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Guideline on Influenza Vaccines

Non-clinical and Clinical Module

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This module will replace the following guidelines and core SmPC:

- Guideline on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorisation Application (EMEA/CPMP/VEG/4717/03 rev. 1)
- Guideline on influenza vaccines prepared from viruses with the potential to cause a pandemic and intended to be used outside of the core dossier context (CHMP/VWP/263499/2006)
- Explanatory note on the withdrawal of the note for guidance on harmonisation of requirements for influenza Vaccines (CPMP/BWP/214/96) and of the core SmPC/PL for inactivated seasonal influenza vaccines (CMDh/128/2003/Rev5 and CMDh/129/2008/Rev3)

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- Points to Consider on the development of live attenuated influenza vaccines (EMEA/CPMP/BWP/2289/01)
- Core SmPC for pandemic vaccines (EMEA/CHMP/VEG/193031/2004)

KEYWORDS	***Influenza, guideline, pandemic, seasonal vaccine, strain change, immunogenicity, zoonotic vaccine***

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1. Introduction

This Guideline on influenza vaccines has been organised with the aim of developing a modular guideline that covers the quality, regulatory, non-clinical and clinical aspects of the development of influenza vaccines. This Non-clinical and Clinical Module is intended to replace five separate guidance documents that were in place previously. Two separate modules cover the quality and regulatory requirements for new influenza vaccines[1]. The content of the new guidance takes into account the lessons learned from the 2009/2010 influenza A(H1N1) pandemic, the experience acquired from requests for CHMP Scientific Advice, as well as prior applications for the approval of pandemic vaccines, vaccines intended for pre-pandemic use and for prevention of seasonal influenza. The revised guidance also reflects current understanding of the predictive value of non-clinical studies for clinical situations and knowledge that individual types of influenza vaccines may differ from each other in terms of their immunogenicity, efficacy and safety.

As a result, this revision has included:

- Re-appraisal of serological testing methods and issues around their standardisation;
- Acknowledgement of the lack of robust evidence to support immunological correlates of protection against influenza;
- Revision of the requirements for annual changes in the antigen composition of seasonal vaccines;
- Review of the evidence regarding the efficacy and safety of various influenza vaccines in different population sub-groups.
- Review of the terms of reference for pandemic mock-up and pre-pandemic vaccines. As further explained in section 5.2, the concept of pandemic mock up vaccines is replaced by pandemic preparedness vaccines, to highlight their role in preparation for future potential influenza pandemics. Pre-pandemic vaccines contain an emerging influenza virus strain of animal origin with pandemic potential (zoonotic virus; a zoonosis is an infectious disease that spreads from animals to humans). Consequently, pre-pandemic vaccines are referred to as zoonotic influenza vaccines throughout this Module (see also section 5.3).

Detailed requirements for the provision of enhanced safety surveillance data are included in the Addendum 'Guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU'.

2. Scope

The scope of the guideline is to address the requirements for non-clinical and clinical data to support an initial Marketing Authorisation for a seasonal, pandemic or zoonotic vaccine, as well as the requirements for strain change applications for already approved vaccines. In addition the guideline also covers recommendations and scientific considerations related to characterisation of the immune response and immunogenicity issues, pre-authorisation clinical studies of protective efficacy and/or post-authorisation studies of vaccine effectiveness, pre- and post-authorisation safety studies and pharmacovigilance plans.

The guidance is relevant to the following types of influenza vaccines:

- Live attenuated influenza vaccines (LAIVs);

[1] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/06/WC500167817.pdf
http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2015/07/WC500189035.pdf

- Inactivated split or subunit vaccines and inactivated whole virion vaccines;
- Vaccines that contain adjuvants

The principles of the requirements are considered to be broadly applicable to:

- Inactivated vaccines that contain alternative vaccine antigens (e.g. do not contain whole haemagglutinin molecules);
- Vaccines that contain recombinant surface antigens;
- DNA vaccines expressing surface antigen(s);
- Virus-like particle (VLP)-based vaccines.

Applicants are recommended to obtain scientific advice from regulatory competent authorities for any new vaccines for which the present guidance may not be wholly applicable.

In this guideline the term new vaccine refers to a new medicinal product which requires a stand-alone marketing authorisation. New vaccines include those which are similar to an existing vaccine in terms of the types of antigens and anticipated interaction with the immune system (e.g. quadrivalent inactivated influenza vaccines that are manufactured similarly to trivalent inactivated vaccines). They also include vaccines that include a novel construct or approach (e.g. influenza vaccines based on a single conserved viral protein).

3. Legal basis and relevant guidelines

This Module has to be read in conjunction with the introduction and general principles of Annex I to Directive 2001/83/EC, as amended, and all other relevant EU and ICH guidelines. These include, but are not limited to:

- Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012)
- Guideline on influenza vaccines – submission and procedural requirements. Regulatory and procedural requirements Module (EMA/56793/2014)
- Guideline on clinical evaluation of new vaccines (EMEA/CHMP/VEG/164653/05)
- Guideline on good pharmacovigilance practices: Module V – Risk management systems (EMA/488220/2012) and Guidance on format of the risk-management plan in the European Union (EMA/465932/2013 Rev.1)
- Guideline on good pharmacovigilance practices (GVP) - Product- or Population-Specific Considerations I: Vaccines for prophylaxis against infectious diseases (EMA/488220/2012)
- Guideline on good pharmacovigilance practices (GVP) Annex I - Definitions (Rev 2) (EMA/876333/2011 Rev 2)

The non-clinical chapter should be complemented for further details with the principles outlined in the following guidelines:

- ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals (EMA/CPMP/ICH/286/95)
- ICH Topic S5(R2) Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (CPMP/ICH/386/95)

- Guideline on the ERA of medicinal products for human use (EMEA/CHMP/SWP/4447/00) and Guideline on environmental risk assessments for medicinal products consisting of, or containing, genetically modified organisms (GMOs) (EMEA/CHMP/BWP/473191/2006 – Corr)
- Directive 2010/63/EU and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

The general requirements for Risk Managements Plans (RMPs) are described here:

- Guideline on good pharmacovigilance practices Module V – Risk management systems (EMA/838713/2011)
- Guidance on format of the risk-management plan in the European Union – in integrated format (EMA/465932/2013 Rev.1).

4. Non-clinical requirements

This chapter provides an overview of the type of non-clinical data that is expected for a marketing authorisation application (MAA) and for subsequent strain change applications, and it applies to all influenza vaccines as detailed in the scope of this module. Specific requirements for adjuvanted vaccines or live attenuated vaccines are exemplified in dedicated paragraphs as appropriate. For further details, this section should be complemented with the principles outlined in the guidelines listed in section 3. Additionally the WHO Guideline on non-clinical evaluation of vaccines (WHO Technical Report Series No. 927, Annex 1)[2] and WHO Guideline on the non-clinical evaluation of vaccine adjuvants and adjuvanted vaccines (Adopted by the 64th meeting of the WHO Expert Committee on Biological Standardization, 21–25 October 2013)[3] may also be informative.

The lots used in non-clinical studies can be either experimental (non-GMP) or manufactured according to Good Manufacturing Practices (GMP) lots. Each lot should be representative of the clinical lots and fully characterized according to the concurrent clinical lot specifications.

The non-clinical safety studies should be conducted in compliance with Good Laboratory Practice (GLP). The immunogenicity evaluations (both pharmacology studies and/or part of the toxicology studies) could be conducted in a non-GLP facility provided the most appropriate scientific standards are guaranteed.

In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes, the 3R principles (replacement, reduction and refinement) should be considered when designing non-clinical studies for an influenza vaccine.

4.1. Requirements for authorisation for all influenza vaccines (MAA)

4.1.1. Primary Pharmacodynamic (PD) studies

Immunogenicity studies

Immunogenicity data originated with small animal species that respond well to human influenza vaccine (e.g. rats, hamsters, guinea pigs, mice and ferrets) should be provided to show and characterise the immunogenicity of the vaccine in the context of protective and/or toxicity studies.

[2] http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf?ua=1

[3] http://www.who.int/biologicals/areas/vaccines/ADJUVANTS_Post_ECBS_edited_clean_Guidelines_NCE_Adjuvant_Final_17122013_WEB.pdf

Immunogenicity studies should include an evaluation of humoral as well as cellular immune responses (depending on reagents availability), and dose-range testing of antigen. The planned clinical administration route should be taken into account when designing such studies, since it may affect the type of immune response induced. Immune responses should ideally be assessed after each dose of vaccine as per intended posology. Data on cross-neutralizing antibodies and cross-reactivity should be obtained from serological studies using heterologous viruses for pandemic, zoonotic or adjuvanted seasonal vaccines.

