Report on Deliberation Results

March 4, 2015

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

[Brand name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype) for Intramuscular Injection "KAKETSUKEN"
[Non-proprietary name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype)
[Applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	September 30, 2014

[Results of deliberation]
In the meeting held on February 26, 2015, the Second Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is classified as a biological product, the re-examination period is 10 years, and the drug substance and the drug product are both classified as powerful drugs.

[Conditions for approval]
The applicant is required to prepare and appropriately implement the risk management plan.

Review Report

February 9, 2015
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype) for Intramuscular Injection "KAKETSUKEN"
[Non-proprietary name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype)
[Applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	September 30, 2014
[Dosage form/Strength]	An emulsion for injection presented in 2 separate vials: a vial of the antigen preparation and a vial of the proprietary emulsion adjuvant to be mixed prior to administration. After mixing, each vaccine dose (0.5 mL) contains 3.75 μg of hemagglutinin (HA) of influenza virus (HA content).
[Application classification]	Prescription drug, (1) Drug with a new active ingredient
[Items warranting special mention]	Orphan drug (Notification No. 0613-1 from the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated June 13, 2012)
[Reviewing office]	Office of Vaccines and Blood Products

Review Results

February 9, 2015

[Brand name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype) for Intramuscular Injection "KAKETSUKEN"
[Non-proprietary name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype)
[Applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	September 30, 2014

[Results of review]

Based on the submitted data, the Pharmaceuticals and Medical Devices Agency (PMDA) concluded that the pandemic vaccine manufactured by the same manufacturing process as that of the proposed product is expected to demonstrate efficacy in preventing novel influenza virus. Its safety is considered acceptable in light of the expected benefits.

As a result of its regulatory review, PMDA concluded that the proposed product may be approved as a manufacturing model for a pandemic vaccine for the indication and dosage and administration shown below, with the following conditions.

[Indication]	Prophylaxis of pandemic influenza
[Dosage and administration]	The usual dosage is 2 intramuscular injections of 0.5 mL of the mixture of the antigen preparation and the proprietary emulsion adjuvant at an interval of ≥2 weeks.
[Conditions for approval]	The applicant is required to prepare and appropriately implement the risk management plan.

Review Report (1)

January 15, 2015

I. Product Submitted for Registration

[Brand name]	Emulsion-adjuvanted, Cell-culture Derived Influenza HA Vaccine (Prototype) for Intramuscular Injection "KAKETSUKEN"
[Non-proprietary name]	Emulsion-adjuvanted, Cell-culture Derived Influenza HA Vaccine (Prototype)
[Applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	September 30, 2014
[Dosage form/Strength]	An emulsion for injection presented in 2 separate vials: a vial of the antigen preparation and a vial of the proprietary emulsion adjuvant to be mixed prior to administration. After mixing, each vaccine dose (0.5 mL) contains 3.75 μg of hemagglutinin (HA) of influenza virus (HA content).
[Proposed indication]	Prophylaxis of pandemic influenza
[Proposed dosage and administration]	The usual dosage is 2 intramuscular injections of 0.5 mL of the mixture of the antigen preparation and the proprietary emulsion adjuvant at an interval of ≥2 weeks.

II. Summary of Submitted Data and Outline of Review by the Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries, etc.

Influenza is an acute respiratory disease caused by infection with an influenza virus belonging to the *Orthomyxoviridae* family. The influenza virus is classified as type A, B, or C by serotype. Of these types, the influenza A virus is further classified into subtypes based on differences in the antigenicity of hemagglutinin (HA) and neuraminidase (NA) on the virus surface (i.e., 16 HA subtypes [H1 to H16] and 9 NA subtypes [N1 to N9]). Host animal species for the influenza A virus include humans and various animals, such as birds, pigs, and horses, differing by subtype except in the case of birds, from which all subtypes have been isolated. Currently, influenza A virus subtypes H1N1 and H3N2 have caused recurring human epidemics. Influenza epidemics are reported to recur because antigenicity changes slightly each year within the same subtypes due to antigenic drift and accordingly, human influenza-specific antibodies cannot neutralize the resulting viruses. Antigenic shift may also produce a new influenza A virus subtype having different antigenicity and species specificity. If the new subtype

virus has human infectivity, the immunity already acquired by the current human population can no longer provide protection against infection with the virus or onset of symptoms. In such cases, an increase in human infections with the virus may potentially result in global epidemics (pandemics).

According to the report from the WHO, fatal cases of human infection with the highly-pathogenic avian influenza H5N1 virus were first confirmed in 1997 in Hong Kong, and human infection with the avian influenza virus presents very serious pathologies including systemic viral infections, bleeding tendencies, multiple organ failure, and cytokine storm, with a mortality rate of approximately 60% (402 of 694 infected individuals died as of January 6, 2015, http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/). In Japan, Article 6, paragraph 7, of the Act on Prevention of Infectious Diseases and Medical Care for Patients Suffering Infectious Diseases defines pandemic influenza as a type of influenza "caused by a pathogenic virus that has newly become capable of human to human transmission and is recognized as likely to significantly affect public life and health through rapid and nationwide spread of the infection due to lack of acquired immunity in the general population." In particular, if the highly-pathogenic avian influenza H5N1 virus newly acquires the ability to cause human to human transmission, leading to the emergence of a pandemic influenza (H5N1), its high lethality may result in serious public health issues. Human infections with H7 and H9 subtypes other than the H5 subtypes have also been reported since 1996 (*Lancet*, 1996;348:902-903, *Lancet,* 1999;354:916-917, *Weekly Epidemiological record*, 2013;88:137-144, WHO GAR. Human infection with influenza A (H7N9) virus in China - update, 9 April 2013. http://www.who.int/csr/don/2013_04_09/en/index.html).These subtype strains may also pose a threat of pandemics. Currently, administration of antiviral agents against influenza virus, such as oseltamivir phosphate or zanamivir hydrate, can be considered an option for treatment of pandemic influenza (H5N1). However, there is a report suggesting potential emergence of a resistant virus associated with the use of oseltamivir phosphate (*N Engl J Med*, 2005;353:2667-2672), thus the use of antiviral agents against influenza virus requires consideration for the potential emergence of resistant strains of the virus.

In August 2004, the Pandemic Influenza Panel in the Infectious Disease Working Group of the Health Science Council prepared a "Report on Pandemic Influenza Preparedness" which presented vaccines against pandemic influenza as the major pillar of the measures in preventing pandemic influenza. Furthermore, "Guidance for Developing Prototype Vaccines in Preparation for Influenza Pandemic" (Notification No. 1031-1 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau [PFSB/ELD], Ministry of Health, Labour and Welfare [MHLW] dated October 31, 2011; "Prototype Guideline" hereinafter) was issued for prompt manufacturing and supply of pandemic influenza vaccines for influenza pandemics involving subtypes other than H5. The Prototype Guideline states that prototype vaccine is a vaccine developed and manufactured in usual conditions using an influenza virus that serves as a vaccine manufacturing model in order to examine a pandemic vaccine manufacturing process in advance. Pandemic vaccine is a vaccine manufactured with the same manufacturing process as that for the prototype vaccine, using a vaccine strain of the influenza virus

that has caused pandemic or a strain obtained by attenuating the virus in question as necessary, during a period where pandemic influenza is very likely to occur, or after declaration of a pandemic by the WHO or any other public organization.

In regulatory application for a prototype vaccine, the manufacturing process and quality control systems for the vaccine are approved as simulation models without specifying vaccine strain subtypes. Another application for a pandemic vaccine will be filed as a new product involving each vaccine strain subtype so that the product application will be reviewed and approved promptly.

In Japan, 3 cell culture influenza vaccines (brand names, Cell Culture Influenza vaccine [Prototype] "Baxter," and Cell Culture Influenza vaccine [Prototype] "Takeda" 1 mL and 5 mL) have been approved for the indication of "prophylaxis of pandemic influenza," as of December 2014.

Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype) for Intramuscular Injection "KAKETSUKEN" (hereinafter referred to as the "prototype vaccine") is an influenza vaccine containing influenza virus HA fraction as the active ingredient. This HA fraction is obtained from the influenza virus which is generated by reverse genetics, proliferated by cell culture, inactivated, and then treated with surfactant. The HA fraction is mixed with an emulsion adjuvant (AS03) developed by GSK Biologicals when used. The application for the prototype vaccine has been filed recently based on the results from the phase III clinical trial in Japanese adults and other studies. The product has not been developed in foreign countries as of [redacted] 20[redacted].

An Application for Orphan Drug Designation of the prototype vaccine was filed based on the PFSB/ELD Notification No. 0331007 dated March 31, 2006. The prototype vaccine was designated as an orphan drug indicated for "prophylaxis of novel (pandemic) influenza" (Designation No., [24 yaku] No. 275) on June 13, 2012.

2. Data relating to quality

2.A Summary of the submitted data

The prototype vaccine (the drug product) is a vaccine containing the active ingredient, influenza virus HA fraction, obtained by proliferating seed influenza virus in duck embryonic stem cell-derived cell lines (EB66 cells), inactivating the purified virus particles by treatment with beta-propiolactone (B-PL) and UV-radiation, followed by lysing the virus with surfactant. It employs an oil-in-water emulsion AS03 adjuvant (AS03) consisting of squalene, tocopherol, polysorbate 80, and phosphate-buffered sodium chloride solution for immunostimulation. The quality-related data for the drug substance and drug product using the Indo05/PR8-RG2 strain were previously evaluated in the review of Cell-culture Derived Influenza Emulsion HA Vaccine H5N1 for Intramuscular Injection "KAKETSUKEN," and an outline of the submitted data, including these review results, is presented below.

2.A.(1) Drug substance

2.A.(1).1) Preparation and control of virus bank

The drug product was developed using the following original strains: Indo05/PR8-RG2 strain, a reassortant strain generated from A/Indonesia/05/2005 (H5N1) strain by reverse genetics at the US Centers for Disease Control and Prevention (CDC), NIBRG-14 strain, a reassortant strain generated from A/Vietnam/1194/2004 (H5N1) strain by reverse genetics at the UK National Institute for Biological Standards and Control (NIBSC), and NIBRG-268 strain, a reassortant strain generated from A/Anhui/1/2013 (H7N9) strain by reverse genetics at the UK NIBSC. When the master virus seed (MVS) is generated to produce pandemic vaccine, an original strain designated by the MHLW is to be used based on Guidelines for the Prevention and Control of Pandemic Influenza (http://www.cas.go.jp/jp/seisaku/ful/keikaku/pdf/gl_guideline.pdf).

The original strains obtained was subcultured on EB66 cells for [redacted] passages to prepare the MVS. The MVS was subcultured on EB66 cells for [redacted] passages to prepare the working virus seed (WVS). In the process of preparing the MVS from the original strains specified for manufacturing vaccine, subcultures may be continued for up to [redacted] passages if the infectivity titer obtained does not meet the criteria after the [redacted]th passage or if a sufficient amount of the virus seed cannot be obtained to produce the drug substance. The vaccine is typically manufactured using the WVS, but the MVS may be used in the event of pandemic or other urgent situations where vaccine manufacturing must be initiated. Table 1-1 lists the control tests for MVS and WVS.

Table 1-1. Virus bank control tests

Items	MVS	WVS
HA test	[redacted]	[redacted]
Infectivity titer test ($CCID_{50}$/mL)	[redacted]	[redacted]
Sterility test	[redacted]	[redacted]
Mycoplasma test (culture test)	[redacted]	[redacted]
Exogenous virus test (to detect RSV-A, RSV-B, HPIV-1, HPIV-2, HPIV-3, HMPV, HAdV, and HRV by PCR)	[redacted]	[redacted]
Antigenicity identification	[redacted]	[redacted]
HA gene base sequencing	[redacted]	[redacted]
Attenuated test[a] (test with embryonated eggs and trypsin dependency test using cell culture)	[redacted]	[redacted]

[redacted]

a. Performed if the gene base sequencing result does not meet the criteria

The stability of the virus seed was tested using the Indo05/PR8-RG2 strain. In antigenicity identification performed with virus that had been subcultured more than the number of passages during the manufacturing of the drug substance (excessively-subcultured virus) using Indo05/PR8-RG2 strain, the excessively-subcultured virus had no change in antigenicity and showed no mutation of amino acid sequences in the HA gene base sequencing, confirming that it maintains a base sequence indicating attenuation. The MVS and WVS of the Indo05/PR8-RG2 strain have so far been confirmed to be stable for at least [redacted] years and [redacted] years, respectively, in the infectivity titer tests ($CCID_{50}$ measurement). Schedules have been planned for both virus seeds to confirm their stability every [redacted] years from now on until [redacted] years have passed. If the virus seeds are used after long-term storage, the infectivity titer will be measured to confirm that the criteria are met. No renewal of the MVS is scheduled at present, but WVS

will be renewed from MVS as necessary, and the tests in Table 1-1 will be performed to confirm that the criteria are met.

2.A.(1).2) Preparation and control of the cell bank

The pre-master cell bank (passage level [redacted], manufacturing No. [redacted]) consisting of EB66 cells, a duck embryonic stem cell-derived cell lines established from embryonated eggs of Pekin ducklings GL30 (*Anas platyrhynchos*) created by Vivalis, is subcultured to prepare the master cell bank (MCB) with passage level [redacted] and working cell bank (WCB) with passage level [redacted]. The MCB, WCB, and the cells cultured exceeding the normal manufacturing condition (CAL, passage level [redacted]) have been confirmed to pass the control tests listed in Table 1-2.

