Published in final edited form as:

Am J Obstet Gynecol. 2012 January ; 206(1): 42.e1–42.e7. doi:10.1016/j.ajog.2011.06.105.

Randomized Clinical Trial of Imiquimod: An Adjunct to Treating Cervical Dysplasia

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Abstract

Objectives—HPV infection is a major risk factor for cervix cancer. Imiquimod is a topical medication that enhances the immune response to HPV-induced genital warts. This study evaluated cervical application of imiquimod as an adjunct to standard treatment for cervical dysplasia.

Study Design—Fifty-six patients were randomized to standard excisional/ablative treatment versus applications of imiquimod followed by standard treatment. The primary endpoint was dysplasia recurrence within two years.

Results—There were no differences in dysplasia recurrence between the two groups. Treatment was well tolerated, with side effects being mild, but significantly worse in women receiving imiquimod for, chills, fatigue, fever, headache, myalgias and vaginal discharge.

Conclusion—This trial does not support the hypothesis that imiquimod, as used in this trial, impacts recurrence of cervical dysplasia, but adequacy of findings are limited by sample size. The trial does support the feasibility and acceptability of the use of imiquimod on the cervix.

Key Terms

Cervical Dysplasia; HPV; Imiquimod; LEEP

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DISCLOSURE: None of the authors have a conflict of interest

Presentation information:

1. Cytokine Activation Following Cervical Application of Imiquimod in Women with Cervical Dysplasia. Presented at the American Society of Colposcopy and Cervical Pathology Biennial Meeting. Lake Buena Vista, FL. March 21, 2002.
2. Immune Modulation in Cervix Cancer Prevention. Presented at the European Research Organization on Genital Infection and Neoplasia Conference. Paris, France. April, 2003

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Introduction

Cervical cancer is the second most common cancer in women worldwide, with more than 270,000 deaths annually.^{1, 2} Cervical dysplasia and invasive cervix cancer are caused by human papillomavirus (HPV). The association between infection and development of cervical cancer is strongest with HPV types 16 and 18.³ Persistent infection with a high risk HPV type is the strongest predictor of cervical dysplasia and cancer.⁴ Pursuant to the notation of this association, HPV testing has begun to be incorporated into cervical cancer screening.⁵ In addition, the HPV vaccines were designed to protect against the oncogenic HPV types 16 and 18.⁶

Current treatments for cervical dysplasia involve the use of freezing, laser, electrosurgery or a surgical scalpel to remove or destroy the cells that have become abnormal or precancerous as a consequence of HPV infection.⁷ Although these approaches are effective, they can lead to complications, including cervical stenosis, bleeding, and pelvic infection.⁸ Therefore, there is increasing interest in HPV immunotherapies for the treatment of HPV associated neoplasia.⁹

Imiquimod is an immune response modifier with antiviral and antitumor activity. It induces the expression of cytokines, such as interferon, tumor necrosis factor, and interleukins 1, 6, and 8.¹⁰ Imiquimod also activates T-cells, resulting in a tumor directed immune response which has been associated with clearance of HPV.¹¹ Imiquimod was approved in 1997 for the treatment of genital warts, most of which are caused by HPV types 6 and 11.¹² Recently, imiquimod has also been found to be effective for treatment of vulvar intraepithelial neoplasia (VIN), which is also associated with HPV infection.^{13, 14}

The purpose of this clinical trial was to evaluate the efficacy and safety of imiquimod for women with cervical dysplasia. The primary goal was to determine if topical treatment with imiquimod could decrease recurrence of cervical dysplasia. A secondary goal was to determine patient acceptability and toxicity of topical imiquimod to the cervix. The current communication presents the results of this study as it relates to these goals.

Materials and Methods

This clinical trial was supported by the NIH and approved by the Mayo Foundation Institutional Review Board. All participants received and signed informed consent forms per Federal regulations, NCT00031759.

Women aged 18 or older, with biopsy proven CIN II, III, or recurrent or persistent CIN I, were randomized to experimental or standard treatment. Other eligibility criteria included; an exclusively ectocervical lesion (determined by colposcopy and/or normal endocervical brushing or endocervical curettage), and a negative pregnancy and HIV test. In addition, all participants were to use adequate contraception for 3 months following enrollment. All biopsies and Pap smears were reviewed by a qualified single pathologist, who was blinded to treatment assignment. At the time of entry into this study, patients were stratified, using dynamic allocation, according to severity of dysplasia, primary vs. recurrent dysplasia, and tobacco use.

