NETSCC, HTA

22nd December 2010

Study Title:

A multi-centre, open-label, clinical, phase 4 trial, following on from a head-to-head comparison study of two H1N1 influenza vaccines in children, to compare firstly, the persistence of antibody against the A/California/7/2009 (H1N1) virus and secondly the immunogenicity and reactogenicity of one dose of a non-adjuvanted trivalent seasonal influenza vaccine, in children who had received a two-dose immunisation regimen of Celvapan or Pandemrix.

Short title:

Swine Flu (Influenza A H1N1) Follow on Vaccine Study

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1. SYNOPSIS

Study Title	**A multi-centre, open-label, clinical, phase 4 trial, following on from a head-to-head comparison study of two H1N1 influenza vaccines in children, to compare firstly, the persistence of antibody against the A/California/7/2009 (H1N1) virus and secondly the immunogenicity and reactogenicity of one dose of a non-adjuvanted trivalent seasonal influenza vaccine, in children who had received either a two-dose immunisation regimen of Celvapan or Pandemrix .**
Short Study Title	Head-to-head comparison of two H1N1 swine influenza vaccines in children aged 6 months to 12 years - an extension study
Internal ref. no.	OVG 2010/03
Clinical Phase	Phase IV
Trial Design	Follow-on study from a randomised control trial
Trial Participants	Children aged approximately 17 months to 14 years and 2 month
Planned Sample Size	Approximately 560
Planned Trial Period	October 2010 – March 2011
Primary Objectives	**1. Persistence of microneutralising antibody titres against H1N1v** To compare the percentage of children with microneutralisation (MN) titres ≥ 1:40, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix. **2. Immunogenicity of trivalent seasonal influenza vaccine** To compare the percentage of children who seroconvert and have a post-vaccination MN titre ≥1:40 or HI titre ≥1:32 (H1N1 strain) or who were seropositive at pre-vaccination and have a 4- fold increase in titre, following one dose of a non-adjuvanted seasonal trivalent influenza vaccine, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

	3. Reactogenicity of trivalent seasonal influenza vaccine To compare the percentage of children experiencing fever, local reactions and non-febrile systemic reactions within the 7 days following one dose of a non-adjuvanted seasonal trivalent influenza vaccine 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix[a].
Secondary Objectives	**Persistence of antibody titres to H1N1v** To compare the percentage of children with HI titre ≥ 1: 32 and the geometric mean HI and MN titres 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix. **Long-term safety monitoring of Pandemrix and Celvapan** Specific adverse events (influenza-like illnesses (ILI)[b], hospitalisations, febrile convulsions, autoimmunity[c] and adverse events of special interest (AESIs[d]) will be assessed in all participants.. **To store serum** For future testing of the immunogenicity of trivalent seasonal influenza vaccine for H3N2 and B strains, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix. Also, should a drifted H1N1 strain emerge next season, this would provide a valuable source of sera to assess cross protection.

[a] The age of study participants will be 11-15 months older than in the original study.
[b] ILI defined, as per the Center for Disease Control and Prevention (CDC) definition, as "temperature of ≥37.8 °C and either cough or sore throat in the absence of a known cause other than influenza" (see reference 2).
[c] See Appendix E
[d] Neuritis, convulsions, anaphylaxis, encephalitis, vasculitis, Guillain-Barré syndrome, Bell's palsy, demyelinating disorders, vaccination failure and narcolepsy – see section 8.1.10

	T cell Responses To study the T cell responses to internal influenza antigens and haemagglutinin (pandemic H1) **Genetics** To identify genes that are differently expressed following one dose of a non-adjuvanted seasonal trivalent influenza vaccine 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.
Primary Endpoints	**1. Persistence of MICRONEUTRALISING antibody titres against H1N1v** The percentage of children with microneutralisation (MN) titres ≥ 1:40, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix **2. Immunogenicity of trivalent seasonal influenza vaccine** The percentage of children who seroconvert and have a post-vaccination MN titre ≥1:40 or HI titre ≥1:32 (H1N1 strain) or who were seropositive at pre-vaccination and have a 4- fold increase in titre, following one dose of a non-adjuvanted seasonal trivalent influenza vaccine, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix **3. Reactogenicity of trivalent seasonal influenza vaccine** The percentage of children experiencing fever, local reactions and non-febrile systemic reactions within the 7 days following one dose of a non-adjuvanted seasonal trivalent influenza vaccine 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix**.**

Secondary Endpoints	**1. Persistence of antibody titres to H1N1v** The percentage of children with HI titre ≥ 1: 32 and the geometric mean HI and MN titres in children 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix **2. Long-term safety monitoring of Pandemrix and Celvapan** Specific adverse events (influenza-like illnesses (ILI), hospitalisations, febrile convulsions, autoimmunity and adverse events of special interest (AESIs) will be assessed in all participants. **3. T cell Responses** The T cell responses to internal influenza antigens and haemagglutinin (pandemic H1) **4. Genetics** The identification of genes differentially expressed in response to vaccination with the seasonal influenza strain.
Investigational Medicinal Products	Non-adjuvanted seasonal trivalent influenza vaccine - Fluarix® (GlaxoSmithKline Biologicals, Dresden, Germany)

2. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
AESI	Adverse Event of Special Interest
CFI	Centre for Infections
CHMP	Committee for Medicinal Products for Human Use
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
CTA	Clinical Trials Authorisation
CTRG	Clinical Trials & Research Governance, University of Oxford
EMEA	European Medicines Agency
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
GP	General Practitioner
HI	Haemaglutination Inhibition
HPA	Health Protection Agency
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
ILI	Influenza Like Illness
IMP	Investigational Medicinal Product
IRB	Independent Review Board
MHRA	Medicines and Healthcare products Regulatory Agency
MN	Microneutralisation

NRES	National Research Ethics Service
OVG	Oxford Vaccine Group
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
RBC	Red blood cells
RDE	Receptor Destroying Enzyme
REC	Research Ethics Committee
RVU	Respiratory Virus Unit
SAE	Serious Adverse Event
SAGE	Strategic Advisory Group of Experts on Immunisation
SAR	Serious Adverse Reaction
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TSG	Oxford Radcliffe Hospitals Trust / University of Oxford Trials Safety Group
VRD	Virus Reference Department
WHO	World Health Organisation

3. BACKGROUND

The first cases of pandemic influenza A (H1N1) 2009 infection were seen in Veracruz, Mexico, in March 2009 and spread rapidly, leading the World Health Organization to declare it the first global pandemic of this century on 11th June 2009. (3, 4) It is thought to have been responsible for 16226 deaths globally as of 21st February 2010.(5)

Children have been identified as a high priority group for immunization during a pandemic influenza outbreak for some time. This is for several reasons; they are effective vectors for disease transmission and have four times higher rates of infection and are hospitalized more frequently than adults.(6-8) This is due, at least in part, to the fact that children had no measurable previous immunity to pandemic H1N1 infection prior to the outbreak, even if in receipt of previous seasonal influenza vaccine.(9)

The UK Government purchased two pandemic influenza vaccines, Celvapan, a non-adjuvanted whole virion vaccine made by Baxter (Vienna, Austria) and Pandemrix™, an $AS03_B$/oil-in-water emulsion-adjuvanted ($AS03_B$) split-virion vaccine made by GlaxoSmithKline Biologicals (Rixensart, Belgium). For the UK national vaccination program the AS03-adjuvanted vaccine was used as the first line. The national vaccination program in children switched from including only high-risk children to all children under 5 years of age in November 2009, when a single dose schedule was also adopted.

In previous influenza pandemics, for example the Spanish Influenza pandemic of 1918, there have been significant second and third waves of pandemic influenza infection in subsequent influenza seasons.(10) It is uncertain which influenza viruses will be prevalent in the Northern Hemisphere in the 2010-2011 influenza season but there is significant concern over the likely reemergence of the A/California/7/2009 (H1N1) virus, the 2009 pandemic influenza strain, as the predominant cause of influenza infection. This is illustrated by the fact that the WHO have recently released their recommendation for which virus strains should be included in trivalent seasonal influenza vaccines for this period, and this includes the A/California/7/2009 virus (H1N1).(11) There are no published data, at present, assessing persistence of seroprotection against the A/California/7/2009 (H1N1) virus for any vaccines.

The immunogenicity and reactogenicity of seasonal influenza vaccines following previous use of adjuvanted or unadjuvanted pandemic influenza H1N1 vaccines are unknown. On 23rd

March 2010 the Australian Government suspended routine immunization of children 5 years of age and under due to a suspected increase in febrile convulsion rates using trivalent unadjuvanted seasonal influenza vaccine (12) (13). A national evaluation of cases of fever with convulsions in young children following seasonal flu vaccination initially found no pattern of increased incidence of this side effect, other than higher numbers in Western Australia (13), although more detailed analysis has now demonstrated similar small increases in the rate of febrile convulsions across all Australian jurisdictions (14). Almost all these additional reactions were caused by Fluvax® or Fluvax® Junior, manufactured by CSL, with an estimated rate of febrile convulsions with these vaccines of up to 9 in 1000 doses compared to less than 1 in 1000 estimated for Influvac® and Panvax® (14). On 1st June 2010 the suspension of the Australian paediatric seasonal influenza immunisation programme was confirmed until further notice (14).

4. RATIONALE FOR FOLLOW-ON STUDY

In Autumn 2009 we undertook a study assessing the safety and immunogenicity of a two-dose schedule of the two Influenza A (H1N1) vaccines purchased by the UK Government, the non-adjuvanted whole virion vaccine and the ASO3-adjuvanted split-virion, in children aged 6 months to 12 years of age. 937 children completed the study by protocol and the main findings were that the adjuvanted vaccine, while reactogenic, was more immunogenic especially in younger children (seroconversion in children under 3 years of age was 98.2% vs. 80.1%, p=0.001) (15).

