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- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*

*The corresponding author has opted to make this information publicly available.

Personal or nonessential information may be redacted at the editor’s discretion.

Questions about these materials may be directed to the Obstetrics & Gynecology editorial office:

obgyn@greenjournal.org.
Date: Jan 08, 2021
To: "BRUNO Oliveira FONSECA"
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-20-3124

RE: Manuscript Number ONG-20-3124

A Randomized Clinical Trial of Topical Imiquimod for the Treatment of High-Grade Squamous Intraepithelial Lesions of the Cervix

Dear Dr. FONSECA:

Your manuscript has been reviewed by the Editorial Board and by special expert referees. Although it is judged not acceptable for publication in Obstetrics & Gynecology in its present form, we would be willing to give further consideration to a revised version.

If you wish to consider revising your manuscript, you will first need to study carefully the enclosed reports submitted by the referees and editors. Each point raised requires a response, by either revising your manuscript or making a clear and convincing argument as to why no revision is needed. To facilitate our review, we prefer that the cover letter include the comments made by the reviewers and the editor followed by your response. The revised manuscript should indicate the position of all changes made. We suggest that you use the "track changes" feature in your word processing software to do so (rather than strikethrough or underline formatting).

Your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Jan 29, 2021, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1:

My questions are below

1. Methods. Please specify how HGSIL diagnosis was made in terms of details of how colposcopy and biopsies were performed.
2. Please describe pathology quality control in diagnosis of HGSIL prior to randomization and during LEEP specimen analysis.
3. How might your findings play out in pregnant and immunosuppressed?
4. What can be extrapolated from treatment of anal dysplasia? Men having sex with men? VIN and VAIN?
5. Please briefly address cost
6. It is interesting that such promising treatment has not done through phase 3 trial yet. Please comment on why. Is this an issue with pharma not getting enough ROI because this drug can't be patented, or some other reason? Why has there not been phase 3 studies yet? Phase 2 Grimm study has been published in 2012. Please comment.
7. Are there different formulations of imiquimod in terms of cream and suppositories?
8. Please comment on your decision to administer imiquimod in clinic by a healthcare professional.
   a. How do you think that will be implemented in clinical practice?
   b. Given that medication was not self-administered, why was placebo not used and why was it not blinded? To me, it seems like an opportunity to reduce bias.
9. Please address possible sources of bias and their effect on your results in your limitations section
10. How was recruitment done? How were patients approached? Clinic? Flyers?
11. Was there an effort to evaluate for patient acceptability of intervention? Why not?
12. End point-why include CIN2 and not CIN3? Can results be analysed for CIN 3 only?
13. What was the rationale behind using CIN 1-3 terminology vs. LAST terminology? Or was it always dual?
14. Sample size calculation was not adjusted for cross over from imiquimod to LEEP group.
15. Wait times to get LEEPs since diagnosis. To make sure I understood it correctly, once a patient was randomized, they either went into 12 week imiquimod group or to LEEP wait list. Control group waited 4 months to get a LEEP, and imiquimod group waited 16 more weeks in addition to that. That seems like a reasonable difference, although randomizing into placebo vs. intervention would have addressed this time lag. Please address.
16. What could be potential recurrence risk after imiquimod vs. LEEP? Do you plan to follow these patients?
17. LEEP is a great endpoint
18. Was there any differences in demographics between included and excluded patients?
19. Lines 156-157. Why was surveillance after LEEP was pap and colpo every 6 months?
20. Were patients incentivized in any way to participate? It seems like since everyone ended up with LEEP, patients had not reason to agree to be in this study since it delayed their treatment and had potential side effects. From what I understood, since wait time for LEEP is so long (4-5 months), patients opted to enroll as that in their minds at least meant they will be getting some sort of treatment.
21. Midway colposcopy as a safety measure in imiquimod arm is helpful
22. Who were the pathologists who confirmed biopsy results? Can you provide more information about colposcopy and how it was done which lead to enrollment?
23. Figure 1. Not able to follow numbers in ITT vs. PP. 8 women were excluded due to loss to follow up and discontinuation, but there was only difference of 7 between ITT (45) and PP (28).
24. Margin positivity (lines 300-305). How was this defined? CIN1-3, or CIN 2-3 only? It should only be HGSIL to be clinically meaningful.
25. What was the compliance with weekly visits in imiquimod group? How many patients completed all visits, etc.?
26. In discussion, please comment on what next steps would be in studying this next.

Reviewer #2:

The manuscript details a randomized controlled superiority trial of application of topical imiquimod crem to CIN2/3 versus initial LEEP. They found that surgical margins were more often negative for HSIL in participants that had undergone topical imiquimod prior to LEEP and more likely to regress, with 60% of experimental participants undergoing regression compared to 22%. The conclusion was that topical imiquimod may be a safe and effective treatment for HSIL.

Comments on methodology:
The premise of the article relies on the fact that the outpatient clinic requires scheduling of LEEPs 2-3 months in advance, therefore a 12-week application of imiquimod followed by LEEP can be compared to when patients can be scheduled for next available LEEP appointment.
1. Why can the clinic accommodate weekly imiquimod applications for patients but cannot accommodate an outpatient LEEP procedure for patients with HSIL until 2-3 months out? This needs to be addressed more fully, as this is a significant weakness that is not generalizable even to the busiest US hospitals.
2. Why were the patients followed with colposcopies q6 months after LEEP? This is not in line with ASCCP guidelines and there is no mention of the results afterwards.
3. Why were LEEPs given to the imiquimod group at 12-week conclusion if the evaluation is a superiority trial and not with a longer time interval to assess for continued regression?

Comments on writing style:
The writing style is very clear, concise, and understandable.

Comments on overall strengths:
The study design has the strengths of randomization and an intention-to-treat analysis, which is crucial given the large number of experimental participants who did not complete the whole treatment. It also was able to compare LEEP samples between control/experimental groups, which is a benefit to confirm that regression occurred (compared to colposcopy-only evaluation).

Comments on weaknesses:
1. Authors assumed a 20% rate of loss-of-follow up. What was the loss of follow-up for straight to LEEP? Can you say this is a non-inferior treatment if you are assuming such a high drop-out rate?
2. Line 203-204 states that 60% of patients treated with imiquimod had histological regression of HSIL by colposcopy - without any mention of the LEEP sample. This line would benefit from the addition of confirmation that regression did not occur, as confirmed by LEEP sample.
3. 7 patients were excluded from the experimental group because of systemic side effects (4 patients) and transportation issues (3 patients), which means that 15% of people dropped out due to side effects. Can you say that it is as safe and effective than LEEP if 15% of candidates could not complete the treatment due to transportation and/or side effects? This seems like a significant number that would hamper the implementation of completion of treatment and generalizability.
4. The analysis of per-protocol seems to be a difficult generalizable study. Focusing on the intention-to-treat lends more credibility to the study. However, the intention-to-treat broken down by HPV subtypes show that actually, the only significant difference is in patients with HPV-16 showing regression.
5. The mean interval between diagnosis of HSIL and LEEP in the control was 16 weeks - four months. This is, by American standards, a very long interval. This is a flawed design, as the comparison is now the control groups = no treatment for 16 weeks (versus immediate surgical intervention) versus imiquimod treatment for 3 months followed by LEEP.

6. The conclusion was that topical imiquimod may be a safe and effective treatment for HSIL. I can not agree with this statement due to multiple factors outlined below. A better conclusion would be that when treatment will be delayed, or when patient highly desires deferral of LEEP, or with HPV16+ or large lesions, imiquimod may lead to regression of lesions with subsequent increased negative margins.
   a. The control group was deferral of intervention/treatment for 4 months, when we know that no interventions will lead to progression of cervical dysplasia with time
   b. Treatment with imiquimod led to at 15% drop-out rate due to side effects and burden of treatment (multiple weekly clinic visits) versus a one-time clinic visit for LEEP
   c. When broken down by HPV subtype, the only significant regression occurred with HPV-16 lesions

7. The note that surgical margins was more likely to be negative in the imiquimod-treatment group was a very interesting note and one of the stronger points of the paper. I would love to see more discussion of this, especially as all patients underwent intensive colposcopy screenings afterwards. Did the difference in surgical margins lead to lower rates of subsequent treatment? It seems to imply that this did not make a difference in the follow-up, however, this would make a significant difference in follow-up with the ASCCP guidelines.

Comments on generalizability:
1. Follow-up post-LEEP was cytology, HPV testing q6 months x2 years, which places a large burden of care on the patients (perhaps contributing to the long wait-time for LEEPs in the clinic) and is not in line with current ASCCP guidelines. As above, it would be interesting to note how decreased rate of positive surgical margins would lead to decreased burden of care
2. The 16-week average time from diagnosis to LEEP seems exceptionally long and not generalizable to the US population

Overall, the study is very interesting however the long time to intervention in the control group hampers the broad conclusions of the paper. A reframing of the study more in line with the conclusions would improve the generalizability and readability.

The study, given that both groups received LEEPs, does not support the conclusion that Imiquimod is equivocal to LEEP. Instead, a long time interval between last Imiquimod treatment and confirmatory LEEP procedure would be required in order to state non-inferiority (for example, if last Imiquimod treatment, then perhaps waiting an additional 16 weeks to see if regression persisted).

