H5N1 Influenza Virus Vaccine, A/Vietnam/1203/2004 (Clade 1) 90 mcg/ml

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List of Abbreviations

A/H5N1	Influenza A Virus of the H5N1 Subtype
AE	Adverse Event
AESI	Adverse Events of Special Interest
ALT	Alanine Aminotransferase
ASPR	Assistant Secretary for Preparedness and Response
BLA	Biologic License Application
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CPMP	Committee for Proprietary Medicinal Products
CRF	Case Report Form
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
FDA	Food and Drug Administration
GMT	Geometric Mean Titer
HA	Hemagglutinin
Hgb	Hemoglobin
HPAI	Highly Pathogenic Avian Influenza
ICH	International Conference on Harmonisation
IM	Intramuscular
IN	Institutional Normal
IND	Investigational New Drug application
LLOQ	Lower Limit of Quantitation
mcg (μg)	Micrograms
Mfg	Manufacturing
mL	Milliliter
NA	Neuraminidase
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NVPO	National Vaccine Program Office
Plt	Platelets
POC	Point of Care

PSURs	Periodic Safety Update Reports
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
ULN	Upper Limit of Normal
VAERS	Vaccine Adverse Event Reporting System
VSD	Vaccine Safety Datalink
VRBPAC	Vaccines and Related Biological Products Advisory Committee
WBC	White Blood Cells
WHO	World Health Organization

1 Background

The potential for a human influenza pandemic is a current public health concern with an immense potential impact. Preparing for the next influenza pandemic requires support and collaboration from multiple partners. On 01 November 2005, the President of the United States requested $7.1 billion in emergency funding for the *National Strategy for Pandemic Influenza*, of which $6.7 billion was designated for the US Department of Health and Human Services (DHHS). In May 2006, the *National Strategy for Pandemic Influenza Implementation Plan* was released. [1] It translated the *National Strategy for Pandemic Influenza* into more than 300 actions, timelines, and metrics for Federal departments and agencies and set clear expectations for State and local governments and other non-Federal entities. One of the Federal priority actions was to "Accelerate the Development of Medical Countermeasures" and included these efforts:

- Establish stockpiles of vaccine and antiviral medications
- Advance technology and production capacity for influenza vaccine
- Develop rapid diagnostics.

Cascading from the National Strategy and National Implementation Plan, one of the key components of the DHHS plan called for increasing capacity to produce pandemic influenza antivirals and vaccines, and increasing stockpiles of these countermeasures. Specific strategic goals for pandemic medical countermeasures are displayed in Table 1.

Table 1: DHHS Pandemic Medical Countermeasure Goals

Vaccine Goal #1	To establish and maintain a dynamic pre-pandemic influenza vaccine stockpile sufficient for 20 million persons (at 2 doses/person): H5N1 vaccine stockpiles
Vaccine Goal #2	To provide pandemic vaccine to all US citizens within 6 months of a pandemic declaration: 600 million doses pandemic vaccine
Antivirals Goal #1	To provide influenza antiviral drug stockpiles for pandemic treatment of 25% of US population: 75 million treatment courses
Antivirals Goal #2	To provide an influenza antiviral drug stockpile for strategic limited containment at onset of pandemic: 6 million treatment courses
Diagnostics Goal #1	To develop new high throughput laboratory and Point of Care (POC) influenza diagnostics for pandemic virus detection

The Pandemic Influenza Medical Countermeasure Program now includes 25 contracts obligating over $3 billion. Table 2 illustrates the multi-pronged approach and diversified portfolio of programs that have been established to help achieve the Implementation Plan's medical countermeasure goals.

