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Safety and efficacy of topical cidofovir to treat high-grade perianal and vulvar intraepithelial neoplasia in HIV-positive men and women

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Objective: To evaluate the safety and efficacy of topical cidofovir for treatment of high-grade squamous perianal intraepithelial neoplasia (PAIN) and vulvar intraepithelial neoplasia (VIN) lesions in HIV-positive individuals.

Design: Phase IIa prospective multicenter trial conducted at eight clinical sites through the AIDS Malignancy Consortium.

Methods: HIV-positive patients with biopsy-proven high-grade PAIN that was at least 3 cm^2 were enrolled. PAIN biopsy specimens were assessed for human papillomavirus (HPV) using PCR and type-specific HPV probing. Participants applied 1% topical cidofovir to PAIN and VIN (if present) for six 2-week cycles. Results were designated as complete response (CR), partial response (PR) (>50% reduction in size), stable disease, or progressive disease (PD).

Results: Twenty-four men and nine women (eight with high-grade VIN as well) were enrolled. Mean age was 44 years and mean $CD4^+$ cell count was 412 cells/μl. HPV DNA (most commonly HPV16) was detected in all pretreatment study specimens. Twenty six (79%) participants completed treatment per protocol: CR, five (15%); PR, 12 (36%), stable disease, seven (21%); PD, two (6%) (one with a superficially invasive cancer and one with new area of high-grade PAIN). Treatment was well tolerated with most common adverse events being mild to moderate affecting lesional skin: pain/burning/irritation (25 patients) and ulceration (13 patients).

Conclusion: Topical cidofovir had 51% efficacy in the short-term treatment of high-grade PAIN and VIN with acceptable toxicity in HIV-positive individuals. Randomized control studies with more prolonged treatment courses and longer follow-up to assess the durability of the response are needed.

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Introduction

HPV-associated cancers of the perianus and vulva in HIV-infected individuals continue to be a significant cause of morbidity in the era of HAART [1]. High-grade squamous perianal intraepithelial neoplasia (PAIN) and vulvar intraepithelial neoplasia (VIN), the precursors to invasive squamous cell cancer, are particularly difficult to treat due to their multifocal presentation and tendency for recurrence [2–4].

Standard therapies for diffuse high-grade perianal or vulvar disease include laser ablation, cryotherapy, electrocautery, and surgical excision with possible skin grafting or skin flaps [2–4], all of which carry significant morbidity such as prolonged postprocedure pain, scarring, risk for infection, incomplete healing, incontinence, and sexual dysfunction [5–8]. Immunocompetent patients have recurrence rates after treatment of 25–50% [5] and HIV-positive patients have recurrence rates of 60–80% [9–11].

Topical treatments such as imiquimod and 5-fluorouracil creams have some efficacy for treatment of high-grade PAIN and anal canal intraepithelial neoplasia (AIN) in HIV-infected men [12–15]. However, treatment with these agents have substantial skin and mucosal toxicity that often leads to discontinuation of treatment. There are few reports of topical treatments applied to high-grade VIN and PAIN in HIV-infected women [7,16].

Cidofovir is a cytidine nucleotide analogue with in-vitro and in-vivo activity against a broad spectrum of herpesviruses as well as adenoviruses, human papillomaviruses (HPVs), polyomaviruses, and human poxviruses [17]. It is currently licensed for intravenous treatment of cytomegalovirus infections. The mechanisms of action of cidofovir on HPV-associated neoplasia are not fully elucidated [18,19].

Several small studies have shown near-complete clearance rates of genital warts, with acceptable toxicity, using topical cidofovir in HIV-infected patients [9,20–23]. Because of the clinical efficacy and tolerability for warts reported in these studies, we conducted a pilot study to evaluate the safety and efficacy of topical cidofovir in HIV-infected patients for treatment of high-grade squamous intraepithelial neoplasia of the perianus and vulva.

Methods

AIDS Malignancy Consortium (AMC) Protocol 046 was a multicenter, single-arm, open-label, pilot trial conducted at eight AMC clinical trial sites (Boston University Medical Center, Boston, Massachusetts, USA; Laser Surgery Care, New York, New York, USA; Montefiore Medical Center, Bronx, New York, USA; University of California, San Francisco, California, USA; Virginia Mason Medical Center, Seattle, Washington, USA; Weill-Cornell Medical College, New York, New York, USA; Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA; University of California, Los Angeles, California, USA). Institutional review boards of the participating institutions approved the study. Each participant gave written-informed consent (clinical trial.gov unique identifier: NCT00550589).