Following events in Australia (13, 14), and regardless of the formal investigation outcome, it is imperative to study the reactogenicity of UK seasonal influenza vaccines in children who had previously received immunization with adjuvanted H1N1 vaccines. It would be particularly important to gain early information on the fever rates in young children in order to assess whether these are higher than expected and carry a potential risk of febrile convulsions.

It is also important to determine the immunogenicity of trivalent seasonal influenza vaccine in children previously given univalent pandemic influenza vaccine. There is emerging data that different priming strategies with adjuvanted or non-adjuvanted vaccines may lead to considerable differences in the response to subsequent influenza vaccines. In the head to head paediatric study (15) unpublished analyses show significantly lower immunogenicity in children who had received seasonal influenza vaccines in the past, despite the receipt of two

doses of either Pandemrix or Celvepan. In addition, unpublished data from a manufacturer study suggests a negative effect of two doses of Pandemrix on immune responses to subsequent seasonal vaccine when given 3 weeks after the second dose (personal communication to E Miller from MHRA). Alternatively, as shown with pandemic H5N1 influenza vaccine, there may be a significant booster response to a subsequent dose following priming 6 or 14 months previously. (16) (17). However, this has not been demonstrated with either Pandemrix or Celvapan, and it is unknown how previous vaccination with these vaccines will affect the immunogenicity of the H1N1 component of an unadjuvanted trivalent seasonal influenza vaccine given a year later.

We therefore propose a follow-on study to compare firstly, the persistence of antibody against the A/California/7/2009 (H1N1) virus after the use of these novel H1N1 influenza vaccines and secondly the immunogenicity and reactogenicity of one dose of a non-adjuvanted trivalent seasonal influenza vaccine in children, after receiving a two-dose immunisation regimen of either Pandemrix or Celvapan.

In previous pandemics, there have been further waves of infection in the subsequent influenza seasons, particularly when the pandemic strain has drifted antigenically. It is important therefore to study the persistence of antibody against pandemic influenza A (H1N1) infection in children, particularly those for whom seasonal influenza vaccine will not be recommended next year. Should a drifted H1N1 strain emerge next season, sera from children vaccinated in 2009 with the A/California/7/2009(H1N1) strain could be used to assess the likely cross protection to such a drifted strain. The existence of this unique cohort of almost 1000 children will allow information on antibody persistence to be generated for both the non-adjuvanted whole virion vaccine (Celvapan) or the ASO3-adjuvanted split-virion vaccine (Pandemrix) and would provide a valuable source of sera to assess cross protection in the event of emergence of a drifted strain.

We therefore propose a follow-on study to compare firstly, the persistence of antibody against the A/California/7/2009 (H1N1) virus after the use of these novel H1N1 influenza vaccines and secondly the immunogenicity and reactogenicity of one dose of a nonadjuvanted trivalent seasonal influenza vaccine in children, after receiving a two-dose immunisation regimen of either Pandemrix or Celvapan.

This follow-on study will also provide an important opportunity to provide data on the long term safety of the Pandemrix and Celvapan vaccines prior to enrolment in the follow-on study.

The study will use a non-adjuvanted trivalent seasonal influenza vaccine, Fluarix® (GlaxoSmithKline Biologicals, Dresden, Germany). It is approved by the EMEA for prophylaxis of influenza in all ages and has been marketed since 1987. It has consistently been shown to meet or exceed the regulatory criteria for immunogenicity against the three strains H1N1, H3N2 and B, and has a good safety profile. (18) Although the option of receiving this vaccine (and having a blood test to assess the immune response to this vaccine) will be offered to all participants in the study, participants (or parents/ guardians, on the participant's behalf) may decline to receive this vaccine and the second blood test. These participants would still be eligible to take part in the study for the first blood test assessing the persistence of antibody from the original study.

Persistence of seroprotection will be assessed by both haemagglutination inhibition (HI) and microneutralisation (MN). Although EMEA guidelines for licensure of influenza vaccine are based on HI assays, the primary objective for this study uses MN titres as its measure. The decision for the preference of MN titres over HI titres was made based on recently published observations by the Centers for Disease Control and Prevention (CDC)(19, 20) and results from the Health Protection Agency's own analysis, which showed that the MN assay generally yields higher titres and detected more seroconversions to A/California/04/2009 than the HI assay (although both generally show high correlation). We therefore used MN titres as the primary outcome measure in the original NIHR funded study (Clinicaltrials.gov registration number: NCT00980850)(1)

The cellular immune response to influenza immunisation will be assessed in children where sufficient blood is available and local laboratory facilities permit. Elispot assays will be carried out using PBMCs isolated from the blood to determine the T cell response to internal influenza antigens, and haemagglutinin (pandemic H1, seasonal H1 and seasonal H3). Exploratory flow cytometry assays may also be used to determine whether the T cells are CD4+ or CD8+, and to examine cytokine secretion.

RNA expression profiles pre and post vaccination will be scrutinised in 20 participants in each group to elucidate genes that are differentially expressed in response to immunisation.

This analysis could highlight genes of particular importance in vaccine responses. Furthermore, comparisons between RNA profiles and correlates of vaccine immunity may identify profiles which could be useful 'biomarkers' of vaccine induced cellular and humoral immunity in future studies.

With appropriate consent, serum samples remaining after the analyses required for this study will be stored for use in future infection and immunity related research studies at the relevant study sites.

5. OBJECTIVES

5.1 Primary objectives

1. Persistence of microneutralising antibody titres against H1N1v

To compare the percentage of children with microneutralisation (MN) titres ≥ 1:40, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

2. Immunogenicity of trivalent seasonal influenza vaccine

To compare the percentage of children who seroconvert and have a post-vaccination MN titre ≥1:40 or HI titre ≥1:32 (H1N1 strain) or who were seropositive at pre-vaccination and have a 4- fold increase in titre, following one dose of a non-adjuvanted seasonal trivalent influenza vaccine, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

3. Reactogenicity of trivalent seasonal influenza vaccine

To compare the percentage of children experiencing fever, local reactions and non-febrile systemic reactions within the 7 days following one dose of a non-adjuvanted seasonal trivalent influenza vaccine 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

5.2 Secondary objectives

Persistence of antibody titres to H1N1v

To compare the percentage of children with HI titre ≥ 1: 32 and the geometric mean HI and MN titres 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

Long-term safety monitoring of Pandemrix and Celvapan

Specific adverse events (influenza-like illnesses (ILI)[e], hospitalisations, febrile convulsions, autoimmunity[f] and adverse events of special interest (AESI's[g]) will be assessed in all participants.

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[e] ILI defined, as per the Center for Disease Control and Prevention (CDC) definition, as "temperature of ≥37.8 °C and either cough or sore throat in the absence of a known cause other than influenza" (see reference 2).

To store serum

For future testing of the immunogenicity of trivalent seasonal influenza vaccine for H3N2 and B strains, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix. Also, should a drifted H1N1 strain emerge next season, this would provide a valuable source of sera to assess cross protection.

T cell Responses

To study the T cell responses to internal influenza antigens and haemagglutinin (pandemic H1).

Genetics

To identify genes that are differently expressed following one dose of a non-adjuvanted seasonal trivalent influenza vaccine 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

6. TRIAL DESIGN

6.1 Summary of trial design

This is a follow-on, multi-centre, open-label, clinical, phase 4 trial to investigate:

a) The persistence of antibody against the A/California/7/2009 (H1N1) virus, 11-15 months after receiving a two-dose immunisation regimen of either a non-adjuvanted H1N1 vaccine (Celvapan, group 1) or the ASO3-adjuvanted H1N1 vaccine (Pandemrix, group 2).

b) The immunogenicity and reactogenicity of one dose of a non-adjuvanted seasonal trivalent influenza vaccine, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan (group 1) or Pandemrix (group 2).

The original NIHR funded study (NCT00980850)(1) evaluating the safety, tolerability and immunogenicity of the Celvapan (non-adjuvanted H1N1 vaccine) and Pandemrix (ASO3-adjuvanted H1N1 vaccine) in children was carried out in Autumn 2009.

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[f] See Appendix E

[g] Neuritis, convulsions, anaphylaxis, encephalitis, vasculitis, Guillain-Barré syndrome, Bell's palsy, demyelinating disorders, vaccination failure and narcolepsy – see section 8.1.10

A summary of the follow-on trial can be seen in the study flowchart (Table 1).

