Author's response to reviews

Title: Superior antigen-specific CD4+ T-cell response with AS03-adjuvantation of a trivalent influenza vaccine in a randomised trial of adults aged 65 and older

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Author's response to reviews: see over
COVER LETTER

Dear Miss Ramos,

We are grateful to you and the reviewers for considering our manuscript for publication in BMC ID. We are pleased to note that Reviewer #1 states that “[t]he study is clear, well-designed and well-written”; Reviewer #2 states that it “provides additional knowledge on the use of adjuvanted inactivated influenza vaccines, which may increase protection in more at risk groups for influenza-associated illness, such as the elderly”; and that both reviewers state that our article is “of importance in its field”.

In light of the reviewers’ commentaries we have made modifications to the manuscript, and these are detailed in the point-by-point replies below.

Yours sincerely,

Innocent Mbawuike.

RESPONSE TO REVIEWER, ELENA PARIANI

Reviewer's report:

The manuscript entitled “Superior antigen-specific CD4+ T-cell response with AS03-adjuvantation of a trivalent influenza vaccine in a randomised trial of adults aged 65 and older” by Robert B Couch et al. presents the results of a clinical trial aimed at the evaluation of T-cell responses and serological antibody responses to a trivalent inactivated AS03-adjuvanted influenza vaccine in adults aged over 65 years. The study is clear, well-designed and well-written. The authors work out properly the objectives of the study using appropriate methodologies and statistical methods, and coming to significant results. The conclusions are supported by the data obtained, and add evidence on the positive effect of AS03 adjuvant system particularly in older people.

Level of interest:

An article of importance in its field

Quality of written English:

Acceptable

Statistical review:

Yes, but I do not feel adequately qualified to assess the statistics.

Minor essential revisions

The following are some comments for the authors’ consideration.

- In the results section of the abstract add p-values in brackets rather than using
“superior” or “higher” only.

Authors:

P-values have been added in the Abstract.

- Page 6, line 12: Change “0.5ml” to “0.5 ml”

Authors:

Amended.

- Page 9, line 24: I would suggest to change “>20-#50mm” to “20-50 mm” and “>50-#100mm” to “50.1-100 mm”. Consider this also for grades reported at page 10, lines 1-2.

Authors:

We would prefer not to make these alterations because the notation for the grading in the manuscript is the same as that used in the clinical trial protocol.

- Page 12, line 6: Please specify the reasons why these two subjects did not complete the study.

Authors:

We now state in the Results that “[t]wo subjects did not complete the study, but were vaccinated. One subject (TIV/AS03[≥65] group) did not complete due to a fatal myocardial infarction; and the other subject (TIV[18–40] group) withdrew consent for a reason not related to any AE.

- Page 17, line 21: Delete “}”

Authors:

Deleted.

- Page 18, line 15: Report the reference of the study described here

Authors:

Amended.

RESPONSE TO REVIEWER, XAVIER SAELENS

Reviewer's report:

The authors compared AS03-adjuvanted TIV with TIV in a cohort of people over 65 years of age. A third group of 18-40 year old adults who received TIV was also included, allowing comparison of immune responses to TIV between young adults and elderly. CD4 T cell responses, as determined by the expression of CD40L, IL-2, TNF, and IFN-
gamma (two or more of either of these) were significantly higher in the AS03-adjuvanted TIV recipients compared to the other two groups on days 21 and 42. This difference remained significant on day 180 after vaccination when comparing the two elderly groups. Vaccine antigen-specific CD8+ T cell responses were not altered after vaccination and the frequency of GrzB+ CD4 and CD8 T cells responsive to vaccine antigen remained constant over the study period. Based on the observation that GrzB+ and IFN-gamma and or IL-2 positive CD4 and CD8 T cell frequencies following restimulation with CMV in CMV carriers were comparable in all groups, it is concluded that the differences in CD4 T cell responses directed against influenza vaccine antigens, are specific to these antigens and do not reflect a bystander effect. Interestingly, there was no correlation between the increased CD4 T responses and influenza virus-specific serum antibody responses, as determined by HI, SPR and MNT assays.

