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Guideline on influenza vaccines

Non-clinical and clinical module

Draft

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Once finalised, 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)
- 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)

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1. Introduction

As announced in the Concept Paper[1], the revision of the guidelines on influenza vaccines has been organised with the aim of developing one single influenza guideline that covers the regulatory, quality[2], non-clinical and clinical aspects. The present module covers the non-clinical and clinical requirements for new influenza vaccines. The content 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 clear 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 types of 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 4.2.2, pandemic mock up vaccines are herewith labelled as 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 4.2.3).

2. Scope

The major areas addressed in this module are:

- Requirements for non-clinical and clinical data to support an initial Marketing Authorisation for a seasonal, pandemic or zoonotic (formerly referred to as pre-pandemic) vaccine;
- Requirements for strain change applications;
- Recommendations for characterisation of the immune response and related immunogenicity issues;
- Situations in which pre-authorisation clinical studies of protective efficacy and/or post-authorisation studies of vaccine effectiveness are required;
- Pre-authorisation and post-authorisation safety data;
- RMP and SmPC aspects.

The guidance is relevant to influenza vaccines that contain:

- Live attenuated influenza viruses;

[1] Please clikc here for the Concept Paper.
[2] Please click here for the Quality Module.

- Inactivated split, subunit or whole virion viruses;
- 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);
- Recombinant surface antigen vaccines;
- DNA vaccines expressing surface antigen(s);
- Viral-like particle (VLP)-based vaccines.

Applicants are recommended to obtain scientific advice from EU Regulators for any novel vaccines for which the present guidance may not be wholly applicable.

3. Legal basis and relevant guidelines

This Module has to be read in conjunction with:

- Directive 2001/83/EC
- Regulation (EC) No 1234/2008
- Guideline on Clinical evaluation of New Vaccines (EMEA/CHMP/VEG/164653/05)
- Guideline on adjuvants in vaccines for human use (CHMP/VEG/134716/2004)
- 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:

- Guideline on Adjuvants (EMEA/CHMP/VEG/134716/2004)
- 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)
- Note for Guidance on preclinical pharmacological and toxicological testing of vaccine (CPMP/SWP/465/95)

4. Applications for influenza vaccines: dossier requirements

This chapter provides an overview of the type of 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[3]) and the intended target population. Further details regarding non-clinical data and the assessment of immunogenicity, efficacy and effectiveness of vaccines, pre-and post-MA, are provided in Chapter 5. In all cases a comprehensive assessment of safety is needed and detailed recommendations for pre-authorisation and post-authorisation data are provided in Chapter 5.

4.1. Non-clinical requirements

- **Requirements for authorisation for all influenza vaccines (MAA)**

See section 5.1.

- **Requirements for applications to change vaccine composition**

Seasonal influenza vaccines

Non-clinical studies are not required.

Pandemic and zoonotic[3] influenza vaccines

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

For LAIVs only animal protection studies are of relevance.

4.2. Clinical requirements

4.2.1. Seasonal vaccines

Seasonal influenza vaccines undergo if appropriate an update of their strain composition annually prior to each influenza season. 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 confirm each year the influenza virus strains recommended for vaccine production in the EU. Based on this recommendation, any strain replacements within authorised vaccines are made via variations (see section on seasonal strain update; see also the quality and the regulatory modules of the influenza guideline).

In exceptional circumstances and based on the perceived emergency of the situation, an approved seasonal influenza vaccine could undergo a variation so that it contains a pandemic strain (for details see the Regulatory Module of the influenza guideline).

- **Requirements for authorisation (MAA) – Immunogenicity and efficacy**

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 is manufactured and has a final HA content similar to that of an EU-authorised inactivated non-adjuvanted vaccine may

[3] See section 4.2.3 for the definition of zoonotic vaccine

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 elderly[4]

For adult and elderly populations, non-inferior immunogenicity should be demonstrated in specific age sub-groups. See Chapter 5 for details of study designs. If an applicant wishes to conduct global studies in which the comparator(s) is/are not approved in the EU it is essential to discuss the plan with EU Regulatory Authorities.

