Reviewer's report

Title: The immune response and efficacy of an inactivated enterovirus 71 vaccine in healthy Chinese children: report from further observation

Version: 1 Date: 6 April 2015

Reviewer: rogier van doorn

Reviewer's report:

The authors describe a substudy of one of the three phase III vaccine trials of an inactivated EV-A71 C4 vaccine that were published from China in 2013-14 in the Lancet and the New England Journal of Medicine (refs 8-10).

A subset of 1100 children was randomized to receive vaccine or placebo and were followed up until day 720 (unblinded after 1 year for publication of the larger trial) and their sera were analysed for neutralizing antibodies (all), IFNgamma and IL4 responses (10 sera from each of 4 agegroups at day 720) and cross-protection (20 sera from day 0 and 360).

The results are interesting, but there is some repetition of the data already presented in the trial paper (particularly the methods section). The authors show a lasting immune response in the 4 agegroups (with unexplained boosting after 1y in all agegroups, and little benefit in the oldest agegroup), some differences among IFNgamma values but no p-values or discussion and cross-protection among all subgenotypes but only on day 360 and in a small subset.

I have added comments, questions and suggestions for improvement below

Major compulsory
-p6 Can the methods section be reduced by referring to the original NEJM publication?
-p10 There is no Results section, is this correct?
-p12-13: The issue of lasting cross-protection is quite an important one for vaccine implementation strategies, yet the authors chose to only use a small selection and only look at day 0 and 360. Why not look at the other timepoints and look at the dynamics of the response, also beyond day 360. It would be interesting to see whether EV-A71 behaves as a single genotype with lasting cross-protection from day 0 to 720, or – like dengue or flu – has an initial broad response across all subgenotypes that wanes over time for the non C4’s. It would also be interesting to include some contemporary CA6 and CA16 strains.

Minor essential
-Line numbers were missing, please add when resubmitting
-Many numbers are presented with 2 decimals, while this level of exactness is often neither justified nor required
- Clarify in abstract and introduction that the IFNgamma-IL4 and subgenotype work was only done on a small subset

-p5 In a published ... efficacy: please clarify there were three different phase 3 trials with three different C4 strains

-p7 ...a phase III trial... : is this the same trial (ref 8) published in NEJM described above? The 1100 participants described here were also part of the enrolled population of this trial (ref 8)?

-p8 Additionally ... EXCEL software: Unclear. Were 20 patients selected from whom samples collected on day 0 and 360 were tested, or were 20 sera - collected on day 0 and 360 from 10 patients - tested? Were 20 patients selected from both vaccinees and placebo-control group, or 20 in total (if so, add how many were vaccinees and how many received placebo)?

-p8 A total ... Asia-Pacific countries: clearly indicate which strains/isolates were used

-p8 ... as described in previously published reports: Reference these reports, particularly development and validation of ELISPOT assays for EV71, which stimulating peptides were used etc

-p9 stimulating peptides: Define

-p9 considered to be susceptible: Define

-p10 recent epidemiological studies: this reference is from 2002, use a more recent reference or delete the word recent

-p11 in clinical trials: Not sure what this addition means or why reference 17 is inserted here

-p11 although ... 100%: Delete, as this is repeated with more accuracy below

-p11 In some cases ... earlier results: What does this mean, infection with a related virus? (Asymptomatic) re-infection with EV71? Can this be discussed in more detail, ie. was this effect caused by a strong increase in a small number of children or was it a common observation?

-p11 the effect of a natural infection ... by the vaccine: What data do the authors have to support this statement?

-p12 synthesized VP1 peptide: Reference

-p12 The result ... enterovirus-infected cases: Was boosting of EV-A71 NAbs observed after infection with other EVs, both in vaccinees and placebo-controls?

-p12 These data ... older children: What data do the authors have to support this statement?

-p12 Notably ... p13 clinical trials: Does this add anything to the results already published in the NEJM paper (ref 8)?

-p12 Any p-values for the IFNgamma/IL4 work? Why are the levels so much lower in the oldest agegroup. How can the authors explain that there is seemingly no difference in the third agegroup? Which differences were significant? Was the sample large enough to be representative?
Whereas ... issue: Reference the recently published papers showing full cross-protection against all EV-A71 subgenotypes after natural infection and among vaccinees (PLoS NTD 7(2):e2067, PLoS One 8(11):e79599, PLoS One 9(6):e100545) and discuss what is new here compared to these. Reference the recent paper from Taiwan showing a C4 to B5 subgenotype switch (PLoS One 10(3):e0116322)

Table 1: Also show data for the placebo group

Figure 1: The dropout rate is quite high, higher than for the entire study it seems, comments on this?

Figure 2 and furthers: Numbering of legends and figures doesn’t match, same with cross-referencing in the body of text

Figure 2 (Figure 3 legends): The boosting of the Nabs level after day 360 in the three youngest agegroups is quite striking, as is the fact that in the oldest agegroups the levels among vaccinees and placebo-controls are virtually indistinguishable after 56 days.

Figure 3 and 4: p values?

Figure 5: The levels among placebo-controls appear to be quite low, considering the levels shown in Figure 2 (Figure 3 legends)

Quality of written English: Needs some language corrections before being published

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