Reviewer #3:

This is a randomized clinical trial of Imiquimod for treatment of cervical CIN 2/3. Patients were randomized to either treatment with LEEP vs weekly Imiquimod for 12 weeks followed by LEEP. Histologic regression was noted significantly more frequently in the group treated with Imiquimod prior to LEEP.

Materials and Methods:
- Lines 130-132 note that cervical cytology was collected before the first application of imiquimod and before the LEEP. Why was this done? It seems irrelevant. The results and their relevance are never discussed.
- Lines 128 and 133: discussion of types of pregnancy tests and whether they were immediately before or 1 week before do not really add anything significant to the paper.

Table 1:
- 22-25% of patients had a negative HPV test but had CIN 2 or 3. This seems like an oddly high number of negative HPV tests, which could affect the rate of regression. There could be patients who were given an incorrect diagnosis of CIN 2 or CIN 3 that then showed "regression" at LEEP. I would be interested to know the index pap smear of these patients or perhaps review the pap smear and biopsies with the final pathology. In Brazil, are the same guidelines used regarding when patients need colposcopy based on pap and HPV testing?

Table 2:
- According to Williams Gynecology, the natural history of CIN 2 is: 43% will regress, 35% will persist, and 5% will progress to invasive cancer in 2 years. For CIN 3, 32% will regress, 56% will persist, and >12% will progress to invasive
cancer. The numbers in the control group are quite different. However, the study was over a shorter time period, so that could affect the difference in outcomes.

Table 3:
- (refer to comment above from Table 1) Regarding the HPV testing, 83-84% of patients with a negative HPV test showed regression.

Other general comments:
- Given that this trial took place in Brazil, the results might not be generalizable to the US population. Is management of abnormal pap smears the same? Are pathological diagnostic standards the same?
- The paper needs grammatical editing.

STATISTICS EDITOR COMMENTS:

Abstract: Need to conform to our RCT template.

lines 187-190: Need to specify the rates of regression of the two cohorts. Should also specify the difference in terms of what is statistically and clinically meaningful. From the study cited, the rates were 39 % vs 73%, a difference of 34% (95% CI = 8-57%). Should format more exactly the stats hypothesis being tested.

General: Based on the samples, all %s should be rounded to nearest integer %, not cited to 0.1% precision.

Table 1: Since the groups were randomly allocated, there is no need to do stats test to compare the cohorts. Any difference is due to random chance. Should format parity as median (range or IQR), since it can only have integer values.

Table 2: Need to clearly separate the primary from secondary outcomes and state first the ITT, then the PP outcome for the primary. Since CIs are included, the column of p-values is redundant and should be omitted.

Table 3: Should be clearly labelled as secondary outcomes. All the NS findings were not powered in the sample size/power calculation and therefore cannot be generalized from these data.

Table 4: Need to label as secondary outcomes, need to include in footnote the number of variables included in the multivariable model.

Tables 5, 6: Again, these are secondary outcomes, not powered in the original design.

EDITORIAL OFFICE COMMENTS:

1. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:
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2. Obstetrics & Gynecology uses an "electronic Copyright Transfer Agreement" (eCTA). When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.
Please check with your coauthors to confirm that the disclosures listed in their eCTA forms are correctly disclosed on the manuscript's title page.

3. For studies that report on the topic of race or include it as a variable, authors must provide an explanation in the manuscript of who classified individuals’ race, ethnicity, or both, the classifications used, and whether the options were defined by the investigator or the participant. In addition, the reasons that race/ethnicity were assessed in the study also should be described (eg, in the Methods section and/or in table footnotes). Race/ethnicity must have been collected in a formal or validated way. If it was not, it should be omitted. Authors must enumerate all missing data regarding race and ethnicity as in some cases, missing data may comprise a high enough proportion that it compromises statistical precision and bias of analyses by race.

Use "Black" and "White" (capitalized) when used to refer to racial categories. The nonspecific category of "Other" is a convenience grouping/label that should be avoided, unless it was a prespecified formal category in a database or research instrument. If you use "Other" in your study, please add detail to the manuscript to describe which patients were included in that category.

4. Clinical trials submitted to the journal as of July 1, 2018, must include a data sharing statement. The statement should indicate 1) whether individual deidentified participant data (including data dictionaries) will be shared; 2) what data in particular will be shared; 3) whether additional, related documents will be available (eg, study protocol, statistical analysis plan, etc.); 4) when the data will become available and for how long; and 5) by what access criteria data will be shared (including with whom, for what types of analyses, and by what mechanism). Responses to the five bullet points should be provided in a box at the end of the article (after the References section).

5. Tables, figures, and supplemental digital content should be original. The use of borrowed material (eg, lengthy direct quotations, tables, figures, or videos) is discouraged. If the material is essential, written permission of the copyright holder must be obtained.

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6. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women’s Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

7. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by
manuscript type: Original Research reports should not exceed 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, references, tables, boxes, figure legends, and print appendices) but exclude references.

8. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:

* All financial support of the study must be acknowledged.
* Any and all manuscript preparation assistance, including but not limited to topic development, data collection, analysis, writing, or editorial assistance, must be disclosed in the acknowledgments. Such acknowledgments must identify the entities that provided and paid for this assistance, whether directly or indirectly.
* All persons who contributed to the work reported in the manuscript, but not sufficiently to be authors, must be acknowledged. Written permission must be obtained from all individuals named in the acknowledgments, as readers may infer their endorsement of the data and conclusions. Please note that your response in the journal's electronic author form verifies that permission has been obtained from all named persons.
* If all or part of the paper was presented at the Annual Clinical and Scientific Meeting of the American College of Obstetricians and Gynecologists or at any other organizational meeting, that presentation should be noted (include the exact dates and location of the meeting).

9. Provide a short title of no more than 45 characters (40 characters for case reports), including spaces, for use as a running foot.

10. The most common deficiency in revised manuscripts involves the abstract. Be sure there are no inconsistencies between the Abstract and the manuscript, and that the Abstract has a clear conclusion statement based on the results found in the paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limit for Original Research articles is 300 words. Please provide a word count.

11. Abstracts for all randomized, controlled trials should be structured according to the journal's standard format. The Methods section should include the primary outcome and sample size justification. The Results section should begin with the dates of enrollment to the study, a description of demographics, and the primary outcome analysis. Please review the sample abstract that is located online here: http://edmgr.ovid.com/ong/accounts/sampleabstract_RCT.pdf. Please edit your abstract as needed.

12. Only standard abbreviations and acronyms are allowed. A selected list is available online at http://edmgr.ovid.com/ong/accounts/abbreviations.pdf. Abbreviations and acronyms cannot be used in the title or précis. Abbreviations and acronyms must be spelled out the first time they are used in the abstract and again in the body of the manuscript.

13. The journal does not use the virgule symbol (/) in sentences with words. Please rephrase your text to avoid using "and/or," or similar constructions throughout the text. You may retain this symbol if you are using it to express data or a measurement.

14. In your Abstract, manuscript Results sections, and tables, the preferred citation should be in terms of an effect size, such as odds ratio or relative risk or the mean difference of a variable between two groups, expressed with appropriate confidence intervals. When such syntax is used, the P value has only secondary importance and often can be omitted or noted as footnotes in a Table format. Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context than citing P values alone.
If appropriate, please include number needed to treat for benefits (NNTb) or harm (NNTh). When comparing two procedures, please express the outcome of the comparison in U.S. dollar amounts.

Please standardize the presentation of your data throughout the manuscript submission. For P values, do not exceed three decimal places (for example, "P = .001"). For percentages, do not exceed one decimal place (for example, 11.1").

15. Please review the journal’s Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available online here: http://edmgr.ovid.com/ong/accounts/table_checklist.pdf.

16. Please review examples of our current reference style at http://ong.editorialmanager.com (click on the Home button in the Menu bar and then "Reference Formatting Instructions" document under "Files and Resources"). Include the digital object identifier (DOI) with any journal article references and an accessed date with website references. Unpublished data, in-press items, personal communications, letters to the editor, theses, package inserts, submissions, meeting presentations, and abstracts may be included in the text but not in the reference list.

In addition, the American College of Obstetricians and Gynecologists' (ACOG) documents are frequently updated. These documents may be withdrawn and replaced with newer, revised versions. If you cite ACOG documents in your manuscript, be sure the reference you are citing is still current and available. If the reference you are citing has been updated (i.e., replaced by a newer version), please ensure that the new version supports whatever statement you are making in your manuscript and then update your reference list accordingly (exceptions could include manuscripts that address items of historical interest). If the reference you are citing has been withdrawn with no clear replacement, please contact the editorial office for assistance (obgyn@greenjournal.org). In most cases, if an ACOG document has been withdrawn, it should not be referenced in your manuscript (exceptions could include manuscripts that address items of historical interest). All ACOG documents (e.g., Committee Opinions and Practice Bulletins) may be found at the Clinical Guidance page at https://www.acog.org/clinical (click on "Clinical Guidance" at the top).

