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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.
RE: Manuscript Number ONG-23-406

Imiquimod for cervical and vaginal intraepithelial neoplasia: A systematic review and meta-analysis

Dear Dr. Hamanishi:

Thank you for sending us your work for consideration for publication in Obstetrics & Gynecology. Your manuscript has been reviewed by the Editorial Board and by special expert referees. The Editors would like to invite you to submit a revised version for further consideration.

If you wish to revise your manuscript, please read the following comments submitted by the reviewers and Editors. Each point raised requires a response, by either revising your manuscript or making a clear argument as to why no revision is needed in the cover letter.

To facilitate our review, we prefer that the cover letter you submit with your revised manuscript include each reviewer and Editor comment below, followed by your response. That is, a point-by-point response is required to each of the EDITOR COMMENTS (if applicable), REVIEWER COMMENTS, and STATISTICAL EDITOR COMMENTS (if applicable) below. The revised manuscript should indicate the position of all changes made. Please use the "track changes" feature in your document (do not use strikethrough or underline formatting). Upload the tracked-changes version when you submit your revised manuscript.

Your submission will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by 04/28/2023, we will assume you wish to withdraw the manuscript from further consideration.

EDITOR COMMENTS:

Dear Dr. Hamanishi and co-authors:

Thank you for your submission.

Your systematic review has gone through our external review process and was discussed by our editorial board. We would like to give your paper additional considerations if you can address our reviewers' comments.

In addition to the comments below, we have the additional requests to consider:
1. There was only 1 RCT for VaIN: therefore, please clarify if these findings were included in the pooled OR, and if so, please justify. Please also include this in Limitations, so reader understands this.
2. Secondary outcomes and Subgroup analyses (pages 10-11): These sections are fairly dense: please simplify to make more reader friendly and avoid repeating data already presented in Tables. Please summarize key findings.
3. Please modify or justify conclusion that imiquimod is a good treatment for VaIN, in light that there is only 1 RCT.
4. "NRS" is not a standard abbreviation: please remove.

We look forward to receiving your revised paper. Thank you again for your submission.

Please also note the following:

* Help us reduce the number of queries we add to your manuscript after it is revised by reading the Revision Checklist at https://journals.lww.com/greenjournal/Documents/RevisionChecklist_Authors.pdf and making the applicable edits to your manuscript.

* Figures 1-3: Please upload as individual figure files on Editorial Manager.

REVIEWER COMMENTS:
Reviewer #1:  
This is a systematic review and meta-analysis regarding the effectiveness and adverse effects of imiquimod for cervical and vaginal intraepithelial neoplasia. Overall, my biggest concern is the limitation of the primary literature that is included in the study. The overall methods of which studies to include in the analyses and the potential bias of including studies that include non-randomized studies and those with VaIN severely limit the quality of the meta-analysis.

- Line 20: The term, "meta-analyzed" is odd phrased.
- Consider modifying "safety outcome requiring treatment discontinuation" to treatment discontinuation due to side effects.
- Line 65 needs to be revised. Unclear.
- Line 93 states that the results from the non-randomized study were used only for the analysis for adverse events. However, Table 3/4 suggests otherwise.
- Line 140: Please provide the rationale for using a dichotomous endpoint ( <=8 vs. >8 wks)
- The non-randomized study added heterogeneity and bias to the meta-analysis, what happens to the safety results when you exclude this study? Would favor limiting the meta-analysis to RCTs.
- Table 2: Grimm et al— it is unclear if the stated weeks is the duration or applied twice for 3-4 wks or week 3 and week 4 out of a 16 week treatment period.
- Line 175-176 is unclear.
- Line 177-178: The detail regarding imiquimod vs surgical treatment is distracting because the stated control arm for this meta-analysis was placebo or no treatment.
- Line 339: With only 1 study on VaIN, the authors are limited to make a meaningful comment about the effectiveness of Imiquimod regarding VaIN.
- Paragraph 126, the references to secondary outcomes is confusing. Please just state what the outcome is vs. "secondary outcome 6 and 7".
- Data in Table 3 and Table 4 appear to be overlapping and very confusing. It is unclear what the purpose of table 3 is.
- Table 4: Recommend adding citations to be more explicit about which study(ies) contributed data for each analysis.

Reviewer #2:  
ONG-23-406  
Imiquimod for cervical and vaginal intraepithelial neoplasia: A systematic review and meta-analysis

The authors present a systematic review and meta-analysis of 8 studies on the use of off-label use of imiquimod for cervical and vaginal intraepithelial neoplasia (CIN and VAIN). The authors included the PRISMA checklist for systematic reviews and stated they registered their review protocol with a third party. The search strategy was provided as an appendix. Study selection followed accepted practices. They identified 8 studies: 6 randomized trials for CIN, 1 non-randomized trial for CIN, and 1 randomized trial for VAIN. Studies were noted to be heterogenous with differing inclusion criteria, allowances for prior treatments, applications of the study drug, frequency of administration, and cessation during menstruation. Additionally, several studies were noted to have some or high risk of bias. The primary efficacy and safety outcomes favored use of imiquimod.

I have the following suggestions for the authors. Additionally, the paper would benefit from a statistical review.

Title - Remove VAIN from title. Only 1 study (out of 8) included patients with VAIN. A systematic review and meta-analysis specifically addressing use of imiquimod for VAIN was referenced (number 7) but the data were not used (3 prospective trials and 1 randomized trial in that analysis). I do not think enough information regarding VAIN is included in this study to draw conclusions.

Abstract - Conclusion: same as above

Methods - Are the authors certain the data were sufficiently uniform to combine into a meta-analysis. It seems the heterogeneity in study design and outcome assessment risks amplifying bias when the data are pooled.

Results - There seems to be a discrepancy in the risk of bias assessment presented in the results and that summarized in the discussion.

Discussion - Remove statement regarding the uniqueness of the project given reference 7. This paper should be compared with other systematic reviews on the topic.

Do the authors have a suggestion for the uniform application of imiquimod?