Table 2: DHHS Pandemic Influenza Medical Countermeasure Programs

	Vaccines	Antivirals	Diagnostics
Advanced Development	•Cell-based •Antigen-sparing •Next Generation •Egg-based Supply	Peramivir	*High Throughput* • Point of Care • Clinical Lab
Acquisitions	H5N1 Vaccine Stockpiles	*Tamiflu*® *& Relenza*® • Federal Stockpiles • State Stockpiles	
Infrastructure Building	•Retrofit Existing Mfg Facilities •Build New Cell-based Mfg Facilities		

Vaccines

Vaccines are the optimal way to control the spread and associated morbidity and mortality of seasonal epidemics or pandemics. Developing vaccines for a pandemic may be divided into two categories: those that are developed against strains of animal influenza viruses that have caused isolated infections in humans, which may be regarded as "pre-pandemic" vaccines; and those that are developed against strains that have evolved the capacity for sustained and efficient human-to-human transmission ("pandemic" vaccines). Because emergence in human populations necessarily reflects genetic changes within the pandemic virus, pre-pandemic vaccines may be a good or poor match for – and offer greater or lesser protection against – the pandemic strain that ultimately emerges. Thus, the DHHS strategy is to simultaneously stockpile a limited amount of pre-pandemic vaccine; build vaccine manufacturing capacity so that pandemic vaccine can quickly be produced should a pandemic occur; and explore approaches utilizing adjuvants to enhance the likelihood that a vaccine administered prior to a pandemic will provide useful protection during a pandemic. Furthermore, this approach will strengthen and integrate both the seasonal and pandemic influenza preparedness needs.

Vaccines – Acquisitions

The Assistant Secretary for Preparedness and Response (ASPR) currently has a vaccine acquisition program that includes four projects with six contracts and obligations over $500 million to procure pre-pandemic vaccine (Table 3).

Although much has been accomplished, continued vigilance and preparation are needed to be ready for Influenza – seasonal epidemics and pandemics.

With the re-emergence of highly pathogenic avian H5N1 influenza virus in poultry and humans in late 2003 in Asia, the National Institutes of Health (NIH) and DHHS in 2004 awarded contracts to Sanofi Pasteur, Inc., Swiftwater, PA (formerly Aventis Pasteur) to

develop and manufacture an egg-based inactivated split H5N1 vaccine at pilot scale for clinical investigation and at commercial scale for stockpiling of pre-pandemic vaccines. With the results of clinical trials conducted by the NIH and others, DHHS has supported sanofi pasteur and other U.S.-licensed influenza vaccine manufacturers to develop their H5N1 vaccine candidates further and manufacture bulk vaccine product using the commercial scale and licensed product process.

Manufacturing these pre-pandemic vaccines not only provides the industry experience in producing novel influenza vaccine candidates at a commercial scale, but also provides a foundation for pre-pandemic vaccine stockpiles. In the early stages of a severe pandemic, and before a well-matched vaccine is available, pre-pandemic vaccines may be used in selected populations to mitigate disease, support essential operations, and maintain social and economic systems.

Table 3: DHHS H5N1 vaccine acquisition projects

Projects	Contracts	Award	Duration	Goals/Results
H5N1 Vaccine Clade 1 - 2004	1	$21M	2004-08	Provide 0.47 M doses at 90 µg/dose
H5N1 Vaccine Clade 1 - 2005	2	$243M	2005-08	Provide 8.0 M doses at 90 µg/dose
H5N1 Vaccine Clade 2 - 2006	3	$241M	2006-08	Provide 4.9 M doses at 90 µg/dose
H5N1 Vaccine 2007	TBD	TBD	2007-09	Provide doses for pre-pandemic stockpile (H5N1)

Currently, 1.3 million doses of H5N1 Influenza Virus Vaccine (90 µg/dose) have been filled in vials. More than 6 million doses (90 µg/dose) of this H5N1 Influenza Virus Vaccine remain in bulk form and await instructions for formulation into final vaccine vials. Additionally, approximately 5 million doses of this H5N1 Influenza Virus Vaccine are currently under production.

Using FDA “strain change” guidance on pandemic vaccine manufacturing, DHHS has encouraged sanofi pasteur and other influenza vaccine manufacturers to seek U.S.-licensure of their H5N1 vaccine products based on their currently licensed influenza vaccine products and extends the Department’s policy on the preferred usage of licensed medical countermeasures for a pandemic like the licensed influenza antiviral drug being stockpiled.

