nature portfolio

Peer Review File

DNA Immunotherapy for Recurrent Respiratory Papillomatosis (RRP): Phase 1/2 study assessing efficacy, safety, and immunogenicity of INO-3107

Corresponding Author: Dr Matthew Morrow

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
1.What are the noteworthy results?
INO-3107 immunotherapy significantly reduced the need for surgeries in patients with Recurrent Respiratory Papillomatosis (RRP), and induced HPV-6 and HPV-11 specific T-cell responses. 81% of patients experienced a reduction in surgery frequency post-treatment.
2.Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.
Compared to existing literature, this work introduces DNA immunotherapy, an innovative approach with considerable clinical potential, demonstrating a high degree of originality.
3.Does the work support the conclusions and claims, or is additional evidence needed?
The study's findings support its conclusions, particularly in terms of reduced surgery frequency and enhanced immune response.
4.Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?
The data analysis and conclusions are generally sound, but the analysis of differences between responders and non-responders could be more detailed.
5.Is the methodology sound? Does the work meet the expected standards in your field?
The methodology adheres to industry standards, particularly the use of DNA immunotherapy combined with electroporation. However, the lack of a detailed power analysis and explanation of sample size determination could affect the robustness of the conclusions.
6.Is there enough detail provided in the methods for the work to be reproduced?
While the methods section provides sufficient detail, more information on the immune response assessments and specific experimental steps is necessary for full reproducibility. A clearer description of how immune responses were evaluated would enhance the transparency and reproducibility of the study.

Review Comments:
Methods Section:
1.Appropriateness of Methods: The study employs a reasonable Phase 1/2 clinical trial design, with clearly defined safety and efficacy endpoints. The use of intramuscular injection followed by electroporation is innovative and appropriate for inducing localized immune responses. The sample size (32 patients) and measurable outcomes (T-cell responses, surgical frequency) are suitable for assessing treatment efficacy. However, the manuscript lacks a detailed power analysis, raising concerns about the sample size and generalizability of the results. Additionally, although the manuscript mentions a subset of non-responders, it does not sufficiently explore their characteristics. A comparative analysis between responders and non-responders should be included.
2.Statistical Deficiencies: While the statistical analysis on page 8 mentions 95% confidence intervals, it lacks clarity on how the variability in patient responses was handled. It is recommended to explain how missing data or incomplete follow-up were addressed in the analysis.
3.Clinical Assessment: The use of CR and PR in the assessment is not the first application, please provide references.

Results Section:

1.Accuracy of Results Description: The results section accurately describes the reduction in surgical frequency after INO-3107 treatment. Most patients (81%) showed clinical responses, with 9 patients experiencing no need for further surgeries. This outcome is clearly presented and demonstrates the potential of INO-3107. Additionally, the article thoroughly describes the clonal expansion of HPV-specific T-cells, confirming the induction of specific immune responses. However, while the article mentions that response was independent of HPV genotype (HPV-6 vs. HPV-11), more in-depth analysis of the differences between HPV subtypes could be provided.
2.Areas for Improvement:
(1)On page 10, where surgical reduction is discussed, more detail should be provided on improvements in clinical symptoms (e.g., voice quality, respiratory function) beyond just the frequency of surgeries.
(2)Regarding immune responses, further exploration of the correlation between the strength of T-cell responses and clinical outcomes is suggested. For example, does a stronger T-cell response correlate with a more significant reduction in surgeries? This relationship is implied but not clearly analyzed in the manuscript.
(3)It would be beneficial to include data on the long-term maintenance of immune responses in follow-up, which could help understand the lasting efficacy of the therapy.

Discussion Section:
1.Depth of Discussion: The discussion thoroughly analyzes the potential of INO-3107 in RRP treatment, particularly focusing on the HPV-specific T-cell responses. However, the long-term maintenance of immune responses and the sustainability of treatment effects are not explored in-depth. For instance, whether the expanded T-cell clones can sustain control of the virus remains an important question. Additionally, while safety is discussed, the applicability of INO-3107 to different age groups (such as pediatric vs. adult patients) could be further addressed.
2.Suggestions and New Ideas: The paper appropriately suggests that larger, randomized trials are necessary for future validation, but it could also propose exploring combination therapies with other immunotherapies or anti-angiogenic treatments, particularly for difficult or recurrent cases of RRP. Furthermore, the potential of INO-3107 in younger patients could be explored, as this group constitutes a significant portion of RRP cases.
3.Limitations: The limitations are reasonably described on page 14, including the small sample size and short follow-up period. These limitations are valid, but the manuscript could further elaborate on potential selection bias and the differences in treatment response between HPV subtypes. Additionally, the need for long-term follow-up data should be emphasized to confirm the sustained effects of the treatment.

References:
1.Accuracy and Completeness: The references are generally accurate, but the second published paper (Norberg et al., 2023)(nihms-2007116) should be cited, particularly when discussing the efficacy of immunotherapies in RRP. This would provide a broader context for the findings and reinforce the discussion on immunotherapy as a promising approach for RRP.
2.Missing Citations: The study could benefit from additional citations related to the role of the tumor microenvironment in immune responses, particularly regarding T-cell exhaustion or immune checkpoint inhibitors.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
The manuscript by Morrow et al. titled „DNA Immunotherapy for Recurrent Respiratory Papillomatosis (RRP): Phase 1/2 study assessing efficacy, safety and immunogenicity of INO-3107“ is an open-label single arm clinical trial performed in 8 centers in the USA. INO-3107, a DNA plasmid encoding E6/E7 oncogenes from HPV-6 and HPV-11, combined with a gene encoding IL-12 to enhance immune response was applied on day 0, weeks 3, 6, and 9 by intramuscular injection accompanied by electroporation. Only one dosage was tested. Altogether 32 patients with RRP were enrolled. Gender and race were not considered in the selection of the patients. All participants were required to undergo surgical intervention approximately 14 days following the initial treatment. The follow-up period lasted for 52 weeks, which included a duration of 43 weeks post the last injection.

The primary endpoints were the safety and tolerability. Both the primary endpoints were confirmed.