Reviewer #3:  
Review of Manuscript ONG-23-406 "Imiquimod for cervical and vaginal intraepithelial neoplasia: A systematic review and meta-analysis"

A meta-analysis which impacts the potential utility of imiquimod therapy for treating/promoting resolution of lower genital tract dysplasia of the cervix and vagina has been submitted. As noted, the authors ultimately identified 8 studies for
inclusion, the majority of which were RCTs and focused on primary treatment of cervical dysplasia. Not surprisingly, the studies utilized multiple different approaches both in administration but also does on how the therapy was utilized. The authors have appropriately included a PRISMA checklist. I have the following questions and comments.

Title - No comments

Précis - No comments other than if space allows consider noting this may be an alternative to excision.

Abstract - Line 27 - Consider denoting how many RCTs and how many single arm trials or those without a direct comparator were included.

Introduction - No major comments. Appropriate commentary on previous publications and context provided, although did you consider seeing if there were studies which included use for treating VIN as it is an accepted off label use (Line 56)?

Methods - Line 82 - What if the regression was to normal? Was that not accounted for?
Line 103 - Was the trade name "Aldara" or "Zyclara" considered for searches?
Line 127 - I presume this was IIT and even if they stopped therapy or crossed over they remained in their assigned group?
Line 139 - How was the cut point for dosing chosen?

Results - Line 172-4 - Consider noting the timing of the LEEP from this RCT.
Line 223-224 - Really wide confidence intervals present.

Discussion - Line 344 - Also consider noting evaluation of other ways like premedication to mitigate side effects

Tables - Table 1 - If available consider adding the range and/or St. deviation for the median or mean age listing.
Table 2 - No comments
Table 3 - Is the commentary on High, medium and low certainty standardly listed here.
Table 4 - No comments

Figures - Figure 1 - Please confirm the numbers are correct for the progression though exclusions.
Figure 2/3 - No comments.

STATISTICAL EDITOR COMMENTS:
Fig 2B and Fig 3: Need to include columns for weight assigned to each row entry. Should also include in all supplemental material with forest plots which do not currently include column of weights.

For the primary outcome, should include funnel plots, which could be in supplemental material.

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Sincerely,
Vivian W. Sung, MD, MPH
Deputy Editor, Gynecology

The Editors of Obstetrics & Gynecology

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.
April 27, 2023
RE: Manuscript Number: ONG-23-406

Dear Professor Sung,

We would like to thank you very much for providing us with the opportunity to revise and resubmit our manuscript. We are delighted that the referees evaluated our work favorably and are pleased with your interest in our paper. We found your and your reviewers’ comments constructive, and we have revised the manuscript in close accord with your recommendations.

We have given our point-by-point responses to the comments in the following pages. We believe that the revised manuscript has been substantially improved by the reviewers’ helpful suggestions and hope that you now find it suitable for publication in Obstetrics & Gynecology.

Sincerely,

Junzo Hamanishi, MD, PhD
Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara cho, Sakyo ku, Kyoto, 606-8507, Japan
Responses to the Editor Comments

Comments to the Author

Thank you for your submission.

Your systematic review has gone through our external review process and was discussed by our editorial board. We would like to give your paper additional considerations if you can address our reviewers' comments.

In addition to the comments below, we have the additional requests to consider:

Comment/Question 1.

1. There was only 1 RCT for VaIN: therefore, please clarify if these findings were included in the pooled OR, and if so, please justify. Please also include this in Limitations, so reader understands this.

Response 1:

We very much appreciate your constructive comments and for giving us the opportunity to clarify. A priori, at the protocol stage, we reasoned that we could include both studies for CIN and VaIN in the pooled OR, because both CIN and VaIN are HPV-related premalignant intraepithelial lesions, that is, these two conditions have biological similarities. We therefore wrote in the protocol that we would calculate the pooled OR for the treatment effects of CIN and VaIN. As it turned out, in our systematic review and meta-analysis, we found three studies for CIN and one study for VaIN. When pooled, the observed heterogeneity between the included studies was very low, I-squared statistics was 0% and upon visual inspection, the 95% confidence intervals of the included studies for CIN and VaIN overlapped to a great extent (Figure 2A).

However, as the editor and the reviewers have pointed out, there was only 1 RCT with a limited sample size for VaIN. The 95% CI was large and we cannot definitively conclude that imiquimod is an effective treatment for VaIN from this study. Therefore, we modified the conclusion sentence for VaIN as follows.

(Page 18, Lines 409-414)

“In conclusion, imiquimod is potentially an alternative therapy to surgery for CIN. Although systemic adverse events are common, treatment discontinuation is not frequently required with appropriate management. For VaIN, imiquimod might be as effective as for CIN; however, this
conclusion must be tempered and interpreted with caution because there was only one small, inconclusive RCT that focused on VaIN.”

We have edited the summary of the results in the discussion.

(Pages 14-15, Lines 308-323)

“...The present study showed that the pooled treatment effects of imiquimod, including CIN and VaIN, were superior to those of placebo or no interventions, both in terms of histological regression and HPV clearance. However, when examined separately according to disease, while the pooled OR for the histological regression of CIN was 4.27 (95% CI 2.11–8.66), treatment effects of imiquimod for VaIN were estimated with much uncertainty (OR for histological regression 2.67, 95% CI 0.36–19.71) since there was only one RCT with small sample size. Although minor adverse events were generally frequent, treatment discontinuation was required in less than 10% of the patients and imiquimod treatment was well tolerated. Our study suggests that imiquimod could be an alternative treatment for patients with CIN who want to avoid surgical treatment in view of the negative effects on future pregnancies. The use of imiquimod for women with CIN who consider future pregnancies is now recommended in the Dutch guidelines.40 Detailed pretreatment counseling about the possible complications and benefits of imiquimod is important.”

Also, we replaced Figure 2 with the results of analyses by type of disease (CIN or VaIN), which were previously presented in the Appendix. We think that this will provide information in a more reader-friendly manner.