We enrolled adult men and women with documented HIV-1 infection and biopsy-proven high-grade PAIN within 12 weeks of study entry covering a surface area of at least 3 cm^2. The perianus was defined as the skin within a 5 cm radius of the anal verge, and thereby included women with high-grade VIN on the posterior perineum. Patients receiving ART had to be on a regimen for at least 12 weeks prior to entry. If they were not receiving ART, the $CD4^+$ T-cell level needed to exceed 200 cells/μl. For patients receiving ART, any $CD4^+$ T-cell level was acceptable.

Cidofovir was provided by Gilead Sciences, Inc (Foster City, California, USA) and compounded by the study compound pharmacy (MasterPharm, LLP, Richmond Hill, New York, USA) into a 1% topical cream packaged in 10 g tubes, sufficient for two treatment cycles. The vanishing cream base was an emulsion of mineral oil, deionized water, cetyl alcohol, ceresin wax, beeswax, and sodium borate. Each batch underwent testing by a third party (Eagle Analytical Services, Houston, Texas, USA) to confirm potency and uniformity. Approval of the compounded product by the Food and Drug Administration was obtained prior to initiating the trial (Investigational New Drug 74 757).

Study protocol

Lesion measurements were taken from the photo-documentation of high-grade PAIN and VIN with the skin on gentle stretch after application of 3–5% acetic acid. A software program (Second Opinion, Torrance, California or DermImage, New York, New York, USA) was used to calculate the total lesion area from the digital images.

Pretreatment specimens for histology and correlative studies included two 3-mm biopsies of the lesions. One specimen was placed in formalin. The other biopsy was flash-frozen for HPV DNA typing. All biopsy sites were photo-documented, so that biopsies collected during and after treatment could be taken from the same location(s).

The participants self-applied the study cream sparingly in a thin layer over the affected areas with a gloved finger once daily and then washed the cream off 6–8 h later. Women with high-grade VIN were instructed to apply

the treatment medication to both the high-grade VIN and PAIN. Treatments occurred for 5 consecutive days followed by no treatment for 9 days (a 14-day treatment cycle). Participants received a treatment diary to record the timing of treatment and any side effects during each week treatment cycle.

Following each treatment cycle, a study clinician reviewed the participant's treatment diary and examined the participant to assess local toxicity and clinical status of lesion(s). Precycle laboratory assessments included serum creatinine, urine protein, and complete blood counts. Absent complications requiring dose delay, the participant received study medication for the next treatment cycle. Therapy continued until completion of six treatment cycles.

Study participants underwent a final evaluation 6 weeks after completion of the sixth treatment cycle. Lesion measurements and two biopsies for histology and molecular studies were taken from the residual lesion, if present, or from the original lesion site.

Response criteria

Complete response (CR) was defined as the absence of any high-grade PAIN or VIN or cancer. A partial response (PR) was defined as no new lesions and a 50% or greater decrease in total lesion area of high-grade PAIN or VIN. Progressive disease (PD) was defined as either at least 25% increase in the total lesion area, or biopsy-proven invasive perianal or vulvar cancer. Stable disease included responses that were not defined in the other response definitions.

Adverse events were graded according to version 4.0 of the NCI Common Terminology Criteria for Adverse Events.

Specimen processing

The formalin-fixed, paraffin-embedded biopsy specimens were sectioned for hematoxylin and eosin histologic confirmation by the study central pathologist (T.M.D.). The frozen specimen biopsies were processed for histologic confirmation, followed by HPV DNA PCR testing. HPV DNA typing was performed using MY09/MY11 consensus HPV-L1 primers as well as primers for amplification of the human β-globin gene, as a control [24].

Pathology review

All study specimens (formalin and frozen) were read by two pathologists. Histologic interpretations were categorized as benign or low-grade PAIN or VIN, high-grade PAIN or VIN, or cancer. If there was a discrepancy in histologic interpretation of a specimen between the two central pathologists, then the specimen was reviewed by a third pathologist. Final determination of histologic interpretation was determined by agreement of at least two pathologists. Each study pathologist was blinded to the interpretation of the others.