Immunogenicity studies in animals might additionally be useful to demonstrate the reproducibility of the manufacturing process in particular during the validation phase of a candidate influenza vaccine manufacturing process. However, when *in vitro* approaches are possible or data can be obtained from clinical studies, this should be preferable to avoid unnecessary animal studies in accordance with the 3Rs principles.

Protection studies

Protection (or challenge) studies should be performed for influenza vaccines with novel mechanisms of action in an adequate animal model, and are intended to provide evidence of protective efficacy for the same or a similar virus strain as the candidate vaccine intended for clinical use. Protection studies should also be performed when suitable human clinical data are not available, e.g. for pandemic vaccines. Ferrets would represent the preferred animal model for influenza challenge studies (provided that the concerned influenza strain replicates well and induces symptomatic infection), as the disease pathogenesis, clinical signs –including febrile response- and mechanisms of immunity closely resemble human disease. Mice are not considered the animal model of choice for protection studies for influenza vaccines.

The virus used to challenge animals should ideally correspond to the wild type virus strain from which the vaccine strain is derived. Animals should be influenza-naïve. Ferrets may need to be primed with hetero-subtypic viruses in certain circumstances (e.g. to mimic lack of naivety in humans for a particular strain or in case of low immunogenic strains). The immunogenicity status of ferrets at baseline should always be discussed and justified in the study protocol.

The design of the study may vary based on the vaccine construct to be studied and should be standardized by the Applicant. Challenge via the intranasal route is the preferred approach, but the intra-tracheal route would also be acceptable if appropriately justified. High doses of challenge virus (~ 10^5 ID_{50} or a lethal dose if known) are preferable. Important endpoints include:

- disease markers such as body temperature, body weight loss, animal behaviour, clinical symptoms (e.g. sneezing or nasal rattling), leukocyte counts, macroscopic and histological examination of organs, and lethality;
- infection markers such as viral shedding (by nasal washes at serial time points), viral peak, kinetics of viral replication and viral clearance (animals should be sacrificed at serial time points and both upper and lower respiratory tracts should be sampled).

Lethality as a single endpoint in a ferret study would generally not be considered sufficiently sensitive to discriminate for protection. Depending on the endpoints used, it may not be necessary to sacrifice the animals at the end of the study based on the 3R principles.

In general claims of cross-protection should be supported by appropriate animal data. Specifically, cross-protection following challenge with heterologous viruses should be assessed for zoonotic/pandemic vaccines or seasonal adjuvanted vaccines, as it could indicate the breadth of protection.

Passive immune transfer studies

Passive immunisation animal studies, which investigate the level of protection induced in naïve animals following passive transfer of antigen-specific sera from immunised animals or sera from vaccinated humans, would be considered supportive of protective immunity with respect to induced humoral immune responses. Such studies are especially relevant for non-replicating pandemic and zoonotic vaccines, where the objective is to determine the antigen-specific neutralizing antibody titre associated with the protection.

4.1.2. Safety pharmacology studies

Dedicated safety pharmacology studies are generally not considered necessary for vaccines. However, the potential for undesirable effects on the cardiovascular or respiratory systems, or on CNS parameters should be considered on a case by case, especially if an adjuvant is included in the formulation or these organs are associated with wild type virus pathology (important in the case of LAIVs). These observations should be included whenever possible in the design of toxicity or immunogenicity studies.

4.1.3. Pharmacokinetics studies

Studies to determine serum concentrations of antigens are not needed. Specific studies may be needed based on the type of vaccine, in case of new formulations or adjuvants, or alternative routes of administration (for example deposition studies at the site of injection, distribution studies or viral shedding studies for LAIVs, see section 4.1.6 on LAIVs).

4.1.4. Toxicology

Toxicology testing should usually be performed with a vaccine that contains the same or similar strain as the candidate vaccine intended for clinical use. The dose levels assessed in all non-clinical safety studies should be in principle at least equivalent to one human dose in volume and antigen content; however careful consideration should be given to the appropriateness of the dose in relation to the experimental animal species chosen.

For new vaccines that have similar manufacturing processes to already authorised vaccines, non-clinical toxicology studies need not be repeated, provided that these studies are of adequate scientific and quality value and a justification on the relevance of the extrapolation to the candidate vaccine is provided.

Acute effects of vaccination, e.g. single dose toxicity studies, should preferably be investigated in repeated dose toxicity studies.

Repeated dose toxicity studies

These studies should investigate the toxicological effects of the candidate vaccine and can be performed in one animal species of relevance (e.g. rats, ferrets, rabbits, etc.) Study design should reflect as much as possible the number of doses and time intervals foreseen in clinical settings; dosing intervals may be shorter (e.g. an interval of 2-3 weeks) considering species-specific differences in the kinetics of the immune response.

Within these studies, every effort should be made to capture signs of immunological toxicity and hypersensitivity reactions (see also section 4.1.6 Additional consideration on adjuvanted vaccines).

Developmental and Reproductive Toxicity

A single study investigating fertility/embryo-foetal/prenatal-postnatal toxicity in one species should be performed. Study design should reflect the intended clinical use of the vaccine as feasible. Vaccination should be performed before mating and during gestation.

For study endpoints see CPMP/ICH/386/95.

Genotoxicity and Carcinogenicity

Genotoxicity and carcinogenicity studies are not normally required for influenza vaccines.

Special consideration should be given to adjuvants (see section 4.1.6 Additional consideration on adjuvanted vaccines) or to other components included in the vaccine formulation.

Local tolerance studies and other toxicity studies

Local tolerance should be evaluated as part of the general toxicity studies after single or repeated administrations. If conducted separately, these studies should be performed in an appropriate animal species (usually rabbits) and ideally the formulation intended for clinical use should be used.

4.1.5. Environmental risk assessment (ERA)

Amino acids, peptides, proteins, carbohydrates and lipids are exempted from the requirement to provide ERA studies because they are unlikely to result in significant risk to the environment. Therefore inactivated vaccines products are exempted due to the nature of their constituents.

4.1.6. Additional considerations

Adjuvanted vaccines

For adjuvanted vaccines, studies should aim at understanding the mechanism of action. Both quantitative and qualitative aspects of the immune response should be addressed.

The development of in vitro model systems is encouraged whenever possible, in order to provide additional relevant information on the adjuvant mechanism of action.

Potential safety concerns to address include local reactogenicity, changes in body temperature and immunotoxicity (e.g. anaphylaxis, unintended immunosuppression or enhancement).

A titration of the optimal ratio adjuvant/antigen is preferred and may provide additional insights. The extent of data extrapolation to humans should be adequately discussed based on the type of adjuvant used, e.g. alum is reported to enhance the immunogenicity of split virion vaccines in mice, ferrets and macaques, but not in humans.

New adjuvanting systems, for which no experience exists in relation to human use, especially when combined with new or modified manufacturing process for the antigens, need to be specifically investigated for their safety profile.

Live attenuated seasonal influenza vaccines (LAIVs)[4]

In addition to the general requirements described in previous sections, the following points should be considered specifically for LAIVs:

Primary PD studies

[4] For non-clinical release testing of monovalent lots of vaccine (e.g. attenuation assays, cold adaptation and temperature sensitivity) please refer to the Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012)

Given the lack of a strong correlation between systemic humoral immune responses and efficacy for LAIV, humoral immunogenicity studies in animals are of limited value, however challenge studies are considered as valid proof-of-concept and should be carried out. Challenge studies should demonstrate that the vaccine tested is able to prevent or significantly suppress replication of a wild-type virus in the lung tissues of animals and significantly decreases the level of replication of the challenge virus in the upper airways.

Vaccine virus shedding should be evaluated by collecting nasal wash samples from vaccinated animals at several time points post vaccination and measuring titres of vaccine virus. Potential transmission of shed vaccine virus to non-vaccinated animals should be explored.

Pharmacokinetics studies

Local deposition and distribution studies as well as studies to characterize the intranasal spray should be performed. These studies – to be performed with a full set of tissues and organs- may provide further evidence to define the pharmacokinetic profile of the vaccine, evaluate the potential gender differences in kinetic disposition, and may provide sufficient exposure data, in conjunction with appropriate toxicology evaluations, to evaluate the potential for safety concerns at clinically relevant exposure levels. One species could be considered sufficient, and the choice of species should be appropriately justified. Distribution studies might include recovery of infectious virus, detection of viral antigens or detection of viral genetic material. Potential haematogenous spread of the vaccine virus should be ruled out.

Neurovirulence

Potential neurovirulence of new vaccine strains should be addressed, and in certain cases be evaluated in an appropriate murine neurovirulence model using a murine neuro-adapted strain as control (see also the Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012)).

Toxicology studies

Special consideration should be given to the choice of the relevant animal model for the detection of foetal or maternal toxicities due to either vaccine virus replication or to a maternal immune response (e.g. ferret).

