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To: Director-General of the Prefectural Health Department (Bureau)

Director of the Evaluation and Licensing Division,
Pharmaceutical and Food Safety Bureau,
Ministry of Health, Labour and Welfare

"Guideline for Development of Prototype Vaccines for Preparation for Pandemic Influenza"

A guideline for development of prototype vaccines for preparation of pandemic influenza aiming at an application for approval of drugs, etc. is organized as provided in the appendix. Please ensure that related parties under your jurisdiction are thoroughly informed.

It should be noted that this guideline presents fundamental principles only based on scientific knowledge at the present time and thus does not rule out other methods if they are based on reasonable grounds reflecting the current academic progress.

(Appendix)

Guideline for Development of Prototype Vaccines for Preparation for Pandemic Influenza

1. Introduction

The Action Plan for Measures against Novel Influenza (amended in February 2009, hereinafter referred to as the "Action Plan") states that one of the actions to be taken in response to a pandemic of novel influenza is "to manufacture pandemic vaccines for all people in Japan within 6 months after determination of virus strains for vaccine production in response to emergence of the novel influenza." As of the end of December 2010, highly pathogenic avian influenza (H5N1) virus is spreading from East Asia mainly to the Middle East and Europe, and its potential viral variants may cause pandemics in humans. In addition to subtype H5 influenza viruses, subtypes H7 and H9 have been reported to have infected humans in Europe and East Asia since 1999, although the number of cases is limited, suggesting a risk of pandemics caused by these subtype viruses.

To manufacture pandemic vaccines against the target subtype viruses in a short period as aimed by the Action Plan, prototype vaccines using model influenza viruses must be developed before occurrence of a pandemic. In addition, such prototype vaccines must be demonstrated to have adequate immunogenicity and acceptable safety in humans in advance. With the prototype vaccines ready for use, effective pandemic vaccines can be manufactured and supplied promptly in response to occurrence of a pandemic by applying the manufacturing method and quality control established for the prototype vaccines. On the other hand, prototype vaccines using model influenza viruses (hereinafter referred to as "prototype vaccines") may differ from vaccines using pandemic virus strains in quality, efficacy, or safety. Because they need to be developed before the occurrence of a pandemic, evaluation of efficacy in the prevention of pandemic influenza has limitations. Despite the above difficulties, preparedness for pandemic influenza is critical, and thus studies must be conducted to the greatest possible extent while no pandemics are occurring, with the expectation that such pandemic vaccines are effective in preventing severe diseases in patients with pandemic influenza. When a pandemic occurs, pandemic vaccines must be immediately evaluated based on the information obtained from prototype vaccines and then rapidly manufactured.

2. Purpose of this guideline

The purpose of this guideline is to present points to be noted for quality, non-clinical, and clinical data to be submitted for applications for marketing approval of prototype vaccines and their derivative pandemic vaccines. Depending on characteristics of a product, measures other than those presented in this guideline may be applicable. Consultation with the Pharmaceuticals and Medical Devices Agency (hereinafter referred to as "PMDA") should be made where necessary.

In addition, development of prototype vaccines does not differ from that of conventional vaccines in terms of basic requirements other than those specified in this guideline or data required for applications for approval. The following guidelines may be used as references: “Guidelines for Nonclinical Studies of Preventive Vaccines for Infectious Diseases” (PFSB/ELD Notification No. 0527-1, dated May 27, 2010); “Guidelines for Clinical Studies of Preventive Vaccines for Infectious Diseases” (PFSB/ELD Notification No. 0527-5, dated May 27, 2010); guidelines issued by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH); and guidelines issued by the World Health Organization (WHO).

3. Scope of this guideline

This guideline shall apply to the prototype vaccines that are designed to induce immune response (immunogenicity) to hemagglutinin (HA) antigens on the influenza virus surface other than those of subtypes H1 and H3 viruses, common viruses that repeatedly cause epidemics among humans, and contain the active substances of inactivated viruses using embryonated eggs or cultured cells as host for virus replications or the active substances manufactured using genetic engineering technology. In addition, it shall also apply to pandemic vaccines of which production strains are not only variants within the same subtype but also those of different subtypes.

