Author's response to reviews

Title: A phase II, open-label, multicentre study to evaluate the immunogenicity and safety of an adjuvanted pre-pandemic (H5N1) influenza vaccine in healthy Japanese adults

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Author's response to reviews: see over
Dear Dr. Norton,

We would like to submit our revised manuscript to *BMC Infectious Diseases*. We thank the editor for granting us an extension of the resubmission deadline.

Ref: 1452825006411612: “A phase II, open-label, multicentre study to evaluate the immunogenicity and safety of an adjuvanted prepandemic (H5N1) influenza vaccine in healthy Japanese adults” by Hideaki Nagai, Hideyuki Ikematsu, Kazuyoshi Tenjinbaru, Atsushi Maeda, Mamadou Drame and François P Roman.

We thank the reviewers for their valuable and constructive comments.

We have carefully considered them and our replies are attached.

All authors have read and approved the final revised version.

We hope the manuscript can be accepted for publication in *BMC Infectious Diseases*.

Yours sincerely,

Dr. Hideaki Nagai
Corresponding author – I submit this revised version of our manuscript on behalf of all authors to *BMC Infectious Diseases*
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A phase II, open-label, multicentre study to evaluate the immunogenicity and safety of an adjuvanted prepandemic (H5N1) influenza vaccine in healthy Japanese adults” by Hideaki Nagai, Hideyuki Ikematsu, Kazuyoshi Tenjinbaru, Atsushi Maeda, Mamadou Drame and François P Roman.

Editorial requests:
1. Please provide the name of the ethics committee that gave approval for this study.

The following information has now been added to the manuscript: The present study (NCT00742885) was approved by the Institutional Review Board (IRB) of each study centre.
- National Hospital Organization Tokyo National Hospital IRB
- Haradoi Hospital IRB

2. Please remove the wording "confidential" from your manuscript.

The watermark “confidential” has been removed from the manuscript.

A. RESPONSES TO COMMENTS OF REVIEWER 1

This paper entitled “A Phase II, open-label, multicenter study to evaluate the immunogenicity and safety of an adjuvanted pandemic (H5N1) influenza vaccine in healthy Japanese adults” by Hideaki Nagai, Hidyuki Ikematsu, Kazuyoshi Tenjinbaru et al reported a clinical study on the safety and efficacy of a H5N1 influenza split-iron vaccine adjuvanted with AS03A in a Japanese population. The study was conducted in two centers with 100 healthy individuals aged from 20-64 years. After the second dose of vaccination, the seroconversion rates and seroprotection rates, in terms serum HI antibody titer against vaccine strain (A/Indonesia/5/2005) reached 91%. The serum HI antibody also showed substantial cross-reactive to other H5N1 stains. During the whole period of study, there was no serious adverse effect found. The authors concluded that the vaccine is safe and elucidates high humoral immune response against vaccine strain H5N1 virus in this Japanese population. On the whole, this is a well designed clinical study and the data here have provided sufficient information to support the authors’ conclusion. I would therefore recommend that the article can be accepted for publication in the journal of BMC Infectious Diseases provided that the following points are required to be clarifications or explanation by the authors before publication.

1. (Minor Essential Revisions) Since the first appearance of H5N1 in 1996, this subtype of influenza virus has evolved into multiple clades in terms of their HA gene. Theoretically, virus from any of this clade could be a candidate to cause pandemic. So, I think the use of “pre-pandemic vaccine” in this article seemed misleading. It would be more appropriate just to use “H5N1 vaccine”.

The authors concur with the reviewer that any H5N1 strain from one of these clades could cause a pandemic. The primary reason for referring to this vaccine as a “prepandemic vaccine” is because the vaccination strategy allows for dosing before and up to just after the onset of a pandemic. In addition, the vaccine confers cross-clade protection to varying extent depending on the level of antigen drift/shift. Experts opinion...
that vaccination with a H5N1 prepandemic vaccine will lead to priming against the Haemagglutinin (HA) of at least one clade, which might be beneficial.\textsuperscript{[1]} Hence, the authors would like continue to refer to the vaccine as a “prepandemic vaccine” in this paper.

2. (Minor Essential Revisions) WHO has to update the vaccine strains every year for seasonal H1N1 and H3N2 influenza due to the rapid evolutionary rate of influenza virus. So, in the Discussion, the authors should provide some information on how much the H5N1 subtype of influenza virus has been evolved in recent years, especially their HA genes. Because the vaccine strain used was an isolate of year 2005, it would be helpful for the authors to explain the pharmaceutical value of a vaccine which produced from a viral isolate five years ago.

