

Report on the Deliberation Results

September 18, 2007

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

[Brand name] Adsorbed Influenza Vaccine (H5N1) “BIKEN”
[Non-proprietary name] Adsorbed Influenza Vaccine (H5N1)
[Applicant] The Research Foundation for Microbial Diseases of Osaka University
[Date of application] January 30, 2007

[Results of deliberation]

In the meeting held on August 31, 2007, the Second Committee on New Drugs concluded that the product may be approved and that this result was to be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

It was decided that the product is classified as a biological product, its re-examination period is 10 years, and both the drug substance and the drug product are classified as powerful drugs.

This English version of the Japanese review report is intended to be a reference material to provide convenience for users. In the event of inconsistency, the Japanese text shall prevail. PMDA shall not be responsible for any consequence resulting from use of this English version.

Review Report

August 15, 2007

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]	Adsorbed Influenza Vaccine (H5N1) “BIKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	January 30, 2007
[Application classification]	1-(1) Drugs with new active ingredients
[Dosage form/Strength]	Injectable suspension with the following constituents per mL: 30 µg (HA content) of a strain of inactivated pandemic influenza virus as the active ingredient, 0.3 mg (on an aluminum content basis) of aluminum hydroxide gel as an adjuvant, and 0.008 mg of thimerosal as a preservative
[Items warranting special mention]	· Minimum Requirements for Biological Products (draft) “Adsorbed Influenza Vaccine” has been submitted. · Orphan drug (Designated on: June 9, 2006)
[Reviewing office]	Office of Biological Products

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Review Results

August 15, 2007

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “BIKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	January 30, 2007 (Application for marketing approval)
[Results of review]	

From the submitted data, it was judged that Adsorbed Influenza Vaccine (H5N1) is expected to have a protective effect against pandemic influenza (H5N1) infection and also to prevent symptoms becoming more severe, and that there should be no serious problem in safety.

In regard to the efficacy, the results of clinical studies in Japan showed inoculation with Adsorbed Influenza Vaccine (H5N1) induced antibody production, indicating the immunogenicity of the product. In addition, the results of challenge tests in mice demonstrated that Adsorbed Influenza Vaccine (H5N1) prevented the onset of disease after infection with a virulent strain of influenza virus (H5N1). These results suggest that Adsorbed Influenza Vaccine (H5N1) may be expected to have a protective effect against pandemic influenza (H5N1) infection and to prevent symptoms becoming more severe. In regard to the safety, there were no serious adverse reactions. Taking into account the seriousness of the target disease, there may be no major problems discouraging approval of the application. However, more complete information should be provided on the use of Adsorbed Influenza Vaccine (H5N1).

Based on the results of the regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product will be approved for the following indications and dosage and administration.

[Indications]	Prophylaxis of pandemic influenza (H5N1)
[Dosage and administration]	The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses.

Review Report (1)

June 29, 2007

I. Product Submitted for Registration

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “BIKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	January 30, 2007 (Application for marketing approval)
[Dosage form/Strength]	Injectable suspension containing the following constituents per mL: 10 µg or 30 µg (HA content) of a strain of inactivated pandemic influenza virus as the active ingredient, 0.3 mg (on an aluminum content basis) of aluminum hydroxide gel as an adjuvant, and 0.008 mg of thimerosal as preservative.
[Proposed indications]	Prophylaxis of pandemic influenza
[Proposed dosage and administration]	The usual dosage is 2 injections of 15 µg (on an HA content basis) per dose administered, subcutaneously or intramuscularly. If necessary, the dose may be changed in the range between 5 and 15 µg, once or twice, administered subcutaneously or intramuscularly.
[Items warranting special mention]	<ul style="list-style-type: none">· Minimum Requirements for Biological Products (draft) “Adsorbed Influenza Vaccine” has been submitted.· Orphan drug (Designated on: June 9, 2006)

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

A summary of the documents submitted by the applicant and the answers to the questions raised by the Pharmaceuticals and Medical Devices Agency (hereinafter referred to as PMDA) are as follows.

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

Influenza is an acute respiratory disease caused by infection with influenza viruses, which belong to the family *Orthomyxoviridae*. Influenza viruses are classified by their serotypes into type A, type B, and type C. Type A influenza viruses are further classified into subtypes (H1 to H16 and N1 to N9) according to differences in the antigenicity of the viral surface hemagglutinin (HA) and neuraminidase (NA). Host animals for the influenza A viruses differ depending on the subtype of the viruses, and include humans, birds, swine, and horses; however, all the subtypes have been isolated from birds.

Subtypes H1N1 and H3N2 of type A influenza virus have been identified as the causes of repeated epidemics in humans. The repeated epidemics of influenza each year are suspected to be caused by the gradual change in the antigenicity of the virus subtypes every year due to antigenic drift, and therefore, by the inability of the influenza-specific antibodies already existing in humans to completely neutralize the viruses. On the other hand, due to antigenic shift, the influenza A viruses may mutate into new subtypes of viruses having different antigenicity and species specificity. There is concern that if this influenza virus becomes infectious to humans, the immunity already acquired in humans may not provide protection against infection, and eventually human-to-human infection with the virus may cause a worldwide pandemic. As a matter of fact, pandemics of influenza caused by novel pandemic strains of influenza viruses have already occurred 3 times in the 20th century: Spanish influenza (subtype H1N1) in 1918, Asian influenza (subtype H2N2) in 1957, and Hong Kong influenza (subtype H3N2) in 1968. These pandemics caused numerous health hazards, reportedly accompanied by reduction in economic and social activities.

Pandemic influenza is defined as “influenza epidemic in human beings caused by infection with an HA and/or NA virus subtype that has not infected humans for decades” in the “Report on Pandemic Influenza Preparedness” (August, 2004). Theoretically, it is possible for any influenza A virus subtypes other than H1N1 and H3N2 to cause pandemic influenza. Since the time human infection with a highly pathogenic H5N1 avian influenza strain was reported in Hong Kong in 1997, infections of humans with highly pathogenic H5N1 avian influenza strain have been reported from all over the world (191 deaths out of 315 cases of infection, as of June 25, 2007; www.who.int/csr/disease/avian_influenza/country/en/index.html). In the human cases of influenza confirmed to be caused by this virus until date, the disease has been observed to be very serious, in addition to a high mortality rate, being frequently complicated by systemic infection, bleeding tendency, multi-organ failure, and cytokine storm which are beyond the realm of influenza routine clinical practice. Therefore, pandemic influenza caused by this virus poses a grave threat never faced before by human beings. The H5N1 influenza virus is considered as a highly likely cause of pandemic influenza, and at present, H5N1 influenza is under surveillance for the trend of its occurrence by the WHO with extensive international cooperation. Also, pandemic influenza preparedness plans are being developed internationally.

In Japan, “Report of Investigative Committee on Pandemic Influenza Preparedness” was published on October 24, 1997, by the “Investigative Committee for Pandemic Influenza Preparedness” established in the then Ministry of Health and Welfare in May 1997. This report pointed out the necessity of development of countermeasures against seasonal influenza, which is the basis of countermeasures against pandemic influenza, of establishment of a system for manufacture of influenza vaccines, and of improvement and development of the manufacturing technology to allow an adequate response to a potential increase in demand in the event of a pandemic. Consolidation of the system has been promoted, including stipulation of “Basic Guidelines for Health Crisis Control” and establishment of

“Coordination Meeting for Health Crisis Control.” The “Law concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infections (hereinafter referred to as the “Infectious Disease Control Law”) was enforced in 1999. Based on this law, “Guidelines for Prevention of Specified Infectious Diseases Related to Influenza” was stipulated and “Liaison Meeting for Comprehensive Countermeasures against Influenza” was established as a sub-committee of “Coordination Meeting for Health Crisis Control.” In addition, the “Report on Pandemic Influenza Preparedness” was prepared in August 2004 by the “Investigative Subcommittee for Pandemic Influenza Preparedness” and published in October 2003 in *Infectious Diseases Committee of Health Sciences Council*. In this report, a direction for the development of a new vaccine against pandemic influenza was proposed. This included: (1) the necessity of development of a vaccine containing an adjuvant (auxiliary immune substance) based on the investigation conducted by Tashiro et al., as described later, (2) development of pandemic influenza vaccine based on the manufacturing method of a “mock-up” vaccine*¹, and (3) obtainment of its marketing approval in accordance with the Pharmaceutical Affairs Law.

* 1 Development of pandemic influenza vaccine based on the manufacturing method of a “mock-up” vaccine

When pandemic influenza occurs, it is not realistic to start development of the vaccine using the pandemic influenza virus strain after it has actually become established in the community, considering the times of development and manufacturing. Therefore, it would be desirable to obtain marketing approval for a mock-up vaccine prepared using a model virus instead of the pandemic influenza virus. If and when pandemic influenza occurs, the pandemic influenza vaccine will be manufactured using the pandemic influenza virus strain and supplied promptly according to the approved manufacturing method of the mock-up vaccine.

Based on the “WHO Global Influenza Preparedness Plan” published by the WHO in May 2005, each country established an action plan to be implemented at the time of occurrence, if it occurs, of pandemic influenza. In Japan, the “Pandemic Influenza Preparedness Action Plan” (hereinafter referred to as “Action Plan”) was formulated in November 2005, with the Ministry of Health, Labour and Welfare (MHLW) playing a central role, and collaboration and cooperation of other ministries and agencies concerned were ensured. This Action Plan will be applied, in principle, for the use of the pandemic influenza vaccine.

In response to the “Report on Pandemic Influenza Preparedness” prepared in August 2004, prior to the development of Adsorbed Influenza Vaccine (H5N1) [hereinafter referred to as the “H5N1 Vaccine”], the development policy for the pandemic influenza vaccine was confirmed at a meeting of MHLW, PMDA, the National Institute of Infectious Diseases, and 4 companies manufacturing influenza vaccine, which are members of Saikin Seizai Kyoukai, held in August 2004. In the Health and Labour Sciences Research conducted from 2001 to 2002 by Tashiro et al., a strain isolated from the highly pathogenic avian influenza, Hong Kong156/97 (H5N1), that was isolated in Hong Kong in 1997 was attenuated by the reverse genetics approach, and an HA vaccine and whole virus vaccine were

prepared experimentally using the attenuated strain, by an already approved manufacturing method (neither of the vaccines contained an adjuvant). In a clinical pharmacology study, no significant increase of the antibody titers was observed after inoculation of animals with these vaccines, although slightly higher neutralizing antibody titers were obtained after inoculation of whole virus vaccine than after that of the HA vaccine (<http://mhlw-grants.niph.go.jp/niph/search/NIST00.do>, Study on safety and efficacy of inactivated influenza H5N1 whole virus vaccine, 2002). Based on these results, it was decided to develop the whole virus vaccine, which shows higher immunogenicity, with the addition of the aluminum adjuvant that has been added to vaccines as an immune-auxiliary substance. A strain of NIBRG-14 obtained by attenuation, using the reverse genetics approach, of A/Viet Nam/1194/2004 (H5N1) isolated in Viet Nam in 2004 by the National Institute for Biological Standards and Control (NIBSC) of the UK was to be used as the strain for the manufacture of the mock-up vaccine.

In the Notification No. 0331007 issued by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated March 31, 2006, drugs designated as orphan drugs were defined as “pharmaceuticals used for the prevention of infectious diseases.” Among these drugs, vaccines to be designated as orphan drugs are defined as “vaccines used for the prevention of new infectious diseases which might occur due to genetic mutation or recur and of which possible occurrence or recurrence cannot be excluded. Also, any of the diseases may have a critical impact on the life and health of people once it occurs, the time of its occurrence and the magnitude of the epidemic are unknown, and there is no occurrence of the disease at the time of application for designation.” On the basis of the H5N1 Vaccine falling under the above category, orphan drug designation for the H5N1 Vaccine was applied to MHLW on April 14, 2006, and the designation was granted to the drug product (Designation No. 186) on June 9, 2006, for the proposed indication of “prophylaxis of influenza (H5N1).”

2. Data relating to quality

As described in the “Report on Pandemic Influenza Preparedness” published in August 2004, the development of the H5N1 Vaccine was urgently sought to prepare for pandemic influenza. Therefore, no sufficient data had been acquired when the application was submitted. Taking into account the information obtained from repeated inquiries to the applicant and the additional data obtained after the application, and on the basis of the revised document submitted in May 2007, the data relating to the quality of the H5N1 Vaccine is summarized below. PMDA is requesting the applicant to submit further additional data and information.

Summary of the submitted data

The H5N1 Vaccine is a vaccine. Pandemic influenza virus to be used as the seed is propagated in embryonated eggs, purified virions are inactivated using formalin, and aluminum hydroxide gel is added as the adjuvant to the inactivated virus.

(1) Drug substance

1) Manufacturing method

a. Origin and control of seed

NIBRG-14 Vero1/E2, an attenuated viral strain of A/Viet Nam/1194/2004 (H5N1) developed by the reverse genetics approach at the National Institute for Biological Standards and Control (NIBSC), UK. NIBRG-14 Vero1/E2/E1, the first subculture of NIBRG-14 Vero1/E2 in special pathogen-free (SPF) embryonated eggs, was supplied by the National Institute of Infectious Diseases, which served as the master seed. It was further propagated in SPF embryonated eggs by the applicant to obtain the NIBRG-14 Vero1/E2/E1/E1 to be used as the working seed. The following 6 parameters were investigated for the in-house in-process control of the master seed and working seed derived from the NIBRG-14 strain: HA titer (hemagglutination reaction), virus content (determination of the infectivity titer in embryonated eggs), sterility, mycoplasma, base sequence of the HA gene, and antigenicity as determined by the hemagglutination inhibition (HI) test.

These seeds were stored at -60°C or lower. The virus content of the master seed was determined after ■ months of storage and the results revealed no change.

The master seed will be renewed from the stock strain supplied by the National Institute of Infectious Diseases if the strain used for vaccine production is changed or the amount remaining is less than ■ bottles, and the working seed will be renewed from the master seed if the strain for vaccine production is changed or the amount remaining is less than that required for vaccine production for ■ months. The renewals shall be conducted using the same procedure as that used for the original seed. In case of significant decrease in the virus content, contamination by other microorganisms, or obvious change of the product caused by the seed, the relevant seed will be discarded and renewed.

At the time of development of the product, the master seed was directly used for the vaccine production until ■, 200■.

b. Manufacturing method

Virus incubation

Thaw ■ bottles of the working seed, dilute using buffer, and use as the seed for vaccine production after adding prednisolone and antibiotics as necessary.

Inoculate the seed for vaccine production into ■ to ■-day-old ■ embryonated eggs, and incubate at ■°C to ■°C under a humidity of ■ ± 10% for ■ to ■ hours. Determine the incubation temperature and time by performing a preliminary investigation on a strain basis. After the incubation, cool the inoculated eggs at ■°C for ■ to ■ hours, and collect the allantoic fluid (■ L).

Preliminary purification and purification process

Add [REDACTED] solution and [REDACTED] solution to the allantoic fluid (broth), adjust pH using buffer, filter through filters with pore sizes of [REDACTED], [REDACTED], and [REDACTED] μm to remove any foreign substances. Further [REDACTED] using the [REDACTED] filter (pore size not larger than [REDACTED] μm), add [REDACTED] solution, and filter the obtained clarified viral solution (70 L) through filters with pore sizes of 3 and 0.8 μm to remove any aggregates.

Collect the virus fraction by the [REDACTED]% to [REDACTED]% sucrose density-gradient centrifugation method using a continuous ultracentrifuge, and dilute to [REDACTED] L using buffer. Pool the virus fraction of [REDACTED] to [REDACTED] batches and use this as 1 lot. Dilute ([REDACTED] L) the virus fraction obtained by the purification using [REDACTED], filter (pore sizes of [REDACTED] and [REDACTED] μm) to remove particles, adjust to [REDACTED] L, and use this solution as the pre-inactivation purified virus suspension. Determine the percent HA content (by SDS-PAGE) using the pre-inactivation purified virus suspension as a part of the in-process control.

Inactivation process

Dilute the pre-inactivation purified virus suspension to obtain a protein content of [REDACTED] to [REDACTED] $\mu\text{g}/\text{mL}$, add [REDACTED]% (v/v) formalin containing buffer to this solution to obtain a final concentration of formalin of [REDACTED]% (v/v), and inactivate the product at [REDACTED] $^{\circ}\text{C}$. Collect the sample [REDACTED] to [REDACTED] days after the start of the inactivation, and perform the inactivation test as an in-process control test. The inactivation period should not be less than [REDACTED] times the “period from the start date of the inactivation to the start date of the inactivation test in which the results conform to the acceptance criteria.” In case the inactivation test fails, continue the inactivation reaction, and perform the inactivation test until the results conform to the acceptance criteria.

Dialysis and final filtration process

Remove any foreign substances in the inactivated virus suspension by centrifugation, concentrate and dialyze using an ultrafiltration membrane (molecular weight cut-off, [REDACTED]) to adjust the solution amount to [REDACTED] kg using phosphate buffered saline (PBS). Further add the formalin and thimerosal stabilizers to obtain final concentration of [REDACTED]% (v/v) and [REDACTED]% (w/v), respectively. Filter this solution through [REDACTED] filter with pore size of [REDACTED] μm , dilute the filtrate to make [REDACTED] kg using the buffer, store at [REDACTED] $^{\circ}\text{C}$, and use this solution as the bulk (stock solution).

c. Critical process steps, key intermediates, and process validation

The inactivation process is defined as a critical process step, because the purified pre-inactivation virus suspension is inactivated in this process; therefore, the inactivation test is specified as an in-process control test. The validation results showed that the [REDACTED] $\mu\text{g}/\text{mL}$ suspension is inactivated [REDACTED] weeks after the start of inactivation and the [REDACTED] $\mu\text{g}/\text{mL}$ suspension is inactivated [REDACTED] weeks after the start of inactivation.

The final filtration process is also defined as a critical process step, because it is the final process in

the manufacture of the bulk, aimed at removal of unnecessary fine particles and ensuring sterility of the product.

No key intermediates are specified.

d. Control of materials of human or animal origin

As for the special pathogen-free (SPF) embryonated eggs used for the seed preparation, 23 pathogens are tested in the SPF chicken listed in the Minimum Requirements for Biological Products for animal use and all eggs used were laid by these chickens after excluding the presence of any of the pathogens. The chickens from which the embryonated eggs are collected are inoculated with vaccines against Newcastle disease, avian infectious bronchitis, fowl pox, infectious bursal disease, Marek's disease, and avian encephalomyelitis, and also vaccines against infectious laryngotracheitis, egg drop syndrome-1976, infectious coryza (types A and C), *Mycoplasma gallisepticum* infection, as needed according to the epidemic situation for the respective diseases. Visual health inspection and counting of the eggs laid are also performed on a daily basis, and the antibodies in the animals after receiving vaccines against the infectious diseases (except infectious bursal disease and fowl pox) are investigated at [REDACTED] and every [REDACTED] months thereafter.

2) Characterization

For the mock-up vaccine stock solution manufactured using the NIBRG-14 strain, analyses such as sucrose density-gradient centrifugation, determination of percent HA content (by SDS-PAGE), comparison of hemagglutination property by the HA using erythrocytes from various animals, electron microscopy, high-performance liquid chromatography (HPLC), and determination of the phospholipid content (after Folch extraction, assay by phosphomolybdic color development) were performed. The bulk was determined to be homogeneous, because sucrose density-gradient centrifugation and HPLC analysis showed a monomodal peak for the HA titer and protein content (OD_{280}). SDS-PAGE was also performed under reducing and non-reducing conditions for the pre-inactivation purified virus suspension, and bands were detected at each molecular weight position of HA, NP, and M1 protein, which represent structural proteins of the influenza virus. Based on the above results of the characterization, the bulk was found to contain influenza virions.

3) Impurities

Ovalbumin and endotoxin from the embryonated eggs are considered as the major impurities potentially originating from the manufacturing process. The removal efficiency of the ovalbumin content was confirmed in the validation to be about 10^{-7} to 10^{-6} times at the preliminary purification process and purification process for [REDACTED] lots. Ovalbumin is also included in the specifications for the bulk, and the actual measured values for the [REDACTED] lots were in the range of 2.16 to 3.55 ng/mL.

As shown in Table 1, batch-to-batch differences of the endotoxin content in the allantoic fluid were

found, which were attributable to the differences in the status of the embryonated eggs. However, endotoxin was removed to not more than 1.63% (residual rate compared to that in the allantoic fluid) during the manufacturing processes, and the actual measured values in the bulk were in the range of 49.3 to 0.18 EU/mL. Bacterial endotoxins test is also included in the specifications for the bulk.

Table 1: Endotoxin content (EU/mL) and residual rate after major manufacturing processes

Stock solution lot No.	Batch No.	Parameter	Incubation process	Preliminary purification process	Purification process		Final filtration process
			Allantoic fluid	Clarified virus solution		Pre-inactivation purified virus suspension	Stock solution
A	G	Endotoxin content	1700	370	→	230	23.3
		Residual rate (%)	100	3.19		0.14	0.02
B	H	Endotoxin content	3100	240	→	73	15.7
		Residual rate (%)	100	1.11		0.03	0.01
C	I	Endotoxin content	6800	680	→	260	49.3
		Residual rate (%)	100	1.56		0.04	0.01
D	J	Endotoxin content	7300	11	→	0.63	0.33
		Residual rate (%)	100	0.02		0.00	0.00
E	K	Endotoxin content	0.26*	0.48	→	0.52	0.52
		Residual rate (%)	100	16.6		1.89	1.63
F	L	Endotoxin content	0.30*	1.0	↗		
		Residual rate (%)	100	31.4			
F	M	Endotoxin content	0.25*	0.68	→	< 0.25	0.18
		Residual rate (%)	100	24.7		< 1.00	0.57
F	N	Endotoxin content	0.25*	0.69	↗		
		Residual rate (%)	100	24.4			

* Not the actual measured value, because 1 batch consists of multiple tanks, and it is the weighted average of the measured values for each tank depending on the amount of each solution.

4) Specifications and test methods

The staining (Gram staining), sterility test, inactivation test (for confirming the absence of virus proliferation after 3 passages of subculture in embryonated eggs), protein content, HA content (by single radial immunodiffusion [SRD]), pyrogen test, bacterial endotoxins test, and ovalbumin content (ELISA method) are included in the specifications for the bulk.

When determining the HA content (by SRD), in the event that SRD reagents [standard antigen and antiserum, see “5) Reference standard or standard substance”] are not available, the HA content is calculated by multiplying the protein content by the percent HA content. The percent HA content is calculated using the pre-inactivation purified virus suspension by performing SDS-PAGE as an in-process control parameter.

