Dear BMJ,

Many thanks for considering our manuscript, we are grateful to you and the reviewers for your comments and respond as follows:

Points made by The BMJ's manuscript committee meeting

1. We are very interested in this work and feel that it will add important information for doctors and parents. That said, the conclusion of safety appears to us to be an overstatement. You can say that based on these findings the SAEs are likely to be less than X but it seems unwise to claim safety.

We agree that our data provide evidence that the risk of systemic reaction to LAIV is no greater in egg-allergic individuals than those without egg allergy, rather than safety per se. We have changed our phraseology in the text accordingly. We would highlight, however, that we have previously shown that the maximum amount of egg protein in LAIV batches used in the UK is at least 10 times lower (and typically 100-1000 times lower) than the amount of egg protein found to trigger local rhinitis symptoms when administered into the nasal airway of children with egg allergy (Turner PJ, Erlewyn-Lajeunesse M. Egg protein in the intranasal live-attenuated influenza vaccine (LAIV) is unlikely to cause egg-mediated allergic reactions in egg-allergic children. J Allergy Clin Immunol Pract 2015; 3:312-3). It is therefore unlikely that LAIV would trigger symptoms due to an IgE-mediated allergic reaction to egg protein.

Furthermore, on the basis of our data, JCVI made the recommendation to the Departments of Health in the UK that "except for those with severe anaphylaxis to egg which has previously required intensive care, children with an egg allergy can be safely vaccinated with Fluenz Tetra® in any setting" (JCVI, 2015). We now quote this wording directly in our paper.

2. Our statistician noted that some of the subgroup analyses (particularly in the youngest group) are underpowered but point towards some increased risk. Paradoxically this is not apparent in the ASthma score but this should also be discussed.

We now include full details of the sub-analyses in the paper (online table E2 and E3) and comment on the trend towards increased lower respiratory symptoms yet no change in ACT score in the discussion.

3. Our statistician also thought that exploration of dose-effect on adverse event should also be done.

We have included our analyses in which we assessed rate of allergic AEFI and delayed AE with LAIV containing >0.3 and >1.0 ng/ml (tables E1 and E2).

4. Given that severely allergic children were excluded, we did not think we would be completely reassured by this paper if we had a severely allergic child, especially when the vaccine is for a disease that isn't uniformly fatal. A serious adverse event with a probability of even 1:1000 would be quite significant, so some toning down is required, especially of 'what this study adds'.

We have attempted to explain some of the confusion regarding what constitutes a 'severe' allergy in the discussion. Anaphylaxis is defined as a "severe, life-threatening generalized or systemic hypersensitivity reaction", and 35% of our cohort had a history of anaphylaxis to egg (20% respiratory and/or cardiovascular involvement). We would therefore suggest that a significant proportion of study participants had 'severe' allergy, and were not found to be at higher risk of AEFI (either of allergic aetiology, or otherwise) following LAIV.

We also highlight the rarity of a food-allergic reaction that it results in ICU admission or death. In the last 21 years, there has only been one fatality due to egg in the UK (and this was in an adult) (Turner et al, JACI 135:4,956–963.e1). There are approximately 3 ICU admissions for every fatality due to food-anaphylaxis in the UK. This implies very few admissions to ICU due to egg allergy over the past 21 years. Indeed, in our study, no child was excluded due to a previous egg allergic reaction requiring ICU admission. The statement by JCVI was based on the absence of data in this very small group of egg-allergic children with prior ICU admission, but as Reviewers 1+4 point out, such children are still likely to tolerate LAIV without problem.

5. We would like you to do a sensitivity analysis with the denominator being the roughly 1/3 of children who had a previous anaphylactic reaction to egg. We think the resulting confidence interval would be informative.

We include this in our results ("In children with a history of anaphylaxis, the equivalent 95% upper CI interval was 1.36%.") and mention in our discussion that the literature now reports 338 children with previous anaphylaxis to egg (equivalent to a 95% upper CI interval of 1.09% for systemic allergic reaction following LAIV).