17. Figure 1: okay
Figure 2: Please provide a high res version of this image and a letter of permission to use in print and electronic formats. Figure 3: Please remove A-C labels, these will be added back per journal style.

When you submit your revision, art saved in a digital format should accompany it. If your figure was created in Microsoft Word, Microsoft Excel, or Microsoft PowerPoint formats, please submit your original source file. Image files should not be copied and pasted into Microsoft Word or Microsoft PowerPoint.

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Figures should be saved as high-resolution TIFF files. The minimum requirements for resolution are 300 dpi for color or black and white photographs, and 600 dpi for images containing a photograph with text labeling or thin lines.

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If you choose to revise your manuscript, please submit your revision through Editorial Manager at http://ong.editorialmanager.com. Your manuscript should be uploaded in a word processing format such as Microsoft Word. Your revision’s cover letter should include the following:

* A confirmation that you have read the Instructions for Authors (http://edmgr.ovid.com/ong/accounts/authors.pdf), and
* A point-by-point response to each of the received comments in this letter. Do not omit your responses to the Editorial Office or Editors’ comments.

If you submit a revision, we will assume that it has been developed in consultation with your co-authors and that each author has given approval to the final form of the revision.

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Jan 29, 2021, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

John O. Schorge, MD
Associate Editor, Gynecology

2019 IMPACT FACTOR: 5.524
2019 IMPACT FACTOR RANKING: 6th out of 82 ob/gyn journals

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/ong/login.asp?a=r). Please contact the publication office if you have any questions.
On behalf of my co-authors, Dr. Ricardo dos Reis and I are pleased to resubmit our revised version of manuscript entitled to submit our manuscript entitled, "Randomized Clinical Trial Evaluating the Efficacy of Topical Treatment with Imiquimod in High-Grade Cervical Intraepithelial Lesions" for your reconsideration as an Original Article in Obstetrics & Gynecology. First of all, we would like to say thank you for your revision and comments. We confirm that the authors read the instructions and we organized a point by point response to each of the received comments.

In this manuscript, a phase II superiority trial, ninety women with cervical HSIL (cervical intraepithelial neoplasia [CIN] 2/3), 49 in the experimental group and 41 in the control group were randomly assigned to 250 mg of 5% imiquimod cream applied to the cervix weekly for 12 weeks followed by loop electrosurgical excision procedure (LEEP) or only LEEP (control group). In the PP population, histological regression was observed in 23 of 38 participants (61%) in the experimental group and 9 of 40 (22%) in the control group (p = 0.001). Surgical margins were negative for HSIL in 36 of 38 participants (95%) in the experimental group and 28 of 40 (70%) in the control group (p = 0.004). In the ITT population, rates of histological regression were also significantly higher in the experimental group. There were no differences in histological regression rates by HPV type or lesion grade. Rates of adverse events (AEs) in the experimental group were 74% (28/38) in the PP population and 78% (35/45) in the ITT population. AEs were mild (grade 1 and 2) and included abdominal pain, low fever, myalgia, fatigue, vaginal discharge, and focal and superficial erosion of the cervix and vagina.

Our manuscript is the first to evaluate the histological regression of high-grade cervical intraepithelial lesions in LEEP histological parts after topical application of the imiquimod immunomodulator directly on the cervix. In addition, new information on the impact of the previous application of imiquimod on the impairment of LEEP margins and the relationship with the histological degree and HPV type of the lesion with the regression rate is available. We look forward to your new comments and criticism of the manuscript.

I declare that this manuscript is not being considered in another journal and will not be submitted to another recant unless a negative final decision is made by
the Editors of Obstetrics and Gynecology. As the lead author, I, Bruno O Fonseca, affirm that this manuscript is an honest, accurate and transparent account of the study being reported, that no importing aspect of the study was omitted and that any discrepancies in the planned protocol of the study were recorded and explained.

This clinical trial was recorded in ClinicalTrials.gov under identification number: NCT03233412 on June 7, 2017, that is, prior to the inclusion of the first patient in the study.

The drug Imiquimod 5% cream (Ixium®) in the form of sachets was provided by Farmoquímica Laboratory S.A., which was not involved in the study design, collection, interpretation or analysis of the data. The authors had full access to all study data and have ultimate responsibility for the decision to submit for publication.

Each author actively participated in the analysis, writing of sections of the manuscript, editing and approval of the final version submitted. All authors gave written permission to quote his name in the manuscript.

This study was approved by the ethics and research council of the Barretos Cancer Hospital on June 22, 2017 under no. 2.133.654. Eligible patients who agreed to participate in the study provided informed consent in writing before inclusion in the study.

Our database was completed by nurse and co-author Naitiele P. Pantano on the RedCap platform and the data accuracy was validated through periodic data qualities.

We would suggest that Figure 3 (Application of the immunomodulator imiquimod in the cervix) is considered as cover art.

We believe this article will be of interest to all members of the Obstetrics & Gynecology community. Please contact me should you have any questions or require additional information.

Thank you for your consideration.

Sincerely,

Bruno O Fonseca
Medical Doctor at Prevention Department
Barretos Cancer Hospital
First author

Ricardo dos Reis
Medical Doctor at Gynecologic Oncology Department
Scientific Director of Research and Educational Institute
Barretos Cancer Hospital
Senior author
Dear Dr. Schorge:

Thank you for your review of our manuscript entitled "Randomized Clinical Trial Evaluating the Efficacy of Topical Treatment with Imiquimod in High-Grade Cervical Intraepithelial Lesions" (ONG-20-3124) for consideration for publication in Obstetrics & Gynecology. We appreciate the reviewers' comments, and we have revised the manuscript based on their recommendations. The comments are addressed below in a point-by-point manner.

Reviewers' comments:

Reviewer #1

Comment 1: Methods. Please specify how HGSIL diagnosis was made in terms of details of how colposcopy and biopsies were performed.

Answer: All patients included in the study participated in our screening program for cervical cancer prevention and had an abnormal Pap smear. They were recruited for colposcopy, which was performed by a gynecologist specializing in colposcopy. Acetic acid (5%) was applied to the cervix, followed by lugol's solution (1%) and colposcopy performed with magnifications of 6X to 40X, and the findings were classified according to the 2011 Colposcopic Terminology of the International Federation for Cervical Pathology and Colposcopy1. Cervical biopsy was performed of any abnormal areas with a biopsy forceps. The sample collected was sent for histopathological evaluation and, in cases of CIN 2 or CIN 3, women were invited to participate in this study at the time of her evaluation in our prevention center at Barretos Cancer Hospital.

This information is included in lines 108-117 in the methods section.

Comment 2: Please describe pathology quality control in diagnosis of HGSIL prior to randomization and during LEEP specimen analysis.

Answer: All pathology slides from biopsy and LEEP were evaluated by two pathologists with specialized training in gynecologic cancers. They sought consensus for discordant cases. Histological diagnosis categories included cervicitis/benign; CIN 1; CIN 2; CIN 3; and invasive lesion. If HSIL could not be graded as CIN 2/3, it was defined as high-grade CIN. In cases of uncertain diagnosis, a complementary immunohistochemical examination for p16 with or without Ki-67 was performed. p16 staining was considered positive when it was strong and diffuse, according to the Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions2. (lines 172-185)
Comment 3: How might your findings play out in pregnant and immunosuppressed?

Answer: There are no available clinical data to endorse the use of imiquimod in pregnant women and/or immunosuppressed patients. Due to the slow evolution of HGSIL to invasive disease\(^3,4\), the best option would be to postpone the clinical or surgical treatment to at least 3 months postpartum. As for the immunosuppressed, it would be contraindicated at first, individualizing each case. We excluded pregnant and immunosuppressed patients of our study protocol. No changes were made to the manuscript.

Comment 4: What can be extrapolated from treatment of anal dysplasia? Men having sex with men? VIN and VAIN?

Answer: Previous studies in individuals with VIN, VAIN and AIN have shown efficacy of imiquimod in treating HPV-induced lesions. In these studies, imiquimod is directly applied to the lesion followed by histological evaluation\(^5-12\). Our study is the first clinical trial to evaluate the histological response after imiquimod by applying it directly to the cervix.

Comment 5: Please briefly address cost

Answer: The application of imiquimod is quickly and easily replicated, and can be performed by a nurse or other trained health professional, not necessarily a doctor, which would reduce costs. In our study, 12 Viba® brushes (Viba-Brush, Rovers Medical Devices, Oss, Netherlands) were required for each patient at a cost of US$1.00 each + 12 imiquimod sachets at a cost of US$4.00 each, totaling US$60.00 per patient in material and medication.

Comment 6: It is interesting that such promising treatment has not done through phase 3 trial yet. Please comment on why. Is this an issue with pharma not getting enough ROI because this drug can't be patented, or some other reason? Why has there not been phase 3 studies yet? Phase 2 Grimm study has been published in 2012. Please comment.

