non-HIV-infected men reported spontaneous

regression of HSIL in 23% of patients [28]. Third, the follow-up period of 24 weeks was too short to draw conclusions about the effect of treatment in the medium-long term. Moreover, the optimal dosage schedule still needs to be determined, as patients who achieved PR may have obtained a benefit from more prolonged treatment. Finally, only serum creatinine and not urinary protein/creatinine ratio was used to assess the possible impact of topical cidofovir on renal function.

The strengths of our study include its prospective design, high biopsy rate, HPV data analysis, high-resolution anoscopy performed by an experienced anoscopist, independent review of specimens by two pathologists, and the use of p16 in the histopathology analysis.

In summary, short-term treatment of anal HSIL in HIV-infected individuals with topical cidofovir was efficacious in 62.5% of cases, with recurrences/metachronous lesions in 13% of patients at week 24. Despite the high frequency of local adverse effects, treatment with cidofovir might be considered in patients diagnosed with multifocal disease that cannot be treated with primary ablative therapy. In this context, targeted infrared coagulation would only be possible after a reduction in the area affected by HSIL.

Although topical cidofovir could play a role in the treatment of anal HSIL, further controlled trials with a greater number of patients, longer treatment courses, and longer follow-up periods are warranted to assess the efficacy and durability of the response.

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Conflicts of interest

The authors declare that they have no financial interests that could cause conflict with the manuscript or their participation in the study. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors might consider relevant to the content of the manuscript have been disclosed. This manuscript has not been published nor is it under consideration for publication elsewhere.

The US Food and Drug Administration (FDA) approved cidofovir in 1997 for the treatment of cytomegalovirus retinitis in patients with AIDS. Therefore, this manuscript discusses an unlabeled use of the drug, not yet approved by the FDA for the treatment of anal dysplasia.

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