Sample size

Thirty-three participants were enrolled to detect a 50% combined PR and CR (with one-sided 0.05 significance level with power of 0.95) compared with an assumed natural regression rate of 20% (allowing a 10% participant drop-out/withdrawal rate).

Statistical analysis

Univariate and multivariate logistic regression analyses were used to compare study participants with a clinical response (PR or CR) to study participants without a significant response (PD or stable disease) for possible predictors of response (pretreatment total lesion size, presence of HPV16, $CD4^+$ cell count, and undetectable HIV viral load). All analyses were performed in SAS version 9.1; SAS Institute, Inc, Cary, North Carolina, USA. Final results are interpreted using an $\alpha = 0.05$ level of significance.

Results

Between February 2008 and August 2009, 47 participants were screened and 33 were enrolled. Reasons for screen failures included the following: participants did not have biopsy-proven high-grade PAIN (six), failure to meet laboratory eligibility criteria (four), clinician judgment of an unacceptable risk for cancer (two), voluntary withdrawal prior to treatment (one), and one potential participant was screened in error. The median age at enrollment was 44 years (range 24–66), 24 (73%) of the participants were men, 12 (36%) were African–American, and eight (24%) were Hispanic. Eight of the nine enrolled female participants had both high-grade VIN and high-grade PAIN. At the time of enrollment, median $CD4^+$ cell count was 412 cells/μl (range 2–1152), median HIV viral load was less than 75 copies/μl, and 97% of the participants were using ART. The median baseline total lesion size was 6.6 cm^2 (standard deviation 5.4, range 3.0–21.3) (Table 1).

The 33 enrolled study participants who completed at least one cycle of treatment constituted the intention-to-treat (ITT) group. Twenty-six of the 33 (78.8%) participants completed the study per protocol and were included in the per-protocol analysis. Four participants were lost to follow-up, two withdrew because of mild adverse events (grade 1 bleeding and anal pain) and one participant was excluded from analysis because the consensus pathology review of the enrollment biopsy was low-grade. Overall, five of the 33 in the ITT group (15%) had a CR, 12 (36%) had a PR, seven (21%) had stable disease, and two (6%) had PD. Response rates for the per-protocol group ($n = 26$) were CR, 19%; PR, 46%; stable disease, 31%;

Table 1. Demographic summary and selected baseline characteristics.

Demographic and baseline characteristics	*N* (%)
Male	24 (73%)
Female	9 (27%)
Race/ethnicity	
White/Hispanic	6 (18%)
White/non-Hispanic	14 (42%)
African–American/Hispanic	2 (6%)
African-American/non-Hispanic	8 (24%)
Asian, multiracial or unknown race/ non-Hispanic	3 (9%)
Age in years	
Mean	44.0
SD	8.9
Median	44.0
Range	24 to 66
CDC risk group	
Homosexual/bisexual contact	19 (58%)
Heterosexual contact	9 (27%)
Homosexual contact and IVDU	1 (3%)
Homosexual/bisexual and heterosexual contact	2 (6%)
Congenital	1 (3%)
Homosexual and heterosexual contact, IVDU and transfusion recipient	1 (3%)
Absolute CD4 cell count at baseline (cells/μl)	
Median	412.0
Range	2–1152
HIV viral load at baseline (copies/μl)	
Median (19/33 or 58% with <75 copies/μl)	< 75
Range	<75 to 203 702
Baseline total lesion area (cm^2)	
Mean	8.3
SD	5.36
Median	6.6
Min–Max	3.0–21.3

CDC, Center for Disease Control; IVDU, intravenous drug user.

Table 2. Human papillomavirus types detected in high-grade perianal intraepithelial neoplasia pretreatment and posttreatment[a], by study participant.