6. We agree with the reviewer who would like to see clarification of what "physician diagnosed allergy" means. About half the children had received flu vaccine in prior years. Does this mean we know they are not allergic to egg products? Do you know which vaccines they had received?

"Physician-diagnosed allergy" means that a medical doctor has diagnosed the child with egg allergy at some stage. Our original SNIFFLE-1 study (JACI 2015;136:376-81) included only children with a very high likelihood of egg allergy. The purpose of this study was to expand the recruitment to encompass a population more similar to that which would be seen by healthcare professionals in primary care and/or the schools setting, where the UK immunisation programme for influenza is based. We cannot expect healthcare professionals, in this setting, to have the time and requisite competencies to assess whether a child has true egg allergy or not. We therefore chose to use the same criteria as might be applied in this setting ie. any physician-diagnosis of egg allergy. However, we did (as Reviewer 4 mentions) conduct a secondary analysis assessing rate of AE in

children who met international consensus criteria for >95% likelihood of clinical egg allergy (Tables E2 and E3).

7. Can you also clarify what is meant by "evidence of wheeze" and where it comes from? From trials? From reporting systems? Can you also clarify what is meant by severe unstable asthma?

We weren't exactly sure what you are asking us to clarify, as we don't use the phrase "evidence of wheeze" in our paper. We have assumed that you are referring to the following sentence in the introduction: "Some guidelines recommend against LAIV in children with recurrent wheeze, due to limited evidence that LAIV may induce wheezing in younger children". This data came from a clinical trial, which we now reference.

8. The risks of vaccine should be weighed against benefits; we do not have that information presented anywhere in the paper.

We have added some background relating to this (as considered by the JCVI) in the introduction.

9. We were also slightly surprised by the uncontrolled design, which means we can't gauge the incidence of reactions relative to children who are not egg allergic.

We have discussed this in the modified discussion. For the reasons stated, we decided it would be a better use of our limited resources to maximise the recruitment of egg-allergic children to assess our primary endpoint (allergic AEFI), with comparison to historical data (Table 2). We note that Reviewers 2,3 and 4 all considered our study design to be appropriate in the circumstances.

10. We were also surprised that parents were willing to subject their children to a treatment that might induce a life threatening reaction. How many parents did you approach to get to their final sample?

1830 children (and their families) were approached, as shown in Figure 1. The Manuscript Committee may be interested to learn that there was great demand from parents to participate, as most parents were keen for their child to receive LAIV within the safe environment of the hospital units participating in the study. Likewise, there was significant positive feedback from online 'blogging'.

11. We also thought that outcomes were not clearly defined, for example: "The primary outcome was the incidence of allergic reaction as an adverse event following immunisation (AEFI) occurring within 2 hours of LAIV administration in eggallergic children"-- determined how?

We have provided some further detail. We used international consensus criteria for allergic AEFI (systemic reactions/anaphylaxis) to define allergic AEs (Ruggeberg et al, Vaccine 2007;25:5675–84; reference 19 in our original submission). The use of the 2 hour window is consistent with international criteria (World Allergy Organization guidelines for the assessment and management of anaphylaxis. WAO J 2011;4:13-37).

12. We thought you should focus on explanations and discuss the "maximum impact" for serious and mild reactions – both are important. Please also make clear -- in the paper and also the abstract-- that seriously allergic children were excluded so results don't apply to them.

We have made some changes to the manuscript which we hope have addressed this.

13. We need more detail in the methods about the harms and adverse events: how were they measured or reported, how were they defined? We also need more detail on exactly what happened clinically. These details of each AE could be included in an appendix document.

We have included further detail in the methods, and a list of acute AEFIs in Table E1.