The following are not included in the specifications, but were investigated: virus content (determination of chicken red cell agglutination [CCA] titer), formaldehyde content, thimerosal content, pH, and leukocyte reduction in mice. The virus content (by CCA) is determined as a part of the in-house in-process control to confirm the percent recovery in the manufacturing process; however, it was excluded from the specifications for the following reasons: the CCA titers vary among the strains used for vaccine production; the results are poorly reproducible; and the HA content (by SRD) included in the specifications ensures an indicator of the virus content. The formaldehyde content, thimerosal content, pH, and others are not included in the specifications for the bulk, because they are

controlled as the specifications of the drug product. The leukocyte reduction in mice was not included in the specifications for the following reasons: the substance which triggers the leucocyte reduction remains unclear; no evidence was provided about its relation to the adverse reactions in humans; and there is poor justification for use of this parameter to evaluate the safety of the bulk.

5) Reference standard or standard substance

As the reference standards for the specifications for the bulk, standard influenza HA antigen (for SRD), reference anti-influenza HA antiserum, and standard albumin for protein assay are used, and all are supplied by the National Institute of Infectious Diseases. The standard influenza HA antigen (for SRD) is stored under light-shielding condition at -20°C to -30°C, except for the initial lot which was set to be stored under light-shielding condition at 10°C or lower. The reference anti-influenza HA antiserum and standard albumin for protein assay are stored at 10°C or lower and under light-shielded condition at 4°C or lower, respectively. No standard substances are used.

6) Stability

Long-term testing was performed under the condition of $\blacksquare^{\circ}\text{C} \pm \blacksquare^{\circ}\text{C}$, and the data for \blacksquare months was submitted at the time of the application, and the data for \blacksquare months and \blacksquare months were submitted at the time of the review. In addition to the 8 parameters in the specifications, a total of 12 parameters, including the CCA titer, pH, thimerosal content, and formaldehyde content, were investigated. No significant changes were noted, and all the acceptance criteria were met. This testing will be continued for \blacksquare months.

Accelerated testing was performed up to 6 months under the conditions of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $60\% \pm 5\%\text{RH}$. In addition to the same \blacksquare parameters as the long-term testing, the fraction test (sucrose density-gradient centrifugation) and electron microscopy were performed. No changes were detected in the chemical properties such as sterility and pH, fraction test, and electron microscopy. However, the protein content and HA content (by SRD) tended to decrease with increasing storage period; the protein content decreased to about $\blacksquare\%$ to $\blacksquare\%$ at \blacksquare months, and the HA content decreased to about $\blacksquare\%$ to $\blacksquare\%$. In contrast, the CCA titer tended to increase.

(2) Drug product

1) Product formulation and manufacturing method

The H5N1 Vaccine is an injectable suspension containing, per mL, 10 or 30 µg (on an HA content basis) of inactivated pandemic influenza virus and 0.3 mg (on an aluminum content basis) of aluminum hydroxide as an adjuvant. As for other excipients, the H5N1 Vaccine contains 0.4 mg of potassium dihydrogen phosphate, 2.5 mg of sodium hydrogenphosphate, and not more than 8.03 mg of sodium chloride. Furthermore, 0.008 mg of thimerosal is added as a preservative. A total of [REDACTED] types of the drug products with 10 or 30 µgHA/mL, filled in 1 or 10 mL glass vials have been submitted for regulatory review.

The bulk is weighed based on the HA content (determined by SRD, or in the event that SRD reagents are not available, it is calculated by multiplying the protein content by the percent HA content obtained by SDS-PAGE), and diluted with PBS. Then thimerosal and aluminum hydroxide, prepared as follows, are added sequentially. Aluminum hydroxide is prepared by mixing and agitating the [REDACTED] solution and the [REDACTED] solution and by [REDACTED] the obtained [REDACTED]. The parameters such as identification, pH, aluminum content, purity (heavy metals, arsenic, and sulfate), abnormal toxicity, bacterial endotoxins, sterility, particle size distribution, and protein adsorption capacity are included in the specifications for aluminum hydroxide.

The final bulk obtained by mixing the excipients with the bulk is allowed to flow under agitation and the drug product is prepared by filling the final bulk into glass vials.

2) Specifications and test methods

The parameters such as pH, aluminum content, protein content, thimerosal content, formaldehyde content, sterility, inactivation, abnormal toxicity, potency (by SRD), extractable volume, description, insoluble foreign matter, osmolarity, and labeling are included in the specifications for the drug product.

For the potency (by SRD), in case SRD reagents are not available, the HA content is calculated using the percent HA content.

These parameters were included in the specifications in reference to those for “influenza vaccine” in the Minimum Requirements for Biological Products of the approved whole virus influenza vaccine not containing any adjuvants; however, the following 4 parameters were omitted: leukocyte reduction in mice, virus content, the weight loss in mice, and potency by the egg neutralization. For the leukocyte reduction in mice, the substance which triggers this phenomenon is not yet specified, and no evidence has been provided about its relation to the adverse reactions in humans, and thus, there is poor justification for the use of this parameter to evaluate the safety of the drug product. In regard to the virus content, the CCA titer cannot be determined in the presence of the adjuvant aluminum hydroxide,

and the potency determined by SRD, which is separately included in the specifications for measurement of the virus content. The weight loss in mice provides a lower sensitivity compared to the abnormal toxicity test using guinea pigs included in the specifications. The potency by the egg neutralization is time-consuming and not really necessary, because SRD is included in the specifications to determine the potency.

3) Reference standard and standard substance

Reference standard used for the specifications for the drug product is the same as that used for the specifications for the bulk.

4) Stability

Because there are ■ types of formulation for the drug product, the parameters were divided into those showing larger variability and those showing little variability in results over time. The data were obtained from the 15-month long-term testing by applying appropriate measurement periods to respective formulations. Under the condition of $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$, in addition to the 13 parameters in the specifications except the labeling, a total of 20 parameters, including bacterial endotoxins, leukocyte reduction in mice, CCA titer, weight loss in mice, ovalbumin content, protein content (supernatant centrifugation) to confirm the adsorbed preparation, and immunogenicity in mice were investigated for 3 lots of the drug product with an HA content of $30\ \mu\text{g}$ ($30\ \mu\text{gHA}$)/1 mL/vial. In addition, 17 of these parameters, excluding the sterility, net volume, and insoluble foreign matter, were investigated for 3 lots of the drug product containing ■ μgHA /■ mL/vial. For 3 lots of 10 mL/vial product of $30\ \mu\text{gHA/mL}$, 8 parameters, including the pH, protein content, sterility, potency (by SRD), description, bacterial endotoxins, protein content (supernatant centrifugation), and immunogenicity in mice were investigated. For 3 lots of ■ mL/vial product of ■ $\mu\text{gHA/mL}$, 7 of these 8 parameters, excluding the sterility, were investigated. As a result, all of the test parameters conformed to the specifications at 12 months, and no significant change was noted. The data at ■ months are currently being collected.

Accelerated testing was conducted for 6 months under the storage condition of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with $60\% \pm 5\%\text{RH}$. The same 20 parameters as those included for the long-term testing were investigated for 3 lots of the $30\ \mu\text{gHA}/1\ \text{mL/vial}$ product, and 15 of these parameters, excluding 5 parameters, namely, the abnormal toxicity, net volume, insoluble foreign matter, leukocyte reduction in mice, and weight loss in mice, were investigated for 3 lots of the ■ $\mu\text{gHA}/■\ \text{mL/vial}$ product. For the 10 mL/vial product of the $30\ \mu\text{gHA/mL}$ formulation, 14 parameters, excluding the aluminum content, thimerosal content, formaldehyde content, viral inactivation, leukocyte reduction in mice, and weight loss in mice, were investigated, and for the ■ mL/vial product of the ■ $\mu\text{gHA/mL}$ formulation, 13 of these 14 parameters, excluding the abnormal toxicity, were evaluated. All of the test parameters were found to conform to the specifications.

Photostability was analyzed for samples of 3 types of the drug product in the sealed vial, namely, that

without packaging, that packaged in a paper box (the proposed commercial packaging), and that light-shielded using aluminum foil, exposed to illumination of 5000 ± 200 lx, at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $60\% \pm 5\%\text{RH}$ for 0, 1, 5, 10, and 15 days. No change was noted in the pH, description, osmolarity, and protein content following light exposure even for the non-packaged product; however, SRD revealed absence of potency at day 5 for the non-packaged product. No change in the HA content was detected by SRD until Day 15 under the conditions of commercial packaging and light-shielding. The results suggested that attention should be paid to storing conditions, such as light-shielding, when the product is used in clinical practice, because the HA content as determined by SRD was affected by light exposure.

Outline of review by PMDA

As for the items for which sufficient additional information is not available and the specifications for the bulk and drug product of which review has not yet been completed, the summary will be provided in the Review Report (2).

(1) Seed lot system

PMDA asked the applicant to explain the handling of other strains and the time necessary to establish a seed lot system, because the applicant used the seed of the NIBRG-14 strain supplied by the National Institute of Infectious Diseases to manufacture the lots for the testing described in the submitted data, without preparing a seed lot system.

The applicant responded as follows.

Separately from the NIBRG-14 strain, the A/Indo/05/2005 (H5N1) PR8-IBCDC-RG2 strain (Indonesian strain) was used for the manufacture of the H5N1 Vaccine. The original seed of Indonesian strain was supplied by the National Institute of Infectious Diseases, and used for preparing the master seed, which was also used as the working seed. For the establishment of a seed lot system, consisting of both the master seed and the working seed, it would take ■ days from the ordering of the SPF eggs until the working seed becomes available, by simple calculation. If the SPF eggs are ready in advance, it would take ■ days. If the master seed is also used as the working seed, it would take ■ days from the ordering of the SPF eggs until the seed becomes available for vaccine production.

PMDA understood that there is a possibility of starting vaccine production shortly after the preparation of the seed (master seed) in case of an emergency, such as the outbreak of a pandemic; however, PMDA recommends the preparation of seed lots because a seed lot system should be established, in principle, under a less urgent situation, namely, a pre-pandemic.

The applicant responded as follows.

It is planned to establish a seed lot system, in principle, except in the event of the necessity of faster

start-up of vaccine production, such as the outbreak of a pandemic, under which condition, the master seed itself may be used as the working seed.

As for the NIBRG-14 strain which was used for the relevant mock-up vaccine, the master seed was prepared by Saikin Seizai Kyokai under contract from the National Institute of Infectious Diseases. The master seed was supplied to the applicant after the National Institute of Infectious Diseases performed some of the seed control tests and confirmed that the HA gene sequence and other properties were the same as those in the original seed. PMDA requested the applicant to prepare the seed lot and perform the control tests in the future, because there may be cases where no exchange of samples would be possible between the National Institute of Infectious Diseases and vaccine manufacturers, including the applicant, at the time of an emergency. The applicant responded that it would be prepared to establish a seed lot system, including conducting the control tests for the seed by themselves through technology transfer from the National Institute of Infectious Diseases.

PMDA also asked the applicant to confirm the virus content, at the time of thawing of the seed, because stability for ■ years or longer was not confirmed in the seed of the NIBRG-14 strain, and it is likely that stability varies among different strains. The applicant responded that the HA test (hemagglutination reaction) and virus content test (determination of infectivity using embryonated eggs) would be performed as a part of the in-process control each time the master seed was thawed to prepare the working seed and each time the working seed is used for the first batch in continuous manufacturing.

PMDA accepted the above response.

(2) Manufacturing process control of the bulk

At the time of the review of the manufacturing process of the bulk, the information submitted after the initial application, about the Indonesian strain manufactured separately, was also evaluated.

1) Setting of the critical process steps

From the point of view of ensuring sterility, the sterile filtration process during the manufacture of the bulk is important because the H5N1 Vaccine is an adsorbed preparation, and sterile filtration cannot be applied after preparation of the final bulk during the manufacture of the drug product. Therefore, PMDA requested the applicant to additionally include in the process control the integrity test of the filter used in the final filtration process, and the applicant took the appropriate action.

In addition, PMDA requested the applicant to set the virus incubation process and purification process as critical process steps, because these processes are considered to have an impact on the quality of the bulk. The applicant added these to the critical process steps and explained the control method as follows. As for the virus incubation process, the HA (hemagglutination reaction) in the allantoic fluid

will be determined as a part of the in-process control. When manufacturing results of each strain for [REDACTED] batches are accumulated, the acceptance criteria will be set at not less than “average – [REDACTED]SD,” and each time the test results for additional [REDACTED] batches are accumulated, the acceptance criteria will be revised. Setting of an upper limit is considered to be unnecessary because the working seed is incubated for only [REDACTED] passages in embryonated eggs, and the proliferative activity is not expected to change. As for the purification process, the HA (hemagglutination reaction) in the pre-inactivation purified virus suspension will be determined as a part of the in-process control. When manufacturing results of each strain for [REDACTED] batches are accumulated, the acceptance criteria will be set at not less than “average – [REDACTED]SD,” and each time additional results are obtained, the acceptance criteria will be revised. The acceptance criteria for the HA in the allantoic fluid and the pre-inactivation purified virus suspension prepared from the NIBRG-14 strain and Indonesian strain, both of which have been already manufactured at this time, are not less than [REDACTED] times and not less than [REDACTED] times, respectively, for the NIBRG-14 strain, and not less than [REDACTED] times and not less than [REDACTED] times, respectively, for the Indonesian strain; these values differed significantly depending on the strain.

PMDA considers it desirable to set the acceptance criteria at this time so that any change in the viral proliferation rate and degree of purification can be detected appropriately. Because there may be significant differences in the characteristics depending on the strain and the pre-investigation time may be insufficient in the event of an emergency, setting of the acceptance criteria after accumulation of the manufacture results for each strain cannot be avoided. Nevertheless, PMDA requested the applicant, in the case that any lots with significant difference from other lots appeared, to take the necessary measures, such as reviewing the root cause and investigating the appropriateness of the release of the lots, even before the acceptance criteria are set. The applicant responded that an appropriate action would be taken, and PMDA accepted this.

2) Virus incubation process

Because it was stated that antibiotics and prednisolone would be used as needed for the seed for vaccine production, which is inoculated into embryonated eggs, PMDA requested the applicant to specify the types and concentrations of the antibiotics and the concentrations of prednisolone.

The applicant responded as follows.

The concentrations of prednisolone and of each antibiotic have been already determined by the antibacterial test, they therefore would be specified in the approval certificate. The necessity of prednisolone and types of antibiotics would be determined as follows. As for the antibiotics used, based on the results of the manufacture of [REDACTED] of [REDACTED], piperacillin sodium for injection, which has a broad antibiotic spectrum against Gram-negative bacteria, Gram-positive bacteria, and Bacteroides of anaerobes, and cefmetazole sodium for injection, which has a high antibacterial activity against β -lactamase producing bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, etc., are added regularly. However, for the manufacture using the

NIBRG-14 strain, there were cases where additional antibiotics were used depending on the bacteria detected at the time of manufacture of the HA vaccine as follows: gentamicin sulfate injection, which exhibits high antibacterial activity against *Pseudomonas aeruginosa*, and minocycline hydrochloride for injection, which exhibits high antibacterial activity against *Maltophilia*, considering the situations, such as when a large amount of vaccines was needed to be manufactured within a short period of time, taking seasons into account, or when some lots had a high endotoxin content. As discussed above, the necessity or types of antibiotics could not be determined uniformly, except for the 2 types of antibiotics that are used regularly. Prednisolone was added for the purpose of [REDACTED] from the experience at [REDACTED]. For the new strains to be used in the future, however, incubation is to be performed under the condition of spike or non-spike of prednisolone before the start of the manufacture, and it should be added in case [REDACTED].

PMDA asked the applicant to explain whether these antibiotics and prednisolone would be removed appropriately in the subsequent manufacturing processes.

The applicant responded as follows.

Influenza HA vaccines are manufactured by the same purification process as that of the H5N1 Vaccine until a clarified virus suspension is obtained. The residual concentrations of antibiotics in the clarified virus suspension of influenza HA vaccines were determined by HPLC, resulting as follows: In cases of [REDACTED] mg/mL of cefmetazole sodium, [REDACTED] mg/mL of piperacillin sodium, and [REDACTED] µg/mL of gentamicin sulfate used for the seed for vaccine production, the concentrations were less than the detection limits of [REDACTED] µg/mL, [REDACTED] µg/mL, and [REDACTED] µg/mL, respectively. After the purification process, the solution is diluted not less than $[REDACTED] \times 10^{[REDACTED]}$ times to obtain the drug product and the theoretical residual concentration in the drug product would be less than [REDACTED] to [REDACTED] pg/mL. The residual amount for minocycline is also being calculated in the same way. The concentration of prednisolone in the seed for vaccine production was [REDACTED] µg/mL, but the residual concentration in the collected allantoic fluid was less than the detection limit ([REDACTED] ng/mL). The theoretical residual concentration in the drug product would be less than [REDACTED] ag*/mL, because the solution is diluted not less than $[REDACTED] \times 10^{[REDACTED]}$ times to obtain the drug product. These results reveal that the antibiotics and prednisolone are appropriately removed.

Although the embryonated eggs are controlled similarly to the process followed in the manufacture of the already approved influenza HA vaccines, the quality assurance of the embryonated eggs is actually limited because the necessity of antibiotics depends on the condition of the embryonated eggs. Therefore, it is understandable that there are cases where antibiotics are/are not necessary and the non-uniform control cannot be avoided. Meanwhile, PMDA requested that regularly used cefmetazole sodium and piperacillin sodium not be used in the future, because they are β-lactam antibiotics, use of

* ag represents attogram. Atto (a) is lower than nano and femto.

which is recommended against by the WHO inactivated influenza vaccine guideline (WHO TRS No. 927, 2005), although the removal status of the current antibiotics will be accepted as satisfactory, provided that the residual concentration of minocycline hydrochloride is confirmed in the data to be submitted for review in the future. The applicant responded that it would not use these antibiotics in the future, that it would search and use other antibiotics with a high antibacterial activity against bacteria producing high levels of endotoxin, which were isolated from embryonated eggs in the past, and that it would investigate the residual amounts in the drug product. PMDA accepted this response.

3) Inactivation process

The influenza virus can be inactivated only in the inactivation process, which is considered as a critical process step that may affect the safety. The inactivation test was to be performed as an in-process control test. The inactivation period was set at not less than ■ times the inactivation treatment period at the time of confirmation of the conformity to the acceptance criteria, however, no upper limit was specified. PMDA asked the applicant whether it was necessary to specify the upper limit from the point of view of ensuring consistent quality.

The applicant responded as follows.

Based on the results obtained so far, the upper limit should not exceed ■ days from the completion day of the shortest inactivation period, and the inactivated virus suspension is controlled as a key intermediate. In addition to the inactivation test, the protein content and formaldehyde content were included in the in-process control parameters, as described below.

It is specified in the application dossier that the inactivation was achieved by allowing the mixture to stand still after the addition of formalin and agitation for ■ seconds. PMDA asked the applicant to explain the justification of the homogeneity with agitation for ■ seconds and the validation of the inactivation process. The applicant additionally performed a validation using phosphate buffer as the mock solution and obtained a sufficiently homogeneous mixture under the agitation condition. Also, as shown in Table 2, the applicant illustrated that all the samples initially collected (■-■ days after the start of the inactivation) conformed to the acceptance criteria in the manufacture of total ■ lots of the bulk, including those obtained using the Indonesian strain. The applicant added explanation that in the protein concentration range of ■ to ■ μg/mL, the samples conformed to the acceptance criteria for the inactivation test at ■ to ■ days, most conforming by ■ days.

Table 2: Conditions of the inactivation process and days to conformity in the inactivation test

Stock solution lot No.	Days to conformity in the inactivation test	Inactivation period* (°C)	Protein content (µg/mL)**		Formaldehyde content
			Target value	Actual measured value	(µg/mL)
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■
■	■	■	■	■	■

FPBMZ, Vietnam strain; FPBMW, Indonesian strain

* Period is shown by days.

** Protein content in the sample

■■■■■■■■

N.T., not tested

PMDA asked the applicant to explain the detection limit of the inactivation test, because the detection sensitivity of infectious viruses possibly lowers due to the coexistence of formalin in the sample.

The applicant responded as follows.

The evaluation was performed by allowing 3 passage subcultures in embryonated eggs so that the sensitivity would be higher than that of the inactivation test used for seasonal influenza HA vaccines. When the detection sensitivity was confirmed by the additional test, the virus detection sensitivity (maximum dilution factor) in the absence and presence of ■% (v/v) formalin was 10■ and 10■, respectively. The detection sensitivity was almost equivalent, although it was slightly lower in the presence of formalin. The testing will be repeated in the future to determine the reproducibility of the results.

In addition, PMDA requested the applicant to control the protein content and formaldehyde content after the completion of the inactivation period to ensure that the inactivation treatment was performed in the validated range. The applicant included these parameters in the in-process control and responded that the immediate acceptance criteria would be average of the actual measured value ± ■SD. The concentration is set to the preset concentration ± ■% for the protein content and ■ to ■ µg/mL for the formaldehyde content. The applicant will revise the acceptance criteria appropriately when the actual manufacturing results are obtained. PMDA accepted the above response.

4) Purification process

PMDA evaluated the validity of the purification process in terms of the purification rate and percent recovery.

Because the methods for measuring the active ingredient differ between the intermediate and the bulk, an increased purity of the active ingredient is of limited value in evaluating the purification rate. Therefore, the purification process was evaluated as follows, based on the impurity content obtained by using a highly quantitative testing approach through the process ξ s .

As shown in “*Summary of the submitted data, (1) Bulk, 3) Impurities, Table 1,*” PMDA asked the applicant to explain the reason why endotoxin content showed almost [REDACTED] times difference between [REDACTED] to [REDACTED] EU/mL for Lots [REDACTED] to [REDACTED] and [REDACTED] to [REDACTED] EU/mL for Lots [REDACTED] to [REDACTED] in [REDACTED] batches of allantoic fluid. The applicant responded that it was likely that some of the embryonated eggs used for [REDACTED] to [REDACTED] had a high endotoxin content, because the manufacture needed to be started within a few months ([REDACTED] 20[REDACTED]) after the manufacturing plan was established urgently, and the surplus eggs that had reached market was used after confirmation that they met the acceptance criteria. PMDA made the following comment on endotoxin content: In case of an emergency, only eggs with a high endotoxin content may be available. However, egg-derived endotoxin is considered to be controlled appropriately because adequate antibiotics are selected to add to the seed for vaccine production, and the endotoxin level is monitored as a part of the in-house in-process control in each purification process and also included in the specifications for the bulk, Actually, the residual rate was not more than [REDACTED]% for the stock solution purified from the allantoic fluid with a high endotoxin content, and the endotoxins were removed at a rate equivalent to or greater than that for Lots [REDACTED] to [REDACTED] (residual rate [REDACTED]% to [REDACTED]%) with a low endotoxin level in the allantoic fluid.

In regard to the removal status of ovalbumin, as shown in “*Summary of the submitted data, (1) Bulk, 3) Impurities,*” the applicant judged that the impurities were appropriately removed at a constant rate. The specific activity (CCA titer/protein content) of the bulk was [REDACTED] to [REDACTED] (average, [REDACTED]; standard deviation, [REDACTED]), and no significant difference was noted among lots. From the above, PMDA has determined that impurities can be removed adequately by the proposed purification process.