However, it shall not apply to live vaccines because a strain change in this type of vaccines can considerably affect immunogenicity and safety depending on characteristics of pandemic strains such as replication competence. This guideline shall not apply to vaccines that are expected to induce immune response to antigens other than HA antigens either. However, this guideline does not intend to discourage development of vaccines outside of the scope, and for development of such vaccines, consultation with PMDA is encouraged where appropriate.

4. Basic principles for development of prototype vaccines

Prototype vaccines should be developed on the assumption that pandemic influenza can be highly fatal and should therefore use novel virus strains, uncommon strains not repeatedly causing epidemics among humans (irrespective of whether infection in humans has been reported, hereinafter the same applies throughout this guideline), and be fully evaluated in terms of the description and strength of the antigens, need for adjuvant, dosage form and quantity as well as inoculation route while there are no pandemics to ensure adequate immunization in the public even if the whole or most of them have not been exposed to pandemic influenza virus strains (hereinafter referred to as “naïve population”). In principle, the manufacturing method and quality control established for the prototype vaccine should be used for pandemic vaccines as well, although parameters in the manufacturing process for the pandemic vaccines may have to be changed depending on characteristics of the pandemic virus strains.

5. Manufacture and quality of prototype vaccines

Points to note for investigations of the manufacture and quality of prototype vaccines are presented below.

- Marketing authorization holders should select virus strains for manufacture of a prototype vaccine (hereinafter referred to as "prototype vaccine production strains") from novel virus strains not repeatedly causing epidemics among humans (novel wild-type virus strains isolated from humans, swine, or birds, post-reassortment influenza virus strains, etc.) to ensure appropriate evaluation on immunogenicity and safety of the pandemic influenza virus strains.
- For vaccines using HA proteins as the main antigens to be targeted, a single radial immunodiffusion (SRD) method is the gold standard HA assay method, but antisera needs to be obtained for the assay. In view of the cases where the anti-sera are not available at the time of manufacture and release of pandemic vaccines, alternative HA assay methods using high performance liquid chromatography (HPLC) or sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and protein assay in combination (to calculate HA content from the percent HA content based on SDS-PAGE and protein amount) should be established in advance and subjected to validation of analytical procedures using prototype vaccines to obtain supporting data.
- If pandemic vaccines are expected to be supplied in multi-dose vials, a preservative and its quantity as well as in-use storage conditions (including in-use storage period) should be specified where appropriate in view of a risk of contamination with adventitious agents and stability after opening (first use). In principle, the preservative quantity to be added should be kept to the minimum necessary. Additional development is encouraged to ensure that an option of single-dose products is also available according to the prevalence in Japan.
- In view of effective immunization in the naïve population and saving of the antigen materials, use of adjuvant should be considered. If used, information on the quality of the adjuvant (e.g., ingredients, quantities, manufacturing method, control method) should be included in the application data.
- Because the performance of operations such as inactivation and purification may differ depending on virus strains, multiple virus strains should be used in the investigations. For example, in addition to prototype vaccine production strains, virus strains of different subtypes or different ones within the same subtype may be used.
- If an animal cell line is used as the replication substrate (or host) of viruses, tumorigenicity and, where necessary, carcinogenicity of the cells should be evaluated. Removal of host cells, host cell DNA, and host cell proteins in the manufacturing process should be evaluated. If the animal cell line to be used poses a concern of tumorigenicity, carcinogenicity should be evaluated in principle.
- An application form for marketing approval can be completed as done for conventional drugs. Acceptance limits for quality control tests of active substances and vaccine products should be provided based on results obtained from the prototype vaccine.

Depending on characteristics or manufacturing method, etc. of an individual prototype vaccine, there are points to note in addition to the above. Consultation with PMDA should be made where necessary.

6. Non-clinical studies of prototype vaccines

For non-clinical studies of prototype vaccines, the points provided below should be noted in addition to the "Guidelines for Nonclinical Studies of Preventive Vaccines for Infectious Diseases" (PFSB/ELD Notification No. 0527-1, dated May 27, 2010).