The following paragraph has been introduced in the Discussion section: “The highly pathogenic avian H5N1 has continued to evolve and diversify over the last decade. The H5 Haemagglutinin (HA) gene, which is the target of choice for the adaptive immune response has been conspicuous in its presence in all isolates since 1996. Data available with the World Health Organization (WHO) indicate that as of March 2009, at least 10 distinct clades have arisen due to genetic re-assortment.\textsuperscript{[2]} A recent modeling study has reported that point mutations in the H5 gene may have led to the evolution of 20 genetically and potentially antigenically distinct strains.\textsuperscript{[3]} The WHO recommends that individual national authorities be consulted and epidemiological and geographical distribution of circulating H5N1 strains be evaluated to decide on the specific H5N1 viruses to be used in H5N1 prepandemic vaccines for respective countries.\textsuperscript{[4]} The vaccine strain (A/Indonesia/5/2005) in this study though first isolated in 2005 was a dominant strain at the time of conduct of this study and was recommended by the WHO for that year; as of February 2010, the WHO has not proposed any new H5N1 strain for vaccine development purposes.\textsuperscript{[4]}

3. (Minor Essential Revisions) Horse red blood cells and chicken red blood cells were used in HI and MN test respectively, is there any specific reason for using difference type of RBC in these two tests?

There is a specific reason for using the different red blood cells for the two tests. Previous experience with the H5N1 vaccine has demonstrated clear benefit in enhancing the sensitivity of the HI assay, hence, the horse RBCs were used for the HI assay. Since no such benefit was observed for the MN assay, chicken erythrocytes were used.

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\textsuperscript{[1]} Webster RG et al. H5N1 Influenza – Continuing evolution and spread. NEJM 2006;355(21):2174-7.


4. (Minor Essential Revisions) Page 9 line 22 to line 26 “A cell suspension, containing a defined number of MDCK cells were then added to the mixture of virus and antiserum and incubated at 33C.” From this description, I found it difficult to understand the rational how the MN assay was performed. It would be helpful if the methodology on MN assay to be explained in more detailed.

While the authors appreciate the reviewer’s comment, they believe that standard methodology was used for conducting the microneutralisation (MN) assay. The critical steps in the assay have already been elucidated in the manuscript and any additional detail may not be of added value to the readers. However, the duration of the incubation period (7 days) has now been mentioned in the manuscript. The authors believe that the reviewer will agree to this observation made by the authors.

**B. RESPONSES TO COMMENTS FROM REVIEWER 2**

This is a clearly written manuscript that describes a study on the immunogenicity and tolerability of a H5N1 vaccine adjuvanted with AS03A in Japanese adults. The data are well-presented and convincing. There are a few minor essential revisions required.

(i) Page 8, section on “assessment of immunogenicity”
Please state if the sera were subjected to 56 ºC treatment to inactivate complement.

For both HI and MN assays, as per standard procedure, the sera were subjected to 56 ºC heat treatment to inactivate the non-specific proteins. This information has now been added to the manuscript.

(ii) Page 9, line 19: “standardized amount of virus” Please describe this in a more precise manner. For example, please state the tissue culture infective doses used.

“Standardised amount of virus” indicates 100 infectious Unit (TCID50) in 0.05mL. This information has now been added to the manuscript.

(iii) Page 9, line 22: “After the incubation period, ..” Please state the length of the incubation period.

The duration of the incubation period was 7 days. This information has now been added to the manuscript.

(iv) Page 9, line 23: “haemagglutination of chicken red blood cells” Please clarify why horse erythrocyte was used in the HI but chicken red blood cells were used here.

Previous experience with the H5N1 vaccine has demonstrated clear benefit in enhancing the sensitivity of the HI assay, hence, the horse RBCs were used for the HI assay. Since no such benefit was observed for the MN assay, chicken erythrocytes were used.

(v) Page 14, section on “Neutralizing antibody response”
Please present the results in a Table.

A table with this data has been added to the manuscript and the text has been amended accordingly.