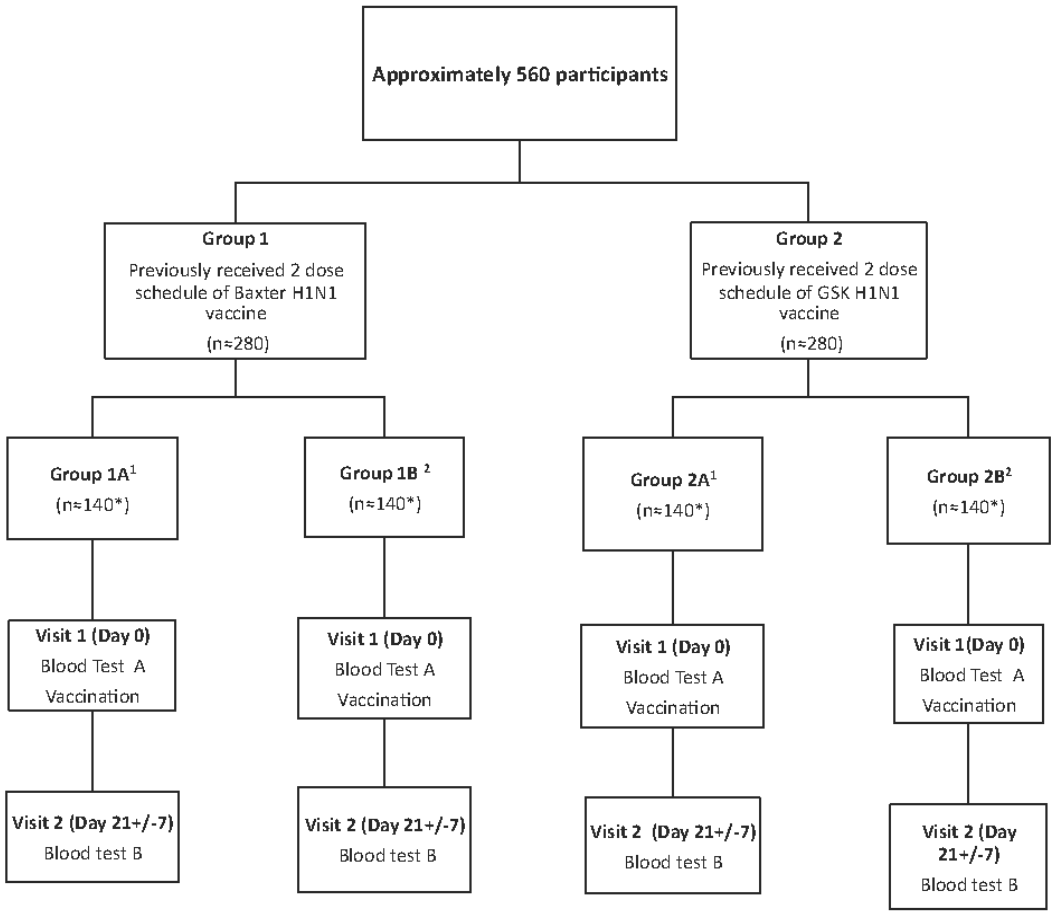

Table 1.
Group 1A and 2A: Aged between 6 months & 3 years old in original study. Group 1B and 2B: Aged between 3 & 12 years old in original study[h]
Based on recruiting 66% of those who took part in original H1N1v vaccine study, see section 9.2 (sample sizes) for further details. Participants will be given the option of having a blood test alone at visit 1, or a blood test, immunisation with seasonal flu vaccine and second blood test at visit 2.

The children in groups 1 & 2 will be divided into two age groups (subgroups A & B) based on the age groups they were in during the original study (NCT00980850)(1), see footnote[h].

Table 1 shows groups and relationship of sample points. Blood A, taken at enrolment, will be used both to demonstrate persistence of antibody against the A/California/7/2009 (H1N1) virus and, for those participants receiving immunisation and a second blood test, as a baseline measurement to compare to Blood B. Blood B will be used to determine

[h] The original study (See reference 1) divided the groups into those under and over 3 years of age. In this extension study we will use the original cohort of patients, 11-15 months after the initial immunisation.

immunogenicity of a non-adjuvanted seasonal trivalent influenza vaccine in the different groups of children.

6.2 Primary and secondary endpoints/outcome measures

6.2.1 Primary endpoints

1. Persistence of MICRONEUTRALISING antibody titres against H1N1v

The percentage of children with microneutralisation (MN) titres ≥ 1:40, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

2. Immunogenicity of trivalent seasonal influenza vaccine

The percentage of children who seroconvert and have a post-vaccination MN titre ≥1:40 or HI titre ≥1:32 (H1N1 strain) or who were seropositive at pre-vaccination and have a 4- fold increase in titre, following one dose of a non-adjuvanted seasonal trivalent influenza vaccine, 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix

3. Reactogenicity of trivalent seasonal influenza vaccine

The percentage of children experiencing fever, local reactions and non-febrile systemic reactions within the 7 days following one dose of a non-adjuvanted seasonal trivalent influenza vaccine 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix**.**

6.2.2 Secondary endpoints

Persistence of antibody titres to H1N1v

The percentage of children with HI titre ≥ 1: 32 and the geometric mean HI and MN titres in children 11-15 months after receiving a two-dose immunisation regimen of either Celvapan or Pandemrix.

Long-term safety monitoring of Pandemrix and Celvapan

Specific adverse events (influenza-like illnesses (ILI), hospitalisations, febrile convulsions, autoimmunity and adverse events of special interest (AESIs) will be assessed in all participants.

T cell Responses

The T cell responses to internal influenza antigens and haemagglutinin (pandemic H1).

Genetics

The identification of genes differentially expressed in response to vaccination with the seasonal influenza strain.

6.3 Trial participants

6.3.1 Overall description of trial participants

We intend to recruit all interested participants who completed the original NIHR funded study (NCT00980850)(1) (n=937) into groups 1 & 2 outlined in table one above. It is anticipated that approximately 66% of these participants are likely to take part in this follow-on study; therefore the study population for groups 1 & 2 is likely to be approximately 560 children, refer to section 9.2 - sample sizes for further details. As the option of only having a blood sample taken at visit 1 will be made available to participants in this study, there will be two cohorts of participants: the 'persistence' cohort (consenting to the baseline blood test alone) and the 'booster' cohort (consenting to the baseline blood test, seasonal influenza vaccine and post-immunisation blood test).

6.3.2 Inclusion criteria

The participants must have completed the original NIHR funded study (NCT00980850)(1) comparing Celvapan with Pandemrix at one of the study sites participating in this follow-on study.

All participants must satisfy all the following criteria to be eligible for the study:

A parent/legal guardian has given written informed consent after the nature of the study has been explained;

Willingness to either

a) undertake a blood test at visit 1 ('persistence' cohort)

b) complete all study procedures ('booster' cohort)

6.3.3 Exclusion criteria

The potential participants may not enter the study if ANY of the following apply:

- Participant(s) in original study (NCT00980850)(1) who had a suspected unexpected serious adverse reaction (SUSAR).
- Participants in the original study (NCT00980850)(1) who did not receive two doses of H1N1 influenza vaccine
- Participants in original study (NCT00980850)(1) who received a third dose of H1N1 influenza vaccine due to an inadequate response to two doses.
- History of severe allergic reaction after previous vaccinations or hypersensitivity to any seasonal influenza vaccine component
- Current egg allergy
- Known or suspected impairment/alteration of the immune system
- Disorders of coagulation
- Immunosuppressive therapy, use of systemic corticosteroids for more than 1 week within the 3 months prior to enrolment
- Receipt of blood, blood products and/or plasma derivatives or any immunoglobulin preparation within 3 months prior to enrolment
- Previous receipt of, or intent to immunize with, any other seasonal influenza vaccine(s) throughout the 2010/2011 influenza season.
- Participation in another clinical trial of an investigational medical product
- Any condition which, in the opinion of the investigator, might interfere with the evaluation of the study objectives. Children with chronic, stable medical illnesses that do not result in immunosuppression (e.g. cerebral palsy, epilepsy, cystic fibrosis, congenital heart disease) will be allowed to participate in the study, unless these conditions will in some way interfere with the completion of study procedures. Children with conditions that may alter the immune response to vaccines (e.g. Trisomy 21) or will affect the ability to accurately describe adverse events (e.g. children over 5 years of age but with severe learning difficulties) will be excluded.

6.3.4 Temporary exclusion criteria

(Applicable to 'booster' cohort only.)

Participants who have experienced fever (>38.0°C) within the previous 24 hours.

Participants receiving another immunisation within 3 days prior to enrolment (21 days for any live vaccine), or planning to receive another vaccine within 7 days of enrolment

6.4 Expenses and benefits

- All participants will be reimbursed £10 for each study visit to cover travel expenses. These payments will be provided to participants at the conclusion of their final study visit (or following the scheduled date for this visit if this were not to be completed).
- Participants will potentially benefit by being offered immunisation with the seasonal influenza vaccine, for which they may not have been entitled if not taking part in the study. This year it will cover pandemic influenza A (H1N1) infection.

6.5 Study procedures

Participants will either have a single blood test taken at visit 1 (persistence cohort) or have a blood test and a dose of a non-adjuvanted seasonal trivalent influenza vaccine administered on visit 1, followed by a second blood test approximately 3 weeks later ('booster cohort'). For the latter cohort a diary card detailing local and systemic effects of the vaccine, any AEs and any medications used to treat these AEs and SAEs will be completed by parents/ guardians for the first week after first immunisation. For the remaining 2 weeks before the subsequent visit (visit 2) the diary card will then be used to record solicited adverse events persisting after the first week following immunisation and any medically significant adverse events occurring.

6.5.1 Recruitment and pre screening

Parents/guardians of participants who completed the original NIHR funded study (NCT00980850)(1) at the sites participating in this follow on study will be sent an invitation letter (by post or e-mail), or contacted by telephone, informing them that we are conducting a follow-on trial. At some centres, at the end of the original study, participant's families were informed of the possibility that they would be approached for a 'follow-on' study, and the opportunity to opt out of this provided. No one expressed that they would not be happy to be approach for this follow-on study. The invitation letter will invite them to take part and direct them to a specifically designed website. The website will allow the parents/guardians of participants to pre-screen themselves and register interest in taking part. Sites will have the option to telephone the parents/guardians of participants prior to sending out reminder cards, or to send out reminder cards without a prior telephone call. If used, reminder cards will be sent two to four weeks after the original invitation.

Once an expression of interest has been received by the study centres an appointment would be made for them to attend at the designated recruitment centre where informed consent would be taken and the first study visit would be carried out.