Deleterious effect on the nasal mucosa induced by the vaccine viruses or excipients should be investigated in appropriate animal models such as ferrets.

Environmental risk assessment (ERA)

The risk of reassortment between wild-type virus and live vaccines virus strains and the potential risk of spread to humans and animals should be addressed.

4.2. Requirements for applications to change vaccine strain composition

Seasonal influenza vaccines

Non-clinical studies are not required to support seasonal strain updates.

Pandemic and zoonotic[5] influenza vaccines

For inactivated vaccines, immunogenicity and protection studies in animals as described in section 4.1.1 could support a strain change application in case human immunogenicity data are not available.

[5] See chapter 5 for the definition of pandemic and zoonotic vaccines

For LAIVs current knowledge regarding lack of correlation between the systemic immune response and protection against clinically apparent influenza indicates that only animal protection studies may be useful.

5. Clinical requirements - dossier content

Chapter 5 provides an overview of the type of clinical data that is expected for a marketing authorisation application (MAA) and for subsequent strain changes according to the type of influenza vaccine under development (i.e. seasonal, pandemic and zoonotic[6]) and the intended target population, as applicable.

Further details regarding the assessment of immunogenicity, efficacy and effectiveness of vaccines, pre-and post-marketing authorisation, are provided in chapter 6. In all cases a comprehensive assessment of safety is needed and methodological considerations for generating safety data for MAAs are provided in section 6.4. Post-authorisation pharmacovigilance aspects are detailed in chapter 7.

5.1. Seasonal vaccines

5.1.1. Requirements for authorisation (MAA)

Seasonal inactivated non-adjuvanted vaccines

This section refers to inactivated split virion and virus subunit vaccines (see further below regarding inactivated whole virion vaccines).

The authorisation of a new inactivated non-adjuvanted seasonal influenza vaccine that has a manufacturing process similar to that of an authorised inactivated non-adjuvanted vaccine already evaluated by EU competent regulatory authorities may be based on comparative studies of safety and immunogenicity in some population sub-groups as detailed below. Although there is no confirmed immunological correlate of protection, it is assumed that demonstration of non-inferior immune responses in some population sub-groups should translate into broadly comparable protection against influenza. The non-inferiority margin should take into account any available data on natural acquisition of antibody in the population under study and available information on the immunogenicity of the comparative vaccine.

The comparative vaccine should be selected according to the type of the test vaccine (i.e. split virion or subunit). The preferred comparators are those vaccines for which there are at least some data available to support their effectiveness.

- Adults, including the elderly7

For adult and elderly populations, non-inferior immunogenicity should be demonstrated in specific age sub-groups (see also section6.1 Clinical Immunogenicity). If an Applicant wishes to conduct global studies in which the comparator(s) is/are not authorised in the EU, some aspects need to be fully justified such as extrapolation and applicability of data to the EU population and reliable leniency with EU GCP requirements. It is essential to discuss the plan with competent regulatory authorities.

- Paediatric population

[6]See section 5.3 for the definition of zoonotic vaccine
[7]The general recommendations included in the guideline on Geriatrics should be follow when elderly are concerned. Please refer to the ICH E7 Studies in Support of Special Populations: Geriatrics Q&A, linked below http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500005218.pdf

Due to the lack of evidence to support the ability of these types of influenza vaccines to elicit protective immune responses and an immune memory response in the youngest age groups, the following recommendations are made at the current time. These recommendations are subject to change as new evidence emerges. Applicants who wish to deviate from these recommendations should provide an adequate justification to support their proposals.

a) For an indication that includes use in children aged from 6 to 36 months, a demonstration of vaccine efficacy, i.e. prevention of influenza in a randomised clinical trial, is required (see also section 6.2 for study design, and section 6.1.3 Essential Immunogenicity studies).

b) For an indication for use in children aged from 3 years up to approximately 9 years, in whom the proportion that is primed is likely to be very variable in different settings, authorisation should usually be based on demonstrating that the immune responses to the selected dose and regimen are at least as good as those observed in children aged 6-36 months in whom efficacy has been satisfactorily demonstrated. To support the bridging of efficacy to the older age group it is recommended that a randomly selected subset of sera obtained from children aged 6-36 months who were included in the efficacy study are re-tested in parallel with sera obtained from children aged 3-9 years (i.e. using the same assay in the same laboratory).

   In cases where vaccine efficacy could not be demonstrated in the 6-36 month olds, the possible basis for an authorisation for use in 3-9 year-olds should be discussed with competent regulatory authorities.

   It might be acceptable in certain circumstances to base authorisation on comparative immunogenicity data (e.g. for authorisation of a quadrivalent vaccine for which a trivalent vaccine is already authorised for use in this age group). These data may be obtained: i) in a prospective randomised trial that compares immune responses to the two vaccines in this age group, or ii) by testing sera obtained from different trials in parallel (see above).

c) For an indication that includes use from approximately 9 years to < 18 years, a demonstration of vaccine efficacy is not required. Authorisation may be based on a direct comparison of immune responses to the candidate vaccine between subjects aged 9-<18 years and young adults or directly against an authorised inactivated non-adjuvanted seasonal influenza vaccine administered to the same age group. For example, a new quadrivalent vaccine could be compared with an authorised quadrivalent vaccine. Alternatively, immune responses to the candidate vaccine in 9-<18 year-olds could be compared with those reported from a different study with the candidate vaccine or an appropriate authorised vaccine provided that sera from the different trials are tested in parallel (see above).

- Immunocompromised individuals

Specific studies in immunocompromised individuals are not required at the time of the marketing authorisation application unless the Applicant wishes to make specific claims for use in sub-populations defined by immune status (see also section 7 on Post-authorisation pharmacovigilance requirements).

This group is diverse, and the ability to mount a response to an influenza vaccine will depend on the underlying type and severity of the immunodeficiency. If this group is studied, immunogenicity data could be obtained from specific subsets or a selected range of immunocompromised patients, resulting in statements in the SmPC that would take into account the actual population(s) studied. Any potential for extrapolation (e.g. of dose regimen) beyond the actual population studied would have to be decided after full review of the data.

Randomised controlled clinical trials to evaluate vaccine efficacy in immunocompromised children are not required. It is not expected that such studies would be feasible to conduct. For example, a placebo

control group would not be appropriate in this population, a fully powered study would be very difficult to enrol and the results would be very difficult to interpret due to the inherent heterogeneity in this patient group. Therefore to support claims in immunocompromised children from the minimum age approved, immunogenicity data could be obtained from relatively small sample sizes of children with a range of immune deficiency types and severity. Direct or indirect (i.e. between studies; see above) comparisons could be made between immunocompromised and age-matched healthy children to indicate whether higher doses and/or different regimens are needed in the immunocompromised.

- Patients with comorbidities

Immunogenicity studies in patients with comorbidities are not required at the time of the marketing authorisation. Some comorbidities may increase the risk of complications from influenza infection but may not impact on the immune response to and protection afforded by vaccination. These data, if generated, may be obtained from specific studies or from subgroups enrolled into age group-specific studies in which exclusion criteria are kept to a minimum. Immunogenicity data do not predict any impact on the risk of complications in those who do develop clinical influenza despite vaccination, which can only be evaluated as part of post-authorisation evaluations of vaccine effectiveness.

- Pregnant women

Some immunogenicity, safety and effectiveness data on the use of inactivated non-adjuvanted seasonal vaccines (split virion and subunit) during pregnancy are currently available to support the use of these vaccines in all stages of pregnancy. Depending on the characteristics of the new vaccine, the available data may or may not support a clear recommendation for use of the vaccine in pregnancy in the SmPC. Applicants are encouraged to obtain vaccine effectiveness data relevant to the use of maternal vaccination to prevent influenza in infancy.

Seasonal inactivated adjuvanted vaccines

- Adults, including the elderly

To authorise the use of a new adjuvanted surface antigen vaccine in adults and/or the elderly an advantage in terms of immune responses is required to justify the inclusion of an adjuvant. Such advantage may be based on a demonstration of superior immunogenicity vs. a non-adjuvanted but otherwise comparable authorised vaccine that has been reviewed by EU competent regulatory authorities. An advantage for the adjuvanted vs. non-adjuvanted formulation could include a higher seroconversion rate, higher antibody titres (based on GMTs or proportions reaching a predefined cut-off titre) or other immune response parameters, including increased breadth or duration of response. The same principles apply to data required to support use in the immunocompromised and subjects with co-morbidities, in addition to other considerations for these subgroups as stated above for inactivated non-adjuvanted vaccines.

