Author's response to reviews

Title: Humoral and cellular responses to a non-adjuvanted monovalent H1N1 pandemic influenza vaccine in hospital employees

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Author's response to reviews: see over
We appreciate the comments from the reviewers and acknowledge the opportunity to reply to their questions regarding our manuscript entitled "Humoral and cellular responses to a non-adjuvanted monovalent H1N1 pandemic influenza vaccine in hospital employees". MS: 4307880411020510.

Below you will find our point-by-point replies to the reviewers' questions and comments. In addition, we have corrected the manuscript following the reviewers’ suggestions and are sending the revised version. The manuscript was also corrected by a professional English editor.

Reviewer 1: Zoltan Vajo

The authors responded to the queries of this reviewer, however, I don’t see the appropriate changes in the revised manuscript.

Q1
First, it would be important to include into the abstract which vaccine was used (manufacturer, etc) and that according to the study, it did not fulfill EMEA/CHMP criteria for licensing.

A1
In the abstract, we have included information regarding the vaccine that was used. See the Abstract (line 37-38) and Methods (lines 120-122).

Q2
The authors' possible explanation to this is speculative, and it is premature to discard current licensing criteria, even in health care workers, and propose new parameters based on a study with 60 participants (see conclusions).

A2
We agree with the reviewer in that our explanation was speculative regarding our proposal of the licensing criteria. We have therefore modified the paragraph in which we commented on the fact that the study participants did not reach the required seroprotection rate for vaccination success according to the international criteria, Results and Discussion (line 299 to 301), and...
Conclusions (line 416-420) and deleted the text: The pandemic vaccine induced a humoral response that did not reach the threshold for successful vaccination.

Q3
I still see no sample size calculation in the revised paper. The explanation the authors offer in their cover letter is not entirely acceptable. First, they determined that the ideal sample size would be 277. However, it was not possible to study that many subjects for the authors. Then, based on their preliminary studies (which are not shown, nor any information about it), based on a mean response of 3.8 and an SD of 4.0, the authors "assumed" normal distribution - however, no distribution calculation is mentioned, and it is unlikely with the mean and SD they provide (3.8 +/- 4.0) that their data in fact had normal distribution. Lastly, the authors reference the EMEA/CHMP criteria, which, however were not meant for an observational study like this. Nonetheless, the authors did not include any of this into the manuscript. The rest of the queries were addressed appropriately.

A3
We apologize for our mistake in our response to the reviewer’s comment asking how we had estimated our sample size. In that response, we explained that the ideal sample size would be 277 because we used a formula to estimate the sample size of a population mean. In the present study, we are analyzing the difference between medians (pre- and post-vaccination). Therefore, calculating the sample size using the previous formula was incorrect. Additionally, we agree that the EMEA/CHMP criteria are not meant for an observational study such as ours. We have re-calculated our sample size based on the asymptotic relative efficiency of the Mann-Whitney U relative to the t-test (worst case scenario, dividing 33 by 0.864), arriving at 38 pairs. As previously explained, we used the data from the first 10 subjects because, to our knowledge, there are no published data describing the mean difference in CD8 cells between pre- and post-H1N1 pandemic influenza vaccination.

We have inserted the following paragraph (lines 247 to 256): To our knowledge, there are no published data describing the mean difference in CD8+ T cells between pre- and post-H1N1 pandemic influenza vaccination. We therefore estimated our sample size based on an analysis of the first 10 pairs of subjects among whom we observed that the difference in response was not normally distributed (median 3.8, IQR (1.44-7.09). We calculated the sample size needed for a two-sample t-test (alpha = 0.05 and beta = 0.8) using a mean of 3.8 and standard deviation of 4, arriving at 33 pairs, and then adjusted the sample size based on the asymptotic relative efficiency of the Mann-Whitney U relative to the t-test (worst case scenario, dividing 33 by 0.864), arriving at 38 pairs. (Introduction to the Theory of Nonparametric Statistics. Randles RH, Wolfe DA. New York, John Wiley & Sons (1979), ISBN: 0-471-04245-5).
Reviewer number 2: Massimiliano Fabbiani

The authors adequately addressed all the raised questions. The manuscript is suitable for publication.

Editorial Requirement
After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further. We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

We have followed your advice and obtained the assistance of a professional editing service. Particular attention was paid to the Abstract.

Sincerely,

Dr. Martha Torres

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