Table 1-2. Cell bank control tests

Items			Cell bank to be tested			
			Current MCB	Current WCB	WCB at time of renewal	CAL
Characterization	Morphology		[redacted]	[redacted]	[redacted]	[redacted]
	Telomerase activity		[redacted]	[redacted]	[redacted]	[redacted]
	Isozyme analysis		[redacted]	[redacted]	[redacted]	[redacted]
	Transmission electron microscopy		[redacted]	[redacted]	[redacted]	[redacted]
	DNA finger print analysis		[redacted]	[redacted]	[redacted]	[redacted]
	Karyotype analysis		[redacted]	[redacted]	[redacted]	[redacted]
	Tumorigenicity		[redacted]	[redacted]	[redacted]	[redacted]
	Carcinogenicity		[redacted]	[redacted]	[redacted]	[redacted]
Purity tests	Sterility		[redacted]	[redacted]	[redacted]	[redacted]
	Tubercle bacillus test		[redacted]	[redacted]	[redacted]	[redacted]
	Mycoplasma test		[redacted]	[redacted]	[redacted]	[redacted]
	Chlamydia test		[redacted]	[redacted]	[redacted]	[redacted]
	In vitro virus test [a]		[redacted]	[redacted]	[redacted]	[redacted]
	In vivo virus test [b]		[redacted]	[redacted]	[redacted]	[redacted]
	Exogenous virus test by PCR	Duck-derived virus,[c] chicken anemia virus, chicken leukemia virus, reticuloendotheliosis virus	[redacted]	[redacted]	[redacted]	[redacted]
		Murine minute virus	[redacted]	[redacted]	[redacted]	[redacted]
		Vesivirus and murine leukemia virus	[redacted]	[redacted]	[redacted]	[redacted]
	Reverse transcriptase test		[redacted]	[redacted]	[redacted]	[redacted]
	Transmission electron microscopy		[redacted]	[redacted]	[redacted]	[redacted]
	Infective retrovirus test		[redacted]	[redacted]	[redacted]	[redacted]
	Murine antibody production test [d]		[redacted]	[redacted]	[redacted]	[redacted]
	Bovine-derived virus test		[redacted]	[redacted]	[redacted]	[redacted]
	Porcine-derived virus test		[redacted]	[redacted]	[redacted]	[redacted]
	Murine xenotropic retrovirus test		[redacted]	[redacted]	[redacted]	[redacted]
	Retrovirus induction test		[redacted]	[redacted]	[redacted]	[redacted]
	Latent DNA virus test [e]		[redacted]	[redacted]	[redacted]	[redacted]
	Murine-derived DNA test		[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

a. Confirmation of cell degeneration, hemoabsorption, and hemagglutination using MRC-5, Vero, and DEF cells
b. Animal inoculation tests (suckling mice, mature mice, guinea pigs, and chicks) and embryonated egg inoculation test
c. 11 virus species
d. Serum analysis was performed for 16 murine-derived viruses by inoculating mice with cell lysate.
e. Electron microscopy and PCR detection of 12 viruses after stimulating cells with chemical substances
f. Co-culture tests with EB66 cells were also performed.
g. No chick test was performed.

Appropriate storage conditions have been specified for the MCB and WCB, and both have been confirmed to exhibit no change in cell viability for [redacted] years and [redacted] years, respectively, after the start of

storage. The MCB should be assessed for cell viability when thawed for preparation of a new WCB, and the WCB should be assessed as well at thawing for vaccine manufacturing or once every [redacted] years. Although the MCB is not scheduled to be renewed at this time, the WCB will be renewed from the MCB when the WCB stock has dropped to a certain level. The tests in Table 1-2 will be performed to confirm that the criteria are met.

2.A.(1).3) Manufacturing process

Table 1-3 shows the manufacturing process for the drug substance.

Table 1-3. Summary of manufacturing process of drug substance

Manufacturing process/critical steps		Intermediates/ critical intermediates	In-process control test
Seed culture	Seed culture 1: [redacted] mL ([redacted]°C, for [redacted] days)		
	Seed culture 2: [redacted] mL ([redacted]°C, for [redacted] days)		
	Seed culture 3: [redacted] L ([redacted]°C, for [redacted] days)		
	Pre-preculture: [redacted] L ([redacted]°C, for [redacted] days) → [redacted] L, [redacted]°C, for [redacted] days		
	Preculture: [redacted] L ([redacted]°C, for [redacted] days) → Adding medium, [redacted] L ([redacted]°C, for [redacted] days)		
Main culture	Stir culture: [redacted] L ([redacted]°C, for [redacted] days)	Main culture	Cultured cell test
↓			
Virus culture	WVS [a] inoculation (m.o.i = 10[redacted] to 10[redacted] [b])		
	Culture ([redacted] ± [redacted]°C [b], for [redacted] to [redacted] days [b])	Virus culture medium	Sterility test, mycoplasma test
Purification	Cell separation (centrifugation Q/Σ = [redacted])		
	Clarification and filtration [redacted] μm)	MF harvest	
	Buffer exchange (ultrafiltration; fraction molecular weight, [redacted])	UF1 harvest	
	Sucrose density gradient centrifugation ([redacted] - [redacted] × g)	SDG pool	Protein content test
↓			
Inactivation 1	B-PL treatment ([redacted]%, [redacted] ± [redacted]°C, for [redacted] - [redacted] hours)	B-PL treatment solution	
Inactivation 2	UV radiation ([redacted] nm, [redacted] J/m^2)	UV inactivation solution	
	Buffer exrchange (ultrafiltration; fraction molecular weight, [redacted])	UF2 harvest	Protein content test
↓			
Surfactant treatment	Triton X-100 treatment ([redacted] w/v%, [redacted] ± [redacted]°C, for [redacted] - [redacted] hours)	Triton X-100 treatment solution	
	Ultracentrifugation ([redacted] to [redacted] × g, for [redacted] hours)	Ultracentrifugation supernatant	
Chromatography 1	[redacted]	[redacted]	
Chromatography 2	[redacted]	[redacted]	
	Buffer exchange (ultrafiltration; fraction molecular weight, [redacted])	UF3 harvest	
Drug substance preparation	Diluted (final HA concentration, [redacted] μg/mL)		
	Supplemented with polysorbate 80 (final concentration, [redacted] μg/mL), d-α-tocopherol succinate (final concentration, [redacted] μg/mL), Triton X-100 (final concentration, [redacted] μg/mL), Filter sterilization ([redacted] μm)	Drug substance	Filter integrity test

a. MVS may be used in some cases.
b. Values specific to virus strain are used.

Process validation has been performed for the drug substance manufacturing process to demonstrate that each step is appropriately controlled.

2.A.(1).4) Safety evaluation for adventitious agents

No biological material other than the host EB66 cells is used in the drug substance manufacturing process.

Table 1-4 gives the results of evaluation of the viral clearance ability in the inactivation process.

Table 1-4. Results of viral clearance test in the virus inactivation process* (LRV)

Virus	Porcine parvovirus	Hepatitis A virus	Pseudorabies virus	Murine leukemia virus
B-PL treatment ([redacted]%, [redacted]°C, for [redacted] hours)	4.72, ≥5.76	≥3.70, ≥3.53	≥5.23, ≥4.97	4.69, 4.60
UV radiation ([redacted] J/m^2)	6.22, 6.09	≥4.36, ≥4.01	5.19, 4.01	2.36, 2.45
Triton X-100 treatment ([redacted]%, [redacted]°C, for [redacted] hours)	N.A.	N.A.	≥4.45, ≥4.45	4.98, 4.40

*Clearance test for each process was held twice and the results of the 2 tests are shown for each process.

2.A.(1).5) History of development of the manufacturing process

Table 1-5 lists major changes made during the development of the manufacturing process of the drug substance using the Indo05/PR8-RG2. As a result of process analysis and quality assessment of the drug substance before and after the changes, the drug substance was confirmed to be comparable before and after the changes from Process A to Process B, as well as those from Process B to Process C. None of these changes were considered to affect the quality of the drug substance.

Table 1-5. Changes in drug substance manufacturing process

	Process A	Process B	Process C
Purpose	[redacted]	[redacted]	Formulation to be marketed
Cell bank used in seed culture	MCB	WCB	
Cell culture scale	[redacted] L	[redacted] L	[redacted] L
Virus seed used in virus culture	MVS	WVS	
Virus culture scale	[redacted] L	[redacted] L	[redacted] L
Virus culture duration	[redacted] days	[redacted] days	
Virus inactivation process	Clarification and filtration → Inactivation 1 → Buffer exchange → Inactivation 2 → Sucrose density gradient centrifugation	Clarification and filtration → Buffer exchange → Sucrose density gradient centrifugation → Inactivation 1 → Inactivation 2	
UV radiation intensity (Inactivation 2 step)	[redacted] J/m^2	[redacted] J/m^2	

2.A.(1).6) Characterization

(a) Structure and characteristics

The major structural proteins of influenza virus include hemagglutinin (HA), neuraminidase (NA), nuclear protein (NP), and matrix protein (M1). The drug substances manufactured using the Indo05/PR8-RG2 strain, NIBRG-14 strain, and NIBRG-268 strain were subjected to SDS-PAGE analysis, single radial immunodiffusion analysis, hemagglutination, and immunogenicity evaluation. The SDS-PAGE analysis under reducing conditions detected bands at approximately [redacted] kDa and approximately [redacted] kDa corresponding to the molecular weights of HA1 and HA2, respectively, in Indo05/PR8-RG2 strain and NIBRG-14 strain, and bands at approximately [redacted] kDa and approximately [redacted] kDa corresponding to the molecular weights of HA1 and HA2, respectively, in the NIBRG-268 strain. Under non-reducing conditions, a band at approximately [redacted] kDa corresponding to the molecular weight

of HA monomer was detected in the Indo05/PR8-RG2 strain and NIBRG-14 strain, and a band at approximately [redacted] kDa in NIBRG-268 strain. Bands at approximately [redacted] kDa and ≥[redacted] kDa, which are considered to correspond to the molecular weights of polymers, were found in all strains. Furthermore, bands at approximately [redacted] kDa and approximately [redacted] kDa corresponding to the molecular weight of NP were detected under reducing and non-reducing conditions. Although the HA bands were observed at different molecular weight bands by strain, each band was identified to correspond to HA or NP via Western blot analysis using a specific antibody or antiserum for each protein. Western blot analysis detected no band for M1, which has been confirmed to be substantially eliminated in the manufacturing process of the drug substance. The single radial immunodiffusion analysis identified distinctive precipitation rings of antigen-antibody complex in the drug substances from Indo05/PR8-RG2 strain, NIBRG-14 strain, and NIBRG-268 strain. The sizes of the rings were linearly dependent on protein concentration.

(b) Impurities

Impurities were studied for the manufacturing process of the drug substance using the Indo05/PR8-RG2 Strain. [redacted] It was also demonstrated that B-PL content is reduced to <[redacted] ppm (limit of detection) and sucrose content to <[redacted] μg/mL (limit of detection), verifying that process-derived impurities are eliminated to sufficiently low concentrations.

For influenza virus strains cultured on chicken eggs as the original strain, the culture will contain chicken egg-derived proteins, but the proteins will be ≥[redacted] × 10[redacted]-fold diluted to sufficiently low concentrations until the manufacturing of the drug substance ([redacted] to [redacted] ng/mL in the worst case wherein all proteins in the original strain are from chicken eggs).

2.A.(1).7) Control of drug substance

Viral inactivation, sterility, host cell-derived DNA content, titer, pH, endotoxin, fraction, protein content, and host cell-derived protein content have been defined as specifications for the drug substance. The titer test must be performed by single radial immunodiffusion (SRD test). However, if a reference antigen or reference anti-influenza HA serum for SRD test is unavailable, a HA content test by SDS-PAGE densitometry should be performed in place of SRD test.

2.A.(1).8) Reference standard or reference material

The reference standard used in the specification test for the drug substance is the reference antigen used in the titer test (SRD test) obtained from the Essential Regulatory Laboratories (ERL), designated by the WHO, and stored under conditions specified by the ERL.

2.A.(1).9) Stability

Table 1-6 lists stability tests for the drug substances using the Indo05/PR8-RG2 strain and NIBRG-14 strain.

Table 1-6. Drug substance stability study

Study	Strain	Number of batches	Storage condition	Storage container	Study period
Long-term	Indo05/PR8-RG2 strain	3	[redacted] ± [redacted]°C, protected from light	Polyethylene single use bags	[redacted] months [a]
	NIBRG-14 strain	3			[redacted] months [a]
Accelerated	Indo05/PR8-RG2 strain	3	[redacted] ± [redacted]°C, protected from light, [redacted] ± [redacted]%RH		[redacted] months
	NIBRG-14 strain	3			[redacted] months
Stress testing (temperature)	Indo05/PR8-RG2 strain	1	[redacted] ± [redacted]°C, protected from light		[redacted] days
Stress testing (shaking)	Indo05/PR8-RG2 strain	1	[redacted] ± [redacted]°C, protected from light, shaken ([redacted] rpm)		[redacted] hours

a. The study is ongoing (will be continued for [redacted] months).

The data from the long-term stability study of [redacted] months for Indo05/PR8-RG2 strain and the data of [redacted] months for NIBRG-14 strain (of [redacted] months for some study items) have been submitted and indicated no definite storage-related change in any study item. [redacted]

The stress study (temperature) found a decrease in titer over time, changes in the band pattern by SDS-PAGE and HA-Western Blotting, and changes in gel filtration HPLC peak ratios. In the stress study (shaking), the titer was stable while shaken for up to [redacted] hours under the actual storage condition.

Accordingly, the shelf life of the drug substance was determined to be [redacted] months after manufacturing when stored in the single-use polyethylene bag at [redacted]°C to [redacted]°C protected from light.

2.A.(2) Drug product

The drug product is an injection consisting of the antigen-containing preparation (antigen preparation) and the proprietary emulsion adjuvant containing adjuvant AS03, which are presented in 2 separate vials, and they are mixed in the same volume before use. A vial of the antigen preparation and a vial of the proprietary emulsion adjuvant are supplied for 10 doses (0.25 mL/dose). The entire contents of the proprietary emulsion adjuvant vial is added to the antigen preparation vial for mixing, resulting in 10 doses freshly prepared for vaccination. The secondary package is a carton box.

2.A.(2).1) Antigen preparation (filled)

The antigen preparation contains 3.75 μg of influenza virus HA fraction (HA content) per 0.25-mL dose as the active ingredient (10 doses per multi-dose glass vial). The antigen preparation contains sodium

chloride, disodium hydrogen phosphate hydrate, potassium dihydrogen phosphate, tocopherol succinate, polyoxyethylene octylphenyl ether (Triton X-100), polysorbate 80, and thimerosal, as excipients.

