Author's response to reviews

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

Authors:

Pierre Van Damme (pierre.vandamme@ua.ac.be)
Robert Arnou (alti2@wanadoo.fr)
Froukje Kafeja (froukje.oosterhuis@ua.ac.be)
Anne Fiquet (afiquet@spmsd.com)
Patrick Richard (prichard@spmsd.com)
Stephane Thomas (sthomas@spmsd.com)
Gilles Meghlaoui (gilles.meghlaoui@wanadoo.fr)
Sandrine I Samson (ssamson@spmsd.com)
Emilio Ledesma (emilio.ledesma@hotmail.com)
Margaret Haugh (mhaugh@spmsd.com)

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Author's response to reviews: see over
Responses to reviewers' comments

Reviewer: Ralf Wagner

Summary of the work:

The authors report on the results of a clinical study that was undertaken in an elderly population to compare two different forms of trivalent seasonal influenza vaccine. The following vaccine products with a strain composition for the 2007/2008 northern hemisphere season have been applied:
- a MF59 adjuvanted subunit vaccine containing 15µg of HA per strain in a dose volume of 0.5ml for intramuscular application (Fluad®, licensed for use in certain EU countries in 1999)
- a non-adjuvanted split virion vaccine containing 15µg of HA per strain in a dose volume of 0.1ml that is applied intradermally by means of a specifically designed device (Intanza®, licensed in the EU in 2009)

One aim of the study was to compare vaccine efficacy according to the criteria established and applied by the European medicines agency for seasonal and also pandemic vaccines. These include seroprotection, seroconversion and rise in GMT measured by either HI- or SRH-test to quantify the HA-specific antibody response. Another aim was to explore the safety profile of the two vaccines by assessing both local and systemic adverse reactions. Based on their data the authors conclude that the intradermally applied vaccine is "not inferior" to the intramuscular vaccine with respect to immunogenicity and safety aspects.

Comments:
The aim and design of the study are well chosen. Given the decreasing activity of the aging immune system ("immunosenescence") it is indeed an important question how vaccination efficacy against seasonal influenza in an elderly population can be improved. One option is to include powerful adjuvanting components into the vaccine formulations. The applicability of such an approach has been confirmed recently for seasonal as well as for pandemic vaccines. Another alternative might be the use of novel routes of administration, such as intradermal application. In the present study these two vaccination strategies have been compared by analysis of HI- and SRH-results (that primarily determine the antibody response directed against the HA protein) for compliance with the CHMP immunogenicity criteria. In principle this is regarded a reasonable procedure since these are the pivotal (and well established) criteria commonly applied for influenza vaccine efficacy determination and hence the data generated are very valuable in regulatory terms. However, it is felt that a couple of specific issues should be addressed by the authors:

- Since the intradermal application is a very novel mode of administration it would be crucial to have a broader view on the immune response elicited by this route – in particular since for the adjuvanted vaccine counterpart such information (eg cytokine stimulation profile, immunity to drift variants…) is available. This is quite an important issue since such immunological effects could have a pronounced impact on the boosterability of immunogenicity induced by annual vaccination and hence on the medium and long-term efficacy of the vaccine. Due to there involvement in the licensing procedure for Intanza® the authors most probably have access to such
data on the activation of different arms of the immune system in response to vaccination. Reference should be made to these data to support the authors´ claim of comparability and/or equivalence of the immunogenicity profile of the two vaccines.