Sanofi Pasteur, Inc. in meeting the US Government’s challenge has developed and has also applied for licensure of the 90 µg H5N1 Influenza Virus Vaccine. This vaccine is a part of stockpile plans within the *National Strategy on Pandemic Preparedness*. The application for licensure is another step in assuring stockpiles of vaccine are available in the event of pandemic declaration.

2 Introduction

On 27 February 2007, the Vaccines and Related Biological Products Advisory Committee (VRBPAC) will meet to review the Biologic License Application (BLA) for H5N1 Influenza Virus Vaccine (A/Vietnam/1203/2004 [Clade 1] 90 μg/mL). H5N1 Influenza Virus Vaccine is a monovalent split virus vaccine containing 90 μg/mL of A/H5N1 HA manufactured by Sanofi Pasteur Inc, Swiftwater, PA.

H5N1 Influenza Virus Vaccine contains thimerosal as a preservative and the 1.0 mL dose is administered intramuscularly in a two dose regimen, approximately 28 days apart. The proposed indication for H5N1 Influenza Virus Vaccine is for active immunization in healthy, adult population 18 to 64 years against the avian influenza A viruses of the H5N1 subtype.

Given that vaccination remains a critical defense against the threat of avian influenza, the National Institute of Allergy and Infectious Diseases (NIAID) took the lead working with licensed manufacturers to generate clinical data that would help support the overall development of safe and effective vaccines against the H5N1 strain. In May 2004, NIAID awarded a contract to sanofi pasteur for the production of a small scale investigational lot of H5N1 Influenza Virus Vaccine for human studies that would be conducted by NIAID. Under the NIAID contract, sanofi pasteur was tasked with producing H5N1 Influenza Virus Vaccine following the same methods used to produce the seasonal influenza vaccine, Fluzone®. In that same year, an investigational new drug (IND) application for the pandemic influenza vaccine product was opened by the NIAID. Over the subsequent three years, a clinical development program in adults, elderly and children was initiated and conducted by NIAID. The results of a clinical trial conducted in adults with H5N1 Influenza Virus Vaccine 90 μg were published in the *New England Journal of Medicine* (NEJM) by Dr. John J. Treanor and colleagues. (Treanor, et.al. 2006, [1])

Following the NEJM publication, the US Department of Health and Human Services (DHHS) requested that sanofi pasteur seek licensure of the vaccine. During a pre-supplemental Biologics License Application (BLA) meeting, Center for Biologics Evaluation and Research (CBER) requested a re-analysis of the serological results by sanofi pasteur. In addition, sanofi pasteur was instructed to submit a separate BLA for a stand alone product independent of the Fluzone® labeling. The BLA was submitted to the Food and Drug Administration (FDA) on 13 October 2006.

In December 2006, NIAID and sanofi pasteur were notified of the 27 February 2007 VRBPAC meeting to discuss the pending application of H5N1 Influenza Virus Vaccine. This briefing document provides information regarding the epidemiology of avian influenza, the mechanism for immunologic protection, clinical development program, clinical data, and provides the concepts behind pharmacovigilance planning in a pandemic.

3 Influenza Pandemic

An influenza pandemic occurs when a novel influenza virus emerges against which the vast majority of the world's population has no immunity. This has been observed only with influenza A viruses and is due to the emergence of a new antigenic variant (antigenic shift) caused by substitution within the hemagglutinin (HA) antigen on the surface of the virus, with or without a concomitant change in neuraminidase (NA), the other surface antigen. If such a virus demonstrates the ability to transmit efficiently from person to person, the result is a global outbreak of the disease that affects a high percentage of individuals in a short period of time and is likely to cause substantially increased morbidity and mortality in all countries of the world.

Most people are immunologically naïve to the novel virus and are therefore more susceptible to influenza infection. The first identifiable influenza pandemic in more than 300 years of detailed records of human influenza occurred in 1847. [1] Since 1847, there have been 3 influenza pandemics: [4]

- The "Spanish influenza", between 1918 to 1919, was due to an A/H1N1 virus related to porcine influenza
- The "Asian influenza", between 1957 to 1958, was due to an A/H2N2 virus
- The "Hong Kong influenza", between 1968 to 1969, was due to an A/H3N2 virus.