(a) Manufacturing process

The manufacturing process of the antigen preparation consists of bulk preparation process and filling process. Concentrated phosphate-buffered sodium chloride solution, polysorbate 80, tocopherol succinate, Triton X-100, and thimerosal are added to water for injection. The drug substance is added to this solution to obtain an HA concentration of [redacted] μg/mL, and then the solution is sterile-filtered to prepare the final bulk. The final bulk is filled in glass vials, each sealed with a rubber stopper and a cap, and the dispensed products are prepared. The bulk preparation process and filling process have been defined as critical steps, and filter integrity test has been defined as an in-process control for the bulk preparation process and sealing performance test as an in-process control for the filling process.

Process validation has been performed for the manufacturing process to confirm that each step is appropriately controlled.

(b) History of development of the manufacturing process

[redacted]

The quality assessment results of the antigen preparation confirmed that the antigen preparation was comparable before and after the manufacturing changes and that the changes did not appear to affect the quality of the drug substance.

(c) Control of the antigen preparation

Description, sterility, abnormal toxicity, titer, protein content, pH, insoluble microparticle, thimerosal content, endotoxin, foreign insoluble matter, and extractable volume have been defined as specifications for the antigen preparation. Although it is specified that SRD test is performed for the titer, if reference antigen or reference anti-influenza HA serum is unavailable for the SRD test, the HA content is to be determined by SDS-PAGE/densitometry in place of the SRD test.

(d) Reference standard and reference material

The specification tests for the antigen preparation uses the same reference standard as that used in the specification test for the drug substance.

(e) Stability

Table 1-7 lists stability tests for the antigen preparation.

Table 1-7. Antigen preparation stability study

Study	Drug substance manufacturing process	Strain	Number of batches	Storage condition	Container	Study period
Long-term	[redacted]	Indo05/PR8-RG2 strain	3[a]	5 ± 3°C, protected from light, upright or inverted	Glass vials (5 mL)	[redacted] months [b]
		NIBRG-14 strain	3	5 ± 3°C, protected from light, upright		[redacted] months
Accelerated		Indo05/PR8-RG2 strain	3[a]	[redacted] ± [redacted]°C, protected from light, [redacted] ± [redacted]% RH, upright or inverted		[redacted] months
		NIBRG-14 strain	3	[redacted] ± [redacted]°C, protected from light, [redacted] ± [redacted]% RH, upright		[redacted] months
Stress testing (temperature)		Indo05/PR8-RG2 strain	1	[redacted] ± [redacted]°C, protected from light		[redacted] days
Stress testing (light exposure)		Indo05/PR8-RG2 strain	1	[redacted] ± [redacted]°C, total illuminance ≥[redacted] lux·h, total near UV radiation energy [redacted] W·h/m^2		

a. One batch is tested under the inverted condition, b. The study is ongoing (will be continued for [redacted] months).

The data from the long-term stability study of [redacted] months for Indo05/PR8-RG2 strain and the data of [redacted] months for NIBRG-14 strain (of [redacted] months for some study items) have been submitted, showing no definite change over time in the study items performed under upright condition. The accelerated study under upright condition found time-course changes in mean particle size, polysorbate 80 content, tocopherol succinate content, insoluble microparticle, and thimerosal content and titer for the Indo05/PR8-RG2 strain, and time-course changes in titer, insoluble microparticle, polysorbate 80 content and tocopherol succinate content for the NIBRG-14 strain. The stress study (temperature) found decreasing titer and increasing trend in insoluble microparticle. The stress study (light) found that the antigen preparation is photolabile. [redacted]

Accordingly, the shelf life of the antigen preparation has been determined to be [redacted] months after manufacturing when filled in glass vials, stored at 2 to 8°C, protected from light.

2.A.(2).2) Proprietary emulsion adjuvant (filled)

The proprietary emulsion adjuvant is an oil-in-water emulsion adjuvant AS03 containing 10.69 mg squalene, 11.86 mg tocopherol, and 4.86 mg polysorbate 80 per 0.25-mL dose (10 doses per multi-dose glass vial). The proprietary emulsion adjuvant contains sodium chloride and potassium chloride as isotonic agents and disodium hydrogen phosphate anhydrous and potassium dihydrogen phosphate as buffering agents.

(a) Manufacturing process

An aqueous phase consisting of buffer containing polysorbate 80 and an oil phase formed by mixing squalene and tocopherol at a volume ratio of [redacted]:[redacted] are mixed to emulsify at a volume ratio [redacted]:[redacted]. The resulting emulsion is sterile-filtered to obtain emulsion bulk. The emulsion bulk is pooled and sterile-

filtered; the resulting final bulk is filled in 2 ml glass vials, each sealed with a rubber stopper and a cap. Description, pH, tocopherol identification, squalene identification, polysorbate 80 identification, endotoxin, sterility, particle size, tocopherol content, squalene content, and polysorbate 80 content have been defined as in-process controls for the emulsion bulk. In addition, the filter integrity test is defined as an in-process control for final bulk preparation process with sterile filtration and sealing performance test as an in-process control for the filling process.

(b) History of the development of manufacturing process

The manufacturing process and formula of the proprietary emulsion adjuvant used in the clinical trials are the same as those of the proprietary emulsion adjuvant used for the quality assessment and the proprietary emulsion adjuvant to be marketed. However, the manufacturing site has changed. Although the emulsion adjuvant was filled in prefilled syringes with needles in the clinical trials, the emulsion adjuvant to be marketed is filled in glass vials. The 2 batches of the emulsion adjuvant used in the clinical trials and the 3 batches of the emulsion adjuvant used for the quality assessment were subjected to batch analysis to confirm the absence of any difference in quality between the emulsion adjuvant used in the clinical trials and that used for the quality assessment.

(c) Control of the proprietary emulsion adjuvant

Description, pH, tocopherol identification, squalene identification, polysorbate 80 identification, endotoxin, extractive volume, sterility, particle size, tocopherol content, squalene content, and polysorbate 80 content have been defined as specifications for the proprietary emulsion adjuvant.

(d) Impurities

A potential impurity in the proprietary emulsion adjuvant is tocopheryl quinone, an oxidation product of tocopherol. Quality tests and stability studies (months) has confirmed that concentrations of tocopheryl quinone in the proprietary emulsion adjuvant are <%.

(e) Stability

Table 1-8 lists stability studies for the proprietary emulsion adjuvant.

Table 1-8. Stability studies for the proprietary emulsion adjuvant

Study	Number of batches	Nitrogen purge when filling	Storage container and stopper	Storage condition	Study period
Long-term	3	No	Glass vial[a] (3 mL), rubber stopper[a]	± °C, dark, inverted	months
	3	Yes/No	Glass vial[a] (3 mL), rubber stopper[a]	± °C, dark, inverted	months[c]
	3	Yes	Glass vial[b] (3 mL), rubber stopper[a]	± °C, dark, inverted	months[c]
Accelerated	3	No	Glass vial[a] (3 mL), rubber stopper[a]	°C, dark, inverted	days
	3	Yes	Glass vial[b] (3 mL), rubber stopper[a]	°C, dark, inverted	days

a. With silicone treatment, b. Without silicone treatment, c. The study is ongoing (will continue up to months).

The long-term stability and accelerated studies resulted in no changes in any of the items studied over time.

The proprietary emulsion adjuvant to be marketed is filled in glass vials (2 mL) without silicone treatment under nitrogen using rubber stoppers without silicone treatment. Stability data up to [redacted] months were obtained from the stability study using batches of the emulsion adjuvant filled in glass vials with silicone treatment (3 mL) in the absence of nitrogen, which is different from the emulsion adjuvant to be marketed. Study data up to [redacted] months using batches of the emulsion adjuvant filled in glass vials with silicone treatment (3 mL) confirm that the presence or absence of nitrogen purge does not affect the stability of the proprietary emulsion adjuvant. Study data using the batches of the emulsion adjuvant filled in glass vials without silicone treatment (3 mL) in the presence of nitrogen has also confirmed that the presence or absence of silicone treatment of glass vials does not affect the stability of the emulsion adjuvant.

The applicant considers that extrapolation of the data from the stability studies using the glass vials (3 mL) is possible for the glass vials (2 mL) of the proprietary emulsion adjuvant to be marketed, based on the relationship between the volume of the vial and that of the upper space. The applicant further explains that rubber stoppers are treated with silicone solely to allow the smooth movement of the stoppers at the capping step. Since the silicon treatment of glass vials does not affect the stability of the proprietary emulsion adjuvant, the use of glass vials with rubber stoppers without silicone treatment for the emulsion adjuvant to be marketed appears to pose no risk to the quality or stability of the emulsion adjuvant.

Accordingly, a shelf life of [redacted] months has been proposed for the proprietary emulsion adjuvant when stored at 2°C to 8°C.

2.A.(2).3) The drug product

For the drug product obtained by mixing the antigen preparation and the proprietary emulsion adjuvant in the same volume, the description, titer, mean particle size, and polydispersity index were assessed immediately after mixing and after storage at room temperature ([redacted]°C to [redacted]°C) under room lighting ([redacted] lux) for [redacted] hours. No changes were observed over time. The titer of the drug product was considered to be stable for at least 24 hours after mixing.

2.B Outline of the review by PMDA

2.B.(1) Pyrogen test

Since pyrogen derived from non-endotoxic virus components has been reported to be present in the whole-virion influenza vaccine (*J Immunol,* 1966;96: 596-605, *Jpn J Med Sci Biol*, 1975;28: 37-52), PMDA requested the applicant to explain whether a pyrogen test should be included in the specifications for the drug substance in order to detect pyrogenic activity derived from any possible residual whole virus.

The applicant responded as follows:
Since the drug product is an influenza virus HA vaccine manufactured by inactivation of the whole virus followed by lysis with surfactants, there appears to be no reason for concerns about fever due to any of the virus components of the drug product. Furthermore, to confirm that pyrogen was controlled throughout the drug substance manufacturing process, 6 batches of the drug substance used in the clinical studies and stability studies were subjected to the Pyrogen Test of the General Tests specified in the Minimum Requirements for Biological Products. The test results confirmed that all the 6 batches met the requirements. The 3 batches used in the stability studies were also confirmed to pass the pyrogen test for up to [redacted] months (the test will be continued for [redacted] months).

On the basis of the above, the proposed drug substance manufacturing process appears to be appropriately controlled and capable of consistent manufacturing, and thus the applicant considers that there is no need to include pyrogen test in the specifications for the drug product.

PMDA concluded that there is no need to include pyrogen test in the specification for the drug substance to determine pyrogenicity of the drug substance based on the pyrogen test results presented and since the manufacturing process is appropriately controlled. PMDA accepted the applicant's response.

2.B.(2) Stability of the antigen preparation

[redacted] Accordingly, it is possible to consider that the antigen preparation has been confirmed to remain stable for at least [redacted] months. [redacted]

PMDA accepted the above explanation.

2.B.(3) Shelf life of the proprietary emulsion adjuvant

The proprietary emulsion adjuvant to be marketed uses vials with rubber stoppers not subjected to silicone treatment. The applicant, on the other hand, has proposed the shelf life based on results of a long-term stability study of the proprietary emulsion adjuvant using vials with rubber stoppers subjected

to silicone treatment. PMDA requested the applicant to explain the appropriateness of the proposed specification.

The applicant responded as follows:
The silicone treatment for rubber stoppers of vials used for the proprietary emulsion adjuvant refers to surface treatment of rubber stoppers with silicone. The surface treatment is applied to rubber stoppers by the manufacturer of the rubber stoppers or at the manufacturing site of the emulsion adjuvant. The applicant had claimed that rubber stoppers of vials for the emulsion adjuvant to be marketed did not undergo the silicone treatment because the manufacturing site did not cover silicone treatment. However, after PMDA pointed out this problem, it came to light that silicone was used as a mold release agent by the manufacturer of the rubber stoppers. The amount of silicone adhering to the rubber stoppers for the emulsion adjuvant and the amount of silicone used in the stability studies were determined, and silicone was detected on both rubber stoppers. The result showed comparable amounts per surface area of the rubber stoppers. On the basis of these findings, there appears to be no qualitative difference between the rubber stoppers used for the emulsion adjuvant of the stability tests and those for the emulsion adjuvant to be marketed. The applicant considers that the stability of the emulsion adjuvant to be marketed can be explained based on the results from the long-term stability studies obtained beforehand. A long-term stability study of the emulsion adjuvant to be marketed has already begun and will be continued for [redacted] months. So far, [redacted]-month data from this study has identified no tendencies different from results obtained for rubber stoppers subjected to silicone treatment.

PMDA accepted the above response from the applicant and concluded that the proposed shelf life of [redacted] months for the proprietary emulsion adjuvant when stored at 2°C to 8°C is acceptable.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

For the primary pharmacodynamic study, immunogenicity and prophylactic efficacy were tested for a vaccine manufactured using the Indo05/PR8-RG2 strain (Indonesia [H5N1] vaccine) generated from the A/Indonesia/05/2005 (H5N1) strain (Indonesia [H5N1] strain) and a vaccine manufactured using the NIBRG-268 strain (Anhui [H7N9] vaccine) generated from the A/Anhui/1/2013 (H7N9) strain (Anhui [H7N9] strain). Equine erythrocytes were used to measure serum HI antigen titers used as immunogenicity index. The effects of the Indonesia (H5N1) vaccine and adjuvant AS03 on the cardiovascular and respiratory systems were studied as safety pharmacology studies.

