Author's response to reviews

Title: The immunity and clinical efficacy of an inactivated enterovirus 71 vaccine in healthy Chinese children: a report from further observations

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Version: 4 Date: 14 July 2015

Author's response to reviews: see over
Response to reviewers

Reviewer 1-Rogier Van Doorn

Authors have made a number of changes as requested but leave others unaddressed or addressed in an unsatisfactory manner. Looking at the paper again and the inconsistencies regarding the numbers of individuals to which the analyses apply I found a few other issues that I overlooked in my first review.

In my opinion the following are absolutely necessary to be addressed before publication in BMC Medicine of these data can be considered.

Abstract and Introduction:

L40: It is still not clarified in abstract and introduction that this work was done on (small) subsets of the initial 1100 (see further below), despite earlier requests to do so and despite authors claims that they did (see cover letter).

350 participants completed the study and the immunology and cross-neutralization work was done on randomly selected small samples.

Thank you very much. In this manuscript, we supplement the details, especially the participants’ number in following-up study, in Abstract, Introduction and Methods.

Methods

L141: rewrite: Additionally, xx individuals (yy in each age group, and per-agegroup zz in the vaccine arm and zz in the placebo arm) were randomly selected using … and serum samples collected on days 0, 56 and 320 were used for cross-neutralization assays.

It is crucial that there is absolutely no confusion about the numbers of individuals and their sera, from different groups and arms.

According to your suggestion, we rewrote this part for clearly understanding in the revision.

Figure 5 legends should also mention this correctly.

The number of participants was added in the legends of all figures.

Also clarify whether individuals were selected from the per protocol analysis (individuals who completed 720d follow-up) population of 350 individuals or not.

Yes.

L152: An ELISPOT … Start a new paragraph here

Thanks. It has been revised in the resubmitted version.

L161-163: Move this sentence to L152 to start the new paragraph with.

Rewrite as follows: xx individuals (yy in each agegroup, and per agegroup zz in the vaccine group and zz in the placebo group) were randomly selected and
the serum sample collected on day 720 was used in the... Clarify whether these are the same individuals as above or not.

Thank you for your help. The sentence has been rewritten in methods (page 8 line 146-150 and page 9 line 158-162).

Figure 3 legends suggest samples from 1100 individuals were used for these tests, change this.

OK. We changed it.

Can the sample sizes for these subanalyses be justified?

This study is the long-term observation of phase III clinical trial. The sample sizes of phase III clinical trial have been justified that a sample of 12000 participants would be needed for the study to have 90% power to detect a difference between the vaccine group and the placebo group, according to the primary outcome (vaccine efficacy), assuming a vaccine efficacy of 75% and allowing for a withdrawal rate of 20% within 11 months following-up. It appears that about 10% of 12000 participants (1100 individuals) is dedicated to the immunogenicity study is rational. However, as this is a newly developed novel vaccine, its associated evaluation shall be further explored.

L171-172: This is not how sample size or power calculations are usually presented. Please rewrite this section and describe how the study was powered to show which differences, and how many enrollees this would require and whether potential drop-out rates (which are considerable, 350 patients completed the study) were taken into account.

Thanks for this comment. As the reasons explained in above question, we revised the related description in this part.

Results and Discussion
L203: Delete “some”

OK. It has been deleted.

L205 and further and Figure 2: specify whether this regards the total of 161+189 (350) individuals (as shown in table 1) that completed the 720d follow up (ie. per-protocol analyses), both in the body of text and in the figure legends, or that data from other/more individuals are shown at the less than 720d follow-up timepoints.