In the abstract, we have added the following sentence for clarification.

(Page 2, Lines 33-35)

“Pooled OR for CIN in the three studies was 4.27 (95% CI 2.11–8.66) while results of one study were available for VaIN (OR, 2.67, 95% CI 0.36–19.71). Pooled probability for primary safety outcome in the imiquimod arm was 0.07 (95% CI 0.03–0.14).”

Accordingly, the abstract was edited to maintain the number of words.

Additionally, we added the above rationale for calculating the pooled OR including CIN and VaIN in the limitations (Pages 17, Lines 382-389).

“First, we presented pooled treatment effects, combining CIN and VaIN. This is because CIN and VaIN are both HPV-related intraepithelial premalignant conditions. As shown in Figure 2A, the observed heterogeneity in primary efficacy outcomes between studies was very small, even when
we combined studies investigating CIN and VaIN. Therefore, we believe that the pooled treatment effects, combining CIN and VaIN, provide useful information for clinicians. However, it should be noted that the treatment effects of imiquimod for VaIN are inconclusive owing to the limited sample size.”

We also added a paragraph in the discussion regarding imiquimod for VaIN (Page 15, Lines 324-333), citing a consensus statement on the management of vaginal intraepithelial neoplasia from four societies which were published in March (Ref 41).

“Imiquimod treatment for VaIN has recently attracted considerable interest. Observational studies have reported a good treatment response to imiquimod for VaIN. A systematic review and meta-analysis including observational studies reported that the pooled complete response to imiquimod for VaIN 2–3 was 76%. A recently published consensus statement on the management of VaIN considered imiquimod as the best topical approach. Although our study cannot conclude that imiquimod is effective for VaIN because of the limited available evidence, it is interesting that the observed heterogeneity of treatment effects of imiquimod in our study was low, suggesting a similar treatment effect on CIN and VaIN, which are both HPV-related premalignancies (Figure 2).”

Reference 41

Comment/Question 2.
2. Secondary outcomes and Subgroup analyses (pages 10-11): These sections are fairly dense: please simplify to make more reader friendly and avoid repeating data already presented in Tables.
Please summarize key findings.
Response 2:
Following your advice, we have edited the Results section of Secondary outcomes and Subgroup analyses and made it more concise, avoiding repeating data presented in Tables.
“Secondary outcomes

We did not conduct meta-analyses for the ORs of each adverse event associated with imiquimod (secondary outcomes 1–5) in comparison with placebo or no intervention, since results in both arms were available in only one study.\textsuperscript{11} No data were available on abdominal pain or abnormal vaginal discharge/genital bleeding for calculation of ORs and absolute risk differences.

The pooled absolute probabilities of adverse events are described in Table 4, and Appendices 5A–F shows the corresponding forest plots. Heterogeneity of results was large, except for vulvovaginal ulceration.

In two RCTs, three cases of telogen effluvium were reported.\textsuperscript{38, 39} Since this symptom was first reported in 2019 and has not been reported in imiquimod treatment for other diseases,\textsuperscript{31} the presence of measurement bias cannot be ruled out. (Table 4).

The pooled OR (95% CI) of HPV clearance was 9.50 (2.98–30.27) (Appendix 5G, Table 3). The absolute risk difference was 0.47 (0.19–0.69). The pooled response rate (95% CI) in the imiquimod arm (sensitivity analysis) was 0.51 (0.35–0.66) (Appendix 5H, Table 4).

Appendix 6 shows results of the post-hoc sensitivity analyses excluding the non-RCT. Appendix 7 describes results of the post-hoc sensitivity analyses for meta-analysis of proportions using the inverse variance method with arcsine transformation, where the individual study weights are available.

Subgroup analyses

Pre-specified subgroup analyses were conducted to explore possible sources of heterogeneity in the results (Appendices 8–11). The pooled OR for histological regression of CIN using three studies was 4.27 (95% CI 2.11–8.66), while only one small, inconclusive RCT was available for VaIN (OR 2.67, 95% CI 0.36–19.71). Due to the limited number of included studies, subgroup analyses of ORs for HPV clearance were not conducted. In some subgroup analyses, we observed heterogeneity, i.e. quite different point estimates among subgroups and substantial lack of overlap in the corresponding CIs. In the subgroup analyses according to the application method, fever (Appendix 9D), abnormal vaginal discharge/genital bleeding (Appendix 9G), and vulvovaginal pain (Appendix 9H) were less frequent in the physician-applied group than in the self-applied group. In the subgroup analyses of the treatment period, abdominal pain was less frequent in the ≤8 weeks group than in the >8 weeks group (Appendix 11F). In the remaining subgroup analyses, forest plots did
not provide visual indication of heterogeneity between subgroups, CIs between the groups greatly overlapped, or subgroup analyses could not be conducted.”

Comment/Question 3.
3. Please modify or justify conclusion that imiquimod is a good treatment for VaIN, in light that there is only 1 RCT.

Response 3:
Following your suggestion, we modified the conclusion.
(Page 18, Lines 412-414)
“For VaIN, imiquimod might be as effective as for CIN; however, this conclusion must be tempered and interpreted with caution because there was only one small, inconclusive RCT that focused on VaIN.”

Comment/Question 4.
4. "NRS" is not a standard abbreviation: please remove.

Response 4:
Following your suggestion, we used the term “non-RCT” instead of “NRS.” The following article published by Obstetrics & Gynecology last year used this terminology to refer to non-randomized controlled trials.

Comment/Question 5.
We look forward to receiving your revised paper. Thank you again for your submission.
Please also note the following:

* Help us reduce the number of queries we add to your manuscript after it is revised by reading the Revision Checklist at https://journals.lww.com/greenjournal/Documents/RevisionChecklist_Authors.pdf and making the applicable edits to your manuscript.

Response 5:
We confirmed the Revision Checklist. Thank you.

Comment/Question 6.
* Figures 1-3: Please upload as individual figure files on Editorial Manager.