The Indonesia (H5N1) vaccine used in clinical studies of the prototype vaccine is the one evaluated as the review of the Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (H5N1) for Intramuscular Injection 'KAKETSUKEN' which included the data from "Mouse immunogenicity study," "Study on prophylactic efficacy of vaccine in naïve ferrets," and "Study on cross-reactive antibody response to H5N1 strains in mouse and ferret serum" as well as "dog telemetry study of Indonesia

(H5N1) vaccine and AS03" and "mechanism of action of AS03" using AS03. The following summary includes these studies as well.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) Studies using Indonesia (H5N1) vaccine

(a) Study on immunogenicity of vaccine in mice (4.2.1.1-1, Study 1[redacted]4, 4.2.1.1-2, Study [redacted]60)

The Indonesia (H5N1) vaccine at 4 different dose levels (1.5, 0.38, 0.09, or 0.02 μg HA), the antigen preparation (from the Indonesia [H5N1] strain) at 4 different dose levels (6.0, 1.5, 0.38, or 0.09 μg HA), a chicken-egg-derived, aluminum-adjuvanted vaccine approved in Japan ("approved vaccine") at 4 different dose levels (1.5, 0.38, 0.09, or 0.02 μg HA) or three types of 50 μL of phosphate buffered saline (PBS; aluminum-adjuvanted, AS03-adjuvanted, or non-adjuvanted) was administered by intramuscular injection to mice (n = 10 females/group) twice, at a 3-week interval. A total of 150 mice divided into 15 groups were used. Serum HI antibody titer and neutralizing antibody titer were measured 20 days after the first injection (the day before the second injection) and 14 days after the second injection.

The Indonesia (H5N1) vaccine groups and approved vaccine groups both exhibited dose-dependent increases in HI antibody titer and neutralizing antibody titer at 14 days after the second injection. The geometric means of the HI and neutralizing antibody titers were comparable between the 0.38 μg HA Indonesia (H5N1) vaccine group and the 1.5 μg HA approved vaccine group. In the antigen preparation groups and antigen-free PBS groups, no increase was found in either the HI antibody titer or neutralizing antibody titer.

These results demonstrated that double vaccination with Indonesia (H5N1) vaccine at 0.38 μg HA can induce HI antibodies and neutralizing antibodies to an extent comparable to that after double vaccination with the approved vaccine at 1.5 μg HA.

(b) Study on prophylactic efficacy of vaccine in naïve ferrets (4.2.1.1-3, Study [redacted])

The Indonesia (H5N1) vaccine or GSK Biologicals (GSK) manufactured chicken–egg-derived HA vaccine (H5N1 strain) (GSK vaccine) at 2 different dose levels (3.8 μg or 1.9 μg HA, containing 0.25 mL or 0.125 mL of AS03, respectively), and the antigen preparation derived from the Indonesia (H5N1) strain (15.0 μg HA), or 0.5 mL normal saline was administered by intramuscular injection to ferrets (n = 6 females/group) twice, at a 3-week interval. A total of 36 animals divided into 6 groups were used. Serum HI and neutralization antibody titers were measured at baseline, at 21 days after the first injection, and at 21 and 27 days after the second injection. At 28 days after the second injection, a lethal dose of wild-type A/Indonesia/05/2005 (H5N1) strain (Indonesia [H5N1] strain) was administered intratracheally. Changes in body weight and survival outcomes were observed for 5 days after the virus challenge, and the lung viral titer was measured at 5 days after the virus challenge or at death.

Data obtained at 21 days after the first injection and at 21 and 27 days after the second injection showed similarly increases in the HI antibody titer and neutralizing antibody titer at all dose levels in the Indonesia (H5N1) vaccine groups and GSK vaccine groups. In the antigen preparation group, no increase was found in either HI antibody or neutralizing antibody titers. The survival rate was 100% (6 of 6 animals) in the 3.8 and 1.9 μg HA Indonesia (H5N1) vaccine groups and 3.8 μg HA GSK vaccine group and 80% (4 of 5 animals) in the 1.9 μg HA GSK vaccine group. Mean rate of body weight loss (mean rate of body weight loss from the day before the virus challenge to 5 days after the virus challenge or to the day of death) ranged from 3.0 to 6.4% in each group. Survival rates were 50% (3 of 6 animals) in the antigen preparation group and 0% in the normal saline group (0 of 6 animals), with mean body weight losses of 11.1% and 14.6%, respectively. Lung viral titers at 5 days after the challenge or at death were below the lower limit of quantitation in both the Indonesia (H5N1) vaccine groups and GSK vaccine groups. Higher viral titers were found in the antigen preparation group and the normal saline group.

These results demonstrated that double vaccination with the Indonesia (H5N1) vaccine protects ferrets against lethal infection with the wild-type strain of the Indonesia (H5N1) strain.

(c) Study on cross-reactive antibody response to H5N1 strains in mouse and ferret serum (4.2.1.1-4, Study [redacted]89, 4.2.1.1-5, Study [redacted]L)

In Study [redacted]89, HI antibody titers against H5N1 strains differing from the vaccine strain were measured in murine serum. These strains were the Vietnam (H5N1) strain, A/Anhui/1/2005 (H5N1) strain, and A/bar-headed goose/Qinghai/1A/2005 (H5N1) strain. The tested murine serum was obtained by administering Indonesia (H5N1) strain vaccine twice in the "(a) Mouse immunogenicity study." The results showed cross-reactivity against all the 3 virus strains (H5N1) differing from the vaccine strain, although the HI antibody titers were lower than that against the vaccine strain (Indonesia [H5N1]).

In Study [redacted]L, HI antibody titers and neutralizing antibody titers against H5N1 strains differing from the vaccine strain were measured in the ferret serum. These strains were the Vietnam (H5N1) strain and A/turkey/Turkey/1/2005 (H5N1) strain. The ferret serum tested was obtained by administering Indonesia (H5N1) strain vaccine twice in the "(b) Study on prophylactic efficacy of vaccine in naïve ferrets." The results showed cross-reactivity against both strains.

These results demonstrated that the Indonesia (H5N1) vaccine was also considered to have cross-reactivity against H5N1 strain subtypes differing from the vaccine strain.

(d) Study on cross-protective efficacy of vaccine in naïve ferrets (4.2.1.1-6, 4.2.1.1-7, Study [redacted]40)

Indonesia (H5N1) vaccine or 0.5 mL normal saline was administered by intramuscular injection to ferrets (n = 6 females/group) twice, at a 3-week interval to a total of 2 groups (12 animals). HI antibody titer and neutralizing antibody titer against the vaccine strain and Vietnam (H5N1) strain, an H5N1 strain

differing from the vaccine strain, were measured in ferret serum before the administration, at 21 days after the first injection, and at 21 and 27 days after the second injection. At 28 days after the second injection, wild-type Vietnam (H5N1) strain was administered intratracheally. Changes in body weight and survival outcomes were observed for 5 days after the virus challenge, and the lung viral titer was measured at 5 days after the virus challenge.

Data obtained at 21 and 27 days after the second administration showed that the HI and neutralizing antibody titers against the vaccine strain and Vietnam (H5N1) strain have increased in the vaccine group. The survival rate at 5 days after the virus challenge was 100% (6 of 6 animals) in both the vaccine and normal saline groups, and the mean rate of body weight loss 5 days after the virus challenge was 7% and 13%, respectively. The lung viral titer at 5 days after the virus challenge was below the lower limit of quantification in the vaccine group, except for 1 animal. The normal saline group had higher viral titers.

These results demonstrated that double vaccination with Indonesia (H5N1) vaccine protects ferrets against infection with an H5N1 strain subtype differing from the vaccine strain.

3.(i).A.(1).2) Study using Anhui (H7N9) vaccine

(a) Study on prophylactic efficacy of vaccine in mice (4.2.1.1-8, Study K[redacted]1)

Anhui (H7N9) vaccine at 2 different dose levels (0.38 or 0.038 μg HA), or 50 μL of AS03-adjuvanted PBS was administered by intramuscular injection to mice (n = 10 or 11 females/group) twice, at a 3-week interval. Each treatment group consisted of two sub-groups: one for antibody titer measurement and one for virus challenge and clinical observation (total of 6 groups, 62 animals). The serum HI antibody titer and neutralizing antibody titer were measured at 13 days after the second injection. At 14 days after the second injection, an estimated lethal dose of wild-type Anhui (H7N9) strain was administered intranasally, and changes in body weight and survival outcomes were observed for 14 days after the virus challenge.

At 13 days after the second injection, the HI antibody titer and neutralizing antibody titer were found to increase in the Anhui (H7N9) vaccine groups in a dose-dependent manner. Survival rates were 100% (11 of 11 animals) in the 0.38 and 0.038 μg HA Anhui (H7N9) groups. Mean rates of maximum body weight loss (mean rate of body weight loss from the timepoint immediately after the virus challenge to that of lowest body weight) were 2.9% and 17.2%, respectively. For the AS03-adjuvanted PBS group, the survival rate was 20% (2 of 10 animals), while the mean rate of maximum body weight loss was 28.2%.

These results demonstrated that vaccination with the Anhui (H7N9) vaccine protects mice against lethal infection with the same Anhui (H7N9) strain as the vaccine strain.

3.(i).A.(1).3) Mechanism of action of AS03 (4.2.1.1-9, 4.2.2.7-1)

This study investigated the mechanism whereby AS03 combined with HA antigen induces humoral and cellular immune response. Immunogenicity was enhanced only when AS03 and HA antigen of H3N2 or H5N1 subtype were administered by intramuscular injection to mice at the same site. Immunogenicity was also enhanced when the antigen was administered 1 hour after AS03 was administered. Immunopotentiation by AS03 administration (NF-κB induction) was localized to the injection site and its draining lymph nodes. The administration of AS03 resulted in increases in cytokine and chemokine production at the injection site; recruitment of antigen-presenting monocytes and dendritic cells, and expression of costimulatory molecules CD80, CD86, and CD40, which enhance proliferation and differentiation of T cells and B cells on antigen-presenting cells in each combination.

The biodistribution of AS03 was studied by intramuscular injection of labeled ovalbumin antigen admixed with labeled AS03 to mice. Little or no co-localization of the antigen and AS03 was found either in muscle tissue or its draining lymph nodes, suggesting that AS03 does not act via direct binding with the antigen.

These results suggest that AS03 may help induce acquired immunity not through control of antigen localization, but through immunopotentiation mediated by cytokine induction.

3.(i).A.(2) Safety pharmacology

3.(i).A.(2).1) Dog telemetry study of Indonesia (H5N1) vaccine and AS03 (4.2.1.3-1, Study [redacted]63, 4.2.1.3-2, Study [redacted]20)

In Study [redacted]63, a single dose of 0.5 mL normal saline was administered intramuscularly to beagle dogs (4 males), and a single dose of 0.5 mL Indonesia (H5N1) vaccine (approximately 5-fold higher than the clinical dose on a body weight basis) was administered intramuscularly 7 days later (n = 4). Blood pressure, heart rate, ECG, and respiration rate were measured without anesthesia 1.5 and 1 hour before and 1, 3, 6, 24, 48, and 168 hours after administration of the vaccine. Arterial blood hemoglobin oxygen saturation was also measured before and 4, 24, 48, and 168 hours after administration of the vaccine. These parameters following the administration of normal saline was compared to those following the administration of the vaccine. The results showed no vaccine effects up to 168 hours after administration.

In Study [redacted]20, a single dose of 0.5 mL normal saline was administered intramuscularly to beagle dogs (4 males), and a single dose of 0.5 mL AS03 (approximately 10-fold higher than the clinical dose on a body weight basis) was administered intramuscularly 7 days later (n = 4). Blood pressure, heart rate, ECG, respiration rate, tidal volume, and minute volume were measured without anesthesia 1 and 0.5 hours before and 1, 3, 6, 24, 48, and 72 hours after administration of the adjuvant. These parameters following the administration of normal saline was compared to those following the administration of AS03. The results showed no effects of AS03 up to 72 hours after administration of the adjuvant.

The effects on the central nervous system (CNS) were evaluated based on clinical observations in the dog telemetry study and rabbit repeated dose toxicity study (4.2.3.2-1, Study [redacted]26, 4.2.3.7.7-1, Study [redacted]56). No CNS effects attributable to the Indonesia (H5N1) vaccine or AS03 was found.

3.(i).B. Outline of the review by PMDA

PMDA concluded that the immunogenicity study, prophylactic efficacy study, and cross-protective efficacy studies using Indonesia (H5N1) vaccine demonstrated the efficacy of the prototype vaccine against the H5N1 subtype and that the prophylactic efficacy study using Anhui (H7N9) vaccine demonstrated immunogenicity and prophylactic efficacy against different subtypes. If the production of a pandemic vaccine is undertaken, the applicant will perform nonclinical studies of the pandemic vaccine to assess its immunogenicity in accordance with "Guidelines for Developing Prototype Vaccines in Preparation for Influenza Pandemic" (PFSB/ELD Notification No. 1031-01 dated October 31, 2011).

3.(ii) Summary of pharmacokinetic studies

No pharmacokinetic study has been performed for the prototype vaccine. Studies to assess the biodistribution of AS03 were performed as described in "3.(i).A.(1).4) Mechanism of action of AS03."

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

A repeated dose toxicity study, a reproductive and developmental toxicity study, and local tolerance studies were performed as toxicology studies of the prototype vaccine, using the Indonesia (H5N1) vaccine (7.5 μg HA/0.5 mL). Other toxicology studies conducted consisted of a repeated dose toxicity study, a genotoxicity study, and a reproductive and developmental toxicity study of AS03. An outline is presented below. (Note that the results of these studies were previously evaluated in the review of Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (H5N1) for Intramuscular Injection "KAKETSUKEN.")

3.(iii).A.(1) Single-dose toxicity (4.2.3.2-1, Study [redacted]26)

The acute toxicity of a single dose of Indonesia (H5N1) vaccine was evaluated after the initial intramuscular administration in the rabbit repeated dose toxicity study (4.2.3.2-1, Study [redacted]26). No deaths were observed, and the approximate lethal dose was considered to be >0.5 mL (at 7.5 μg HA, approximately 17-fold higher than the proposed clinical dose on a body weight basis).

3.(iii).A.(2) Repeated-dose toxicity (4.2.3.2-1, Study [redacted]26)

Normal saline or Indonesia (H5N1) vaccine 0.5 mL was administered by intramuscular injection to rabbits (n = 10/sex/group) in the thigh 3 times at 2-week intervals. In each group, 10 animals were evaluated at 3 days after the final injection; the remaining 10 animals were evaluated after a recovery period of 28 days. In the vaccine group, hematological findings included elevated white blood cell count and fibrinogen, clinical chemistry findings included elevated globulin and decreasing A/G ratio, and histopathological findings included fasciitis, perivascular lymphocyte infiltration, and splenic lymphoid

follicular hyperplasia. All of these changes tended to resolve. Any changes other than those at the injection sites were considered attributable to injection site reactions or immune response against the vaccine. The no-observed-adverse-effect level (NOAEL) was considered to be 0.5 mL (approximately 17-fold higher than the proposed clinical dose on a body weight basis).