Patient	HPV type(s), pretreatment	HPV types, posttreatment	Response
1	16	45	CR
35	16	Generic only	CR
38	26/69	Generic only	CR
3	26/69, 33	Generic only	PR
10	33	None	PR
14	82	82	PR
22	16	16, 58	PR
26	16	72	PR
29	66	66, 54, 35	PR
31	16, 72	90/10, 16	PR
32	16	73	PR
8	16	Generic only	SD
36	53	Generic only	SD
39	16	18	SD
43	16	16	SD
46	16	16	SD
4	Generic only	16	PD
13	16	16	PD
5	16	16	exclude

CR, complete response; HPV, human papillomavirus; PAIN, perianal intraepithelial neoplasia; PD, progressive disease; PR, partial response; SD, stable disease.
[a]For patients with a complete response, the HPV is from the site of the pretreatment high-grade PAIN.

and PD, 8%. Of the two participants with PD, one was diagnosed with invasive cancer and the other with an increase in high-grade PAIN lesion size. The overall clinical benefit rate (CR + PR) for participants among the ITT population was 17/33 (51.5%), one-sided 95% confidence interval (36.1, 100%). The response rate for participants in the per-protocol group was 17/26 (65.4%), one-sided 95% confidence interval (47.4, 100%). The outcomes for the eight women treated for both high-grade VIN and PAIN were one CR (13%); three PR (38%); one stable disease (13%); one PD (13%); two lost to follow-up (25%).

Thirty of 33 baseline specimens were available for HPV typing. The other specimens had insufficient tissue for analysis or significant freezing artifact limiting HPV testing. All 30 evaluable specimens were HPV positive. HPV 16 was detected in 16 (53%) of the evaluable baseline biopsy specimens. Nineteen study participants had pretreatment and posttreatment specimens evaluable for HPV typing. Comparing HPV types detected pretreatment and posttreatment; five patients had the same HPV types (four with HPV16), three had the same HPV type with additional HPV types detected post-treatment, nine had different HPV types detected, and only one patient had no HPV detected posttreatment (Table 2).

Risk factors including median $CD4^+$ cell count, total lesional area, detection of HPV16 in pretreatment specimen, and having undetectable HIV viral load were assessed for association with clinical response (CR or PR). No significant factors were associated with clinical response with univariate or logistic regression analysis, but this could be due to small numbers in each strata.

Adverse events were reported for 32 (97.0%) participants (Table 3). Most were localized to the skin at the lesions undergoing treatment: 25 study participants complained of mostly mild and self-limiting pain/burning/irritation of the affected skin. Clinicians reported mild-to-moderate ulceration of the lesional skin in 13 study participants. Three patients experienced severe adverse events. One participant had an invasive perianal squamous cell carcinoma at study completion, which was treated with surgical excision. Another study participant had bacterial pneumonia and herpes zoster; a third participant had methicillin-resistant *Staphylococcus aureus* cellulitis on her thigh, distant from the site of treatment, that required parenteral antibiotics.

Table 3. Adverse events.[a]

Adverse events (by patient)	Grade		
	Mild (grade 1)	Moderate (grade 2)	Severe (grade 3)
Pain/burning/pruritis of the anus/perianus/perineum	7	18	
Bleeding from perianus	7		
Ulceration of the perianus/perineum	8	5	
Invasive perianal cancer			1
Proteinuria	7		
Anemia	3		
Neutrophil count decreased	0	3	
HSV		3	
Upper respiratory infection		2	
Lung infection		2	1
Shingles			1
Methicillin-resistant *Staphylococcus aureus* skin infection (thigh)			1
Vaginal infection	0	3	
Syphilis	0	1	
Diarrhea	4	2	
Constipation	2	1	
Nausea/vomiting	2	4	
Perirectal abscess		1	

HSV, herpes simplex virus. Only categories with either (at least) (3) mild events or any moderate event were included.
[a]CTC Adverse Event Categories v4.

Discussion

There are few reports of topical treatments applied to preinvasive lesions of the vulva and perianus in persons with HIV [12–15] and no reports of topical cidofovir for this purpose in HIV-positive men or women. We report the first prospective, open-label, multisite study evaluating topical cidofovir for the treatment of high-grade PAIN and VIN in HIV-infected patients. Our results show that over 65% of participants who completed the therapy had at least a PR, and although local skin reactions were very common, most study participants completed the treatment course.