Secondary endpoints were several. First – efficacy. Here the number of surgical interventions for one year after the first application of the treatment was compared to the number of surgical interventions a year before the start of the treatment. Since the decision to treat or observe for RRP is highly subjective, and at least before the treatment it has been done without specified and controlled conditions, I consider this a weak point of the study. Additionally, one year of pre- and after-treatment observation is not long considering the random intervals of the lesions' occurrence. Last but not least, the parameter for selecting the patients for complete, partial and overall responses is very tight considering already mentioned variable intervals of occurrence of the surgery necessitating lesions and the length of the follow-up.

Even more important is the fact that there has been surgery performed after the beginning of the treatment even in cases with no lesions (not reported, information is missing, if I understand correctly (see comment below)), and this intervention can influence the frequency of recurrences since antigens released from the lesions during the surgery might add to the treatment effect. This should be discussed as a limitation of the study.

However, efficacy was assessed additionally by changes in the RRP Severity Score and by antigen-specific cellular immune responses. T-cell phenotype and lytic ability were measured by flow cytometry.

Additional comments

The manuscript is missing a more detailed methodological section. If not possible because of limits for the length of text it should be included in a supplement (HPV detection and typing, ELISPOT method, peptides for stimulation (the reference is for HPV16/18 specific peptides; in HR HPVs E6/7 have different characteristics, so discussion why HPV6/11 E6/7 derived peptides were used would be useful.)). Also, a clear specification of why for some tests fewer patients were available is needed. Such details are not even specified in the protocol of the study. The methodological section doesn´t provide enough detail for the work to be reproduced.

Immunology endpoints included clearance of HPV6 and 11 from the resected tissue. I don´t see why this result is listed between immunology endpoints as well as the level of ctHPV DNA. Further, serology of HPV-specific antibodies and analyses of pro-inflammatory and immunosuppressive elements in blood were mentioned as immunology endpoints. However, results on humoral antigen-specific response tested by ELISA and level of cfHPV DNA were not evaluated in this manuscript and should be taken away. Similarly, the profiling of differentially expressed miRNAs which is mentioned in the protocol is not mentioned in the manuscript since it was not evaluated.

Even though the number of samples (patients) analyzed by the particular method is mentioned in the correlative analysis paragraph, it should also be specified in tables and figure legends for each parameter and /or method together with reasons for their lacking.

In Figure 3D, graphs in the lower panel specification of the genes to which population of cells they belong will make it easier for the readers.

In Figure 3D the legend is not clear. The shapes in the network are not specified.

In Figure 4A it would be easier for the readers to attribute the genes to certain phenotypes/functions of the cells.

In conclusion, the trial phase 1/2 of RRP with DNA-based treatment has shown the safety and tolerability of the drug. It also showed promising data on the treatment response as documented by the increased number of cytotoxic and memory CD8+ T cells and by the number of specific TCRβ clones in responders both in PBMC and tissue and by the detection of changes in the expression of the proinflammatory response genes. Since no effective treatment exists for RRP except for invasive procedures, it is important to look for new ways how to lower the morbidity of RRP patients. As mentioned in the comments the weak point is the “non-standardized” pretreatment information about the number of surgeries reported only a year before the start of the treatment and short follow-up. Similar results were shown also for preventive vaccination which has a similar “treatment” effect in those patients (see doi: 10.1001/jamaoto.2022.1190) and this should be mentioned in the introduction. However, this type of research is very important and this data substantiates further clinical testing. Longer follow-up should show if the observed effect of the treatment will last or if it is just temporary.

Reviewer #4

(Remarks to the Author)
The manuscript by Morrow et al. assess the efficacy, safety, and immunogenicity of a DNA vaccine for treatment of HPV-associated papillomatosis. The manuscript centers around a recently completed Phase1/2 study evaluating a total of 32 patients. The clinical results are interesting and encouraging to further pursue DNA vaccine-based treatment approaches for this disease. While the study is overall interesting, the immunological analyses as well as overall data presentation requires work. While the immunological analyses take up the majority of real estate, there's an overall dearth on the employed methodologies making it hard to judge the work and interpret the data properly. Please find below a list of issues that need to be addressed:

1) While I appreciate the authors trying to provide big panels for the reviewers, having figures spread out over multiple pages makes it hard to evaluate the data and also raises concerns how the data will be ultimately presented when strict page-limits for figures are enforced. Two examples: Figure 4 spans 4 pages, and Extended Figure 3 takes the win with over 10 pages!!! Furthermore, several panels in main figures consist of several not sub-panels (e.g. Figure 4B and 4C consist of 5 sub-panels), making it hard to figure out where what is shown.

2) Line 110 lacks the reference to Derkay et al
3) Please clarify what the components of INO-3107 are. Even after reading the entire manuscript the reader will not know what the contained antigens are.
4) Related to point 3; What does stimulation with HPV-6/11 peptides mean? Only vaccine contained antigens, all E proteins,

all HPV proteins? What kind of peptides? The referenced study was working on HPV16/18 but also didn't say whether they are 10-mers, 15-mers, 20-mers or what. This is crucial to properly interpret the data.
5) How was the combined HPV6 and 11 response determined? Stimulation with a pool of peptides or mathematical combination of the individual responses?
What's the purpose of the /10E6 PBMC y-axes labels? Fold-change is already a relative term. It would be however helpful to show the number of SFU/10E6 PBMCs at baseline to get an idea about the magnitude of the responses.
6) The experiments shown in Figure 2B onwards require further clarification, especially the authors refer to a previous manuscript for procedures. What am I looking at? I assume these are in vitro expanded cells, as TIM-3 is usually not present in human PBMCs ex vivo, especially not with such a beautiful signal. Which brings me to my question what can we learn from these data? Do these data suggest a difference in the type of immune response elicited or are they simply just a readout of more cells being present prior to the expansion? I.e. what do these data tell you about the phenotype of the cells in vivo? Are the 6 immunological "non-responders" in the summary Figure 2B truly all zero, or did the authors equate negative responses to zero?
Also, how do the authors reconcile their ELISPOT data from 2A with 2B? Figure 2A suggests on overage a 8-16 fold increase, whereas Figure 2B suggests that the average increase is 1%. How representative are the flow plots in Figure 2B? BTW, I do not see any trend with TIM3 and LAG3 regarding response.
7) How was the TCR determined from PBMCs? DNA or RNA-based? Sort-purified T cells or total PBMCs? How many cells were analyzed?
8) What samples have been used for airway tissue analysis? Pre-treatment samples are likely from resected papillomas. Where are the post-treatment samples from? I couldn't find any description when and how these were obtained.
9) There is no Table 3 and the actual Figure 3D is not mentioned in the figure legend nor text.
10) Any stats to substantiate the claim of increases in enrichment scores in Ext. Fig. 6A?
11) The absent and present associations shown in Figure 4B should be shown/quantified. Does it reach statistical significance?
12) Line 349: What does increase in strength of correlation mean? A p value but no r value is shown to quantify the strength.
13) Regarding the T cell analyses, the authors focus on CD8+ T cells throughout their story. Is the vaccine only eliciting CD8+ T cells?
14) Lines 93-98 highlight several exploratory immunological endpoints of the study that were not addressed at all such as clearance of HPV from tissues, humoral immune responses, inflammatory and suppressive elements in blood, and cfHPV DNA. What about these?