3.(iii).A.(3) Genotoxicity

No genotoxicity study has been performed.

3.(iii).A.(4) Carcinogenicity

No carcinogenicity study has been performed.

3.(iii).A.(5) Reproductive and developmental toxicity (4.2.3.5.1-1, Study [redacted]029)

Normal saline or Indonesia (H5N1) vaccine 0.2 mL was administered by intramuscular injection to rats (n = 44 females/group) at 28 and 14 days before mating, on gestation days 3, 8, 11, and 15, and at 7 days after delivery. One half of the maternal animals were subjected to caesarian section, and the fetuses were examined on gestation day 20. The findings showed no teratogenicity in any of the groups. The remaining maternal animals were subjected to necropsy at 25 days after delivery; the results showed no effects on maternal functions. No effect was found on the development of the litters. The NOAELs for maternal general toxicity, maternal function, embryos/fetuses, and litters all were considered to be 0.2 mL (approximately 60-fold higher than the proposed clinical dose on a body weight basis).

3.(iii).A.(6) Local tolerance (4.2.3.6-1, Study [redacted]27, 4.2.3.6-2, Study [redacted]30)

The local tolerance of Indonesia (H5N1) vaccine administered intramuscularly was evaluated in a single dose study and a repeated dose study.

In the single dose study, 0.5 mL of normal saline, Indonesia (H5N1) vaccine, or the antigen preparation from Indonesia (H5N1) strain (15 μg HA/0.5 mL) was injected into the vastus lateralis muscle of rabbits (n = 6 males/group). A 0.5-mL dose of the vaccine is used for a single injection in humans. Although fasciitis was observed at the injection site of animals in both the vaccine group and the antigen preparation group at 3 days after administration, a trend toward recovery was observed in the vaccine group, and the antigen preparation group was found to have recovered at 7 days after administration.

In the repeated dose study, 0.5 mL of normal saline, Indonesia (H5N1) vaccine, or the antigen preparation from the Indonesia (H5N1) strain was injected twice into the same site in the vastus lateralis muscle of rabbits (n = 6 males/group) at a 3-week interval. Fasciitis was observed at the injection site in both the vaccine group and the antigen preparation group at 3 days after the second injection, with conditions slightly more pronounced in the vaccine group than in the antigen preparation group. For the findings in question, a trend toward recovery was observed in the vaccine group, while the antigen preparation group was found to have recovered on the 28th day after the second injection.

Since the severity of fasciitis was lower in the antigen preparation group than in the vaccine group in the above studies, the findings in the fascia was considered to be largely due to the effects of AS03.

3.(iii).A.(7) Other toxicity studies (studies of AS03)

3.(iii).A.(7).1) Repeated dose toxicity (4.2.3.7.7-1, Study [redacted]56, 4.2.3.7.7-2, Study [redacted]33)

In a repeated dose toxicity study of adjuvant AS03, 0.5 mL of normal saline or AS03 was injected to the thigh muscle of rabbits (n = 10/sex/group) 4 times at 2-week intervals. Hematology tests after the first and fourth injections found a high neutrophil count and elevated fibrinogen levels; histopathology at 3 days after the final injection identified splenic lymphoid follicular hyperplasia and fasciitis at the injection site, sciatic perineuritis, and perivascular cell infiltration. All findings showed signs of recovery, and any changes other than those at the injection site were considered attributable to local injection site reaction. The NOAEL was considered to be 0.5 mL (approximately 34-fold higher than the proposed clinical dose on a body weight basis).

3.(iii).A.(7).2) Genotoxicity (4.2.3.7.7-3, Study 2[redacted]4, 4.2.3.7.7-4, Study [redacted]87, 4.2.3.7.7-5, Study [redacted]69)

A bacterial reverse mutation assay, a murine lymphoma TK assay, and a rat micronucleus assay were performed to investigate the genotoxicity of AS03. All results were negative.

3.(iii).A.(7).3) Reproductive and developmental toxicity (4.2.3.7.7-6, Study [redacted]129, 4.2.3.7.7-7, Study H[redacted]1)

A study evaluating early embryonic development to implantation and a study evaluating the effects of AS03 on female fertility, embryonic/fetal development, prenatal/postnatal development, and maternal functions were performed to investigate reproduction toxicity.

Study H[redacted]1 evaluated early embryonic development to implantation. Normal saline or AS03 0.1 mL was administered by intramuscular injection to rats (n = 20 females/group) daily from Day 0 to Day 6 of gestation (a total of 7 doses). General conditions and injection site reactions were observed and body weights and food consumption measured up to Day 14 of gestation. Animals studied were subjected to necropsy on Day 14 of gestation to evaluate the number of corpora lutea, implantations, live conceptuses, and dead conceptuses. There were no abnormal findings in early embryonic development. Reduced body weight gain and feed consumption were found, but no other abnormalities were observed. The NOAEL for reproductive potential in female rats appeared to be 0.1 mL (approximately 60-fold higher than the proposed clinical dose on a body weight basis).

Study [redacted]129 evaluated the effects of AS03 on female fertility, embryonic/fetal development, prenatal/postnatal development, and maternal functions. AS03 0.2 mL was administered intramuscularly to rats (n = 48 females/group) 28 days before mating, and 0.2 mL of AS03-PBS mixture (0.1 mL of AS03) was administered intramuscularly on gestation days 6, 8, 11, and 15 and at 7 days after delivery. In the negative control group, 0.2 mL of PBS was administered intramuscularly 28 days before mating,

on gestation days 6, 8, 11, and 15, and at 7 days after delivery. One half of the pregnant animals in each group were subjected to caesarean section on gestation day 20 to examine the fetuses. The results showed no teratogenicity in any group. The remaining maternal animals were subjected to necropsy at 25 days after delivery; the results showed no effects on maternal functions. The results also showed no effects on litter development. NOAELs for general toxicity of mother animals, maternal function, embryos/fetuses, and litters all are considered to be 0.1 mL (approximately 60-fold higher than the proposed clinical dose on a body weight basis).

3.(iii).B. Outline of the review by PMDA

Based on the document submitted, PMDA concluded that there is no particular toxicity problems with the drug product.

4. Clinical data

4.(i).A Summary of the submitted data

The results from a Japanese Phase I study, Phase II study, and Phase III study performed with Indonesia (H5N1) vaccine were submitted for the safety and efficacy evaluation data. Table 4-1 provides a summary of the respective clinical trials. Table 4-2 gives the list of Indonesia (H5N1) vaccine used in clinical trials. The summary below shows the data from these clinical trials previously evaluated in the review of Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (H5N1) for Intramuscular Injection "KAKETSUKEN."

Table 4-1. Summary of clinical trials

Phase	Study	Design	Endpoint	Subject	Number of vaccinated subjects		Dosage regimen
I	295P1	Non-randomized, open-label, dose escalation	Safety Immunogenicity	Healthy adult men (≥20 and ≤40 years of age)	1/2MA group[a]: 20 MA group: 20 HA group: 20	60 subjects in total	0.5 mL per dose (0.25 mL only in 1/2MA) 2 IMs with a 21 ± 2-day interval
II	295P2	Randomized, double-blind	Safety Immunogenicity	Healthy adults (≥20 and <65 years of age)	MA group: 62 HA group: 62 MB group: 63 HB group: 61	248 subjects in total	0.5 mL per dose 2 IMs with a 21 ± 7-day interval
III	295P3	Uncontrolled	Safety Immunogenicity	Healthy adults (≥20 and <65 years of age)	MA group: 369	369 subjects	0.5 mL per dose 2 IMs with a 21 ± 7-day interval

a. In this group, MA is administered in a 0.25 mL volume per dose

Table 4-2. Vaccines used in the clinical trials

Study drug	Description
MA	A vaccine freshly prepared by mixing equal volumes of an antigen preparation containing 3.75 μg of HA antigen and the adjuvant AS03A[a]
HA	A vaccine freshly prepared by mixing equal volumes of an antigen preparation containing 7.5 μg of HA antigen and the adjuvant AS03A
MB	A vaccine freshly prepared by mixing equal volumes of an antigen preparation containing 3.75 μg of HA antigen and the adjuvant AS03B[b]
HB	A vaccine freshly prepared by mixing equal volumes of an antigen preparation containing 7.5 μg of HA antigen and the adjuvant AS03B

a. 0.25 mL of AS03 adjuvant containing 10.69 mg squalene, 11.86 mg tocopherol, and 4.86 polysorbate 80
b. 0.25 mL of AS03 adjuvant containing 5.35 mg squalene, 5.93 mg tocopherol, and 2.43 polysorbate 80

4.(i).A.(1) Phase I study (5.3.5.2.1 and 5.3.5.2.2, Study 295P1, study period from [redacted] 20[redacted] to [redacted] 20[redacted])

A non-randomized, open-label, dose-escalation study was conducted at one center in Japan to investigate the safety and immunogenicity of the prototype vaccine in healthy Japanese adult men ≥20 and ≤40 years of age (target sample size: 60 [n = 20/group]). Subjects received 2 intramuscular injections of 0.25 mL of the MA formulation (1/2MA group), 0.5 mL (MA group) of the MA formulation, or 0.5 mL of the HA formulation (HA group) in the lateral supraspinatus at an interval of 21 ± 2 days.

The 60 subjects (n = 20/group) enrolled in this study all received the study drug at least once. All were included in the safety analysis population and the full analysis set (FAS). The FAS was defined as the major immunogenicity analysis population.

For immunogenicity, the endpoint was defined as an HI antibody titer against the HA antigen of the vaccine strain (Indonesia strain) at 21 days after the second vaccination (measured by equine and chicken erythrocytes). Table 4-3 presents the results for seroconversion rate (percentage of subjects with a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or subjects with a pre-vaccination HI titer ≥ 1:10 and a minimum 4-fold post-vaccination increase in HI titer), seroprotection rate (percentage of subjects with an HI titer ≥ 1:40), and geometric mean titer (GMT) ratio (pre-vaccination/post-vaccination ratio of geometric mean HI titers) at 21 days after the second vaccination.

Table 4-3. HI antibody response against the vaccine strain at 21 days after the second vaccination[a] (FAS)

Species of erythrocyte	Group	N	Number of subjects with seroconversions	Seroconversion rate (%) [95% CI]	Number of positive subjects	Seroprotection rate (%) [95% CI]	Geometric mean titer ratio [95% CI]
Horse	1/2MA	20	20	100.0 [83.2, 100.0]	20	100.0 [83.2, 100.0]	21.11 [15.89, 28.05]
	MA	20	20	100.0 [83.2, 100.0]	20	100.0 [83.2, 100.0]	25.99 [20.12, 33.58]
	HA	20	20	100.0 [83.2, 100.0]	20	100.0 [83.2, 100.0]	18.38 [13.38, 25.25]
Chicken	1/2MA	20	9	45.0 [23.1, 68.5]	9	45.0 [23.1, 68.5]	5.66 [4.29, 7.46]
	MA	20	18	90.0 [68.3, 98.8]	19	95.0 [75.1, 99.9]	8.57 [6.27, 11.73]
	HA	20	15	75.0 [50.9, 91.3]	17	85.0 [62.1, 96.8]	7.46 [5.54, 10.06]

N. Number of subjects analyzed

a. Protocols specified that all antibody titers below the lower limit of quantitaton (antibody titer 10) will be defined here as antibody titer 5.

With respect to safety, the percentages of subjects exhibiting at least 1 adverse event during the observation period (from the first vaccination to 21 days after the second vaccination) were 95.0% (19 of 20 subjects) in the 1/2MA group; 90.0% (18 of 20 subjects) in the MA group; and 85.0% (17 of 20 subjects) in the HA group. The percentages of subjects exhibiting at least 1 adverse reaction during the observation period were 85.0% (17 of 20 subjects) in the 1/2MA group, 90.0% (18 of 20 subjects) in the MA group, and 85.0% (17 of 20 subjects) in the HA group. Overall, 4 grade 3 adverse events occurred in 3 subjects. The group-by-group breakdown of the events is as follows: 3 events in 2 subjects of the 1/2MA group (arthralgia [2 events] and fatigue [1 event]) and 1 event in 1 subject of the HA group (pyrexia). The causal relationship of all the events to the vaccination could not be ruled out but the adverse events all resolved.

Adverse events and adverse reactions occurring in ≥10% of subjects in all treatment groups during the observation period are listed in Table 4-4.

Table 4-4. Adverse events and adverse reactions occurring in ≥10% of subjects in any treatment group during the observation period (Safety analysis population)

Events		1/2MA group N = 20				MA group N = 20				HA group N = 20			
		Adverse event		Adverse reaction		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%	n	%	n	%
Local reaction	Injection site pain	16	80.0	16	80.0	15	75.0	15	75.0	15	75.0	15	75.0
	Injection site erythema	4	20.0	4	20.0	1	5.0	1	5.0	0	0	0	0
	Injection site induration	3	15.0	3	15.0	1	5.0	1	5.0	0	0	0	0
	Injection site swelling	2	10.0	2	10.0	1	5.0	1	5.0	1	5.0	1	5.0
Systemic reaction	Myalgia	2	10.0	2	10.0	9	45.0	9	45.0	2	10.0	2	10.0
	Fatigue	6	30.0	6	30.0	6	30.0	6	30.0	4	20.0	4	20.0
	Pyrexia	1	5.0	1	5.0	4	20.0	4	20.0	5	25.0	5	25.0
	Headache	4	20.0	4	20.0	3	15.0	3	15.0	1	5.0	1	5.0
	Chill	0	0	0	0	3	15.0	3	15.0	2	10.0	2	10.0
	Hyperhidrosis	1	5.0	1	5.0	2	10.0	2	10.0	3	15.0	3	15.0
	Arthralgia	2	10.0	2	10.0	1	5.0	1	5.0	2	10.0	2	10.0
Laboratory results	White blood cell count decreased	2	10.0	2	10.0	2	10.0	1	5.0	2	10.0	2	10.0
	Blood creatine phosphokinase increased	1	5.0	0	0	2	10.0	0	0	2	10.0	0	0

N. Number of subjects analyzed; n. number of onsets

Throughout the follow-up period (from 22 days after the second vaccination to 6 months after the second vaccination), no deaths, adverse events leading to study discontinuation, serious adverse events, or cases of potential immune-mediated disease were reported in any of the groups.