Answer: It is unclear to the authors why a Phase 3 has not yet been performed. In this specific study, we received the donation of medication (Imiquimod) from the laboratory Farmoquímica S.A., but our scientific team has no direct relationship with the pharmaceutical industry. We would like to emphasize that our findings confirm the results of the Grimm et al. study\(^13\) published in this journal in 2012. In addition, our specific results regarding the increase of negative surgical margins in LEEP specimens after treatment with Imiquimod, maybe it could be a good opportunity for a phase 3 study.
Comment 7: Are there different formulations of imiquimod in terms of cream and suppositories?

Answer: We are not aware of different formulations of imiquimod. The only commercialized formulation is imiquimod 5% cream. The suppository formulation is not commercialized, unless manipulated, which is generally restricted for research.

Comment 8: Please comment on your decision to administer imiquimod in clinic by a healthcare professional.

Answer: Our decision to apply imiquimod by a health professional was due to: a) direct application to the lesion on the cervix and avoid the spread of imiquimod cream in the vaginal canal; b) Give participants greater certainty that the medication was being used correctly, as it was being applied by a health professional. Of note, we found a lower number of complications related to imiquimod application compared to Grimm et al. study (74% x 97%) and, as for effectiveness, we reached in a NNT similar of Grimm manuscript (2.63 (PP) / 3.25 (ITT) x 2.9). Also in our study the number of applications were less (12 vs. 16 weeks).

Comment 8-a: How do you think that will be implemented in clinical practice?

Answer: As for the implementation in clinical practice, we understand, from the experience of this study, to be simple, fast and replicable, since the brush used is already sold worldwide and imiquimod has shown excellent adhesion to the cervical mucosa. Also, for the application it is necessary a simple training with the brush and imiquimod cream. It is not necessary to be performed by a doctor, a nurse or other health professional can be trained. Besides that, we have a training video showing the application process (included in supplementary material).

Comment 8-b: Given that medication was not self-administered, why was placebo not used and why was it not blinded? To me, it seems like an opportunity to reduce bias.

Answer: Our institution did not feel it would be ethical to submit one group to placebo treatment, based on previous publication that showed the efficacy of imiquimod treatment in HSIL. Our first objective was to evaluate the regression rate between the group with imiquimod before LEEP surgery compared to patients managed by only LEEP surgery.

Comment 9: Please address possible sources of bias and their effect on your results in your limitations section.

Answer: We believe that the time delay between diagnosis and LEEP procedure in the control group was a limitation. This waiting time for LEEP (10 – 22 weeks)
could affect the regression rate in the control group. We do not believe that this time could cause the progression for invasive cancer in one patient. We would like to emphasize that we have also one progression for invasive cancer in the experimental arm too. We know from the literature that the average time for CIN 3 evolution to invasive lesion ranges from 8 to 12 years. To evaluate if this period between diagnosis and LEEP procedure could affect our results, we decided to analyze if this delay could interfere in lesion regression, lesion persistence or lesion progression. This analysis showed that this time interval was not significant \( p = 0.09 \), showing that the time had no statistical interference in the evolution of cervical lesions in this study. This information is included in the Results section (lines 313-314) and was added to the Discussion (lines 398-401).

**Comment 10:** How was recruitment done? How were patients approached? Clinic? Flyers?

**Answer:** Recruitment was carried out at the clinic when the patient presented for the results of the cervical biopsies. She was invited to participate in this study, knowing that, regardless of her decision, she would undergo standard treatment with LEEP, preceded or not by immunomodulatory treatment with imiquimod. If she agreed to participate, a consent form and subsequent randomization via RedCAP\textsuperscript{14} were applied. Flyers were not used and patients were invited to participate during the normal consultation.

**Comment 11:** Was there an effort to evaluate for patient acceptability of intervention? Why not?

**Answer:** Yes, weekly, prior to the applications, the patients were asked about acceptance and any discomfort related to the treatment. We reminded the patients that they could discontinue the clinical treatment at any time. Except for those 4 participants who interrupted the applications due to systemic adverse effects, all the others reported good treatment acceptability, with strict attendance to all scheduled applications. Everything was recorded in the participant's medical record.

**Comment 12:** End point-why include CIN2 and not CIN3? Can results be analysed for CIN 3 only?

**Answer:** Both CIN 2 and 3 were included and the percentage of CIN 3 in our study was 66% (table 1). Also, we analyzed if the imiquimod efficacy was different between CIN 2 and CIN 3 and we found that the efficacy in terms of regression was the same in both lesion types. We decided to analyze the efficacy of imiquimod only in CIN 3, table below. As we can find, the efficacy only in CIN 3 is statistical significant and the response difference is higher compared to efficacy in CIN 2 plus CIN 3. This table was added as supplementary material.
Table 2.1. Secondary outcome. Histological evolution of CIN 3 biopsies after evaluation of the EZT surgical specimen.

Analysis by intention-to-treat

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control n (%)</th>
<th>Experimental n (%)</th>
<th>Response difference (95% IC)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression*</td>
<td>2 (9.5)</td>
<td>15 (62.5)</td>
<td>53% (30 – 76%)†</td>
<td>0.001†</td>
</tr>
<tr>
<td>Persistence**</td>
<td>18 (85.7)</td>
<td>9 (37.5)</td>
<td>48.2% (23 – 73%)§</td>
<td>0.001‡</td>
</tr>
<tr>
<td>Progression***</td>
<td>1 (4.8)</td>
<td>0 (0.0)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Total</td>
<td>21 (100.0)</td>
<td>24 (100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis by per-protocol

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control n (%)</th>
<th>Experimental n (%)</th>
<th>Response difference (95% IC)</th>
<th>P</th>
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<tr>
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<td>Total</td>
<td>21 (100.0)</td>
<td>24 (100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n (%): absolute number (percentage)
95% IC: 95% confidence interval
The value of ‘p’ was calculated to compare the control and experimental groups.
* Regression: Grade 1 cervical intraepithelial injury or complete remission.
** Persistence: Grade 2 or 3 cervical intraepithelial lesion
*** Progression: Invasive cervical carcinoma
† Calculated by the % regression of the experimental group subtracted from the % of the control group.
‡ Pearson’s chi-squared test
§ Calculated by the % persistence of the control group subtracted from the% of the experimental group.

Comment 13: What was the rationale behind using CIN 1-3 terminology vs. LAST terminology? Or was it always dual?

Answer: Although the LAST terminology is the most recent classification, we decided to use the CIN 1-3 terminology as it continues to be the most commonly used in cervical dysplasia.

Comment 14: Sample size calculation was not adjusted for cross over from imiquimod to LEEP group.

Answer: The sample size calculation was adjusted for cross over from imiquimod to LEEP group. This information was added at statistical analysis in the methods section (Lines 197-204).
Comment 15: Wait times to get LEEPs since diagnosis. To make sure I understood it correctly, once a patient was randomized, they either went into 12 week imiquimod group or to LEEP wait list. Control group waited 4 months to get a LEEP, and imiquimod group waited 16 more weeks in addition to that. That seems like a reasonable difference, although randomizing into placebo vs. intervention would have addressed this time lag. Please address

Answer: The patients, after being randomized, had their LEEP scheduled immediately, and those randomized to the control group were scheduled in the first available slot on the agenda (ranging between 10 and 16 weeks after diagnosis) and those in the experimental group were scheduled in a minimum interval of 12 weeks after diagnosis, so that it was possible to carry out the immunomodulator applications.

As we described in the methods part (Lines 131-133), the Barretos Cancer Hospital cares for a high volume of public patients from all regions of Brazil; therefore the wait time for LEEP is usually around 2 to 3 months.

As we included in the response to comment 9, we believe that the time (delay) between diagnosis and LEEP procedure in the control group was a limitation of our study. We do not believe that this time could affect lesion progression. We know from the literature that the average time for CIN 3 evolution to invasive lesion ranging from 8 to 12 years. To evaluate if this period between diagnosis and LEEP procedure could affect our results, we decided to analyze if this delay could interfere in lesion regression, lesion persistence or lesion progression. This analysis showed that this time interval was not significant (p = 0.09), showing that the delay had no statistical interference in the evolution of cervical lesions in this study. This information is included in lines 313-314 at results part and we added at the discussion part, as limitation (lines 398-401).

As stated in our answer to comment 8-b, our institution did not feel it would be ethical to submit one group to placebo treatment, based on previous publications that showed the efficacy of imiquimod treatment in HSIL. Our first objective was to evaluate the regression rate between the group with imiquimod before LEEP surgery compared to patients managed by only LEEP surgery.

Comment 16: What could be potential recurrence risk after imiquimod vs. LEEP? Do you plan to follow these patients?

Answer: Yes, we are performing follow-up for these women for 2 years, performing colposcopy, cytology and COBAS HPV testing every six months (when HPV positive in the previous evaluation). We will have this information from all women soon, however, with the data collected so far, the percentage of recurrence has been the same among the patients with CIN 2 and CIN 3 at LEEP specimen, in the control and experimental groups. However, as in the experimental group the most of the LEEP specimens showed histological regression, the recurrence rate in this group has been exceptionally lower.
compared to control group. As soon as we complete this follow-up, we will forward it for publication.

Comment 17: LEEP is a great endpoint

Answer: Thank you for your comment.

Comment 18: Was there any differences in demographics between included and excluded patients?