Comments:
This is an interesting study that provides additional knowledge on the use of adjuvanted inactivated influenza vaccines, which may increase protection in more at risk groups for influenza-associated illness, such as the elderly.

Level of interest:
An article of importance 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 interest

Major compulsory revision

1. I am not sure that the conclusion that the significant increases in CD4 T cell responses in the AS03 group are merely split antigen-specific, as suggested by the comparison with the CMV responses. The authors should compare the flu and CMV-specific responses of the CD4 T cells that express CD40L, IL-2, TNF, and IFN-gamma (two or more of either of these). This is important because Fig. 4 shows that GrzB and IFN-gamma/IL-2 expressing CD4 and CD8 T cells do not differ between the three TIV groups.

Authors:
We understand this comment as suggesting that a potential bystander effect should also have been evaluated in CMV-specific CD4+ T cells expressing at least two of the immune-activation markers among CD40L, IL-2, TNF-α, and IFN-γ. However, this evaluation was not performed in the clinical trial. Granzyme B and at least IFN-γ, and/or
IL-2 were selected as markers because this reflected a classical bystander activation where antigen non-specific T cells would become cytotoxic (e.g. see Ehl et al, J Exp Med. 1997, 185:1241-1251). CD4+ T cells were analysed in addition to CD8+ T cells because of the more recent insights which implicate CD4+ T cells in bystander activation, as reviewed by Onur Boyman (Eur J Immunol 2010, 40:936-939; and referenced in the manuscript). Nevertheless, we have qualified the conclusion of the analysis in the Results section by clearly stating that it was based on “the frequencies of CMV-specific CD4+ and CD8+ T cells expressing Granzyme B and at least IFN-γ and/or IL-2”; such that it now states that “[t]here was no suggestion of bystander activation because from the evaluation of the frequencies of CMV-specific CD4+ and CD8+ T cells expressing Granzyme B and at least IFN-γ and/or IL-2 in CMV-seropositive subjects, because these frequencies appeared unaffected by vaccination (Additional File 1)” (inserted text underlined).

**Minor essential revision**

2. The authors should mention if the volunteers were allowed to use medication to try to mitigate the adverse events that appeared in some cases following vaccination and, if so what kind of medication.

Authors:

Participants were allowed to take medication (e.g. analgesics and antipyretics) during the study and this was recorded. Therefore information about such medication has been added. It is now stated in the Methods that;

“The use of medication during the study was recorded from Day 0 to Day 180, and was reviewed by the investigator for any potential relationship with a study measurement. The medication was considered as prophylactic when it was administered in the absence of any symptom and in anticipation of a reaction to the vaccination.”

And it is now stated in the Results that;

“[t]he taking of medication during the study was reported by 64%, 53% and 54% of the subjects in the TIV/AS03(≥65), TIV(≥65), and TIV(18–40) groups, respectively. This included antipyretic medication, which was reported by 30%, 25% and 40% of the subjects in the TIV/AS03(≥65), TIV(≥65), and TIV(18–40) groups, respectively. No use of prophylactic antipyretic medication was reported”

3. In the introduction or the discussion, it would be helpful if the authors could clarify the (surmised) mechanism of action of the AS03 adjuvant.

Authors:

It is now stated in the Introduction that: “Preclinical experiments suggest that AS03 enhances the adaptive responses to vaccine antigens by triggering a transient innate response local to the injection site [23].”
4. Page 7, line 15: please be more precise on “short term”: how many hours of stimulation 5. Page 8, line 20: please provide details on the antibodies used for cell staining

Authors:

Short-term is now defined in the Study endpoints Section of the Methods, and was equivalent to 20 hours antigen-stimulation in the *ex vivo* PBMC cultures (as already described in the *Ex vivo* short-term T-cell re-stimulation assay section).