- Paediatric population

The need to demonstrate superior immune responses for the adjuvanted vaccine vs. an appropriate non-adjuvanted vaccine also applies to paediatric subjects. In addition, as for inactivated non-adjuvanted vaccines, use of the adjuvanted vaccine in children aged ≤36 months should be supported by clinical efficacy. Immune responses to selected regimens in older paediatric age groups should be at least non-inferior to those documented in the age group in which efficacy was demonstrated. Alternatively, it might be acceptable in certain circumstances to base authorisation on non-inferior immune responses vs. another adjuvanted vaccine for which efficacy has been documented.

- Pregnant women

There are no adequate and well-controlled studies in pregnant women with seasonal adjuvanted vaccines. However safety and effectiveness data are available in pregnant women exposed to adjuvanted monovalent pandemic vaccines in particular during second and third trimesters. Any available and relevant data in pregnant women should be taken into account when developing the SmPC. However, depending on the characteristics of the new vaccine and of the new adjuvant, the available data may or may not support a clear recommendation for use of the vaccine in pregnancy in the SmPC (see also section 6.4 Clinical Safety).

LAIVs

Currently, due to lack of any robust correlation between immune response parameters and protection against clinical disease, seasonal live attenuated influenza vaccines can only be authorised based on a demonstration of vaccine efficacy in specific age and other population subsets.

For already authorised LAIVs, subject to prior agreement with competent regulatory authorities, immunological bridging studies may be used to support changes in formulation or delivery device.

Other types of vaccines- New vaccines

In principle if a new vaccine for prevention of seasonal influenza is developed that does not have appropriate comparators already authorised or reviewed by EU competent regulatory authorities (e.g. whole virion vaccines, recombinant antigen vaccines), demonstration of efficacy against relevant clinical outcomes in appropriate populations would be required to support authorisation. Applicants are recommended to discuss alternative strategies with competent regulatory authorities during the early stages of clinical development, for example to discuss the possibility of demonstrating efficacy in some age and population sub-groups and extrapolating to others based on immune response data.

5.1.2. Requirements for applications to change vaccine strain composition

Seasonal influenza vaccines undergo annually, if appropriate, an update of their strain composition prior to each influenza season (seasonal strain update). Twice a year, typically in February for the northern hemisphere and in September for the southern hemisphere, WHO experts recommend the influenza A and B virus strains that should be used in the production of seasonal influenza vaccines for the coming season. Following WHO recommendations for the northern hemisphere, EU experts evaluate each year the influenza virus strains recommended for vaccine production in the EU. Based on this recommendation, any strains replacements within authorised vaccines are made via variations (see also the Guideline on influenza vaccines – submission and procedural requirements - Regulatory and procedural requirements module (EMA/56793/2014)).

In principle, there is no need to provide clinical data to support seasonal strain updates (for quality requirements see the Guideline on Influenza Vaccines - Quality Module EMA/CHMP/BWP/310834/2012)). However other post-authorisation measures should be in place (see section 6.3 on Vaccine effectiveness and section 7 on Post-authorisation pharmacovigilance requirements; see Addendum to this Module on ‘Guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU’).

In exceptional circumstances and based on the perceived emergency of the situation, an approved seasonal influenza vaccine might undergo a variation so that it contains a pandemic strain (for details see the Guideline on influenza vaccines – submission and procedural requirements - Regulatory and procedural requirements module (EMA/56793/2014)).

5.2. Pandemic vaccines

Pandemic vaccines are indicated for immunisation against pandemic influenza viruses and are intended for use only following the recognition of a pandemic at the level of the EU.

5.2.1. MAAs submitted prior to the recognition of a pandemic (pandemic preparedness vaccines)

In order to prepare for a pandemic, Applicants are recommended to submit a MAA for a 'pandemic preparedness vaccine', formerly known as a 'mock up' pandemic vaccine. The MAA should be supported by data on relevant strain(s). When a pandemic is recognised in the EU, the Marketing Authorisation Holder (MAH) for each authorised pandemic preparedness vaccine should submit a variation application under article 21 of Regulation (EC) No 1234/2008 to include the declared pandemic strain in the pandemic vaccine (for details see the Guideline on influenza vaccines – submission and procedural requirements - Regulatory and procedural requirements module (EMA/56793/2014), and section 5.2.1.2 of this Module).

5.2.1.1. Requirements for authorisation (MAA)

The MAA for a pandemic preparedness vaccine should include data obtained with a vaccine that is the same as the intended final pandemic vaccine in terms of construct (including amount of antigen, excipients and adjuvant, if any) and mode of manufacture. This 'core dossier' should provide data on the safety and immunogenicity of the vaccine construct when it contains a potential pandemic strain that is poorly immunogenic and to which the vast majority of humans are immunologically naïve (e.g. H5N1). This strategy allows identification of a dose regimen that is likely to be suitable should the next pandemic be due to such a strain. Safety and immunogenicity data for the same vaccine construct but containing other potential pandemic strains and seasonal strains may be included in the core dossier as supportive evidence, if relevant (see below).

Pandemic preparedness vaccines - inactivated

MAAs should be based on safety and immunogenicity data generated as described above. Applicants are strongly encouraged to investigate two or more versions of the same construct that contain poorly immunogenic strains to which most humans are naive in order to gain a better understanding of the likely performance of the vaccine in case of an actual pandemic. Any safety or efficacy data generated previously with the same or similar (based on manufacturing processes) vaccine construct(s) authorised or reviewed by EU competent regulatory authorities (e.g. seasonal or zoonotic vaccines) should be included in the core dossier as supportive evidence.

As a minimum, the core dossier should contain safety and immunogenicity data from healthy adults aged 18 years and above, preferably including at least some data from subjects in various age brackets from 60 years onwards. As far as may be possible and depending to some extent on the perceived risk, data on safety and immunogenicity of the vaccine should be obtained from other age and population groups, including in particular healthy children[8].

Some immunogenicity and effectiveness data on the use of monovalent adjuvanted pandemic vaccines (H1N1v split virion and subunit) during pregnancy are available to support the use of these vaccines in all stages of pregnancy. Any available and relevant data in pregnant women should be taken into account when developing the SmPC.

It is expected that vaccine effectiveness will be evaluated during the pandemic in accordance with plans detailed in the Risk Management Plan (RMP) (see section 6.3 and chapter 7). During the actual

[8]The paediatric data can also be obtained with the corresponding zoonotic vaccine, if available.

pandemic, safety and effectiveness data should be collected in populations that were and were not included in safety and immunogenicity studies in the MAA (e.g. pregnant women, for whom safety data may be collected by means of registries).

Pandemic preparedness vaccines – live attenuated

Humoral systemic immune responses to LAIVs do not show a strong correlation with protection against clinical influenza. Nevertheless the investigation of appropriate dose regimens for LAIVs containing potential pandemic strains could include studies in which subjects presumed to be naive to the selected vaccine virus receive a single dose of the LAIV followed (after an appropriate interval) by a dose of an inactivated non-adjuvanted vaccine containing the same strain. The immune responses to the first and second doses could provide useful information on the ability of a single dose of the LAIV to prime various age groups against a poorly immunogenic strain to which most, if not all, are naive. This study design is considered useful as an indirect proof of the potential for protection of a pandemic LAIV in the absence of efficacy data in the interpandemic period, but it should not be considered as indicative for the definition of the LAIV posology in pandemic settings. The approach to strain selection should be the same as outlined above for inactivated vaccines.

Subjects participating in clinical trials with LAIVs in the inter-pandemic period or pandemic alert phase should be kept in appropriate clinical isolation conditions (see also WHO Technical Report Series TRS 941 Annex 5). It is not expected to be feasible to conduct such studies in the paediatric population during an inter-pandemic period.

If there are efficacy and/or effectiveness data generated with the same LAIV containing seasonal strains in any population, the information could be considered supportive for the same construct containing a pandemic strain. Seasonal efficacy data gathered in young immunologically naïve subjects may be of particular value. The expectations for post-authorisation studies are as for inactivated pandemic preparedness vaccines.

5.2.1.2. Requirements for applications to change vaccine strain composition

The pandemic strain change application applies to pandemic preparedness vaccines (see also Guideline on influenza vaccines – submission and procedural requirements - Regulatory and procedural requirements Module (EMA/56793/2014)) and should be submitted upon identification of the actual pandemic virus strain after official pandemic declaration. It may include quality data only, although it would be preferable that some clinical data indicative of the likely immunogenicity of the pandemic strain are included in the strain change variation dossier. If this is not possible then such data would be required as conditions and/or specific obligations to the MA including reporting of the results to competent regulatory authorities within the timelines agreed. At the same time the plans for estimating vaccine effectiveness should be activated and results should be reported in the pre-agreed timeframes (see also section 6.3 Vaccine Effectiveness and 7 Post-authorisation pharmacovigilance requirements).

If the need for a strain change is envisaged in interpandemic periods, advice should be sought from competent regulatory authorities on the data requirements.