Answer: Only one participant in the control group (40/41) and four participants in the experimental group (45/49) were excluded from the ITT demographic analysis, which did not generate a statistically significant difference in the demographic analysis.

Comment 19: Lines 156-157. Why was surveillance after LEEP was pap and colpo every 6 months?

Answer: The standard follow-up after LEEP in Brazil is every 6 months with colposcopy, cytology and COBAS HPV testing. This approach is performed because we would like to evaluate, with great accuracy, the recurrence rate and HPV clearance among the groups studied. These data were not mentioned because we have not yet completed the proposed follow-up of 2 years after LEEP in all patients. As soon as we have the complete follow-up we will forward it for publication.

Comment 20: Were patients incentivized in any way to participate? It seems like since everyone ended up with LEEP, patients had not reason to agree to be in this study since it delayed their treatment and had potential side effects. From what I understood, since wait time for LEEP is so long (4-5 months), patients opted to enroll as that in their minds at least meant they will be getting some sort of treatment.

Answer: There was no incentive for patients to participate, except for the fact that they contributed to the research and the enthusiasm for the use of medication with innovative potential. The idea of receiving at least some treatment prior to LEEP was not evident to us, as patients did not know the date of LEEP prior to randomization. We would like to emphasize that all patients signed a consent form before study enrollment. We explained to all patients, before entering in the study, that it was a study evaluating a new drug for CIN 2 and CIN 3 treatment but we emphasize that the standard treatment would not be changed according to group allocation.
Comment 21: Midway colposcopy as a safety measure in imiquimod arm is helpful

Answer: Thank you for your comment. The midway colposcopy was very important because our intention with this approach was to detect lesion progression. Another important point is that there are persistent lesions identified in LEEP specimens that were not identified at colposcopy

a) Lesion regression at colposcopy: 84.4% (ITT).

b) Lesion regression at LEEP specimen: 53.3% (ITT).

We created a new table below showing the evolution of colposcopy findings according to the imiquimod treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Initial colposcopy</th>
<th>Intermediate colposcopy</th>
<th>Pré-LEEP colposcopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Normal findings(^1)  (ITT/PP)</td>
<td>1 (2.2)/0</td>
<td>11(27.5)/ 11(28.9)</td>
<td>29 (64.4)/ 27 (71.0)</td>
</tr>
<tr>
<td>Findings grade 1 (minor)(^*) (ITT/PP)</td>
<td>17 (37.8)/ 14 (36.8)</td>
<td>19 (47.5)/ 18 (47.4)</td>
<td>9 (20.0)/ 7 (18.4)</td>
</tr>
<tr>
<td>Findings grade 2 (major)(^*) (ITT/PP)</td>
<td>27 (60.0)/ 24 (63.2)</td>
<td>9 (22.5)/ 9 (23.7)</td>
<td>7 (15.6)/ 4 (10.6)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (100.0)/ 38 (100.0)</td>
<td>39 (100.0) / 38(100.0)</td>
<td>45 (100.0)/ 38 (100.0)</td>
</tr>
</tbody>
</table>

ITT: Intention to treat analysis
PP: Analysis Per Protocol
* In the ITT analysis, 6 participants did not complete the 6th week of imiquimod application and therefore did not undergo intermediate colposcopy.
Data were shown in ‘n’ (percentage of regression)
\(^1\)According to the 2011 Colposcopic Terminology of the Internacional Federation for Cervical Pathology and Colposcopy

Comment 22: Who were the pathologists who confirmed biopsy results? Can you provide more information about colposcopy and how it was done which lead to enrollment?

Answer: The pathologists for the study were staff pathologists from BCH with expertise in cervical dysplasia and cancer. Please see response to Comment #1 which describes how the colposcopy was performed. This information is included in lines 108-117 in methods.
**Comment 23:** Figure 1. Not able to follow numbers in ITT vs. PP. 8 women were excluded due to loss to follow up and discontinuation, but there was only difference of 7 between ITT (45) and PP (28).

*Answer:* As described in lines 254-256 “In the experimental group, after treatment allocation, one patient was excluded from PP and ITT analysis because she became pregnant during treatment and she had not yet undergone LEEP.”

This patient was *not* included in the ITT and PP analysis because it was not possible to evaluate the imiquimod response since we did not have the LEEP specimen. For this reason, there were 45 participants in the ITT analysis and 38 in the PP analysis.

*We think that it was important to include this observation at the bottom of figure 1.*

**Comment 24:** Margin positivity (lines 300-305). How was this defined? CIN1-3, or CIN 2-3 only? It should only be HGSIL to be clinically meaningful.

*Answer:* The margin was considered positive when there was CIN2 and/or CIN3 present in any margins of the LEEP specimen.

**Comment 25:** What was the compliance with weekly visits in imiquimod group? How many patients completed all visits, etc.?

*Answer:* In general, the participants attended the appointments accurately, and of the 38 participants analyzed per protocol, 26 (68.4%) attended the scheduled applications without any delay, 9 (23.7%), did not comply with the schedule on only one application, therefore they were rescheduled. Two patients (5.3%) did not comply with the schedule on 2 applications and 1 (2.6%) did not comply with the schedule applications for 6 visits, and all of them were rescheduled. Although there were delays in applications in some patients, all of them received all 12 applications.

**Comment 26:** In discussion, please comment on what next steps would be in studying this next.

*Answer:* We have a new project in progress where we will measure immunological biomarkers during imiquimod applications, which will help us understand the immune response plateau and consequent dose adjustment in high-grade cervical intraepithelial lesions.
Reviewer #2:

Comments on methodology

Comment 1: Why can the clinic accommodate weekly imiquimod applications for patients but cannot accommodate an outpatient LEEP procedure for patients with HSIL until 2-3 months out? This needs to be addressed more fully, as this is a significant weakness that is not generalizable even to the busiest US hospitals.

Answer: We believe that the time (delay) between diagnosis and LEEP procedure in the control group was a limitation. This waiting time for LEEP (10 – 22 weeks) could affect the regression rate in the control group. We do not believe that this time could cause the progression for invasive cancer in one patient. We would like to emphasize that we have also one progression for invasive cancer in the experimental arm too. We know from the literature that the time average for CIN3 to invasive lesion ranging from 8 to 12 years3 4. To evaluate if this period between diagnosis and LEEP procedure could affect our results, we decided to analyze if this delay could interfere in lesion regression, lesion persistence or lesion progression. This analysis showed that this time interval was not significant (p = 0.09), showing that the time had no statistical interference in the evolution of cervical lesions in this study. This information is included in line 313-314 at results part and we added the information below at the discussion part, as limitation (lines 398-401).

Comment 2: Why were the patients followed with colposcopies q6 months after LEEP? This is not in line with ASCCP guidelines and there is no mention of the results afterwards.

Answer: The follow-up schedule is based on the Brazilian guidelines. Please see response to Reviewer #1, Comment #19. This information is included in line 168-170 at methods.

Comment 3: Why were LEEPs given to the imiquimod group at 12-week conclusion if the evaluation is a superiority trial and not with a longer time interval to assess for continued regression?

Answer: Thank you for your question. The response of imiquimod cream 5% starts 1-4h after its topical application15, with a half-life of 30 hours16, therefore 3 days after the last dose we have already reached in the maximum local stimulus. However, the time of permanence of the immune response after stimulus is unknown and in this study we did not have conditions to evaluate this aspect. We have a new project in progress where we will measure immunological biomarkers during imiquimod applications, which will help us in the evaluation of the immune response plateau and consequent dose adjustment in high-grade cervical intraepithelial lesions.
Comments on writing style:

Comment 1: The writing style is very clear, concise, and understandable.

Answer: Thank you for your comment.

Comments on overall strengths:

Comment 1: The study design has the strengths of randomization and an intention-to-treat analysis, which is crucial given the large number of experimental participants who did not complete the whole treatment. It also was able to compare LEEP samples between control/experimental groups, which is a benefit to confirm that regression occurred (compared to colposcopy-only evaluation).

Answer: Thank you for your comment.

Comments on weaknesses:

Comment 1: Authors assumed a 20% rate of loss-of-follow up. What was the loss of follow-up for straight to LEEP? Can you say this is a non-inferior treatment if you are assuming such a high drop-out rate?

Answer: Thank you for your comment. We assumed this high dropout rate because our protocol included patients who live up to 300 km away from the hospital and who would have to show up weekly for imiquimod. In addition, these patients have few resources and often do not have their own vehicle, requiring a public transport system. However, we observed that, of the patients residing in the city of Barretos-SP (where our hospital is located), there was no abandonment due to transportation. Ideally, treatment should preferably be carried out in the patient's city, which is readily possible. The brush used in the application (Viba-Brush, Rovers Medical Devices, Oss, Netherlands) is sold worldwide, the imiquimod cream adheres very well to the cervix and the application has high replicability, and it can be performed by a non-health professional doctor.

Comment 2: Line 203-204 states that 60% of patients treated with imiquimod had histological regression of HSIL by colposcopy - without any mention of the LEEP sample. This line would benefit from the addition of confirmation that regression did not occur, as confirmed by LEEP sample.