Reviewer #5

(Remarks to the Author)
Thank you for the opportunity to review this manuscript, in which the authors described the results of a phase 1/2 trial of intramuscular INO-3107 in patients with HPV-associated recurrent respiratory papillomatosis. The trial was sponsored by Inovio Pharmaceuticals. Of note, a prior publication reported on the first 21 of the total 32 patients enrolled in the trial. The manuscript is very well written (please note a typo on line 196) and presents detailed methods and results related to the correlative analyses performed. There were no safety issues with INO-3107, which showed encouraging signals of activity, both clinical and immunological, that certainly warrant the planned further trials. The limitations of this work are duly acknowledged by the authors, the main one being the single-arm design. A noteworthy feature of the manuscript and accompanying protocol is that no description is provided of the product composition (the same goes for the protocol available in ClinicalTrials.gov). I would suggest that at least a high-level description be provided. It should be noted that my expertise is limited to the clinical and statistical aspects of clinical-trial methodology, particularly in oncology.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
1、The expanded discussion of your findings is well-written, and I believe it strengthens the manuscript's overall impact. Your incorporation of relevant literature helps contextualize your results effectively.
2、The revised figures and tables are clearer and better organized, significantly improving the readability of the manuscript. I would recommend one final check for consistency in labeling and formatting, particularly with the figure legends, to ensure complete clarity for readers unfamiliar with the subject matter.
3、The conclusion section is now much more concise and focused, which is excellent. It provides a clear take-home message. It might be helpful to briefly mention potential avenues for future research in this area, as it would provide a natural progression from your findings and demonstrate the broader implications of your work.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3

(Remarks to the Author)
The authors have incorporated my comments and I have no further comments. I agree with the publication of the article and wish the authors that the results will be confirmed in further clinical trials.

Reviewer #4

(Remarks to the Author)
This manuscript has substantially improved and now provides the methodological details required to assess/interpret the presented results and conclusions. I appreciate the authors consolidating the main figures and will let the editorial office worry about the extended figures. I have two minor points that should be addressed:

In Figure 2 and the corresponding extended Figure: Is the information about the total HPV6+11 response important? The totality of the response is not mentioned anywhere. Also, based on the response to my previous question about the origin of this value (i.e. it being the mathematical sum), I would highly recommend removing it as it is highly misleading. The respective E proteins of HPV6 and HPV11 are 81-84% identical and thus the majority of readout HPV6 response will also respond to HPV11, meaning that the mathematical sum vastly overestimates the total response. I would simply remove this from the main figure and extended data.

Can you add the following qualifier to your statement in line 358? "…., we did not increases in enrichment scores in 4 out of 5 of these patients"

Authors Responses to Journal and Peer Review Comments

Please find below point-by-point Author Responses to Reviewer comments. We are very pleased to say we were able to address all comments, inclusive of clarifications, new text and new figures as requested by the Reviewers – all of which are identified both in the Author Response Document as well as in the redlined and cleaned version of the revised manuscript.

We have split each set of Reviewer comments into their own pages for ease of comprehension.

We look forward to responses to these revisions, and if anything else further is needed, please do let us know.

Thank you again for your consideration and review of our manuscript.

Reviewer #1 (Remarks to the Author):

1.What are the noteworthy results?
INO-3107 immunotherapy significantly reduced the need for surgeries in patients with Recurrent Respiratory Papillomatosis (RRP), and induced HPV-6 and HPV-11 specific T-cell responses. 81% of patients experienced a reduction in surgery frequency post-treatment.
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The study's findings support its conclusions, particularly in terms of reduced surgery frequency and enhanced immune response.
4.Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?
The data analysis and conclusions are generally sound, but the analysis of differences between responders and non-responders could be more detailed.
5.Is the methodology sound? Does the work meet the expected standards in your field?
The methodology adheres to industry standards, particularly the use of DNA immunotherapy combined with electroporation. However, the lack of a detailed power analysis and explanation of sample size determination could affect the robustness of the conclusions.
6.Is there enough detail provided in the methods for the work to be reproduced?
While the methods section provides sufficient detail, more information on the immune response assessments and specific experimental steps is necessary for full reproducibility. A clearer description of how immune responses were evaluated would enhance the transparency and reproducibility of the study.

Author Response: We thank the Reviewer for these comments and have addressed them using detailed responses to the more specific questions the Reviewer has provided below via additions to the manuscript as per the Reviewers suggestions.

Review Comments:
Methods Section:
1.Appropriateness of Methods: The study employs a reasonable Phase 1/2 clinical trial design, with clearly defined safety and efficacy endpoints. The use of intramuscular injection followed by electroporation is innovative and appropriate for inducing localized immune responses. The sample size (32 patients) and measurable outcomes (T-cell responses, surgical frequency) are suitable for assessing treatment efficacy. However, the manuscript lacks a detailed power analysis, raising concerns about the sample size and generalizability of the results.

Author Response: We thank the Reviewer for the comment. The objectives of this Phase 1/2 trial were to estimate effects of treatment, and on this basis, no power analysis is applicable. A sample size of 32 is appropriate for a rare disease such as RRP for the basis of estimating effects. The efficacy assessment results are structured on a per-patient basis using the patient's own previous clinical history of disease and surgical interventions as a control to compare outcomes against. The confirmatory Phase 3 trial of INO-3107 will be powered based on a statistical hypothesis.

1.Appropriateness of Methods: Additionally, although the manuscript mentions a subset of non-responders, it does not sufficiently explore their characteristics. A comparative analysis between responders and non-responders should be included.