5.2.2. MAAs submitted after the recognition of a pandemic (emergency procedure)

It may become necessary to authorise a pandemic vaccine once a pandemic is duly recognised in the EU. If a MAA for a pandemic vaccine is submitted in such circumstances, the dossier will be evaluated within an ‘emergency procedure’ (see Guideline on influenza vaccines – submission and procedural requirements - Regulatory and procedural requirements Module (EMA/56793/2014)). If an emergency

procedure is envisaged, discussions should be initiated with regulatory competent authorities as early as possible.

The dataset required for authorisation of inactivated or live attenuated influenza vaccines will vary on a case by case and will take into account all information already available that is of relevance to each construct. Thus it should be anticipated that more data would be required to support approval of a new vaccine construct than would be needed for an established and well-known construct.

5.3. Zoonotic vaccines

Zoonotic influenza vaccines are intended for immunisation in the context of outbreaks of zoonotic influenza viruses with pandemic potential, including use in specific groups like veterinarians or laboratory personnel and when there is anticipation of a possible pandemic due to the same or a similar strain. Zoonotic influenza vaccines stand for pre-pandemic vaccines in Regulation(EC) No 1234/2008.

5.3.1. Requirements for authorisation (MAA)

The MAA should include strain-specific and population-specific data. For example, if a zoonotic vaccine containing A/Indonesia/05/2005 (H5N1) has been studied in adults, it shall be indicated for the prevention of influenza due to A/Indonesia/05/2005 (H5N1) in adults only. Applicants may submit data with the same vaccine construct containing other zoonotic influenza strains as supportive evidence, if relevant.

Due to the usual epidemiology of influenza zoonotic strains, it is not expected that clinical efficacy can be established at the time of the marketing authorisation application for zoonotic vaccines. However, if there is any usage of the vaccine in outbreak situations it is possible that valuable information might be obtained on efficacy and safety and every effort should be made to capture the data and report on the experience gained.

In all cases, immune responses to the vaccine should be fully characterised within each age group for which an indication is sought.

It is recommended that the MAA contains data on antibody persistence and responses to booster doses in cohorts of vaccinees from each age and risk group for which an indication is claimed. If not in the MAA, such data would be required post-authorisation (see also section 6.1 on clinical immunogenicity, paragraph on persistence).

5.3.2. Requirements for applications to change vaccine strain composition

It may become necessary to replace the zoonotic strain that was in the vaccine at the time of the MA by another zoonotic strain if, for example, there are data indicating low or negligible cross-reactivity and cross-protection against drift variants. Two scenarios could occur that have different implications for data requirements as follows:

a) Replacement of the strain in the authorised vaccine with a different strain of the same subtype (e.g. supplanting the original H5N1 with another H5N1 clade). In this case the MAH may submit a strain change variation that includes only the manufacturing and quality data related to the new strain (see the Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012)), if appropriately justified. However, whenever feasible, it is recommended that the new version of the vaccine is administered to subjects who previously received the initial vaccine to assess the degree of cross-priming, although such data may be submitted after the strain change variation has been approved.

b) Replacement of the HA/NA subtype (e.g. supplanting the original H5N1 strain with an H7N7 strain). In this case advice should be sought from competent regulatory authorities on the data requirements, but in principle immunogenicity and safety studies are required.

6. Clinical requirements - scientific aspects

The following sections provide guidance on the clinical data that may be generated to support a marketing authorisation. The actual extent of the clinical data, choice of assays and parameters applied to data interpretation should be appropriately justified. Applicants are encouraged to discuss their proposals for the clinical development programme with competent regulatory authorities. The sections apply to:

- Non-adjuvanted and adjuvanted haemagglutinin-based vaccines, including split virus, subunit and whole virus inactivated vaccines propagated in embryonated chicken eggs or cell culture.
- Recombinant haemagglutinin-based protein vaccines, RNA and DNA vaccines that express HA and VLP-based vaccines.
- Live attenuated influenza vaccines (sections 6.2, 6.3 and 6.4).

6.1. Clinical Immunogenicity

6.1.1. Immunological assays and parameters to be assessed

The assessment of the immunogenicity of influenza vaccines is traditionally based on two tests, the haemagglutination inhibition assay [HI] that detect antibody directed against the HA antigen, and the single radial haemolysis assay [SRH]. Neither the HI nor the SRH assays are standardised. It has been shown that they are both subject to considerable inter-laboratory variability. In any one clinical development programme all HI and SRH assays should be conducted in designated and adequately qualified laboratories. Whenever possible, a single laboratory or a limited number of laboratories should conduct all assays and the same assay methodology should be employed throughout. If feasible, long term storage of sera is recommended to allow for re-testing as and when improved assays are developed (e.g. they could be re-tested as part of the validation process). Applicants should employ validated assays and in-house controls, unified laboratory protocols and standard reagents. Where international standards are available they should be used.

The Virus Neutralisation (VN) assay quantifies functional antibody. The assay is usually based on detecting the ability of human serum at various dilutions to prevent viral replication in microplates (i.e. using a microneutralisation technique [MN]). It is essential that neutralizing antibody titres are determined in all studies, at least in a representative subset of the study population and preferably in all subjects. However, as for HI and SRH, there is no standardization of techniques, and there are insufficient data to recommend a specific method. Critical assay parameters known to affect the results include the type of readout, the duration of incubation, and the use of trypsin. The various aspects of the methodology used should be adequately justified and explored for impact on the results. Unless otherwise justified the first serum sample dilution should not be greater than 1:10. Whenever possible the same assay methodology and adequately experienced laboratory should be used throughout any one clinical development programme. It is recommended that Applicants should liaise with an appropriate reference laboratory of choice for retesting samples by each method to provide some indication of the reliability of the data.

Measurement of cell-mediated immunity (CMI) is encouraged at least in randomly selected subsets across the whole intended age range. An evaluation of CMI may be particularly informative in the

elderly (e.g. aged 75years and older) due to the recognised effects of immunosenescence and observations that antibody titres as measured by HI and VN that are higher than those in younger adults may not predict protection.

It is recommended that studies should monitor the quantity and quality of T-cell responses. For example, antigen-specific T-cell frequencies should be estimated (e.g. including Th1, Th2, T regulator cells, memory T cells and relevant cytokines). In addition, a thorough analysis of CD4+ and CD8+ responses, as well as the activation of memory B cells, would allow for a better characterisation of the effect of vaccination on antibody responses and clinical protection.

Applicants may consider evaluating anti-neuraminidase NA antibodies at least in randomly selected subsets. If conducted, the assay used should be validated and should be performed in appropriately experienced laboratories.

Applicants may also consider documenting antibody kinetics as an indicator of past priming and of maturation of the immune response. Such data may be particularly useful in studies that commenced with vaccination of immunologically naïve subjects.

Due to the pathogenicity and epidemiology of influenza zoonotic strains, human sera obtained from recipients of zoonotic and pandemic vaccines should be evaluated to determine:

- Cross-reactivity: i.e. cross-reaction of antibodies elicited by the selected vaccine strain to naturally occurring drift variants of the same virus subtype (e.g. H5N1) as measured in vitro.
- Cross-priming: i.e. evidence of an anamnestic response after boosting with a related but drifted strain following initial vaccination with the selected vaccine strain, based on comparison with the immune response to a first dose of the drifted strain in a previously unvaccinated control group.
- Cross-protection: if this is claimed, evidence should be based on demonstrating cross-reactive immune responses in sera from vaccinees supplemented by non-clinical data (see also the Non-clinical section).

Due to ongoing drift it is anticipated that additional data could be generated as appropriate after initial authorisation of the vaccine.

Study protocols should specify and give details of the methodologies that will be used to evaluate immune responses to vaccination as well as the rationale for the timing of sampling. If changes to methodologies are necessary during the clinical development programme, adequate cross-validation data should be provided.

For further details on assay validation please refer to the Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012).

6.1.2. Analysis and presentation of immunological data

All influenza vaccines

The immunological data obtained from each study generated using HI and/or SRH and VN assays should be presented in detail by vaccine strain and using a standard approach in each study report. As a minimum, for results of each assay conducted:

- GMTs (with 95% confidence intervals) and pre-/post-vaccination ratios (GMRs) should be calculated.

- Reverse cumulative distribution curves should be provided. These should be supplemented by tables presenting percentages of vaccinees with titres above a range of cut-off levels on a logarithmic scale (e.g. titres above 1:10, 1:100 and 1:1000).
- Seroconversion rates should be reported. Seroconversion may be defined in several ways including at least an x-fold increment in titre over baseline and/or appearance of a measurable titre in a subject with previously undetectable or non-quantifiable antibody.
- Analyses in study population subsets according to factors such as age and pre-existing antibody status should be provided.
- Immune responses to revaccination should be reported based on immunological status prior to the additional dose.
- Where more than one strain has been used in any assay the data should be shown separately as described above.
- Any available data on antigen specific T-cell responses including CD4+ T-cells and CD8+ cytotoxic T-lymphocytes (CTLs) and relevant cytokines should be presented taking into account baseline status.