Response 6:
We have uploaded the figures as individual files.
Responses to the comments of Reviewer: #1

Comment/Question 1.

This is a systematic review and meta-analysis regarding the effectiveness and adverse effects of imiquimod for cervical and vaginal intraepithelial neoplasia. Overall, my biggest concern is the limitation of the primary literature that is included in the study. The overall methods of which studies to include in the analyses and the potential bias of including studies that include non-randomized studies and those with VaIN severely limit the quality of the meta-analysis.

Response 1

We appreciate this opportunity to clarify the issues raised by the reviewer. First, we included only randomized controlled studies in the meta-analysis of the efficacy of imiquimod vs placebo, but included both randomized and non-randomized studies to estimate the proportions of side effects in the imiquimod arm. The imiquimod arms from randomized-controlled trials and the imiquimod arms from non-randomized prospective studies can provide important information about the frequencies of side effects. We agree that we may not have been very clear in these different types of analyses and their respective source studies. We therefore added:

(Page 10, Lines 201-203)

"In total, results of four studies were available to compare treatment effects of imiquimod with those of placebo or no-intervention.10, 11, 19, 36 The remaining four studies were used for the meta-analyses of proportions in the imiquimod arm.12, 18, 35, 37"

We have also added post-hoc sensitivity analyses excluding the non-randomized studies following Reviewer #2 Comment/Question 7 below.

Second, we completely agree with the reviewer’s concern about including the VaIN studies and about the fact that we found only one relevant RCT. Please see our response to the Editor’s Comment/Question 1.

At the protocol stage, we reasoned that we could include both studies for CIN and VaIN in the pooled OR, because both CIN and VaIN are HPV-related premalignant intraepithelial lesions, that is, these two conditions have biological similarities. We, therefore, wrote in the protocol that we would calculate the pooled OR for the treatment effects of CIN and VaIN. As it turned out, in our systematic review and meta-analysis, we found three studies for CIN and one study for VaIN. When pooled, the observed heterogeneity between the included studies was very low, I-squared statistics was 0% and
upon visual inspection, the 95% confidence intervals of the included studies for CIN and VaIN overlapped to a great extent (Figure 2A).

However, there was only 1 RCT with a limited sample size for VaIN. The 95% CI was large and we cannot definitively conclude that imiquimod is an effective treatment for VaIN from this study. Therefore, we modified the conclusion sentence for VaIN as follows.

(Page 18, Lines 412-414)
"For VaIN, imiquimod might be as effective as for CIN; however, this conclusion must be tempered and interpreted with caution because there was only one small, inconclusive RCT that focused on VaIN."

Also, we replaced Figure 2 with the results of analyses by type of disease (CIN or VaIN).

Comment/Question 2.
- Line 20: The term, "meta-analyzed" is odd phrased.
Response 2:
Following the reviewer’s advice, we have modified the sentence as follows.
(Page 1, Lines 22-23)
“We estimated pooled odds ratios (ORs) of imiquimod compared with placebo or no intervention.”

Comment/Question 3.
- Consider modifying "safety outcome requiring treatment discontinuation" to treatment discontinuation due to side effects.
Response 3:
We have modified the abstract, main text and Tables accordingly.

Comment/Question 4.
- Line 65 needs to be revised. Unclear.
Response 4:
We have modified the sentence as follows.
(Page 4, Lines 75-79)
“For VaIN, several treatment modalities have been used, including surgical treatment, carbon dioxide laser, 5-fluorouracil, and radiation therapy. However, recurrence is common, and a standard treatment for VaIN 2–3 has not been established. 13-16 Imiquimod is one of the optimal treatments for VaIN 2–3.7, 17”

Comment/Question 5.
- Line 93 states that the results from the non-randomized study were used only for the analysis for adverse events. However, Table 3/4 suggests otherwise.

Response 5:
We thank the reviewer for the careful review of the manuscript. We included only randomized studies when investigating the treatment effects of imiquimod compared versus placebo or no intervention. But we included the non-randomized studies when we conducted meta-analyses of absolute proportions of treatment response. However, as the reviewer has pointed out, this was not clearly stated in the protocol. We, therefore, decided to exclude the non-randomized studies from the meta-analysis of proportions of treatment response (Figure 2B, Appendix 5H, and corresponding subgroup analyses). We corrected the manuscript and Table 4 accordingly.

Comment/Question 6.
- Line 140: Please provide the rationale for using a dichotomous endpoint ( <=8 vs. >8 wks)

Response 6:
The cut-off for the dichotomization was pre-specifed in the protocol (PROSPERO: CRD42022377982). This is based on our clinical experience (Ref 17), where most patients with VaIN achieved a complete response after 8 weeks of imiquimod treatment. We have added the following sentence to Methods. (Page 8, Lines 164-165)

“The cut-off for the dichotomization was pre-specified based on our clinical experience.17”
Comment/Question 7.
- The non-randomized study added heterogeneity and bias to the meta-analysis, what happens to the safety results when you exclude this study? Would favor limiting the meta-analysis to RCTs.

Response 7:

We very much appreciate the reviewer’s constructive comments. Following their advice, we have added post-hoc sensitivity analyses excluding the non-randomized study. The results are presented in Appendix 6. There are small differences between the results of the original analyses and those of the post-hoc sensitivity analyses. The following sentences were added to the manuscript, accordingly.

(Page 8, Line 157)

“In a post-hoc sensitivity analyses, we excluded non-RCTs from the analyses.”

(Page 13, Lines 278-281)

“Appendix 6 shows results of the post-hoc sensitivity analyses excluding the non-RCT.”

Comment/Question 8.
- Table 2: Grimm et al—it is unclear if the stated weeks is the duration or applied twice for 3-4 wks or week 3 and week 4 out of a 16 week treatment period.

Response 8:

We have modified Table 2 accordingly.

Comment/Question 9.
- Line 175-176 is unclear.

Response 9:

Following the reviewer’s advice, we have modified the sentences as follows.