The impact of pandemic influenza is better appreciated when compared with the more familiar patterns associated with inter-pandemic disease. Between pandemics, influenza is characterized by extremely low viral transmission in the summer [5] followed by an annual increase in winter seasonal activity. [6] The winter epidemic is variable in intensity and duration, usually produces clinically recognizable disease in the population.

In contrast, influenza pandemics are not limited to the winter season, and are characterized by several waves of infection following the emergence of the virus. [4] In the 1918-1919 pandemic, the first wave occurred in spring 1918 in the USA. The second began in August 1918 and had a higher mortality rate. The third appeared in spring 1919. The reason for these waves of infection is unclear. During the successive waves, virus virulence increased. [4] These characteristics have important implications for planning against the next pandemic.

The pandemics of the 20th century occurred at intervals ranging from 11 to 39 years. It is now approximately 39 years since the last pandemic in 1968. Pandemic influenza can occur at any time of year and may spread rapidly throughout the world. The three influenza pandemics of the 20th century demonstrate what can be expected when the next one occurs.

The estimated clinical attack rate was remarkably similar in the last three pandemics: about 25% of the world's population. The 1918 to 1919 pandemic killed 50 to 100 million people versus around one million people in 1957 to 1958 and 800,000 people in1968 to 1969.

Between pandemics, the vast majority of influenza-related deaths occur in the elderly, although infants and young children may also succumb. A similar pattern of age-specific

mortality occurred in the first wave of 1918 pandemic influenza. However, during the second wave, this pattern changed radically. Mortality among 0 to 4 year-olds rose considerably, but death rates in all other age groups less than 40 years old increased more dramatically, peaking at almost 15% in the 25 to 29 year age group. In contrast, in those over 50 years old, death rates were lower in the second wave than in the first and were especially low in the over 80s. [4]

Pandemic influenza is characterized by the sudden onset of severe typical influenza symptoms: high fever, headache, myalgia, arthralgia, anorexia, nausea, vomiting and cough lasting two to four days. Although most patients recover, some die rapidly due to tracheo-bronchitis associated with dyspnoea. After initial recovery, some patients subsequently develop pneumonia.

Although antiviral drugs may be beneficial, vaccines will form the main prophylactic measure against pandemic influenza and will play a major role in the plans to prepare for a pandemic. The World Health Organization (WHO) Influenza Surveillance Program provides representative influenza viruses for antigenic and genetic analysis and from this information, the WHO is able to make recommendations on vaccine composition. [7] The WHO reference laboratories, such as the Center for Disease Control and Prevention (CDC) Influenza Branch, have a key-role to play in detecting new influenza viruses that are likely to cause pandemics and advising on suitable vaccines strains and their use. As of 30 January 2007, the WHO current pandemic alert level is 3 which is defined as no or very limited human-to-human transmission. [7]

Conventional inactivated influenza vaccines may be unsuitable against pandemic influenza when given as a single dose. In naïve populations, the 15 µg-dose of a conventional split vaccine without adjuvant is poorly immunogenic. Recent studies of "pandemic like" vaccines have shown the advantages of adjuvanted vaccines and a two dose schedule, especially in unprimed individuals. [9, 10, 11, 12, 13, 14]

In order to accelerate the development of the pandemic vaccine, the European Committee for Proprietary Medicinal Products (CPMP) has developed guidelines for licensing pandemic influenza vaccines. [15, 16] The guidelines recommend the development of a "mock-up" pandemic vaccine, produced from a novel influenza virus. Speed in vaccine development is vital and this guideline provides the basis for a fast-track licensing procedure for pandemic vaccines within the European Union. The procedure involves the submission and approval of a core pandemic dossier during the inter-pandemic period, followed by a fast-track approval of the pandemic vaccine, based on the submission of pandemic variation.

The Center for Biologics Evaluation and Research has also issued a draft guidance regarding the clinical data required for licensure of a pandemic vaccine. It recommends that licensure of pandemic influenza vaccines may be sought either as a supplement to an existing BLA or as a new BLA using the accelerated approval regulations (21 CFR Part 601 Subpart E). Clinical trials are needed to support the appropriate dose and regimen of the pandemic influenza vaccine.