6.5.2 Informed consent

A participant information sheet (in either paper or electronic form) will be provided to the participant's parent or legal guardian. At visit 1, a verbal version of the participant information will be presented to the participant's parent or legal guardian detailing no less than:

- The exact nature of the study;
- The implications and constraints of the protocol;
- The known side effects and any risks involved in taking part.
- It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant's parent or legal guardian will be allowed as much time as required to consider the information, and the opportunity to question the researcher, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will be obtained by means of a dated signature of the person legally responsible for the participant and signature of the person who presented informed consent. A copy of the signed Informed Consent will be given to the participant's parent or legal guardian. The original signed form will be retained at the study site. The informed consent discussion will be conducted by a nurse or doctor who has been trained in the consent process. The written informed consent form and any other written information will be revised whenever important new information becomes available that may be relevant to the consent. Any revised written informed consent form and written study information will be submitted to an ethics committee for approval before use.

In addition to informed consent from the parent/legal guardian, for participation in the study assent will also be sought and documented for those aged over 7 years before any study specific procedures are performed

The participant's parent or legal guardian will be informed in a timely manner if new information becomes available that may affect the decision to participate in the clinical trial. The communication of this information will be documented.

6.5.3 Screening and eligibility assessment

On arrival at visit 1, prior to gaining full written consent for the trial, if verbal consent is given a local anaesthetic (Ametop or Emla) according to local practice at each site) will be applied to the candidate. This will be done for the families' convenience to save them time during the

visit as, to be effective, the local anaesthetic cream, dependent on brand, needs to be applied for at least 30 minutes prior to venepuncture. This is the same procedure as took place in the original NIHR funded study (NCT00980850)(1) so participants will already understand the reason for this.

Following the attainment of informed consent, potential participants will be assessed by a study doctor to determine whether the candidate satisfies the inclusion/ exclusion criteria and to aid in the analysis of data. This assessment will include:

- Demographics: The date of birth, ethnicity and gender.
- Medical History: This will include history of asthma and details of any influenza-like illnesses (ILI), hospitalisations, febrile convulsions, autoimmunity and adverse events of special interest (AESI's)
- Concomitant Medication: All immunosuppressive medication and non-steroidal anti-inflammatory medications.
- Physical Examination.
- Axillary temperature (booster cohort only)

The details of this assessment will be recorded in the CRF. If the inclusion/ exclusion criteria are satisfied (including willingness to have a blood sample taken) and the informed written consent has been obtained the participant will be enrolled.

6.5.4 Baseline assessments

1. Perform blood draw collecting up to 7 ml in children under 3 years of age in the current study and 10ml in children ≥ 3 years of age[i].
2. Administer vaccination with a non-adjuvanted seasonal trivalent influenza vaccine. For all children administer 0.5 ml of vaccine.*
3. Record vaccination details in participant's 'red book' and/or the study vaccination card.*
4. Observe the participant for at least 20 minutes after vaccination for any immediate reactions.*
5. Fill out an 'unscheduled vaccination' form for the participant's Primary Care Trust.*
6. Fill out a notification to the participant's GP of the vaccine administered.*
7. Provide participant with study centre contact details (including 24 hour telephone advice line contact details for study staff member).
8. Instruct participant on notifying study centre of any serious adverse events/reactions.*

[i] As opposed to the age group they were enrolled into in the original study.

9. Instruct participants to use antipyretics only to treat fever or other adverse reactions, rather than prophylactically.*
10. Provide participant's parent or legal guardian with a Diary Card to detail local and systemic effects and AEs in first seven days after immunisation and any ongoing solicited reactions or doctor's visit/visit to Emergency Department from day 8 to the next visit.*
11. Provide the parent/guardian with local anaesthetic cream (Ametop or Emla according to local practice at each site) and instructions for use prior to visit 2 so that they can apply it to the child's skin in the appropriate amount of time prior to the visit.*
12. Schedule Visit 2, 21 (-7/+14) days after Visit 1.*

* Applicable to 'booster' cohort only

Visit 2

(Booster cohort only)

21 days (-7/+14 days) after visit 1 date.

1. If no local anaesthetic cream (Ametop or Emla according to local practice at each site) has been applied by parents prior to arrival then apply now.
2. Review diary card and obtain interim history and check eligibility criteria, specifically assessing for:
    a. serious adverse events
    b. adverse events requiring a visit to a physician or emergency department or potentially leading to the withdrawal of the participant
    c. newly prescribed vaccines
    d. any solicited AEs continuing on after day 7 post-immunisation or any medically significant AEs (as recorded in the diary card).
3. Perform blood draw collecting up to 7 ml in children under 3 years of age in the current study and 10ml in children ≥ 3 years of age[j].
4. Give Feedback form and reply paid envelope to parents, to be returned anonymously.

Every endeavour should be made to respect the timelines indicated above, however if a participant is not able to undertake a study visit within these timelines (e.g. due to intercurrent illness) then as long as the visit is able to be done in a reasonably timely manner they will not be excluded from the study (determined on an individual case basis by the clinical study team)..

[j] As opposed to the age group they were enrolled into in the original study.

6.5.5 Blood sampling

The volume of blood samples obtained from infants less than 3 years of age will be up to 7 ml, the volume after 3 years of age will be up to 10 ml. If the initial attempt at venepuncture is unsuccessful, (i.e. less than 4 ml obtained), then, depending on the judgment of the staff member, assent will be sought from the parents and child (as appropriate according to age) to have a further attempt. Following the initial attempt at venepuncture, a parent may decline any of these further attempts and their child will still be eligible to remain in the study. A local anaesthetic cream (Ametop or Emla according to local practice at each site) or cold spray (ethyl chloride) will be applied for an appropriate period of time prior to each venepuncture. For children in the booster cohort the parent/guardian will be provided with the anaesthetic cream and instructions for use prior to Visit 2 so that they can apply it to the child's skin in the appropriate amount of time prior to the visit.

Wherever possible (depending on the volume of blood obtained from the participant), a minimum of 4ml of blood should be available for serological analysis. For children in the booster cohort (for whom it is anticipated that blood would be available following immunisation with seasonal influenza vaccine) additional T cell or RNA analyses may be performed at local sites according to blood volume and local capacity (see Table 2).

	Serology only	Serology and T cells* and/or RNA analysis**
4 – 7 ml obtained	All into serology	NA
≥7 ml obtained	All into serology tube	At least 4 ml for serology tube 3 ml into Heparinised tube (if T cell analysis being performed). 2.5 ml into PAX tube (if RNA analysis being performed)

Table 2: Allocation of blood samples at bedside.

* Approximately 100 participants will have T cell analyses performed at the Oxford site.

** At least 20 participants in each group will have RNA analyses performed.

Allocation into the serology only, serology and T cells or serology and RNA analysis subsets will depend on blood volume obtained at V1, local capacity and other logistical

considerations (time and day of sampling etc). Enrolment into the T cell and RNA analysis groups is to be allocated and monitored locally (e.g. by controlling the number of T cell and RNA blood tube 'packs' distributed and used).

6.5.6 Diary card for recording local and systemic side effects

For the Booster cohort the participant's parent or guardian will be instructed to complete a diary card to record daily temperatures and describe local and systemic symptoms, all adverse events (AEs), and usage of analgesic/antipyretic medication for seven days following each vaccination starting on the day of administration. They will be asked to bring the completed diary cards to visit 2. If the parents forget to bring the diary card they will be given a stamped addressed envelope in order to post the diary card back to the study site as soon as possible. The research staff will review the diary cards with the parents/ guardians at this visit and any discrepancies clarified at this time. The diary cards will be entered by site staff onto the study electronic database. Data clarification will occur at the local site, contacting the participant's parent or guardian where necessary.

6.5.7 Follow-up of sub-optimal vaccine responses.

Participants found to have MN titres below 1:40 or HI titres below 1:32 for Influenza A H1N1 will be offered an additional dose of the seasonal flu vaccine, to be arranged by the study sites. This will not form part of the study evaluations.

6.6 Laboratory methods

6.6.1 Serological analysis

Blood samples taken from participants will be stored at room temperature for up to 60 minutes, and then stored at 2ºC to 8ºC. Samples collected at each study site will be centrifuged at 3000 rpm for 10 minutes within 24 hours at the study site and separated into at least two aliquots for storage at or below -30ºC. One aliquot will be shipped to the Centre for Infections Virus Reference Department (VRD) for testing, the other aliquot should remain at the study site for storage. All samples will be analysed by microneutralisation (MN) and hemagglutination inhibition (HI) with the NIBRG121 virus (rg virus based on A California/7/2009 (vH1N1).

- **Microneutralisation (MN)**

The Microneutralisation assay will only be performed for the analysis of serological responses to the pandemic H1N1 strain.

A protocol has already been set up in the laboratory and data from the initial paediatric vaccine study shows that this assay is more sensitive (i.e. detects more 4-fold increases and generally higher GMTs) than the HI when analyzing seroresponses after vaccination with pandemic monovalent vaccine. However, MN is not routinely used for analysis of seasonal vaccines for several reasons:

There is no defined correlate of protection for the MN, whereas such values are defined for HI and SRH. Secondly, this test is technically more demanding and time consuming than the HI. Lastly, the cross-reactivity between strains of currently circulating (seasonal) viruses and resulting pre-existing immunity complicates the development of specific and sensitive MN protocols and potentially confuses interpretation of results from vaccine trials.

The Microneutralisation assay for measurement of responses to pandemic H1N1 influenza will be performed in 96- well format according to previously described protocols (20) and SOPs developed at RVU.