Reply: This clinical trial did not aim to answer this question and so we feel the information is not necessary in this manuscript. However, we could imagine that the ID route induces different cellular mediated immune responses in parallel to the increased humoral responses, highlighting the added value of the ID route on cellular mediated immunity. Abadie et al have recently shown that ID and IM routes of vaccination differ both quantitatively and qualitatively in their capacity to stimulate cellular immunity in mice vaccinated with the live-attenuated MVA (Modified Vaccinia Ankara) virus (Abadie V, et al. PLoS One. 2009 Dec 7;4(12):e8159.). First, the MVA-specific T-cell response was significantly stronger in the lymph nodes (LNs) draining the skin than in those draining the muscle. Second, the relative proportion of multiple cytokine production (IFNγ, IL-2 and/or TNFα) by the draining LN (DLN)-derived CD8+ T cells was much higher in the ID group than in the IM group. These observations were associated with differences in the type of innate cells infiltrating the DLNs between the two modes of vaccine delivery: ID immunization induced an influx of neutrophils, dendritic cells and macrophages, but the IM route recruited only dendritic cells. Among these cells, antigen-presenting cells recruited via the ID route expressed higher levels of MVA-derived antigens and were much more potent in stimulating MVA-specific T-cells than those induced via the IM route. Based on these findings and although Intanza is an inactivated vaccine and their experiments were done on mice, we could speculate that Intanza has a higher capacity to stimulate cellular immunity than the standard IM comparator, in particular in the LNs draining the injection site.

- Next, the definition of “non-inferiority” (upper limit of 95% CI # 1.5) as implemented by the authors needs further justification. From the data provided it is clear that the GMTs achieved with the adjuvanted vaccines are somewhat higher and it is at current difficult to conclude/predict whether this has any impact on vaccine efficacy or not. Therefore, more information on the scientific rationale for setting the limit to 1.5 should be provided.

Reply: The rationale for setting the limit to 1.5 for the GMT ratio (Group 2 to Group 1) was based on clinical relevance and the expected variability of the endpoints. With this limit, the maximum GMT ratio to satisfy the non-inferiority criterion was calculated to be 1.2. The limit is also consistent with the results from the clinical development trials for Intanza 15µg. This limit is now recommended by the FDA in their guidance for clinical data needed to support the licensure of seasonal inactivated influenza vaccines that can be found at: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091990.pdf. We have added explanation and cite this document in the manuscript.

- Further, more detailed information on the pre-immune status of study participants is urgently required. It is most likely that especially in the elderly population there is a significant level of seroprevalence against seasonal influenza strains. In the manuscript it is stated that such “baseline antibody titers were slightly different in the two groups”. This is an important point since any bias in seroprevalence before
vaccination can have a drastic impact on titers achieved by vaccination. The authors need to clarify this point appropriately. In this context, the procedure followed for the “post-hoc ANCOVA adjusting for baseline” titers shall be described in more detail.

**Reply:** The pre-vaccination GMTs have been added into Table 2. The post-hoc analysis of covariance (ANCOVA) model used is a general linear model with one continuous outcome variable (baseline titre in a log scale) and one variable factor (Group). No interaction term was included in the model; therefore the same regression slope applies to the two groups and the estimate of the group effect does not depend on the level of the covariate at which it is estimated. This is the most commonly used ANCOVA model and therefore we did not include the above information in the initial version of the paper. We have now added the following information: ‘(ANCOVA model with group and pre vaccination titre; no interaction)’.

- Lastly, although all safety signals that have been detected are rather mild it is clear from the presented results that injection site adverse events were more frequent with the intradermally administered vaccine. Hence the two vaccine preparations are not fully equivalent in this respect. Therefore it might be reasonable to clarify that the two vaccines do not have exactly the same safety profile but that the higher rate of injection site reactions is acceptable due to the mild symptoms and the short duration.

**Reply:** We have added a sentence at the end of the discussion section to state this.

**Reviewer:** Alexander C Schmidt

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.

Reply: We have more clearly stated that the SRH method was listed as a secondary endpoint. The clinical study report synopsis is available at: http://www.clinicalstudyresults.org/drugdetails/?company_id=80&sort=c.company_name&page=2&drug_id=9708

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?

Reply: We have added more information about the model on page 9.

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?

Reply: We have added a table (new table 2) to provide the requested information.

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

Reply: We have added this information into the legend for Figure 1.

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

Reply: The data in the table (now Table 5) is for three days following vaccination as defined by EMA, and the data in the figure is for seven days – so there were no reports of injection-site induration >5 cm for >3 consecutive days in the first three days.

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

Reply: This has now been added in the (new) Table 3.
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.