Author Response: We thank the Reviewer for the comment. Per the Reviewer's feedback, Responder vs Non-Responder analyses (inclusive of isolation of Non-Responders, regression analyses of Responders and Non-Responders together, and head-to-head comparisons) now appear in the following sections: Figure 7A, B, C, Extended Figure 2, Extended Figure 3, Extended Figure 4, Extended Figure 7. To the Reviewers point, all analyses suggest differences between Responder and Non-Responder immune responses, which have been highlighted in the text across the entirety of the manuscript. A new passage with respect to airway tissue analyses was incorporated, which now states the following in the Results: "When assessed by clinical response, responders had significantly higher total CD8+, CD8+ Tem and CD8+ T cytotoxic scores at end of study tissue as compared to pre-treatment, while non-responders did not."

2.Statistical Deficiencies: While the statistical analysis on page 8 mentions 95% confidence intervals, it lacks clarity on how the variability in patient responses was handled. It is recommended to explain how missing data or incomplete follow-up were addressed in the analysis.

Author Response: Thank you for this question. The variability of response was handled by using the difference for each subject (post-treatment minus pre-treatment) as the analysis variable, which is a standard acceptable practice for a clinical trial for a rare disease such as RRP and has been used by other groups studying this disease. All subjects had complete follow-up in this trial. There were no missing or incomplete safety or efficacy data. Immunogenicity data was not available for all subjects, as detailed in the manuscript. Results reported are based on observed data; no imputation was used. These aspects have been clarified in the manuscript. These statements now appear in the Endpoints and Assessments as well as in the Results section of the manuscript.

3.Clinical Assessment: The use of CR and PR in the assessment is not the first application, please provide references.

Author Response: The definitions of CR and PR as described in the manuscript are aligned with methodology from groups working on T-cell based therapies for RRP. A reference has been added to the manuscript to clarify this.

Results Section:
1.Accuracy of Results Description: The results section accurately describes the reduction in surgical frequency after INO-3107 treatment. Most patients (81%) showed clinical responses, with 9 patients experiencing no need for further surgeries. This outcome is clearly presented and demonstrates the potential of INO-3107. Additionally, the article thoroughly describes the clonal expansion of HPV-specific T-cells, confirming the induction of specific immune responses. However, while the article mentions that response was independent of HPV genotype (HPV-6 vs. HPV-11), more in-depth analysis of the differences between HPV subtypes could be provided.

Author Response: We agree with the Reviewer that there was not sufficient clarity with respect to genotypic differences in clinical response to INO-3107. The text in the Results section for efficacy has been amended to state that HPV-6 ORR was 66.7% and HPV-11 ORR was 83.4%.

2.Areas for Improvement:
(1)On page 10, where surgical reduction is discussed, more detail should be provided on improvements in clinical symptoms (e.g., voice quality, respiratory function) beyond just the frequency of surgeries.

Author Response: We thank the Reviewer and have included additional details as requested. The Results section addressing RRP severity scores now details that both anatomical and symptom scores were obtained, there was no other assessment of voice quality (Voice Handicap Index) or of respiratory function beyond the reporting of signs or symptoms of respiratory distress as symptom scores or as adverse events. Anatomical disease severity was measured by the modified Derkay Score. Scores were obtained prior to surgery at or near Day 0, and again at each visit when laryngoscopy was performed. There was a mean change in total site score of -10.7 (95% CI -16.3, -5) between Day 365 and Day 0, and a mean change in total symptom score of -0.4 (95% CI -0.7, 0.0) between Day 365 and Day 0. The mean change in total clinical score (total site and total symptom score) was -11 (95% CI -16.7, -5.3), which demonstrates clinical benefit.

(2)Regarding immune responses, further exploration of the correlation between the strength of T-cell responses and clinical outcomes is suggested. For example, does a stronger T-cell response correlate with a more significant reduction in surgeries? This relationship is implied but not clearly analyzed in the manuscript.

Author Response: We agree with the Reviewer that strength of response as compared to clinical outcome is important to address. In this updated re-submission, the manuscript now describes in Figure 7B that stronger T-cell responses are observed in patients exhibiting a clinical response as compared to those who did not exhibit a clinical response and in 7C that there is a direct correlation between strength of T-cell response in airway tissue at the end of the study relative to the change in surgical frequency ($p=0.019$). Extended Figure 7 further details the strength of CD8+ T-cell response comparing responders to non-responders. The Results section has been edited to make this comparison clearer for readers as follows: Further analysis also revealed that the degree of change in T-cell infiltration in airway tissues after treatment with INO-3107 associates strongly with the change in surgery frequency on trial (Figure 7C $p=0.019$, and Extended Figure 7D $p=0.015$).

(3)It would be beneficial to include data on the long-term maintenance of immune responses in follow-up, which could help understand the lasting efficacy of the therapy.

Author Response: We agree with the Reviewer that long-term maintenance of immune response is important in the treatment of this disease. As it stands, this clinical study was designed to end 52 weeks following entry into the trial. Data for the Week 52 timepoint is presented in Figure 2C with respect to peripheral T-cell immunity and is described in the associated text in the Results section which discusses peripheral T-cell data. Data for tissue is noted in End of Study assessments displayed in Figures 3 through 7 and described through the result of the Results section. Immune assessments past Week 52 were not performed as the study had ended. The Discussion section of the manuscript has been amended to include that future clinical trials will address prolonged efficacy, immune response and redosing of INO-3107.

Discussion Section:
1.Depth of Discussion: The discussion thoroughly analyzes the potential of INO-3107 in RRP treatment, particularly focusing on the HPV-specific T-cell responses. However, the long-term maintenance of immune responses and the sustainability of treatment effects are not explored in-depth. For instance, whether the expanded T-cell clones can sustain control of the virus remains an important question.

Author Response: We agree with the Reviewer, and this point has been added to the discussion as follows: “The maintenance of this immune response should therefore be expected to be associated with prolonged impact on disease state, although this remains to be confirmed”.

1.Depth of Discussion: Additionally, while safety is discussed, the applicability of INO-3107 to different age groups (such as pediatric vs. adult patients) could be further addressed.