(Page 10, Lines 201-206)

“In total, results of four studies were available to compare treatment effects of imiquimod with those of placebo or no-intervention.10, 11, 19, 36 The remaining four studies were used for the meta-analyses of proportions in the imiquimod arm.12, 18, 35, 37”
Comment/Question 10.
- Line 177-178: The detail regarding imiquimod vs surgical treatment is distracting because the stated control arm for this meta-analysis was placebo or no treatment.

Response 10:
We agree with the reviewer’s advice, and have removed this sentence from the manuscript.
(Page 10, Lines 206-208)

Comment/Question 11.
- Line 339: With only 1 study on VaIN, the authors are limited to make a meaningful comment about the effectiveness of Imiquimod regarding VaIN.

Response 11:
We have now modified the conclusions.
(Page 18, Lines 412-414)
“For VaIN, imiquimod might be as effective as for CIN; however, this conclusion must be tempered and interpreted with caution because there was only one small, inconclusive RCT that focused on VaIN.”
Please see our response to the Editor’s Comment/Question 1.

Comment/Question 12.
- Paragraph 126, the references to secondary outcomes is confusing. Please just state what the outcome is vs. "secondary outcome 6 and 7".

Response 12:
Following the reviewer’s advice, we have modified this paragraph as follows. The changes based on this suggestion are underlined.
(Pages 7-8, Lines 142-171)
“We conducted intention-to-treat analyses of treatment effects. For adverse events, patients who started the allocated treatment were analyzed since including patients who did not start the allocated treatment can lead to the underestimation of the risk of adverse events. For the primary efficacy outcome (histological regression of the disease), fever, arthralgia/myalgia, abdominal pain, abnormal vaginal discharge/genital bleeding, and HPV clearance, we calculated odds ratios (ORs) with 95% confidence intervals (CIs) using a random-effects model (inverse variance model). For primary safety outcome and vaginal ulceration, we conducted meta-analyses of proportions in the imiquimod treatment arms (generalized linear mixed model).24 For vulvovaginal pain, we
calculated ORs with 95% CI using a fixed effects Mantel Haenszel method with no continuity correction since this outcome was expected to be rare in the control group.\textsuperscript{25} We also used the estimated ORs and corresponding uncertainty, together with the point estimate of the pooled event rate in the control arms to estimate absolute risk difference of events. In a post-hoc sensitivity analyses, we excluded non-RCTs from the analyses. We also performed sensitivity analyses using the inverse variance method with arcsine transformation to provide individual study weights, because these weights are not available in meta-analyses of proportions using a generalized linear mixed model.\textsuperscript{24} To explore possible sources of heterogeneity, we conducted preregistered (i.e. at the protocol) subgroup analyses by type of disease (CIN or VaIN), application method (self-applied or physician-applied), average weekly dose ($\leq$18.75 or $>$18.75 mg/week of imiquimod), and treatment period ($\leq$8 weeks or $>$8 weeks). The cut-off for the dichotomization was pre-specified based on our clinical experience.\textsuperscript{17} The difference between subgroups was evaluated via the p-value for the test for subgroup differences, and by visually inspecting the point estimates and 95% CIs of subgroups in forest plots. In sensitivity analyses, we meta-analyzed proportions of patients that experienced the primary efficacy outcome, fever, arthralgia/myalgia, abdominal pain, abnormal vaginal discharge/genital bleeding, vulvovaginal pain, and HPV clearance."

Comment/Question 13.
- Data in Table 3 and Table 4 appear to be overlapping and very confusing. It is unclear what the purpose of table 3 is.
  
  Response 13:
  We appreciate the reviewer’s helpful comments. Table 3 describes the comparison of imiquimod versus placebo/no intervention and the odds ratios are presented. On the other hand, Table 4 shows the proportions of patients experiencing events in the imiquimod arm. To clarify this point, we have changed the titles of these Tables.
  
  “Table 3. Comparison of imiquimod versus placebo/no intervention”
  “Table 4. Proportions of patients experiencing events in the imiquimod arm”

Comment/Question 14.
- Table 4: Recommend adding citations to be more explicit about which study(ies) contributed data for each analysis.
  
  Response 14:
  We have added citations to Table 3 and Table 4.
Responses to the comments of Reviewer #2

Comment/Question 1.
ONG-23-406
Imiquimod for cervical and vaginal intraepithelial neoplasia: A systematic review and meta-analysis

The authors present a systematic review and meta-analysis of 8 studies on the use of off-label use of imiquimod for cervical and vaginal intraepithelial neoplasia (CIN and VAIN). The authors included the PRISMA checklist for systematic reviews and stated they registered their review protocol with a third party. The search strategy was provided as an appendix. Study selection followed accepted practices. They identified 8 studies: 6 randomized trials for CIN, 1 non-randomized trial for CIN, and 1 randomized trial for VAIN. Studies were noted to be heterogenous with differing inclusion criteria, allowances for prior treatments, applications of the study drug, frequency of administration, and cessation during menstruation. Additionally, several studies were noted to have some or high risk of bias. The primary efficacy and safety outcomes favored use of imiquimod.

I have the following suggestions for the authors. Additionally, the paper would benefit from a statistical review.

Response 1:
We wish to express our appreciation to Reviewer #2 for their helpful comments, which have helped us significantly improve the paper. Our point-by-point responses to the comments are described below.

Comment/Question 2.
Title - Remove VAIN from title. Only 1 study (out of 8) included patients with VAIN. A systematic review and meta-analysis specifically addressing use of imiquimod for VAIN was referenced (number 7) but the data were not used (3 prospective trials and 1 randomized trial in that analysis). I do not think enough information regarding VAIN is included in this study to draw conclusions.

Abstract - Conclusion: same as above

Response 2:
We very much appreciate the reviewer’s insightful comments. The three studies cited in Ref 7, which Reviewer #2 has mentioned above, are studies without control arms. Since the reported frequency of adverse events would be very low if adverse events are not actively monitored, we decided not to
include studies without control arms at the protocol stage. Therefore, these three studies do not meet our eligibility criteria.