Answer: Thank you for your comment. We apologize for the mistake. The text was adjusted and we made it clear that the regression rate seen in the interim analysis was evidenced in the LEEP specimens.

Now in lines 216-219.
Comment 3: 7 patients were excluded from the experimental group because of systemic side effects (4 patients) and transportation issues (3 patients), which means that 15% of people dropped out due to side effects. Can you say that it is as safe and effective than LEEP if 15% of candidates could not complete the treatment due to transportation and/or side effects? This seems like a significant number that would hamper the implementation of completion of treatment and generalizability.

Answer: Thank you for your comment and question. We would like to emphasize that the treatment with imiquimod should preferably be carried out in the patient's city of residence. There was no abandonment related to the transport of any participant residing in the city of Barretos (where our hospital is located). As for dropouts due to systemic side effects, four women (8.7%) dropped out of a total of 46 participants in the experimental group (45 participants analyzed by ITT plus one patient who became pregnant), none of them had serious side effects.

Comment 4: The analysis of per-protocol seems to be a difficult generalizable study. Focusing on the intention-to-treat lends more credibility to the study. However, the intention-to-treat broken down by HPV subtypes show that actually, the only significant difference is in patients with HPV-16 showing regression.

Answer: Thank you for your comment. When stratifying by type of HPV, due to the low sample for each specific type of HPV, the number of regressions does not reach statistical significance, although it is numerically greater. To obtain proven statistical significance for all types of HPV, we would need another study with a specific sample calculation. Therefore, it was not possible to say whether it would be effective on a specific type of HPV, except for the HPV 16 in the intention to treat population analyzed, when the sample was larger.

Comment 5: The mean interval between diagnosis of HSIL and LEEP in the control was 16 weeks - four months. This is, by American standards, a very long interval. This is a flawed design, as the comparison is now the control groups = no treatment for 16 weeks (versus immediate surgical intervention) versus imiquimod treatment for 3 months followed by LEEP.

Answer: Thank you for your comment. You are correct about the interval between HSIL diagnosis and LEEP but we would like to emphasize that our institution (Barretos Cancer Hospital) is a reference oncology hospital that attends public oncology patients and cares for a high volume of patients from all regions of Brazil; therefore, the wait time for LEEP was usually around 2 to 3 months. We are currently adapting the hospital structure that allows LEEP with an average wait time of 2 weeks. Patients in the control group underwent LEEP as soon as possible. For this reason, according to your suggestion we prefer to change the name of control group for “Wait for LEEP” instead LEEP only. We change it at line 127.
We believe that the time (delay) between diagnosis and LEEP procedure in the control group was a limitation. This waiting time for LEEP (10 – 22 weeks) could affect the regression rate in the control group. We do not believe that this time could cause the progression for invasive cancer in one patient. We would like to emphasize that we have also one progression for invasive cancer in the experimental arm too. We know from the literature that the time average for CIN3 evolution to an invasive lesion ranging from 8 to 12 years. To evaluate if this period between diagnosis and LEEP procedure could affect our results, we decided to analyze if this delay could interfere in lesion regression, lesion persistence or lesion progression. This analysis showed that this time interval was not significant (p = 0.09), showing that the time had no statistical interference in the evolution of cervical lesions in this study. This information is included in line 313-314 at results part and we added the information below at the discussion part, as limitation (lines 398-401).

Comment 6: The conclusion was that topical imiquimod may be a safe and effective treatment for HSIL. I can not agree with this statement due to multiple factors outlined below. A better conclusion would be that when treatment will be delayed, or when patient highly desires deferral of LEEP, or with HPV16+ or large lesions, imiquimod may lead to regression of lesions with subsequent increased negative margins.

Answer: Thank you for your comment. Taking into account their positioning, we agree to modify the conclusion to “... when necessary to postpone LEEP or when the patient highly desires deferral the procedure, weekly topical treatment with imiquimod for 12 weeks is effective in HSIL regression, especially in those with HPV 16, where it shows statistical significance in histological regression. Besides that, there is a possibility to use in large lesions, where the rate of free surgical margins can increase.” (line 338-342 at discussion and line 59-62 at abstract).

a. The control group was deferral of intervention/treatment for 4 months, when we know that no interventions will lead to progression of cervical dysplasia with time.

Answer: Thank you for your comment. This waiting time for LEEP (10 – 22 weeks) could affect the regression rate in the control group. We do not believe that this time could cause the progression for invasive cancer in one patient. We would like to emphasize that we have also one progression for invasive cancer in the experimental arm too. We know from the literature that the time average for CIN3 evolution to an invasive lesion ranging from 8 to 12 years. To evaluate if this period between diagnosis and LEEP procedure could affect our results, we decided to analyze if this delay could interfere in lesion regression, lesion persistence or lesion progression. This analysis showed that this time interval was not significant (p = 0.09), showing that the time had no statistical interference in the evolution of cervical lesions in this study. This information is included in line 313-314 at results part and we added the information below at the discussion part, as limitation (lines 398-401). We are currently adapting the hospital structure that allows LEEP with an average wait of 2 weeks.
b. Treatment with imiquimod led to a 15% drop-out rate due to side effects and burden of treatment (multiple weekly clinic visits) versus a one-time clinic visit for LEEP.

Answer: Thank you for your comment. We would like to emphasize that the treatment with imiquimod should preferably be carried out in the patient's city of residence. There was no abandoning related to the transport of any participant residing in the city of Barretos (where our hospital is located). As for dropouts due to systemic side effects, four women (8.7%) dropped out of a total of 46 participants in the experimental group (45 participants analyzed by ITT plus one patient who became pregnant), none of them had serious side effects.

c. When broken down by HPV subtype, the only significant regression occurred with HPV-16 lesions.

Answer: Thank you for your comment. As we answered above, when stratifying by type of HPV, due to the low sample for each specific type of HPV, the number of regressions does not reach statistical significance, although it is numerically greater. To obtain proven statistical significance for all types of HPV, we would need another study with a specific sample calculation. Therefore, it was not possible to say whether it would be effective on a specific type of HPV, except for the HPV 16 in the intention to treat population analyzed, when the sample was larger.

Comment 7: The note that surgical margins was more likely to be negative in the imiquimod-treatment group was a very interesting note and one of the stronger points of the paper. I would love to see more discussion of this, especially as all patients underwent intensive colposcopy screenings afterwards. Did the difference in surgical margins lead to lower rates of subsequent treatment? It seems to imply that this did not make a difference in the follow-up, however, this would make a significant difference in follow-up with the ASCCP guidelines.

Answer: Thank you for your comment and question. Glad you found it interesting, we also consider this data very important, due to its originality.

All patients are followed up every six months and the vast majority of them have been followed up for at least one year. In our preliminary analysis, we observed less recurrence of HSIL in patients in the experimental group compared to those in the control group probably due to more negative margins and smaller lesions in the imiquimod group. We are collecting this data and expect to publish it in about one year.
Comments on generalizability:

Comment 1: Follow-up post-leep was cytology, HPV testing q6 months x2 years, which places a large burden of care on the patients (perhaps contributing to the long wait-time for LEEPs in the clinic) and is not in line with current ASCCP guidelines. As above, it would be interesting to note how decreased rate of positive surgical margins would lead to decreased burden of care.

Answer: Thank you for your comment. Yes, we have established this frequency of follow-up because we would like to have a highly accurate analysis of the outcomes in both groups. We also expect these results from the follow-up in the future. If we conclude a significant decrease in the rate of recurrence in the imiquimod group, maybe the imiquimod could be an option of treatment instead excisional treatment. Maybe the frequency of appointments after LEEP could reduced. We are collecting this data and expect to publish it in about 1 year.

Comment 2: The 16-week average time from diagnosis to LEEP seems exceptionally long and not generalizable to the US population.

Answer: Thank you for your comment. You are correct about the long interval between HSIL diagnosis and LEEP and we agree that it is not generalizable for all women. We would like to emphasize that our institution (Barretos Cancer Hospital) is a reference oncology hospital that attends public oncology patients and cares for a high volume of patients from all regions of Brazil; therefore, the wait time for LEEP was usually around 2 to 3 months. We are currently adapting the hospital structure that allows LEEP with an average wait of 2 weeks.

We believe that the time (delay) between diagnosis and LEEP procedure in the control group was a limitation. This waiting time for LEEP (10 – 22 weeks) could affect the regression rate in the control group. We do not believe that this time could cause the progression for invasive cancer in one patient. We would like to emphasize that we have also one progression for invasive cancer in the experimental arm too. We know from the literature that the time average for CIN3 evolution to an invasive lesion ranging from 8 to 12 years\(^3,4\). To evaluate if this period between diagnosis and LEEP procedure could affect our results, we decided to analyze if this delay could interfere in lesion regression, lesion persistence or lesion progression. This analysis showed that this time interval was not significant (\(p = 0.09\)), showing that the time had no statistical interference in the evolution of cervical lesions in this study. This information is included in line 313-314 at results part and we added the information below at the discussion part, as limitation (line 398-401).

Overall, the study is very interesting however the long time to intervention in the control group hampers the broad conclusions of the paper. A reframing of the study more in line with the conclusions would improve the generalizability and readability.