- Paediatric population

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 section 5.2.2 for study details).

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 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 demonstrated. Alternatively, 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).

c) For an indication that includes use from approximately 9 years (see also section on dose finding and paragraph on serological markers for priming), comparative immunogenicity data are acceptable and a demonstration of vaccine efficacy is not required. The comparison could be made against immunogenicity data obtained with the candidate vaccine in older age groups (e.g. young adults) or directly against an authorised inactivated non-adjuvanted seasonal influenza vaccine. For example, a new quadrivalent vaccine could be compared with an authorised quadrivalent vaccine.

- Immunocompromised individuals

Immunogenicity studies may be conducted before or after the initial MA.

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. Sponsors may consider obtaining immunogenicity data from specific subsets or a wider range of immunocompromised patients, resulting

4 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 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500005218.pdf

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 use 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. Results could be compared with age-matched healthy children to indicate whether higher doses and/or different regimens are needed in the immunocompromised.

- Patients with comorbidities

Some co-morbidities may increase the risk of complications from influenza infection but may not impact on the immune response to and protection afforded by vaccination. Hence a demonstration of comparable immune responses to healthy adults of the same age range can be inferred to mean that protection against disease may be similar. These data 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

There are considerable data on the use of inactivated unadjuvanted vaccines (split virion and subunit) during pregnancy. Sponsors are encouraged to obtain data on the ability of maternal vaccination to prevent influenza in infancy.

Seasonal inactivated adjuvanted vaccines

To authorise the use of a novel 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 vaccine that has been used in the EU. Demonstrating non-inferiority would not be considered sufficient. 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.

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, the adjuvanted vaccine should be shown to have clinically relevant efficacy at least in children aged 6-36 months. 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.

LAIVs

Currently, due to lack of any clear 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.

Other types of vaccines- Novel vaccines

In principle if a novel vaccine for prevention of seasonal influenza is developed that does not have appropriate comparators already authorised or used in the EU (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 (e.g. immunocompromised patients and pregnant women) based on immune response data.

- **Requirements for applications to change vaccine composition (seasonal strain update)**

In principle, there is no need to provide clinical data to support seasonal strain updates. Vaccine performance should be monitored by means of product-specific effectiveness studies and enhanced safety surveillance that should be included in the RMP. The reactogenicity profile of influenza vaccines after annual strain updates should be investigated in the population indicated for each vaccine (including children if applicable) in order to confirm acceptable tolerability of the newly recommended strain(s) (for details see Annex I on Enhanced safety surveillance).

See also section 5.2.3 on Vaccine effectiveness and section 5.2.5 on Post-authorisation pharmacovigilance requirements.

4.2.2. Pandemic vaccines

Pandemic vaccines are indicated for immunization against pandemic influenza viruses and are intended for use only following the recognition of a pandemic at the level of the EU, in the framework of Decision No 2119/98/EC.

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 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 (see paragraph on pandemic strain update).

- **Requirements for authorisation (MAA) – Immunogenicity and efficacy**

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 but contains a potential pandemic strain. This "core dossier" should provide data on the safety and efficacy of the vaccine construct when it contains a potential pandemic strain that is poorly immunogenic and for 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 should be included in the core dossier as supportive evidence.

Pandemic Vaccines - inactivated

MAAs should be based on 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 construct in case of an actual pandemic. Any efficacy data generated previously with the same or similar vaccine construct(s) authorised or used in the EU (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 aged >60 years. 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 particularly healthy children[5].

It is expected that vaccine effectiveness will be evaluated in the post-authorisation phase (i.e. during the pandemic) in accordance with plans that are included in the RMP (see section 5.3). During the actual 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 data may be collected by means of registries).