4.(i).A.(2) Phase II study (5.3.5.1.1 and 5.3.5.1.2, Study 295P2, study period from [redacted] 20[redacted] to [redacted] 20[redacted])

A multicenter, randomized, double-blind, parallel-group study was conducted in healthy Japanese adults of ≥20 and <65 years of age (target sample size: 224, n = 56/group) at 4 Japanese institutions to investigate the immunogenicity and safety of the prototype vaccine and the appropriateness of the proposed dose.

Subjects were to receive 2 intramuscular injections of 0.5 mL of the MA, HA, MB, or HB vaccine formulation in the lateral supraspinatus at an interval of 21 ± 7 days.

All 248 subjects (62 in the MA group, 62 in the HA group, 63 in the MB group, and 61 in the HB group) enrolled in this study received the study drug at least once. All were included in the safety analysis population. Excluding 2 subjects (one from the HA group and one from the HB group) whose blood was not sampled after the first vaccination, the remaining 246 subjects were included in the FAS, which was defined as the major immunogenicity analysis population.

The primary immunogenicity endpoint was defined as an HI antibody titer against the HA antigen of the vaccine strain (Indonesia strain) at 21 days after the second vaccination (measured by equine erythrocytes), assessing whether the 3 criteria in the Prototype Vaccine Guidelines (seroconversion rate,

>40%; seroprotection rate, >70%; and GMT ratio, >2.5) were met. The results of the study are presented in Table 4-5.

Table 4-5. HI antibody response against the vaccine strain at 21 days after the second vaccination[a] (measured by equine erythrocyte) (FAS)

Group	N	Number of subjects with seroconversions	Seroconversion rate (%) [95% CI]	Number of positive subjects	Seroprotection rate (%) [95% CI]	GMT ratio [95% CI]	Criteria[b]
MA	60	60	100.0 [94.0, 100.0]	60	100.0 [94.0, 100.0]	33.90 [28.82, 39.88]	Passed
HA	59	59	100.0 [93.9, 100.0]	59	100.0 [93.9, 100.0]	40.48 [34.39, 47.64]	Passed
MB	61	61	100.0 [94.1, 100.0]	61	100.0 [94.1, 100.0]	28.56 [24.69, 33.04]	Passed
HB	60	59	98.3 [91.1, 100.0]	59	98.3 [91.1, 100.0]	30.55 [25.44, 36.70]	Passed

N. Number of subjects analyzed (excluding subjects not having antibody titer result after the second administration)
a. Protocols specified that antibody titers below the lower limit of quantitation (antibody titer 10) will be defined here as antibody titer 5.
b. Considered passed if all three criteria of the Prototype Vaccine Guidelines were met

With respect to safety, the percentages of subjects exhibiting at least 1 adverse event during the observation period (from the first vaccination to 21 days after the second vaccination) were 91.9% (57 of 62 subjects) in the MA group; 88.7% (55 of 62 subjects) in the HA group; 95.2% (60 of 63 subjects) in the MB group; and 90.2% (55 of 61 subjects) in the HB group. The percentages of subjects exhibiting at least 1 adverse reaction during the observation period were 90.3% (56 of 62 subjects) in the MA group; 87.1% (54 of 62 subjects) in the HA group; 95.2% (60 of 63 subjects) in the MB group; and 88.5% (54 of 61 subjects) in the HB group. Overall, 14 grade 3 adverse events occurred in 8 subjects. The group-by-group breakdown of the events is as follows: 11 events in 5 subjects of the MA group (pyrexia [4 events], injection site erythema [1 event], injection site induration [1 event], injection site swelling [1 event], headache [1 event], chill [1 event], fatigue [1 event], and dehydration [1 event]); 2 events in 2 subjects of the HA group (pyrexia and influenza); and 1 event in 1 subject of the MB group (injection site erythema). The causal relationship of all events except influenza in the HA group to the vaccination could not be ruled out but the adverse events all resolved.

Table 4-6 lists adverse events and adverse reactions occurring in ≥5% of subjects in any of the groups during the observation period.

Table 4-6. Adverse events and adverse reactions occurring in ≥5% of subjects in any treatment group during the observation period (Safety analysis population)

	Events	MA group N = 62				HA group N = 62				MB group N = 63				HB group N = 61			
		Adverse event		Adverse rection		Adverse event		Adverse reaction		Adverse event		Adverse reaction		Adverse event		Adverse reaction	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Local reactions	Injection site pain	53	85.5	53	85.5	52	83.9	52	83.9	49	77.8	49	77.8	45	73.8	45	73.8
	Injection site erythema	19	30.6	19	30.6	19	30.6	19	30.6	10	15.9	10	15.9	12	19.7	12	19.7
	Injection site induration	17	27.4	17	27.4	15	24.2	15	24.2	10	15.9	10	15.9	11	18.0	11	18.0
	Injection site swelling	17	27.4	17	27.4	15	24.2	15	24.2	8	12.7	8	12.7	10	16.4	10	16.4
	Injection site pruritus	8	12.9	8	12.9	3	4.8	3	4.8	8	12.7	8	12.7	2	3.3	2	3.3
	Injection site warmth	4	6.5	4	6.5	2	3.2	2	3.2	0	0	0	0	1	1.6	1	1.6
Systemic reactions	Fatigue	36	58.1	36	58.1	36	58.1	34	54.8	27	42.9	27	42.9	27	44.3	25	41.0
	Myalgia	23	37.1	22	35.5	23	37.1	23	37.1	21	33.3	21	33.3	22	36.1	21	34.4
	Headache	21	33.9	20	32.3	26	41.9	25	40.3	28	44.4	25	39.7	20	32.8	20	32.8
	Arthralgia	18	29.0	18	29.0	17	27.4	17	27.4	11	17.5	11	17.5	8	13.1	7	11.5
	Chill	13	21.0	12	19.4	17	27.4	17	27.4	7	11.1	7	11.1	5	8.2	5	8.2
	Pyrexia	8	12.9	8	12.9	18	29.0	18	29.0	5	7.9	4	6.3	3	4.9	3	4.9
	Hyperhidrosis	4	6.5	4	6.5	7	11.3	7	11.3	13	20.6	13	20.6	5	8.2	5	8.2
	Nasopharyngitis	0	0	0	0	1	1.6	0	0	2	3.2	0	0	5	8.2	1	1.6
	Nausea	4	6.5	2	3.2	2	3.2	2	3.2	0	0	0	0	3	4.9	2	3.3

N. Number of subjects to be analyzed; n. number of onsets

Throughout the observation period and the subsequent open-label follow-up period (from 22 days after the second vaccination to 6 months after the second vaccination), no deaths, adverse events leading to study discontinuation, serious adverse events, or cases of potential immune-mediated disease were reported in any of the groups.

4.(i).A.(3) Phase III study (5.3.5.2.3 and 5.3.5.2.4, Study 295P3, study period from [redacted] 20[redacted] to [redacted] 20[redacted])

A multicenter, open-label, uncontrolled study was conducted in healthy Japanese adults of ≥20 and <65 years of age (target sample size: 333) at 5 Japanese institutions to investigate the immunogenicity and safety of the prototype vaccine. Subjects received 2 intramuscular injections of 0.5 mL of the MA vaccine in the lateral supraspinatus at an interval of 21 ± 7 days.

All 369 subjects enrolled in the present study received the study drug at least once. All were included in the safety analysis population. Excluding 5 subjects whose blood was not sampled after the first vaccination, the remaining 364 subjects were included in the FAS, which was defined as the major immunogenicity analysis population.

The primary immunogenicity endpoint was defined as an HI antibody titer against the HA antigen of the vaccine strain (Indonesia strain) at 21 days after the second vaccination (measured by equine erythrocytes), assessing whether the 3 criteria in the Prototype Vaccine Guidelines (seroconversion rate, >40%; seroprotection rate, >70%; and GMT ratio, >2.5) were met. Table 4-7 gives the results of this assessment.

Table 4-7. HI antibody response against vaccine strain on 21st day after second vaccination[a] (measured by equine erythrocyte) (FAS)

N	Number of subjects with seroconversions	Seroconversion rate (%) [95% CI]	Number of positive subjects	Seroprotection rate (%) [95% CI]	GMT ratio [95% CI]	Criteria[b]
364	364	100.0［99.0, 100.0］	364	100.0［99.0, 100.0］	43.73［41.15, 46.47］	Passed

N. Number of subjects analyzed
a. Protocols specified that all antibody titers below the lower quantitative limit (antibody titer 10) will be defined here as antibody titer 5.
b. Considered passed if all three criteria of the Prototype Vaccine Guidelines were met

With respect to safety, the percentage of subjects exhibiting at least 1 adverse event during the observation period (from the first vaccination to 21 days after the second vaccination) was 93.5% (345 of 369 subjects). The percentage of subjects exhibiting at least 1 adverse reaction during the observation period was 93.5% (345 of 369 subjects). A total of 25 grade 3 adverse events occurred in 20 subjects (injection site erythema [8 events], pyrexia [6 events], injection site swelling [4 events], positional vertigo [1 event], dental caries [1 event], periodontitis [1 event], chill [1 event], malaise [1 event], arthralgia [1 event], and headache [1 event]). The causal relationship of all events, except caries and periodontitis, to the vaccination could not be ruled out, but the adverse events all resolved.

Table 4-8 lists the adverse events and adverse reactions occurring in ≥5% of subjects during the observation period.

Table 4-8. Adverse events and adverse reactions occurring in ≥5% of subjects during the observation period (Safety analysis population)

Number of subjects analyzed		N = 369			
Event term		Adverse event		Adverse reaction	
		n	%	n	%
Local reaction	Injection site pain	320	86.7	320	86.7
	Injection site erythema	126	34.1	126	34.1
	Injection site swelling	106	28.7	106	28.7
	Injection site induration	86	23.3	86	23.3
	Injection site pruritus	42	11.4	41	11.1
	Injection site warmth	27	7.3	27	7.3
Systemic reaction	Fatigue	157	42.5	156	42.3
	Headache	133	36.0	131	35.5
	Myalgia	124	33.6	122	33.1
	Arthralgia	97	26.3	96	26.0
	Chill	93	25.2	93	25.2
	Pyrexia	86	23.3	85	23.0
	Hyperhidrosis	44	11.9	44	11.9
	Malaise	22	6.0	21	5.7

N. Number of subjects analyzed; n, number of onsets

No deaths, adverse events leading to study discontinuation, serious adverse events, or cases of potential immune-mediated disease were reported during the observation period. No deaths were reported throughout the follow-up period (from 22 days after the second vaccination to 6 months after the second vaccination). Two serious adverse events arose in 2 subjects (acute abdomen [1 event] in 1 subject and thyroid cancer [1 event] in 1 subject). The 2 events were considered unrelated to the vaccine. In addition, 1 non-serious event of Basedow's disease occurred in 1 subject as a potential immune-mediated disease, but was considered unrelated to the vaccine.

4.(i).B Outline of the review by PMDA

4.(i).B.(1) Efficacy

The applicant explained the efficacy of the prototype vaccine as follows:

Essentially, to evaluate the efficacy of an influenza vaccine, its preventive effects must be evaluated. However, it appears difficult to evaluate preventive effects in humans, since no pandemic influenza currently prevails. Accordingly, it was decided that the immunogenicity of the prototype vaccine would be evaluated with respect to HI antigen titer against vaccine strains, based on the Prototype Vaccine Guidelines. Since HI antibody titer measurement with equine erythrocytes is considered more sensitive than that with avian erythrocytes against virus strain antigens derived from avian influenza virus such as H5N1 subtypes (*Virus Res*, 2004;103: 91-95), Studies 295P2 and 295P3 used HI antibody titer measured with equine erythrocytes as the primary endpoint. As a result of Study 295P3, the seroconversion rate, seroprotection rate, and the GMT ratio at 21 days after the second MA vaccination, all met the criteria presented in the Prototype Vaccine Guidelines.

In Study 295P3, the sustainability of antibody titers were examined from the 21st day to the 180th day after the second MA vaccination in several subjects. Antibody titers on the 180th day after the second vaccination were found to be reduced compared to those on the 21st day (Table 4-9).

Table 4-9. Changes in HI antibody response from 21st through 90th and the 180th day after the second vaccination[a] (equine erythrocytes) (Study 295P3, FAS)

Time point (after the second vaccination)	N	Number of subjects with seroconversion	Seroconversion rate (%) [95% CI]	Number of positive subjects	Seroprotection rate (%) [95% CI]	GMT ratio [95% CI]
21 days	99	99	100.0 [96.3, 100.0]	99	100.0 [96.3, 100.0]	47.36 [42.13, 53.25]
90 days	99	98	99.0 [94.5, 100.0]	98	99.0 [94.5, 100.0]	24.35 [21.79, 27.22]
180 days	96	74	77.1 [67.4, 85.0]	74	77.1 [67.4, 85.0]	10.91 [9.35, 12.74]

N. Number of subjects analyzed (excluding subjects not having antibody titer result after second vaccination)
a. Protocols specified that all antibody titers below the lower limit of quantitation (antibody titer 10) will be defined here as antibody titer 5.

In Study 295P1, immunogenicity against virus strains of clades differing from that of the vaccine strain (Indonesia strain) was assessed at 21 days after the second MA vaccination. As a result, the vaccine was confirmed to have HI antigen titer meeting the criteria presented in the Prototype Vaccine Guidelines against A/Vietnam/1194/2004 (H5N1) strains (clade 1), A/bar-headed goose/Qinghai/1A/2005 (H5N1) strain (clade 2.2), and A/Anhui/1/2005 (H5N1) strains (clade 2.3) (Table 4-10), demonstrating cross-immune response against viruses of different clades.