We are very sorry about the unclearly description leading to these questions. So we rewrote these parts and added the numbers for different assay in the figure legends. We hope it is helpful to understand the results. Briefly, 1100 participants received the vaccine or placebo for vaccine efficacy analysis (Figure 4), and 350 participants completely provide the blood samples within the whole two-year following-up. Of these 350 participants, sera from 350, 160 and 40 participants are collected, respectively for neutralizing antibody measurement (Figure 2 and Table 1), cross-neutralization assay (Figure 5) and cellular immune responses assay (Figure 3).
L220-228: Describe and discuss the 4 important observations from Figure 2 more clearly. Figure 2 shows 1) a clear difference in immunogenicity between vaccine and placebo in younger age groups between day 0 and 56. 2) Figure 2 also shows a steady decrease of NAbs among vaccinees between day 56 and 360, presumably waning of the immune response against the vaccine followed by 3) a significant boosting of the NAbs among vaccinees in all agegroups at 540 days (why, caused by what and why do we not really see this among individuals who received placebo?) vs 4) an expected more or less steady increase over time among individuals who received placebo, although in the older agegroups the levels among vaccinees and individuals who received placebo look similar throughout.

*We modified the part of results and discussion, according to your suggestion.*

L246-251: As above, the authors should more clearly describe and discuss separate observations from each figure. In this figure higher IFN and IL4 responses are seen in all vaccinee groups, there is however an unexpected high IFN response in individuals who received placebo in one agegroup. The authors suggest this is caused by either a small sample size or natural viral circulation. It is unclear why natural virus circulation would only have an effect on one agegroup, though. The statement “These data are sufficient to implicate … “ is therefore too strong

*Yes, we rewrote this part (page 14 line 269-275).*

L271-273: These numbers appear to be different from what is described on page 8. To avoid any confusion describe as above the exact number of individuals whose samples were used, and include the numbers in each agegroup and per agegroup in each arm. Rewrite e.g. as: We randomly selected xx individuals (yy in each agegroup, and per agegroup zz in the vaccine and zz in the placebo arm) and used sera collected on days 0, 56 and 320; these sera were subjected to...

*Yes, we rewrote it in the methods (page 8 line 146-150) and in the results (page 15 line 293-297) to avoid repeated.*

Figure 4: The authors should indicate the population at risk in the Kaplan Meier plots. Is this the (dynamic) population under study (1100 in the beginning, 350 at the end) or the 350 that finished the follow-up? If a dynamic population is used, population at risk should be indicated per month. Authors should also look at the y-axis, as the size of one event on the y-axis (10 events required for the curve to drop to 99%) suggests there were ~1000 participants in each arm.

*Thank you very much. We apologized for this mistake and corrected in revision. The 1100 participants who received the vaccine or placebo were analyzed for vaccine efficacy.*
Figure 5: Further to my previous comments... the levels of NAbs (e.g. against C4) among the small number of placebo controls shown here are much lower than the levels of the 1100 placebo controls shown in figure 2. The authors explanation is not satisfactory. Data are shown here from a randomly selected small subset (Figure 5) out of a population of 1100 with much higher titers (Figure 2).

Thank you very much. Figure 2 shows the dynamic profile of the antibody levels in 350 children immunized with the vaccine for a 2 years follow-up period, which specifies GMTs of each individual subset groups. Whereas Figure 5 indicates the cross-neutralizing reactivity to the total sera taken from the 160 individuals by different genotype viral strains. There are 2 main reasons for us to select a small subset of 160 from 1100: one is that only 350 of 1100 completely provided the blood samples within the 2 years follow-up study; another is that only 20 participants are included in the 36-71 months of age vaccine subset group, which leads to the maximum selection of 20 participants in the remaining 7 subset vaccine and placebo groups.

Also, the y-axes of Figure 2 and 5 should be the same (Fig 2 now starts at 0, Fig 5 at 2)

We agree with the reviewer. So the y-axes of Fig 2 and Fig 5 have been the same in revision.

Figure 1: No explanation is given yet for the seemingly discrepant dropout rates between the 12000 patient study and the study on a subset of 1100 (in which the dropout between the 2nd dose and 1y follow up appears to be much larger). Was it possible for participants to drop out of one study (this one) but continue in the other (the NEJM paper)?

The phase III trial is designed for the safety and efficacy observation on 12000 participants and immunogenicity measurement in a subgroup of 1100 of 12000 with blood taken, which leads to the lower drop-out (see the attached NEJM paper). While in contrast, the blood samples are taken from the subsequent 1100 participants for the 2 years follow-up study for the purpose of GMTs measurement, cross-neutralization assay and cellular immune response, which thereby leads to the high drop-out.