Pandemic vaccines – live attenuated

Humoral immune responses to LAIVs do not correlate well 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 and 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. 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 or pandemic alert phase should be kept in appropriate clinical isolation conditions (see also WHO Technical Report Series TRS 941 Annex 5).

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 carrying a pandemic strain. The expectations for post-authorisation studies are as for inactivated vaccines.

- **Requirements for applications to change vaccine composition (pandemic strain change)**

The pandemic strain change 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.

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

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

It may become necessary to authorise a new pandemic vaccine while a pandemic is considered incipient or is already recognised in the EU. If a MAA for a pandemic vaccine is submitted in such circumstances, the evaluation process will be accelerated (emergency procedure).

The dataset required for authorisation of inactivated or LAIVs 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 novel vaccine construct than would be needed for an established and well-known construct.

In case an emergency procedure is envisaged, it is recommended to initiate discussions with regulatory competent authorities as early as possible.

4.2.3. 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 1234/2008.

- **Requirements for authorisation (MAA) - Immunogenicity and efficacy**

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.

Due to the usual epidemiology of influenza zoonotic strains, it is not expected that clinical efficacy can be established pre-authorisation 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 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 on persistence in chapter 5).

- **Requirements for applications to change vaccine composition (zoonotic strain change)**

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): the MAH may submit a strain change variation that includes only the manufacturing and quality data related to the new strain (see quality module), 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.

b) Replacement of the HA/NA subtype (e.g. supplanting the original H5N1 strain with an H7N7 strain): advice from EU Regulatory Authorities should be sought on the regulatory framework and data requirements but in principle immunogenicity and safety studies are required.

5. Requirements for influenza vaccines: scientific aspects

5.1. Non-Clinical aspects

This section applies to all influenza vaccines as detailed in the scope of this module. Specific requirements per vaccine type 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.

The lots used in nonclinical studies can be either experimental (non-GMP) or 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 nonclinical 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[6].

5.1.1. 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. Immunogenicity studies should include an evaluation of humoral as well as cellular immune responses, dose-range testing of antigen and persistence of immunity. 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 single and multiple doses. 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 (see section 5.1 and footnote 5).

- **Protection studies**

Protection (or challenge) studies should be performed with new influenza vaccines in a relevant animal model and are intended to provide evidence of protective efficacy for the specific vaccine virus strain intended for clinical use upon relevant virus challenge. Ferrets represent the preferred animal model for influenza challenge studies as the disease pathogenesis, clinical signs –including febrile response-

[6] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/02/WC500161024.pdf

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 heterologous 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 sponsor. 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; the use of lower doses to encompass animal welfare should be appropriately justified. 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.

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 formulations.

- **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 pandemic and zoonotic vaccines, where the objective is to determine the antigen-specific neutralizing antibody titre associated with the protection.

5.1.2. Safety pharmacology studies

Dedicated safety pharmacology studies are generally not considered necessary for vaccine products, 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 (in the case of a LAIV). These observations should be included whenever possible in the design of toxicity or immunogenicity studies.

5.1.3. 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, novel adjuvants or alternative routes of administration (for example deposition studies at the site of injection, distribution studies or viral shedding studies for LAIV vaccines – see section on LAIVs).

5.1.4. Toxicology

Toxicology testing should usually be performed with a vaccine that contains the same strain intended for the candidate vaccine 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 constructs and manufacturing processes to already authorised vaccines, routine non-clinical toxicity 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 (e.g. a vaccination schedule with 2 or 3 doses at a 3-4 weeks interval) and should consider potential species-specific differences.

Within these studies, the applicant should investigate the risk of immunological toxicity, hypersensitivity reactions and autoimmunity reactions where appropriate, e.g. in the case of preservatives and adjuvants (see also section Additional consideration on adjuvanted vaccines).

- **Developmental and Reproductive Toxicity**

For novel vaccines, 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 also 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 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.

5.1.5. 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 (see EMEA/CHMP/SWP/4447/00). For GMO influenza vaccines see section Additional considerations on LAIVs.