The study, given that both groups received LEEPs, does not support the conclusion that Imiquimod is equivocal to LEEP. Instead, a long time interval
between last Imiquimod treatment and confirmatory LEEP procedure would be required in order to state non-inferiority (for example, if last Imiquimod treatment, then perhaps waiting an additional 16 weeks to see if regression persisted).

Answer: Thank you for your comment. As we discussed and answered in the comments above, we agree that the delay between diagnosis and LEEP is a study limitation. Therefore, we made this question very clear in the text (lines 398-401) and we modified the conclusion of the study to: "... In this study, we found when necessary to postpone LEEP or when the patient highly desires deferral the procedure, weekly topical treatment with imiquimod for 12 weeks is effective in HSIL regression, especially in those with HPV 16, where it shows statistical significance in histological regression. Besides that, there is a possibility to use in large lesions, where the rate of free surgical margins can increase. (line 338-342 at discussion and line 59-62 at abstract). Besides that we changed the name of the control group for “Wait for LEEP”.

Reviewer #3

Materials and Methods:

Comment 1: Lines 130-132 note that cervical cytology was collected before the first application of imiquimod and before the LEEP. Why was this done? It seems irrelevant. The results and their relevance are never discussed.

Answer: Thank you for your comment and question. We chose to collect cervical cytology immediately before any of the treatments, as we knew that the control group would wait for LEEP without any treatment and we would liked to assess whether there would be a difference between the pap smear done just before LEEP and the previous cytology diagnosis. We observed that there was no difference. After your comment, we chose to remove this information from the manuscript, which really turned out to be irrelevant.

Comment 2: Lines 128 and 133: discussion of types of pregnancy tests and whether they were immediately before or 1 week before do not really add anything significant to the paper.

Answer: Thank you for your comment. We agree with your comment and remove the types of pregnancy tests used from the manuscript. We changed in lines 141-142. We included only this part: “Patients in both groups had a blood pregnancy test performed 1 week before LEEP”.

Table 1:

Comment 1: - 22-25% of patients had a negative HPV test but had CIN 2 or 3. This seems like an oddly high number of negative HPV tests, which could affect the rate of regression. There could be patients who were given an incorrect diagnosis of CIN 2 or CIN 3 that then showed "regression" at LEEP. I would be interested to know the index pap smear of these patients or perhaps
review the pap smear and biopsies with the final pathology. In Brazil, are the same guidelines used regarding when patients need colposcopy based on pap and HPV testing?

Answer: Thank you for your comment and question. The Brazilian cervical cancer screening program is based only on the Pap smear. Our group of patients underwent the COBAS HPV test only on the date of entry into the study, that is, 3 months after the collection of cytology in the screening program and 1 month after the initial colposcopy and confirmatory HSIL biopsy. This time between the diagnosis of HSIL and the test for HRHPV may justify the high rate of negative tests. This justification was added to the article. (lines 136-142). All Pap smears and biopsies used for the diagnosis were reviewed (lines 247-253) and those patients where HSIL was not confirmed, they were removed from the analysis (Figure 1). In Brazil we used the same guidelines as in worldwide, the 2011 Colposcopic Terminology of the International Federation for Cervical Pathology and Colposcopy1.

We are sending the photos of the slides of the pap smears and cervical biopsy (attached via editorial manager) of all patients with negative HRHPV of the two groups evaluated.

<table>
<thead>
<tr>
<th>ID</th>
<th>HPVAR</th>
<th>Pap smear</th>
<th>Initial biopsy</th>
</tr>
</thead>
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<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>negative</td>
<td>ASC-H</td>
<td>CIN 2</td>
</tr>
<tr>
<td>9</td>
<td>negative</td>
<td>ASC-H</td>
<td>High grade CIN</td>
</tr>
<tr>
<td>11</td>
<td>negative</td>
<td>ASC-H</td>
<td>High grade CIN</td>
</tr>
<tr>
<td>35</td>
<td>negative</td>
<td>ASC-H</td>
<td>CIN 3</td>
</tr>
<tr>
<td>53</td>
<td>negative</td>
<td>ASC-H</td>
<td>High grade CIN</td>
</tr>
<tr>
<td>61</td>
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<td>ASC-H</td>
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</tr>
<tr>
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<td>ASC-H</td>
<td>High grade CIN</td>
</tr>
<tr>
<td>86</td>
<td>negative</td>
<td>ASC-H</td>
<td>High grade CIN</td>
</tr>
<tr>
<td>87</td>
<td>negative</td>
<td>ASC-H</td>
<td>CIN 2</td>
</tr>
<tr>
<td><strong>Experimental group</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>negative</td>
<td>ASC-H</td>
<td>CIN 3</td>
</tr>
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</tr>
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<td>CIN 2</td>
</tr>
<tr>
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<td>CIN 2</td>
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</tr>
<tr>
<td>89</td>
<td>negative</td>
<td>ASC-H</td>
<td>CIN 2</td>
</tr>
</tbody>
</table>
Comment 1: According to Williams Gynecology, the natural history of CIN 2 is: 43% will regress, 35% will persist, and 5% will progress to invasive cancer in 2 years. For CIN 3, 32% will regress, 56% will persist, and >12% will progress to invasive cancer. The numbers in the control group are quite different. However, the study was over a shorter time period, so that could affect the difference in outcomes.

Answer: Thank you for your comment. Yes, in our study the patients were followed-up after diagnosis and before LEEP, only 10 to 22 weeks, so we believe that it justifies the difference in the numbers.

Below is a supplementary table with detailed data for CIN-2 and CIN-3.

In the table below, the histological regression in 3-5 months in the group treated with imiquimod is higher than expected in 2 years without treatment, as it showed in Williams Gynecology. The rate of regression is similar between CIN 2 and CIN 3 when treated with the immunomodulator, in both ITT and PP analyses.

### Table 2.2 (supplementary). Secondary outcome. Histological evolution after evaluation of the EZT surgical specimen.

<table>
<thead>
<tr>
<th>Variável</th>
<th>Control group n(%)</th>
<th>Experimental group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIN 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression*</td>
<td>2/8(25)</td>
<td>7/12(58.3)</td>
</tr>
<tr>
<td>Persistence**</td>
<td>6/8(75)</td>
<td>5/12(41.7)</td>
</tr>
<tr>
<td>Progression***</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>CIN 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression*</td>
<td>2/21(9.5)</td>
<td>16/30(53.3)</td>
</tr>
<tr>
<td>Persistence**</td>
<td>18/21(85.7)</td>
<td>13/30(43.3)</td>
</tr>
<tr>
<td>Progression***</td>
<td>1/21(4.8)</td>
<td>1/30(3.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variável</th>
<th>Control group n(%)</th>
<th>Experimental group n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIN 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression*</td>
<td>2/8(25)</td>
<td>7/11(63.6)</td>
</tr>
<tr>
<td>Persistence**</td>
<td>6/8(75)</td>
<td>4/11(36.4)</td>
</tr>
<tr>
<td>Progression***</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>CIN 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression*</td>
<td>2/21(9.5)</td>
<td>15/24(62.5)</td>
</tr>
<tr>
<td>Persistence**</td>
<td>18/21(85.7)</td>
<td>9/24(37.5)</td>
</tr>
<tr>
<td>Progression***</td>
<td>1/21(4.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

n (%): absolute number (percentage)

* Regression: Grade 1 cervical intraepithelial injury or complete remission.

** Persistence: Grade 2 or 3 cervical intraepithelial lesion

*** Progression: Invasive cervical carcinoma
Table 3:

Comment 1: (refer to comment above from Table 1) Regarding the HPV testing, 83-84% of patients with a negative HPV test showed regression

Answer: Thank you for your comment. According to the comment on table 1.

Other general comments:

Comment 1: Given that this trial took place in Brazil, the results might not be generalizable to the US population. Is management of abnormal pap smears the same? Are pathological diagnostic standards the same?

Answer: Thank you for your comment and question. In our opinion, we believe that our findings might be generalizable to the US population because the entire protocol (imiquimod application, HPV test, cytology, colposcopy and LEEP) can be applied in this population. Besides that, we have a movie showing the imiquimod application process and we attached the movie as accessory material. Screening in Brazil is based exclusively on pap smear. It starts at the age of 25 with an annual frequency that becomes triennial after 2 consecutive negative exams. The findings of ASC-H, HSIL and AGC are indications of colposcopy. The protocol for LSIL is to repeat the cytology in 6 months when the patient is over 30 years old and in 1 year when the age is less than 30 years. In the ASC-US cytological result, cytology is repeated in 3 years if the age is below 25 years, in 1 year if the age is between 25-30 years and in 6 months if the age is over 30 years. The HPV test is not carried out in the country's protocols, so it is used only in research protocols.

Are the patterns of pathological diagnosis the same? The standards used in the diagnosis are the same. They follow the international guidelines for associated HPV squamous intraepithelial lesions, described in LAST² and reiterated by Female Genital Tumors: WHO Classification of Tumors¹⁷ and maintained in the latest WHO edition published in August 2020.

Comment 2: The paper needs grammatical editing.