Author Response: We agree with the Reviewer, and while there are no data for the pediatric population at this time, we recognize the burden of RRP disease in the pediatric population. Because we do not yet have clinical data that support the investigation of INO-3107 in children, we have opted at this time to leave reference to this population out, however in the introduction we recognize that this is a disease that affects both adults and children. To that end, the manuscript now states “Future investigations could include studies of INO-3107 in the pediatric population” in the Discussion section.

2.Suggestions and New Ideas: The paper appropriately suggests that larger, randomized trials are necessary for future validation, but it could also propose exploring combination therapies with other immunotherapies or anti-angiogenic treatments, particularly for difficult or recurrent cases of RRP. Furthermore, the potential of INO-3107 in younger patients could be explored, as this group constitutes a significant portion of RRP cases.

Author Response: We agree with the Reviewer, although as the Reviewer is likely aware, single-agent data with immune checkpoint inhibitors for the treatment of RRP has, to date, not been successful. It is true, though, that in cases of pulmonary disease, an approach including INO-3107 with a potential second therapy could be considered, if it were to be determined that such an approach would be warranted. We also agree that the pediatric population should be addressed in the future, and we have added a sentence to this end.

3.Limitations: The limitations are reasonably described on page 14, including the small sample size and short follow-up period. These limitations are valid, but the manuscript could further elaborate on potential selection bias and the differences in treatment response between HPV subtypes. Additionally, the need for long-term follow-up data should be emphasized to confirm the sustained effects of the treatment.

Author Response: We thank the Reviewer for noting additional limitations regarding selection bias and the need for long-term data. With respect to selection bias, while it is true that investigators may have chosen appropriate patients for this trial (as is always the case in any clinical trial), the study population consisted of both HPV-6 and HPV-11 infected patients, all patients required at least two surgeries in the prior year, were enrolled across 8 sites to reduce potential bias introduced by a single site study, and had a range of 2-8 surgeries the prior year, ensuring that each patient was suffering from moderate to severe disease and met the definition of recurrent disease. These statements are now included in the manuscript in the Study Design and Patients section. With respect to HPV subtype, data indicate that response was similar between subtypes, as noted in the figures and the updated text. With respect to long-term follow up, we recognize this need and look forward to the opportunity to collect these data to demonstrate durability of clinical response and the manuscript now reflects this.

References:

1.Accuracy and Completeness: The references are generally accurate, but the second published paper (Norberg et al., 2023)(nihms-2007116) should be cited, particularly when discussing the efficacy of immunotherapies in RRP. This would provide a broader context for the findings and reinforce the discussion on immunotherapy as a promising approach for RRP.

Author Response: We thank the Reviewer for this feedback and have included the Norberg reference in the discussion in the context of immunotherapy as a promising approach for RRP in the Introduction.

2.Missing Citations: The study could benefit from additional citations related to the role of the tumor microenvironment in immune responses, particularly regarding T-cell exhaustion or immune checkpoint inhibitors.

Author Response: We thank the Reviewer for this feedback and have included references to tumor microenvironment as well as monoclonal antibodies targeting immune-related molecules inclusive of PD1/PDL1, TGFβ and VEGFA in the discussion as well as specifically calling out these metrics in the Discussion.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Author Response: We thank the Reviewer for facilitating training in peer review.

Reviewer #3 (Remarks to the Author):

The manuscript by Morrow et al. titled „DNA Immunotherapy for Recurrent Respiratory Papillomatosis (RRP): Phase 1/2 study assessing efficacy, safety and immunogenicity of INO-3107“ is an open-label single arm clinical trial performed in 8 centers in the USA. INO-3107, a DNA plasmid encoding E6/E7 oncogenes from HPV-6 and HPV-11, combined with a gene encoding IL-12 to enhance immune response was applied on day 0, weeks 3, 6, and 9 by intramuscular injection accompanied by electroporation. Only one dosage was tested. Altogether 32 patients with RRP were enrolled. Gender and race were not considered in the selection of the patients. All participants were required to undergo surgical intervention approximately 14 days following the initial treatment. The follow-up period lasted for 52 weeks, which included a duration of 43 weeks post the last injection.

The primary endpoints were the safety and tolerability. Both the primary endpoints were confirmed.

Secondary endpoints were several. First – efficacy. Here the number of surgical interventions for one year after the first application of the treatment was compared to the number of surgical interventions a year before the start of the treatment. Since the decision to treat or observe for RRP is highly subjective, and at least before the treatment it has been done without specified and controlled conditions, I consider this a weak point of the study. Additionally, one year of pre- and after-treatment observation is not long considering the random intervals of the lesions' occurrence. Last but not least, the parameter for selecting the patients for complete, partial and overall responses is very tight considering already mentioned variable intervals of occurrence of the surgery necessitating lesions and the length of the follow-up.

Author Response: We appreciate the Reviewer’s response and recognize that the decision to perform surgery is subjective. However, on this trial we ensured that the decision to perform surgery among individual patients was made by their treating laryngologist, in order to maintain consistency in the time period prior to and following administration of INO-3107. The clinical trial protocol mandated that prior to surgery, both anatomical and symptom severity (modified Derkay) scores be obtained, and that the rationale for surgery be clearly documented in the case report forms. This information has been expanded in the manuscript in the Methods and Results sections.

With respect to the duration of pre-exposure history and post-exposure follow-up, while the decision to enroll was based on a minimum of two surgeries in the prior year, each patient

had up to three years of prior history, documenting the severity of their disease as consistent across those years (data not shown). With respect to longer term follow up, we recognize this need and look forward to the opportunity to collect these data to demonstrate durability of clinical response, which is now highlighted in the manuscript in the Discussion.

Reviewer #3, Continued:

Even more important is the fact that there has been surgery performed after the beginning of the treatment even in cases with no lesions (not reported, information is missing, if I understand correctly (see comment below)), and this intervention can influence the frequency of recurrences since antigens released from the lesions during the surgery might add to the treatment effect. This should be discussed as a limitation of the study.