5.1.6. Additional considerations

- **Adjuvanted vaccines**

The principles to be applied for the development of adjuvanted vaccines are laid out in the Guideline on Adjuvants (EMEA/CHMP/VEG/134716/2004) and in the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines.

For adjuvants it is especially important to address the level of antibodies induced, the type and magnitude of immune responses (e.g. Th1/Th2 balance and evidence of cross-protection to heterologous viruses) and the persistence of immune responses[7].

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 be investigated include local reactogenicity, fever, immunotoxicity (e.g. anaphylaxis, unintended immunosuppression or enhancement) or autoimmune disorders.

A titration of the optimal ratio adjuvant/antigen should also be performed. 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, separately and in combination with the influenza virus antigen, within the same toxicity study if possible. This includes the need for a standard battery of genotoxicity studies and an appropriate assessment of potential effects on reproduction and development.

- **Live attenuated seasonal influenza vaccines (LAIV)[8]**

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

Primary PD studies

- Given the limited correlation between humoral 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 test is able to prevent 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- should define the pharmacokinetic profile of the vaccine, evaluate the potential gender differences in kinetic disposition, and provide sufficient exposure data, in conjunction with appropriate toxicology evaluations to evaluate the potential for safety concerns at clinically relevant exposure levels. One

[7] Further details are included in the WHO guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines.
[8] For non-clinical release testing of monovalent lots of vaccine please refer to the quality module of the influenza guideline (e.g. attenuation assays, cold adaptation and temperature sensitivity)

species could be considered sufficient, and the choice of species 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.

Safety pharmacology studies

- Potential neurovirulence of vaccine strains should be evaluated in appropriate murine neurovirulence models using murine neuro-adapted strains as controls. Crossing of the blood-brain-barrier might be an indication of potential neurovirulence.

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 for reassortment of wild-type virus with vaccines virus strains and the potential risk of spread to humans and animals should be addressed (see EMEA/CHMP/BWP/473191/2006 – Corr).

5.2. Clinical aspects

5.2.1. Clinical Immunogenicity

The recommendations included in the following 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, DNA vaccines that express HA and VLP-based vaccines.

- **Immunological testing**

Assays and parameters to be assessed

The assessment of the immunogenicity of influenza vaccines is based mainly on two tests that detect antibody directed against the HA antigen. Neither the haemagglutination inhibition [HI] nor serial radial haemolysis [SRH] assays are standardized. It has been shown that they are both subject to very considerable inter-laboratory variability. In any one clinical development programme it is recommended that all HI and SRH assays are conducted in a single designated laboratory. 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-testing as part of the validation process). Sponsors 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 methodology. Critical assay parameters known to affect the

results include the type of readout, the duration of incubation, the use of naturally permissive vs. non-permissive cell lines 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 sample dilution should not be greater than 1:10. As for HI and SRH, it is recommended that the same assay and experienced centralised laboratory are used throughout any one clinical development programme. It is also recommended that sponsors 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) should be envisaged at least in randomly selected subsets across the whole intended age range (e.g. for vaccines proposed for use in the elderly at least some data should be generated in subjects aged 75-84 and > 85 years). 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. An evaluation of CMI may be particularly informative in the elderly due to the recognised effects of immunosenescence on CMI and observations that even very high antibody titres as measured by HI and VN may not predict protection.

Sponsors are encouraged to evaluate anti-neuraminidase NA antibodies at least in randomly selected subsets.

Applicants are recommended to submit studies of antibody kinetics as indicator of past priming and of maturation of the immune response as they may be useful component of the evaluation.

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 to challenge with a related but drifted strain following initial vaccination with the selected vaccine strain based on comparison with a first dose of the drifted strain vaccine in an unvaccinated control group.
- Cross-protection: if this is claimed, evidence should be based on cross-reactivity data supplemented by non-clinical data (see also the Non-clinical section).

Due to ongoing drift it is anticipated that 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 needed during the clinical development programme, adequate cross-validation data should be provided.