- ***Serum pre-treatment***

  Elimination of complement (e.g. from Fetal Calf Serum in culture medium) will be performed by incubation of study sera and appropriate quality control sera (provided and chosen according to test virus by RVU; usually serum of ferret, sheep or human, with/without neutralization activity) at +56°C / 30min. This step will be performed simultaneously for all study samples and control sera.

- ***MN Test***

  The MN analysis with the NIBRG121 virus will be performed as follows: A two-fold dilution series will be set up for each of the samples and control sera. After addition of a pre-titred virus (usually around 100x$TCID_{50}$ per well or 0.1-1 virus particle per cell) neutralisation will be performed by incubation of the virus/serum mixture at room temperature for 1h. We will routinely perform a 6-step dilution (covering titres 20 to 640), but will determine endpoint titres for each sample by further titrating those specimen that show titres> 640.

  After neutralization, a suspension of MDCK cells will be added and the plates will be incubated for 16h at 37oC in a CO2 incubator. The remaining infectivity of virus after neutralisation is determined in an EIA

format using a mAb to detect expression of viral nucleoprotein. The amount of nucleoprotein expression is determined photometrically (OD450) using a plate reader

- ***Reading***

  An OD reading for each dilution step for each sample will be used to calculate the titre. The titre will be reported as the reciprocal dilution at which 50% of the virus is neutralized (e.g. titre of 100). The microneutralisation analysis will be performed in duplicate (in separate runs on 2 days) for each sample.

  The two titres for each sample must not differ by more than a two-fold serial dilution. In cases, where samples don't fall within this limit, a third analysis is performed and the two closest titres (which must be within a two-fold serial dilution) will be reported.

- **Hemagglutination inhibition (HI)**

  All sera will be analysed by HI using A/California/7/2009 (H1N1)-like virus (NIBRG121).The principle of the HI test is based on the ability of specific anti-influenza antibodies to inhibit hemagglutination of red blood cells (RBC) by influenza virus HA. The sera to be tested have to be previously treated to eliminate the non-specific inhibitors and the anti-species HAs. The experiment will be performed in accordance to protocols and SOP's established by RVU.

  - ***Serum pre-treatment***

    Elimination of non-specific inhibitors will be performed by incubation of the unknown serum samples and quality control sera (serum of ferret or human immunized with influenza virus) with neuraminidase (RDE II; 18 h / +36°C followed by heat-inactivation 1h / +56°C).

    Preparation will be performed simultaneously for serum obtained pre- and post-vaccination.

  - ***HI Test***

    For the HI analysis virus samples will be titrated in an 8-step two-fold dilution series, starting at a 1:8 dilution of serum sample (or quality control sera) and incubated with the HA antigen suspension (previously titrated to adjust the dilution at 4 HAU (Hemagglutination units)/25 µL; 50% endpoint). The HA antigen is not added to the well dedicated to the RDE quality control.

The mixture is incubated for 1 hour at room temperature and 25 µL of the 0.5% RBC suspension (turkey blood) are added. The reaction is left for ½ hour for the turkey blood and 1h for the guinea pig blood at room temperature before reading.

- ***Reading***

  The serum titre is equal to the highest reciprocal dilution, which induces a complete inhibition of hemagglutination. The titre of each quality control serum is close to the previously assigned value (within one serial two-fold dilution limits).

  The RBC controls (RBC suspension without antigen) and the RDE controls do not produce any agglutination.

  Each serum sample is titrated in duplicate and individual titers will be reported (two for each sample). These must not differ by more than a two-fold serial dilution. In cases, where samples don't fall within this limit, a third analysis is performed and the two closest titres (which must be within a two-fold serial dilution) will be reported.

- ***Reporting***

  The collaborator(s) will receive results for both assays in form of an Excel table by email.

6.6.2 Assays of cellular immunity

Where sufficient blood is available (≥7ml, see Table 2), and depending on local facilities, Elispot assays will be carried out using PBMCs isolated from the blood to determine the T cell response to internal influenza antigens, and haemagglutinin from pandemic H1,seasonal H1 and seasonal H3. Exploratory flow cytometry assays may also be used to determine whether the T cells are CD4+ or CD8+, and to examine cytokine secretion

6.6.3 Genetics

In at least 20 participants in each group at the Oxford site, at the point of venepuncture, 2.5ml of the 7-10ml whole blood sample will be drawn into a PAXgene vacutainer and gently inverted. These samples will then be stable at room temperature and should be transported to the laboratory with 24 hours. The site laboratory will freeze these samples at -20°C to -70°C until time of RNA extraction and analysis. Allocation into the subgroup for this analysis is outlined in section 6.5.5.

6.7 Definition of end of trial

The end of trial is the date at which the processing of samples for the purposes of this study has been completed.

6.8 Discontinuation/ withdrawal of participants from study treatment

Notwithstanding the participant's being enrolled into the 'persistence' or 'booster' cohorts, each participant has the right to withdraw study at any time. The investigators recognise the need to respect the intention to treat population as much as possible, therefore will endeavor to keep consenting participants in the trial, according to their selected cohorts, as much as reasonably possible. However, an investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the study or retrospective having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up

Withdrawn participants will not be replaced.

Data generated from participants that later withdraw will still be included in the analysis on an intention to treat basis.

The reason for withdrawal will be recorded in the end of study CRF if the participant offers an explanation.

If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

6.9 Source data

Source documents are original documents and records from which participants' data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (i.e.., there is no other written or electronic record of data).

All documents will be stored safely in confidential conditions. With the exception of the study diary card (where the participant's first name only will be listed) and correspondence sent to the relevant child health computer department and general practitioner all documents leaving the study sites will refer to the participant by the study participant number/code, not by name.

7. TREATMENT OF TRIAL PARTICIPANTS

7.1 Description of study treatment

The study will use a non-adjuvanted trivalent seasonal influenza vaccine, Fluarix®. It is approved by the EMEA for prophylaxis of influenza in all ages and has been marketed since 1987. It has consistently been shown to meet or exceed the regulatory criteria for immunogenicity against the three strains H1N1, H3N2 and B, have a good safety profile. (14) This season's influenza vaccine will include the A/California/7/2009 virus.

All children in the booster cohort will receive 0.5ml of vaccine.

The vaccine will be administered intramuscularly via a 23 gauge, 25 mm needle into the non-dominant upper arm.

7.2 Storage of study vaccine

The non-adjuvanted seasonal trivalent influenza vaccine will be supplied directly to the study sites by the manufacturer GlaxoSmithKline. As per MHRA advice, no clinical trial labels will be used. The investigator (or delegate) will make an inventory and acknowledge receipt of all shipments of study medication/vaccine.

All vaccine supplies must be stored between +2 and +8°C. Vaccines that have been stored differently from the manufacturer's recommendations must not be used unless the manufacturer provides written authorization for use. In the event that the use cannot be authorized, vaccine supply must be replaced with fresh stock supplied by the manufacturer.

7.3 Vaccine administration

The investigator will be responsible for the administration of the vaccine to subjects enrolled into the booster cohort according to the procedures stipulated in this study protocol. All vaccines will be administered only by personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

The vaccine must be visually inspected before use.

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines.

Any axillary temperature ≥ 38°C or serious active infection is reason for delaying vaccination.

Standard immunization practices should be observed and care should be taken to administer the injection intramuscularly. A 23 gauge, 25 mm needle is to be used for administration. As with all injectable vaccines, appropriate medical treatment and supervision should be readily available in case of rare anaphylactic reactions following administration of the study vaccine. Epinephrine 1:1000 should be available in case of any anaphylactic reactions. Care must be taken to ensure the vaccine is not injected into a blood vessel.

7.4 Vaccine compliance

The sponsor will be responsible for adequate and accurate accounting of vaccine usage. The investigator or designee will administer the study vaccines only to individuals included in this study following the procedures set out in this study protocol. The date, dosage, and time of the vaccinations will be recorded. The investigator will track vaccines received, used and wasted and will retain all unused or expired products until the sponsor is satisfied that the vaccine accountability records are correct. Thereafter, all unused vaccines are to be destroyed at the investigational site. An overall summary of vaccines supplied, received, wasted, used and returned will be prepared at the conclusion of the study.

7.5 Accountability of the study treatment

All vaccine doses will be accounted for within an accountability log. Unused vaccine at the end of the trial will be disposed of with written documentation describing this process.

7.6 Concomitant medication

Any immunosuppressant and non-steroidal medication taken at the time of enrolment into the study is to be recorded on the CRF.

8. SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participants administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

8.1.2 Adverse reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose.

The phrase "responses to a medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

8.1.3 Medically significant adverse event

All adverse reactions taking place between Visit 1 and Visit 2 requiring medical consultation with the General Practitioner, Emergency Department, or leading to a subject's withdrawal (excluding pre-planned visits and GP or Emergency Department visits for routine medical care) will be considered to be medically significant adverse events. Adverse events solicited in the diary card that are ongoing after day 7 (as recorded in the diary card provided) will similarly be recorded in the CRF.

The following information will be recorded for medically significant AEs: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. Follow up information should be provided as necessary.

8.1.4 Unexpected adverse reactions

An adverse reaction, the nature or severity of which is not consistent with the summary of product characteristics.

8.1.5 Severe adverse events

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.1.6 Serious adverse event (SAE)

A serious adverse event is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening, NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events including febrile convulsions. NOTE: Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.1.7 Serious adverse reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting investigator, believed with reasonable probability to be due to one of the study treatments, based on the information provided.

8.1.8 Expected serious adverse events/reactions

No serious adverse events or reactions are expected.