Patients randomized to standard treatment received the excisional or ablative treatment recommended by their provider. Options included a loop electrosurgical excision procedure (LEEP), laser, cryotherapy, or conization, as dictated by the size, location, and severity of the lesion and according to the usual treatment practice of the attending physician.

Patients randomized to the experimental treatment received 5 applications of the immune response modifier, imiquimod. One single use packet, containing 50mg of 5% imiquimod in an oil in water cream base, was used for each treatment. After insertion of a vaginal speculum, the cream was applied directly to the ectocervix using a cotton tipped applicator. The speculum was withdrawn and a contraceptive diaphragm was placed as a medication barrier. The patient was instructed to leave the diaphragm in place for at least 6, but no more than 10, hours. The diaphragm was then removed by the research nurse, a vaginal speculum was re-inserted, and the cervical region was gently flushed with saline. Treatment was repeated every 3 to 4 days (about twice a week), but was suspended during menses. Side effects were graded following each of the five applications of imiquimod, by a single research nurse. Toxicities were graded per NCI Common Terminology Criteria (CTC) version 2.0. Two to four weeks following the fifth topical treatment, ablative or excisional therapy was carried out as described for the standard treatment group above.

Patients were to return for repeat evaluation three to four months following definitive treatment. In addition to a Pap smear, a cervicovaginal swab was obtained to test for HPV. Colposcopy was performed at the discretion of the physician, with abnormal appearing areas on the cervix being biopsied per usual clinical practice. Subsequent evaluations were scheduled at three- to four-month intervals until patients had two consecutive normal Pap smears, then at least twice yearly for two years.

The primary endpoints were the rates of persistent and recurrent CIN, and progression to cervical cancer within two years. Recurrent or persistent dysplasia was defined as those participants who were diagnosed with CIN after treatment who exhibited at least one of the same HPV type(s) as they had at study entry. If CIN was diagnosed with a new HPV type, or there was no detectable HPV type, this was coded as new disease.

In a previous trial involving a similar population of women with cervical dysplasia, the rate of recurrent or persistent disease following standard treatment was 26% to 29%, depending on the treatment employed.¹⁵ Power was based on a 25% recurrence rate within two years following treatment. A sample size of 152 patients would provide a 90% chance of detecting a 50% decrease (12% recurrent or persistent disease) in treatment failures. The study was closed due to slow accrual, prior to reaching the planned number of protocol subjects.

HPV detection was accomplished using two complimentary methods. The first method was a PCR amplification and automated DNA sequencing strategy, developed in a Mayo Clinic research laboratory. This strategy combines the use of broad range primers for detection of multiple HPV types and type specific primers to insure the most sensitive detection of HPV types 16 and 18. The second method was the Roche HPV Linear Array Genotyping System (Roche Molecular Systems, Inc., Alameda, CA). This noncommercial Roche product is a reverse line blot containing 27 different HPV probes. For both HPV detection methods, DNA was extracted from samples using a modified Isoquick procedure. The extracted DNA was amplified by PCR. Reactions showing agarose gel bands of the appropriate size were subjected to purification and then submitted to a core laboratory for automated DNA sequencing (PE ABI 377 DNA Sequencer, PE Applied Biosystems, Foster City, CA). Sequences were compared to HPV sequences in the Gene Bank data base using commercial software (Wisconsin Package Version 9.1, Genetics Computer Group, Madison, WI). An HPV type was assigned if the sequence of the amplified region showed at least 90% homology to a known HPV type. Negative control samples were interspersed with test specimens in order to monitor the possible development of contamination problems during preparation, amplification, or detection. Samples with negative or equivocal results using the standard primer set were re-tested using different primers^{15, 16} and or using nested PCR techniques.

Results

Between September 1999 and January 2003, 56 women were enrolled and randomized to experimental treatment (N=28) or standard treatment (N=28). One woman was seen for a single visit at which time she consented to participate in the study, but did not return for study treatment. Another patient received her first treatment with imiquimod, but withdrew after experiencing chills and headaches. Both patients remain in the analysis according to their assigned treatment group. Patient flow is described in the CONSORT diagram (Figure 1).

Patient characteristics are listed in table 1. They were well balanced between groups except for tobacco use, which was higher in the experimental group. The mean and median follow-up times were 3.1 years (SD=1.36 years) and 3.3 years, respectively. There was no difference in follow-up time by study arm ($p=0.67$).