For further details on assays validation please refer to the quality module.

- **Analysis and presentation of immunological data**

<u>All influenza vaccines</u>

The immunological data obtained from each study should be presented in detail by vaccine strain and using a standard approach in each study report. As a minimum:

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

- Reverse cumulative distribution curves of HI and VN titres 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 (seroconversion may be defined in several ways including at least an x-fold increment in HI or VN titres over baseline and/or appearance of a measurable titre in a subject with previously undetectable 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 based on comparisons of the recipients' pre- and post-dose immunological status.
- 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, immunogenicity studies should describe the percentages that achieve an immune response above a pre-defined and appropriately justified threshold level. Additional analyses should evaluate percentages of vaccinees reaching alternative and higher titres and should report on whether the lower bound of the 95% confidence interval around the point estimates also exceeds each cut-off value assessed. Additionally, seroconversion rates and GMRs (see above) should be reported. The VN data should be analysed in a similar fashion with appropriate cut-offs applied to titres and data trends should be compared with the HI results.

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

- **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. Lack of such data should always be justified.

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 children and in the elderly and to identify appropriate age-specific dose regimens.

Dose finding studies in children should be assessed by means of the same parameters as in other age groups and should attempt to support a broad range of exploratory analyses in subgroups. Primary immunization schedules should at least be investigated in children aged 6 to 36 months, who are most likely to be antigen naïve, including an assessment of the ability of the first dose to prime (see further below). It is recommended that scientific advice is sought early in the development programme.

If a sponsor is pursuing development of a non-adjuvanted inactivated seasonal vaccine in the paediatric population, it is essential to document the immune response and conduct adequate dose

finding studies before deciding whether the vaccine appears suitably immunogenic to merit proceeding with an efficacy study.

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.

Persistence of protection and revaccination

For seasonal vaccines, persistence of immune response following primary vaccination should be investigated at least up to 12 months after completion of the initial regimen.

In any population(s) studied in which annual revaccination is not recommended antibody persistence could be followed beyond 12 months. In such populations, sponsors 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.

The choice of revaccination schedule should be investigated and justified. Especially in the case of adjuvanted vaccines and LAIVs, the immune response to revaccination with these types of vaccines should be compared to revaccination with a non-adjuvanted vaccine based on antibody levels and cell-mediated immune response data. For example, it is important to understand whether children who have received a dose of adjuvanted vaccine that efficiently primes the immune system can be satisfactorily re-vaccinated in subsequent years with non-adjuvanted vaccines. The study population should be followed up following primary vaccination as described above.

For pandemic and zoonotic vaccines immunogenicity data should be collected for at least 6 months following primary vaccination in the first instance 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 the need to maintain antibody titres due to continued risk of exposure.

Serological markers for priming

There is no serological marker available indicating effective priming in any age group, whether by natural exposure or through vaccination. Any evidence of adequate priming associated with the first dose of a vaccine should be provided. Such evidence might come from demonstrating an anamnestic response to antigen challenge administered at least 6-12 months after the first dose in comparison with an unvaccinated age-matched cohort. The exercise should be repeated following the primary series if it is decided that more than one dose of a vaccine is needed to secure priming.

These data are important to elucidate appropriate vaccination schedules (e.g. if the data show that a single dose of an adjuvanted vaccine is followed by an anamnestic response to a non-adjuvanted vaccine containing the same strain[s]).

Immunological correlates of protection

A correlate of protection may be defined as an immune response that is responsible for and statistically interrelates with protection from clinical disease[9]. 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 infection 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, which potentially may vary according to individual characteristics, populations, specific age

[9] Plotkin, CID 2013; Plotkin and Gilbert, CID 2012.

groups (e.g. no correlate has ever been identified or clinically validated in the paediatric population) and vaccine types.

During new vaccine development programmes sponsors should make every effort to obtain data that could support identification and validation of correlates of protection. 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.