Author Response: We understand the Reviewers concern and wish to clarify. In our study, no subject had surgery performed at the start of the trial in cases where there was no disease present. Every patient enrolled on the trial required a surgery secondary to clinically relevant disease as documented by a baseline anatomical and symptom severity (modified Derkay) score. The surgery performed at or before Day 0 was what defined the timing of the first dose of INO-3107, and not vice-versa. This important issue is noted in the Methods section which states "Patients must have been judged by the investigator to be appropriate candidates for an upcoming surgical intervention." Because every surgery matters to patients with RRP, no surgery was performed that was not absolutely clinically necessary during the conduct of the trial (i.e., surgery was not conducted during the course of the trial if no lesion was present). With respect to antigen stimulation post-surgery, indeed this mechanism of action may be contributing to the effect of INO-3107, but it is clear that in the absence of INO-3107, such an effect would not be seen, as the clinical benefit seen in this trial is not consistent with a surgical effect alone.

Additional comments

1. The manuscript is missing a more detailed methodological section. If not possible because of limits for the length of text it should be included in a supplement (HPV detection and typing, ELISPOT method, peptides for stimulation (the reference is for HPV16/18 specific peptides; in HR HPVs E6/7 have different characteristics, so discussion why HPV6/11 E6/7 derived peptides were used would be useful.)). Also, a clear specification of why for some tests fewer patients were available is needed. Such details

are not even specified in the protocol of the study. The methodological section doesn´t provide enough detail for the work to be reproduced.

Author Response: We thank the Reviewer for highlighting these additional details, which are now included in the methods section of the manuscript. HPV-6 and/or HPV-11 were detected historically and/or at baseline by HPV DNA genotyping followed by HPV-specific qPCR. For ELISpot, PBMCs were stimulated *in vitro* with pools of peptides spanning HPV-6 or HPV-11 E6/E7 antigens relevant to treatment with INO-3107. Variability in sample number included in analyses was due to sample availability and/or the type of comparison that was being made.

2. Immunology endpoints included clearance of HPV6 and 11 from the resected tissue. I don´t see why this result is listed between immunology endpoints as well as the level of ctHPV DNA. Further, serology of HPV-specific antibodies and analyses of pro-inflammatory and immunosuppressive elements in blood were mentioned as immunology endpoints. However, results on humoral antigen-specific response tested by ELISA and level of cfHPV DNA were not evaluated in this manuscript and should be taken away. Similarly, the profiling of differentially expressed miRNAs which is mentioned in the protocol is not mentioned in the manuscript since it was not evaluated.

Author Response: We agree with the Reviewer and have edited the manuscript to include only endpoints for which data were generated and are discussed in the text.

3. Even though the number of samples (patients) analyzed by the particular method is mentioned in the correlative analysis paragraph, it should also be specified in tables and figure legends for each parameter and /or method together with reasons for their lacking.

Author Response: We thank the Reviewer for highlighting these missing details, which have now been added to the figure legends and elaborated upon in the methods section of the manuscript. Any analyses involving fewer than the total number of study patients (N = 32) were due to sample availability and/or determined by the type of comparison that was being made.

4. In Figure 3D, graphs in the lower panel specification of the genes to which population of cells they belong will make it easier for the readers.

Author Response: We thank the Reviewer for highlighting that clarity is needed regarding this panel. We have moved this panel to Extended Data Figure 6 and specified gene function in the figure legend as follows: CIITA, and CD74 are expressed in HLA class II

positive cells, FLT3LG is also expressed in HLA class II positive cells, with emphasis on dendritic cells. CSF1 is involved in the proliferation, differentiation, and survival of monocytes and macrophages, which are cell types that express CD14. XCR1 is expressed on dendritic cells.

5. In Figure 3D the legend is not clear. The shapes in the network are not specified.

Author Response: We thank the Reviewer for highlighting the need to expand the legend for Figure 3D. Legends have been added to both this figure (now Figure 5B) and Extended Data Figure 5B.

6. In Figure 4A it would be easier for the readers to attribute the genes to certain phenotypes/functions of the cells.

Author Response: We thank the Reviewer for this suggestion. We have specified gene functions in the figure legend (now Figure 6), as they pertain to each CD8+ T-cell phenotype graphed, as follows: CD3D/E/G and CD8A are T-cell identification markers. CD27 encodes a receptor required for activation of CD8+ T-cells. EOMES and TBX21 are transcription factors expressed in activated CD8+ T-cells. CTSW, GRZA, GZMK, FASLG and PRF1 encode lytic proteins that induce T-cell killing of target cells. Both CX3CR1 and CXCR6 chemokines promote survival, maintenance and migration of CD8+ T-cells.

7. In conclusion, the trial phase 1/2 of RRP with DNA-based treatment has shown the safety and tolerability of the drug. It also showed promising data on the treatment response as documented by the increased number of cytotoxic and memory CD8+ T cells and by the number of specific TCRβ clones in responders both in PBMC and tissue and by the detection of changes in the expression of the proinflammatory response genes. Since no effective treatment exists for RRP except for invasive procedures, it is important to look for new ways how to lower the morbidity of RRP patients. As mentioned in the comments the weak point is the “non-standardized” pretreatment information about the number of surgeries reported only a year before the start of the treatment and short follow-up.

Author Response: Thank you, please see the Author response above.

8. Similar results were shown also for preventive vaccination which has a similar “treatment” effect in those patients (see doi: 10.1001/jamaoto.2022.1190) and this should be mentioned in the introduction.

Author Response: We thank the Reviewer for calling our attention to this this publication. The introduction has been edited to call out this approach, with this study specifically cited.

9. However, this type of research is very important and this data substantiates further clinical testing. Longer follow-up should show if the observed effect of the treatment will last or if it is just temporary.

Author Response: Thank you; please see our response, above.

Reviewer #4 (Remarks to the Author):

The manuscript by Morrow et al. assesses the efficacy, safety, and immunogenicity of a DNA vaccine for treatment of HPV-associated papillomatosis. The manuscript centers around a recently completed Phase1/2 study evaluating a total of 32 patients. The clinical results are interesting and encouraging to further pursue DNA vaccine-based treatment approaches for this disease. While the study is overall interesting, the immunological analyses as well as overall data presentation requires work. While the immunological analyses take up the majority of real estate, there's an overall dearth on the employed methodologies making it hard to judge the work and interpret the data properly. Please find below a list of issues that need to be addressed:

1) While I appreciate the authors trying to provide big panels for the reviewers, having figures spread out over multiple pages makes it hard to evaluate the data and also raises concerns how the data will be ultimately presented when strict page-limits for figures are enforced. Two examples: Figure 4 spans 4 pages, and Extended Figure 3 takes the win with over 10 pages!!! Furthermore, several panels in main figures consist of several not sub-panels (e.g. Figure 4B and 4C consist of 5 sub-panels), making it hard to figure out where what is shown.