PMDA then asked the applicant to explain the percent recovery, and made the following comments based on the information shown in response. The protein content and variation coefficient of the stock solution obtained from 1 egg was [REDACTED] \pm [REDACTED] μ g/egg and less than [REDACTED]%, respectively, from the actual manufacturing result provided so far for [REDACTED] lots of NIBRG-14 strain. The percent recovery of protein was judged to be stable. The HA content of the stock solution originating from 1 egg was about [REDACTED] μ gHA for the NIBRG-14 strain, on the basis that the percent HA content was [REDACTED]% \pm [REDACTED]% (result for [REDACTED] lots of NIBRG-14 strain), and therefore about [REDACTED] doses would be obtained from 1 egg in the case the

clinical dose was set at 15 µgHA/dose. From the manufacturing result of ■ lots of the Indonesian strain, the HA content of the stock solution originating from 1 egg was about ■ µgHA/egg, corresponding to ■ doses. The difference among the strains was thought to be associated with the proliferation in the embryonated eggs. From the above, PMDA has considered that the active ingredient with stable percent recovery would be obtained from the same strain using the proposed manufacturing process, but that, however, it is necessary to take into account proliferative activity of the strain to be used in the embryonated eggs at the time of establishing the manufacturing plan.

(3) Characterization of the bulk

PMDA considers it necessary to determine whether or not influenza HA vaccine of the H5N1 subtype produced by the reverse genetics approach retains the whole-virus structure with a lower antigenicity than the seasonal influenza HA vaccine (*Phil. Trans. R. Soc. Lond.*, 2001; 356: 1953-1960) and a higher antigenicity than the HA vaccine (*Virus Res.*, 2004; 103: 163-171, *Lancet*, 2003; 362: 1959-1966). Because the HPLC analysis revealed the peak of virions was outside the range of the molecular weight for the column, PMDA asked the applicant to explain about impurities to be detected by the analysis. The applicant showed that egg-derived impurities could be detected based on the analysis results of samples of allantoic fluid added to the bulk, and stated that impurities in the molecular weight range which could be covered by the HPLC column used were detectable. PMDA considers that it is difficult to detect the degradation of virions with high sensitivity by the HPLC column, taking into consideration the size of the virions. Actually, when the analysis was performed by adding the stock solution of influenza HA vaccine to the stock solution of the H5N1 Vaccine, a peak of HA was detected at a position very close to that of the virions. PMDA considers that a comprehensive evaluation using other methods, such as the sucrose density-gradient centrifugation, is required to detect the degradation of virions. The sucrose density-gradient is described in the next section, “(4) Stability of the bulk.”

The applicant determined the bands detected on SDS-PAGE as HA, HA1, and HA2 proteins of influenza virus based only on the molecular weight. PMDA asked the applicant whether it was necessary to confirm the bands were actually HA1 and HA2 proteins, such as by amino acid sequence analysis and use of specific antibodies, and to check the inclusion of the influenza virus in the bulk.

The applicant performed additional analyses and explained as follows.

When the bands detected on SDS-PAGE, the prospective HA1 and HA2, under the reducing condition were transferred to a membrane, and the analysis up to 5 N-terminal amino acid residues was performed, only amino acids (No. 343-347) of HA of NIBRG-14 strain were detected from the band corresponding to the molecular weight of HA2. Signals of multiple amino acids were detected from the band corresponding to the molecular weight of HA1, and the largest peak was consistent with the amino acid sequence (No. 17-21) of the HA protein, except for cysteine which can not be identified by the analysis. When western blotting was performed on SDS-PAGE under the non-reducing and

reducing conditions using sheep anti-HA serum for SRD, bands were detected corresponding to the molecular weights of about 76 kDa, 53 kDa, and 23 kDa. The above findings strongly suggested that the bands detected on SDS-PAGE corresponded to HA, HA1, and HA2 proteins. PMDA accepted the above explanation of the applicant.

(4) Stability of the bulk

As for the long-term stability of the bulk, the data at ■ months was additionally submitted, and no significant abnormalities were noted in relation to the parameters tested. The HA content (by SRD) and CCA titer, indicators of the content of the active ingredient, showed no tendency towards decrease: in fact, the CCA titer increased. PMDA asked the applicant to explain this finding.

The applicant responded as follows.

The CCA titer corresponds to the HA antigen (virus) content, however, an increase in the content caused by storage is unlikely. Even though the HA content was determined by the SRD based on a different principle, it showed no tendency towards increase. In the light of these findings, it was considered that HA antigen did not change. Since there might be some factors affecting the determination of the CCA titer, it is planned to additionally investigate the factors and submit the results in the future. PMDA accepted this response of the applicant and will review the results when they are submitted in the future.

PMDA requested the applicant to demonstrate the stability of the higher-order structure, because the retention of the higher-order structures of the whole virions is an important factor in the efficacy of the product, even though the content of the active ingredient has already been evaluated by the long-term testing as being stable. The applicant submitted the results of the additionally performed electron microscopy and fraction test (determination of the HA titer by the fraction pattern) by sucrose density-gradient centrifugation for the sample at ■ months of long-term storage, and explained that there was no change even after ■ months of storage as compared with that stored for ■ months. Because a shoulder observed on the low density side of the virion peak at ■ months of storage was clearly larger than that observed after ■ months of storage in the fraction test results by the sucrose density-gradient centrifugation, PMDA asked the applicant to explain the impact of this change on the efficacy and safety.

The applicant explained as follows.

The detection of the HA titer at the lower sucrose density may suggest the change in the description relating to the precipitation rate of the virus, especially the degradation of virions. However, almost no degradation products were detected by electron microscopy and some lots showed a similar shoulder in the fraction test even at █ months. Therefore, it is considered that the change in question is attributable to the test method or the characteristic of the stock solution, and has no impact on the efficacy and safety.

PMDA considers as follows.

No change was noted in HA titer integration value in the fraction obtained by sucrose density-gradient centrifugation, although the accelerated testing revealed a decrease in the HA content to █% to █% after █ months of the storage. This finding suggests that degradation of virions corresponding to an HA content decrease of about █% to █% may be unable to be detected by the method. However, comprehensive evaluation based on the results of the fraction by sucrose density-gradient centrifugation, electron microscopy, and determination of the HA content (by SRD) would be the only way because it is also difficult to detect degradation of virions with high sensitivity by HPLC analysis and other methods [see previous section “(3) Characterization of the bulk”]. In addition, the low antigenicity of the H5N1 influenza virus makes it difficult to appropriately confirm using animals the immunogenicity (efficacy) which reflects the higher-order structure of the bulk not containing adjuvant. Nevertheless, it is necessary to adequately take into account the limitations of detection of the higher-order structure of virions when evaluating quality and stability test results.

PMDA requested the applicant to specify an expiration period for the bulk, because this was not proposed at the time of the application. The applicant specified the expiration period as █ months based on the additionally submitted results, and PMDA accepted this. When the stability at █ months is confirmed with the ongoing long-term testing, a partial change approval application to extend the expiration period to █ months will be filed.

PMDA also requested the applicant to investigate the stability of the bulk manufactured from a new strain, at least by the testings to evaluate the active ingredient of the bulk (e.g., protein content, HA content [by SRD], fraction by sucrose density-gradient centrifugation), until sufficient information is accumulated to determine the differences among strains, because the stability of a product manufactured from a different strain may differ. The applicant accepted this request and responded that it would perform the long-term testing for up to █ years also in the Indonesian strain which was used for the manufacture.

(5) Manufacturing process of the drug product

No in-process control tests were included in the manufacturing process of the drug product and the filling process was the only critical process. However, in response to the PMDA's request, the final

bulk adjustment process was added to the critical process step, in the light of sterility assurance of the drug product. As for the filling process, PMDA requested the applicant to add confirmation of the fill volume and sealing performance to the in-process control tests, and the applicant responded that it would take an appropriate action.

The sterility and fill volume in the filling process were evaluated as the process validation. However, because the H5N1 Vaccine is a suspension, the following are also considered to be important: the agitation condition; homogeneity of the fill volume of the active ingredient at filling; and adsorption of the adjuvant. Therefore, PMDA requested the applicant to appropriately define these conditions and perform the process validation, if necessary. The applicant responded that it would submit the additional test results to confirm homogenous filling of other adsorbed preparations and the mock solution manufactured using the same filling machine as that would be used for the manufacture of the H5N1 Vaccine. The applicant also stated that it would justify the filling condition by performing the concurrent validation at the time of the actual manufacture in the future, because the drug product would not be actually manufactured unless the direction from the national government to manufacture the drug product is issued in the event of a pandemic. PMDA accepted this response.

(6) Stability of the drug product

The long-term testing results of the drug product at 15 months were additionally submitted, and no specific change in quality was noted.

PMDA considers that the immunogenicity in mice as a useful test parameter to directly confirm the efficacy, however, the quantitative performance is not high because no decrease in immunogenicity in mice was detected, even though decrease of about 10% to 20% in the protein content and potency (by SRD) were shown in the results at 6 months of accelerated testing. On the other hand, while the result of the immunogenicity test in mice is expressed as ED₅₀, the antibody titer is determined as quantitative numerical values in the test, and it is considered possible to evaluate the values in terms of the immunogenicity in mice in a more quantitative manner by taking into consideration the data of the reference sample evaluated at the same time. PMDA requested the applicant to show the immunogenicity in mice as quantitative values, and to evaluate it by referring to the antibody titer relative to the reference sample in order to correct the daily difference and individual variation between animals in the measurement of the antibody titer.

The applicant showed the immunogenicity in mice as the antibody titer relative to the reference sample according to the parallel assay method and explained as follows. The parallel assay method is the analytical method applicable to cases having parallelism and linearity of the regression line. The parallelism and linearity of the test results were not adequate or appropriate for evaluation; however, no change due to the storage period was noted, although there was a large variability when the relative titer was calculated. In regard to the precision of the immunogenicity test in mice of which result was

expressed as ED₅₀ in the application dossier, the variation coefficient was ■%, and the approximately 10% to 20% of decrease in the protein content and potency (by SRD) may not be detected.

PMDA considers as follows.

As the applicant explained, the evaluation using the relative value to the reference sample is difficult, and the effects of the individual differences in mice are considered to be significant in the evaluation using ED₅₀. In addition, the quantitative performance of the immunogenicity test in mice cannot be adequate when the precision of the test method is concerned. Meanwhile, the SRD potency test provides quantitative results for evaluating the efficacy, but it does not directly evaluate higher-order structures or immunogenicity, because it is a testing system employing immunophoresis after solubilizing virions. Based on the fact that the immunogenicity test results in mice revealed no change despite the decrease of about 10% to 20% in the potency as detected by SRD, it would be possible to say that no impact of a decrease in potency of this level was observed on the immunogenicity in mice. Nevertheless, it is more appropriate to consider that the decrease actually could not be detected, given the weak quantitative performance of the immunogenicity test in mice. From the discussion above, it is concluded that there is no test method which can adequately evaluate the stability relating to the efficacy, and that there is no choice but to confirm the absence of change in quality by using results of tests employing multiple methodologies, including assay methods for HA antigen content such as SRD, to ensure the stability of the drug product.

The applicant stated that it would specify the expiration period of the drug product as 15 months to ensure the stability based on the long-term testing results, and PMDA accepted this. However, PMDA requested the applicant to confirm stability assurance by performing long-term testing when the product is manufactured from a new strain, because the stability of a drug product produced from a different strain may differ. The applicant responded that it would take an appropriate action.

(7) Analytical validation of the test methods

The test methods have not been analytically validated enough or at all for many of the specifications for the bulk and drug product presented at the time of application. When evaluating the test results, it could not be determined whether the variability in values was caused by the test methods or the samples. Also, some compendial test methods might need to be evaluated for specificity and influence of coexisting substances. PMDA requested the applicant to determine whether the test methods for the specifications for the bulk and drug product were adequately evaluated. The applicant performed additional validation for about 20 parameters and submitted the data. The applicant stated that the data of the ongoing validation would be submitted when they become available.

PMDA accepted the response.

3. Non-clinical data

(i) Summary of pharmacology studies

Summary of the submitted data

(1) Primary pharmacodynamics

1) Antibody titer determination

The following 3 types of test solutions were prepared: a solution prepared by adding 0.3 mg/mL (on an aluminum content basis) of aluminum hydroxide gel to 30 µg HA/mL of whole inactivated influenza virus (bulk) (Solution A, with the same composition as the product presented for review), a solution prepared by adding 0.9 mg/mL of aluminum hydroxide gel to 30 µg HA/mL of the bulk (Solution B), and a solution of 30 µg HA/mL of the bulk not containing aluminum (Solution C). Each solution was diluted to obtain solutions containing 0.024 µg HA/100 µL, 0.12 µg HA/100 µL, 0.6 µg HA/100 µL, and 3 µg HA/100 µL. Each of these solutions was injected twice, at an interval of 3 weeks, intramuscularly into the femur or subcutaneously into the back of 5-week-old female BALB/c mice (10 animals per group), at the dose of 100 µL/animal. Phosphate buffered saline (PBS; 100 µL) was used as the control solution and administered similarly to the animals. Blood samples were collected 2 weeks after the second administration to measure the HI and neutralizing antibody titers. The dose-dependency of the HA antigen, presence/absence of aluminum hydroxide, amount of aluminum hydroxide, and the difference in the administration route were investigated.

· HI antibody titer and neutralizing antibody titer

Both the mean HI antibody (geometric mean) and mean neutralizing antibody (geometric mean) titers increased in a dose-dependent manner within the investigated dose range of 0.024 to 3 µg of HA in the vaccine. The proportion of mice with an HI antibody titer $\geq 1:40$ was 90% or more in the animals receiving the vaccine containing aluminum hydroxide at the dose of 0.6 µg HA, as compared with 50% to 60% in the animals receiving the vaccine not containing aluminum hydroxide at the same dose. On the other hand, the proportion of mice with a neutralizing antibody titer $\geq 1:160$ was 80% or more in the animals receiving the vaccine containing aluminum hydroxide at the dose of 0.6 µg HA, as compared with 50% in the animals receiving the vaccine not containing aluminum hydroxide at the same dose.

As shown above, both the HI antibody and neutralizing antibody titers were significantly higher in the animals receiving the vaccine containing aluminum hydroxide gel than in those receiving the vaccine not containing aluminum hydroxide gel. In addition, there were no significant differences in the extent of increase in the HI or neutralizing antibody titers between the animals receiving vaccine A and B with different contents of aluminum hydroxide gel. In regard to the administration route, significantly higher titers of both the HI and neutralizing antibodies were observed after intramuscular administration than after subcutaneous administration.

2) Challenge test

To female BALB/c mice aged 8 weeks (20 animals per group), 100 μ L of the H5N1 Vaccine (vaccine containing 30 μ g of HA/mL of inactivated pandemic influenza whole virus and 0.3 mg/mL of aluminum hydroxide gel on an aluminum content basis) or 100 μ L of aluminum hydroxide gel as control (0.3 mg/mL on an aluminum content basis) was subcutaneously administered twice, at a 3-week interval between the doses. Exsanguination was performed on 10 animals in each group 2 weeks after the second administration to determine the titers of the HI and neutralizing antibodies; the remaining 10 animals of each group were transnasally infected with 20MLD₅₀ of a virulent H5N1 strain (A/Viet Nam/JP1203/04), followed by observation of the animals for 2 weeks to evaluate the survival rate.

In the animals vaccinated with the H5N1 Vaccine, although mild piloerection was noted a few days to around 10 days after the infection, no serious symptoms were observed, and all the 10 animals recovered completely. Meanwhile, in the animals of the control group, mild piloerection began to be noted a few days after the infection, and, serious symptoms such as decreased spontaneous locomotor activity, loss of appetite, coarse fur, emaciation, and nervous symptoms (hind-limb paralysis, circling, etc.) appeared with the passage of time, and 9 animals died: 1 on Day 7, 2 on Day 8, 5 from Days 9 to 11, and 1 on Day 14 after the transnasal infection. The HI antibody titers in the animals of the control group were less than 10 (below the detection limit), while those in animals vaccinated with the H5N1 Vaccine ranged from 10 to 40 (geometric mean, 16.2); and the neutralizing antibody titers in the animals of the control group were all less than 20 (below the detection limit), while those in the animals vaccinated with the H5N1 Vaccine ranged from 20 to 640 (geometric mean, 149.3).

These results demonstrated the protective effect of the H5N1 Vaccine against the occurrence of more severe symptoms and death attributable to challenge with a virulent strain of the virus.

(2) Summary of the results of pharmacology safety studies

1) Effects on the central nervous system

To male Crl:CD (SD) rats aged 5 weeks (6 animals per group), 0.25 mL/kg or 0.5 mL/kg of the H5N1 Vaccine was injected subcutaneously into the back as a single dose. No effects of the H5N1 Vaccine on the general condition or behavior of the rats were observed at either 0.25 mL/kg or 0.5 mL/kg (corresponding to 25 times or 50 times the clinical dose).

2) Effects on the cardiovascular and respiratory systems (telemetry)

To male beagle dogs aged 8 months (4 animals per group), 0.25 mL/kg or 0.5 mL/kg (corresponding to about 25 times or 50 times the clinical dose) of the H5N1 Vaccine was injected subcutaneously into the back twice, at a 2-week interval between the doses. The blood pressure (systolic, diastolic and mean blood pressure), heart rate, PR interval, QRS width, and the QT interval and QTc were evaluated as the cardiovascular parameters, and the respiratory rate and arterial blood gas analysis parameters

(pO₂, pCO₂, pH, and arterial hemoglobin O₂ saturation [%]) were evaluated as the respiratory parameters.

· **Effects on the cardiovascular system**

Significant prolongation of the QTc was observed 4 hours after the second administration of the H5N1 Vaccine at the dose of 0.25 mL/kg, and significant shortening or prolongation of the QT interval and the QTc was observed 2 hours after the second administration of the H5N1 Vaccine at the dose of 0.5 mg/kg. However, since administration of the H5N1 Vaccine at 0.5 mg/kg also produced an increase in the heart rate with the shortening of the QT interval, this may be associated with the change in the heart rate. Since there was no change in the QTc as compared with the pre-treatment value, the above-mentioned changes were not considered to be caused by the administration of the H5N1 Vaccine. There were no effects of the H5N1 Vaccine on any of the other parameters evaluated.

· **Effects on the respiratory system**

Significantly high or low values of pO₂, pCO₂, pH, and arterial hemoglobin O₂ saturation (%) were sporadically observed before or after the first or second administration (0.25 mL/kg or 0.5 mL/kg), but all these changes were considered to be within the acceptable physiological variation range for normal animals. There was no effect of the H5N1 Vaccine on general condition or behavior of dogs at any observation time-point after the first or second administration.

From these findings, it was concluded that the H5N1 Vaccine has no effects on the central nervous system, cardiovascular system, or the respiratory system.

Outline of review by PMDA

(1) Cross-protective effect of the H5N1 Vaccine against infection

PMDA concluded that there were no major problems with the submitted data supporting the efficacy of the H5N1 Vaccine. From this standpoint, PMDA asked the applicant to show the results of non-clinical studies, if any were conducted in addition to the above challenge tests, on the cross-protective effect of the H5N1 Vaccine against infection with other epidemic H5N1 strains. The applicant responded as follows.

A study was conducted in mice vaccinated with the H5N1 Vaccine by the National Institute of Infectious Diseases, in order to investigate the cross-immune responses and cross-protective effect of the H5N1 Vaccine against infection with an epidemic strain (Clade 2) with different antigenicity from that of the strain used for this vaccine (Clade* 1: NIBRG-14 strain). The results were reported as a

* The origin of the term Clade is “branch” in Greek, which means a branch of a dendrogram in taxonomy. In the case of the H5N1 viruses, it refers to a group of viruses classified based on the homology of gene sequences in the dendrogram.

shared research report entitled “Study on efficacy of a vaccine for pandemic influenza virus in mice” (<http://mhlw-grants.niph.go.jp/niph/search/NIST00.do>, 54th meeting of the Japanese Society for Virology, 2006; Presentation Summary No. 3 B26) of Regulatory Science of Pharmaceuticals and Medical Devices “Research on the efficacy and safety of a vaccine for pandemic influenza” aided by a Health and Labour Sciences Research Grant in 2006. The results demonstrated that after administration in mice, the H5N1 Vaccine induced neutralizing antibodies against Clade 2 viruses (A/Indonesia/6/05:subClade 1 and A/Turkey/12/06:subClade 2), although the serum neutralizing antibody titer was lower than that against Clade 1 virus (Clade 1 virulent A/Viet Nam/JP1203/04 similar to the parent strain A/Viet Nam/1194/2004 of a strain NIBRG-14). Thus, the H5N1 Vaccine also induced cross-immune responses with the production of neutralizing antibodies against Clade 2 viruses.

In a study, in which mice vaccinated with the H5N1 Vaccine and control mice were infected with the Clade 2 virus, and pulmonary and nasal lavage fluid specimens obtained 6 days after the infection were examined for the presence/absence of the virus, the virus was detected in both or either of the pulmonary and nasal lavage fluid specimens in all the animals of the control group (8 of the 8 animals), whereas, no virus was detected in 7 of the 8 animals of the group vaccinated with the H5N1 Vaccine. In the follow-up observation of the individual animals, all the mice in the control group showed a significant decrease in the body weight after infection with the virulent strain of the virus, and 5 of the 8 animals died. On the other hand, the mice vaccinated with the H5N1 Vaccine showed no change in the body weight after infection, even though neither HI nor neutralizing antibodies were detected, or showed only a transient decrease in the body weight but recovered early, and there were no deaths. These results revealed that the H5N1 Vaccine has a cross-protective effect against viruses with different antigenicity. In this study, since mild clinical symptoms, such as piloerection, were observed even in mice with no decrease in the body weight, it was considered that infection with the virus had been established in these mice. Therefore, the vaccine may exert its efficacy by suppressing viral proliferation and removing the virus at an early stage of the infection rather than by protecting against the viral infection itself at an initial stage of the infection (such as viral invasion). This finding suggests that the protective effect of the H5N1 Vaccine, derived from Clade 1 virus (NIBRG-14), against infection with the Clade 2 virus, with different antigenicity, may be exhibited in humans.

In response to the above observations by the applicant, PMDA made the following comments. Although it is difficult to obtain clinical study data demonstrating the protective effect of the H5N1 Vaccine against infection with pandemic influenza viruses at the present time, which has not yet occurred, and it is impossible to determine whether all the results obtained in the mouse experimental model would also be applicable to humans, these data on the cross-protective effect of the H5N1

H5N1 strains which have been isolated since 2003 are classified into Clade 1 and Clade 2. The Clade 1 and Clade 2 show considerable differences in the antigenicity. The Clade 2 viruses are further classified into subClade viruses 1, 2, and 3.