For example, the Prentice criteria could be used to assess whether an immunologic correlate of protection can be determined. To model the relationship between the occurrence of influenza and antibody titre level, the logistic regression model advocated by Dunning[10] would be appropriate as it accommodates both antibody titres and factors independent of antibody titres.

Human challenge studies could provide useful information for establishing correlates of protection. Data derived from passive immunisation studies in animals may also assist in identifying immune markers correlating with protection.

5.2.2. Clinical efficacy

In instances in which an efficacy study is considered to be feasible and necessary (see chapter 4), this section considers the design of such studies.

- **Methodological considerations**

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 it is necessary to select an appropriate non-influenza control vaccine that may provide some benefit in the intended target age group.

Alternatively, subject to adequate justification, sponsors 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). However, if the efficacy of the selected comparator has been well documented in prior studies and is considered to be clinically useful then, subject to adequate justifications and following discussions with competent regulatory authorities, it may be acceptable to plan a primary analysis based on showing non-inferior efficacy.

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 (see EMEA/CHMP/VWP/164653/2005*)*. 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.

Great care in age stratification is required, especially in trials that enrol young children and the elderly. Both age and previous exposure to influenza vaccines should be considered in the trial design to

[10] Dunning, Statistics in Medicine 2006.

ensure adequate representation of children who are most likely to be naive to influenza. For the purpose of demonstrating efficacy in the elderly it is important to include persons in the older age range (75 – 84 years and >85 years of age) and ensure that the frail elderly are represented (e.g. those living at home but receiving home care services, or those living in nursing homes).

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. If any immunological test will be applied only to a subset of the population then it is strongly recommended that there is randomisation of subjects at baseline to provide adequate samples for specific tests.

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[11] (ILI) that are laboratory confirmed by PCR or culture or both.

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, sponsors should include a discussion of the anticipated efficacy across other influenza types [e.g. if A(H1N1) predominates in the study the sponsor 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.

If the sponsor proposes an alternative primary endpoint or wishes to propose co-primary endpoints this should be discussed with EU Regulatory Authorities before initiating the efficacy study. For example, in patients with laboratory-confirmed influenza a composite endpoint consisting of influenza-related pneumonia, hospitalisation and influenza-related mortality could be considered as an alternative primary endpoint for studies conducted in the elderly.

An important secondary endpoint is the estimate of efficacy against influenza due to well-matched strains[12]. 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 only 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 sponsor wishes but such data would not be considered supportive in an assessment of vaccine efficacy.

Duration of the study

[11] 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

[12] 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_ganda_recommendation.pdf).

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.

5.2.3. Vaccine effectiveness

This section focusses on effectiveness studies for seasonal influenza vaccines. However, the design principles that are outlined would also apply to effectiveness studies that are conducted during a pandemic situation.

Due to antigenic drift vaccine effectiveness cannot be presumed from historical data. In addition, although a substantial number of effectiveness studies for inactivated trivalent flu vaccines have been published in the past, these studies have differed in terms of study design, case definitions, primary endpoints and target populations. Published results often reflect data from unspecified vaccines and the estimates are not usually product-specific. Considering the diversity of seasonal influenza vaccines (trivalent and quadrivalent vaccines, split, subunit and whole virion vaccines, egg or cell culture derived vaccines, virosomal vaccines and vaccines with and without adjuvants), product-specific effectiveness data are necessary.

Thus, investigations of vaccine effectiveness for individual influenza vaccines should be routinely performed as part of the post-marketing surveillance. In line with the Guideline on good pharmacovigilance practices - *GVP P.I: Vaccines for prophylaxis against infectious diseases*[13], post-authorisation effectiveness studies should be included in the RMP as additional pharmacovigilance activities for all influenza seasonal vaccines including novel influenza vaccines and seasonal strain updates (see *Guideline on good pharmacovigilance practices (GVP) Module V – Risk management systems*[14], sections V.B.9.4 and V.B.10.2).