6.1. Evaluation of preventive effect

Because a prototype vaccine is supposed to be developed while there is no pandemic influenza, evaluation of the preventive effect in humans is not feasible. The prototype vaccine or prototype vaccine production strains should be inoculated in an appropriate animal model (e.g., ferrets) to investigate immune response, and challenge studies should also be conducted to evaluate the preventive effect. Based on a relationship between the preventive effect and immune response indicated by results from the challenge studies, immunological measures may be specified for clinical studies. Vaccines using recombinant HA protein or a part of this molecule as the antigen to be targeted, including seasonal influenza vaccines, have limited results of use globally and may not exert an adequate preventive effect as expected owing to differences in carbohydrate structure of HA protein. The preventive effect should be fully evaluated in animals.

- In the challenge studies, the preventive effect and immune response such as antibody production should be fully evaluated. In these studies, cross-immunity or whether the prototype vaccine is also effective in preventing infections caused by different virus strains within the same subtype of the prototype vaccine production strain should be investigated in view of potential emergence of variants of the prevalent virus strain during a pandemic.
- Challenge studies of vaccines manufactured using strains of a different subtype should be conducted to evaluate the preventive effect in preparation for cases where a subtype of the pandemic vaccine strain may be different from that of the prototype vaccine strain.
- Need for adjuvant should be investigated as well.

6.2. Immunogenicity evaluation

- Immunogenicity should be evaluated in appropriate animals such as mice and ferrets, which are highly responsive to human influenza vaccines, selected in view of the route of administration and composition of the vaccine product. Through this evaluation, dosage, number of doses, route of administration, and need for adjuvant should be examined before start of clinical studies.
- Immunogenicity of vaccines manufactured using strains of a different subtype should be evaluated in preparation for cases where a subtype of the pandemic vaccine strain may be different from that of the prototype vaccine strain.

6.3. Safety evaluation

- In general toxicity studies of the prototype vaccine, safety should be adequately evaluated in view of all possible situations where pandemic vaccines are used, including dosage, number of doses, and route of administration. In addition, a safety pharmacology core battery should be evaluated in a part of the general toxicity studies or independent studies.
- Because pandemic vaccines are supposed to be inoculated in pregnant women and women of childbearing potential too, reproduction toxicity of the prototype vaccine should be evaluated.
- If novel adjuvant is used, toxicity of itself should be evaluated as done for the other vaccines.

7. Clinical studies of prototype vaccines

In clinical studies of a prototype vaccine where immunogenicity and safety are evaluated, the points provided below should be noted in addition to the "Guidelines for Clinical Studies of Preventive Vaccines for Infectious Diseases" (PFSB/ELD Notification No. 0527-5, dated May 27, 2010).

7.1. Study population

Clinical studies in healthy adults should be conducted. When results from the clinical studies in healthy adults become available, dosage and administration in healthy children and elderly should be immediately investigated based on them, and immunogenicity and safety should be evaluated.

7.2. Dose setting

Dose-finding studies should be conducted to determine appropriate dose levels, dosing interval, and number of doses. For the number of doses, because pandemic vaccines are supposed to be used in the naïve population, in whom adequate immune response may not be induced by one dose, two-dose regimens may have to be investigated.

If adjuvant is used, the appropriate amount and an antigen/adjuvant dose ratio should be investigated in clinical studies in principle. The results should be used to justify composition of the vaccine product.

7.3. Evaluation method of immunogenicity

Evaluation of true efficacy or a preventive effect of pandemic vaccines is difficult at the time of development when no pandemic influenza is prevalent. Furthermore, a relationship between results on immunogenicity measures and the preventive effect of pandemic vaccines has not been established at the present time. The second-best strategy is to specify appropriate immunogenicity measures that enable objective evaluation, in view of characteristics of the prototype vaccine subject to clinical development and mechanism of immunization of pandemic vaccines expected from these characteristics.