Author Response: We apologize to the Reviewer for involving multiple pages per figure. The manuscript has been updated to reflect a single Figure per page, and multi-page figures have now been broken into separate individual figures for ease of interpretation.

2) Line 110 lacks the reference to Derkay et al

Author Response: We thank the Reviewer for calling our attention to this oversight. The reference and number have been added to the manuscript.

3) Please clarify what the components of INO-3107 are. Even after reading the entire manuscript the reader will not know what the contained antigens are.

Author Response: The components of INO-3107 are now stated in the Treatment section of the manuscript.

4) Related to point 3; What does stimulation with HPV-6/11 peptides mean? Only vaccine contained antigens, all E proteins, all HPV proteins? What kind of peptides? The referenced

study was working on HPV16/18 but also didn't say whether they are 10-mers, 15-mers, 20-mers or what. This is crucial to properly interpret the data.

Author Response: We thank the Reviewer for highlighting these missing details for peptide stimulation, which have now been added to the methods section of the manuscript. Briefly, PBMCs were stimulated *in vitro* with pools of 15-mer peptides overlapping by 11 amino acids and spanning HPV-6 or HPV-11 E6/E7 antigens which are the antigens encoded by INO-3107.

5a) How was the combined HPV6 and 11 response determined? Stimulation with a pool of peptides or mathematical combination of the individual responses?

Author Response: We thank the Reviewer for highlighting these missing details, which have now been added to the methods section of the manuscript. Outputs from HPV-6 and HPV-11 peptides stimulations independent of one another were summed together in order to provide readers with a view of what an "overall" response to INO-3107 components looks like when taking all components into account.

5b)What's the purpose of the /10E6 PBMC y-axes labels? Fold-change is already a relative term. It would be however helpful to show the number of SFU/10E6 PBMCs at baseline to get an idea about the magnitude of the responses.

Author Response: We thank the Reviewer for this question. Y-axes for Figure 2A and Extended Data Figure 1 (top panel) have been updated. Additional graphs displaying screening versus peak post-treatment IFN-γ responses have been added to Extended Data Figure 1.

6a) The experiments shown in Figure 2B onwards require further clarification, especially the authors refer to a previous manuscript for procedures. What am I looking at? I assume these are in vitro expanded cells, as TIM-3 is usually not present in human PBMCs ex vivo, especially not with such a beautiful signal. Which brings me to my question what can we learn from these data?

Author Response: We thank the Reviewer for their comment. We added clarity to the legend for Figure 2B and details regarding procedures for flow cytometry to the Methods section of the manuscript. Figure 2B supports characterization of pre- and post-treatment peripheral CD8+ T-cell responses to *in vitro* stimulation with HPV peptides as assessed by multiparametric flow cytometry as now described in the Methods section. The data show post-treatment increases in CD8+ T-cells that can recognize antigen, activate in response

to that antigen, and display lytic potential. Altogether these data illustrate the enhanced capability of RRP patients to mount an HPV-specific, peripheral cytolytic T-cell response upon treatment with INO-3107. This assay has been reported on previously including, but not limited to, the following references:

Mau, T., et al. Interim Results of a Phase 1/2 Open-Label Study of INO-3107 for HPV-6 and/or HPV-11-Associated Recurrent Respiratory Papillomatosis. *Laryngoscope* **133**, 3087-3093 (2023).

Aggarwal, C., et al. Immunotherapy Targeting HPV16/18 Generates Potent Immune Responses in HPV-Associated Head and Neck Cancer. *Clin. Cancer. Res.* **25**, 110-124 (2019).

Trimble, C. L., et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. *Lancet* **386**, 2078-2088 (2015).

6b) Do these data suggest a difference in the type of immune response elicited or are they simply just a readout of more cells being present prior to the expansion? I.e. what do these data tell you about the phenotype of the cells in vivo? Are the 6 immunological "non-responders" in the summary Figure 2B truly all zero, or did the authors equate negative responses to zero?

Author Response: We thank the Reviewer for this question. The data are descriptive of the HPV-specific CD8+ T-cell response (specifically those being CD38+PD1+Prf+ as noted in the figure) as being increased over baseline for all but six patients, as noted by the Reviewer. The figure answers the question of "does treatment with INO-3107 induce HPV specific CD8+ T-cells of cytolytic phenotype above what is noted prior to treatment" i.e., "is there an increase in immune response" for which the answer is yes, aside from those six patients. For the six patients that do not exhibit an increase, two of them have true values of zero, and four have negative responses. The four non-zero values are set to zero based on the data presentation format, which is focused on observation of increased response relative to pretreatment. A statement has been added to the figure legend to clarify this.

6c)Also, how do the authors reconcile their ELISPOT data from 2A with 2B? Figure 2A suggests on overage a 8-16 fold increase, whereas Figure 2B suggests that the average increase is 1%. How representative are the flow plots in Figure 2B?

Author Response: The Reviewer is correct in interpretation of the data. The authors suggest that the assays as conducted do not generate 1:1 outputs that are able to be compared against one another as the Reviewer is suggesting due to the following: ELISpot looks at total PBMCs of a single function (IFNg production). Flow Cytometry is looking only at CD8+ T-cells and more specifically for co-expression of three independently regulated markers. Overall – they are different assays, with different sensitivities, asking different questions as currently conducted. If Flow Cytometry had been run to assess IFNg production and the numbers were discrepant against the ELISpot output, the Authors agree that a deep dive and reconciliation would be in order. But as these assays ask different questions of different cell types, it does not seem that a reconciliation is necessary for these data. Regarding the second part of the question, the Authors are not aware of a metric of representation to describe Figure 2B, but are comfortable stating that what is displayed in the figure is an accurate representation of the study population.

6d) BTW, I do not see any trend with TIM3 and LAG3 regarding response.

Author Response: Reference to TIM3 and LAG3 have been removed from the manuscript per the Reviewer's suggestion.

7) How was the TCR determined from PBMCs? DNA or RNA-based? Sort-purified T cells or total PBMCs? How many cells were analyzed?

Author Response: We thank the Reviewer for highlighting these missing details, which have now been added to the methods section of the manuscript. Briefly, RNA-based TCR sequencing was performed on a minimum of 1×10^6 viable PBMCs.