- **Principles of Study design**

It is always 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. Adherence to the same study protocol is recommended to enable comparisons among products and studies. Studies will have to be conducted in accordance with Good Epidemiological Practice (GEP) guidelines and with guidelines of ENCePP. Sponsors may liaise with organisations who have experience in influenza effectiveness studies and who have implemented a functioning infrastructure to conduct multicentre studies.

In general, prospective observational studies are preferred as retrospective studies may introduce additional bias. ECDC has published a core protocol for a case-control study (controls ILI, influenza negative) to evaluate effectiveness of influenza vaccines. The study protocol has already been tested to provide robust results both during the H1N1 pandemic 2009/2010 and several non-pandemic influenza seasons. Thus, the ECDC case-control study protocol is strongly recommended. If this study design is not feasible other study protocols may also be used, e.g. the cohort study protocol also

[13] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/12/WC500157839.pdf
[14] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500129134.pdf

published by ECDC[15] or a screening method where IVE is estimated by comparing the vaccination coverage between ILI patients positive for influenza and a reference group (lit to be included). Within the cohort studies, nested test-negative case–control studies should be conducted to estimate IVE against medically attended laboratory-confirmed influenza.

Continuous investigation of individual influenza seasons is warranted to monitor effectiveness of each individual seasonal vaccine and to investigate the impact of possible virus drift on effectiveness.

The duration of the protection provided by influenza vaccines remains unknown. To characterise the potential wane of vaccine protection after vaccination, data should be collected throughout the season and in sequential seasons.

- **Endpoints and case definition**

Endpoints

1. Case control design

The primary endpoint for case control studies and nested case control studies should be laboratory confirmed influenza.

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 are for example:

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

It is recommended to include laboratory-confirmed influenza outcomes in all studies (nested case-negative case control design, see above).

Case definition

Influenza case definition combined with laboratory confirmation results has the highest specificity for influenza, and laboratory confirmation is therefore essential to estimate the true vaccine effectiveness. Cases should meet the EU ILI and influenza case definition. 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 essential.

[15] For further details see:
http://ecdc.europa.eu/en/publications/Publications/0907_TED_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Case_Control_Studies.pdf
http://ecdc.europa.eu/en/publications/Publications/0907_TER_Influenza_AH1N1_Measuring_Influenza_Vaccine_Effectiveness_Protocol_Cohort_Database_Studies.pdf

- **Target population**

Effectiveness of influenza vaccination has to be investigated in the target population for the respective vaccines (e.g. children in case of live attenuated vaccine; pregnant women, chronically ill patients and elderly patients for inactivated vaccines).

The age effect has to be taken into account by adjustment stratified analysis of children, adolescents, individuals <65 years, ≥65 years and > 80 years of age. Patients with certain underlying diseases are known to be at increased risk of serious influenza-related complications. It is therefore important to assess the effectiveness of influenza vaccines in these risks groups. Thus, additional sub analyses should be performed concerning underlying medical conditions including frailty.

- **Selection of cases/ cohorts**

Standardised approaches for recruitment of subjects are warranted to reduce a possible selection bias.

As a minimum the following information has to be collected:

- date of vaccination and invented name of vaccine received
- data on onset of ILI symptoms
- 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.

- **Study Analyses and data management**

For study analyses including statistical evaluation and data management reference is made to the respective core study protocols published by ECDC. Careful consideration for important confounding factors is important, such as healthy vaccinee effect and confounding by indication. Consideration should also be given to other important confounding factors such as health-seeking behaviour and age specific differences in vaccination coverage and previous influenza vaccination.

- **Presentation of results**

It is acknowledged that some outcomes will be available in real time, whereas others will not become available before the end of the season. Therefore, IVE for different outcomes might be calculated at different time periods. Study results in terms of crude and adjusted influenza effectiveness should be presented on an annual basis and as soon as they will become available. The results should be submitted together with the strain update for the next season with an updated RMP in order to allow for timely adjustments of the study protocol for the evaluation of vaccine effectiveness for the next season or additional training of participating centres. Vaccine effectiveness analyses should be presented for the different influenza subtypes. Sub group analysis for different target populations as specified above should be provided.