Pandemic and zoonotic vaccines

As a minimum, for each of HI and/or SRH and VN data, the percentages that achieve an immune response above pre-defined and appropriately justified threshold levels should be reported. Additional analyses should evaluate percentages of vaccinees reaching alternative (including higher) titres and, in each case, should report on whether the lower bound of the 95% confidence interval around the point estimate exceeds the selected cut-off value. Additionally, seroconversion rates and GMRs (see above) should be reported. The findings should be compared between HI and/orSRHand VN data to describe any consistent trends that may occur.

Data on cross-reactive antibody and from cross-priming studies should be reported along the lines specified above.

6.1.3. Essential immunogenicity studies

Dose finding studies

Applications for marketing authorisation for influenza vaccines should include data supporting the chosen dose, schedule and vaccine formulation for the different target groups for which an indication is sought (as defined by age or medical condition as relevant). Lack of such data should always be justified. This requirement is applicable in general to all vaccine types, with some specific comments provided below. Applicants should consider the need to obtain advice from competent regulatory authorities early in the development programme.

If an adjuvant is used, enhancement of the immune response, potentially resulting in reduced antigen content, should be demonstrated in association with an acceptable safety profile. Data to support the selected antigen-adjuvant ratio should be provided. It is particularly important to assess the benefit of adding an adjuvant in vaccines for children and for the elderly and to identify appropriate age-specific dose regimens.

If an Applicant is pursuing development of a non-adjuvanted inactivated seasonal vaccine in the paediatric population, it is essential to document the immune response, and to conduct adequate dose finding studies before deciding whether to proceed to an efficacy study. Dose finding studies in children should be conducted and assessed as in other age groups and should attempt to support a broad range

of exploratory analyses in subgroups. Primary immunisation schedules should be investigated at least in children aged 6 to 36 months, who are most likely to be influenza naïve, including an assessment of the ability of the first dose to prime. If the vaccine appears to be poorly immunogenic in children aged ≤36 months (i.e. with markedly lower immune responses compared to older children or young adults) it may not be appropriate to conduct an evaluation of vaccine efficacy in this age group. If the dose finding study suggests that for a specific vaccine construct the immune responses differ significantly for one of the strains in a seasonal vaccine, or according to the specific strain included in a pandemic or zoonotic vaccine, it is recommended that the findings are discussed with competent regulatory authorities before proceeding with the clinical development programme.

Persistence of immune responses and possible need for revaccination

For seasonal influenza vaccines the possible need to document antibody persistence and the immune responses to booster doses at the time of the marketing authorisation application should be considered and discussed with competent regulatory authorities. The provision of such data is considered of particular interest for the cases described below, for which it may be appropriate that the following data should be obtained at the time of the marketing authorisation application or only after the grant of the marketing authorisation:

a. Adjuvanted seasonal vaccines

For adjuvanted seasonal vaccines, persistence of immune response following primary vaccination should be investigated up to 12 months after completion of the initial regimen, i.e. before the dose of the following season is administered, to investigate the need for annual revaccination.

In any population(s) studied in which annual revaccination is not recommended antibody persistence could be followed beyond 12 months. In such populations, Applicants should consider boosting subsets of study participants at one and two years following primary immunisation in order to investigate the effect, need for and timing of a booster dose.

b. Inactivated pandemic and zoonotic vaccines

For pandemic and zoonotic vaccines immunogenicity data should be collected for at least 6 months following primary vaccination to evaluate persistence of immunity and/or booster responses as applicable (i.e. in case of non-persistent antibodies), as this is informative in case of subsequent pandemic waves and/or in case of need to maintain antibody titres due to continued risk of exposure.

Immunological correlates of protection

For inactivated influenza vaccines containing viral HA, an HI titre of 1:40 was previously suggested to represent a reasonable statistical correlate for an efficacy of 50-70% against clinical symptoms of influenza based on challenge studies in healthy adults. Since then, evidence has emerged to indicate that there remains a need to better define correlates of protection against influenza, which potentially may vary according to individual characteristics, populations, specific age groups (e.g. the paediatric population) and vaccine types.

During new influenza vaccine development programmes Applicants should make every effort to obtain data that could support identification and validation of correlates of protection against clinically manifest influenza. During efficacy studies various immune response parameters as described above should be investigated at least in population subsets and analyses should be conducted to explore the correlation between immune response parameters and protection against disease.

6.2. Clinical efficacy - methodological considerations

In instances in which an efficacy study is considered to be necessary and feasible (see chapter 5 on dossier content), this section considers the design of such studies.

6.2.1. Study design and choice of control

Clinical endpoint studies should be designed as prospective double-blind randomised controlled studies. Secondary contact studies may provide supportive evidence for protective efficacy.

Studies should preferably be designed to demonstrate superiority of the vaccine over an unvaccinated group. To achieve a double blind design and to avoid (or at least minimise) the use of placebo injections, whenever possible anon-influenza control vaccine should be selected that may provide some benefit in the intended target age group.

Alternatively, subject to adequate justification, Applicants could choose to conduct an active controlled study i.e. in which the control vaccine is an approved influenza vaccine. In this case the study may be designed to show superiority of the test vaccine over an authorised product (e.g. an adjuvanted vaccine vs. a non-adjuvanted vaccine). Depending on the characteristics of the test vaccine and of the selected comparator, and subject to adequate justifications, it may be acceptable to plan a primary analysis based on showing non-inferior efficacy. The choice of non-inferiority margins should be appropriately justified by the Applicant.

Randomisation may be based on individuals or clusters, although the potential for bias should be discussed for the latter. The numbers of subjects within each clinical trial should be adequate to ensure that the trial is able to fulfil its objectives. Exclusion criteria should be kept to a minimum. Stratification into age categories or into groups with other characteristics (e.g. patients with comorbidities or frail elderly) that may cause them to respond to the vaccine differently should be employed to ensure that a representative cross-section of the population is studied. However it is not expected that the study is powered to demonstrate efficacy in subgroups.

Great care in age stratification is required, especially in trials that include or are confined to young children or the elderly. In studies in children, both age and previous exposure to influenza vaccines should be considered to ensure adequate representation of subjects who are most likely to be naive to influenza. In studies in the elderly the study population should include those living at home but receiving home care services or living in nursing homes. Every effort should be made to enrol a representative sample of subjects above 75 years and to stratify according to age.

The protocols for protective efficacy studies should pre-define when and in which subsets samples will be obtained for immunological evaluation and should state the assays to be used. Whenever an immunological test is to be applied to sera from a subset of the population, the selection process should ensure that the sample is broadly representative of the total study population.

6.2.2. Clinical endpoints

To establish the efficacy of the test vaccine in preventing influenza, the primary endpoint should be based on all cases of influenza-like illness[9] (ILI) that are laboratory confirmed by PCR or culture or both. If the Applicant proposes an alternative primary endpoint or wishes to propose co-primary endpoints, this should be discussed with competent regulatory authorities before initiating the efficacy study. For example, in patients with laboratory-confirmed influenza a composite endpoint consisting of

[9] For definitions see ECDC website: http://www.ecdc.europa.eu/en/publications/publications/0907_ted_influenza_ah1n1_measuring_influenza_vaccine_effectiveness_protocol_case_control_studies.pdf

influenza-related pneumonia, hospitalisation and influenza-related mortality could be considered as an alternative primary endpoint for studies conducted in the elderly.

It is to be expected that the vast majority of cases documented in any one study, even if conducted over more than one season, will most likely be due to one strain or subtype (i.e. due to A/H1N1 or A/H3N2 or a specific B lineage). Hence it is not expected that any one study will be able to provide estimates of strain-specific efficacy and studies will not be powered for such analyses. Depending on the strain that actually predominates in the documented cases, Applicants should include a discussion of the anticipated efficacy across other influenza types [e.g. if A(H1N1) predominates in the study the Applicant should discuss any available evidence that might support extrapolation of the efficacy observed to A(H3N2)]. Additionally, attempts should be made to estimate strain-specific effectiveness in the post-authorisation period.

An important secondary endpoint is the estimate of efficacy against influenza due to strains that are well-matched to those in the vaccine[10]. If the efficacy study is conducted in season(s) in which there is a poor match between the recommended vaccine strains and the predominant circulating strains this can be expected to affect the estimate of vaccine efficacy based on the recommended primary endpoint. Whenever this occurs the estimate of vaccine efficacy against influenza due to well-matched strains would be of considerable importance when evaluating the overall potential benefit of the vaccine, assuming that sufficient cases due to such strains occur during the efficacy study to allow for an estimate of efficacy to be made.