Reviewer: 1

• Figure 1 indicates that 13 children were excluded as not eligible. Why were these children excluded? How many had very severe anaphylaxis to egg requiring intensive care? How many had had asthma symptoms in the preceding 72 hours?

We have included these details in the caption to Figure 1. No child was excluded due to prior ICU admission.

• In the final paragraph, the authors state that the current study supports published guidance that indicates that children with "very severe anaphylaxis to egg requiring intensive care" and those with "evidence of the active wheezing in the 72 hours prior two LAIV" should not receive LAIV. It would seem more appropriate to state that although such children were excluded, it is not known whether or not they are at greater risk for vaccine reactions and may not be at increased risk.

We have mentioned this in our discussion in relation to prior ICU admission. We are unable to address the safety of LAIV in children with acute wheeze, as this was not assessed in our study, nor do we think most physicians would consider it appropriate to administer LAIV to children with acute lower respiratory symptoms.

• The actual guidance cited in reference number 18 states that even "children with a history of severe anaphylaxis to egg which has previously required intensive care, should be referred to specialists for immunisation in hospital" and that although LAIV "should be deferred in children with a history of active wheezing in the past 72 hours or those who have increased their

use of bronchodilators in the previous 72 hours. If their condition has not improved after a further 72 hours then, to avoid delaying protection in this high risk group, these children should be offered an inactivated influenza vaccine." It seems important to state that even children in these groups should be vaccinated with these precautions and that the precautions themselves may be unnecessary.

We have quoted the guidance and referenced it in our paper – we weren't sure as to the extent BMJ would like us to quote guidance from DoH relating to influenza vaccination rather than comment on our actual data. Should the BMJ consider this to be an important statement, we would be happy to add it (perhaps as a 'box') to highlight it.

Reviewer: 2

• Page 10, lines 38-44: The grammar of the sentence "Secondary outcomes were: incidence..." appears to be suboptimal as generally a colon should not follow a verb and the list of secondary outcomes is difficult to understand with clarity (I believe there were 2 in total and that the ACT outcome was the change between prior to vaccination and 1 month following?).

The reviewer has understood our secondary outcomes, so we haven't made any change as yet. Please advise us if the BMJ committee would like any changes to be made.

• Page 11, line 13: I believe there is a missing close parentheses after "12 months"

Many thanks, we have fixed this.

• Page 11, line 19: "prior influenza administration..." would be better stated as "prior receipt of any influenza vaccination (IIV or LAIV) and prior receipt of LAIV" or something similar

We have changed the wording used.

• The non-significantly higher rate of wheeze in younger children is not unexpected given the pathophysiology of childhood wheeze. It is not clear how this analysis addresses the question of whether LAIV induces a higher rate of wheeze in children less than 5 years of age. The authors may wish to clarify the utility of their finding, perhaps by contrasting it with the ACT score in the various age groups, which provides a pre- and post-vaccination assessment within the same children, controlling for age effects.

We have commented on this in our discussion.

• Table 2: Rates of parent-reported wheeze and medically-attended wheeze in children following LAIV are available as "wheeze" and "medically-attended wheeze" in Ambrose et al EJCMID 2012, Ashkenazi et al PIDJ 2006, and Fleming et al PIDJ 2006. The time intervals for collection appear to differ from the Turner et al study however.

The references provided by the Reviewer use very different definitions ('wheeze to 42 days post LAIV') as we mention in our original manuscript, in contrast to the 3 days following LAIV in this study. We therefore feel that such a comparison would not be valid.

• Limitations of the current study should be discussed, in particular that the study was not a randomized placebo-controlled study and thus event rates can only be compared to historical data and/or a clinical assessment of relevance.

We have included this in our discussion in the modified manuscript.

• The authors may wish to clarify that the selection of LAIV with a high ovalbumin content represent something of a worse-case scenario given that much of the vaccine used has much lower ovalbumin content.

