Reviewer’s report

**Title:** Evaluation of non-inferiority of intradermal versus adjuvanted seasonal influenza vaccine using two serological techniques: a randomised comparative study

**Version:** 2  **Date:** 4 March 2010

**Reviewer:** Alexander C Schmidt

**Reviewer’s report:**

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**General comments:**

This report describes a well designed and important clinical trial comparing the safety and immunogenicity of an intradermally administered trivalent split virion influenza vaccine to an intramuscularly administered MF59-adjuvanted influenza vaccine in adults 65 years of age and older. The study is designed as a non-inferiority study since influenza vaccination is recommended for this age group and a placebo arm would be considered unethical. The study design applies a commonly used non-inferiority margin of 1.5 for the post vaccination geometric mean titer ratio between treatment arms and describes the immunogenicity parameters outlined by European regulators for the evaluation of new batches of seasonal influenza vaccine. In addition to HI titers as a correlate of protection and primary endpoint of this study, the less commonly used single radial hemolysis (SRH) method is used as a secondary endpoint. Using the primary endpoint, non-inferiority of the intradermal vaccine is established for influenza A H1N1 and influenza B but not H3N2. Using SRH, non-inferiority is shown for all three strains.

The manuscript is well organized and well written. A little more detail on demographics and inclusion of pre-vaccination GMTs would improve the manuscript and help the reader. I do not have the statistical expertise to judge the post-hoc analysis presented in Table 3 but the statistician I spoke to indicated that additional info was needed on how the data was modelled to adjust for pre vaccination titers. I would like to recommend that a statistician review the paper if the other reviewer is not an expert in this area.

**Minor essential revisions:**

1. The paper should state more clearly that the original hypothesis, i.e., that the immunogenicity of the intradermal vaccine was non-inferior to that of the adjuvanted vaccine for each virus strain in terms of antibody titers using the HI method, had to be rejected since only 2 of 3 strains were non-inferior when using the primary endpoint as defined in the protocol (without correction). I agree with the authors that the two vaccines look largely comparable with regards to immunogenicity but the authors should clearly state whether the SRH was listed
as an endpoint and whether a linear regression analysis was listed in the protocol as a method.

2. Could you please describe what model was used for the regression analysis? Was all the data from both treatment groups fit into one model or was this done separately for each treatment group? Were corrections for groups made separately? Was an interaction term included?

4. Page 10, line 25
Please provide demographic characteristics for both treatment arms and indicate whether they differed statistically (age, sex, significant medical condition and type if available, influenza vaccination in the previous year). A table might be easiest?

5. Figure 1:
Please indicate statistically significant difference for H1N1 seroprotection rate in Figure 1 & the legend.

6. Figure 2:
Fig. 2 indicates occurrence of induration in ID vaccinees whereas Table 4 does not. Please explain or correct.

7. Table 1:
I think pre-vaccination GMTs should be included. This would be very informative.

Discretionary revisions:

8. Page 2, line 16
I find the sentence "Geometric mean antibody titres induced by the intradermal vaccine for all three virus strains were in the same range as those induced by the adjuvanted vaccine assessed by HI and SRH methods." not very helpful. It should probably be deleted since the range of GMTs is not given and the 95% CIs for the H3N2 GMTs by HI do not overlap. The next sentence contains all the info needed, i.e., that using the primary endpoint non-inferiority was demonstrated for 2 of 3 strains.

9. Page 4, line 11: I would add the 2009 ACIP recommendations (PMID: 19644442) as a reference - or replace reference #4 with the new recommendations.

10. Page 5, line 9: Please update this sentence to indicate that Intanza has received EU marketing authorization for the elderly.

11. Page 8, line 5: Was SRH listed as a secondary endpoint in the clinical protocol or was it later added as an alternative to HI serology? SRH is not listed as a secondary endpoint on ClinicalTrials.gov.

12. Page 8, line 25
The EMEA assessment criteria were defined primarily for annual re-licensure, rather than for efficacy or non-inferiority studies. It would be good to indicate this either here or in the discussion.

13. Page 9, last line:
A little more detail would be helpful to the readers I think. Did the pre-vaccination titers differ significantly between treatment groups? I think it would be very helpful if the pre-vaccination GMTs were provided as part of the results.

14. Page 10, line 16:
Could you please provide a little more detail on this volunteer, e.g. timing and severity of solicited and unsolicited AEs in the first three days post vaccination and also preceding day 20? Any known cause for the cardiac arrest? MI?

15. Page 11, line 7
As above, what is meant by "in the same range"? Ranges are not indicated in Table 2, the CIs for post-vaccination GMT by HI do not overlap for H3N2, and the GMT ratio is >1.5. I think this sentence should be deleted.

Was the Pearson's correlation coefficient determined on log transformed data? Was a coefficient determined for H3N2 and B? What model was used?

17. Page 12, line 8.
If the difference in seroprotection rates was statistically significant for H1N1, this should be indicated in Figure 1 and the figure legend.

18. Page 12, line 21
Please indicate % erythema, swelling, induration and pruritus by treatment group.

19. Page 13, line 5
Please indicate % fever >38.5C by treatment group

20. Page 13, line 19
As above, I find the description of anti-HA antibodies by HI as "similar" is a little awkward when the non-inferiority hypothesis had to be rejected for H3N2. Could be expressed in a more differentiated way.

21. Page 14, line 6
I would describe the HI as a correlate of protection rather than a correlate of efficacy, especially when discussing seroprotection in a influenza-experienced population.

22. Page 14, line 10
Shouldn't the sentence starting with "Results from the SRH assay..." be preceded by "As for the B strain, ..."?
23. Please include in the discussion a paragraph on why influenza B GMTs and GMT ratios (post over pre GMT) were lower in the current study compared to the Holland study (NCT00296829). Strain differences? Population differences?

24. Please include in the discussion a paragraph on how the immunogenicity of the MF59 adjuvanted vaccine in the current study compares to earlier randomized studies.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests