Author's response to reviews

Title: Long-term outcome of the humoral and cellular immune response of an H5N1 adjuvanted influenza vaccine in elderly persons: two-year follow-up of a randomised open-label study

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Author's response to reviews: see over
Dear editors,

Please find attached the revised manuscript entitled “Long-term outcome of the humoral and cellular immune response of an H5N1 adjuvanted influenza vaccine in elderly persons: two-year follow-up of a randomised open-label study”. This manuscript has been revised in response to the peer review comments.

Responses to the peer review comments and changes made to the manuscript are detailed below. These changes have also been highlighted in yellow in the revised manuscript.

We hope that this revised version of our manuscript is now acceptable for publication in Trials.

Yours Sincerely,

On behalf of the authors

Paul Gillard
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Referee 1

Gillard et al. have conducted a phase II, open label study in healthy older adults in which they evaluated the safety and immunogenicity of a single and double dose of AS03 adjuvanted and non-adjuvanted H5N1 vaccine in older adults. The primary study objectives have been reported in a paper published in 2011 (Heijmans et al. J Infect Dis 2011; 203: 1054-62). This paper reports on the long-term persistence (up to 2 years) of the humoral and cellular immune response after H5N1 vaccination. The study demonstrates that in combination with the AS03 adjuvant a single 3.8 mcg dose of H5N1 vaccine suffices to induce a strong and durable immune response. There is no need for a higher antigen dose in the older population which simplifies the vaccination schedule in the context of mass vaccination during a pandemic.

The study has been properly conducted and the paper is very well written and merits to be published. I only have few minor comments.

Major Compulsory Revisions: none

Minor Essential Revisions:

1. Results - HI antibody response: the authors mention ‘all CHMP criteria’, whereas only SCR and SPR results are reported, not the GMT ratio, which is the third CHMP criterion.

Response: This sentence has been rephrased (p. 10 line 3–4):

“For SCR and SPR, CHMP criteria were no longer fulfilled at both month 12 and month 24 for the HI immune response against any of the two strains tested (Figure 2B-C and Figure 3B-C).”

2. References: Reference 13: typo: μg (instead of mug)

Response: This reference has been corrected.

3. Reference 16: author name ‘von SF’ should be ‘von Sonnenburg F’

Response: This reference has been corrected.

4. Legend: Figure 6: the symbols ‘alpha’ and ‘gamma’ (of TNF-alpha and interpheron-gamma) are not correctly depicted, but represented as squares in the text.

Response: The symbols are now written in full, to prevent the incorrect depiction.

Discretionary Revisions:

1. Objectives: A pandemic vaccine could boost the immune memory that has been generated after priming with a pre-pandemic vaccine. Do the authors have any evidence if memory B-cells have been elicited?
Response: In the current study, no assessment of memory B-cell response was performed. However, in a long-term booster study performed in a younger adult population from Asia, the significant responses observed with booster immunisation up to 3 years after priming allow to infer that a memory response is induced (Gillard et al, BMC Infect Dis 2014, 14:142).

This information has also been added to the discussion of the manuscript (p. 13 line 17–20):

No assessment of memory B-cell response was performed in the current study. However, in a long-term booster study with AS03\textsubscript{A}-adjuvanted H5N1 vaccine performed in a younger adult population from Asia, the significant responses observed after one dose of heterologous booster vaccine up to 3 years after priming allow to infer that a memory response is induced [23].

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Referee 2

The manuscript “Persistence of the humoral and cellular immune response of an H5N1 adjuvanted influenza vaccine in elderly persons: a two-year follow-up study of a randomized open-label study” adds long-term follow-up data to a previously published clinical trial. The induction of long lasting immunity is critical for influenza vaccines, which might be used in pre-pandemic settings in order to provide sufficient protection at the time, when the pandemic hits the population. Therefore the data presented is highly relevant.

Major Compulsory Revisions

1. The title indicates that specific immune responses against H5N1 persist for 2 years. However, the data show that antibody levels decline substantially over the follow-up period. This should be better reflected in the title, the abstract and the discussion.

Response: We agree that the decline in antibody levels was not mentioned clearly in the abstract, and we have added a sentence to the conclusions section of the abstract (p. 3 line 1):

“Antibody levels declined substantially in all groups.”

The decline in antibody levels was already mentioned several times in the Discussion section, but we have now also added a sentence to the Conclusions (p. 13 line 23):

“During the 2-year follow-up period, antibody levels declined substantially in all groups.”

Additionally, we propose to adapt the article title as follows:
“Long-term outcome of the humoral and cellular immune response of an H5N1 adjuvanted influenza vaccine in elderly persons: two-year follow-up of a randomised open-label study”

2. The usage of two influenza strains (homologous and heterologous to the vaccine strain) needs to be explained in the Material + Methods section.

Response: The following sentence has been added to the ‘Immunogenicity evaluation’ section of the Materials and Methods (p. 7 line 4–5):

“We assessed the immunogenicity against two influenza strains: the vaccine-homologous A/Vietnam/1194/2004 strain and the heterologous A/Indonesia/05/2005 strain.”

We have also clarified that the cellular immune response was only assessed for the A/Vietnam/1194/2004 strain (p. 8 line 1–2).

3. The results for the two different influenza strains need to be discussed in more details. The heterologous strain is not mentioned at all in the introduction and in the discussion.

Response: The following sentence has been added to the last paragraph of the Introduction (p. 5 line 5–7):

“We assessed the persistence of the immune response against the vaccine-homologous A/Vietnam/1194/2004 strain, as well as against the vaccine-heterologous A/Indonesia/05/2005 strain.”