We did not observe a dose-effect relationship. Furthermore, the batches of LAIV used in the UK still contain 100-1000 fold less ovalbumin than that permitted by the regulator. JCVI have advised that batches available in the UK in future years are going to continue to have low levels of ovalbumin present. Furthermore, our previous data suggest that even in a worst-case scenario, LAIV is unlikely to trigger an IgE-mediated allergic response in the nose (Turner and Erlewyn-Lajeunesse, JACI In Pract 2015; 3:312-3) as mentioned above.

• It is notable that ACT score improved following LAIV in children 2-11 years. A reduction in wheeze following LAIV has been observed in randomized studies (Fleming et al, PIDJ 2006). This may be due to short-term non-specific antiviral immunity induced by innate immunity mechanisms (see Zhu et al, Vaccine 2010).

We thank the Reviewer for this comment. However, we note that the 'reduction' was a "percentage point difference in incidence of -0.1% (90% CI = -2.4 to 2.2; 95% CI = -2.8 to 2.6)" in the study by Fleming et al, an effect which we would argue is not clinically or statistically significant (as the authors of the paper themselves point out).

Reviewer: 3

• P13 line 15-22 "For this study, we sourced vaccine with detectable ovalbumin. In 667 (86%) children, the LAIV batch used contained >0.3 ng/ml ovalbumin, of whom 511 (66%) received a dose containing >1ng/mL ovalbumine" The amount of

ovalbumine is greater than 0.3 ng/ml or 0.1 ng/ml. Please describe the upper value of the amount of ovalbumine or the range of values.

We now provide the upper value in the text.

• P14 Line 37 "Therefore, no child experienced a systemic reaction following LAIV"
This should be reworded to say "Therefore, no child experienced a systemic reaction "caused" by LAIV

We have amended the wording.

• P15 line 19 "Sixty-two children (8.1%, 95% CI for population 6.3-10.3%) experienced lower respiratory symptoms …P15 line 27 To assess this, in an additional exploratory analysis, we compared the rate of lower respiratory symptoms in children with asthma or recurrent wheeze: children under 5 years were slightly more likely to develop lower respiratory symptoms compared to those over 5 years, although this did not reach statistical significance (22/149 (15%) vs 26/296 (8.7%),P=0.07)". I understand that only 14 [62 – (22+26)=14] children with lower respiratory symptoms did not have asthma or recurrent wheeze. Please also provide the percentage stratified by age (under 5 and ≥5). The group "over 5 years" should probably be labeled "5 years and older"

We have clarified these data in the results.

• P15 line 52 "There was no significant change in ACT score for children 12 years and over (p=0.12). In those aged 2-11 years, there was a small but significant improvement in ACT following LAIV (p<0.001). A similar improvement was also noted when the analysis was restricted to children under 5 years (p<0.001)." Please provide the actual change in ACT scores for the different groups.

We have now provided this information.

Reviewer: 4

• Given the scope of the trial and the reach this report may have to other westernized nations, I would ask the authors to consider expansion of possible case numbers (lines 38-42) in two ways. The first would be to be to elucidate number of egg allergic individuals in the UK, in Europe, Australia, Canada, and the US (these numbers are easily available in the Allergy literature, and will serve to emphasize that this affects hundreds of thousands of kids, so as not to minimize the issue of why a change in policy based on these data may be urgently indicated. The second would be to stratify the number of estimated egg allergic children with asthma or history of transient wheeze (or reactive airways), which is the highest risk group for influenza related complications. Again approximate estimations should be easy to compile, and again I think this strengthens the message of this manuscript. The policy implications for these findings are very large, and thus strengthening the scope of problem that these data could solve will serve to maximize the potential impact of the manuscript.

As the Reviewer highlights, we have provided an indication of case numbers within the UK population already. We do not ourselves consider it appropriate or necessary to do the same for Australia, Canada or USA (or indeed, any other country, especially given WHO intentions to use LAIV in the developing world to control influenza), as we believe that the implications of our findings should be immediately apparent to other countries faced with similar LAIV implementation issues. Nonetheless, we will endeavour to provide estimates for other countries if the BMJ considers this request to be helpful.