There were no significant complications associated with the excisional or ablative treatments. Statistically significant differences in side effects, potentially related to imiquimod, were seen between groups for headache, myalgias, fatigue, fever, rigors and vaginal discharge. The grade and frequency of the toxicities for each group are shown in table 2. One patient experienced a near syncopal event while receiving imiquimod, which required intravenous hydration in an emergency room. This occurred when the patient was starting treatment with an antidepressant medication; she had similar hypotensive problems in the past associated with the use of antidepressants. She completed the remaining applications of imiquimod without further episodes of hypotension.

Overall, there was no difference in recurrence between the experimental and standard therapy group. There were 13 women with a second diagnosis of dysplasia within 2 years of study entry, 7 in the study group and 6 in the control group. Eight women had a recurrence that included at least one HPV type matching the HPV type(s) present at study entry, 4 in the imiquimod group and 4 in the control group (Table 3). The average time to recurrence in the study group was 10 months versus 6 months in the control group. Five women had new HPV types or no HPV found at the time of second diagnosis of dysplasia, 3 in the study group and 2 in the control group (Table 4). These patients are regarded as having new dysplasia and are not included in subsequent discussions of recurrent dysplasia. No patients were diagnosed with invasive cancer during the study treatment or the follow-up period.

The diagnosis of recurrent dysplasia occurred in subjects who had mild (3/8, 38%), moderate (2/8, 25%) and severe dysplasia (3/8, 38%) dysplasia at study entrance. More patients who entered the trial with a diagnosis of recurrent dysplasia had a recurrence during the follow up (6/8, 75%), compared to those with a first time diagnosis (2/8, 25%)(Table 3).

All but one patient tested positive for HPV at study entry (Table 1). HPV types were gained and lost over the course of follow up (Table 5). Although the majority of patients tested positive for HPV at their last examination (31/49, 63%), a large percent lost at least one of the original HPV types. In fact, only 3 patients in the experimental arm and 3 patients in the standard treatment arm retained all original HPV types at their last follow up exam.

Comment

This study failed to provide data to support the use of imiquimod as an adjunctive treatment for cervical dysplasia. The overall recurrence rate of 14% and second diagnosis of dysplasia rate of 23% are consistent with previous investigations.¹⁵ Of note, in this trial, recurrent disease was defined specifically as dysplasia with the same HPV types as original diagnosis,

whereas, in most clinical trials all subsequent dysplasia is defined as recurrence without awareness of HPV changes.

This study does, however, provide supportive data for the safety and tolerability of topical imiquimod application to the cervix. Imiquimod is used as treatment for many skin conditions and is, overall, well tolerated. In trials of imiquimod for external genital warts 34% of patients using topical imiquimod had mild to moderate local site reactions, including pruritus, burning, erythema, excoriation, erosion, and edema.¹⁷ Topical application of imiquimod for external genital warts also produced systemic side effect in <10% of patients. These included fatigue, headache, nausea, and myalgias.¹⁷ Similar side effects were seen with application for VIN, where the most common patient reported side effects were vulvar pain and pruritus. Systemic adverse events were rare, but included flu-like symptoms.¹³

In a recent randomized clinical trial of imiquimod for treatment of VIN, patients in the imiquimod group experienced more headache, weariness, muscular aches, and flulike symptoms, although these effects were not significantly different from the control group, overall the imiquimod group had more side effects.¹⁴ In the current study, there were also more side effects experienced in the imiquimod group. However, despite more side effects with imiquimod, only one patient did not complete the planned treatments due to the toxicities, which supports its tolerability. As the side effects are flu-like in nature, they did provide corroborating evidence of immune modification. In addition, treatments are available to offset the toxicity experienced with imiquimod. A recent trial investigated the use of NSAIDs to treat common side effects of imiquimod therapy. This trial found that NSAID use did not interfere with immunomodulatory properties of imiquimod and can safely be used to manage side effects.¹⁸

Another objective of this study was to observe the effect of imiquimod on the frequency of clearance of HPV DNA. Persistent infection with a high risk HPV type is the strongest predictor of cervical dysplasia and cancer.⁴ Imiquimod is an immune response modifier which affects the immune system in many ways. Imiquimod stimulates toll-like receptors on the surface of immature dendritic cells leading to the activation of NF κ B, resulting in the production of cytokines, such as INF- α , TNF- α , IL-2, IL-6, IL-8, IL-12, and chemokines. In addition, imiquimod causes an activation of antigen-presenting cells and leads to activation of the type 1 T-cell immune response.^{11, 12, 14, 19}