Vaccine against infection presented in the above study provides very important information in terms of the clinical efficacy of the H5N1 Vaccine. The applicant quoted the finding from a shared research report of the Health and Labour Sciences Research that “even though the mouse blood antibody titers were below the detection limit or not significantly elevated after administration of the vaccine, the vaccine can protect the mice against the cross-antigenic viral challenge.” Similar results were also stated in a report of a viral challenge test in ferrets (*Nat. Rev. Microbiol.*, 2006; 4: 565-566). These data suggest that post-vaccination immune responses other than specific antibody production and cellular immunity may be involved in the protective effect of the product against the infection.

(ii) Summary of pharmacokinetic studies

No pharmacokinetic studies were conducted.

(iii) Summary of toxicology studies

Summary of the submitted data

(1) Single-dose toxicity

Single-dose toxicity of the H5N1 Vaccine was examined following subcutaneous administration in rats and dogs.

Rats were given the H5N1 Vaccine at doses of 2 and 10 mL/kg, corresponding to 200 and 1000 times the clinical dose (30 µg HA/mL drug product was used, which contains 15 µg of HA protein/dose), respectively (the same applies hereinafter). Pathological examination revealed subcutaneous induration at the injection site in the 2 dose groups. No abnormalities were observed in other organs. It was thus concluded that the minimum lethal dose in rats was ≥ 10 mL/kg and that no significant systemic toxicity appears after single administration of the H5N1 Vaccine at either dose.

Dogs were given the H5N1 Vaccine at doses of 1 and 5 mL/kg, corresponding to 100 and 500 times the clinical dose, respectively. At necropsy, subcutaneous induration at the injection site was observed in the 2 dose groups. At 5 mL/kg, hypertrophy of the lymph follicles of the spleen was noted in females, which was considered to represent an immunological reaction to the H5N1 Vaccine. No such reaction was noted at 1 mL/kg, suggesting the reaction to be of no toxicological significance at clinical doses. It was thus concluded that the minimum lethal dose in dogs was ≥ 5 mL/kg and that no significant systemic toxicity appears after single administration of the H5N1 Vaccine at either dose.

(2) Repeat-dose toxicity

Repeat-dose toxicity of the H5N1 Vaccine was examined in a 4-week subcutaneous administration study in rats, in which the H5N1 Vaccine was administered at doses of 0.25 and 0.5 mL/kg, corresponding to 25 and 50 times the clinical dose, respectively, 5 times at intervals of 1 week. At 0.5 mL/kg in both sexes, subcutaneous induration was noted macroscopically and granulomatous inflammation was observed histopathologically at the injection site. Furthermore, hyperplasia of the

germinal centers of the axillary lymph nodes was noted at both doses in both sexes, which was considered to represent an immunological reaction to the H5N1 Vaccine. Blood biochemistry revealed that a high γ -globulin values and low A/G ratio were noted at both doses. These changes were also considered to be related to immunological reaction. The no-observed-adverse-effect level (NOAEL) was estimated to be ≥ 0.5 mL/kg under the conditions employed in this study.

(3) Reproductive and developmental toxicity

The effect of the H5N1 Vaccine on fertility and early embryonic development to implantation was evaluated in a 4-week subcutaneous administration study in rats, by histopathological examination, etc. of the reproductive organs of animals of both sexes. The H5N1 Vaccine was considered to have no adverse effects on the reproductive function under the conditions employed in this study.

In a study of the effect of the H5N1 Vaccine on embryo-fetal development, doses of 0.25 and 0.5 mL/kg, corresponding to 25 and 50 times the clinical dose, respectively, were administered 3 times, on Days 7, 12, and 17 of gestation. No toxic effects of the H5N1 Vaccine were observed on the general condition of the dams. From the above results, NOAEL was estimated to be ≥ 0.5 mL/kg for the reproductive function in dams and embryo-fetal development under the conditions employed in this study.

A study of the effect of the H5N1 Vaccine on pre- and postnatal development of offspring and maternal function is underway.

(4) Local irritation

The potential of the H5N1 Vaccine to produce local irritation at the intramuscular and subcutaneous administration sites was examined in rabbits following administration of a single dose and two repeat doses.

For evaluating the effect after intramuscular administration, BK-PIFP (drug product not containing aluminum hydroxide gel) and adsorbed diphtheria-purified pertussis-tetanus combined vaccine (DPT vaccine) were used as the reference control substances, physiological saline as the negative control substance, and acetic acid solution (0.75 % and 6 %) as the positive control substance. The dosing volume in all cases was 0.5 mL. The site of injection was the lateral great muscle of the femoral region of the hind leg. For single-dose administration, the H5N1 Vaccine, BK-PIFP, and DPT vaccine were injected into the left hind leg and physiological saline was injected into the right hind leg. In the positive control group, 6 % acetic acid was injected into the left hind leg and 0.75 % acetic into the right hind leg. For repeat-dose administration, the first doses of the H5N1 Vaccine, BK-PIFP, and DPT vaccine were injected into the left hind leg and the corresponding second doses were administered into the right hind leg (on Day 14 or 15 after the first administration). The specimens were collected 2 and 7 days after the injections (Days 3 and 8, respectively) in the single-dose experiment, and 2 and 7 days

after the second injection (Days 17 and 22, respectively, after the first injection) in the repeat-dose experiment, for macroscopic and histopathological examinations. Macroscopic examination revealed minimal abnormalities after single-dose administration of the H5N1 Vaccine. Histopathological examination revealed retention of the administered substance and inflammatory cell infiltration, degeneration of muscle fibers, hemorrhage, and mineral deposition in the muscle fibers of minimal or mild severity on Day 3 after the injection. On Day 8, in addition to the same lesions as described above but of mild to moderate severity, mild regeneration of muscle fibers was noted. The lesions observed after single administration of the H5N1 Vaccine were more severe than that after administration of physiological saline, but less severe than that after administration of 0.75 % acetic acid. The severity of the lesions was considered to be similar to that after injection of DPT vaccine. After administration of two repeat doses of the H5N1 Vaccine, macroscopic examination showed minimal to mild changes. Histopathological examination revealed inflammatory cell infiltration and degeneration of muscle fibers in addition to retention of the administered substance and hemorrhage at the second injection site. The changes observed after two repeat doses of the H5N1 Vaccine were considered to be similar to those after injection of DPT vaccine. From the above results, the potential of the H5N1 Vaccine to cause local irritation at the site of intramuscular injection was considered to be equal to that of DPT vaccine.

For evaluating the effect after subcutaneous administration, BK-PIFP and DPT vaccine were used as the reference control substances and physiological saline was used as the negative control substance. The dosing volume in all cases was 0.5 mL. In the single-dose experiment, the H5N1 Vaccine, BK-PIFP, and DPT vaccine were injected into the abdominal wall on the left side and physiological saline was injected into the abdominal wall on the right side. The specimens were collected 2 and 7 days after the injections (Days 3 and 8, respectively). In the repeat-dose experiment, the H5N1 Vaccine, BK-PIFP, and DPT vaccine were administered into the abdominal wall on the left side as the first dose and into the abdominal wall on the right side as the second dose 14 days later (Day 15). The specimens were collected 2 and 7 days after the second administration (Days 17 and 22 after the first administration). Macroscopic examination revealed edema at the sites of injection of the H5N1 Vaccine and of DPT vaccine after the second administration. Histopathological examination revealed similar degrees of inflammatory cell infiltration on Day 8 after single administration of the H5N1 Vaccine and of DPT vaccine. At the site of the first injection in the repeat-dose group, minimal inflammatory cell infiltration was observed on Day 17 after the injection of the H5N1 Vaccine. At the site of the second injection, inflammatory cell infiltration accompanied by edema and necrosis was observed on Day 17 after the injection, although the change was milder than that caused by DPT vaccine. On Day 22, the same extent of inflammatory cell infiltration was observed for DPT vaccine and the H5N1 Vaccine, which was accompanied by fibrosis and necrosis, and fibrosis, respectively. From the above data, local irritation caused by the H5N1 Vaccine was considered to be slightly milder than that caused by DPT vaccine.

Outline of review by PMDA

Through adequate pre-application guidance and advice obtained from PMDA, controversial toxicological issues had already been resolved by the time of the application, and no problematic points were raised by PMDA at the time of the review.

4. Clinical data

Summary of the submitted data

The results of 1 Japanese Phase I study and 1 Japanese Phase II/III study were submitted as efficacy and safety evaluation data. These studies and their major efficacy data are summarized in Table 3.

Table 3: Summary of clinical studies and their efficacy data (Study BK-PIFA/001, Study BK-PIFA/002)

Area	Study Number	Phase	Subjects	Dosage and administration	Number of subjects	Primary endpoint	Major findings
Japan	BK-PIFA/001	I	Healthy male adults (20-40 years of age)	1.7 µg/5 µg/15 µg 2 injections at an interval of 21 (± 1) days, subcutaneous or intramuscular	20/group	Safety/immunogenicity	337 adverse events in 105 of 120 subjects (87.5%), 246 adverse reactions in 92 of 120 subjects (76.7%). Seroconversion rate (neutralizing antibody titer) 1.7 µg group, subcutaneous 47.4%/intramuscular 40.0% 5 µg group, 75.0%/65.0% 15 µg group, 78.9%/95.0%
	BK-PIFA/002	II/III	Healthy adults (20-64 years of age)	5 µg/15 µg 2 injections at an interval of 21 (± 7) days, subcutaneous	5 µg group, 150 15 µg group, 150	Immunogenicity	Seroconversion rate (neutralizing antibody titer) 5 µg group, 44.0% 15 µg group, 70.9%

(1) Japanese phase I study (Study Number, BK-PIFA/001; Attachment, 5.3.5.1-1; Publication, None; Study Period, ■■■ 20■■■ to ■■■ 20■■■)

An open-label study was conducted at a single trial site in Japan to evaluate the safety and immunogenicity of the H5N1 Vaccine in healthy male adults.

The investigational vaccine (1.7, 5, or 15 µg in terms of HA content) was to be administered subcutaneously or intramuscularly in the upper arm twice at an interval of 21 ± 1 days.

All 120 vaccinated subjects (20 per group) were included in the safety analysis. Excluding 1 subject who discontinued the clinical study 9 days after the first administration (one subject receiving 15 µg subcutaneously), the remaining 119 subjects were included in the efficacy analysis. The efficacy endpoint was the seroconversion rate calculated as the proportion of subjects changing to positive for anti-viral antibodies. Seroconversion was defined as follows: (a) a post-vaccination neutralizing antibody titer ≥ 40 or ≥ 80 and a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration, and (b) a post-vaccination HI antibody titer ≥ 20 or ≥ 40 and a four-fold or greater rise in post-vaccination HI antibody titer compared with the baseline level assayed prior to the first administration. An antibody titer < 10 was expressed as “5.” The seroconversion rates at the post-study investigation (investigation conducted 21 ± 2 days after the second administration) are summarized in Table 4, with 1 subject who received 1.7 µg subcutaneously being excluded from calculation of the seroconversion rate because the post-study data for this subject was not available.

Table 4: Seroconversion rates (Study BK-PIFA/001, post-study investigation)

Dose group	Route of administration	Number of subjects included	HI antibody titer (equine erythrocytes)				HI antibody titer (chicken erythrocytes)				Neutralizing antibody titer			
			Post-vaccination HI antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level		Post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level		Post-vaccination HI antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level		Post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level		Post-vaccination neutralizing antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level		Post-vaccination neutralizing antibody titer ≥ 80 and a four-fold or greater rise compared with baseline level	
			Number of subjects	Seroconversion rate (%)	Number of subjects	Seroconversion rate (%)	Number of subjects	Seroconversion rate (%)	Number of subjects	Seroconversion rate (%)	Number of subjects	Seroconversion rate (%)	Number of subjects	Seroconversion rate (%)
1.7 μg	Subcutaneous	19	3	15.8	2	10.5	8	42.1	4	21.1	9	47.4	5	26.3
	Intramuscular	20	3	15.0	0	0.0	7	35.0	3	15.0	8	40.0	4	20.0
5 μg	Subcutaneous	20	7	35.0	3	15.0	13	65.0	9	45.0	15	75.0	13	65.0
	Intramuscular	20	9	45.0	6	30.0	13	65.0	11	55.0	13	65.0	11	55.0
15 μg	Subcutaneous	19	8	42.1	4	21.1	12	63.2	6	31.6	15	78.9	13	68.4
	Intramuscular	20	11	55.0	9	45.0	17	85.0	12	60.0	19	95.0	13	65.0

A total of 337 adverse events were reported by 105 of 120 subjects (87.5%): 42 events in 15 of 20 subjects (75.0%) in the subcutaneous 1.7 µg group, 40 events in 13 of 20 subjects (65.0%) in the intramuscular 1.7 µg group, 61 events in 20 of 20 subjects (100.0%) in the subcutaneous 5 µg group, 44 events in 18 of 20 subjects (90.0%) in the intramuscular 5 µg group, 86 events in 19 of 20 subjects (95.0%) in the subcutaneous 15 µg group, and 64 events in 20 of 20 subjects (100.0%) in the intramuscular 15 µg group. Major adverse events included the following: injection site erythema, 5 events reported by 4 of 20 subjects (20.0%) in the 1.7 µg subcutaneous group, 3 events reported by 3 of 20 subjects (15.0%) in the 1.7 µg intramuscular group, 7 events reported by 5 of 20 subjects (25.0%) in the 5 µg subcutaneous group, 3 events reported by 3 of 20 subjects (15.0%) in the 5 µg intramuscular group, 12 events reported by 11 of 20 subjects (55.0%) in the 15 µg subcutaneous group, and 6 events reported by 6 of 20 subjects (30.0%) in the 15 µg intramuscular group; injection site pain, 2 events reported by 2 of 20 subjects (10.0%) in the 1.7 µg subcutaneous group, 6 events reported by 5 of 20 subjects (25.0%) in the 1.7 µg intramuscular group, 9 events reported by 6 of 20 subjects (30.0%) in the 5 µg subcutaneous group, 8 events reported by 5 of 20 subjects (25.0%) in the 5 µg intramuscular group, 11 events reported by 9 of 20 subjects (45.0%) in the 15 µg subcutaneous group, and 18 events reported by 13 of 20 subjects (65.0%) in the 15 µg intramuscular group; pyrexia, 2 events reported by 1 of 20 subject (5.0%) in the 1.7 µg intramuscular group, 3 events reported by 3 of 20 subjects (15.0%) in the 5 µg subcutaneous group, 1 event reported by 1 of 20 subject (5.0%) in the 5 µg intramuscular group, and 4 events reported by 4 of 20 subjects (20.0%) in the 15 µg subcutaneous group; injection site swelling, 2 events reported by 2 of 20 subjects (10.0%) in the 1.7 µg subcutaneous group, 2 events reported by 2 of 20 subjects (10.0%) in the 1.7 µg intramuscular group, 2 events reported by 2 of 20 subjects (10.0%) in the 5 µg subcutaneous group, 4 events reported by 4 of 20 subjects (20.0%) in the 15 µg subcutaneous group, and 4 events reported by 4 of 20 subjects (20.0%) in the 15 µg intramuscular group. Injection site erythema, injection site pain, injection site swelling, and pyrexia were all judged to be adverse reactions except for 1 event of pyrexia reported by 1 subject in the 1.7 µg intramuscular group. None of these adverse events were serious and all disappeared either with no treatment or with concomitant medication.

The severity of adverse events was determined using 4-grade classification systems: the severity of adverse events reported as local reactions (at the administration site) was graded from Grade A to Grade D, and that of adverse events occurring elsewhere from Grade 1 to Grade 4. Grade D and Grade 4 represented the most severe of the 4 grades for each classification system.

Grade C adverse events at the administration site were reported by subjects receiving subcutaneous administration: injection site erythema reported by 1 subject in the 5 µg subcutaneous group and injection site swelling reported by 1 subject in the 15 µg subcutaneous group. Both adverse events were judged to be adverse reactions.

Grade 3 adverse events, other than those occurring at the administration site, were as follows: nasopharyngitis, 1 event reported by 1 subject in the 1.7 µg subcutaneous group; enteritis infectious, 1

event reported by 1 subject in the 1.7 µg subcutaneous group; syncope vasovagal, 1 event reported by 1 subject in the 5 µg subcutaneous group; blood phosphorus decreased, 1 event reported by 1 subject in the 5 µg intramuscular group; pyrexia, 1 event reported by 1 subject in the 15 µg subcutaneous group; blood creatine phosphokinase increased, 1 event reported by 1 subject in the 15 µg intramuscular group; and blood phosphorus decreased, 1 event reported by 1 subject in the 15 µg intramuscular group. Among these adverse events, pyrexia in the 15 µg subcutaneous group and blood creatine phosphokinase increased in the 15 µg intramuscular group were judged to be adverse reactions.

There were no deaths or serious adverse events in this study.

One subject in the 1.7 µg subcutaneous group discontinued the clinical study due to an adverse event. A Grade 1 urticaria occurred in this subject 15 days after the first administration and the clinical study was discontinued 21 days after the first administration, i.e. prior to the second administration. This adverse event disappeared without treatment 42 days after the first administration.

(2) Japanese phase II/III study (Study Number, BK-PIFA/002; Attachment, 5.3.5.1-2; Publication, None; Study Period, ■■■ 20■■■ to ■■■ 20■■■)

A multi-center, randomized, double-blind, parallel-group comparative study involving 2 dose groups was conducted at 9 study sites in Japan as an investigator-initiated clinical trial to evaluate the safety and immunogenicity of the H5N1 Vaccine in healthy adults.

The H5N1 Vaccine (5 or 15 µg in terms of HA content) was to be administered subcutaneously in the extensor side of the upper arm twice at an interval of 21 ± 7 days.

All 300 vaccinated subjects (5 µg group, 150 subjects; 15 µg group, 150 subjects) were included in the Full Analysis Set (FAS) and also in the safety analysis. Excluding 34 subjects (14 subjects in the 5 µg group and 20 subjects in the 15 µg group) with non-eligibility, treatment protocol violations, and other protocol deviations, the remaining 266 subjects (5 µg group, 136 subjects; 15 µg group, 130 subjects) were included in the Per Protocol Set (PPS).

The primary endpoints of immunogenicity were HI antibody titer against H5 antigen and neutralizing antibody titer against H5N1 influenza virus. The seroconversion rate (the proportion of subjects seroconverted to positive) was calculated based on the following definition of seroconversion: (a) a post-vaccination HI antibody titer ≥ 20 or ≥ 40 and a four-fold or greater rise in post-vaccination HI antibody titer compared with the baseline level assayed prior to the first administration, and (b) a post-vaccination neutralizing antibody titer ≥ 40 or ≥ 80 and a four-fold or greater rise in post-vaccination neutralizing antibody titer compared with the baseline level assayed prior to the first administration. An antibody titer < 10 was expressed as “5.” The seroconversion rates obtained for FAS are summarized in Table 5. Two subjects in the 15 µg group who discontinued the study after the first administration were excluded from the analysis of the post-study investigation (investigation conducted 21 ± 7 days after the second administration).

Table 5: Seroconversion rates (Study BK-PIFA/002, FAS)

Dose group	Assessment timing	Number of subjects included	HI antibody titer (equine erythrocytes)		HI antibody titer (chicken erythrocytes)		Neutralizing antibody titer	
			Post-vaccination HI antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level	Post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level	Post-vaccination HI antibody titer ≥ 20 and a four-fold or greater rise compared with baseline level	Post-vaccination HI antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level	Post-vaccination neutralizing antibody titer ≥ 40 and a four-fold or greater rise compared with baseline level	Post-vaccination neutralizing antibody titer ≥ 80 and a four-fold or greater rise compared with baseline level
5 μ g, seroconversion rate (95% CI)	Prior to second administration	150	30.0% (22.8%-38.0%)	18.7% (12.8%-25.8%)	20.0% (13.9%-27.3%)	3.3% (1.1%-7.6%)	22.0% (15.7%-29.5%)	10.7% (6.2%-16.7%)
	Post-study investigation	150	43.3% (35.3%-51.7%)	22.7% (16.2%-30.2%)	14.7% (9.4%-21.4%)	2.7% (0.7%-6.7%)	44.0% (35.9%-52.3%)	25.3% (18.6%-33.1%)
15 μ g, seroconversion rate (95% CI)	Prior to second administration	150	50.0% (41.7%-58.3%)	32.7% (25.2%-40.8%)	33.3% (25.9%-41.5%)	18.7% (12.8%-25.8%)	36.0% (28.3%-44.2%)	24.7% (18.0%-32.4%)
	Post-study investigation	148	68.9% (60.8%-76.3%)	41.9% (33.8%-50.3%)	27.0% (20.1%-34.9%)	10.8% (6.3%-17.0%)	70.9% (62.9%-78.1%)	53.4% (45.0%-61.6%)

The seroconversion rates in the FAS for HI antibody titer (equine erythrocytes) and neutralizing antibody titer determined at the post-study investigation were higher than those prior to the second administration in both dose groups. Also, the seroconversion rate in the 15 µg group was higher than that in the 5 µg group, regardless of assessment timing (number of administrations experienced) and the type of antibody titer investigated. The seroconversion rates in the PPS were comparable to those in the FAS.

The geometric mean titer (GMT) increase was examined as the secondary efficacy endpoint. For HI antibody titer (equine erythrocytes), the GMT increase was augmented in parallel with the number of vaccine administrations and tended to be higher in the high-dose group. In contrast, little difference was noted in the GMT increase for HI antibody titers assayed with chicken erythrocytes, regardless of assessment timing (number of administrations experienced) and the dose of vaccine administration. For neutralizing antibody titer, the GMT increase was augmented in parallel with the number of vaccine administrations regardless of the vaccine dose and tended to be higher in the high-dose group.

Table 6: Geometric mean titer (GMT) increase (Study BK-PIFA/002, FAS)

Endpoint	Dose group	Assessment timing	Number of subjects	GMT increase (fold)	
				Geometric mean	Geometric standard deviation
HI antibody titer (equine erythrocytes)	5 µg	Prior to second administration	150	1.97	2.441
		Post-study investigation	150	2.45	2.459
	15 µg	Prior to second administration	150	3.16	2.611
		Post-study investigation	148	4.27	2.410
HI antibody titer (chicken erythrocytes)	5 µg	Prior to second administration	150	1.58	1.842
		Post-study investigation	150	1.38	1.751
	15 µg	Prior to second administration	150	2.26	2.434
		Post-study investigation	148	1.89	2.312
Neutralizing antibody titer	5 µg	Prior to second administration	150	1.70	2.245
		Post-study investigation	150	2.64	2.419
	15 µg	Prior to second administration	150	2.38	2.632
		Post-study investigation	148	4.76	2.664

The severity of adverse events was determined using 4-grade classification systems as in Study BK-PIFA/001: the severity of adverse events reported as local reactions (at the administration site) was graded from A to D, and that of adverse events occurring elsewhere from 1 to 4. Grade D and Grade 4 represented the most severe of the 4 grades in each classification system.