• Methods: Line 11 (page 9): please elaborate on what you mean by "physician diagnosed egg allergy". This makes all the difference in terms of how any policy may be interpreted. What were the criteria—history of reaction, documented failed egg oral challenge, positive skin test or sIgE >95%PPV, or other? This must be explicitly stated to provide diagnostic validity and provide a basis for who will ultimately be included in a policy shift. Also, what type of physician makes this diagnosis--I assume you imply Allergy specialist here but if a GP or pediatrician made some of these diagnoses, but again the validity of who makes the diagnosis can vary, and this would potentially affect your pre-test probability of the child being at risk for possible egg related adverse reactions attributable to this as a vaccine excipient (e.g., I trust an allergist's diagnosis far more than a non-allergist's).

We have provided clarification (something also requested above), as well as including further details on the specialist knowledge of the physician making the diagnosis (the diagnosis was verified by an allergy specialist in 673 (86%) children). We have referenced the criteria used to define 95% PPV in our original submission. We would also highlight that our original SNIFFLE-1 study (JACI 2015;136:376-81) included only children with a very high likelihood of egg allergy. The purpose of this study was to expand the recruitment to encompass a population more similar to that which would be seen by healthcare professionals in primary care and/or the schools setting, where the UK immunisation programme for influenza is based.

• Line 17, page 11: please provide evidence to substantiate what I would say is a ridiculous claim of airborne reaction to egg. I have no doubt this is a route of exposure to which reactivity is attributed, but short of directly snorting/inhaling dehydrated egg powder or water in which egg protein was boiled and protein leached, I am not aware of any case reports or rigorously conducted studies showing that any egg protein can be inhaled in vapor and be realistically attributed to causing a reaction. This route has been reported for shellfish, and debunked for peanut. This group itself would be reportable.

We did not want to use this manuscript as a platform to discuss the frequency of true reactions to 'airborne egg'. We share the sceptism of the Reviewer, although the he may be interested to learn that this is a recognised occurrence in industry (eg. Leser C, Hartmann AL, Praml G, Wüthrich B. J Investig Allergol Clin Immunol. 2001;11(2):89-93.) Nonetheless, what is important

here is that parents who consider their child to react to 'airborne egg' may be more concerned that their child would react to LAIV. It is for this reason that we included this in our sub-analyses.

• Line 22 (same page): why were recipients previously tolerant of LAIV included? Was a pre-specified sensitivity analysis planned to exclude these cases?

The purpose of this study was to expand the recruitment to encompass a population more similar to that which would be seen by healthcare professionals in primary care and/or the schools setting, where the UK immunisation programme for influenza is based. It is possible that prior LAIV may increase risk of AE to subsequent LAIV due to increased sensitisation. It was therefore essential to include children who have previously received LAIV, as LAIV is given annually.

• Line 30-40: What was the estimated power for this sample size? I understand the difficulty of finding a confidence interval around a highly improbable event, but I think precursory power should be reported. For comparison, can you list what the rate of reaction to LAIV in the general population is, and show power based on difference in those populations, which would be the better comparison, rather than trying to show power to demonstrate the effective rate you expected to find in this population would be "nil"? Though this is a bit of post hoc dabbling, it could be considered a secondary analysis, and you'd be showing there was adequate power for this. For affecting policy, I would argue that it's less important (but not unimportant) to show that this vaccine causes virtually no reactions in the egg allergic, and more important to show it causes no higher rate of reaction than would be expected in the general population.

We would agree that this is "post hoc dabbling" and therefore unhelpful and not justified. Furthermore, the literature reports over 1,000,000 doses of LAIV in non-allergic individuals, in contrast to the sample size in this study.