By activating the local immune response, imiquimod assists in clearance of HPV infection.²⁰ A recent randomized clinical trial, which found imiquimod to be effective in the treatment of VIN, discovered that HPV viral clearance was strongly associated with histologic regression of disease.¹⁴ In addition, clearance of HPV infection resulted in the normalization of immune cell counts at the site of the lesion.¹¹ In the current study there was a higher loss of original HPV types in the imiquimod group and fewer patients in the imiquimod group were virus positive at follow up. This supports the known activity of imiquimod to assist in the clearance of HPV. In addition, the time to recurrence was longer in the imiquimod group. However, any differences identified between groups were small and due to the small numbers, statistical comparisons were not done.

The sequential HPV analyses performed in this trial proved valuable in the analysis of dysplasia occurring after patients completed treatment. Over the course of patient follow-up, the patterns of HPV infection showed a high level of variability. The acquisition and eradication of HPV types renders a true understanding of the course of infection almost impossible without HPV typing. Only 62% of the apparent episodes of recurrent dysplasia, in fact, represented the same infection that was present at study entry. Thus, 38% of subsequent diagnoses of dysplasia were actually new disease which would have been

misrepresented without type specific HPV testing. This finding is especially interesting as HPV typing is further explored as a clinical tool.

Testing for high risk HPV types as part of screening for cervical cancer was accepted by the FDA in 2003. Currently the American Cancer Society and the American College of Gynecology (ACOG) recommend that HPV testing be added to cytology for women > 30 years of age. The approved HPV screening used clinically is a pooled test, which identifies 13 high risk types of HPV.²¹ It is important to emphasize that, with the pooled high risk HPV test results obtained through clinical laboratories, clinicians have no way to determine whether a patient has persistence of a high risk type or types, or a series of newly acquired infections that her body is dealing with effectively. Thus, while the pooled HPV test may be the most appropriate test for screening purposes, the current study suggests a potential benefit of type specific HPV testing in clinical trials pertaining to cervical dysplasia. Demonstration of a new disease versus recurrent dysplasia has different implications for patient counseling and, at times, for management decisions. Evaluation of type specific high risk virus persistence also helps to identify the cohort of patients at highest risk for recurrent dysplasia and cancer.

The primary limitation of this study was the small sample size. The goal accrual was 152 patients, however only 56 were enrolled. The twice daily clinic visits for five applications of imiquimod to the cervix made it difficult to accrue participants and speaks to the difficulty of integrating this type of treatment into community practice. Nonetheless, the small sample size limits the sensitivity to recognize a difference in recurrent dysplasia between groups.

In addition the study did not investigate the most effective dosage or application technique. The dosing regimen of 50mg of 5% imiquimod applied to the cervix on 5 separate occasions (2× weekly) was utilized because of market availability and concerns for safety. The technique of application of imiquimod to the cervix with washing was done in order to maximize potential effectiveness and minimize side effects, mimicking prescribed use for genital warts.

The dosage and administration of topical imiquimod varies based on the indication. For external anogenital warts, the treatment schedule is 3 times a week for 6-10 hours until clearance or for maximum of 16 weeks.¹⁷ The regimen used in a recent trial of imiquimod for the treatment of VIN was 250mg of 5% imiquimod twice a week for 16 weeks.¹⁴ Therefore, it may be that a higher and more frequent dosing schedule would have netted different results. Although the main activity of imiquimod is local, small amount are absorbed systemically. The drug is retained in the skin for prolonged periods with an elimination half-life of about 1 day.¹⁷ It remains unclear whether there is a difference in systemic absorption with cervical application.

While this study does not support the effectiveness of cervical imiquimod, 50 mg, over 5 applications as an adjunct to treatment for dysplasia, the tolerability of the treatment allows for future exploration of other dosing regimens and treatment durations. Data that continue to emerge about imiquimod's immune modulating properties and benefits in other HPV related conditions will hopefully guide further research in this area.

Acknowledgments

Source of financial support: This work was supported by the following United States National Institutes of Health Grants: CA124477 and CA079429.