And, as Reviewer #2 has pointed out, there was only 1 RCT with a limited sample size for VaIN. The 95% CI was large and we cannot conclude that imiquimod is an effective treatment for VaIN from this study. Therefore, we modified the conclusion sentence for the abstract as follows.

(Pages 2-3, Lines 46-50)

“Imiquimod was found to be effective for CIN whereas data on VaIN were limited. Although local and systemic complications are common, treatment discontinuation is infrequent. Thus, imiquimod is potentially an alternative therapy to surgery for CIN.”

For clarification, Figure 2 was replaced with the results of analyses by type of disease (CIN or VaIN), which were previously presented in the Appendix.

We have also added a paragraph discussing imiquimod for VaIN, citing reference 7. (Page 15, Lines 324-333)

“Imiquimod treatment for VaIN has recently attracted considerable interest. Observational studies have reported a good treatment response to imiquimod for VaIN. A systematic review and meta-analysis including observational studies reported that the pooled complete response to imiquimod for VaIN 2–3 was 76%. A recently published consensus statement on the management of VaIN considered imiquimod as the best topical approach. Although our study cannot conclude that imiquimod is effective for VaIN because of the limited available evidence, it is interesting that the observed heterogeneity of treatment effects of imiquimod in our study was low, suggesting a similar treatment effect on CIN and VaIN, which are both HPV-related premalignancies (Figure 2).”

As we discussed in our response to the Editor’s Comment/Question 1, we included studies on VaIN to calculate the pooled OR, but have added an explanation of limitations regarding VaIN. Therefore, we would like to include both CIN and VaIN in the title because the pooled OR includes both types of studies.
Comment/Question 3.

Methods - Are the authors certain the data were sufficiently uniform to combine into a meta-analysis. It seems the heterogeneity in study design and outcome assessment risks amplifying bias when the data are pooled.

Response 3:

We very much appreciate this opportunity to clarify. At the protocol stage, we reasoned that we could calculate the pooled OR. As it turned out, in our systematic review and meta-analysis, the observed heterogeneity of primary efficacy outcome and primary safety outcome between the included studies was low or moderate. Therefore, we think that the pooled results of these outcomes are very meaningful.

For secondary adverse events, however, the observed heterogeneity was high and these results should be interpreted with caution. To indicate the uncertainty of the pooled proportions, the observed ranges of probability were described in Table 4.

We also added the following sentence to the limitations.

(Pages 17-18, Lines 390-394)

“Second, although we described the pooled probabilities of secondary adverse events, the observed heterogeneity was high except for vaginal ulceration. To account for the uncertainty in pooled results, we also show the observed probability ranges (Table 4). Despite this uncertainty, we believe that we have provided the best available evidence on the adverse effects of vaginal use of imiquimod.”

Comment/Question 4.

Results - There seems to be a discrepancy in the risk of bias assessment presented in the results and that summarized in the discussion.

Response 4:

We confirmed that there was no discrepancy between the results and the discussion. However, the results summarized in the discussion may have been ambiguous. We have edited the following sentences.

(Pages 16-17, Lines 366-370)

“Among the studies used for the main analysis of primary efficacy outcome, the overall risk of bias was not high\textsuperscript{10,11,19} except for a preliminary discontinued RCT\textsuperscript{36} (Appendix 3A). Regarding the
post-hoc risk of bias assessment for the prevalence of adverse events (Appendix 3C), the risk of bias in the representativeness of the study samples was judged to be low in all studies.”

Also, the following sentence might have been confusing, we have removed this sentence from the manuscript.
(Page 17, Lines 379-381)

“However, this bias could be high for long-lasting adverse events since the first case of telogen effluvium has been reported recently.37, 38”

Additionally, we found a misstatement in the discussion regarding the certainty of evidence. This might have been confusing. We have modified the sentence as follows.
(Page 16, Lines 361-362)

“We did not downgrade the certainty of the evidence because studies with a low risk of bias accounted for 85% of the weights.”

**Comment/Question 5.**

**Discussion - Remove statement regarding the uniqueness of the project given reference 7. This paper should be compared with other systematic reviews on the topic.**

Response 5:
We appreciate the reviewer’s comment. Following their advice, we have removed this sentence from the manuscript. (Page 14, Line 308-309)
Also, we have added a paragraph discussing imiquimod for VaIN, citing reference 7, as explained in our response to Revier #2 Comment/Question 2.

**Comment/Question 6.**

**Do the authors have a suggestion for the uniform application of imiquimod?**

Response 6:
We appreciate this question. The results of this study suggest that applying imiquimod once weekly by a physician seems effective (Appendix 9A) while the frequency of adverse events was relatively low (Appendix 9D, 9G, 9H), and further investigation should be needed. We think that the effectiveness and safety of this application method should further be investigated. However, we have not yet applied imiquimod with this method and we cannot make a strong suggestion. In our small experience, applying imiquimod three times a week by physicians is showing a very good response (Ref 17).
However, the results of this study suggest that the frequency of administration may be reduced. We think that physician-applied methods are useful for monitoring adverse events.
Responses to the comments of Reviewer: #3

Comment/Question 1.
Review of Manuscript ONG-23-406 "Imiquimod for cervical and vaginal intraepithelial neoplasia: A systematic review and meta-analysis"

A meta-analysis which impacts the potential utility of imiquimod therapy for treating/promoting resolution of lower genital tract dysplasia of the cervix and vagina has been submitted. As noted, the authors ultimately identified 8 studies for inclusion, the majority of which were RCTs and focused on primary treatment of cervical dysplasia. Not surprisingly, the studies utilized multiple different approaches both in administration but also does on how the therapy was utilized. The authors have appropriately included a PRISMA checklist. I have the following questions and comments.

Response 1:
We thank Reviewer #3 for the positive comments. We have responded to their comments as follows.

Comment/Question 2.
Title - No comments

Response 2: No change requested.