• Results: Line 32, page 13: I'd argue to exclude the sensitized children who have never eaten egg before, or at least analyze them as a separate subgroup. These kids are an artifact of past (and erroneous, in my opinion) thinking that we can paternalistically prevent kids from "reacting", and few if any of these PPV's are derived from population based samples and at best represent a poor understanding of posterior probability limited to a clustered sample and not otherwise able to generalize to other settings. Health Nuts in Australia is the only such study actually. The clinical definition of allergy is sensitization in the setting of clear symptoms typical of IgE mediated reactivity. Inclusion of this group may represent a realistic group managed as "egg allergic", but a harsh critic could argue these kids are standard risk until it's definitively proven they react to egg (as opposed to a view that they are highly probable to react to egg). Thus, I'd rather see these kids analyzed as a subgroup, or a sensitivity analysis showing the rate with and without these kids included to make sure the population is enriched with true reactors.

We included this group for the reasons stated above. Only 56 (7.2%) were in this group, and thus we do not feel their inclusion have skewed our analyses. Furthermore, we performed sub-analyses (now formally reported in Tables 2+3) and found no change in rate of AEs in children with >95% likelihood of true clinical allergy.

• Line 29 page 14: what were these kids tested to? I presume LAIV but this is not stated, and it should be. Were they tested to other excipients in the vaccine as well?

We have clarified this in the text.

• Discussion: I am not sure I'd make such a big deal over a rate of ~1% of localized IgE mediated reactions, and would like to see what the rate is in the non-egg allergic population (and that you show this is not significantly different, thus reassuring the reader this is mere background noise that happens to a few in the course of receiving vaccination). I also think the point of there being lower ovalbumin in successive lots each year is moot. This happened with IIV too, yet the contraindication remained until the US/Canadian effort debunked this was unsafe in 2009-2012. I'd challenge you to find any actual proof that ovalbumin in any type of influenza vaccine was ever actually attributable to a bona-fide IgE mediated reaction, as opposed to historical paranoia that this "could" be a risk factor without any actual proof of danger. The issue here is that this is not a "clean" vaccine, and it has lots of excipients equally or more likely to cause a reaction than the minute quantity of egg. I think you can be much more forward here in saying this is definitively safe, and de-emphasize the ovalbumin levels.

• Please do more to emphasize the safety of LAIV in the child with reported wheeze. The use of the ACT as an objective outcome measure was quite brilliant. You should be able to comment if there was enough power to find an effect greater than the index MCID. I agree wholeheartedly with the ridiculous US contraindication, but the CDC and ACIP take a very conservative approach to this concept. Please trust me in that you will be doing the US a huge favor by really hammering home these data that you have. While this may not be enough in and of itself to convince the CDC to change the policy, it may be enough to encourage a US group to explore this subgroup more deeply, which would help make that policy change.

• I'd also encourage you to revise your assessment that this vaccine would not be safe for those with severe anaphylaxis. Yes, you didn't discretely study this, but that doesn't mean that it is not equally as safe. I'd argue that any child who is at risk for anaphylaxis is potentially at risk for ventilation given the right circumstances and these are not two separate populations of kids with anaphylaxis. They are one, and thus the findings would have exceptionally high probability of extrapolating. You have no data to say it's not safe, though equally you have no data to prove it is. You could argue practically that the number of kids meeting that criteria are low, and maybe then you should consider showing the number of expected cases that would be excluded from the policy to show how minimal that would be. These kids are essentially collateral damage from study exclusion, and the probability they'd be fine and benefit from LAIV has to be considered.

Given the comments of the BMJ Editorial committee, we are reluctant to over-emphasise our data in terms of demonstrating safety. The purpose of our submission is to report our study data and provide the evidence which underpins the revised UK

guidance on use of LAIV in children with egg allergy +/- asthma. We would be delighted if our data can be used to drive a similar change in policy in North America, but do not feel it is our role to do so in this manuscript.