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Figure 1. CONSORT Diagram

Table 1
Demographics and study characteristics

	Imiquimod (N = 28)	Control (N = 28)	p value
Age			0.9280
Mean (SD)	30 (8.92)	29 (9.17)	
Nationality			0.3130
Hispanic	0 (0%)	1 (4%)	
Non-Hispanic white	28 (100%)	27 (96%)	
Disease at Study Entry			0.7891
Primary	13 (46%)	14 (50%)	
Recurrent or persistent	15 (54%)	14 (50%)	
Severity of dysplasia			0.6797
I	11 (39%)	10 (36%)	
II	10 (36%)	13 (46%)	
III	7 (25%)	5 (18%)	
Tobacco Use			0.2646
1 = Yes	12 (43%)	8 (29%)	
2 = No	16 (57%)	20 (71%)	
Ablative Therapy			0.3636
Cryotherapy	1 (4%)	1 (4%)	
LEEP	6 (23%)	2 (8%)	
Laser/LEEP	19 (73%)	21 (88%)	
Missing data	2	4	
HPV status at study entry			N/A
Single HPV			
16	5	7	
18	0	1	
Other	13	6	
Mixed type with 16	5	7	
Mixed type with 18	1	2	
Other mixed	4	4	
HPV negative	0	1	

Toxicities**Table 2**

Side Effect/Grade	Imiquimod N=28	Control N=28	p-value
Myalgia			<0.0001
1	12 (42.9%)	0	
2	3 (10.7%)		
Rigors			<0.0001
1	13 (46.4%)	0	
Fatigue			0.0001
1	10 (35.7%)	0	
2	3 (10.7%)		
Vaginal Discharge			0.0012
1	9 (32.1%)	0	
Headache			0.0054
1	4 (14.3%)	0	
2	3 (10.7%)		
Fever			0.021
1	5 (17.9%)	0	
Abdominal Pain			0.08
1	3 (10.7%)	0	
Anorexia			0.16
1	2 (7.1%)	0	
Nausea			0.16
1	1 (3.6%)	0	
3	1 (3.6%)		
Dizziness			0.16
1	2 (7.1%)	0	
Inflammation			0.16
1	1 (3.6%)	0	
2	1 (3.6%)		
Insomnia			0.33
1	1 (3.6%)	0	
Stomatitis			0.33
2	1 (3.6%)	0	
Dehydration			0.33
2	1 (3.6%)	0	
Hot Flashes			0.33
1	1 (3.6%)	0	
Photosensitivity			0.33

Side Effect/Grade	Imiquimod N=28	Control N=28	p-value
1	1(3.6%)	0	
Sinus Tachycardia			0.33
1	1 (3.6%)	0	
Syncope			0.33
3	1 (3.6%)	0	
Arachnoiditis			0.33
1	1 (3.6%)	0	
Vaginal Bleeding			0.33
2	1 (3.6%)	0	
Mouth Dryness			0.33
1	1 (3.6%)	0	
Dermatology			0.33
1	1 (3.6%)	0	
Sexual Function			0.33
1	2 (7.1%)	1 (3.6%)	
2	1 (3.6%)		

Table 3
Summary of Recurrent Dysplasia Diagnoses

	Imiquimod	Control	Total
Recurrence of Dysplasia	4	4	8
Average Time to Recurrence (months)	10	6	
Characteristics at Study Entry			
Severity of Dysplasia			
CIN 1	1	2	3
CIN 2	1	1	2
CIN 3	2	1	3
Recurrent/persistent dysplasia	3	3	6
First diagnosis of dysplasia	1	1	2
Tobacco Use	1	1	2
HPV status at Recurrence			
Lost one HPV Type	2	2	4
All original types persist	2	2	4

Table 4
HPV Types in New versus Recurrent Dysplasia

Imiquimod	
Recurrent Dysplasia	
HPV initial	HPV at Recurrence
18,52,54	18,52
16,31	16
74	74
16	16
New Dysplasia	
HPV initial	HPV at Second Diagnosis
16	neg
16	neg
51,54	61
Control	
Recurrent Dysplasia	
HPV initial	HPV at Recurrence
16	16
16	16
31,51	31
15,51,84,54	84
New Dysplasia	
HPV initial	HPV at Second Diagnosis
39,18	66
39,42,84	neg

Table 5
Change in HPV Status

	Imiquimod	Control
Study Entry		
Virus Positive	28/28	27/28
Last Follow up *		
Virus Positive	15/26 (58%)	16/23 (70%)
Lost all original HPV Types	19/26 (73%)	16/23 (70%)
Lost at least one original HPV Type	23/26 (88%)	18/23 (78%)
Gained new HPV type	8/26 (31%)	9/23 (39%)
All original type(s) persist	3/26 (12%)	3/23 (13%)
Missing HPV Follow up data	2	5

* Average time to final check of HPV status was 12 months in control group, 17 months in imiquimod group