Reply: This has been removed as suggested.

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.

Reply: Reference #4 has been replaced with a publication in Vaccine (Poland and Morse 2010;28: 2799–2800).

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

Reply: This sentence has been modified to indicate that Intanza received the European marketing authorisation for adults 60 years old and older in 2009.

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.

Reply: This method is listed as a secondary endpoint in the clinical study report synopsis which is posted at: http://www.clinicalstudyresults.org/drugdetails/?company_id=80&sort=c.company_name&page=2&drug_id=9708

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.

Reply: We have added this information in this section (see page 7 ligne 26).

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.

Reply: We have added the pre-vaccination GMTs into (new) Table 3.

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?

**Reply:** We have added the medical history about this patient in this section, and although we have the information about this patient, there is no medical interest in providing information about the timing and severity of AEs.

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.

**Reply:** We have deleted the sentence as requested.

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

**Reply:** We used a general linear model with one continuous variable (log baseline titres) and one variable factor (group). No interaction term was included so that the same regression slope applied to both groups and the estimate of the group effect did not depend on the value of the co-variable at which the effect was measured. This is the most commonly used ANCOVA model so we did not think it was necessary to include all this information; however we have included more information about the model in the methods section (page 9).

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.

**Reply:** We have modified the legend for Figure 1 to indicate which comparison was statistically significant.

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

**Reply:** We have added the percentages for these events, as requested.

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

**Reply:** We have given the percentages of participants who had fevers ≤38.5°C and >39.6°C in the manuscript.

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.
**Reply:** We have modified this in the manuscript – at this particular place we were stating the two methods, HI and SRH, gave similar results.

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 an influenza-experienced population.

**Reply:** We have changed the term to correlate of protection.

22. Page 14, line 10
Shouldn't the sentence starting with "Results from the SRH assay..." be preceded by "As for the B strain, ..."?

**Reply:** We do not think the sentence should start as the reviewer has suggested, so we have not modified it.

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?

**Reply:** We cannot compare the GTMs as they were generated with a different composition of the seasonal trivalent vaccine. Generally speaking, it is not recommended to compare GTMs from different vaccine clinical trials because of the different composition of the vaccines, the different demographic characteristics of the populations studied (including the pre-vaccination titres) and to the assay used. We, therefore, do not think we should include this information in the manuscript. For information, the composition for the vaccines used in these two trials was:

**Southern hemisphere, 2005: Vaccine Composition**
A/New Caledonia/20/99(H1N1)-like virus;
A/Wellington/1/2004(H3N2)-like virus;
B/Shanghai/361/2002-like virus

**Northern hemisphere, 2007-2008: Vaccine Composition**
A/Solomon Islands/3/2006 (H1N1)-like virus;
A/Wisconsin/67/2005 (H3N2)-like virus;
B/Malaysia/2506/2004-like virus

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.

**Reply:** We think this is out of the scope of the paper, and unless the editor thinks we should include this in the discussion, we would prefer not to.

**Reviewer:** Michael Pfleiderer

**Reviewer's report:**

European Medicines Agency's acronym is EMA, no longer EMEA
Reply: We have modified this throughout the manuscript.

Minor Essential Revisions:

Preimmunization titres of study subjects should be indicated in table 2 as well as in the corresponding text paragraphs

Reply: We have added the pre-vaccination GMTs in the table (it is now Table 3).

A post hoc analysis as regards non-inferiority of seroprotection rates should be performed. Seroprotection is the more relevant parameter compared to GMTs.

Reply: We do not think this is necessary since the data in Figure 1 and the text on page 12 provides an indirect comparison.

Clinical significance of the non-inferiority margin chosen should be well justified

Reply: The choice of the non-inferiority margin was driven by the clinical relevance and the expected variability of the endpoints and the desired accuracy of the conclusions of the study. With a 1.5 non-inferiority margin for the GMT ratio (post-vaccination Group 2 to Group 1 GMT ratio), assuming a common SD and a number of evaluable subjects as defined in the protocol, the maximum observable GMT ratio to meet the non-inferiority criterion was around 1.2. In addition, a non-inferiority of 1.5 was consistent with the development studies with Intanza 15µg. This 1.5-margin is now recommended in the FDA Guidance for Industry on “Clinical data needed to support the licensure of seasonal inactivated influenza vaccines” and the reference has been added to the manuscript.

