Author’s response to reviews

**Title:** Safety and immunogenicity of a varicella vaccine without human serum albumin (HSA) versus a HSA-containing formulation administered in the second year of life: a phase III, double-blind, randomized study

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**Author’s response to reviews:**

Dr. Maria Zalm, PhD
Editor-in-Chief
BMC Pediatrics
09 January 2019
Dear Dr. Zalm,

On behalf of all the authors, I would like to resubmit the manuscript entitled "Safety and immunogenicity of a varicella vaccine without human serum albumin (HSA) versus a HSA-containing formulation administrated in the second year of life: a phase III, double-blind, randomized study", for publication in BMC Pediatrics.

We are grateful that you consider our manuscript acceptable for publication and we thank the peer reviewers for their constructive feedback on the manuscript. We have included a point-by-point response to the reviewers’ comments in this letter below and are providing the revised manuscript with all changes marked using the ‘Track Changes’ tool as well as a clean version of the revised manuscript, as requested.

Responses to reviewers’ comments:

Specific Comments Reviewer 1

1. There is even distribution of subjects from different regions, e.g. only 71 (11.5%) from Mexico. Any specific reason for that?

As highlighted by the reviewer, the number of recruited participants was lower in Mexico compared to the other countries; as reflected by a lower percentage in the Table 1. Similar recruitment between countries is rarely if ever a requirement in vaccine immunogenicity trials. Multiple countries are used to enhance recruitment and operational feasibility. Some centers, notably due to late initiation in the trial, failed to reach the required recruitment rate leading to a slight disproportionate number of children in these countries. We have not made any changes to the manuscript on this point but could add a footnote to the table if required by the editors.

2. Is the 2 dose schedule used in this study recommended in the Immunization program of these areas since only 1 dose is recommended in some countries?

Although the two-dose schedule is recommended to achieve optimal vaccine coverage and protection, this schedule, as mentioned by the reviewer, is only recommended in a few countries and implemented as such in the routine childhood immunization program. However, to add complete information on the safety and immunogenicity profile of the varicella vaccine produced without human serum albumin (HSA), this study evaluated the two-dose schedule but assessed the immunogenicity and safety after each dose vaccination. We have not made any changes to the manuscript on this point.

3. Although fever > 39 was observed in almost equal numbers, any fever (> 38) was a little more in VAR-HAS group. Explanation for this
As pointed out by the reviewer, the incidence of fever > 38.0°C related to vaccination is slightly higher for the VAR-HSA group compared to the VAR+HSA group, irrespective of the period post-vaccination. However, the exploratory analyses for the 15 day-period post-dose 1 vaccination did not reveal any significant differences between the two groups, although this analysis was not adjusted for multiplicity. In addition, it is unlikely that this slight difference in low grade fever between the two vaccine groups would have any significant clinical implications or might affect differentially children administered with either vaccine. To convey to the reader this information captured from the tables, we have now adjusted the whole discussion: “Our results showed that the incidence of fever >39.0°C following the administration of one dose of varicella vaccine produced without HSA was not significantly different from that observed after vaccination with the HSA-containing varicella vaccine, which has been in use for more than three decades was the primary objective of this trial. The 39.0°C threshold in the present study was selected based on its clinical significance in the targeted age group, in view of the potential consequences in terms of need for medical advice, complications, or hospitalizations [9]. Moreover, the incidence of fever of any severity was also found to be similar between groups after each vaccination and no increase was observed with the administration of a second dose. A previous trial seemed to indicate a slight numerical increase in the incidence of fever ≥37.5°C for the varicella vaccine without HSA, but the statistical significance of the difference could not be evaluated, as the study was not powered to detect differences in terms of fever between groups [6]. Our study included a larger number of children of the same age (1231 vs 244 in the previous study) and was powered to assess any statistically-significant increase in rate of fever >39.0°C in the 15-day period post-first vaccination. While temperature ≥38.0°C is commonly reported following administration of the HSA-containing varicella vaccine [10], severe fever (>39.0°C) is uncommon and only occurring in the first seven days post-vaccination [3, 4, 10]. In the current study, although a slight increase in the incidence of fever >38.0°C related to vaccination was observed for the varicella vaccine without HSA, exploratory analyses showed that the incidence of fever of any severity was similar between the two groups in the 15-day period following first vaccination. Similar fever rates were found for low grade fever (>38.0°C) between the two vaccine groups for the 8-day post-first vaccination, in contrast with previous observations [6]. No increase in the incidence of fever was observed after the second dose and data related to the intensity, onset, duration and outcome of the reported fever cases post-each vaccination did not indicate any clinically significant difference between the two vaccines.”