A total of 1786 adverse events were reported by 285 of 300 subjects (95.0%), including 871 events in 146 of 150 subjects (97.3%) in the 5 µg group and 915 events in 139 of 150 subjects (92.7%) in the 15 µg group. A total of 1664 adverse reactions were reported by 280 of 300 subjects (93.3%), including 805 reactions in 142 of 150 subjects (94.7%) in the 5 µg group and 859 reactions in 138 of 150 subjects (92.0%) in the 15 µg group.

Assuming that Grades A, B, C, and D correspond to Grades 1, 2, 3, and 4, forty adverse events of Grade 3 or higher were reported in the 5 µg group, while 110 adverse events of Grade 3 or higher were reported in the 15 µg group. No Grade 4 adverse events were reported in either group.

In addition, 39 adverse reactions of Grade 3 or higher were reported in the 5 µg group, while 110 adverse reactions of Grade 3 or higher were reported in the 15 µg group. No Grade 4 adverse reactions were reported in either group.

Tables 7 to 9 summarize adverse events and reactions by dose group: subjective symptoms/objective signs by System Organ Class and those with an incidence of 5% or higher by Preferred Term (Table 7); local reactions by System Organ Class and by Preferred Term (Table 8); and laboratory abnormalities by Preferred Term reported in at least 5 subjects in either dose group (Table 9).

**Table 7: Adverse events and reactions by System Organ Class and those by Preferred Term
with an incidence of 5% or higher in either dose group
(subjective symptoms/objective signs) (Study BK-PIFA/002, safety analysis set)**

System Organ Class/ Preferred Term	Adverse events in the 5 µg group (number of subjects, incidence)				Adverse events in the 15 µg group (number of subjects, incidence)			
	Adverse reactions		Adverse events		Adverse reactions		Adverse events	
Number of subjects included	150				150			
Adverse events, number of subjects	40		69		34		60	
Adverse events, incidence	26.7%		46.0%		22.7%		40.0%	
Infections and infestations	2	1.3%	17	11.3%	1	0.7%	14	9.3%
Nasopharyngitis	1	0.7%	11	7.3%	1	0.7%	10	6.7%
Psychiatric disorders	0	0.0%	1	0.7%	0	0.0%	1	0.7%
Nervous system disorders	14	9.3%	20	13.3%	11	7.3%	12	8.0%
Headache	12	8.0%	17	11.3%	10	6.7%	10	6.7%
Eye disorders	0	0.0%	1	0.7%	0	0.0%	1	0.7%
Ear and labyrinth disorders	0	0.0%	0	0.0%	1	0.7%	1	0.7%
Cardiac disorders	1	0.7%	1	0.7%	0	0.0%	0	0.0%
Vascular disorders	0	0.0%	0	0.0%	0	0.0%	1	0.7%
Respiratory, thoracic and mediastinal disorders	5	3.3%	13	8.7%	5	3.3%	10	6.7%
Gastrointestinal disorders	6	4.0%	12	8.0%	0	0.0%	6	4.0%
Skin and subcutaneous tissue disorders	3	2.0%	5	3.3%	0	0.0%	1	0.7%
Musculoskeletal and connective tissue disorders	6	4.0%	7	4.7%	2	1.3%	7	4.7%
Reproductive system and breast disorders	0	0.0%	1	0.7%	0	0.0%	1	0.7%
General disorders and administration site conditions	20	13.3%	23	15.3%	22	14.7%	25	16.7%
Malaise	14	9.3%	16	10.7%	19	12.7%	19	12.7%
Injury, poisoning and procedural complications	0	0.0%	0	0.0%	0	0.0%	4	2.7%

Table 8: Adverse events and reactions by System Organ Class and by Preferred Term**(local reactions) (Study BK-PIFA/002, safety analysis set)**

System Organ Class/ Preferred Term	Adverse events in the 5 µg group (number of subjects, incidence)				Adverse events in the 15 µg group (number of subjects, incidence)			
	Adverse reactions		Adverse events		Adverse reactions		Adverse events	
Number of subjects included	150				150			
Adverse events, number of subjects	141		141		137		137	
Adverse events, incidence	94.0%		94.0%		91.3%		91.3%	
Musculoskeletal and connective tissue disorders	2	1.3%	2	1.3%	4	2.7%	4	2.7%
Muscular weakness	2	1.3%	2	1.3%	4	2.7%	4	2.7%
General disorders and administration site conditions	141	94.0%	141	94.0%	137	91.3%	137	91.3%
Injection site bruising	4	2.7%	4	2.7%	4	2.7%	4	2.7%
Injection site erythema	123	82.0%	123	82.0%	129	86.0%	129	86.0%
Injection site induration	10	6.7%	10	6.7%	16	10.7%	16	10.7%
Injection site irritation	1	0.7%	1	0.7%	1	0.7%	1	0.7%
Injection site pain	106	70.7%	106	70.7%	109	72.7%	109	72.7%
Injection site paraesthesia	1	0.7%	1	0.7%	1	0.7%	1	0.7%
Injection site pruritus	87	58.0%	87	58.0%	103	68.7%	103	68.7%
Injection site rash	0	0.0%	0	0.0%	1	0.7%	1	0.7%
Injection site warmth	56	37.3%	56	37.3%	78	52.0%	78	52.0%
Injection site swelling	84	56.0%	84	56.0%	87	58.0%	87	58.0%
Injection site joint movement impairment	0	0.0%	0	0.0%	1	0.7%	1	0.7%
Injection site eczema	0	0.0%	0	0.0%	1	0.7%	1	0.7%

Table 9: Adverse events and reactions by Preferred Term reported in at least 5 subjects in either dose group**(laboratory abnormalities) (Study BK-PIFA/002, safety analysis set)**

System Organ Class/Preferred Term	Adverse events in the 5 µg group (number of subjects, incidence)				Adverse events in the 15 µg group (number of subjects, incidence)			
	Adverse reactions		Adverse events		Adverse reactions		Adverse events	
Number of subjects included	150				150			
Adverse events, number of subjects	19		26		7		14	
Adverse events, incidence	12.7%		17.3%		4.7%		9.3%	
Laboratory abnormalities	19	12.7%	26	17.3%	7	4.7%	14	9.3%
Alanine aminotransferase increased	4	2.7%	5	3.3%	0	0.0%	1	0.7%
Aspartate aminotransferase increased	7	4.7%	8	5.3%	0	0.0%	1	0.7%
Neutrophil count increased	3	2.0%	5	3.3%	1	0.7%	2	1.3%
White blood cell count increased	5	3.3%	7	4.7%	3	2.0%	5	3.3%

There were no deaths during this clinical study.

A serious adverse event was reported by 1 subject in the 15 µg group. Radial nerve palsy in the right upper arm occurred 20 days after the first administration, but a causal relationship between the H5N1 Vaccine and this event was ruled out. The second administration was performed in this subject as scheduled and the event disappeared after persisting for 23 days.

One subject in the 15 µg group discontinued the clinical study due to an adverse event. The second administration was not performed in this subject due to a common cold (Preferred Term, nasopharyngitis).

Outline of review by PMDA

(1) Efficacy

As a result of the regulatory review described below, PMDA has concluded that the immunogenicity of the H5N1 Vaccine against influenza virus strain NIBRG-14 has been sufficiently demonstrated by the submitted study results.

This PMDA conclusion will be further considered, taking Expert Advisors' comments into account.

The details of the efficacy review are described below.

1) Efficacy endpoints

A guideline for seasonal influenza vaccine established by the Committee for Proprietary Medicinal Products (CPMP) of the European Agency for the Evaluation of Medicinal Products (EMA) (CPMP/BWP/214/96; <http://www.ema.europa.eu/pdfs/human/bwp/021496en.pdf>) requires that at least 1 of the 3 serological assessment criteria for HI titer, as presented in Table 10, be met in assessing the efficacy of influenza vaccine prepared against a particular virus strain recommended once a year for the coming season. In addition, a European Medicines Agency (EMA) guideline on pandemic influenza vaccines coming into effect in 2007 (CHMP/VWP/263499/2006; <http://www.ema.europa.eu/pdfs/human/vwp/26349906enfin.pdf>) requires that all 3 criteria, as defined in the guideline CPMP/BWP/214/96, be fulfilled.

Table 10: EMA criteria for influenza vaccines (HI antibody titer)

	18-60 years of age	Over 60 years of age
Seroconversion rate	> 40%	> 30%
GMT increase	> 2.5	> 2.0
Response rate	> 70%	> 60%

Seroconversion rate: Proportion of subjects either "with a pre-vaccination HI antibody titer < 10 and a post-vaccination HI antibody titer ≥ 40 " or "with a four-fold or greater rise in HI antibody titer" (%)

GMT increase: Magnitude of increase in post-vaccination geometric mean titer (GMT) from the pre-vaccination level, with an HI antibody titer below the detection limit (< 10) expressed as "5."

Response rate: Proportion of subjects with a vaccination HI antibody titer ≥ 40 (%)

In Study BK-PIFA/002, the applicant defined assessment criteria for seroconversion as "a post-vaccination antibody titer ≥ 20 or ≥ 40 and a four-fold or greater rise in post-vaccination antibody titer compared with the baseline level assayed prior to the first administration" for HI antibody titer and as "a post-vaccination antibody titer ≥ 40 or ≥ 80 and a four-fold or greater rise in post-vaccination antibody titer compared with the baseline level assayed prior to the first administration" for neutralizing antibody. The seroconversion rates for both HI antibody titer and neutralizing antibody titer were used as primary endpoints.

PMDA asked for the applicant's view on the reasons for defining seroconversion rates as primary efficacy endpoints.

The applicant responded that seroconversion rates were used as primary endpoints for the following reasons: (a) In Study BK-PIFA/001, an HI antibody titer or neutralizing antibody titer ≥ 10 was seen in some of the subjects prior to the first administration of the H5N1 Vaccine, but whether such antibodies existing prior to vaccination were actually specific to H5 influenza virus was not clear. Therefore, the applicant considered a seroconversion rate, as defined on the basis of both a change from pre-vaccination seronegativity to post-vaccination seropositivity and a certain (e.g., four-fold or greater) rise in antibody titer, to be most appropriate as the primary endpoint for the immunogenicity of the H5N1 Vaccine in assessing acquisition of the ability to produce antibodies specific to H5 influenza virus; and (b) the applicant consulted existing foreign guidelines on pandemic influenza vaccines including the one established by the EMEA.

PMDA considers as follows.

Although the associations between immunological indices such as antibody titer and protection against infection/development are not sufficiently clear for pandemic influenza, it is impossible to assess the protective effect of the H5N1 Vaccine against infection with/development of its target disease because no outbreak of pandemic influenza has actually occurred as yet. Accordingly, it may be understandable that the efficacy of the H5N1 Vaccine must be assessed in terms of its immunogenicity. In discussing protective effects of the H5N1 Vaccine against infection, it is important to assess HI antibody titer according to the EMEA/CPMP guideline on seasonal influenza vaccines based on a reported clinical investigation (*J. Hyg., Camb.*, 1984; 92, 301-312) and empirical association with the clinical efficacy (protection against infection) of an influenza vaccine. The antibody response rate, which is 1 of the 3 serological criteria required by the EMEA/CPMP guideline for seasonal influenza vaccine, is defined by assuming the pre-existence of people retaining antibodies against existing influenza viruses in a particular population at a certain percentage and that the value of the antibody response rate may be essentially comparable to that of the seroconversion rate in assessing the immunogenicity of a pandemic influenza vaccine aimed at primary immunization of populations immunologically naive to a pandemic influenza virus, unlike a seasonal influenza vaccine, because the existence of antibodies specific to the pandemic influenza virus prior to vaccination is theoretically impossible. Under such situations, the applicant's decision to use the seroconversion rate and GMT increase as primary and secondary endpoints for the immunogenicity of the H5N1 Vaccine, respectively, is appropriate. Also, it is necessary to assess HI antibody titer assayed with chicken erythrocytes, because the EMEA/CPMP guideline adopts HI antibody titer assayed with avian (chicken or turkey) erythrocytes (*Develop. Boil. Standard.*, 1977; 39: 273-281) as a serological criterion.

In the protocol for Study BK-PIFA/002, PPS had been defined as the major analysis set for immunogenicity to ensure an accurate efficacy assessment of the H5N1 Vaccine. However, prior to

locking data sets, the investigator* stated in the statistical analysis plan that the major analysis set for immunogenicity was FAS, without changing the protocol description from PPS to FAS. The investigator justified this change in the analysis set as follows: (a) it was considered valid to define FAS as the major analysis set for immunogenicity of the H5N1 Vaccine because Study BK-PIFA/002 was a confirmatory study; (b) it was considered that changing the analysis set from PPS to FAS would not lead to overestimation of the immunogenicity of the H5N1 Vaccine. PMDA does not consider this change to have seriously influenced efficacy assessment in Study BK-PIFA/002, because the seroconversion rates in the PPS and FAS were quite comparable. Nevertheless, the protocol should have been revised because definition of the major analysis set is an important issue in clinical study design.

2) Efficacy

In Study BK-PIFA/002, the seroconversion rate for HI antibody titer (chicken erythrocytes), according to the definition of seroconversion by the applicant, was as low as 2.7% in the 5 µg group and 10.8% in the 15 µg group, even at the post-study investigation.

PMDA interpreted the definition of “seroconversion” stated in the EMEA/CPMP guideline (CPMP/BWP/214/96) as either “pre-vaccination HI antibody titer < 10 and post-vaccination HI antibody titer ≥ 40” or “pre-vaccination HI antibody titer ≥ 10 and a four-fold or greater rise in post-vaccination HI antibody titer compared with the baseline level assayed prior to the first administration.” PMDA then directed the applicant to recalculate seroconversion rates for HI antibody titer according to this alternative definition of seroconversion. Based on the results submitted by the applicant, PMDA confirmed that the seroconversion rate for HI antibody titer (chicken erythrocytes) at the post-study investigation did not meet the requirement of the EMEA/CPMP guideline (above 40%) (Table 11). PMDA further confirmed that both the GMT increase (Table 12) and the response rate (Table 13) for HI antibody titer (chicken erythrocytes) failed to fulfill the EMEA/CPMP criteria.

Table 11: Seroconversion rates (according to PMDA’s interpretation) (Study BK-PIFA/002, FAS)

Dose group	Assessment timing	Number of subjects included	HI antibody titer (chicken erythrocytes)		HI antibody titer (equine erythrocytes)	
			“With post-vaccination HI antibody titer ≥ 40” or “with a four-fold or greater rise in HI antibody titer”		“With post-vaccination HI antibody titer ≥ 40” or “with a four-fold or greater rise in HI antibody titer”	
			Number of subjects	Seroconversion rate (%) (95% CI)	Number of subjects	Seroconversion rate (%) (95% CI)
5 µg	Prior to second administration	150	5	3.3 (1.1-7.6)	28	18.7 (12.8-25.8)
	Post-study investigation	150	4	2.7 (0.7-6.7)	34	22.7 (16.2-30.2)
15 µg	Prior to second administration	150	28	18.7 (12.8-25.8)	49	32.7 (25.2-40.8)
	Post-study investigation	148	16	10.8 (6.3-17.0)	62	41.9 (33.8-50.3)

* Refer to those who conducted the investigator-initiated clinical trial.

Table 12: Geometric mean titer (GMT) increase (Study BK-PIFA/002, FAS) (Reproduction of Table 6)

Endpoint	Dose group	Assessment timing	Number of subjects	GMT increase (fold)	
				Geometric mean	Geometric standard deviation
HI antibody titer (equine erythrocytes)	5 µg	Prior to second administration	150	1.97	2.441
		Post-study investigation	150	2.45	2.459
	15 µg	Prior to second administration	150	3.16	2.611
		Post-study investigation	148	4.27	2.410
HI antibody titer (chicken erythrocytes)	5 µg	Prior to second administration	150	1.58	1.842
		Post-study investigation	150	1.38	1.751
	15 µg	Prior to second administration	150	2.26	2.434
		Post-study investigation	148	1.89	2.312
Neutralizing antibody titer	5 µg	Prior to second administration	150	1.7	2.245
		Post-study investigation	150	2.64	2.419
	15 µg	Prior to second administration	150	2.38	2.632
		Post-study investigation	148	4.76	2.664

Table 13: Antibody response rate**(Study BK-PIFA/002, FAS) (Additionally submitted by the applicant and modified by PMDA)**

Endpoint	Dose group	Assessment timing	Number of subjects included	Antibody response rate	
				Number of subjects	% (95% CI)
HI antibody titer (chicken erythrocytes)	5 µg	Prior to second administration	150	7	4.7 (1.9-9.4)
		Post-study investigation	150	6	4.0 (1.5-8.5)
	15 µg	Prior to second administration	150	28	18.7 (12.8-25.8)
		Post-study investigation	148	17	11.5 (6.8-17.8)
HI antibody titer (equine erythrocytes)	5 µg	Prior to second administration	150	30	20.0 (13.9-27.3)
		Post-study investigation	150	36	24.0 (17.4-31.6)
	15 µg	Prior to second administration	150	52	34.7 (27.1-42.9)
		Post-study investigation	148	64	43.2 (35.1-51.6)

PMDA asked for the applicant's view on the reasons for a smaller increase in HI antibody titer (chicken erythrocytes) generated by the H5N1 Vaccine than that achieved with conventional seasonal influenza vaccines.

The applicant responded that the apparently small rise in HI antibody titer (chicken erythrocytes) might be ascribable to the reported lower immunogenicity of an H5 influenza vaccine in humans compared with that of seasonal influenza vaccines (*Lancet*, 2001; 357: 1937-1943).

PMDA further examined the appropriateness of the assay system used for determination of HI antibody titer as follows. In 16 of the 298 subjects (5.4%) receiving the second administration of the H5N1 Vaccine in Study BK-PIFA/002, the post-study HI antibody titer (chicken erythrocytes) decreased to a quarter or below of that prior to the second administration (Table 14). PMDA asked for the applicant's view on the cause of this finding and the applicant responded as follows.

The drop in post-study HI antibody titer (chicken erythrocytes) may be ascribed firstly to avian influenza virus strain NIBRG-14 used as the virus production strain and the target antigen for HI antibody titer assay. This strain has experienced a smaller number of passages in embryonated chicken eggs and exhibits a lower affinity (weaker binding) to chicken erythrocytes than other strains. Another possible reason is an additional criterion for hemagglutination inhibition positivity introduced into the HI antibody titer assay (chicken erythrocytes) in Study BK-PIFA/002, that is, a percentage of the erythrocyte population precipitates moving on the surface of the tilted assay plate, in an amount approximately equal

to 50% or more: no such drop in post-study HI antibody titer (chicken erythrocytes) was noted in any of the subjects in Study BK-PIFA/001.

In further consideration of the fact that no such decreases in post-study HI antibody titer (equine erythrocytes) or post-study neutralizing antibody titer were noted in any of the subjects investigated in Study BK-PIFA/002, PMDA considers it reasonable to ascribe the decrease in HI antibody titer (chicken erythrocytes) to the assay system used.

As discussed above, PMDA considers a drop in HI antibody titer (chicken erythrocytes) to a quarter or below of the pre-vaccination level found after administration of the H5N1 Vaccine in a limited number of subjects (Table 14) to suggest a non-constant and fluctuating sensitivity of HI antibody titer assay using chicken erythrocytes. The impact of this issue on efficacy assessment of the H5N1 Vaccine is not negligible. Thus, PMDA considers that application of HI antibody titer assayed with chicken erythrocytes, conventionally used as the criterion for the clinical efficacy of seasonal influenza vaccine, to efficacy assessment of the H5N1 Vaccine is not necessarily appropriate.

Table 14: List of subjects with a drop in antibody titer to a quarter or below of the pre-vaccination level and changes in antibody titer (Study BK-PIFA/002)

Case No.	Dose group	HI antibody titer (chicken erythrocytes)			Neutralizing antibody titer			HI antibody titer (equine erythrocytes)		
		Prior to first administration	Prior to second administration	Post-study investigation	Prior to first administration	Prior to second administration	Post-study investigation	Prior to first administration	Prior to second administration	Post-study investigation
B01-A	5 µg	5	20	5	20	40	40	5	20	20
B01-B		5	20	5	20	10	20	5	10	10
B01-C		5	20	5	20	20	40	5	5	5
B03-D		5	20	5	20	10	20	5	5	5
B04-E		5	20	5	10	40	40	5	10	20
B08-F		5	20	5	10	20	40	5	5	10
B09-G		5	20	5	40	40	80	5	20	20
B01-H		5	40	10	10	80	40	5	40	40
B02-I	15 µg	5	20	5	20	20	40	5	5	5
B02-J		5	20	5	40	40	40	5	80	80
B04-K		10	20	5	5	40	40	5	80	80
B06-L		5	20	5	20	20	20	5	5	5
B06-M		10	80	20	20	160	160	5	40	40
B07-N		5	20	5	40	40	80	20	40	40
B07-O		5	20	5	10	20	40	5	10	20
B09-Q		5	20	5	20	80	80	5	80	80

While the EMEA/CPMP guideline for seasonal influenza vaccines consistently uses HI antibody titer assayed with avian (chicken or turkey) erythrocytes as a serological efficacy criterion (*Develop. Biol. Standard.*, 1977; 39: 273-281), an FDA guidance on licensure of pandemic influenza vaccines dated May 2007 (<http://www.fda.gov/cber/gdlns/panfluvac.pdf>) states that not only erythrocytes of avian origin but also those from other animal species may be used in antibody titer assay for efficacy assessment of a pandemic influenza vaccine candidate. Because the relationship between HI antibody titers assayed with erythrocytes of different origin, equine and avian, has not been thoroughly investigated to date, there may still be uncertainty about whether it is reasonable to simply substitute an HI antibody titer assayed with

avian erythrocytes ≥ 40 as a criterion for seroprotection level with an HI antibody titer assayed with equine erythrocytes ≥ 40 . However, PMDA considers it reasonable to assume that an HI antibody titer assayed with equine erythrocytes may be equivalent to the value assayed with avian erythrocytes, judging from the principle of HI antibody titer assay. Assuming the equivalence of HI antibody titers assayed with erythrocytes from different species, the seroconversion rate (41.9%) and the GMT increase in HI antibody titer assayed with equine erythrocytes in the 15 μg group (4.27) meet the requirement of the EMEA/CPMP guideline, while the response rate (43.2%) is below the level required by this guideline. PMDA therefore considers that it may be reasonable to expect protective effects of the H5N1 Vaccine against pandemic influenza virus infection based on the presented HI antibody titer data.