8.1.9 Suspected unexpected serious adverse reaction (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information.

8.1.10 Adverse event of special interest (AESI)

When taking the medical history adverse events of special interest that have occurred since participation in the original study will be determined. AESIs are those AEs previously recommended by the CHMP (Committee of Medicinal Products for Human Use) for inclusion as part of Risk Management Plans to be submitted with the Marketing Authorisation Application for a Pandemic Influenza Vaccine (EMEA/359381/2009), i.e.: neuritis, convulsions, anaphylaxis, encephalitis, vasculitis, Guillain-Barré syndrome, Bell's palsy, demyelinating disorders, and vaccination failure. AESI's are therefore only relevant for long term follow up from vaccine received in original study. In light of EMEA review of the relationship between Pandemrix and narcolepsy announced in August 2010, narcolepsy will also be considered an AESI.

8.1.11 Potentially immune mediated diseases (pIMDs)

Adverse events that constitute pIMDs are those diseases and conditions listed in Appendix E.

8.2 Reporting procedures for all adverse events

In the seven days following vaccine administration the following solicited symptoms will be recorded by the participant's parents/guardian in their study diary:

- injection site reactions (local tenderness, swelling or erythema)
- Fever (≥ 38°C per axilla)
- Non febrile systemic reactions, i.e:
  - reduced feeding, reduced activity, irritability, persistent crying, vomiting or diarrhoea, receiving medication for pain or temperature (17 month to day before 5th birthday).
  - malaise, headache, nausea/ vomiting, diarrhoea, reduced appetite, muscle pain or joint pain, receiving analgesic/ antipyretic medication (5 to 14 year olds).

In addition parents/ guardians will be requested to record any other general symptoms in the 7 days post vaccination in the diary card.

They will be asked to bring the completed diary cards to visit 2. The research staff will review the diary cards with the parents/ guardians at this visit and any discrepancies clarified at this time. Medically significant adverse events that have occurred in the period between visit 1 and visit 2 will be recorded on the CRF, whether or not these are attributed to the study

medication. Adverse events solicited that are ongoing after day 7 will similarly be recorded in the CRF. The diary cards will be entered by site staff onto the study electronic database. Data clarification will occur at the local site, contacting the participant's parent or guardian where necessary.

The relationship of medically significant AEs to the study medication will be assessed by a medically qualified investigator according to the following criteria:

- Related - If the causal relationship between the IMP and the SAE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.
- Not related - If there is no causal relationship between the IMP and the SAE i.e. the event is caused by something other that the IMP e.g. underlying disease, a concomitant medication.

Verbal consent will be sought from participants to follow up all AEs considered related to the study medication, AEs leading to the participant's withdrawal from the study, AESIs, pIMD and pregnancies until resolution or the event is considered stable. If obtained this verbal consent will be documented in participant's case report form (CRF).

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from treatment (see section 6.6). A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

The rates of adverse events experienced by participants will be reviewed by a data monitoring committee (see section 11 below).

8.3 Reporting procedures for serious adverse events (SAEs)

All SAEs must be reported to the chief investigator or delegate for review within one working day of discovery or notification of the event. The chief investigator or delegate will then forward these on to CTRG and to the relevant vaccine manufacturer within 24 hours of receipt. All SAE information must be recorded on a signed SAE form and relayed to the chief investigator by fax or email. Additional information received for a case (follow-up or

corrections to the original case) need to be detailed on a new SAE form and faxed to the chief investigator or delegate for review and forwarding to the CTRG.

The CI will report all SUSARs to the sponsor, MHRA, the Research Ethics Committee concerned and Host NHS Trusts. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The CI will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.
In addition to the expedited reporting above, the CI shall submit once a year throughout the clinical trial or on request a safety report to the Competent Authority (MHRA in the UK), Ethics Committee, Host NHS Trust and sponsor.

The CTRG will ensure that all SAEs are reviewed by medical monitors on a weekly basis and at the next meeting of the Oxford Radcliffe Hospitals Trust / University of Oxford Trials Safety Group (TSG), who will meet at regular intervals and consider:

- Occurrence and nature of adverse events
- Whether additional information on adverse events is required
- Consider taking appropriate action where necessary to halt trials
- Act / advise on incidents occurring between meetings that require rapid assessment (e.g. SUSARs)

If deemed appropriate, the TSG will refer the SAEs experienced in the study to the data monitoring committee for review.

8.4 Reporting of pregnancy

Although pregnancy tests will not be performed in this study due to the age range of the participants, if the investigators were to become aware of a study participant receiving a study vaccine within 30 days prior to pregnancy or during pregnancy, then they would inform the chief investigator or delegate, who will inform the sponsor, the ethics committee, the MHRA and the vaccine manufacturer of this occurrence.

9. STATISTICS

9.1 Description of statistical methods

9.1.1 Dealing with those revaccinated due to being negative after two doses

Those revaccinated will be excluded from recruitment, but for persistence a random sample will be selected for inclusion and it will be assumed that their HI and MN titre would have remained the same as it was after two doses. The proportion selected will be set to be equal to the proportion of those who were not boosted who were recruited, this will enable an unbiased estimate of persistence to be calculated post vaccination.

9.1.2 Demographics

Demographic data (age, sex, study site, time since immunisation) will be tabulated for all participants (persistence and booster cohorts) and separately for these two cohorts.

9.1.3 Persistence

For persistence the following end points are used:

- HI seropositive: persistence titre >= 1 in 32
- HI geometric mean: persistence geometric mean calculated from logged antibody titres
- MN seropositive: persistence titre >= 1 in 40
- MN geometric mean: persistence geometric mean calculated from logged antibody titres– only if at least 80% non censored at the upper end

Each of these end points will be calculated for each group with 95% confidence intervals. Titres below the assay limits will be given a value of half the limit.

The proportions seropositive in groups 1 and 2 will be compared using Fisher's exact test both overall and by age group (A and B, corresponding to age less than 3 years or greater than/equal to three years in the original study). Geometric means will be compared between groups 1 and 2 by normal errors regression on logged antibody titres.

The above endpoints will be calculated for all participants (persistence and booster cohorts) and separately for the persistence and booster cohorts, with comparisons in these measures between the two cohorts made to ensure that the booster cohort is representative of the overall study population with regard to these endpoints.

Additional persistence analyses

For groups 1 and 2 proportions positive and logged antibody titres will be modelled in more detail to look at the effect of variables such as sex, study site, age at vaccination, time since vaccination, post second dose vaccination titre, pre first dose titre, previous seasonal influenza vaccination, interval between vaccine doses. The decline from post second dose can be modelled using the paired data by looking at the geometric mean fold change post primary to 9 months.

For both groups the effect of adjusting for age, sex, study site, and previous seasonal vaccination will be investigated using multivariable logistic and normal errors regression.

9.1.4 Long term safety

Long term safety assessments for groups 1 and 2 will be estimated as proportions with 95% CI's and compared between groups using Fisher's exact test.

9.1.5 Immunogenicity of trivalent seasonal vaccine

For these analysis the following definitions are used:

- HI seropositive: post vaccination titre >= 1 in 32
- HI seroresponse: post vaccination titre has at least a 4 fold rise from pre-vaccination and is >= 1:32
- HI geometric mean: post vaccination geometric mean calculated from logged antibody titres
- HI geometric mean fold rise: pre to post vaccination calculated from logged antibody titres
- MN seropositive: post vaccination titre >= 1:40
- MN seroresponse: post vaccination titre has at least a 4 fold rise from pre-vaccination and is >= 1:40
- MN geometric mean: post vaccination geometric mean calculated from logged antibody titres – only if at least 80% non censored at the upper end.
- MN geometric mean fold rise: pre to post vaccination calculated from logged antibody titres – only if at least 80% non censored at the upper end

Each of these end points will be calculated for each group with 95% confidence intervals. Titres below the assay limits will be given a value of half the limit.

Comparisons between groups 1 and 2 (and within age strata A and B) will be performed using Fisher's exact test. Post vaccination geometric mean HI and MN titres will be calculated.

Additional Immunogenicity of trivalent seasonal vaccine analysis

For groups 1 and 2 the effect of post second pandemic vaccine dose titres on post trivalent seasonal vaccine responses will be examined in multivariable models.

Other covariates will include age, sex, prevaccination titre, time to blood, study site, previous seasonal influenza vaccination and time since vaccination.

9.1.6 Safety of trivalent seasonal vaccine

End points for safety are as follows:

- Proportion with each local / systemic reaction from the diary card within 7 days post vaccination. These will be shown according to severity where the most severe level reached within the 7 days is used, see appendix A for full list of reactogenicity data being collected on diary cards.

95% confidence intervals will be calculated with stratification by age <5 years and ≥5 years (age in *current* study) for each group.

Comparisons of groups will be by Fisher's exact test using any severity and severe as the outcomes. Comparisons will be performed within age strata (<5, 5+ years of age in *current* study) and overall for redness, swelling, tenderness and fever.

9.2 Sample size

Based on the number of individuals who received two doses in the head to head trial and on possible recruitments rates the approximate numbers available in each group are shown below using the age splits of <3 / 3+ years and <5 / 5+ years (<3/ 3+ is the age range as per participant's age in the original study which will be used for immunogenicity analysis and <5/5+ is the age range at the time of the current study for the reactogenicity analysis).

	Recruitment rate				
Age	40%	50%	60%	66%	70%
<3	90	110	130	140	150
3+	100	120	140	160	170
total	190	230	270	300	320

	Recruitment rate				
Age	40%	50%	60%	66%	70%
<5	90	115	140	150	165
5+	90	115	140	150	165

total	180	230	280	300	330

9.2.1 Sample size tables

The scenarios of 50% and 66% recruitment will be considered for a reaction rate of 10% and for proportions positive of 50% (persistence) and 80% (post vaccination).