Comment/Question 3.
Précis - No comments other than if space allows consider noting this may be an alternative to excision.

Response 3:
Following the reviewer’s advice, we have changed the Précis as follows. The same change is also added to the conclusion of the abstract and the main text.

(In Précis, Page 1, Lines 5-6)
“Imiquimod is potentially an alternative treatment to surgery for cervical intraepithelial neoplasia.”

(In the abstract, Page 2-3, Lines 48-50)
“Thus, imiquimod is potentially an alternative therapy to surgery for CIN.”

(In the main text, Page 18, Lines 409-410)
“In conclusion, imiquimod is potentially an alternative therapy to surgery for CIN.”
Comment/Question 4.

Abstract - Line 27 - Consider denoting how many RCTs and how many single arm trials or those without a direct comparator were included.

Response 4:

We followed the reviewer’s suggestion and have modified the abstract as follows.

(Page 2, Lines 29-31)

“Four studies contributed to the pooled OR for the primary efficacy outcome. An additional four studies were available for meta-analyses of proportions in the imiquimod arm.”

We excluded single-arm trials to maintain the quality of included studies. Meta-analyses of proportions were conducted using the data of the imiquimod arm in controlled studies.

Since our explanation might not be enough, we modified the text as follows.

(Pages 6, Line 106-111)

“Single-arm studies were excluded to maintain the quality of included studies. The results of the non-RCTs were used only for the analysis of adverse events. Studies that did not compare the treatment effects of imiquimod with those of a placebo or no intervention were only used to estimate absolute event rates in the imiquimod arm (for example, studies in which the interventions in the control arm was surgery).”

Comment/Question 5.

Introduction - No major comments. Appropriate commentary on previous publications and context provided, although did you consider seeing if there were studies which included use for treating VIN as it is an accepted off label use (Line 56)?

Response 5:

We very much appreciate the reviewer’s thoughtful comments. We also referred to studies of imiquimod for VIN, and cited some of them as reference (Ref 4, 5, and 6). However, since the purpose of our study was to evaluate the efficacy and safety of vaginal use of imiquimod, we did not include studies on VIN in our analyses.
Comment/Question 6.

Methods - Line 82 - What if the regression was to normal? Was that not accounted for?

Response 6:
We appreciate this helpful comment. Regression to normal was also included, but the explanation was not enough. Following their advice, we have modified the sentence.

(Page 5, Lines 92-96)

“The primary outcomes were histological regression of the disease (primary efficacy outcome), including downgrading from high-grade lesions (CIN 2–3 and VaIN 2–3) to normal findings or low-grade lesions (CIN 1 and VaIN 1), and treatment discontinuation due to side effects (primary safety outcome).”

Comment/Question 7.

Line 103 – Was the trade name “Aldara” or “Zyclara” considered for searches?

Response 7:
We thank Reviewer #3 for their insightful comment. Since we did not include the trade name “Aldara” or "Zyclara", we conducted an additional search using the following search terms. As a result, 10 additional records (2 in PubMed, 2 in CENTRAL, 3 in ClinicalTrials.gov, 3 in WHO registry, and 0 in ISRCTN registry) were identified in the search, none of which were clinical studies on imiquimod for CIN or VaIN. Therefore, we believe that our search strategy provided in the article was adequate.

<PubMed search>
#1 "aldara"[All Fields]
#2 "zyclara" [All Fields]
#3 "genital diseases, female"[MeSH Terms]
#4 "genital neoplasms, female"[MeSH Terms]
#5 ("vagina"[MeSH Terms] OR "vagina"[All Fields] OR "vaginal"[All Fields] OR "vaginally"[All Fields] OR "vaginals"[All Fields] OR "vaginitis"[MeSH Terms] OR "vaginitis"[All Fields] OR "vaginitides"[All Fields]) AND ("carcinoma in situ"[MeSH Terms] OR ("carcinoma"[All Fields] AND "situ"[All Fields]) OR "carcinoma in situ"[All Fields] OR ("intraepithelial"[All Fields] AND "neoplasia"[All Fields]) OR "intraepithelial neoplasia"[All Fields])
#6 "uterine cervical dysplasia"[MeSH Terms] OR ("uterine"[All Fields] AND "cervical"[All Fields] AND "dysplasia"[All Fields]) OR "uterine cervical dysplasia"[All Fields] OR ("cervical"[All Fields]
AND "intraepithelial"[All Fields] AND "neoplasia"[All Fields]) OR "cervical intraepithelial neoplasia"[All Fields]
#7 (#1 OR #2) AND (#3 OR #4 OR #5 OR #6)

<CENTRAL>
#1 All text: aldara
#2 All text: zyclara
#3 MeSH descriptor: [Uterine Cervical Dysplasia] explode all trees
#4 MeSH descriptor: [Genital Neoplasms, Female] explode all trees
#5 MeSH descriptor: [Genital Diseases, Female] explode all trees
#6 #1 or #2
#7 #3 or #4 or #5
#8 #6 and #7

<ClinicalTrials.gov>
Other terms, “aldara”
Other terms, “zyclara”

<WHO>
“aldara”
“zyclara”

<ISRCTN>
Text search, “aldara”
Text search, “zyclara”
Comment/Question 8.
Line 127 - I presume this was IIT and even if they stopped therapy or crossed over they remained in their assigned group?

Response 8:
We very much appreciate the reviewer’s constructive comments. To examine adverse effects, we included women who started the allocated treatment since the incidence of adverse effects might be underestimated by including women who did not start the allocated treatment.
To simplify the study protocol, we did not conduct intention-to-treat analyses when investigating treatment effects. However, as Reviewer #3 has pointed out, intention-to-treat analysis is more appropriate for investigating the treatment effects of imiquimod. Therefore, following their advice, we decided to perform intention-to-treat analyses when investigating the treatment effects of imiquimod. However, results were not much different from the previous results, and thus the discussion and the conclusions of this study were not affected.