Other secondary endpoints should include all-cause mortality, hospitalisation, ILI syndromes, all-cause pneumonia and, in children, otitis media. If reductions in secondary attack rates in household or school settings are to be assessed, they should be based on laboratory confirmed influenza cases.

Since baseline serostatus does not predict protection nor does it reliably indicate priming, the protocol should pre-define a secondary analysis that examines rates of influenza according to prior exposure to influenza vaccines.

Endpoints relating to a daily lifestyle (absenteeism, use of health care resources, costs) may be collected if the Applicant wishes, but such data would not be considered supportive in an assessment of vaccine efficacy.

6.2.3. Duration of the study

It is difficult to establish *a priori* the number of seasons to be included in a clinical trial, due to uncertainties related to strain match and attack rate. Previous studies have required one or more seasons in order to collect sufficient cases to support a robust estimate of vaccine efficacy. However, if the study is conducted in a population that will require re-vaccination every year, this must be planned for within the protocol and the statistical analysis plan. If the study is conducted in a population subset and in countries in which routine vaccination is not recommended for that specific group, then the data collected in the second season after vaccination may potentially be informative regarding persistence of protection and cross-protection.

There are only limited data on the efficacy of adjuvanted and live attenuated influenza vaccines over multiple seasons and data on the need for revaccination over consecutive seasons should be addressed in clinical trials.

[10]To help define 'well-matched strains' please see the WHO website: available candidate vaccine viruses (http://www.who.int/influenza/vaccines/virus/en/) and full technical report following WHO recommendations (http://www.who.int/influenza/vaccines/virus/recommendations/2014_15_north/en/); associated FAQ (http://www.who.int/entity/influenza/vaccines/virus/recommendations/201402_qanda_recommendation.pdf).

6.3. Vaccine effectiveness

In line with the Guideline on good pharmacovigilance practices - GVP P.I: Vaccines for prophylaxis against infectious diseases[11], post-authorisation effectiveness studies should be included in the Risk Management Plan as additional pharmacovigilance activities for all influenza seasonal and pandemic vaccines including currently authorised and new influenza vaccines (see Guideline on good pharmacovigilance practices (GVP) Module V – Risk management systems[12], sections V.B.9.4 and V.B.10.2).

It is acknowledged that adequate brand name specific active surveillance of effectiveness may not be feasible for any vaccine in any season, hence a justification for lack of data or limited data for a specific season should be presented by the MAH and it will be considered on a case by case basis (see also section 6.3.6). If needed or justified, a meta-analysis of results for subsequent seasons can be presented to increase the power and to estimate effectiveness for a specific vaccine across seasons.

It is preferable that studies are conducted in the EU/EEA. However, data from other regions may be acceptable if the extrapolation to the EU population can be justified.

This section considers the design of effectiveness studies that could be applied to the evaluation of seasonal influenza vaccines and to the evaluation of pandemic vaccines during a pandemic situation.

6.3.1. Principles of Study design

Studies will have to be conducted in accordance with Good Epidemiological Practice (GEP) guidelines and with guidelines of ENCePP. Applicants are encouraged to liaise with organisations/institutions/public health authorities who have experience in influenza effectiveness studies and who have implemented a functioning infrastructure to conduct multicentre studies.

For evaluation of effectiveness of influenza vaccines it would be preferred to consider study protocols which have already been tested both in pandemic and seasonal settings, e.g. the case control study protocol published by ECDC[13], or a prospective cohort study utilizing population-based databases/registers[14], e.g. with validation of clinical outcomes by PCR in a subset of subjects. If these are not feasible, a screening method where influenza vaccine effectiveness (IVE) is estimated based on the proportion of laboratory positive cases who had been vaccinated and the vaccination coverage in the general population could be considered[15]. Within the cohort studies, nested test-negative case–control studies should be conducted to estimate IVE against medically attended laboratory-confirmed influenza.

6.3.2. Endpoints and case definition

Endpoints

It is recommended to include laboratory-confirmed influenza outcomes in any study design. Other endpoints could be:

1. Case control design/ test negative case control design

[11] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/12/WC500157839.pdf
[12] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500129134.pdf
[13] http://ecdc.europa.eu/en/publications/Publications/0907_TED_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Case_Control_Studies.pdf
[14] http://ecdc.europa.eu/en/publications/Publications/0907_TER_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Cohort_Database_Studies.pdf
[15] Valenciano M, Kissling E, Ciancio BC, Moren A. Study designs for timely estimation of influenza vaccine effectiveness using European sentinel practitioner networks. *Vaccine*(2010)

The primary endpoint for case control/ test negative case control studies should be laboratory confirmed influenza.

Based on the study setting (general population or hospital) secondary outcomes may address the ability of vaccines to prevent pneumonia and influenza related hospitalisation (influenza related or associated with respiratory or cardiac disease) or death.

2. Cohort design

Endpoints of interests may include:

- Medically attended respiratory infection (MAARI);
- medically attended ILI;
- all cause deaths;
- respiratory deaths;
- hospitalisations for pneumonia and influenza;
- hospitalisations for all respiratory conditions;
- Laboratory-confirmed cases of MAARI/hospitalised pneumonia and influenza and
- ICU admissions.

In addition to providing a vaccine effectiveness estimate, MAHs are encouraged to undertake antigen analyses in a sample of specimens in the same effectiveness studies. It is recommended to document age of subjects, vaccination status (including history of), severity of disease in vaccine breakthrough cases, geographic area and week of onset of ILI during the season or pandemic period. These data are considered important to put vaccine effectiveness data into perspective (e.g. match of the vaccine with circulating strains, antigenic drift during the season or pandemic period).

Case definition

Cases should meet the EU ILI and influenza case definition (see case definition by ECDC). An influenza case definition that includes laboratory confirmation has the highest specificity for influenza, and is essential to avoid misclassification of cases. Laboratory confirmation of influenza by reverse transcription polymerase chain reaction (RT-PCR) or culture using an established method in (community) reference laboratories which undergo periodic external quality assessments for virus detection and characterisation methods is required.

6.3.3. Target population

The effectiveness of an influenza vaccine has to be investigated in the population for which it is indicated for use (e.g. children in case of LAIV). It should be acknowledged that data can only be collected according to the use of the product in the relevant country(ies).

As far as appropriate and feasible, the age effect has to be taken into account by adjusted stratified analysis of children, adolescents, individuals <65 years, ⩾65 years and >75 years of age.

Patients with certain underlying diseases or conditions (e.g. pregnancy) are known to be at increased risk of serious influenza-related complications. It is therefore encouraged to assess the effectiveness of influenza vaccines in these risks groups as far as it may be possible. Multivariate approach in the analysis of data should be considered in order to control for different confounding factors.

Applicants are encouraged to obtain vaccine effectiveness data relevant to the use of maternal vaccination to prevent influenza in infancy.

6.3.4. Selection of cases / cohorts

Standardised approaches for recruitment of subjects in studies with active data collection (e.g. test negative case control studies) are warranted to reduce a possible selection bias. As a minimum the following information has to be collected:

- date of vaccination and commercial name of vaccine received;
- data on onset of ILI symptoms;
- data of specimen collection;
- laboratory method for ascertainment;
- the identity of influenza strains confirmed to be causative;
- data on potentially important confounders such as previous influenza (multiple years if feasible) vaccination, the presence and severity of any chronic condition, smoking history, health-seeking behaviour, any hospitalisation for chronic conditions in the previous 12 months;
- Clinical information such as hospital admission due to severe influenza.

In studies utilizing population-based databases (e.g. cohort studies), a plan to address possible bias (e.g. by inclusion of background information available from existing databases or via sensitivity analysis) should be included.

6.3.5. Presentation of results

Study results in terms of crude and adjusted influenza effectiveness should be presented on an annual basis and as soon as they become available. Vaccine effectiveness analyses should be presented by influenza subtype. Exploratory sub group analysis for different target populations as specified above should be provided, if feasible. It is acknowledged that some outcomes might be available in real time (interim results), whereas others will not become available before the end of the season (final results), since IVE for different outcomes might be calculated at different time periods.

6.3.6. Interpretation of results

Results of effectiveness studies by brand should to some extent contribute with important information to the overall clinical evidence available for each influenza vaccines, especially new vaccines. Whilst every effort to collect yearly and brand-specific effectiveness data should be made, difficulties to reach this objective are acknowledged and should be taken into account at the time of assessment. Moreover, estimates should always be considered within the context of the multitude of factors aside from the vaccine that could determine the effectiveness of the vaccine. Therefore, results of vaccine effectiveness studies may yield potential signals that require further investigation to determine the drivers of estimated effectiveness. In the majority of cases, results from different seasons would have to be collected before any conclusion could be drawn. However, if a specific concern (e.g. of a quality nature with a specific vaccine) has been identified or is strongly suspected by the deviation of the results from the expected pattern, regulatory actions may be considered.