Table: Precision of estimates: 95% CIs around various percentages and sample sizes

	Percentage		
Sample Size	10%	50%	80%
90	5%-18%	39%-61%	70%-88%
100	5%-18%	40%-60%	71%-87%
110	5%-17%	40%-60%	71%-87%
120	5%-17%	41%-59%	72%-87%
140	6%-16%	41%-59%	72%-86%
160	6%-16%	42%-58%	73%-86%
180	6%-15%	42%-58%	73%-86%
230	6%-15%	43%-57%	74%-85%
300	7%-14%	44%-56%	75%-84%

So the proposed sample sizes enable reactions or proportions positive to be estimated to within between +/-5% to +/-10% depending on the observed percentage, recruitment rate and group being considered.

Table: Detectable differences - 66% recruitment scenario

Comparison	Percentage in first group	Percentage detectable as different below	Percentage detectable as different above
Group 1 (N=140) v 2 (N=140) age <3 (at time of original study)	10% 50% 80%	2% 33% 64%	23% 67% 92%
Group 1 (N=160) v 2 (N=160) age >=3 (at time of original study)	10% 50% 80%	2% 34% 65%	22% 66% 92%

Table: Detectable differences - 50% recruitment scenario

Comparison	Percentage in first group	Percentage detectable as different below	Percentage detectable as different above
Group 1 (N=110) v 2 (N=110) age <3 (at time of original study)	10% 50% 80%	1% 31% 62%	25% 69% 94%
Group 1 (N=120) v 2 (N=120) age >=3 (at time of original study)	10% 50% 80%	1% 31% 63%	24% 69% 93%

For the primary hypothesis comparing persistence in each age group 50% in one group would be detectable as different from about 67% in the other group. After vaccination 80% would be detectable as different from 92%. For other comparisons slightly larger differences are detectable.

9.3 The level of statistical significance

The level of statistical significance will be taken as 5%.

9.4 Criteria for the termination of the trial

The study uses the non-adjuvanted trivalent seasonal influenza vaccine, Fluarix®. It is approved by the EMEA for prophylaxis of influenza in all ages and has been marketed since 1987. It has consistently been shown to meet or exceed the regulatory criteria for immunogenicity against the three strains H1N1, H3N2 and B and to have a good safety profile. It is unlikely that any safety issues should lead to termination of the trial, however the data monitoring committee will have the authority to recommend termination of the trial. In addition, the investigator has the right to discontinue this study at any time. If the clinical study is prematurely terminated, the investigator is to promptly inform the participants and should assure appropriate therapy and follow-up for the participants.

9.5 Procedure for accounting for missing, unused, and spurious data

The reason for missing data (consent withdrawn, lost to follow-up, removed from study due to serious side effects, death, or unable to obtain any laboratory results) will be indicated but missing data will not be imputed. Amount of missing data between the four groups and other demographic characteristics will be compared.

9.6 Procedures for reporting any deviation(s) from the original statistical plan

Any additional analysis or deviation(s) from the analysis plan will be documented and updated according to the statistical standard operating procedure.

9.7 Study datasets

9.7.1 For persistence

All individuals recruited and with a blood sample taken at visit 1.

9.7.2 For immunogenicity of trivalent seasonal vaccine

Modified ITT: All individuals enrolled into the booster cohort vaccinated and with a post vaccination blood sample taken. This is the primary analysis for this objective

Per Protocol: All individuals vaccinated and with a post vaccination blood sample taken and with no major protocol deviations.

9.7.3 For safety of trivalent seasonal vaccine

Modified ITT: All individuals vaccinated and with at least one safety result.

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and the study sites standard operating procedures.

Regular monitoring will be performed according to ICH GCP. Monitoring of this study will be conducted by freelance monitors in collaboration with the quality assurance manager of the Oxford Vaccine Group and local staff at each study centre. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures and an approved monitoring plan, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

A trial steering committee will be formed that will include, but not be limited to, the chief investigator, a statistician, a quality assurance manager and project manager.

A Data Monitoring Committee (DMC) will be convened that will primarily have responsibility for reviewing the adverse event rates and serious adverse events experienced by

participants in this study. The DMC will be independent of the study team and will report to the trial steering committee. The DMC will include, but not be limited to, a paediatric infectious disease specialist, a statistician and a consultant with expertise in public health.
This committee will be in addition to the trial safety group (TSG), who will provide review of serious adverse events as part of routine procedures for the CTRG.

12. ETHICS

12.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

12.3 Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.
The Investigator will submit and, where necessary, obtain approval from the above parties and the sponsor for all substantial amendments to the original approved documents.

12.4 Participant confidentiality

The trial staff will ensure that the participants' anonymity is maintained. With the exception of the study diary card (where the participant's first name only will be listed) and correspondence sent to the relevant child health computer department and general practitioner all documents leaving the study sites will refer to the participant by the study participant number/code, not by name. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

12.5 Compensation for harm

In the very unlikely event that a participant suffered any harm during the duration of the trial, compensation for harm arising from the study vaccine would be provided by the vaccine manufacturer.

The sponsor will provide compensation from harm arising from participation in the study that is not due to the study treatment.

Negligent Harm: Indemnity and/or compensation for negligent harm arising specifically from an accidental injury for which the University is legally liable as the Research Sponsor will be covered by the University of Oxford. The NHS will owe a duty of care to those undergoing clinical treatment, with Trust Indemnity available through the NHS Litigation Authority Scheme.

Non-Negligent Harm: Indemnity and/or compensation for harm arising specifically from an accidental injury, and occurring as a consequence of the Research Subjects' participation in the trial for which the University is the Research Sponsor will be covered by the University of Oxford.

13. DATA HANDLING AND RECORD KEEPING

The Chief Investigator will have ultimate responsibility for management of data with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study.

All study files (paper and electronic) with demographic and clinical details on the participants will be kept in a locked research office at each participating study centre. The study details will subsequently be entered on to a computer with an electronic database protected by a password. All blood samples will be identified by study number and study initials..

Information on study participants will be recorded on hard copy case report forms (CRFs) held locally to be entered into a web based electronic CRF (eCRF, OpenClinica™ database stored on a secure University of Oxford server). The eCRFs will include the following:

i. Subject contact details (to be retained locally)
ii. Inclusion and exclusion criteria
iii. Medical history

iv. Immunosuppressive treatment at study start
v. Each vaccination and each blood test
vi. Post vaccination follow up at 3 weeks
vii. Study termination record for subjects completing per protocol and for earlier withdrawals
viii. Age specific diary cards for completion by parents

Each study site will be responsible for generating and retaining its own source documents if required.

Each study participant will have a unique study number, which will be allocated following the taking of informed consent. For each participant, sufficient labels with the same study number will be generated (by Oxford Vaccine Group and Health Protection Agency) to label all CRFs, diary cards, vaccine vials and blood sample tubes.

13.1 Web based eCRF

The investigators will enter the data into the volunteers' CRFs, which will be in a paper and/or electronic format (using an OpenClinica™ database stored on a secure University of Oxford server). As above, this includes safety data, laboratory data (both clinical and immunological) and outcome data. Data is entered in a web browser on PCs in the trial site building and then transferred to the OpenClinica Database by encrypted (Https) transfer.

OpenClinica is clinical trials software for electronic data capture (EDC) and clinical data management (CDM) which enables compliance with regulatory guidelines such as 21 CFR Part 11.

13.2 Data locking

At the end of the study, the database will be locked and a data extract provided to the study statistician for analysis according to a pre-defined statistical analysis plan.

14. FINANCE AND INSURANCE

The involved parties will be insured, in accordance with the Clinical Trials regulations, against financial loss resulting from personal injury and/or other damages, which may arise as a consequence of this study. For details see contract agreements.

15. PUBLICATION POLICY

The Investigator will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator prior to submission.

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APPENDIX A: SAFETY DATA

Reactogenicity Data Collected in Diary Cards

- Percentage of children experiencing each of fever (≥ 38°C per axilla), local tenderness, local swelling or local erythema within the 7 days following one immunisation with the non-adjuvanted seasonal trivalent influenza vaccine in.
- Percentage of children experiencing each of: reduced feeding, reduced activity, irritability, persistent crying, vomiting or diarrhoea, receiving medication for pain or temperature within the 7 days following one immunisation with the non-adjuvanted seasonal trivalent influenza vaccine. (17 month to 5 year olds).
- Percentage of children experiencing each of: malaise, headache, nausea/ vomiting, diarrhoea, reduced appetite, muscle pain or joint pain, receiving analgesic/ antipyretic medication within the 7 days following one immunisation with the non-adjuvanted seasonal trivalent influenza vaccine (5 to 14 year olds).

In children aged under 5 years the severity of solicited systemic reactions will be graded according to the following criteria:

Reduced Feeding:
0 None
1 Mild Eating less than normal for 1-2 feeds
2 Moderate Missed 1-2 feeds completely
3 Severe Refused most or all feeds

Reduced Activity
0 None
1 Mild Less interested in surroundings, toys etc
2 Moderate No interest in above and sleeping through feeds
3 Severe Sleeping most of the time

Increased Irritability
0 None
1 Mild Continuously irritable for less than 1 hour

2 Moderate Continuously irritable for 1 to less than 3 hours
3 Severe Continuously irritable for 3 or more hours

Persistent Crying
0 None
1 Mild Cried continuously for less than 1 hour
2 Moderate Cried continuously for 1 to less than 3 hours
3 Severe Cried continuously for 3 or more hours

Vomiting
0 None
1 Mild 1-2 episodes without interfering with routine
2 Moderate Several episodes & cannot keep any food down
3 Severe: Frequent episodes & taking nothing by mouth

Diarrhoea
0 None
1 Mild More loose stools than usual
2 Moderate Frequent runny stools without much solid material
3 Severe Multiple liquid stools without much solid material

In children aged 5 years or above the severity of solicited systemic events will be assessed on the following scale:

Generally unwell (malaise)
0 = No
1 = Mild (transient with no limitation on normal activity)
2 = Moderate (some limitation in daily activity)
3 = Severe (unable to perform normal daily activity).