The serological criteria required for seasonal influenza vaccines are established by assuming the pre-existence of a certain level of immunity to the influenza virus strain used in vaccine production. The EMEA/CHMP guideline for pandemic influenza vaccines (CHMP/VWP/263499/2006) states that all 3 assessment criteria defined in the preceding EMEA/CPMP guideline should be fulfilled, because pandemic influenza vaccines are intended for primary immunization of immunologically naive populations. Since association between fulfillment of all 3 assessment criteria and achievement of protective effects against virus infection has not been confirmed to date, PMDA considers that the failure to fulfill all 3 assessment criteria required for pandemic influenza vaccines does not necessarily imply absence of a protective effect against infection with pandemic influenza pathologically different from that with a conventional seasonal influenza.

It may also be important to assess immunogenicity of the H5N1 Vaccine in terms of not only HI antibody titer (inhibition indicator of red blood cell agglutination via HA protein) but also neutralizing antibody titer (inhibition indicator of viral adsorption and proliferation), considering that the H5N1 Vaccine is a whole virus vaccine retaining the overall virion structure, but not an HA vaccine with the virion disrupted. In fact, the EMEA/CHMP guideline for pandemic influenza vaccines (CHMP/VWP/263499/2006) states that “a demonstration that the candidate vaccine elicits neutralising antibodies directed against the vaccine strain is very important,” besides requiring fulfillment of all 3 assessment criteria defined in the EMEA/CPMP guideline for seasonal influenza vaccines (CPMP/BWP/214/96).

Whether a correlation exists between neutralizing antibody titer and HI antibody titer (chicken erythrocytes) or between neutralizing antibody titer and a protective effect against influenza virus infection is not clear to date. Accordingly, it is impossible to judge the protective effect of the H5N1 Vaccine against infection based on the submitted study results of neutralizing antibody titer. Nevertheless, PMDA considers that a vaccine eliciting a minimum four-fold rise in post-vaccination neutralizing antibody titer as compared with the pre-vaccination level may be judged as having a certain clinical significance, because the above-mentioned immunological response suggests an activation of the immune system by acquired immunity equivalent to actual virus infection. PMDA's view is based on the following points:

- (a) The protective effect of the H5N1 Vaccine against virus infection in humans may well be expected, considering that a neutralizing antibody titer assay measures the ability of antibodies to inhibit viral adsorption and proliferation;
- (b) A four-fold or greater difference in antibody titer between acute-phase and convalescent sera (*Harrison's Principles of Internal Medicine* 16th ed) is generally accepted as the serological diagnostic criterion for virus infection; and
- (c) "A fourfold or greater rise in neutralization antibody titer for H5N1 based on testing of an acute serum specimen (collected 7 days or less after symptom onset) and a convalescent serum specimen. The convalescent neutralizing antibody titer must also be 1:80 or higher," which is defined as a diagnostic criterion for a confirmed case of human infection with influenza (H5N1) virus, according to "WHO case definitions for human infections with influenza A(H5N1) virus" (August 29, 2006).

Since in Study BK-PIFA/002 a four-fold or greater increase in neutralizing antibody titer was noted at post-study investigation in 44.7% of the subjects in the 5 µg group and 70.9% of the subjects in the 15 µg group (Table 15), PMDA has concluded that the H5N1 Vaccine did in fact induce antibody production after 2 administrations to human subjects, revealing the immunogenicity of the H5N1 Vaccine.

Table 15: Proportion of subjects with a four-fold or greater rise in antibody titer (Study BK-PIFA/002, FAS)

Endpoint	Dose group	Assessment timing	Number of subjects included	Subjects with a four-fold or greater rise in antibody titer, %	
				Number of subjects	%
HI antibody titer (equine erythrocytes)	5 µg	Prior to second administration	150	45	30.0
		Post-study investigation	150	65	43.3
	15 µg	Prior to second administration	150	75	50.0
		Post-study investigation	148	102	68.9
HI antibody titer (chicken erythrocytes)	5 µg	Prior to second administration	150	30	20.0
		Post-study investigation	150	22	14.7
	15 µg	Prior to second administration	150	50	33.3
		Post-study investigation	148	40	27.0
Neutralizing antibody titer	5 µg	Prior to second administration	150	34	22.7
		Post-study investigation	150	67	44.7
	15 µg	Prior to second administration	150	55	36.7
		Post-study investigation	148	105	70.9

GMT increase = post-vaccination antibody titer/baseline antibody titer prior to the first vaccination

Percentage of subjects with a four-fold or greater GMT increase (%) =

(number of subjects with a four-fold or greater GMT increase/number of subjects included) × 100

Taken together, HI antibody titer data with equine erythrocytes suggested a potential protective effect against infection, although HI antibody titer data with chicken erythrocytes was insufficient to demonstrate such a protective effect, and neutralizing antibody titer data revealed the definite induction of antibody production following the administration of the H5N1 Vaccine. Thus, PMDA considers the administration of the H5N1 Vaccine to potentially result in acquisition of immunity against pandemic influenza. Highly pathogenic avian influenza (H5N1) is currently prevalent mainly in South-East Asia, with a persistent and expanding epidemic demonstrated by case identification in Europe. Along with growing concern regarding rapid spread into human populations via human-to-human transmission, there

is growing expectation that administration of the H5N1 Vaccine will enhance acquisition of immunity against pandemic influenza.

(2) Safety

PMDA considered that, although adverse reactions occurred at high frequencies with local reactions noted particularly at a high frequency upon the first administration, no serious adverse reactions occurred during clinical studies of the H5N1 Vaccine and concluded that there are no particular problems with respect to its tolerability based on the submitted safety data, considering that the target disease of the H5N1 Vaccine is extremely serious.

This PMDA conclusion will be finalized, taking Expert Advisors’ comments into account.

The details of the safety review are described below.

In Study BK-PIFA/002, the incidence of adverse events by dose group (871 events in 146 of 150 subjects [97.3%] in the 5 µg group vs. 915 events in 139 of 150 subjects [92.7%] in the 15 µg group) and adverse reactions (805 events in 142 of 150 subjects [94.7%] in the 5 µg group vs. 859 events in 138 of 150 subjects [92.0%] in the 15 µg group) tended to be comparable or even higher in the 5 µg group, with no dose-dependency noted. The incidence of local reactions, comprising the majority of adverse events associated with the H5N1 Vaccine, failed to demonstrate dose-dependency. One serious adverse event (radial nerve palsy in the right upper arm (Preferred Term, radial nerve palsy), 20 days after the first administration) occurred in the 15 µg group, but a causal relationship between the H5N1 Vaccine and this event was ruled out. Comparison of the incidence of adverse events assessed after the first and second administration revealed that adverse events tended to occur more frequently upon the first administration in both dose groups (Table 16).

Table 16: Frequency of adverse events by administration timing (Study BK-PIFA/002, safety analysis set)

	Adverse events		Adverse reactions	
	First administration, number of subjects (incidence, 95% CI)	Second administration, number of subjects (incidence, 95% CI)	First administration, number of subjects (incidence, 95% CI)	Second administration, number of subjects (incidence, 95% CI)
5 µg group	140/150 (93.3%, 88.1%-96.8%)	123/150 (82.0%, 74.9%-87.8%)	136/150 (90.7%, 84.8%-94.8%)	117/150 (78.0%, 70.5%-84.3%)
15 µg group	132/150 (88.0%, 81.7%-92.7%)	111/148 (75.0%, 67.2%-81.7%)	129/150 (86.0%, 79.4%-91.1%)	111/148 (75.0%, 67.2%-81.7%)
	Adverse events (Local reactions)		Adverse reactions (Local reactions)	
	First administration, number of subjects (incidence, 95% CI)	Second administration, number of subjects (incidence, 95% CI)	First administration, number of subjects (incidence, 95% CI)	Second administration, number of subjects (incidence, 95% CI)
5 µg group	132/150 (88.0%, 81.7%-92.7%)	112/150 (74.7%, 66.9%-81.4%)	132/150 (88.0%, 81.7%-92.7%)	112/150 (74.7%, 66.9%-81.4%)
15 µg group	128/150 (85.3%, 78.6%-90.6%)	108/148 (73.0%, 65.1%-79.9%)	128/150 (85.3%, 78.6%-90.6%)	108/148 (73.0%, 65.1%-79.9%)

The applicant explained the higher incidence of adverse events upon the first administration of the H5N1 Vaccine as follows.

The applicant confirmed that the incidence of adverse events, occurring at an incidence of 3 % or higher, other than nasopharyngitis tended to be higher upon the first administration compared with that upon the second administration. In particular, the incidence of injection site reactions (injection site erythema, injection site pain, injection site pruritus, and injection site warmth) differed by 10% or even more between the first and second administrations. These findings suggested that the H5N1 Vaccine caused stronger inflammatory reactions at the injection site upon the first than upon the second administration, but the reason is not clear (Table 17). It was likely that not only injection site but also systemic inflammatory reactions associated with immunity tended to be stronger upon the first than the second administration, although this was not confirmed because of the limited number of subjects included in Study BK-PIFA/002. In contrast, no major difference in the severity of adverse events was noted between the first and second administrations.

**Table 17: Incidence of major adverse events (Preferred Term) with each administration
(Study BK-PIFA/002, safety analysis set)**

Symptoms (Preferred Term)	First administration, number of subjects (%)		Second administration, number of subjects (%)	
	5 µg group (150 subjects)	15 µg group (150 subjects)	5 µg group (150 subjects)	15 µg group (148 subjects)
Nasopharyngitis	4 (2.7)	4 (2.7)	8 (5.3)	6 (4.1)
Headache	11 (7.3)	6 (4.0)	9 (6.0)	5 (3.4)
Malaise	8 (5.3)	17 (11.3)	10 (6.7)	5 (3.4)
Injection site erythema	108 (72.0)	114 (76.0)	92 (61.3)	94 (63.5)
Injection site induration	7 (4.7)	13 (8.7)	3 (2.0)	4 (2.7)
Injection site pain	89 (59.3)	95 (63.3)	70 (46.7)	71 (48.0)
Injection site pruritus	76 (50.7)	90 (60.0)	51 (34.0)	54 (36.5)
Injection site warmth	48 (32.0)	65 (43.3)	30 (20.0)	47 (31.8)
Injection site swelling	65 (43.3)	68 (45.3)	56 (37.3)	62 (41.9)

**Table 18: Incidence of major adverse reactions (Preferred Term) with each administration
(Study BK-PIFA/002, safety analysis set)**

Symptoms (Preferred Term)	First administration, number of subjects (%)		Second administration, number of subjects (%)	
	5 µg group (150 subjects)	15 µg group (150 subjects)	5 µg group (150 subjects)	15 µg group (148 subjects)
Nasopharyngitis	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.7)
Headache	9 (6.0)	6 (4.0)	4 (2.7)	4 (2.7)
Malaise	7 (4.7)	17 (11.3)	9 (6.0)	5 (3.4)
Injection site erythema	108 (72.0)	114 (76.0)	92 (61.3)	94 (63.5)
Injection site induration	7 (4.7)	13 (8.7)	3 (2.0)	4 (2.7)
Injection site pain	89 (59.3)	95 (63.3)	70 (46.7)	71 (48.0)
Injection site pruritus	76 (50.7)	90 (60.0)	51 (34.0)	54 (36.5)
Injection site warmth	48 (32.0)	65 (43.3)	30 (20.0)	47 (31.8)
Injection site swelling	65 (43.3)	68 (45.3)	56 (37.3)	62 (41.9)

PMDA recognized that one serious adverse event (radial nerve palsy in the right upper arm [Preferred Term, radial nerve palsy], 20 days after the first administration) occurred in the 15 µg group but a causal relationship between the H5N1 Vaccine and this event was ruled out. PMDA also confirmed that, although adverse events were noted at high frequencies with local reactions noted particularly at a high frequency upon the first administration, no serious adverse reactions actually occurred. Considering that

the target disease of the H5N1 Vaccine is extremely serious, PMDA concluded that there are no particular problems with respect to its tolerability based on the submitted safety data.

Compared with local reactions associated with seasonal influenza vaccine (11.4% according to the package insert of Influenza HA Vaccine “Biken HA,” revised in July 2006 [10th Edition]), local reactions associated with the H5N1 Vaccine tend to occur more frequently (Study BK-PIFA/002, 94.0% [141 of 150 subjects] in the 5 µg group and 91.3% [137 of 150 subjects] in the 15 µg group). The applicant ascribed this to (a) the H5N1 Vaccine being a whole virus vaccine, (b) use of aluminium hydroxide as an adjuvant, (c) difference in methods of surveying adverse reactions, and (d) difference in virus strains used for vaccine production. PMDA considers the applicant’s explanation to be acceptable.

Since serious adverse reactions associated with seasonal influenza vaccine include shock or anaphylactoid symptoms, acute disseminated encephalomyelitis (ADEM), Guillain-Barre syndrome, convulsion (including febrile convulsion), hepatic dysfunction, jaundice, and asthmatic attacks, PMDA believes that information on such adverse reactions should be carefully collected after marketing.

(3) Clinical positioning

While reported cases of influenza suspected to be via human-to-human transmission are extremely rare at present except for those caused by H1N1 and H3N2 subtype pandemic strains (*N. Engl. J. Med.*, 2005; 352: 333-340, *J. Infect. Dis.* 2000; 181: 344-348), the total number of confirmed human cases of influenza (H5N1) available from the WHO website is 315 including 191 deaths (as of June 25, 2007), which demonstrates persistently high fatality with this pathogen in humans and a steady annual increase in both cases and deaths (Table 19; http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_06_15/en/index.html). Considering the fatality rate documented for “Spanish flu” influenza virus (H1N1), 2.5% or higher, during the 1918-1919 pandemic (Marks, G. & Beatty, W. K. *Epidemics.*, 1976; New York: Scribner.), the lethality of the present H5N1 strain may be reduced in a possible future human pandemic; however, recent serologic surveys in Vietnam and Thailand have revealed no evidence of asymptomatic infections (*N. Engl. J. Med.*, 2005; 353: 1374-1385). Therefore, it is currently anticipated that human infection with a pandemic influenza virus strain derived from the avian influenza virus A/H5N1 strains will involve more severe symptoms.

Table 19: Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) reported to WHO (as of June 25, 2007)

	2003	2004	2005	2006	2007	Total
Cases	4	46	98	115	52	315
Deaths	4	32	43	79	33	191
Fatality (%)	100.0	69.6	43.9	68.7	63.5	60.6

As of the time of this regulatory review, the following information has become available on possible treatment methods for H5N1 influenza with their efficacies.

Oseltamivir and zanamivir are reportedly effective in animal models infected with H5N1 influenza virus (*J. Infect. Dis.*, 1998; 178: 1592-1596, *Antimicrob. Agents Chemother.*, 2001; 45: 1216-1224, *Nature*, 2005; 437: 1108). These antivirals have also been reported to inhibit proliferation of H5N1 influenza virus strains isolated from infected humans, suggesting their prophylactic and therapeutic efficacy (*J. Infect. Dis.*, 1998; 178: 1592-1596, *Antimicrob. Agents Chemother.*, 2001; 45: 1216-1224, *Antimicrob. Agents Chemother.*, 2001; 45: 743-748, *J. Infect. Dis.*, 2005; 192: 665-672). In fact, however, their NA-inhibiting and antiviral activities *per se* are not extraordinarily high. Of additional concern, 95% or more of H5N1 influenza virus strains isolated in Vietnam and Thailand were reportedly resistant to amantadine and rimantadine (*J. Infect. Dis.*, 2006; 193: 1626-1629).

The following reports are available on clinical use of oseltamivir. Out of 8 patients with H5N1 influenza receiving oseltamivir, 4 died and oseltamivir-resistant virus strains were isolated from 2 of them (*N. Engl. J. Med.*, 2005; 353: 2667-2672). Antiviral treatment with oceltamivir in patients with H5N1 influenza identified in Vietnam in 2004 led to the following outcomes: H5N1 influenza virus had disappeared from clinical samples within 2 or 3 days after initiation of oseltamivir administration in surviving patients, while early initiation of oseltamivir administration in patients who died had failed to stop disease progression or sufficiently reduce viral count in the pharynx (*Emerg. Infect. Dis.*, 2005; 11: 201-209, *N. Engl. J. Med.*, 2004; 350: 1179-1188).

Judging from the above-mentioned findings, treatment methods for human infection with H5N1 influenza virus are still extremely uncertain and far from established, although some successful attempts have been reported. Therefore, PMDA considers prophylaxis to be the most important for possible countermeasures against pandemic influenza virus infection with an anticipated high fatality rate in humans. Since the immunogenicity of the H5N1 Vaccine in humans was demonstrated, as discussed under “(1) Efficacy” and favorable data was obtained with the H5N1 Vaccine in an immunization/challenge test in an animal model [see “3. Non-clinical data, (i) Summary of pharmacology studies”], PMDA considers administration of the H5N1 Vaccine to potentially reduce clinical symptoms and decrease fatalities associated with pandemic influenza infection.

The Pandemic Influenza Preparedness Action Plan of the Japanese Government (hereinafter referred to as “Action Plan”) states that, at WHO Phase 4 when human-to-human transmission of a new subtype of influenza virus is confirmed, the following countermeasures will be implemented: cooperation with reference laboratories of WHO, World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) for identification and analysis of the virus strain, acquisition of the virus subtype in question, development of candidate virus strains for production of vaccines, and commencement of manufacturing of pandemic vaccines in Japan. PMDA asked the applicant to roughly estimate how long it will take from obtaining the master seed to production of the first vaccine lot. The applicant responded that it will take 19.7 to 22.2 weeks, including the period required for national assay, and 13.7 to 16.2 weeks with omission of the national assay.

Although the first wave of the “Spanish flu” pandemic (1918-1919) was highly contagious but not especially fatal, the second wave was characterized by a 10-fold increase in the fatality rate. In the “Asian flu” pandemic in 1957-1958, a second wave occurred 2 to 3 months after the disappearance of the first wave, causing increased fatalities. PMDA judges that, based on the applicant’s response and experiences of the previous influenza pandemics mentioned above, the causative virus strain isolated during the first wave of a future pandemic can be used for production of a prophylactic vaccine in time for the onset of the second wave considered to be associated with higher fatality.

Historically, the milder clinical symptoms and lower fatality associated with the “Hong Kong flu” pandemic (1968-1969) as compared with those associated with “Spanish flu” (1918-1919) and “Asian flu” (1957-1958) pandemics have been at least partly ascribed to the fact that the immediately preceding “Asian flu” pandemic was caused by the H2N2 strain sharing the N2 subtype with influenza virus A/H3N2, the causative agent of the subsequent “Hong Kong flu” pandemic, and immunity against N2 antigen acquired during the former pandemic protected the exposed populations from the subsequent infection with the H3N2 strain (*J. Infect. Dis.*, 2005; 192: 233-248). Since non-clinical data confirmed that the H5N1 Vaccine exhibits cross-protection against H5N1 influenza virus strains [see “3. Non-clinical data, *Outline of review by PMDA*, (i) Summary of pharmacology studies, (1) Cross-protective effect of the H5N1 Vaccine against infection”], PMDA considers administration of the H5N1 Vaccine to potentially reduce clinical symptoms and decrease fatalities associated with pandemic influenza infection even when the virus strain used for vaccine production is not identical with that causing the actual pandemic.

The Action Plan states that, at WHO Phase 3 when human infection with a new subtype of influenza virus is confirmed, the Japanese Ministry of Health, Labour and Welfare will review candidate virus strains for production of pre-pandemic vaccines according to the availability of clinical isolates of highly pathogenic avian influenza virus at risk for causing a pandemic in humans to start manufacturing and stockpiling pre-pandemic vaccine sources. Although the efficacy of the H5N1 Vaccine in actual pandemic influenza infection remains uncertain due to antigenic variation of the causal virus, infection prophylaxis by administration of the H5N1 Vaccine is currently expected to serve as an emergency countermeasure at the pre-pandemic stage (the period prior to the first wave of a pandemic, WHO Phases 4 and 5) and against the first wave of the pandemic.

Judging from the non-clinical and clinical data submitted and based on the historical experiences mentioned above, PMDA considered the H5N1 Vaccine to potentially exhibit a protective effect against pandemic influenza or prevent symptoms becoming more severe, and concluded that the H5N1 Vaccine should be positioned as a prophylactic vaccine against pandemic influenza virus infection.

This PMDA conclusion will be finalized, taking Expert Advisors’ comments into account.

(4) Indications

Based on the data for the H5N1 Vaccine manufactured using influenza virus strain NIBRG-14 and also considering the discussion described under “(3) Clinical positioning,” PMDA has concluded that it is acceptable to state in the INDICATIONS section that the vaccine is indicated for “prophylaxis of pandemic influenza.” However, the following measures are considered to be necessary. Since no data on efficacy and safety of the H5N1 Vaccine in younger subjects (under 20 years of age) and elderly people (65 years of age or older) were submitted for this regulatory review, it is appropriate to include the description “Immunogenicity and safety have not been established in pediatric and geriatric populations.” in the PRECAUTIONS section in the package insert of the product. Also, assessing immunogenicity and safety in children and elderly people after marketing is essential, as discussed below [see “(6) Post-marketing considerations”].

PMDA asked the applicant to specify which individuals, if any, are not eligible for immunization with the H5N1 Vaccine.

The applicant responded as follows.

As is the case with vaccination against seasonal influenza, individuals falling under Paragraphs 2 to 4 and 6, Article 2 of Enforcement Regulations of Preventive Vaccination Law in Japan (Paragraph 2, Individuals with apparent pyrexia; Paragraph 3, Individuals evidently developing a severe acute disease; Paragraph 4, Individuals with an obvious history of anaphylactic reaction to any ingredient of an injection used for immunization against the target disease; and Paragraph 6, Individuals not falling under Paragraphs 2 to 5 but in a condition not eligible for immunization) may not be eligible for immunization with the H5N1 Vaccine.

PMDA concluded that it is appropriate to include the following statement in the package insert of the product: “The vaccine should be used with caution, with consideration of the health status and constitution of the recipient, after careful consultation and assessment of eligibility for vaccination followed by fully informing the recipient of the necessity, adverse reactions, and usefulness of vaccination to secure the prior consent of the recipient, and only if the potential benefits outweigh the potential risks.”

This PMDA conclusion will be further considered, taking Expert Advisors’ comments into account.

(5) Dosage and administration

The proposed DOSAGE AND ADMINISTRATION is “The usual dosage is 2 injections of 15 µg in terms of HA content per dose, administered subcutaneously or intramuscularly. If necessary, a single injection of 5 to 15 µg per dose or 2 doses may be administered subcutaneously or intramuscularly.”

PMDA has concluded that, based on the clinical study data, the DOSAGE AND ADMINISTRATION should be “The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses,” with the antigen content per dose (0.5 mL) specified as “15 µg (in terms of HA antigen)” in the columns of ingredients and their quantity and manufacturing method in the approval form.

This PMDA conclusion will be finalized, taking Expert Advisors’ comments into account.

The details of the review are described below.

1) Route of administration

In Study BK-PIFA/001, no major difference in the frequency of adverse events was noted between different routes of administration (Table 20).