For example, neutralizing antibody titers indicative of an extent to which viruses are absorbed or inhibited from replicating is deemed as a critical measure. In addition, measures of cellular immunity may be desirable if activation is expected, and secondary efficacy measures such as anti-neuraminidase antibody level in serum, of which a relationship to a preventive effect in humans remains unknown, may be also desirable for data collection.

Antibody titer assay should be standardized by comparison with the reference standard as long as standard anti-sera are available.

For the prototype vaccine prepared for development of pandemic vaccines that are expected to exert the effect by inducing immune response to HA proteins, the main antigens to be targeted, and to be subcutaneously or intramuscularly inoculated, immunogenicity can be provisionally evaluated based on either of hemagglutination-inhibition antibody (HI) titer or single radial hemolysis (SRH) antibody titer, both of which are shown to be correlated with the preventive effect by seasonal influenza vaccines. The reference values to use can be found in the Note for guidance on harmonization of requirements for influenza vaccines (CPMP/BWP/214/96) presented by the European Medicines Agency (EMA). Because pandemic vaccines are supposed to be inoculated in the population naive to pandemic influenza viruses, who would be highly likely to have serious symptoms if infected with the concerned viruses, they are expected to induce robust immune response. Aiming at meeting the expectation, clinical studies of the prototype vaccine should be conducted in the study population comprising groups of at least 50 participants each, and the results should meet criteria for 3 measures (seroconversion rate, increase in antibody titer, and proportion of antibody-positive participants) in principle. Of note, if SRH antibody titer is used in evaluation, its correlation to HI antibody titer should be checked.

◆ Adults (aged 20 to less than 65 years)

- Seroconversion rate (proportion of participants in whom the HI antibody titer was increased from <1:10 before vaccination to ≥1:40 after that or in whom it was ≥1:10 before vaccination and increased ≥4 fold after that; or proportion of participants in whom the SRH antibody titer was increased from ≤4 mm^2 before vaccination to 25 mm^2 after that [or the standardized value equivalent to the HI antibody titer ≥1:40 if it is different from 25 mm^2] or in whom it was >4 mm^2 before vaccination and increased by 50% in area) >40%
- Increase in geometric mean HI or SRH antibody titer after vaccination >2.5 fold
- Proportion of antibody-positive participants (proportion of participants achieving the HI antibody titer ≥1:40 or SRH antibody titer >25 mm^2 [or the standardized value equivalent to the HI antibody titer ≥1:40 if it is different from 25 mm^2]) >70%

- Elderly (aged 65 years or more)
  - Seroconversion rate >30%
  - Increase in geometric mean HI or SRH antibody titer after vaccination >2.0 fold
  - Proportion of antibody-positive participants >60%

- To minors aged less than 20 years including children, the criteria in adults should be applied with modifications in principle.

Even for prototype vaccines using HA proteins as the main antigens to be targeted, neutralizing antibody titers should be evaluated. For ones expected to activate cellular immunity, the corresponding measures should be evaluated. Measures such as anti-neuraminidase antibody level in serum may provide supplementary data. That is, such data can be used to support immunogenicity of prototype vaccines including one that does not meet a part of the above 3 criteria based on HI antibody titer.

Cross-immunity or whether antibodies raised by the prototype vaccine also recognize viruses of different strains within the same subtype should be evaluated as well using such viruses based on not only HI or SRH antibody titer but also neutralizing antibody titer.

In principle, duration of post-vaccination immunity and booster effect should be also investigated although the concerned data are not essential for applications for approval.

7.4. Safety evaluation

Safety should be evaluated in a population with a sample size sufficient to have at least 1 participant who experiences an adverse event with the incidence of 1% at a 95% probability. Safety follow-up should be performed for 6 months after vaccination for a main purpose of monitoring occurrence of autoimmune disease, etc., although it depends on characteristics of the prototype vaccine under development.

If novel adjuvant is used or the amount of adjuvant is not less than that in other documented vaccines, a plan for clinical studies to evaluate safety of the adjuvant should be considered according to its characteristics.