We believe that our results are particularly important because treatment of extensive high-grade PAIN and VIN in HIV-infected patients is difficult. Surgical and ablative therapies are associated with significant morbidity. Often, the lesions recur and the repeat treatments cause additive morbidity. An effective topical treatment that is well tolerated has clear clinical benefits over the current surgical options. Even though complete clearance occurred in only 15%, the PR in an additional one-third of treated patients is clinically beneficial. Many patients with flat low-grade disease may not require treatment and a reduction in high-grade lesion area may enable a targeted and less morbid excision or ablative procedure [7].

Prior reports of locally applied cidofovir have focused on the treatment of genital and/or perianal warts (condylomas) in HIV-infected patients and VIN in immunocompetent women. In studies of HIV-infected participants with genital warts treated with 1% cidofovir cream or gel, overall response rates of 70–90% were reported, with significantly less pain and a lower relapse rate compared with ablative procedures [9,10,17,20–23]. A series of 12 immunocompetent women with VIN3 treated with topical cidofovir found that four women had a CR and three had PR [25].

The diagnosis of cancer in one of our participants is concerning and may represent an occult lesion that was present prior to treatment and not sampled by biopsy. However, it could also be failure of the study agent to prevent progression of the precancerous lesion to invasive cancer or nonadherence to treatment. Because these are typically heterogeneous and multifocal lesions, one small biopsy as indicated per protocol might not have identified the cancer. Wide local excision of high-grade VIN lesions is associated with detection of occult cancer in 3–5% of immunocompetent patients [5]. In a placebo-controlled study of imiquimod 5% treatment of immunocompetent patients with high-grade VIN, one of 26 (4%) patients in the treatment arm and two of 26 (8%) patients in the placebo arm were diagnosed with cancer of the vulva within 7 and 12 months, respectively [26]. Similar progression rates have been seen in other studies [13,25,27]. As in our study, the cancers detected in these studies could represent occult disease or true progression to cancer and highlight the inherent diagnostic limitations that should be considered when designing future studies.

This is the first study to evaluate HPV genotyping in HIV-infected patients with high-grade PAIN. HPV was detected in all lesions in the study, and HPV-16 was detected in 50% of these lesions. De Vuyst *et al.* [28] reports similar HPV genotypes in high-grade AIN in HIV-positive individuals, in which case HPV was identified in 97% of high-grade AIN and HPV16 was

identified in 55%. It is unclear why 13 of 19 (68%) study participants had a new HPV type detected posttreatment. This may represent acquisition of a new HPV infection, activation of a previously subclinical HPV infection, or detection of a concurrent HPV type that was not identified in the pretreatment specimen.

Strengths of our study include its prospective multicenter trial design, inclusion of HIV-infected men and women, uniformity of the compounded study drug, HPV data analysis, colposcopic evaluation of lesion size with validated measurement software to better delineate lesions and response, and independent pathology review of specimens to determine inclusion and response in the trial.

Study limitations include the small sample size, lack of placebo arm, limited duration of treatment, and short-term follow-up. In the absence of a placebo arm we were not able to distinguish the 'true' response rate from spontaneous regression. However, prior studies of anal, perianal, and vulvar lesions have shown very low rates of natural regression, especially in the setting of HIV. None of the 26 immunocompetent participants in the placebo arm of the imiquimod study of high-grade VIN [25] and only one of 26 (4%) HIV-infected patients in the placebo arm of the imiquimod study of AIN2–3 [13] demonstrated regression of high-grade intraepithelial disease. Therefore, it is likely that the rate of spontaneous regression in HIV-infected individuals is low and, thus, the short-term responses to topical cidofovir are likely not due to natural regression, but due to the study drug. The standardization of treatment cycles in this study did not allow for prolonged treatment courses for patients with PRs. In addition, the short-term follow-up (6 weeks after completion of treatment) prevents our commenting on the durability of treatment response. High recurrence rates (>50%) following other forms of treatment for high-grade AIN have been documented for HIV-infected patients, even in the ART era [29]. Lastly, long-term toxicity or toxicity with repeated use of topical cidofovir are not known.

In summary, topical cidofovir had 51% efficacy in the short-term treatment of high-grade PAIN and VIN in HIV-infected individuals. Phase 3 trials should be conducted with more prolonged treatment courses and longer follow-up to assess the durability of the response.

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Conflicts of interest

There are no conflicts of interest.

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