8) What samples have been used for airway tissue analysis? Pre-treatment samples are likely from resected papillomas. Where are the post-treatment samples from? I couldn't find any description when and how these were obtained.

Author Response: The Reviewer is correct that pre-treatment samples are from resected papillomas. Post-treatment samples are either resected papilloma (if any were present) or biopsy of initial disease site when possible. Post-treatment samples are from End of Study. The Methods section now more accurately reflects this.

9) There is no Table 3 and the actual Figure 3D is not mentioned in the figure legend nor text.

Author Response: The Reviewer is correct, and the text has been edited to properly call out Figure 3 data in the text as well as the Figure Legend.

10) Any stats to substantiate the claim of increases in enrichment scores in Ext. Fig. 6A?

Author Response: The claim of enrichment score is observational; no statistical increase is observed, as noted in the text.

11) The absent and present associations shown in Figure 4B should be shown/quantified. Does it reach statistical significance?

Author Response: We thank the Reviewer for pointing out our oversight. Figure 4B, which is now Figure 7A based on Reviewer and Editorial feedback, now shows p and r values. The manuscript has been edited to call the data a trend instead of an association, and it is stated no significance is seen.

12) Line 349: What does increase in strength of correlation mean? A p value but no r value is shown to quantify the strength.

Author Response: We agree with the Reviewer that "strength of correlation" was not the appropriate choice of words for what the sentence was attempting to convey. It has been edited to state that the pattern of significance between assessments was persistent, instead of suggesting a change in strength of correlation.

13) Regarding the T cell analyses, the authors focus on CD8+ T cells throughout their story. Is the vaccine only eliciting CD8+ T cells?

Author Response: We agree with the Reviewer that the manuscript is focused predominantly on CD8+ T-cells. This is due to CD8+ T-cell activity being proposed as being key to MoA of the treatment, which is the cytotoxic-based elimination of HPV infected cells. However, as the Reviewer points out, clarification that this treatment is able to induce CD4+ T-cells as well is important to state. Therefore, we have edited the manuscript to reflect this in the text and called out previous reports that CD4+ T-cell activity has been noted in testing of INO-3107.

14) Lines 93-98 highlight several exploratory immunological endpoints of the study that were not addressed at all such as clearance of HPV from tissues, humoral immune responses, inflammatory and suppressive elements in blood, and cfHPV DNA. What about these?

Author Response: We agree with the Reviewer and have edited the manuscript to include only endpoints that are discussed in the text.

Reviewer #5 (Remarks to the Author):

Thank you for the opportunity to review this manuscript, in which the authors described the results of a phase 1/2 trial of intramuscular INO-3107 in patients with HPV-associated recurrent respiratory papillomatosis. The trial was sponsored by Inovio Pharmaceuticals. Of note, a prior publication reported on the first 21 of the total 32 patients enrolled in the trial. The manuscript is very well written (please note a typo on line 196) and presents detailed methods and results related to the correlative analyses performed. There were no safety issues with INO-3107, which showed encouraging signals of activity, both clinical and immunological, that certainly warrant the planned further trials. The limitations of this work are duly acknowledged by the authors, the main one being the single-arm design. A noteworthy feature of the manuscript and accompanying protocol is that no description is provided of the product composition (the same goes for the protocol available in ClinicalTrials.gov). I would suggest that at least a high-level description be provided. It should be noted that my expertise is limited to the clinical and statistical aspects of clinical-trial methodology, particularly in oncology.

Author Response: We thank the Reviewer for pointing out the typo, which has been corrected. A full description of the product composition is also now included in "Treatment" section of the manuscript.

Authors Responses to Journal and Peer Review Comments (NCOMMS-24-47683-A)

Please find below point-by-point Author Responses to Reviewer comments. We very much appreciate the remarks and additional comments on the revised manuscript. All remaining recommendations have been incorporated.

If anything further is needed, please let us know.

Thank you for your review and acceptance of our manuscript.

Reviewer #1 (Remarks to the Author):

1、The expanded discussion of your findings is well-written, and I believe it strengthens the manuscript's overall impact. Your incorporation of relevant literature helps contextualize your results effectively.

Author Response: We thank the Reviewer for their review and remarks.

2、The revised figures and tables are clearer and better organized, significantly improving the readability of the manuscript. I would recommend one final check for consistency in labeling and formatting, particularly with the figure legends, to ensure complete clarity for readers unfamiliar with the subject matter.

Author Response: We thank the Reviewer for their comment and have completed a final check for consistency.

3、The conclusion section is now much more concise and focused, which is excellent. It provides a clear take-home message. It might be helpful to briefly mention potential avenues for future research in this area, as it would provide a natural progression from your findings and demonstrate the broader implications of your work.

Author Response: We thank the Reviewer for their comment and have added text to the Conclusion with respect to how potential future research may have broader implications for the field.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Author Response: We thank the Reviewer for facilitating training in peer review.

Reviewer #3 (Remarks to the Author):

The authors have incorporated my comments and I have no further comments. I agree with the publication of the article and wish the authors that the results will be confirmed in further clinical trials.

Author Response: We thank the Reviewer for their review and remarks.

Reviewer #4 (Remarks to the Author):

This manuscript has substantially improved and now provides the methodological details required to assess/interpret the presented results and conclusions. I appreciate the authors consolidating the main figures and will let the editorial office worry about the extended figures. I have two minor points that should be addressed:

In Figure 2 and the corresponding extended Figure: Is the information about the total HPV6+11 response important? The totality of the response is not mentioned anywhere. Also, based on the response to my previous question about the origin of this value (i.e. it being the mathematical sum), I would highly recommend removing it as it is highly misleading. The respective E proteins of HPV6 and HPV11 are 81-84% identical and thus the majority of readout HPV6 response will also respond to HPV11, meaning that the mathematical sum vastly overestimates the total response. I would simply remove this from the main figure and extended data.

Author Response: We thank the Reviewer for the comment and have removed the total HPV6+11 response in Figure 2 and the Extended Figure which has been moved to the Supplement per Journal requirements.

Can you add the following qualifier to your statement in line 358? "…., we did not increases in enrichment scores in 4 out of 5 of these patients"

Author Response: We thank the Reviewer for the comment and have updated the statement in the manuscript.