Table 20: Frequency of adverse events by route of administration in Study BK-PIFA/001

		Between first and second administrations				Between second administration and post-study investigation				Between first administration and post-study investigation			
		Number of subjects included	Number of subjects	Number of events	Incidence (%)	Number of subjects included	Number of subjects	Number of events	Incidence (%)	Number of subjects included	Number of subjects	Number of events	Incidence (%)
Subcutaneous	Adverse events	60	46	112	76.7	58	35	77	60.3	60	54	189	90.0
	Adverse reactions	60	40	85	66.7	58	30	56	51.7	60	51	141	85.0
Intramuscular	Adverse events	60	41	73	68.3	60	36	75	60.0	60	51	148	85.0
	Adverse reactions	60	34	55	56.7	60	24	50	40.0	60	41	105	68.3

Furthermore, when immunogenicity was compared between different routes of administration, post-administration antibody titers in the intramuscular group tended to be higher than those in the subcutaneous group, but the difference between these 2 groups was minor. Based on this finding, PMDA considers both subcutaneous and intramuscular routes to possibly be used in administration of the H5N1 Vaccine. However, currently available Study BK-PIFA/001 information is limited concerning intramuscular administration of the H5N1 Vaccine. PMDA has therefore concluded that it is appropriate to state in the PRECAUTIONS section in the package insert that “Experiences with intramuscular injection are limited.”

2) Antigen content per dose

PMDA considers it desirable to specify the antigen content per dose of the H5N1 Vaccine as 15 µg, expecting a rise in antibody titer to the possible extent, because the threshold of antibody titer in relation to the protective effect against infection is unknown. Furthermore, as mentioned in “(2) Safety,” Study BK-PIFA/002 demonstrated no dose-dependent increase in incidences of either adverse events or adverse reactions (adverse events, 97.3% in the 5 µg group, 92.7% in the 15 µg group; adverse reactions, 94.7% in the 5 µg group, 92% in the 15 µg group). Thus, PMDA thinks that there are no particular problems in view of the safety with defining the antigen content as 15 µg per dose, the highest dose assessed in the clinical studies. To assure appropriate use of the H5N1 Vaccine in clinical practice, its dosage should be specified in terms of volume of the injection solution, not in terms of antigen content per dose. PMDA has therefore concluded that the dosage should be 0.5 mL per dose, with the antigen content per dose (0.5 mL) specified as “15 µg (in terms of HA antigen)” in the columns of ingredients and their quantity and manufacturing method in the approval certificate.

3) Dosage and administration

While the interval between 2 injections was scheduled to be 21 ± 1 days in Study BK-PIFA/001 and 21 ± 7 days in Study BK-PIFA/002, the actual interval was 20-22 days in the former and 14-27 days in the latter. PMDA asked for the applicant’s view on the possible effects of differences in the injection interval (up to 13 days) on efficacy and safety. The applicant responded that the injection interval does not seem to seriously affect the efficacy or the safety of the H5N1 Vaccine, because Study BK-PIFA/002 demonstrated that there were no differences in antibody production or the incidence of adverse reactions depending on the injection interval. PMDA accepted the applicant’s response.

As described in “(2) Antigen content per dose,” PMDA considers it desirable to specify the antigen content per dose of the H5N1 Vaccine as 15 µg and the dosage as 2 injections, expecting a rise in antibody titer to the extent possible with the threshold of antibody titer in relation to the protective effect against infection unknown.

However, as discussed below [see “(6) Post-marketing considerations 3) Considerations for antigen saving”], it may become impossible to ensure a sufficient production of the H5N1 Vaccine at the onset of a pandemic. Furthermore, social activities of the population will be restricted in association with such a pandemic (Action Plan), and thus, it may become difficult for individuals to receive multiple injections of the H5N1 Vaccine. As suggested from the experience of the “Hong Kong flu” pandemic (*J. Infect. Dis.*, 2005; 192: 233-248), it is assumed that there would arise the necessity of examining a possible strategy, which allows immunization with a virus strain causing the pandemic to prevent symptoms becoming more severe, despite the reduction of the antigen content per dose. PMDA therefore considers that 1 or 2 injections of the H5N1 Vaccine at an antigen content of 5 to 15 µg per dose may be acceptable, depending on the situation.

(6) Post-marketing considerations

1) Populations not included in clinical studies

a. Children

A WHO report (*Weekly epidemiological record.*, No. 26, 2007, 82, 41-48) analyzed 256 cases of human infection with H5N1 influenza virus strain reported between November 25, 2003 and November 24, 2006 and demonstrated the patient number and fatality rate to be the highest in the age group between 10 and 19 years (Table 21).

Table 21: Fatality rate among laboratory-confirmed human cases of H5N1 infection by age group

(Source: *Weekly epidemiological record.*, No. 26, 2007, 82, 41-48)

Age group	< 5	5-9	10-19	20-29	30-39	40-49	≥ 50	Total
Fatality rate (%)	44.4 (12/27)	48.7 (19/39)	75.8 (50/66)	63.0 (34/54)	65.9 (27/41)	42.9 (6/14)	40.0 (6/15)	60.2 (154/256)

Based on the WHO data suggesting the possibility of a high incidence of infection and a high fatality rate in the younger population upon the onset of pandemic influenza, and considering previous reports on seasonal influenza indicating that (a) the risk of death and sequelae due to influenza-related encephalopathy tended to be higher in the younger population; (b) an increase in excess mortality in elderly people (*N. Engl. J. Med.*, 2001; 344: 889-896) and a rapid increase in influenza mortality in infants (*Nippon Eiseigaku Zasshi [Japanese Journal of Hygiene]* 2002; 57: 571-584) were noted after discontinuation of mass vaccination of schoolchildren in the 1980’s; (c) vaccination is effective in preventing encephalopathy (Steering Committee of Japanese Society for Pediatric Infectious Diseases, *Shoni Knsen Meneki [Infection and Immunity in Childhood]*. 1999; 11: 429-431); and (d) children constitute a vulnerable population (the clinical trial directive EU/2001/20 Official J. European Communities 1.5.2001 L 121/34), PMDA considers development of the H5N1 Vaccine for recipients 20 years of age or younger to be essential and advocates that it be conducted as soon as possible.

PMDA asked the applicant about the development schedule for the H5N1 Vaccine in children. The

applicant responded that the development of the H5N1 Vaccine for children had not been planned at the time point of this regulatory review.

Administration of the H5N1 Vaccine in children is not recommended because no pediatric clinical data, particularly information on safety, was submitted for this regulatory review; however, PMDA has concluded that children should not be excluded from the target population of the H5N1 Vaccine and lack of sufficient information on safety in children should be clearly stated in the “Pediatric Use” section of the package insert, considering that the target disease of the H5N1 Vaccine is extremely serious. On the other hand, PMDA considers that detailed post-marketing information on immunogenicity and safety in children should be collected, and in particular, that the safety of the H5N1 Vaccine should be assessed as soon as possible.

b. Elderly people

Study BK-PIFA/002 demonstrated that the seroconversion rate tended to be higher in the younger group (20-39 years of age) than in the older group (40-65 years of age), except for data obtained prior to the second administration in the 5 µg group. Based on this finding, PMDA asked for the applicant’s view on the effect of age on the efficacy of the H5N1 Vaccine.

The applicant responded that, although no data was available on administration of the H5N1 Vaccine to elderly people as of the time of this regulatory review, the immunological response might be weaker in elderly people, judging from the fact that seroconversion rate in the older group (40-65 years of age) was lower than that in the younger group (20-39 years of age).

Based on a reported tendency for a smaller post-vaccination rise in antibody titer in elderly people as compared with that in young people receiving seasonal influenza vaccine (*Vaccine*, 2006; 24: 1159-1169), PMDA considers a similar concern to apply to the H5N1 Vaccine as well. PMDA has also concluded that, although administration of the H5N1 Vaccine to elderly people is not recommended because no safety data for this population are currently available, elderly people should not be excluded from the target population of the H5N1 Vaccine and lack of sufficient information on safety in elderly people should be clearly stated in the “Use in the Elderly” section of the package insert, considering that the target disease of the H5N1 Vaccine is extremely serious. PMDA further considers it necessary to quickly collect post-marketing information on the immunogenicity and safety of the H5N1 Vaccine in elderly people.

c. Women of child-bearing age

PMDA asked the applicant to explain clinical and non-clinical data currently available on the safety of the H5N1 Vaccine in women of child-bearing age and in pregnant women.

The applicant responded as follows.

The H5N1 Vaccine is a protein preparation manufactured by inactivating pandemic influenza virions propagated in embryonated chicken eggs and its potential to affect reproductive and developmental processes may be minor. Furthermore, no currently available non-clinical data demonstrate effects of the H5N1 Vaccine on fertility or maintenance of pregnancy, nor was any harm, such as embryonic lethality, fetal growth inhibition, or teratogenicity, documented. However, based on the fact that data on the safety of the H5N1 Vaccine administered to pregnant women is not sufficient, currently it is reasonable to exclude pregnant or potentially pregnant women, in principle, from among the recipients of the H5N1 Vaccine and to limit administration to cases in which the potential benefits outweigh the potential risks, as in vaccination with the currently available influenza HA vaccine. In addition, the final results of the ongoing study on pre- and postnatal development and maternal functions will be reported as soon as they are made available.

PMDA accepted the applicant's response.

2) Efficacy and safety of the H5N1 Vaccine manufactured with a vaccine strain other than NIBRG-14

The applicant submitted for this regulatory review the data on the quality, efficacy (immunogenicity), and safety of the H5N1 Vaccine manufactured with strain NIBRG-14 as a mock-up vaccine against pandemic influenza. However, the efficacy and safety of the H5N1 Vaccine manufactured with a vaccine strain derived from another pandemic influenza virus strain, including those of the H5N1 subtype, have not been investigated. Therefore, whether the efficacy and safety of such a vaccine are identical with those of the original H5N1 Vaccine manufactured with strain NIBRG-14 or how much these vaccines differ, if at all, with respect to efficacy and safety remains obscure. PMDA considers it necessary to confirm the efficacy (immunogenicity) and safety of the H5N1 Vaccine manufactured with a different vaccine strain in place of strain NIBRG-14 in the near future.

3) Considerations for antigen saving

PMDA has concluded that, based on the clinical study data, the DOSAGE AND ADMINISTRATION should be "2 injections of 15 µg HA per dose" [see "(5) Dosage and administration"].

The H5N1 Vaccine is manufactured by purifying and inactivating virus propagated in embryonated chicken eggs and production of the vaccine antigen depends on the number of chicken eggs available. The possible prevalence of highly pathogenic avian influenza in chickens concomitant with an outbreak of pandemic influenza in humans is a situation in which securing a sufficient number of chicken eggs for vaccine production is assumed to be difficult or even impossible. When the amount of available vaccine antigen is limited, reducing the antigen content per dose to increase the number of vaccine recipients may achieve a greater protective effect against pandemic influenza virus infection at the population level at the onset of the pandemic, a strategy preferable in view of

public health rather than immunizing a limited number of recipients according to the pre-defined DOSAGE AND ADMINISTRATION. The Japanese Ministry of Health, Labour and Welfare plans to take hold of the vaccine supply and discuss the priority for vaccination at the onset of an actual pandemic (“Guidelines on Vaccination against Pandemic Influenza”). The submitted clinical data demonstrates that the H5N1 Vaccine induced antibody production in humans when administered twice at a dose of 5 µg of antigen per injection, although with a smaller rise in antibody titer than that achieved when administered twice at a dose of 15 µg of antigen per injection. This finding suggests that administration of the H5N1 Vaccine with the antigen content per dose reduced from 15 µg in an actual influenza pandemic may exhibit a certain priming effect. PMDA therefore considers further assessment of administration of the H5N1 Vaccine at a reduced dose to be desired, including clinical studies to confirm a booster effect of re-vaccination after a certain period following the initial low-dose vaccination.

4) Cross-protective effect

Non-clinical data confirmed that the H5N1 Vaccine exhibited cross-protection against H5N1 influenza virus strains with different antigenicities [see “3. Non-clinical data, *Outline of review by PMDA*, (i) Summary of pharmacology studies, (1) Cross-protective effect of the H5N1 Vaccine against infection”], which suggests that administration of the H5N1 Vaccine has the potential to reduce clinical symptoms and decrease fatalities associated with pandemic influenza infection even when the virus strain used for vaccine production is not identical to that causing the actual pandemic due to antigenic variation. PMDA considers it desirable to further assess the cross-reactivity of antibodies induced by administration of the H5N1 Vaccine in humans with influenza virus strains other than that used for vaccine production.

III. Results of Compliance Review Concerning the Documents Appended to the New Drug Application and Conclusion by PMDA

1. PMDA conclusion regarding the results of document compliance review

Document compliance review was conducted in accordance with the provisions of the Pharmaceutical Affairs Law for documents appended to the new drug application. As there were no major problems, it was concluded that there should be no problem with conducting a regulatory review based on the application dossier.

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

GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Law for the dossiers appended to the new drug application (Study BK-PIFA/001, 5.3.5.1-1 and Study BK-PIFA/002, 5.3.5.1-2). The results revealed: failure to submit the audit protocol to the

head of the study site, deficiencies in records concerning the quality and control of the investigational vaccine, inadequate monitoring activities, non-compliance with the procedure for preparation of the clinical study reports (concerning the investigator), and deficiencies in management of the Institutional Review Board of some study sites related to the audit report and monitoring report. However, as there were no major problems, it was concluded that there should be no problem with conducting a regulatory review based on the application dossier.

IV. Overall Evaluation

As for the efficacy of the H5N1 Vaccine, although it is difficult at present to assess its protective effect against pandemic influenza infection, the H5N1 Vaccine induced, after 2 injections at a dose of 15 µg per injection, an increase in HI antibody titer (equine erythrocytes) fulfilling 2 of these 3 assessment criteria, all of which should be fulfilled according to the EMEA/CHMP guideline for pandemic influenza vaccines (CHMP/VWP/263499/2006). In addition, it induced an increase in neutralizing antibody titer fulfilling the same 2 assessment criteria. PMDA therefore considers the administration of the H5N1 Vaccine to potentially result in acquisition of immunity against pandemic influenza. Also, the H5N1 Vaccine was confirmed to show a protective effect against challenge with a highly virulent H5N1 influenza virus strain in mice. Based on these findings, PMDA concluded that the H5N1 Vaccine has the potential to exhibit a protective effect against pandemic influenza virus infection or prevent symptoms becoming more severe.

As for the safety of the H5N1 Vaccine, adverse reactions occurred at high frequencies, with local reactions noted particularly at a high frequency upon the first administration, but no serious adverse reactions occurred. Considering that the target disease of the H5N1 Vaccine is extremely serious, PMDA concluded that no particular problems are noted with respect to its tolerability.

PMDA considers it necessary to collect detailed post-marketing information on the immunogenicity and safety of the H5N1 Vaccine in children, and in particular to assess the safety as soon as possible. In addition, the immunogenicity and safety in elderly people as well as the efficacy (immunogenicity) and safety of the H5N1 Vaccine manufactured with a different vaccine strain in place of strain NIBRG-14 should be confirmed. Also, it is desirable to assess the antigen reduction as well as the cross-protective effects, assuming the use of the H5N1 Vaccine in an actual pandemic.

Review Report (2)

August 15, 2007

I. Summary of the Product

[Brand name]	Adsorbed Influenza Vaccine (H5N1) “BIKEN”
[Non-proprietary name]	Adsorbed Influenza Vaccine (H5N1)
[Applicant]	The Research Foundation for Microbial Diseases of Osaka University
[Date of application]	January 30, 2007

II. Contents of Review

The Pharmaceuticals and Medical Devices Agency (hereinafter referred to as PMDA) discusses below the matters related to quality that had not been reviewed at the time of preparation of the Review Report (1), and the matters related to the study results submitted after Expert Discussion.

Also, PMDA sought the comments of the Expert Advisors on the Review Report (1). A summary of the review based on the discussion of the Expert Advisors and also a summary of the review of the data submitted after the Expert Discussion are described below.

The Expert Advisors participating in the Expert Discussion have remarked that Item 1 and Item 2 (1) of “Immediate measures for the issue of conflict of interest involving outside experts in PMDA,” dated May 8, 2007, is not applicable to the H5N1 Vaccine.

1. Quality

The items for which the review was not completed by the time of preparation of the Review Report (1) and the test results submitted after the Expert Discussion are as follows.

(1) Reference standard

PMDA asked the applicant the reason for changing the storage condition of the standard influenza HA antigen (for SRD), which is used to determine the HA content (by SRD) of the bulk and the potency (by SRD) of the drug product, from “10°C or lower” to “-20°C to -30°C.”

The applicant responded as follows.

The reference standard in question was supplied from the National Institute of Infectious Diseases. First ■ years and ■ months, and then ■ years after the manufacture, the National Institute of Infectious Diseases and the manufacturers developing pandemic influenza vaccines examined the reference standard based on the NIBSC standard antigen (International Reference Standard). The reference standard stored under refrigeration had about ■% less HA content (conversion according to

the International Reference Standard) as compared to the same reference standard stored under freezing, suggesting a possible stability problem with storage under refrigeration. Therefore, after discussions between the National Institute of Infectious Diseases and the vaccine manufacturers, the storage condition for the reference standard was changed to that under freezing (-20°C to -30°C).

PMDA considered that the values of the HA content (by SRD) of the bulk and the potency (by SRD) of the drug product presented in the application dossier might not be accurate, because the reference standard stored under refrigeration was used as the reference sample for all the tests including the long-term testing, and asked the applicant to explain this point.

The applicant responded as follows.

It is considered that there is no stability problem with the reference standard, even though stored under refrigeration, because no change was noted over time in the diffusion ring diameter as determined by SRD for ■ months after the manufacture.

PMDA considers as follows.

Taking into account the precision of the test method, it is likely that the measurement of the diffusion ring diameter is not sensitive enough to detect change in the HA content. The HA content determined using the reference standard stored under refrigeration decreased by about ■% as compared with that determined with the reference standard stored under freezing in SRD with higher precision based on the International Reference Standard. This result cannot be ignored. That is to say, PMDA does not agree the applicant's claim that there is no problem with the reference standard used. Furthermore, PMDA asked the applicant whether there was any problem with the reference standard used for other lots. The applicant responded that there might be a problem in relation to the uniformity of the HA content also for the reference standard used for the subsequent lot, and that a new lot of reference standard was currently being prepared at the National Institute of Infectious Diseases.

There was not enough time to establish a quality control system for the reference standard, including stability evaluation and optimization of the storage condition, because the development of the product was sought urgently. PMDA requested the National Institute of Infectious Diseases, through the Ministry of Health, Labour and Welfare, to establish a quality control system for the reference standard as soon as possible. PMDA also requested the applicant to re-evaluate the stability of the product and the test methods employing SRD for determination of the HA content and potency, after the quality of the reference standard is ensured, and to revise the acceptance criteria as needed. The applicant responded that it would take an appropriate action.

(2) Specifications and test methods for the bulk

PMDA requested the applicant to revise the acceptance criteria of the specifications for the bulk,

such as protein content, HA content, bacterial endotoxins test, ovalbumin content, and pyrogen test, because the justification for these acceptance criteria was not clear. The applicant revised them appropriately based on the actual measured values. The acceptance criteria of the HA content were decided to be revised when the quality of the reference standard is established, as stated in “(1) Reference standard.”

PMDA accepted the above response.

(3) Specifications and test methods for the drug product

PMDA asked the applicant whether the content uniformity test is required, because the product is an injectable suspension. The applicant responded that it would additionally include the content uniformity test in the specifications. PMDA also requested the applicant to perform the insoluble particulate matter test, because the Japanese Pharmacopoeia 15th edition specifies in the General Rules for Preparations that the test should be performed also for injectable suspensions. The applicant responded that it would additionally include the insoluble particulate matter test in the specifications after confirming that the test is feasible with solubilization of the drug product achieved by adding nitric acid and heating.

PMDA also requested the applicant to revise the acceptance criteria of the specifications for the drug product, such as protein content, potency, aluminum content, thimerosal content, and formaldehyde content, based on the actual measured values. The applicant revised them appropriately. The acceptance criteria of the potency were decided to be revised when the quality of the reference standard is established, as stated in “(1) Reference standard.”

PMDA accepted the above response.

(4) Biological materials and their control

PMDA asked the applicant to explain the countermeasures against the adventitious viral contamination during the manufacturing process of the product.

The applicant responded as follows.

Most viruses that possibly contaminate embryonated eggs can be controlled by vaccination of adult chickens. These viruses are also detectable by evaluating the health condition of adult chickens and counting the eggs laid. Inactivation of these adventitious viruses is considered to be feasible, because the clearance index of the vaccine strain (NIBRG-14 strain) during the inactivation process (formalin concentration ■■■% (v/v), ■■■°C, not less than ■■ days) by formaldehyde is more than 8.7, the viral clearance index of each of the Japanese encephalitis, measles, and rubella viruses by ■■■% (v/v) formalin treatment (■■■°C, ■■ days) is > ■■ to > ■■, and the clearance index of type I herpes simplex and human polio by ■■% (v/v) formalin treatment (■■■°C, ■■ days) is > ■■ and ■■.

respectively.

PMDA considers as follows.

Although vaccination of adult chickens and antibody tests are undertaken, no acceptance criteria of the antibody titer increase have been developed. The viral contamination cannot be completely controlled by vaccination only. As for the viral clearance evaluation results, the viral clearance capability of the manufacturing process of the product has not been evaluated adequately, because the Japanese encephalitis, measles, and rubella viruses are enveloped viruses, and the clearance index of the polio virus, a non-enveloped virus which may have high formalin resistance, is the result obtained at [REDACTED] % (v/v) of formalin concentration. Therefore, the countermeasures taken against adventitious viral contamination of the product cannot be considered adequate. PMDA requested the applicant to strengthen the control system of the embryonated eggs, including development of acceptance criteria of the antibody titer after vaccination of adult chickens, and to perform virus tests on intermediates (bulk, etc.) until virus clearance test results are obtained using an appropriate model virus. Furthermore, PMDA also requested the applicant to control mycoplasma contamination, because neither control nor clearance evaluation was performed on any other raw materials or intermediates, except for the control of mycoplasma in the virus seed.

The applicant responded as follows.

Adult chickens will strictly be controlled by defining frequency of antibody tests and acceptance criteria. For the inactivation process, clearance tests will be performed using chicken leukemia virus (enveloped), chicken adenovirus (non-enveloped), and mycoplasma, as recommended by the WHO (WHO TRS No. 927, 2005) for evaluation of inactivation process of inactivated influenza vaccines derived from egg; the results will be submitted around [REDACTED], 20[REDACTED]. Until the safety against adventitious infectious substances is confirmed by the above tests, adventitious virus test and mycoplasma test will be performed on the bulk.

PMDA called for information about the detection sensitivity of the adventitious virus test and mycoplasma test, which are planned to be performed. The applicant responded that the Newcastle disease virus, which has the potential to infect humans, can be detected, however, the accurate sensitivity is unclear. The applicant stated that it would confirm the detection sensitivity, including for other viruses and 2 types of chicken mycoplasma, and submit the results by [REDACTED], 20[REDACTED].