Headache
0 = None
1 = Mild (transient with no limitation on normal activity)
2 = Moderate (some limitation in daily activity)
3 = Severe (unable to perform normal daily activity).

Vomiting

0 None

1 Mild 1-2 episodes without interfering with routine

2 Moderate Several episodes & cannot keep any food down

3 Severe: Frequent episodes & taking nothing by mouth

Diarrhoea

0 None

1 Mild More loose stools than usual

2 Moderate Frequent runny stools without much solid material

3 Severe Multiple liquid stools without much solid material

Reduced feeding

0 None

1 Mild Eating less than normal for 1-2 meals

2 Moderate Missed 1-2 meals completely

3 Severe Refused most or all meals

Myalgia

0 = None

1 = Mild (transient with no limitation on normal activity)

2 = Moderate (some limitation in daily activity)

3 = Severe (unable to perform normal daily activity).

Arthralgia

0 = None

1 = Mild (transient with no limitation on normal activity)

2 = Moderate (some limitation in daily activity)

3 = Severe (unable to perform normal daily activity).

In both age groups, local erythema and swelling will be classified as absent, less than 2.5 cm and greater than or equal to 2.5 cm, while local tenderness will be assessed on the following scale:

0 = None

1 = Mild (transient with no limitation on normal activity)

2 = Moderate (some limitation in daily activity)

3 = Severe (unable to perform normal daily activity).

APPENDIX B: STUDY TIMELINES

Stage	Timing (Planned start date - second week in November (13th), depending on vaccine availability and regulatory approval)
Visit 1	Week 1 to 3
Visit 2	Weeks 3 to 7
Laboratory testing	Weeks 7 to 14
Analysis and initial report	Week 15 - 18
Completion of study for initial reporting	Week 18 (21st March 2011, if commence 13th November 2010)

APPENDIX C: STUDY PERSONNEL

CFI

Professor Elizabeth Miller:	Principal investigator for CFI site and overall trial co-ordinator
Nick Andrews:	Trial statistician
Liz Sheasby:	Quality Assurance at the CFI site
Pauline Kaye:	Trial data manager
Dr. Katja Hoschler:	Responsible for overseeing serological testing for the trial
Teresa Gibbs:	Senior administrator responsible for overseeing data entry and verification

OVG

Professor Andrew Pollard:	Chief investigator of study
Dr Matthew Snape:	Principal investigator for OVG site
Tessa John:	Clinical Team Leader at OVG site
Simon Kerridge:	Quality Assurance at the OVG site
Ben Thompson:	Project Manager at OVG site
Philip de Whalley:	Research Fellow

Jenner Institute

Dr Sarah Gilbert:	Cellular immune response analysis

University of Southampton Wellcome Trust Clinical Research Facility

Dr Saul Faust:	Principal investigator at Southampton site
Dr Woolf Walker	Research Fellow
Michelle Casey	Senior Paediatric Research Sister
Emma Lim	Research Fellow

St George's Vaccine Institute

Dr Paul Heath:	Principal investigator at St George's site.
Dr Clarissa Oeser.	Research Fellow
Dr Shamez Ladhani.	Consultant Paediatrician
Dr Ifeanyichukwu Okike:	Research Fellow
Dr Stephan Kohlhoff	Consultant Paediatrician
Nigel Butter	Research Nurse

Bristol Children's Vaccine Centre

Professor Adam Finn:	Principal investigator at Bristol site
Dr Jolanta Bernatoniene:	Consultant Paediatrician
Dr Edward Clarke:	Clinical Lecturer in Paediatric Infectious Diseases
Dr Ruth Allen:	Manager, Medicines for Children South West
Natalie Fineman:	MCRN Research Nurse team leader

Royal Devon and Exeter Hospital

Dr Richard Tomlinson:	Principal Investigator at Royal Devon and Exeter

APPENDIX D: IMD

Immune Mediated Disorders (IMD)

Event Category	Immune-Mediated Disorder	MedDRA PT
Neuroinflammatory disorders	Cranial nerve disorders	Optic neuritis
		III nerve paralysis
		III nerve paresis
		IV nerve paralysis
		IV nerve paresis
		VI nerve paralysis
		Facial palsy
		Facial paresis
		VII nerve paralysis
		XI nerve paralysis
		Vagus nerve paralysis
		Acoustic nerve neuritis
		Glossopharyngeal nerve paralysis
		Trigeminal palsy
		Trigeminal nerve paresis
		Tongue paralysis
		Hypoglossal nerve paresis
		Anosmia
		Neuritis cranial
		Cranial neuropathy
		Paresis cranial nerve
		Cranial nerve paralysis
		Cranial nerve palsies multiple
	Multiple sclerosis	Multiple sclerosis
		Primary progressive multiple sclerosis
		Progressive multiple sclerosis
		Marburg's variant multiple sclerosis
		Secondary progressive multiple sclerosis
		Multiple sclerosis relapse
		Progressive relapsing multiple sclerosis
		Relapsing-remitting multiple sclerosis
	Demyelinating disease	Demyelination
		Leukoencephalomyelitis
		Acute disseminated encephalomyelitis
		Concentric sclerosis
		Neuromyelitis optica
		Chronic inflammatory demyelinating polyradiculoneuropathy
		Demyelinating polyneuropathy
	Transverse myelitis	Myelitis transverse
		Myelitis
	Guillain-Barré syndrome	Guillain-Barré syndrome
		Miller Fisher syndrome
	Myasthenia gravis	Myasthenia gravis
		Ocular myasthenia
	Encephalitis	Encephalitis
		Encephalomyelitis

Event Category	Immune-Mediated Disorder	MedDRA PT
		Encephalitis post immunisation
		Encephalitis toxic
	Neuritis	Neuritis
		Cervical neuritis
		Mononeuritis
		Mononeuropathy multiplex
		Brachial plexopathy
		Radiculopathy
		Radiculitis
		Radiculitis brachial
		Radiculitis cervical
Musculoskeletal disorders	Systemic lupus erythematosus	Systemic lupus erythematosus
	Cutaneous lupus	Cutaneous lupus
	Sjogren's syndrome	Sjogren's syndrome
	Scleroderma	Scleroderma
		Systemic sclerosis
		CREST syndrome
		Morphoea
	Dermatomyositis	Dermatomyositis
	Polymyositis	Polymyositis
	Rheumatoid arthritis	Rheumatoid arthritis
		Juvenile arthritis
	Polymyalgia rheumatica	Polymyalgia rheumatica
	Reactive arthritis	Arthritis reactive
		Reiter's syndrome
	Psoriatic arthritis	Psoriatic arthropathy
	Ankylosing spondylitis	Ankylosing spondylitis
	Undifferentiated spondyloarthropathy	Spondyloarthropathy
	Mixed connective tissue disease	Mixed connective tissue disease
Gastrointestinal disorders	Crohn's disease	Crohn's disease
	Ulcerative colitis	Colitis ulcerative
	Ulcerative proctitis	Proctitis ulcerative
	Celiac disease	Coeliac disease
Metabolic disorders	Autoimmune thyroiditis	Autoimmune thyroiditis
	Hashimoto's thyroiditis	
	Grave's or Basedow's disease	Basedow's disease
	Insulin-dependent diabetes mellitus	Type 1 diabetes mellitus
	Addison's disease	Addison's disease
Skin disorders	Psoriasis	Psoriasis
	Vitiligo	Vitiligo
	Raynaud's phenomenon	Raynaud's phenomenon
	Erythema nodosum	Erythema nodosum
	Autoimmune bullous skin diseases	Pemphigus
		Pemphigoid
		Dermatitis herpetiformis
Other	Stevens-Johnson syndrome	Stevens-Johnson syndrome
		Erythema multiforme
		Toxic epidermal necrolysis
	Autoimmune hemolytic	Anemia heamolytic autoimmune

Event Category	Immune-Mediated Disorder	MedDRA PT
	anemia	
	Thrombocytopenias	Thrombocytopenia
		Autoimmune thrombocytopenia
		Idiopathic thrombocytopenic purpura
		Thrombocytopenic purpura
		Thrombotic thrombocytopenic purpura
	Antiphospholipid syndrome	Antiphospholipid syndrome
	Vasculitis	Vasculitis
		Diffuse vasculitis
		Leukocytoclastic vasculitis
		Behcet's syndrome
		Temporal arteritis
		Takayasu's arteritis
		Microscopic polyangiitis
		Polysrteritis nodosa
		Wegener's granulomatosis
		Allergic granulomatous angiitis
		Henoch-Schonlein purpura
		Kawasaki's disease
	Pernicious anemia	Pernicious anaemia
	Autoimmune hepatitis	Autoimmune hepatitis
	Primary biliary cirrhosis	Biliary cirrhosis primary
	Primary slerosisng cholangitis	Cholangitis sclerosing
	Autoimmune glomerulonephritis	Glomerulonephritis
	Autoimmune uveitis	Uveitis
	Autoimmune myocarditis	Autoimmune myocarditis
	Sarcoidosis	Sarcoidosis