Subanalyses should be performed based on a finer age stratification (e.g. 65 - 69, 70 - 89, 80 and above) as well as by dividing study subjects into groups with and without seroprotective titres pre-vaccination. This will provide the basis for a more comprehensive interpretation of potential differences as regards immunogenicity/efficacy of the two vaccines/routes of administration investigated.

Reply: Analyses according to age (<75 years and ≥75 years) were performed for both assays, HI and SRH. These analyses showed slightly higher immune responses in the younger subgroup in both vaccine groups (see tables below). The percentages of subjects achieving post-vaccination threshold of 10, 40, 80, 160, and 640 (1/dil) according to their pre-vaccination titres (i.e. <10, ≥10 and <40, ≥40 [1/dil]) were calculated. We did not perform an analysing according to seroprotective titres pre-vaccination (<40 and ≥40 [1/dil])

Discussion should be modified/amended accordingly.
## Post-Vaccination GMT per Age Group - HI Method (Per Protocol Set)

<table>
<thead>
<tr>
<th>1/dil</th>
<th>Flu-ID 15µg Group (N=390)</th>
<th>Addigrip® Group (N=385)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/Solomon (H1N1)</td>
<td>A/ Wisconsin (H3N2)</td>
</tr>
<tr>
<td>&lt; 75 years (N=242)</td>
<td>&gt;=75 years (N=148)</td>
<td>&lt; 75 years (N=242)</td>
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<tr>
<td>Post-Vaccination GMT [95% CI]</td>
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<td></td>
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<tr>
<td>&lt; 75 years (N=242)</td>
<td>&gt;=75 years (N=148)</td>
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<td>1/dil</td>
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<td>A/ Wisconsin (H3N2)</td>
</tr>
<tr>
<td>&lt; 75 years (N=214)</td>
<td>&gt;=75 years (N=171)</td>
<td>&lt; 75 years (N=214)</td>
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<td>Post-Vaccination GMT [95% CI]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 75 years (N=214)</td>
<td>&gt;=75 years (N=171)</td>
<td>&lt; 75 years (N=214)</td>
</tr>
<tr>
<td>129.8 [110.8;152.1]</td>
<td>80.6 [65.6;98.9]</td>
<td>299.6 [261.2;343.7]</td>
</tr>
<tr>
<td>150.0 [130.2;172.8]</td>
<td>94.5 [79.2;112.7]</td>
<td>385.5 [334.9;443.8]</td>
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### Post-Vaccination GMT per Age Group - SRH Method (Per Protocol Set)

#### Flu-ID 15µg Group (N=389)

<table>
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<th>mm²</th>
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<th>A/ Wisconsin (H3N2)</th>
<th>B/Malaysia</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 75 years (N=241)</td>
<td>&gt;=75 years (N=148)</td>
<td>&lt; 75 years (N=241)</td>
<td>&gt;=75 years (N=148)</td>
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<tr>
<td>Post-Vaccination GMT [95% CI]</td>
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<td>40.0 [33.2;48.1]</td>
<td>43.4 [38.5;49.0]</td>
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</table>

#### Addigrip® Group (N=382)

<table>
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<tr>
<th>mm²</th>
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<th>A/ Wisconsin (H3N2)</th>
<th>B/Malaysia</th>
</tr>
</thead>
<tbody>
<tr>
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<td>&gt;=75 years (N=169)</td>
<td>&lt; 75 years (N=213)</td>
<td>&gt;=75 years (N=169)</td>
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<tr>
<td>Post-Vaccination GMT [95% CI]</td>
<td>65.4 [59.0;72.5]</td>
<td>42.3 [35.9;49.8]</td>
<td>51.4 [45.9;57.6]</td>
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</table>