Answer: Thank you for your comment. We made grammatical corrections as requested.

Statistics Editor Comments:

Comment 1: Need to conform to our RCT template.

Answer: Thank you for your comment. Ok, the summary was adequate according to the template.
Comment 2: lines 187-190: Need to specify the rates of regression of the two cohorts. Should also specify the difference in terms of what is statistically and clinically meaningful. From the study cited, the rates were 39 % vs 73%, a difference of 34% (95% CI = 8-57%). Should format more exactly the stats hypothesis being tested.

Answer: Thank you for your comments. The text has been modified and supplemented (lines 197-204).

Comment 3: General: Based on the samples, all %s should be rounded to nearest integer %, not cited to 0.1% precision.

Answer: Thank you for your comment. It has been modified and corrected.

Comment 4: Table 1: Since the groups were randomly allocated, there is no need to do stats test to compare the cohorts. Any difference is due to random chance. Should format parity as median (range or IQR), since it can only have integer values.

Answer: Thank you for your comments. The table was modified as suggested. We opted to keep the p value if you do not mind because it shows that the randomization process was correct.

Comment 5: Table 2: Need to clearly separate the primary from secondary outcomes and state first the ITT, then the PP outcome for the primary. Since CIs are included, the column of p-values is redundant and should be omitted.

Answer: Thank you for your comment and correction. The table was modified as suggested. Now the table is clearer.

Comment 6: Table 3: Should be clearly labelled as secondary outcomes. All the NS findings were not powered in the sample size/power calculation and therefore cannot be generalized from these data.

Answer: Thank you for your comment. The table was modified as suggested. We decided to specify the group of treatment as primary outcome and the other variables as secondary outcomes.

Comment 7: Table 4: Need to label as secondary outcomes, need to include in footnote the number of variables included in the multivariable model.

Answer: Thank you for your comment. The table was modified as suggested.
Comment 8: Tables 5, 6: Again, these are secondary outcomes, not powered in the original design

Answer: Thank you for your comment. The table was modified as suggested. We agree that the secondary outcomes were not powered in the original design, we calculated our sample size based on primary outcome, CIN 2 and CIN 3 regression with imiquimod. We believe that we can keep this analysis of regression based on HPV type, surgical margins status, type of positive margins and initial histology (CIN 2 and CIN 3) because we specify that these variables are secondary outcomes. Besides that, we believe that these results bring important information for the reader. We would like to say thank you for the suggestion to mention that these variables are secondary outcomes.

Editorial Office Comments:

1. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:
   A. OPT-IN: Yes, please publish my point-by-point response letter.
   B. OPT-OUT: No, please do not publish my point-by-point response letter.

Answer: A. OPT-IN: Yes, please publish our point-by-point response letter.

2. Obstetrics & Gynecology uses an "electronic Copyright Transfer Agreement" (eCTA). When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.

Please check with your coauthors to confirm that the disclosures listed in their eCTA forms are correctly disclosed on the manuscript's title page.

Answer: We have confirmed that the authors have no conflicts of interest to disclose.

3. For studies that report on the topic of race or include it as a variable, authors must provide an explanation in the manuscript of who classified individuals' race, ethnicity, or both, the classifications used, and whether the options were defined by the investigator or the participant. In addition, the reasons that race/ethnicity were assessed in the study also should be described (eg, in the Methods section and/or in table footnotes). Race/ethnicity must have been collected in a formal or validated way. If it was not, it should be omitted. Authors must enumerate all missing data regarding race and ethnicity as in some cases, missing data may
comprise a high enough proportion that it compromises statistical precision and bias of analyses by race.

Use "Black" and "White" (capitalized) when used to refer to racial categories. The nonspecific category of "Other" is a convenience grouping/label that should be avoided, unless it was a prespecified formal category in a database or research instrument. If you use "Other" in your study, please add detail to the manuscript to describe which patients were included in that category.

Answer: race was not included as a variable

4. Clinical trials submitted to the journal as of July 1, 2018, must include a data sharing statement. The statement should indicate 1) whether individual deidentified participant data (including data dictionaries) will be shared; 2) what data in particular will be shared; 3) whether additional, related documents will be available (eg, study protocol, statistical analysis plan, etc.); 4) when the data will become available and for how long; and 5) by what access criteria data will be shared (including with whom, for what types of analyses, and by what mechanism). Responses to the five bullet points should be provided in a box at the end of the article (after the References section).

Answer: This table was included

5. Tables, figures, and supplemental digital content should be original. The use of borrowed material (eg, lengthy direct quotations, tables, figures, or videos) is discouraged. If the material is essential, written permission of the copyright holder must be obtained.

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When you submit your revised manuscript, please upload 1) the permissions license and 2) a copy of the original source from which the material was reprinted, adapted, or modified (e.g., scan of book page(s), PDF of journal article, etc.).

If the figure or table you want to reprint can be easily found on the internet from a reputable source, we recommend providing a link to the source in your text instead of trying to reprint it in your manuscript.

Answer: all tables, figures, and supplemental digital content are original.

6. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women’s Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-obstetrics-data-definitions and the gynecology data definitions at https://www.acog.org/practice-management/health-it-and-clinical-informatics/revitalize-gynecology-data-definitions. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.
Answer: N/A

7. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, references, tables, boxes, figure legends, and print appendixes) but exclude references.

Answer: Thank you for your comment. We tried to adjust our manuscript to the page numbers indicated in the journal. Now the manuscript is with 5,637 words, abstract is with 309 words, introduction is with 228 words and Discussion is with 772 words. We apologize if one section is with more words than is permitted because we believe that if we remove more words, probably it will lose the meaning.

8. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:
* All financial support of the study must be acknowledged. * Any and all manuscript preparation assistance, including but not limited to topic development, data collection, analysis, writing, or editorial assistance, must be disclosed in the acknowledgments. Such acknowledgments must identify the entities that provided and paid for this assistance, whether directly or indirectly. * All persons who contributed to the work reported in the manuscript, but not sufficiently to be authors, must be acknowledged. Written permission must be obtained from all individuals named in the acknowledgments, as readers may infer their endorsement of the data and conclusions. Please note that your response in the journal's electronic author form verifies that permission has been obtained from all named persons. * If all or part of the paper was presented at the Annual Clinical and Scientific Meeting of the American College of Obstetricians and Gynecologists or at any other organizational meeting, that presentation should be noted (include the exact dates and location of the meeting).

Answer: these parameters were met

9. Provide a short title of no more than 45 characters (40 characters for case reports), including spaces, for use as a running foot. The short title “Imiquimod for the treatment of CIN2/3” was added as a running foot

10. The most common deficiency in revised manuscripts involves the abstract. Be sure there are no inconsistencies between the Abstract and the manuscript, and that the Abstract has a clear conclusion statement based on the results found in the paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limit for Original Research articles is 300 words. Please provide a word count.

Answer: these parameters have been met

11. Abstracts for all randomized, controlled trials should be structured according to the journal's standard format. The Methods section should include the primary outcome and sample size justification. The Results section should begin with the dates of enrollment to the study, a description of demographics, and the primary outcome analysis. Please review the sample abstract that is located online

12. Only standard abbreviations and acronyms are allowed. A selected list is available online at http://edmgr.ovid.com/ong/accounts/abbreviations.pdf. Abbreviations and acronyms cannot be used in the title or précis. Abbreviations and acronyms must be spelled out the first time they are used in the abstract and again in the body of the manuscript.

13. The journal does not use the virgule symbol (/) in sentences with words. Please rephrase your text to avoid using "and/or," or similar constructions throughout the text. You may retain this symbol if you are using it to express data or a measurement.

14. In your Abstract, manuscript Results sections, and tables, the preferred citation should be in terms of an effect size, such as odds ratio or relative risk or the mean difference of a variable between two groups, expressed with appropriate confidence intervals. When such syntax is used, the P value has only secondary importance and often can be omitted or noted as footnotes in a Table format. Putting the results in the form of an effect size makes the result of the statistical test more clinically relevant and gives better context than citing P values alone.

If appropriate, please include number needed to treat for benefits (NNTb) or harm (NNTh). When comparing two procedures, please express the outcome of the comparison in U.S. dollar amounts.

Please standardize the presentation of your data throughout the manuscript submission. For P values, do not exceed three decimal places (for example, "P = .001"). For percentages, do not exceed one decimal place (for example, 11.1").

15. Please review the journal's Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available online here: http://edmgr.ovid.com/ong/accounts/table_checklist.pdf.
16. Please review examples of our current reference style at http://ong.editorialmanager.com (click on the Home button in the Menu bar and then “Reference Formatting Instructions” document under “Files and Resources). Include the digital object identifier (DOI) with any journal article references and an accessed date with website references. Unpublished data, in-press items, personal communications, letters to the editor, theses, package inserts, submissions, meeting presentations, and abstracts may be included in the text but not in the reference list.

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17. Figure 1: okay

Figure 2: Please provide a high res version of this image and a letter of permission to use in print and electronic formats.
Answer: these parameters were met.

Figure 3: Please remove A-C labels, these will be added back per journal style.
Answer: these parameters were met.

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