5.2.4. Clinical safety

- **Requirements pre-authorisation**

Investigation of clinical safety should be performed 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.

Size of the database

As a general rule, the total size of the safety population for any influenza vaccine should consist of at least 3000 individuals. Table 1 outlines the usual anticipated safety database depending on the population studied before filing a MAA and the proposed age range for the indication:

Table 1

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 subject 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.

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 sponsor'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 LAIV 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

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

that adequate precautions can be introduced into study protocols. These precautions should then be reviewed once shedding data become available.

- **Requirements post-authorisation**

Depending on the indication(s) authorised, the MAH should propose in the RMP relevant additional pharmacovigilance activities (e.g. post-authorisation studies) to address e.g. identification of rare and very are 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 Guideline on good pharmacovigilance practices: Vaccines for prophylaxis against infectious diseases (GVP P.I). It is recommended to seek scientific advice concerning design and conduct of post-authorisation studies.

5.2.5. Post-authorisation pharmacovigilance requirements

Any influenza vaccine authorisation application should include a risk management plan as part of the initial submission. The general requirements for RMPs are described in the Guideline on good pharmacovigilance practices: Module V – Risk management systems (EMA/838713/2011) and Guidance on format of the risk-management plan in the European Union – in integrated format (EMA/465932/2013 Rev.1). 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).

For any influenza vaccine and 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, it should be demonstrated through appropriate measures 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.

For seasonal influenza vaccines the RMP should include plans to address the following:

- Enhanced surveillance of vaccine safety: safety and reactogenicity of the new strains 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 authorities. Detailed requirements for the provision of enhanced surveillance data are included in Annex I - Guidance on enhanced safety surveillance for seasonal influenza vaccines in the EU.
- Product-specific effectiveness studies (see section on Vaccine Effectiveness).

For 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.

For 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:

- In the event of a declared pandemic, it would be important to monitor the effectiveness of any vaccines administered before the start of a pandemic that is expected to provide protection based on evidence of cross-reactivity and/or cross-protection. Such data would be informative for planning future prepandemic vaccination strategies and, if data become available early enough showing evidence of protection, it could allow for any available pandemic vaccine to be directed primarily to previously unvaccinated cohorts.
- Pregnant women are likely to be among the first groups targeted in pandemic vaccination campaigns (e.g. registries). Vaccination of pregnant women might additionally protect neonates from infection. As the immune system undergoes changes during pregnancy, altered immune responses, especially T-cell response, may affect the ability of the vaccine to prime. Effectiveness and safety should be monitored and such studies should be planned for and agreed during the MA procedure.
- The accumulation of immunogenicity, efficacy and safety data 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.
- The subjects enrolled into these studies should be followed carefully for the development of influenza. Specific case and case detection definitions should preferably be developed and used consistently during a pandemic. However, these may need reconsideration as the clinical presentation might change during the late pandemic phases. Protocols should describe the populations to be studied and methods to estimate vaccine effectiveness. Clinical outcomes should at least include age specific morbidity and mortality, including rates of hospitalisation. Data from these subjects should be used to develop possible serological criteria for protection.
- In addition to the assessment of rates of local and systemic reactions in the immediate post-vaccination period, there are specific longer-term 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 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 Annex I), i.e. data should be provided rapidly and collected within a month from the start of vaccination.

6. Core 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 Quality Module on the Labelling, the Annex to the Guideline on clinical evaluation of new vaccines: summary of product characteristics requirements (EMEA/CHMP/VWP/382702/2006), and the QRD templates published on the EMA website.

7. Annex I – Enhanced safety surveillance for seasonal influenza vaccines

This guidance was adopted as standalone document (EMA/PRAC/222346/2014) in April 2014 and can be found here. It will be annexed to this module foAnnex