8. Other investigations with prototype vaccines

In principle, pandemic vaccines are supposed to be approved without clinical study results; besides, the prototype vaccine has limited immunogenicity and safety data. When the prototype vaccine is approved, a plan of post-marketing surveillance, etc. should be prepared to evaluate immunogenicity, efficacy, and safety of pandemic vaccines in practical settings. Post-marketing investigations of pandemic vaccines are described in Section “9.3 Post-marketing investigations.”

9. Investigations with pandemic vaccines

To ensure that pandemic vaccines are rapidly manufactured and supplied when their need arises, prior development of the prototype vaccine is encouraged. Once the prototype vaccine is approved, pandemic vaccines in the same composition except for the vaccine production strains, which are subject to change, are manufactured by applying most parts of the manufacturing method and quality control established for the prototype vaccine in principle. The strain change is allowed once the following procedures are completed: pandemic vaccines prepared by changing strains in the prototype vaccine should be immediately evaluated based on quality and non-clinical study data in principle; and if acceptable, they can be granted a new marketing approval under a name different from that of the prototype vaccine in an expedited manner.

Investigation items to note for the strain change are as follows:

9.1. Quality

- Although virus seeds for pandemic vaccines should be confirmed to be free from adventitious agents, no test data may be available at the start of manufacture. To ensure that preliminary test results are available before the start of manufacture, a plan using multiple rapid techniques such as polymerase chain reaction (PCR) in parallel should be formulated.
- Results of manufacture and quality control of pandemic vaccines including the preliminary test results should be submitted to PMDA as they become available. In an application form for marketing approval of pandemic vaccines to be submitted, the manufacturing method and acceptance limits should be entered based on quality control results obtained during manufacture of the pandemic vaccines. If the manufacturing method and acceptance limits need to be changed from those for the prototype vaccine, the details and reasons for the changes should be immediately submitted.
- In the case where standard antigens or anti-sera necessary for the SRD method were not available at the start of manufacture or release of pandemic vaccines using HA proteins as the main antigens to be targeted, and thus an alternative assay method has been used, a switchover to the SRD method should be made as soon as the standard antigens or anti-sera become available.
- Pandemic vaccines should also be subjected to stability studies. If results failing to meet the acceptance limits are obtained during the planned storage period, this matter should be reported to PMDA.

9.2. Non-clinical studies

At least 1 lot of pandemic vaccines should be used in animal studies to evaluate the immunogenicity before approval, and the results should be compared with those of the prototype vaccine. Non-clinical immunogenicity data from at least 3 lots should be submitted in the end to demonstrate consistency

among the lots, but timing of the studies can be determined in view of the situation of pandemic influenza. Consultation with PMDA should be made as appropriate.

9.3. Post-marketing investigations

After approval of pandemic vaccines, post-marketing surveillance, etc. should be implemented according to the plan at the time of approval of the prototype vaccine to evaluate immunogenicity and safety of the pandemic vaccines as well as, if possible, efficacy. For actual implementation of the post-marketing surveillance, etc., the situation of pandemic influenza should be considered.

9.3.1. Post-marketing immunogenicity evaluation

Immunogenicity should be evaluated in adults. Pandemic vaccines should be confirmed to have adequate immunogenicity in children and elderly, who may exhibit different immune responses from those in adults. If possible, immunogenicity evaluation in high-risk populations such as pregnant women, patients with chronic diseases, and immunocompromised individuals is desirable.

9.3.2. Post-marketing safety evaluation

Pharmacovigilance surveillance should be separately conducted in a population of 3,000 individuals or more to detect at least 1 individual who has experienced an adverse event with the incidence of 0.1% at a ≥95% probability. The populations to be covered by the surveillance should be specified to collect safety information from children and elderly as well as high-risk populations in whom safety evaluation with the prototype vaccine was difficult. Items subject to information collection should be specified based on safety information on the prototype vaccine, seasonal influenza vaccines, etc. available to that point and characteristics of the target populations.