4. Data from previous study (Ref no 6) has not been included, please include this also.

Upon relevant suggestion from the reviewer, we have now included the results from the previous study which we refer to throughout the manuscript. It is now clear to the reader what was the numerical difference observed in terms of fever rates between the two vaccine groups in this original study, although as mentioned in the present manuscript, that study was not powered to statistically compare any difference in reactogenicity outcomes between the two vaccines. We have added this information to the introduction: “Unexpectedly, after the first vaccination, a slightly higher rate of fever ≥37.5°C, but not in fever >39.0°C, was observed in children receiving the vaccine produced without HAS (28.1% - 95% CI: 20.3-37.0%) as compared to the HSA-containing varicella vaccine (18.0% - 95% CI: 11.7-26.0%) [6].”
Specific Comments Reviewer 2

1. Page 5, last line: I think that Figure 1 is not necessary

We would like to stress that the purpose of this section is not to add to the scientific content of the paper nor to be promotional, but merely to simplify the main scientific messages of the study in order to make it quickly understandable to an audience with limited medical expertise. In addition, more and more initiatives encouraging the sharing of scientific results with broader audience have been launched these latest years in many journals. The inclusion of such section is part of this new trend promoting transparency and knowledge sharing in the publication field. Our preference would be to leave figure 1 in place.

2. Page 7, Line 17: Did the authors carry out molecular analysis to determine whether the varicella skin rash was caused by the vaccine or not?

As mentioned by the reviewer, we acknowledge that some cases of varicella skin rashes may have been caused by concomitant natural infection with varicella virus. In the present study, molecular analysis performed on varicella cases among vaccinated patients revealed that such patients were infected with the wild type varicella virus. To inform the reader about this fact, we have added in the section of the discussion which deals with varicella cases: “It is worth noting that molecular analysis performed on the varicella cases among vaccinated children in the present study revealed that such patients were infected with the wild type virus”.

3. Page 7, Line 23: The authors demonstrated that blood samples were collected before and 42 days after vaccination. Were all of post-vaccination samples collected at 42 days after the two doses of vaccination?

The related statement within the manuscript, section immunogenicity assessment, is “Blood samples were collected from sub-cohorts of participants pre-vaccination and at 42 days post-each vaccination”. However, to make clear to the reader that blood samples were collected 42 days post-dose 1 vaccination and 42 days post-dose 2 vaccination, we have adapted the wording: “Blood samples were collected from sub-cohorts of participants pre-vaccination, 42 days post-dose 1 vaccination and 42 days post-dose 2 vaccination”.

4. Page 9: Table 1 should be moved to after the main text

The table 1 has been placed at the end of the manuscript, as per reviewer’s suggestion.

5. Additional tables should be used as the regular tables. And tables A1_2 and A1_3 should demonstrate as Table A1_1. Difference should be described in the Table. Although all data were not statistically difference, this should be added in explanations.
We have moved the Table 1 to the tables pertaining to the main text. The Table 1 displays results that were part of the first objective of the study and were then statistically powered, especially for the >39.0°C fever rate (in the 15-day period post-first vaccination). However, and as mentioned in the legend of the Table 1 as well as in the related text section, apart from the >39.0°C fever rate, all other comparisons were exploratory, without adjustment for multiplicity. Because of this, the results should be interpreted with caution. All other comparisons in the incidence of fever between the two vaccine groups (Tables 2 and 3) of this trial are purely descriptive and have therefore been kept as additional tables.

6. It is difficult to understand Figure 2. Did they authors use data collected from patients administrated of forbidden vaccine, or the patients with concomitant infection? The author should clearly explain this issue in the text.

For the safety analysis, we used data from the total vaccinated cohort as indicated in the statistical analysis section: “The safety analysis was performed on the total vaccinated cohort, including all participants receiving at least one study vaccine dose.” However, for the immunogenicity assessment, we used data from around the first 400 subjects (previously randomly assigned to the treatment groups) and selected among them eligible participants to be included in the according-to-protocol (ATP) cohort for immunogenicity. For this purpose, we used more stringent criteria and excluded patients such as those with concomitant infection, no-compliance with vaccination schedule, etc as described in Figure 2. This protocol scheme is reflected in the “ATP cohort for immunogenicity” box of the Figure 2. However, to improve readability of the figure, we have now removed in the box the mention on “XX excluded: analysis not planned (XXX)” and have adjusted the text: “Analysis planned: XXX and XX excluded: …”. We have also adjusted the related sections in the manuscript: In immunogenicity assessment: “Blood samples were collected from sub-cohorts of participants (± first 400 subjects, after randomization to the treatment groups) pre-vaccination and 42 days post-dose 1 vaccination and 42 days post-dose 2 vaccination. In statistical analysis: “The ATP cohort consisted of eligible and evaluable participants (see exclusion criteria in Figure 2) with available results who were seronegative at pre-vaccination.”

We believe these responses and the according changes in the revised manuscript address the reviewers’ comments and we look forward to receiving your final decision.

Sincerely,

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