Table 4-10. HI antibody cross-immune response against viruses of different clades after second vaccination with MA[a] (equine erythrocytes) on Day 42 (Study 295P1, FAS)

Virus strain	N	Number of subjects with seroconversion	Seroconversion rate (%) [95% CI]	Number of positive subjects	Seroprotection rate (%) [95% CI]	GMT ratio [95% CI]
Vietnam	20	19	95.0 [75.1, 99.9]	20	100.0 [83.2, 100.0]	8.57 [6.50, 11.30]
Qinghai	20	20	100.0 [83.2, 100.0]	20	100.0 [83.2, 100.0]	21.11 [14.57, 30.58]
Anhui	20	20	100.0 [83.2, 100.0]	20	100.0 [83.2, 100.0]	17.15 [13.28, 22.14]

N. Number of subjects analyzed
a. Protocols specified that all antibody titers below the lower limit of quantitation (antibody titer 10) will be defined here as antibody titer 5.

PMDA considers as follows:

The analysis of immunogenicity at 21 days after the second MA vaccination in Study 295P3 showed that the seroprotection rate, seroconversion rate, and GMT ratio based on HI antibody titer (the primary endpoint) met the criteria presented in the Prototype Vaccine Guidelines. At 180 days after the second vaccination, the criteria presented in the Prototype Vaccine Guidelines were also met. Thus, double vaccination with the prototype vaccine is expected to have preventive effects against pandemic influenza virus. Although the number of subjects evaluated was limited, the results suggested that the prototype vaccine induces a cross-immune response against virus strains from different clades, demonstrating the possibility of helping safeguard against the infection with pandemic influenza virus strains from a clade differing from that included in the vaccine.

4.(i).B.(2) Safety

4.(i).B.(2).1) Results of clinical trials

Based on the results from the clinical trials, the applicant explained the safety of the prototype vaccine as follows:

In Studies 295P3, 295P2, and 295P1, no clinically significant adverse events were observed in any treatment groups up to 21 days after the second vaccination of the prototype vaccine. It therefore appears to be well-tolerated.

In Study 295P3, 2 serious adverse events (acute abdomen and thyroid cancer) were reported in 2 subjects, as well as 1 potential immune-mediated disease (Basedow's disease) in 1 subject during the follow-up period (from 22 days after the second vaccination to 6 month after the second vaccination). All the 3 events were considered unrelated to the prototype vaccine, and there appears to be no concerns for long-term safety.

PMDA concluded that the prototype vaccine is a well-tolerated safety profile.

4.(i).B.(2).2) Narcolepsy

Based on reports from foreign countries concerning the development of narcolepsy after vaccination with the A/H1N1 2009 influenza vaccine containing AS03, the applicant gave the current opinion on development of narcolepsy following vaccination with the prototype vaccine as described below:

Epidemiological studies were performed on development of narcolepsy following vaccination with influenza vaccines (Pandemrix H1N1 and Arepanrix H1N1) containing AS03 manufactured by GSK. Studies from France and Finland reported an increased risk of narcolepsy in adults and a Swedish study reported a similar risk in young adults (aged 21-30 years) (http://ansm.sante.fr/S-informer/Points-d-information-Points-d-information/Vaccins-pandemiques-grippe-A-H1N1-et-narcolepsie-Resultats-de-l-etude-europeenne-et-de-l-etude-cas-temoins-francaise-Point-d-information, *PLoS One*, 2012;7: e33536, http://www.lakemedelsverket.se/upload/nyheter/2011/Fallinventeringsrapport_pandermrix_110630.pdf). A large scale study was performed by the Vaccine Adverse Event Surveillance and Communication (VAESCO)

consortium through a network of research institutions and public health organizations in 8 European countries (the UK, Italy, the Netherlands, Sweden, Denmark, Norway, Finland, and France) in which Pandemrix H1N1 was used. The study concluded that narcolepsy signals were detected in Finland and Sweden, but that no statistically significant increase in the risk was found in other participating countries (http://ecdc.europa.eu/en/publications/publications/vaesco report final with cover.pdf). In addition, of 24 events of narcolepsy found in a study performed in Quebec, Canada, 8 developed after vaccination, but this result indicated a very low risk for children and adolescents aged <20 years (1 in 1,000,000 individuals vaccinated), precluding causality between Arepanrix H1N1 and narcolepsy (*PLoS One*, 2014;9:e108489).

Accordingly, it appears that no causal relationship has been established to date between influenza vaccines containing AS03 and narcolepsy. Taking into consideration the limit of epidemiological studies, however, it seems difficult to draw a conclusion regarding causal relationship between the vaccines in question and narcolepsy which cannot be ruled out completely. Thus, "narcolepsy" will be included in the "Important potential risks" section of the risk management plan (draft) for the prototype vaccine. The applicant also plans to include in the package insert (draft) information on reports indicating that risks of narcolepsy increased in individuals receiving influenza vaccines containing AS03 in foreign countries.

The applicant has obtained information indicating that the European Medicines Agency (EMA) has imposed a requirement to conduct non-clinical and epidemiological studies on GSK (the manufacturing authorization holder of Pandemrix H1N1) to further investigate the association between Pandemrix H1N1 and narcolepsy. These studies are currently ongoing.

PMDA considers that circumstances in foreign countries warrant continued monitoring and appropriate measures should be taken when new information becomes available in the future.

4.(i).B.(3) Clinical positioning

PMDA's view on the clinical positioning of the prototype vaccine is as follows:

Currently, there is no established treatment for pandemic influenza. The potential for drug resistance in virus strains has been reported with antiviral agents against influenza [see "1. Origin or history of discovery and usage conditions in foreign countries, etc."]. Thus, preventing the onset/aggravation of pandemic influenza through vaccinations occupies a critical position not just from the public health perspective, but also from the clinical view. Based on the results of clinical trials with the Indonesia (H5N1) vaccine, the prototype vaccine was shown to generate high immunogenicity in humans as required by the Prototype Vaccine Guidelines and to have preventive effects against more than 1 subtype (H5N1 and H7N9) in ferrets and mice [see "3.(i) Summary of pharmacology studies"]. Thus, vaccination with a pandemic vaccine produced by the same manufacturing process as that for the prototype vaccine is expected to prevent the onset/aggravation of pandemic influenza and the prototype vaccine can be an important option in the event of an outbreak of pandemic influenza.

Since the prototype vaccine is manufactured using cell culture technologies, manufacturing is expected to take less time than vaccines manufactured with embryonated egg culture. Additionally, this manufacturing process is not influenced by the availability of eggs, and the prototype vaccine can be administered to individuals with egg allergy. These advantages make the prototype vaccine appropriate for situations in which pandemic influenza calls for immediate and extensive measures.

4.(i).B.(4) Indications

Based on the results of clinical trials and the clinical positioning above, PMDA concluded an appropriate indication of the prototype vaccine is "prophylaxis of pandemic influenza."

4.(i).B.(5) Dosage and administration

4.(i).B.(5).1) Vaccination dose

The applicant explained the reason for selecting the antigen and adjuvant contents of the prototype vaccine as follows:

Since the seroconversion rate and seroprotection rate were 100% in all of the MA, HA, and MB groups and 98.3% in the HB group, the GMT ratios were compared between the vaccination groups (Table 4-11). In comparison of the groups receiving AS03A-adjuvanted vaccines, the GMT ratio in the HA group was higher than that in the MA group. In the groups receiving AS03B-adjuvanted vaccines, there was no significant difference in GMT ratio between the MB group and the HB group. Comparisons of the groups receiving vaccine with the same antigen content showed that the MA groups had a trend of higher rate than that of the MB groups, and the HA group had higher trend than that of the HB group. Since the above results suggested that the antibody content does not have great effect on the immunogenicity and that the immunogenicity is potentiated depending on the adjuvant content, it seemed desirable to select the MA or HA formulation from the view of immunogenicity.

With respect to safety, the groups receiving AS03A-adjuvanted vaccine formulations (the HA and MA groups) were found to have a trend toward a higher incidence of adverse reactions than the groups receiving AS03B-adjuvanted vaccine formulations (the HB and MB groups), but all the 4 vaccine formulations seemed be tolerable. Comparisons of the groups receiving HA and MA recommended from the view of immunogenicity showed that specific adverse events (adverse events reported from the time point of vaccination with each vaccine formulation to 6 days after vaccination [Day 0 to Day 6, Day 21 to Day 27]) tended to be more common in the HA group than in the MA group. The incidence of the events reported in subjects in the MA group and HA group was as follows: pyrexia in 12.9% (8 of 62 subjects) and 27.4% (17 of 62 subjects), respectively; headache in 33.9% (21 of 62 subjects) and 41.9% (26 of 62 subjects), respectively; and chills in 21.0% (13 of 62 subjects) and 27.4% (17 of 62 subjects), respectively.

Table 4-11. Comparison of geometric mean ratio of antibody titer change between vaccine formulations with different antibody contents[a] (Study 295P2)

Adjuvant	AS03A		AS03B	
Study drug group	MA (N = 60)	HA (N = 59)	MB (N = 61)	HB (N = 60)
GMT ratio[95% CI]	33.90 [28.82, 39.88]	40.48 [34.39, 47.64]	28.56 [24.69, 33.04]	30.55 [25.44, 36.70]

N. Number of subjects analyzed (excluding subjects not having antibody titer result after second vaccination)
a. Protocols specified that all antibody titers below the lower limit of quantitation (antibody titer 10) will be defined here as antibody titer 5.

Based on the above considerations, the MA formulation was selected as the recommended clinical dose that was expected to have higher immunogenicity with fewer safety problems.

Influenza pandemics will require a rapid manufacturing of large vaccine volumes. Since the MA formulation contains less antigen than the HA formulation, it will allow more vaccine manufacturing from limited amounts of antigen, thus a useful characteristic.

Study 295P3 demonstrated the immunogenicity and well-tolerated safety profile of the MA formulation. Thus, PMDA concluded that the selection of the MA formulation as the recommended clinical dose is acceptable, in light of its suitability as a vaccine to be manufactured in the event of a pandemic.

4.(i).B.(5).2) Vaccination interval

The vaccination interval of the prototype vaccine was explained by the applicant as follows:
In Study 295P3, the vaccination interval was defined as 21 ± 7 days. For the immunogenicity of the prototype vaccine, the 21- to 28-day interval produced higher GMT ratio than the 14- to 20-day interval, but the subject group with 14- to 20-day interval and the group with 21- to 28-day interval both met the criteria presented in the Prototype Vaccine Guidelines (Table 4-12). Thus, it appears that the interval range from 14 to 28 days does not need to be limited further. Although no subjects received vaccination at intervals of >28 days, the results from the subgroup analysis in question indicates that sufficient immunogenicity can be obtained with an interval of >28 days.

Table 4-12. Subgroup analysis of HI antibody response[a] against the vaccine strain after second vaccination on Day 42 by vaccination interval (equine erythrocyte) (Study 295P3)

Vaccination interval	N	Number of subjects with seroconversion	Seroconversion rate (%) [95% CI]	Number of positive subjects	Seroprotection rate (%) [95% CI]	GMT ration [95% CI]
14 to 20 days	91	91	100.0 [96.0, 100.0]	91	100.0 [96.0, 100.0]	35.06 [31.21, 39.39]
21 to 28 days	273	273	100.0 [98.7, 100.0]	273	100.0 [98.7, 100.0]	47.07 [43.92, 50.45]

N. Number of subjects analyzed
a. Protocols specified that all antibody titers below the lower limit of quantitation (antibody titer 10) will be defined here as antibody titer 5.

PMDA considers as follows:

The result of a subgroup analysis showed a fold-increase in GMT for the prototype vaccine administered at 14- to 20-day intervals, meeting the criterion presented in the Prototype Vaccine Guidelines. The vaccination at a 14- to 20-day interval tended to produce lower GMT ratio than that at a 21- to 28-day interval. However, since a difference in the vaccination interval is unlikely to cause any marked decline in immunogenicity at an interval ranging from 14 to 28 day, the vaccination interval of 14 to 28 days is acceptable. A textbook on vaccine clinical practice used around the world (*Plotkin Vaccines, 6th ed.,*

Elsevier, 2013) states that an interval of at least 21 to 28 days between vaccinations is appropriate for inducing initial antigen-specific immune response in an optimal manner. According to the textbook, delayed vaccination that failed to follow the recommended booster immunization schedule will not affect antibody response against the vaccine, because the initial vaccination generates immunologic memory. Based on these facts and the results of clinical trials, the dosage and administration defines the vaccination interval as "≥2 weeks" without specifying the upper limit of the interval. Since the clinical trial protocol specified the vaccination interval of 21 days, the applicant considered it appropriate to specify that "the usual vaccination interval is 3 weeks" in the "Precautions for dosage and administration" section.

4.(i).B.(5).3) Populations eligible for vaccination

The applicant explained the vaccination subjects of the prototype vaccine as follows:

Although children aged <20 years or elderly subjects aged ≥65 years were not studied in clinical trials performed previously, a clinical study of the Indonesia (H5N1) vaccine to examine the dosage and administration to be used in children aged ≥6 months and <20 years, as well as a study to investigate the safety and efficacy of the vaccine in the elderly aged ≥65 years, are currently ongoing. The clinical studies will end in [redacted] 20[redacted] for children and in [redacted] 20[redacted] for elderly subjects. Another application will be filed or necessary precautions will be provided based on new information from these studies.

Furthermore, the following information has been obtained to date regarding safety in pregnant women receiving AS03-adjuvanted influenza vaccines including the prototype vaccine.

Clinical studies of the prototype vaccine did not included pregnant women. In Study 295P2, one subject proved to be pregnant after the second vaccination with Indonesia (H5N1) vaccine but there were no adverse pregnancy outcomes. Post-marketing surveillance of the AS03-adjuvanted influenza vaccine from GSK (Pandemrix H1N1) showed no increase in the incidence of congenital abnormalities, miscarriages, or low weight newborns. In addition, a large cohort study of PandemrixH1N1 in Denmark (*BMJ*, 2012;344: e2794) and a historical cohort study in Sweden (*Eur J Epidemiol.,* 2013;28: 579-588) suggested no association between the AS03-adjuvanted influenza vaccine and adverse reactions in pregnant women.