(Page 11, Lines 235-240)
“Primary efficacy outcome (histological regression of the disease)
The pooled OR (95% CI) for the primary efficacy outcome was 4.05 (2.08–7.89) (Figure 2A, Table 3). Heterogeneity between studies was low (I² = 0%). The absolute risk difference was 0.34 (0.18–0.46). Appendix 4 presents a funnel plot of this analysis. The pooled response rate (95% CI) in the imiquimod arm (sensitivity analysis) was 0.61 (0.52–0.70) (Figure 2B, Table 4).”

Also, we have modified the text as follows.
(Page 7, Line 142-146)
“We conducted intention-to-treat analyses of treatment effects. For adverse events, patients who started the allocated treatment were analyzed since including patients who did not start the allocated treatment can lead to the underestimation of the risk of adverse events.”

Comment/Question 9.
Line 139 - How was the cut point for dosing chosen?

Response 9:
The cut-off for the dichotomization was pre-specified in the protocol (PROSPERO: CRD42022377982). This is because in our clinical experience, applying 12.5mg of imiquimod three...
times a week showed a good response in women with high-grade VaIN (Ref 17). The cut-off point is half of our weekly dose.

We have added the following sentence to Methods.

(Page 8, Lines 164-165)

“The cut-off for the dichotomization was pre-specified based on our clinical experience.”

Comment/Question 10.

Results - Line 172-4 - Consider noting the timing of the LEEP from this RCT.

Response 10:

We have added this information to the footnotes of Table 1.

Comment/Question 11.

Line 223-224 - Really wide confidence intervals present.

Response 11:

We appreciate this opportunity to clarify. As Reviewer #3 has pointed out, the confidence intervals are, indeed, wide. We showed the confidence intervals to show the imprecision of the point estimate.

Comment/Question 12.

Discussion - Line 344 - Also consider noting evaluation of other ways like premedication to mitigate side effects

Response 12:

We agree with the reviewer’s proposal and have revised the sentence.

(Page 18-19, Lines 416-417)

“Further studies are needed to optimize the method of imiquimod administration and mitigate adverse events.”
Comment/Question 13.

Tables - Table 1 - If available consider adding the range and/or St. deviation for the median or mean age listing.

Table 2 - No comments

Response 13:

We have revised Table 1 in accordance with the reviewer’s suggestion. However, it might be confusing since participants’ age descriptions were different between the studies: mean ± standard deviation, median (interquartile range), or median (range). If the editor would consider the previous simplified version more appropriate, we would like to revise Table 1 again.

Comment/Question 14.

Table 3 - Is the commentary on High, medium and low certainty standardly listed here.

Response 14:

Correct. We followed the Cochrane methodology. For example, the same description is used in a recently published Cochrane Review.


Comment/Question 15.

Table 4 - No comments

Response 15: No change requested.

Comment/Question 16.

Figures - Figure 1 - Please confirm the numbers are correct for the progression through exclusions.

Figure 2/3 - No comments.

Response 16:

We very much thank Reviewer #3 for the clarification. The numbers in the figures were correct, but the results of the full-text screening were, indeed, difficult to understand. We have revised Figure 1 and added the total number of excluded records in the full-text screening (n = 34). The total number of excluded records in the first screening was also added (n = 580).
Responses to the Statistical Editor Comments

Comment/Question 1.

Fig 2B and Fig 3: Need to include columns for weight assigned to each row entry. Should also include in all supplemental material with forest plots which do not currently include column of weights.

Response 1:

We are very thankful to the Statistical Reviewer for their important and constructive suggestions. We agree with the Editor that showing the weight of each study in the meta-analysis would be useful. However, the method (GLMM) we used for the analysis does not provide study weights. Alternatively, we could have used the inverse variance method after a logit transformation. However, this method is biased for the cases of small event rates. Another alternative would be to use the arcsine transformation, but the individual study results are hard to interpret with this method. Moreover, Schwarzer et al. write (Added as Ref 24):

"Accordingly, we support the viewpoint of previous works, recommending the use of GLMMs for the meta-analysis of single proportions. From our perspective, the only disadvantage of a GLMM is that individual study weights are not available, which we consider as a minor drawback."

Thus, we have decided to use a GLMM as our primary analysis. To allow, however, our readers to assess the impact of the studies in the meta-analysis as the Editor suggests, we have now added a sensitivity analysis using arcsine in the appendix (Appendix 7). There, we also show the study weights.

Accordingly, we have added a description of this analysis.

Methods (Page 8, Lines 158-160)

“We also performed sensitivity analyses using the inverse variance method with arcsine transformation to provide individual study weights, because these weights are not available in meta-analyses of proportions using a generalized linear mixed model.24”

Results (Page 13, Lines 279-281).

“Appendix 7 describes results of the post-hoc sensitivity analyses for meta-analysis of proportions using the inverse variance method with arcsine transformation, where the individual study weights are available.”
Comment/Question 2.

For the primary outcome, should include funnel plots, which could be in supplemental material.

Response 2:

We appreciate this helpful suggestion. In our analysis we have followed the Cochrane handbook's advice (https://handbook-5-1.cochrane.org/chapter_10/10.4.3.1_recommendations_on_testing_for_funnel_plot_asymmetry.htm), where it states that "As a rule of thumb, tests for funnel plot asymmetry should be used only when there are at least 10 studies included in the meta-analysis because when there are fewer studies the power of the tests is too low to distinguish chance from real asymmetry". Thus, we did not test for funnel plot asymmetry since our analyses included less than 10 studies. At the same time, we agree with the editor that a funnel plot is a good visualization tool. Thus, following this comment, we have now decided to include a funnel plot for the primary outcome as Appendix 4, but not to perform the test for funnel plot asymmetry, following Cochrane's recommendations.

We have added the following sentence to the manuscript.

Results (Page 11, Lines 238-239)

“Appendix 4 presents a funnel plot of this analysis.”

We believe that our responses and changes adequately address the Reviewers’ and Editors’ comments.

Thank you again for your comments on our paper. We trust that the revised manuscript is suitable for publication.