In addition, the following paragraph has been added to the Discussion (p. 12–13 line 24–5):

“The HI immune response against the heterologous A/Indonesia/05/2005 strain was lower than against the A/Vietnam/1194/2004 strain in all groups, and GMTs were below the assay cut-off of 10 except for the 2xH5N1-AS group at month 12. Neutralizing antibody GMTs against A/Indonesia/05/2005 decreased compared to the post-vaccination timepoint, but remained well above the assay cut-off and also tended to be higher than the pre-vaccination GMTs (although the 95% CIs were overlapping for the 1xH5N1-AS group).”

4. Statistical information needs to be added to all Figures. There is no indication of statistically significant differences between groups or time points.

Response: As mentioned in the Statistical analysis section, the persistence analysis was performed descriptively; thus, no p-values were calculated. The commonly used criteria for significance of differences between groups or timepoints can be evaluated by looking at the overlap of the 95% confidence intervals. Comparative analyses with p-values were only planned and performed for the co-primary objective, i.e. the comparison of the single and double vaccine dose with respect to the GMT ratio and seroconversion rates at days 21 and 42 for HI antibodies and at day 42 for neutralising antibodies (reported in Heijmans et al, J Infect Dis 2011, 203:1054-62).
Minor Essential Revisions

1. The follow-up study was done only with participants in Belgium. Is that in accordance with the original study protocol? If so, this should be stated in the Material + Methods section.

**Response:** The fact that the follow-up studies at month 12 and month 24 were only performed in Belgium was in accordance with the amended study protocol; the amendment was made due to the fact that the cohort of subjects recruited in Italy was too small to warrant further follow-up. This information has been added to the manuscript (p. 6 line 2–3):

*The long-term persistence of immune response and safety data at month 12 and month 24, reported here, were only evaluated for participants in Belgium, as specified in the amended study protocol; the cohort of subjects recruited in Italy was too small to warrant follow-up.*

2. page 9, last paragraph: “... response against any of the two strains tested (Figure 2B-C)”. Figure 2 shows results for one of the strains. Please correct the text or the reference to the Figure.

**Response:** A reference to Figure 3 has been added, as reference to the results for the heterologous strain (p. 10 line 4).

3. The scales of the figures are quite arbitrary. I suggest harmonizing the scaling (e.g. Figure 3 B and C compared to Figure 2 B and C and Figure 5 A compared to Figure 6A)

**Response:** The scaling of Figures 2 and 3, and Figures 4 and 5 has been harmonized.

Discretionary Revisions

1. In order to fully evaluate the residual antibody responses after 12-24 months a comparison with pre-vaccination data would be useful. I understand that these data have been previously published, but nevertheless it might be interesting to see whether/ how far above baseline antibody titers are 12-24 months later. Maybe this information could at least be integrated in the text.

**Response:** The pre-vaccination data have been added to the Results section:

- For the homologous HI immune response (p. 9–10 line 24–2):
  
  *In comparison, pre-vaccination GMTs were 11.5 (95% CI: 8.7–15.1) for 1xH5N1-AS, 9.7 (95% CI: 7.4–12.5) for 2xH5N1-AS, 9.3 (95% CI: 6.3–13.6) for 1xH5N1 and 6.8 (95% CI: 5.4–8.7) for 2xH5N1.*

- For the heterologous HI immune response (p. 10 line 13–16):
  
  *GMTs remained higher than baseline for the adjuvanted groups while those of the non-adjuvanted groups were in the same range as baseline values; pre-vaccination GMTs were 5.1 (95% CI: 5.0–5.2) for 1xH5N1-AS, 5.1 (95% CI: 5.0–5.3) for 2xH5N1-AS, 5.1 (95% CI: 4.9–5.3) for 1xH5N1 and 5.0 (95% CI: 5.0–5.0) for 2xH5N1.*
- For the homologous neutralising antibody response (p. 10 line 20–22):
  “The antibody GMTs remained higher than the pre-vaccination values, which were 131.8 (95% CI: 95.4–182.1) for the 1xH5N1-AS group and 115.6 (95% CI: 90.0–148.4) for the 2xH5N1-AS group.”

- For the heterologous neutralising antibody response (p. 11 line 1–2):
  “Pre-vaccination GMTs were 50.4 (95% CI: 38.6–65.8) for 1xH5N1-AS and 36.6 (95% CI: 27.8–48.1) for 2xH5N1-AS.”

2. Cut-off levels of the assays should be shown in the Figures, e.g. as dashed lines (e.g. Figure 2 and 3 cut-off 10 for GMTs).
Response: The assay cut-off has been added to the GMT graphs in Figures 2, 3, 4 and 5.

3. It would be interesting to see, whether antibody titers decline at slower after vaccination with adjuvanted vaccine. I suggest to calculate “half-life times” for antibody titers, if that is feasible.
Response: We acknowledge the question but we have not performed the suggested calculation for which there are few timepoints to allow a robust calculation. We do believe a half-life time calculation will not bring more information than the persistence serological data published in this paper as well as in similar publications. In addition, the established seroprotection parameter against influenza is antibody titre measured several weeks after vaccination. The persistence antibody responses are probably not the only immunological parameter to consider for the protection against pandemic influenza virus and available data suggest that the Th1-cell frequencies have been shown to be a good early predictor of seroprotection after booster vaccination (Pedersen et al, J Infect Dis 2012, 206:158-166).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Editorial requests
1. Please include the date your study was registered with your trial registration number at the end of your Abstract.
Response: This information has been added to the abstract:

2. Please move your funding statement to the Acknowledgements section.
Response: The funding statement has been moved to the requested location.

3. Please include an additional file title and legend section after your figure legend section.
Response: The title of Additional file 1 has been added after the figure legend section.