PMDA considers as follows:

If a highly pathogenic influenza virus becomes transmissible from human to human resulting in an influenza pandemic, serious outcome could be expected. Thus, although no information has been obtained as of now from clinical studies in children, in whom influenza infection is frequently reported (*WHO Weekly epidemiological record,* 2013;88:137-144), as well as infants, pregnant women, and elderly people who are considered to be included in the high risk population, these populations should not be excluded from the populations eligible for vaccination with the prototype vaccine and the dosage and administration should include no conditions such as age to limit the populations for vaccination.

Currently available information on the pregnant woman receiving the AS03-adjuvanted influenza vaccine suggests no concerns regarding the safety of vaccine during pregnancy. However, since there has been no experience of vaccination with the prototype vaccine in pregnant women, the package insert should include the following precautions: “Since safety and efficacy have not been established in pregnant women, the prototype vaccine should be administered only if the potential benefits of vaccination outweigh the risks,” and “Safety in children and elderly individuals has not been established.” Furthermore, it is necessary to conduct appropriate risk communication through information provision via the package insert and other information materials, based on the immunogenicity data and safety information obtained from the clinical studies that are ongoing in children or elderly subjects.

Based on the above, PMDA considers it appropriate to define the dosage and administration of the prototype vaccine as follows: “The usual dosage is 2 intramuscular injections of 0.5 mL of the mixture of the antigen preparation and the proprietary emulsion adjuvant at an interval of ≥2 weeks” and to describe the usual vaccination interval in the “Precautions for vaccination” section.

4.(i).B.(6) Post-marketing commitments

In the post-marketing surveillance plan proposed by the applicant, based on the Prototype Vaccine Guidelines, a use-results survey will be conducted to investigate the safety of a pandemic vaccine produced by the same manufacturing process as that of the prototype vaccine (target sample size: 3,000) as well as a post-marketing clinical study to investigate immunogenicity (target sample size: 150 [50 adults, 50 children, and 50 elderly individuals]). The proposed observation period of the use-results survey is 3 weeks after vaccination of the prototype vaccine, with a separate observation period defined for narcolepsy. Assuming that practical problems with the post-marketing surveillance may occur, such as restrictions in visiting medical institutions during a pandemic, the applicant is seeking measures such as conducting retrospective research after concluding a contract with medical institutions. However, there are limitations in anticipating possible problems and preparing measures against them at present, and if the situation makes it difficult to conduct the research, the applicant will comprehensively cooperate with the administrative bodies for the implementation of related initiatives and programs to collect safety information on pandemic vaccine.

PMDA considers as follow:

Unlike general pharmaceutical products, a pandemic vaccine will be used under the vaccination system developed in accordance with the government’s pandemic influenza preparedness plan. Thus, it is assumed that research procedure may be constrained depending on the situation where the pandemic vaccine is actually used. While paying attention to trends in regulatory policies including “Guidelines for the Prevention and Control of Pandemic Influenza” prepared by the Japanese government (http://www.cas.go.jp/jp/seisaku/ful/keikaku/pdf/gl_guideline.pdf), and the applicant should take appropriate measures by revising the post-marketing surveillance plan.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

PMDA is currently investigating on this issue and the assessment results and conclusion will be presented in Review Report (2).

IV. Overall Evaluation

PMDA concluded that a pandemic vaccine produced by the same manufacturing process as that of the prototype vaccine is expected to be effective in the prophylaxis of pandemic influenza and that its safety is tolerable, as described in "II.4.(i).B.(1) Efficacy" and "II.4.(i)B.(2) Safety." PMDA considers that the prototype vaccine may be approved as a production model for pandemic vaccines if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

Review Report (2)

February 5, 2015

I. Product Submitted for Registration

[Brand name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype) for Intramuscular Injection "KAKETSUKEN"
[Non-proprietary name]	Emulsion-adjuvanted Cell-culture Derived Influenza HA Vaccine (Prototype)
[Applicant]	The Chemo-Sero-Therapeutic Research Institute (KAKETSUKEN)
[Date of application]	September 30, 2014

II. Content of the Review

The outline of the comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) is described in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 20-8/2008 dated December 25, 2008).

(1) Efficacy and Indications

PMDA has concluded that the prototype vaccine is expected to have preventive effect against pandemic influenza since the immunogenicity of the prototype vaccine (3.75 μg HA antigen and AS03 adjuvant) manufactured with the Indonesia (H5N1) strain used in the Japanese Study 295P3 met all 3 criteria (seroconversion rate, >40%; seroprotection rate, >70%, and geometric mean titer [GMT] ratio, >2.5-fold) in "Guideline on the Development of Prototype Vaccine against Pandemic Influenza" (PFSB/ELD Notification No. 1031-01 of MHLW, dated October 31, 2011; "Prototype Vaccine Guidelines" hereinafter). PMDA has concluded that it is appropriate to determine the indication for the prototype vaccine to be "prophylaxis of pandemic influenza." PMDA's conclusion was supported by the expert advisors.

(2) Safety

Based on results from Studies 295P3, 295P2, and 295P1, PMDA has concluded that the prototype vaccine presents well-tolerated safety profile.

It has been reported that in foreign countries, narcolepsy developed after vaccination with the influenza A/H1N1 2009 virus vaccine containing the same adjuvant (AS03) as that used in the prototype vaccine (*BMJ* 346 doi: http://dx.doi.org/10.1136/bmj.f794, 2013). According to the applicant explanation, it is difficult to draw a conclusion on the association between AS03-adjuvanted influenza vaccines and

narcolepsy, although epidemiological studies have been performed on the development of narcolepsy following vaccination with AS03-adjuvanted influenza vaccines manufactured by GSK Biologicals (GSK) (Pandemrix H1N1 and Arepanrix H1N1).

PMDA understands the applicant's explanation but considers it necessary to list "narcolepsy" as an "important potential risk" in the risk management plan of the prototype vaccine and to take appropriate measures for risk communications. The measures include provision of information in the package insert, such as cases involving the development of narcolepsy after vaccination with AS03-adjuvanted influenza vaccines in foreign countries. In addition, circumstances in foreign countries should be monitored continuously and appropriate actions should be taken when new information becomes available.

The expert advisors supported the above conclusion by PMDA.

The expert advisors commented that a safety monitoring system should be established in advance so that information on very rare events such as narcolepsy, recognized as a potential risk of the prototype vaccine, can be collected in the same way as in normal times even during a pandemic.

PMDA asked the applicant to address these issues. The applicant responded to take appropriate actions.

(3) Dosage and administration

PMDA's conclusions on the dosage and administration are as follows:

- Study 295P3 demonstrated the immunogenicity of the MA vaccine formulation (containing 3.75 μg HA antigen and AS03A adjuvant) and its tolerable safety profile. The antigen and adjuvant contents would be useful in manufacturing of a pandemic vaccine. For these reason, the MA vaccine formulation is selected for the recommended clinical dose is acceptable.

- Based on the description in a textbook on vaccine clinical practice used around the world (*Plotkin Vaccines, 6th ed.,* Elsevier, 2013) and the results of Study 295P3 performed at the vaccination interval of 21 ± 7 days, it is appropriate to specify the vaccination interval of "≥2 weeks" without defining the upper limit of the vaccination interval, and to provide the statement to the effect that "the usual vaccination interval is 3 weeks" in the section of "Precautions for dosage and administration of the vaccination."

- Precautions for the populations eligible for vaccination are necessary. Since cases of pandemic influenza are assumed to have serious outcomes, the populations eligible for vaccination must not exclude children in whom influenza infection is frequently reported, and high risk populations consisting of infants, pregnant women, and elderly individuals. The package insert should include precautions stating the following: "Since safety and efficacy have not been established in pregnant

women, the vaccine should be administered only if the potential benefits of vaccination outweigh risks," "Safety in children and elderly individuals has not been established."

Based on the above, the expert advisor supported the PMDA conclusion that it is appropriate to specify the dosage and administration of the prototype vaccine as follows: "The usual dosage is 2 intramuscular injections of 0.5 mL of the mixture of the antigen preparation and the proprietary emulsion adjuvant at an interval of ≥2 weeks" and to describe a typical vaccination interval in the Precautions for Vaccination section.

(4) Risk management plan (draft)

For actual use of a pandemic vaccine manufactured by the same manufacturing process as that of the prototype vaccine, the applicant plans a use-results survey to investigate the safety of the pandemic vaccine (target sample size: 3000), as well as a post-marketing clinical study to investigate the immunogenicity of the pandemic vaccine (target sample size: 150 [50 adults, 50 children, and 50 elderly]). PMDA has concluded that attention should be paid to the above studies and trends in regulatory policies, including "Guidelines for the Prevention and Control of Pandemic Influenza" prepared by the Government (http://www.cas.go.jp/jp/seisaku/ful/keikaku/pdf/gl_guideline.pdf) and needs to gather information and conduct studies, with necessary measures including restrictions on procedures for post-marketing surveillance and others.

The expert advisors supported the above conclusion by PMDA.

In addition, the expert advisors commented that infant data should also be collected when immunogenicity data are collected from children in post-marketing clinical studies of a pandemic vaccine. PMDA asked the applicant to address these issues and the applicant agreed to take appropriate actions.

Based on the above discussion, PMDA has concluded that the current risk management plan for the prototype vaccine is appropriate. The plan includes the safety specifications and efficacy specifications listed in Table 1 and the additional pharmacovigilance and risk minimization activities presented in Tables 2, 3, and 4.

Table 1. Safety and efficacy specifications in risk management plan

Safety specifications		
Important identified risks	Important potential risks	Important missing information
None	• Shock, anaphylaxis • Acute disseminated encephalomyelitis (ADEM) • Guillain-Barre syndrome • Convulsion • Liver dysfunction, jaundice • Asthmatic attack • Thrombocytopenic purpura/thrombocytopenia • Angiitis (allergic purpura, allergic granulomatous angiitis, leukocytoclastic vasculitis, etc.) • Interstitial pneumonia • Encephalitis, encephalopathy, myelitis • Mucocutaneous ocular syndrome (Stevens-Johnson syndrome) • Nephrotic syndrome • Narcolepsy	• Safety in vaccinated subjects
Efficacy specifiations		
• Immunogenicity in vaccinated individuals		

Table 2. Summary of additional pharmacovigilance and risk minimization activities in the risk management plan

Additional pharmacovigilance activities	Additional risk minimization activities
• Early post-marketing phase vigilance • Post-marketing clinical study of pandemic vaccine • Drug use-results survey to assess the safety of pandemic vaccine (target sample size: 3,000)	• Early post-marketing phase vigilance

Table 3. Outline of the draft drug use-results survey

Objective	To study safety of a pandemic vaccine
Survey method	Adjusted depending on pandemic vaccine vaccination system
Target population	Individuals receiving the prototype vaccine (pursuant to the National Action Plan for Pandemic Influenza and New Infectious Diseases)
Observation period	For 21 days after the first and second vaccinations of the prototype vaccine
Target sample size	3,000 individuals
Major survey items	Enrollment information on vaccinated individuals, characteristics of vaccinated individuals (sex, age, medical history, and complications, including immunodeficiency), vaccination status of the prototype vaccine, vaccination status of other vaccines, use of concomitant drugs, adverse events (fever, injection site reaction, narcolepsy and related events, and others)

Table 4. Outline of the draft post-marketing clinical study

Objective	To confirm the immunogenicity and safety of a pandemic vaccine in populations eligible for vaccination
Study design	Uncontrolled study
Target population	Individuals receiving the prototype vaccine (including members of high risk populations such as pregnant women, patients with chronic disease, and immunodeficient individuals, if possible)
Observation period	For 21 days after the first and second vaccinations of the prototype vaccine
Target sample size	50 adults, 50 children (including infants), and 50 elderly people (150 individuals in total)
Major survey items	Confirming whether the HI antibody titer after the second vaccination meets the criteria of the Prototype Vaccine Guidelines, as well as safety.

(5) Quality

1) Stability of the drug substance

In long-term stability studies of the drug substance (Review Report (1), Table 1-6), the results of the long-term stability study of NIBRG-14 strain for [redacted] months ([redacted] months in some study items) were submitted. All items met the specification values.

Based on the results of the Indo05/PR8-RG2 strain, PMDA confirmed that the shelf life for the drug substance previously defined as [redacted] months does not need to be changed.

2) Stability of the antigen preparation

In long-term stability studies of the antigen preparation (Review Report (1), Table 1-7), the results of the long-term stability study of Indo05/PR8-RG2 in upright and inverted condition for 24 months and the results of NIBRG-14 strain for [redacted] months ([redacted] months in some items) were submitted. Both strains met the specification in all items.

Based on the results of the Indo05/PR8-RG2 strain, PMDA considers it reasonable to define the shelf life for the antigen preparation as 24 months, although it has been defined as [redacted] months.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based compliance inspection and data integrity assessment

A document-based compliance inspection and data integrity assessment was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, there were no particular problems. Thus, PMDA concluded there should be no problem with conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

The data that should be submitted in the new drug application (5.3.5.1.1, 5.3.5.1.2, 5.3.5.2.3) in accordance with the provisions of the Pharmaceutical Affairs Act had underwent GCP on-site inspection as the data submitted for the already approved product. As a result of the GCP on-site inspection, PMDA had concluded there should be no problem with conducting a regulatory review based on the submitted application documents. No GCP on-site inspection was therefore conducted for this application.

IV. Overall Evaluation

As a result of the above review, PMDA has concluded that the prototype vaccine may be approved as a production model for a pandemic vaccine with the indication and dosage and administration statements shown below. Since the prototype vaccine is an orphan drug, the re-examination period is 10 years. Both drug substance and drug product are classified as a powerful drug, and the product is classified as a biological product.

[Indication]

Prophylaxis of pandemic influenza

[Dosage and administration]

The usual dosage is 2 intramuscular injections of 0.5 mL of the mixture of the antigen preparation and the proprietary emulsion adjuvant at an interval of ≥2 weeks.