Subtype

Influenza virus is largely divided into types A, B, and C according to serotype, and type A influenza virus is further classified according to extents of antigenicity of hemagglutinin (HA) and neuraminidase (NA) on the virus surface. Up to date, 16 types of HA and 9 types of NA have been reported, and by their combination, theoretically a total of 144 subtypes from H1N1 to H16N9 can exist.

Adjuvant

Adjuvant is a substance that enhances immune response to the antigen when mixed with it.

Virus seed

Virus seed is a virus strain used in biological products such as vaccines. A homogenous virus suspension or lyophilizate prepared through passage and replication of the virus in embryonated eggs or cultured cells under conditions that maintain its genetic properties.

Hemagglutination inhibition (HI) antibody titer

HI antibody titer is determined by measuring serum concentrations of antibodies recognizing HA proteins, which are on the surface of influenza virus and cause agglutination of erythrocytes by binding to them, and the determined HI antibody titer can be used in evaluation of immune response. When animal erythrocytes are added to serially diluted serum specimens previously incubated with a constant amount of the virus antigen, the erythrocytes agglutinate responding to the virus antigen remaining uncaptured by antibodies in the serum specimen. The maximum dilution factor of the serum specimen that inhibits agglutination of erythrocytes is used as the antibody titer.

Single radial hemolysis (SRH) assay

SRH assay measures serum concentrations of antibodies against influenza virus using the reaction that antibodies specific to influenza virus lyse virus-coated erythrocytes in the presence of complement, and the measured values can be used in evaluation of immune response. A serum specimen is added to a well in agarose gel containing virus-coated erythrocytes and complement and allowed to diffuse, and an area with hemolysis around the well is measured.

Single radial immunodiffusion (SRD) assay

SRD assay is a gold-standard method to measure HA antigen amounts and used in quality control of influenza vaccines. A specimen is added to a well in agar gel containing antibodies against influenza virus and allowed to diffuse, and a diameter of the precipitin ring formed by antigen-antibody complex is measured as the antigen amount in the specimen.

Geometric mean antibody titer

The geometric mean antibody titer is the n-th root of the product of n titer values in n participants ($\sqrt[n]{X1 \times X2 \times \cdots \times Xn}$) and used as the mean titer in a group of n participants.

Cross-immune response

Cross-immune response is immune response to substances with a structure similar to the epitope (antigenic determinant) of the immunogen. Cross-immune response occurs on a structure analogous to the antigen used as the immunogen.

Seasonal influenza vaccines

Seasonal influenza vaccines are influenza vaccines manufactured using prevalent strains. Usually, trivalent influenza vaccines comprised of 2 type A influenza virus strains (mainly, H1 and H3 strains) and 1 type B influenza virus strain are manufactured.

Pandemic influenza

Pandemic influenza is infection with novel influenza viruses causing a global pandemic as defined in Article 6-7 of the Act on the Prevention of Infectious Diseases and Medical Care for Patients with Infectious Diseases.

Pandemic strains

Pandemic strains are type A influenza virus strains against which vaccination is recommended by the WHO during a period with a novel influenza pandemic highly predicted (after WHO declaration of phase 4 or 5) or during an ongoing pandemic (after WHO declaration of phase 6).

Pandemic vaccines

Pandemic vaccines are vaccines that use pandemic strains or virus strains with antigenicity equivalent to that of the pandemic strains or strains highly predicted to be prevalent and are manufactured by applying the manufacturing method approved for the prototype vaccine in Japan with modifications.

Priming effect

The priming effect refers to an effect that induces immunological memory (primary immunization) to a specific antigen in an individual immunologically naive to the antigen.

Booster effect

The booster effect refers to an effect that increases the antibody titer in a primarily immunized individual in response to one additional (booster) dose which is administered a certain period after the primary series.

Immunogenicity

Immunogenicity is the vaccine’s ability to induce humoral (specific antibody) and/or cellular immunity and/or immunological memory.

Prototype vaccine

Simulated vaccine. The prototype vaccine is a simulated influenza vaccine manufactured and developed using model influenza viruses for production before occurrence of a pandemic on assumption that the production strains would be changed (including changes of subtypes) in response to occurrence of pandemic influenza where necessary.