PMDA accepted the above response.

(5) Others

The results of sucrose density-gradient centrifugation and electron microscopy obtained at [REDACTED] months in the long-term testing of the bulk were stated to be the same as those performed after [REDACTED] months [see “Review Report (1), 2. Data relating to quality, *Outline of review by PMDA*, (4)

Stability of the bulk”]. However, it was found that the result at ■ months was actually obtained ■ months after the manufacture (after the start of the long-term testing), and it was revealed that the stability of the higher-order structure could not be evaluated adequately. PMDA requested the applicant to further evaluate the stability, including that of the higher-order structure, in the ongoing long-term testing of the stock solution manufactured from the Indonesian strain. The applicant responded that the relevant test results would be submitted at the partial change approval application for extension of the expiration period. PMDA accepted this response.

In regard to the issues for which it was stated in the Review Report (1) that additional confirmation would be made, the applicant responded as follows.

• **“(2) Manufacturing process control of the bulk, 2) Virus incubation process”**

As for the use of antibiotics, the applicant responded as follows.

The residual concentration of minocycline hydrochloride for injection in the clarified virus solution was determined to be less than ■ μg/mL of the quantitation limit. Because this solution is diluted not less than ■ × 10■ times in the subsequent processes, the theoretical residual concentration in the drug product would be less than 0.05 pg/mL, and it was confirmed that the antibiotic was removed appropriately. Also, β-lactam antibiotics will not be used, and the data regarding the residual amount of the new candidate antibiotics, such as pazufloxacin mesilate injection, fosfomycin sodium for injection, and dibekacin sulfate for injection, will be submitted by the time of the approval.

PMDA accepted the above response.

• **“(2) Manufacturing process control of the bulk, 3) Inactivation process”**

As for the sensitivity of the inactivation test performed after the inactivation process, the test results for the detection limit in the presence and absence of formalin were submitted. The results showed that the detection sensitivity of the test in the presence of ■% (v/v) formalin was 10■ times lower than that in its absence, and 10■ times lower than the detection limit for the inactivation test (in the presence of ■% [v/v] formalin) of the bulk. The applicant explained that the decrease in the sensitivity was not significant, and any residual infectious viruses would be detected adequately, because the inactivation test is included in the specifications for the bulk and drug product.

PMDA accepted the above response.

• **“(5) Analytical validation of the test methods”**

The analytical validation data of the test methods was submitted additionally. In regard to the critical in-process control tests and the specifications including the inactivation test described above, the

applicant explained the validity of the test methods by showing the results of the additional investigations, mainly the specificity, intermediate precision, detection limit, and so on. PMDA accepted these explanations.

2. Toxicology studies

The study of the effect of the H5N1 Vaccine on pre- and postnatal development of offspring and maternal function, which was reported as ongoing at the time of preparation of the Review Report (1), was completed and the data were submitted. The product was injected subcutaneously at doses of 0.25 and 0.5 mL/kg, corresponding to 25 and 50 times the clinical dose, a total of 7 times, namely, 3 times on Days 7, 12, and 17 of gestation and 4 times on Days 0, 7, 14, and 21 after delivery. No changes attributable to the H5N1 Vaccine were observed in either the general condition, etc. of the dams or the development, etc. of the F1 offspring. From these results, NOAEL was estimated to be 0.5 mL/kg or above, both for the dams and for the F1 offspring.

PMDA concluded there is no major problem with the toxicology study data, including the above study results that were submitted after the application, taking into account the discussion at the Expert Discussion.

3. Efficacy

Although HI antibody titer data obtained with chicken erythrocytes failed to suggest a protective effect of administration of the H5N1 Vaccine against pandemic influenza virus infection, (a) HI antibody titer data obtained with equine erythrocytes suggested a potential protective effect against infection, and (b) neutralizing antibody titer data clearly demonstrated antibody production induced by administration of the H5N1 Vaccine. PMDA has therefore concluded that administration of the H5N1 Vaccine has the potential to result in acquisition of immunity against pandemic influenza, thereby reducing clinical symptoms and decreasing fatalities associated with the disease.

The Expert Advisors made the following comments.

Although the efficacy of a vaccine should generally be demonstrated by confirmation of its protective effect against infection, it is difficult to assess protection against pandemic influenza virus infection in advance. Furthermore, associations between HI antibody titer or neutralizing antibody titer and protection against infection are not sufficiently clear for pandemic influenza, which is a limitation in the efficacy assessment of the H5N1 Vaccine on the basis of these antibody titers. Nevertheless, the efficacy of the H5N1 Vaccine must be assessed in terms of immunogenicity because no other indices are as yet available. Considering the submitted non-clinical data in mice, including a protective effect against infection, the conclusion by PMDA that a protective effect of the H5N1 Vaccine against virus infection in humans may well be expected to be reasonable. Thus, the conclusion was supported by the Expert Advisors. Methods for antibody titer assay were also discussed. PMDA considers it difficult to compare the immunogenicity of the H5N1 Vaccine with

that of similar drug products available in Japan and overseas due to the absence of a domestically standardized method (i.e., standardized among the vaccine manufacturing sites in Japan) or an internationally standardized method. This was accepted by the Expert Advisors.

The Expert Advisors made the following comment: Long-term changes in post-vaccination antibody titer should be confirmed by follow-up assessment. Since follow-up assessment of antibody titer had been scheduled 90 days and 180 days after administration of the H5N1 Vaccine based on discussion at clinical trial consultation during its development, PMDA asked the applicant to immediately present the available follow-up data.

The applicant responded as follows.

A clinical study was conducted in the subjects of Study BK-PIFA/002 to measure HI antibody titers (equine erythrocytes) and neutralizing antibody titers 90 days and 180 days after the first administration of the investigational vaccine. Although the final follow-up report according to the statistical analysis plan of this study will be completed around the middle to the end of August, the following limited data is currently available: although neither neutralizing antibody titers nor HI antibody titers (equine erythrocytes) changed greatly in the 5 µg group, neutralizing antibody titer slightly decreased in the 15 µg group 180 days after the first vaccination.

Based on the data presented above, the applicant explained the considerations in use of the H5N1 Vaccine as either a pre-pandemic or a pandemic vaccine as follows:

When the H5N1 Vaccine is used as a pre-pandemic vaccine and subsequent occurrence of a pandemic is predicted approximately 6 months after the pre-pandemic vaccination, re-assessment of antibody titers in the recipients is recommended and an additional administration of the H5N1 Vaccine may be helpful depending on the re-assessment data. When the H5N1 Vaccine is used as a pandemic vaccine, its protective effect is expected to be maintained during the pandemic period of approximately 6 months. In addition, an opportunity for spontaneous infection during the pandemic period would elicit a booster effect to maintain the antibody titer level over a post-vaccination period longer than 6 months.

Changes in antibody titer

Assessment timing	Dose group	Number of subjects*	Neutralizing antibody titer (fold)		HI antibody titer (fold)	
			Geometric mean	Standard deviation	Geometric mean	Standard deviation
Prior to first administration	5 µg	150	14.5	1.95	5.4	1.38
	15 µg	150	15.3	1.76	5.2	1.25
Prior to second administration	5 µg	150	24.6	2.46	10.6	2.64
	15 µg	150	36.5	2.50	16.5	2.63
Post-study investigation	5 µg	150	38.2	2.61	13.2	2.61
	15 µg	148	72.5	2.46	22.4	2.41
90 days after first administration	5 µg	143	38.3	2.94	14.0	1.78
	15 µg	144	78.5	2.67	18.2	1.94
180 days after first administration	5 µg	142	38.5	2.51	14.4	1.83
	15 µg	148	56.6	2.53	18.7	1.94

* Antibody titer data prior to first and second administrations, and on post-study investigation, were obtained in the FAS in Study BK-PIFA/002, while follow-up data were obtained 90 days and 180 days after the first administration in the subjects of Study BK-PIFA/002 who gave informed consent to participate in the follow-up study.

PMDA considers as follows.

The Pandemic Influenza Preparedness Action Plan of the Japanese Government and Guidelines on Vaccination against Pandemic Influenza state that, at WHO Phase 4 when human-to-human transmission of a pandemic influenza virus is confirmed, the following countermeasures will be implemented: commencement of vaccination with pre-pandemic vaccines, acquisition of a pandemic influenza virus strain for vaccine production immediately followed by manufacturing of pandemic vaccines, and commencement of vaccination with pandemic vaccines. Neutralizing antibody titer tended to decrease over time after vaccination, as already stated. Therefore, it is necessary to investigate a need for re-vaccination after a prolonged interval from the initial vaccination (e.g., an interval from the initial vaccination with a pre-pandemic or pandemic vaccine to the first wave of a pandemic at WHO Phase 6 or an interval from vaccination with a pandemic vaccine during or after the first wave of a pandemic to a subsequent second or third wave of the identical pandemic) based on the completed final report of the aforementioned clinical study, and to implement an additional clinical study to confirm the efficacy and safety of booster vaccination (re-vaccination) after a certain period following the initial vaccination.

4. Safety

Although adverse reactions occurred at high frequencies with injection site reactions noted particularly at a high frequency upon the first administration, such frequent development of intense local (injection site) reactions can be readily predicted because the H5N1 Vaccine is not a split vaccine (a vaccine consisting of a mix of virus components but retaining no virion structure) like seasonal influenza vaccines but does contain inactivated whole virions with a potentially higher immunogenicity as the active ingredient as well as aluminium hydroxide as an adjuvant. On the other hand, no serious adverse reactions occurred except at the injection site. Considering that the target disease of the H5N1 Vaccine is extremely serious, PMDA concluded that there are no

particular problems with respect to its tolerability based on the submitted safety data.

The above PMDA conclusion was supported by the Expert Advisors. Moreover, the Expert Advisors made the following comments.

Although adverse reactions associated with the H5N1 Vaccine are tolerable, their frequencies are much higher than those of other vaccines previously approved in Japan. Therefore, safety information such as the type and frequency of individual adverse reactions reported in the clinical studies should be provided to the recipients of the H5N1 Vaccine. Also, information on risks and benefits associated with vaccination with the H5N1 Vaccine should be fully provided prior to actual vaccination, because to what extent the immunity against pandemic influenza acquired by vaccination with the H5N1 Vaccine is actually effective in preventing infection has not yet been confirmed. Moreover, the Expert Advisors stated: Quick and detailed safety data collection is crucial, because the H5N1 Vaccine will be administered to an extremely large number of recipients within a short time when actually used [see “Review Report (1), 4. Clinical data, *Outline of review by PMDA*, (6) Post-marketing considerations”].

5. Clinical positioning and indications

Based on the results of assessment of the immunogenicity and safety of the H5N1 Vaccine, a mock-up vaccine produced with influenza virus strain NIBRG-14, PMDA has concluded that it has the potential to be useful as a vaccine against H5N1 influenza. Furthermore, considering the submitted non-clinical data and based on historical experiences of previous influenza pandemics such as the “Spanish flu,” “Asian flu,” and “Hong Kong flu” [see “Review Report (1), 4. Clinical data, *Outline of review by PMDA*, (3) Clinical positioning”], PMDA considers the H5N1 Vaccine to potentially exhibit a protective effect against pandemic influenza or prevent symptoms becoming more severe and concluded that the H5N1 Vaccine should be positioned as a prophylactic vaccine against pandemic influenza virus infection. Thus, PMDA has concluded that there are no problems with stating in the INDICATIONS section that the product is indicated for “prophylaxis of pandemic influenza.”

Although the Expert Advisors supported the above PMDA conclusion, they stated that the subtype of pandemic influenza as the target of the product should be defined more clearly in the INDICATIONS section.

In the actual production of a pandemic influenza vaccine according to the procedure described for the H5N1 Vaccine, the Japanese Ministry of Health, Labour and Welfare and relevant authorities are expected to cooperate with reference laboratories of WHO, World Organization for Animal Health (OIE), and Food and Agriculture Organization (FAO) for identification and analysis of a pandemic

influenza virus strain and to develop attenuated candidate virus strains for production of vaccines. The applicant will then produce the vaccine using a vaccine strain designated by the Ministry of Health, Labour and Welfare. Therefore, there may actually be no trouble if the subtype of the pandemic influenza virus used for vaccine production is not specified in the INDICATIONS section. On the other hand, it is impossible at present to predict the seriousness of the disease associated with infection with a pandemic influenza virus belonging to a subtype other than H5N1, making assessment of the risk-benefit balance difficult. Therefore, the Expert Advisors stated that the product should be indicated exclusively for influenza of subtype H5N1.

PMDA communicated these comments to the applicant and the applicant responded that the statement in the INDICATIONS section will be changed as follows: the product is indicated for “prophylaxis of pandemic influenza (H5N1).”

PMDA accepted the applicant’s response.

6. Dosage and administration

A rise in antibody titer to the extent possible is desirable, because the threshold of antibody titer in relation to the protective effect against infection is unknown. In addition, Study BK-PIFA/001 demonstrated that the post-administration rise in antibody titer in the intramuscular group tended to be greater than that in the subcutaneous group, but that the difference between these two groups was minor and there were no major differences in the incidence of adverse events between different routes of administration. Based on these facts, PMDA has concluded that the DOSAGE AND ADMINISTRATION should be “The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses,” with the antigen content per dose (0.5 mL) specified as “15 µg (in terms of HA antigen)” in the columns of Ingredient and their quantity and Manufacturing method in the approval certificate. PMDA has also concluded that it is reasonable to state in the PRECAUTIONS section of the package insert that “Experiences with intramuscular injection are limited.”

Although the Expert Advisors supported the above PMDA conclusion, they stated that descriptions in the DOSAGE AND ADMINISTRATION section of the package insert should be improved so that information specific to each route of administration, including efficacy and safety information, is provided unambiguously to on-site medical personnel.

PMDA requested the applicant to modify descriptions in the DOSAGE AND ADMINISTRATION section of the package insert, taking the above comment into account. The applicant handled them appropriately.

7. Post-marketing considerations

PMDA considered it necessary to collect post-marketing information on the following topics in order to further assess populations not included in the clinical studies and the usefulness of the H5N1 Vaccine as a countermeasure against pandemic influenza.

- a. Children
- b. Elderly people
- c. Efficacy and safety of the H5N1 Vaccine manufactured with a vaccine strain other than NIBRG-14
- d. Cross-protective effects
- e. Antigen saving

The Expert Advisors made the following comments: Whether information collection on these 5 topics is possible as a part of post-marketing surveillance of the H5N1 Vaccine used according to the Pandemic Influenza Preparedness Action Plan and whether additional clinical studies are necessary should be assessed as soon as possible; in particular, prompt information collection on the use of the H5N1 Vaccine in children and elderly people, populations experiencing particularly high fatalities, is urgently needed.

PMDA communicated these comments to the applicant, and the applicant responded as follows.

- a. To facilitate aggressive vaccination with the H5N1 Vaccine in children, a clinical study to confirm the immunogenicity and safety of the H5N1 Vaccine in children should be conducted as soon as possible after marketing approval. The study will be conducted after discussion of the detailed study design, including stratification of the pediatric subjects by age and the vaccine dose employed, with the Ministry of Health, Labour and Welfare and relevant experts.
- b. Although a post-vaccination rise in antibody titer may be smaller in elderly people than in healthy adults, this difference may not be serious enough to reject the efficacy (immunogenicity) of the H5N1 Vaccine in such elderly recipients. Since vaccination against pandemic influenza is expected to be conducted as part of a mass vaccination program organized by administrative agencies in almost all cases and a post-vaccination health status survey and post-vaccination antibody titer assay by the National Institute of Infectious Diseases are planned for some vaccine recipients, the applicant intends to collect information on the immunogenicity and safety of the H5N1 Vaccine in elderly people by helping such post-vaccination surveillance. The applicant will also cooperate in future clinical studies or clinical research of the H5N1 Vaccine in elderly people sponsored by the national government by supplying the investigational vaccine and other possible means.
- c. Based on the data on the quality of stock solutions produced with two different virus strains,

NIBRG-14 and Indonesia, the immunogenicity and safety of the H5N1 Vaccine produced with a vaccine strain other than NIBRG-14 may be comparable to those of the original. Furthermore, since Study BK-PIFA/002 demonstrated no major difference in the incidence or severity of adverse reactions between the 5 µg and 15 µg groups receiving different amounts of antigen per dose, adverse reactions associated with the H5N1 Vaccine may be ascribable mainly to immune responses against the aluminium hydroxide gel used as adjuvant. Accordingly, it may reasonably be expected that a difference in virus strain used for production of the H5N1 Vaccine would not greatly affect safety. However, should an ongoing immunogenicity test in mice demonstrate an apparent difference in immunogenicity between the drug products produced with two different virus strains, NIBRG-14 and Indonesia, additional clinical studies will be conducted to investigate the safety and efficacy (efficacy in particular) of these drug products in humans.

- d. Cross-neutralisation against pandemic influenza virus strains other than that used for production of the H5N1 Vaccine will be investigated after marketing approval is granted, in collaboration with the National Institute of Infectious Diseases, using serum specimens collected from the subjects of Study BK-PIFA/002.
- e. Countermeasures to provide a maximum protective effect against infection at the population level within a limited production volume of the H5N1 Vaccine, including a clinical study to confirm the immunogenicity of low-dose multiple injections of the H5N1 Vaccine, will be assessed after discussion with the Ministry of Health, Labour and Welfare and relevant experts.

PMDA accepted the applicant's response and directed the applicant to conduct the clinical study of the H5N1 Vaccine in children as soon as possible, collect information on the clinical use of the H5N1 Vaccine in elderly recipients without delay, and to actively assess detailed procedures for items d. and e. after discussion with relevant authorities.

The applicant understood the above directions.

The Expert Advisors made the following comments.

Information on the efficacy and safety of the H5N1 Vaccine should be fully provided prior to vaccination, including the fact that it is unknown to what extent the immunity against pandemic influenza acquired by vaccination with the H5N1 Vaccine is actually effective in preventing infection. Also, quick and detailed safety data collection is crucial, because the H5N1 Vaccine is administered to an extremely large number of recipients within a short time when actually used. The Guidelines on Vaccination against Pandemic Influenza states that the vaccination is planned, as a rule, as a mass vaccination organized by prefectural governments (for pre-pandemic vaccines) or municipal governments (for pandemic vaccines). In conducting actual vaccination, the following are planned in accordance with the Guidelines: Currently available information is to be provided

extensively to vaccine recipients by, for example, distribution of leaflets describing efficacy and adverse reactions of the vaccine and holding an explanatory meeting as necessary to educate target individuals regarding vaccination. Vaccine recipients are to be ordered to stay at the vaccination site for at least 30 minutes after injection to watch for serious adverse reactions, such as anaphylactic shock, and safety data collection from some recipients using a health status questionnaire, in a manner similar to a post-immunization health status survey following a routine immunization, is to be conducted as post-vaccination surveillance. In addition, when a vaccine recipient is aware of symptoms suspected to be adverse reactions associated with injection of a pandemic influenza vaccine or a doctor identifies a vaccine recipient suspected to have such adverse reactions, immediate communication of this fact must be made to the prefectural or municipal government organizing mass vaccination with subsequent prompt reporting by the alerted local government to the Ministry of Health, Labour and Welfare using the National Epidemiological Surveillance of Infectious Disease (NESID) system in an emergency. PMDA considers it to be necessary that administrative agencies take the initiative on information delivery and safety data collection with respect to pandemic influenza vaccines, because they are in charge of selecting vaccination targets and the organization of mass vaccination.

Based on these facts, PMDA requested the applicant to actively assess and discuss with administrative agencies what to do as part of the responsibilities of the marketing authorization holder of the H5N1 Vaccine when the vaccine is actually used as part of the national countermeasures against pandemic influenza.

The applicant understood the above request.

On the other hand, the Expert Advisors made the following comment with respect to post-marketing surveillance of the efficacy of the H5N1 Vaccine: Although detailed future assessment is necessary on the extent to which detailed surveying is possible in situations of an actual influenza pandemic, data on the protective effect against infection should be collected to the extent possible. In fact, in cooperation with medical institutions and local governments involved in vaccination against pandemic influenza, collection of blood specimens from some of the vaccine recipients after obtaining informed consent for antibody titer assay at the National Institute of Infectious Diseases is planned according to the Guidelines on Vaccination against Pandemic Influenza. PMDA considers it desirable to assess procedures for evaluation of actual protective effects against pandemic influenza as well.

8. Others

(1) Pre-vaccination seropositivities

The Expert Advisors stated that the clinical significance of pre-vaccination seropositivities noted in the clinical studies of the H5N1 Vaccine should be investigated.

PMDA asked for the applicant's view on the meaning of pre-vaccination seropositivity against H5 antigen.

The applicant responded as follows.

Pre-vaccination seropositivities for HI antibody titer may be ascribed to (a) some serum factor non-specifically binding to viruses, and (b) cross-reactivity of pre-existing antibodies against seasonal influenza virus with H5 antigen. For neutralizing antibody titer, pre-vaccination positivities may be ascribed to (c) possible involvement of pre-existing antibodies against N1 antigen, but (d) the possibility of cross-reaction between some pre-existing serum antibodies with a relatively low specificity and the hemagglutinin protein moiety of the H5 antigen cannot be ruled out.

PMDA also asked for the applicant's view on the difference in the safety of the H5N1 Vaccine between 12 subjects with pre-vaccination seropositivity against H5 antigen (HI antibody titer assayed with chicken erythrocytes) and others with pre-vaccination seronegativity.

The applicant responded that no major differences in local reactions at the injection site or symptoms seen elsewhere were noted between the seropositive and seronegative subjects.

PMDA considers the applicant's response to be reasonable overall but advocates further post-marketing assessment of domestic (i.e., among the vaccine manufacturing sites in Japan) and international standardization of antibody titer assays.

(2) Outcome of pregnancy identified during clinical study

A female subject was found to be pregnant during Study BK-PIFA/002 and thus discontinued the study. PMDA asked the applicant to explain the outcome of the woman after discontinuation of the study and confirmed the outcome of the woman as follows. The woman had a spontaneous delivery at a gestational age of 40 weeks and 3 days without any health problems up to the delivery. No findings suggestive of effects of the investigational vaccine administration were observed in either the mother or the child.

III. Overall Evaluation

As a result of the above review, PMDA has concluded that the H5N1 Vaccine may be approved for the following indications and dosage and administration after modifying the description. Both the drug substance and the drug product are designated as powerful drugs and the drug product is classified as a biological product. As the H5N1 Vaccine is an orphan drug, PMDA determined that a re-examination period of 10 years is appropriate.

[Indications]

Prophylaxis of pandemic influenza (H5N1)

[Dosage and administration]

The usual dosage is 2 injections of 0.5 mL per dose administered intramuscularly or subcutaneously, with an interval of approximately 3 weeks between the doses.