Please find the table below.
Table 3: Seropositivity rates and seroconversion rates of H5N1 neutralising antibodies against A/Indonesia/05/2005 and A/Vietnam/1194/2004 strains on Days 0, 42 and 182 (ATP cohort for persistence)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Time point</th>
<th>N</th>
<th>Seropositivity % (95% CI)</th>
<th>N</th>
<th>Seroconversion % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Indonesia</td>
<td>PRE</td>
<td>99</td>
<td>11.1 (5.7-19.0)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Day 42</td>
<td>100</td>
<td>100 (96.4-100)</td>
<td>99</td>
<td>97.0 (91.4-99.4)</td>
</tr>
<tr>
<td></td>
<td>Day 182</td>
<td>99</td>
<td>100 (96.3-100)</td>
<td>98</td>
<td>93.9 (87.1-97.7)</td>
</tr>
<tr>
<td>A/Vietnam</td>
<td>PRE</td>
<td>100</td>
<td>50.0 (39.8-60.2)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Day 42</td>
<td>100</td>
<td>95.0 (88.7-98.4)</td>
<td>100</td>
<td>47.0 (36.9-57.2)</td>
</tr>
<tr>
<td></td>
<td>Day 182</td>
<td>99</td>
<td>92.9 (86.0-97.1)</td>
<td>99</td>
<td>58.6 (48.2-68.4)</td>
</tr>
</tbody>
</table>

Seroconversion rate for MN antibodies: Percentage of subjects with at least four-fold increase post-vaccination neutralising antibody titres

C. RESPONSES TO COMMENTS FROM REVIEWER 3

I reviewed the article entitled “A phase II, open-label, multicentre study to evaluate the immunogenicity and safety of an adjuvanted pandemic (H5N1) influenza vaccine in healthy Japanese adults”. The data would be of interest for Japanese people and local government.

Major Compulsory Revisions:

1. Trials in other countries using the mentioned pandemic H5N1 influenza split-virion vaccine adjuvanted with AS03A has been reported, please compare the immunogenicity and persistency induced in Japanese population with the data in other population.

The following paragraph has been added to the Discussion section: “In a study in Asian adults, following two doses of the AS03-adjuvanted H5N1 vaccine, seroprotection rates in terms of HI antibodies against the vaccine homologous (A/Vietnam/1194/2004) and heterologous (A/Indonesia/5/2005) strains were 94.3% and 50.2%, respectively. For neutralising antibodies against the A/Vietnam/1194/2004 and A/Indonesia/5/2005 (Clade 2.1) strains, seroconversion rates were 96% and 91.4%, respectively [9]. In another study in Europe, two doses of the H5N1 vaccine elicited strong immune responses against vaccine heterologous A/turkey/Turkey/1/2005 (Clade 2.2) and A/Anhui/1/2005 (Clade 2.3) strains (neutralising seroconversion rates: 75–85%). The study also reported persistence of neutralising seroconversion rates in 60–70% of subjects, up to six months after vaccination [12].”

2. Please compare the immunogenicity and persistency of vaccine in seropositive population and seronegative population before vaccination.

The following statement has been included to the Results section: “Trends for higher HI antibody responses were observed against both vaccine homologous and heterologous strains 21 days after the second vaccine dose, in subjects who were seropositive before vaccination. This must be interpreted with caution given the low number of seropositive subjects before vaccination (N = 4 to 6).”
3. Please present cumulative distribution curves for titers of HI antibodies for different age group and at different time point, to more clearly show the immunogenicity and persistency of the vaccine.

The primary purpose of this study was to evaluate whether the immune response elicited by the AS03A-adjuvanted 3.75µg HA study vaccine against vaccine homologous and heterologous strains complied with the United States and European regulatory criteria for pandemic influenza vaccines. The immunogenicity analysis was performed with the aim to substantiate this objective; the information requested by the reviewer was not part of the planned analysis and is unavailable. The authors believe that the immunogenicity data presented in this manuscript are sufficient to qualify the adequate immune response in this population.

4. Although the titer was low, 5% of the participants were seropositive against all the three H5N1 viruses before vaccination, please analyze the possible reason for this high seropositivity.

The following statement has been included to the Discussion section: “Moderate levels of baseline seropositivity have already been observed in other populations, particularly for the A/Vietnam strain. In a previous study in a large Asian population, ≤7.2% of subjects was seropositive for the A/Vietnam strain prior to vaccination [9]. The fact that the seropositivity was observed in individuals aged >55 years may concur with repeated exposure to conserved epitopes of seasonal influenza antigens (either through vaccination or natural infection), possibly at the origin of a moderate cross-reactogenic response; this is supported by several reports that showed cross-reactivity between human and avian strains [27-30].”