6.4. Clinical safety

Clinical safety should be investigated pre-authorisation in all clinical studies according to the requirements of CHMP Note for Guidance on the Clinical Evaluation of New Vaccines (CPMP/EWP/463/97).

Follow-up should be performed for at least 6 months post-vaccination (last dose) to ascertain additional serious adverse events.

As a general rule, the total size of the safety population for any influenza vaccine should consist of at least 3000 individuals. Applicants are encouraged to discuss the proposed size of the safety database with competent regulatory authorities during the clinical development programme since alternative requirements may be considered depending on the vaccine type and construct.
Table 1 outlines the usual anticipated safety database for a new vaccine before filing a MAA depending on the population studied and the proposed age range for use:

Table 1. Safety database for a new vaccine

Indication of the vaccine	**Size of the safety database required to detect ADRs occurring at a frequency as stated below[16]:**
Adults from 18 to 65 years Or Children from 6 months to 17 years Or Elderly >65 years of age	≤ one in one thousand persons vaccinated (i.e. rare ADRs) e.g. a database of approximately 3000 subjects might be sufficient in the only or in one of these specified age groups; data in other groups may be less as detailed below
Specified age groups in addition to any one of the above e.g. infants, children, adolescents, elderly	≤one in one hundred (i.e. uncommon ADRs) e.g. a database of approximately 300 subjects from each additional specified age group might be sufficient
Specified risk groups in addition to any one of the above e.g. immune compromised individuals, chronically ill patients	≤one in one hundred (i.e. uncommon ADRs) e.g. a database of approximately 300 subjects from each additional specified risk group might be sufficient

There should be appropriate stratification within each age group investigated. For example, if only children are investigated, a total sample size database of at least 3000 individuals is expected, of which at least 300 subjects for each specified paediatric age group (infants, toddlers, young children, children 9-11, 12-14 and 15-17 years) is considered sufficient if no unexpected differences in reactogenicity or adverse reactions among age groups have been detected.

If the indication is intended to include both adults and children, a total safety database of 3000 adults is expected plus 300 individuals for each of the infant, children and adolescent paediatric groups (i.e. ~900 paediatric individuals in total), provided no unexpected serious adverse reactions is observed across paediatric age groups.

A substantial amount of safety data available on the use of inactivated non-adjuvanted seasonal vaccines (split virion and subunit) and monovalent adjuvanted pandemic vaccines during pregnancy

[16]Applicants are encouraged to discuss the proposed size of the safety database with competent regulatory authorities during the clinical development programme.

are available to support the use of these vaccines in all stages of pregnancy. Therefore adequate safety data in adults should suffice to support use of such vaccines in pregnancy. For other types of influenza vaccines or other types of adjuvants, the extent of the safety data that should be provided to support use during pregnancy should be discussed with competent regulatory authorities.

If a particular type of serious adverse event is identified and there is concern that it may be vaccine-related, then additional safety data may need to be generated.

Safety experience obtained with an individual Applicant's adjuvant in combination with other antigens could be considered supportive. Advice should be sought from competent regulatory authorities.

In addition to the requirements mentioned above, for LAIVs the amount (titres) and duration of vaccine virus shedding should be well characterised during the clinical programme. Any potential risk to close contacts, especially those who are immunocompromised, as a result of vaccine strain transmission should be fully assessed based on its virological characterisation before commencing clinical trials so that adequate precautions can be introduced into study protocols. These precautions should then be reviewed once shedding data become available.

7. Post-authorisation pharmacovigilance requirements

Any influenza vaccine MAA should include a Risk Management Plan (RMP) as part of the marketing authorisation application. Specific aspects of pharmacovigilance planning for vaccines are described in the Guideline on good pharmacovigilance practices (GVP) - Product- or Population-Specific Considerations I: Vaccines for prophylaxis against infectious diseases (EMA/488220/2012).

Depending on the indication(s) authorised, the Applicant should propose in the RMP relevant additional pharmacovigilance activities (e.g. post-authorisation studies) to address e.g. identification of rare and very rare adverse events, ad hoc emerging safety concerns and safety of populations not studied in clinical trials, such as immunocompromised or patients with underlying conditions(see also the GVP P.I). It is recommended to discuss with competent regulatory authorities concerning design and conduct of post-authorisation studies.

7.1. Any influenza vaccine

Based on the authorised indication(s), the RMP should include as a minimum studies to address the following:

- If immunocompromised are not studied pre-authorisation and if the Applicant wishes to make specific claims regarding use in such patients, data in immunocompromised should be generated post-authorisation by means of immunogenicity studies or effectiveness studies or both (see also section 6.3 on effectiveness studies). It would be relevant to demonstrate if a higher number of doses or a booster dose is required in immunocompromised compared to a primary schedule for the healthy population.
- The elderly and frail population should be an essential part of the post-marketing monitoring program envisaged.

7.2. Seasonal influenza vaccines

The RMP should include plans to address the following:

- Enhanced surveillance of vaccine safety: safety and reactogenicity of the new strains (as introduced via seasonal strain updates, section 5.1.2) should be evaluated in terms of local (e.g. swelling at the injection site) and systemic adverse reactions (e.g. fever, myalgia) in the different

age groups based on the indication, particularly in young children if applicable. Such data should be collected as soon as possible at the beginning of the vaccination campaign each year. Timely results should be provided to competent regulatory authorities. Detailed requirements for the provision of enhanced safety surveillance data are included in the Addendum 'Guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU'.

7.3. Zoonotic influenza vaccines

The RMP should include plans to address the following:

- Whenever the opportunity arises, such as during any government-directed use of vaccine within cohorts in individual countries, further information should be collected from observational studies to expand the safety and the immunogenicity database.
- If there is exposure of vaccinees to circulating influenza strains with a potential to cause a pandemic (e.g. persons dealing with avian influenza outbreaks in flocks or close contacts of documented cases of human infection due to such viruses) information on breakthrough cases should be collected. It is especially recommended to collect additional data in populations which have been studied to a lesser extent in the pre-authorisation clinical trials.
- Monitoring the effectiveness of any vaccine that is administered during the pandemic alert phase. Such data would be informative for planning future prepandemic vaccination strategies and, if data become available early enough before a pandemic is duly recognised showing evidence of protection, it could allow for any available pandemic vaccine to be directed primarily to previously unvaccinated cohorts.

7.4. Pandemic influenza vaccines

The RMP should include plans to address the key challenges described in chapter P.I.A.4. Aspects related to vaccination programmes of Guideline on good pharmacovigilance practices (GVP) - Product- or Population-Specific Considerations I: Vaccines for prophylaxis against infectious diseases (EMA/488220/2012). In addition, the RMP should also consider:

- Pregnant women are likely to be among the first groups targeted in pandemic vaccination campaigns. Vaccination of pregnant women might additionally protect neonates from infection. Effectiveness and safety should be monitored and such studies should be planned for at the time of the marketing authorisation procedure (e.g. pregnancy registries for safety).
- The accumulation of immunogenicity, effectiveness and safety data during a pandemic should ideally be a co-operative effort between companies and public health authorities. Facilities for the rapid sharing of these data should be in place since the information will likely have implications for all the vaccines in use in a pandemic as well as for future pandemics. Rapid sharing and rapid review of these data will be important since it may be necessary to implement changes in the vaccine, in the vaccination schedule or programme during the pandemic.
- Concerning safety data, in addition to the assessment of rates of local and systemic reactions in the immediate post-vaccination period, there are specific longer-term and rare and very rare adverse events that need to be evaluated, such as the risk of narcolepsy or Guillain-Barré syndrome. For pandemic vaccines, large-scale safety data are expected to be generated from the field use.
- Before submitting the pandemic strain change variation, MAHs should discuss and agree with competent regulatory authorities the plans for the enhanced safety surveillance to be performed during the pandemic period. Provision of such data should follow as a minimum the requirements

for the seasonal influenza vaccines (see the Addendum 'Guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU').

8. SmPC, PL and labelling for influenza vaccines

There is no Core SmPC for individual influenza vaccines. Individuals SmPCs should be tailored according to the data for each product.

See also the available general guidance on the Product Information, such as the SmPC guideline, the Annex to the Guideline on clinical evaluation of new vaccines: summary of product characteristics requirements (EMEA/CHMP/VWP/382702/2006), the QRD templates published on the EMA website, and the Guideline on Influenza Vaccines – Quality Module (EMA/CHMP/BWP/310834/2012).