These trials are encouraged to include an assessment of immunogenicity and safety. Although this draft guidance is not considered binding, it outlines specific criteria for immunogenicity and safety as indicated below:

1. Immunogenicity:

Data to support the selected dose and regimen should be based on the evaluation of immune responses elicited by the vaccine. The hemagglutination inhibition (HI) antibody assay has been used to assess vaccine activity and may be appropriate for the evaluation of the pandemic influenza vaccine. Appropriate endpoints may include: (i) the percent of subjects achieving an HI antibody ≥ 1:40, and (ii) rates of seroconversion, defined as a four-fold rise in HI antibody titer post-vaccination.

The geometric mean titer (GMT) should be included in the results. These data and the 95% confidence intervals (CI) of the point estimates of these evaluations should be provided with the BLA clinical supplement.

Considerable variability can be introduced into the laboratory assay used to measure HI antibodies as a result of a number of factors including differences in viral strains, red blood cell types, and the presence of non-specific inhibitors in the assay medium.

Thus, suitable controls and assay validation are important for interpreting HI antibody results. Other immunologic assays, such as the microneutralization assay, might also be used to support the approval of a pandemic influenza vaccine as a clinical supplement to the BLA.

2. Safety:

Local and systemic reactogenicity events should be well defined in all age groups for whom approval of the vaccine is sought. Appropriate grading scales to describe the severity of the adverse events should be included in the study protocol.

Serious adverse events should be monitored and collected for all subjects throughout the duration of the studies. The protocol should include a clinic visit or telephone contact at least six months post-vaccination to ascertain additional serious adverse events and new onset of chronic illnesses that may have occurred in the interim. [17]

The data submitted in the BLA meet the requirements as outlined in the draft guidance.

4 Avian Influenza H5N1 Disease

Avian influenza is a contagious disease caused by viruses that normally infect only birds and less commonly, pigs. An outbreak of avian influenza, especially of the highly pathogenic form can be devastating for the poultry and farming industry. The Avian influenza A viruses of the H5N1 subtype are causing widespread infections in bird populations throughout Southeast Asia, with spread into Central Asia, Africa, and Europe. [18]

The disease can spread from country to country through migratory birds, including wild waterfowl, sea birds, and shore birds. There have been a number of instances of transmission of these viruses to humans, resulting in severe disease or death. [19]

These viruses possess a new H5 subtype of hemagglutinin, against which at present there is little immunity in human populations. The A/H5N1 viruses have the potential to cause extremely severe respiratory illness in humans, and have been known to repeatedly "jump the species barrier". Many of the viruses isolated from humans have been found to be genotypically resistant to the adamantanes, [20] (antiviral agents) and resistance to oseltamivir (Tamiflu®) has also been documented. [21]

Although human-to-human transmission appears at present to be rare, [22] a recent bird-flu outbreak in an Indonesian village where seven family members died, has raised the level of concern that the virus may be able to pass directly between people. With no animal identified as yet as the source of infection, the family cluster in Indonesia raises the suspicion of human-to-human transmission. [23] There is also a possibility that in this current situation, avian and human influenza viruses could exchange genes if an individual was simultaneously infected with viruses from both species. This could give rise to a new subtype of the influenza virus to which humans would not have natural immunity, and could result in the next influenza pandemic in humans.

As of 11 January 2007, the cumulative number of laboratory-confirmed human cases of Avian Influenza A-(H5N1) reported to the World Health Organization (WHO) was 264, including 158 (59.85%) deaths in human adults and children in Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey, and Vietnam. [24]

The development of an effective vaccine against influenza A (H5N1) virus is a matter of considerable urgency.

5 Epidemiology of Avian Influenza H5N1 Disease in North America

The cornerstones of pandemic preparedness include enhanced surveillance for the identification of emerging viruses, expanded capacity to produce relevant vaccines, antiviral medications for prevention and treatment of infections caused by pandemic viruses, and improved public health infrastructure to manage and coordinate control efforts.

Incidence of avian Influenza H5N1 disease

To date, highly pathogenic avian influenza H5N1 has not been recorded in the New World, although outbreaks of related avian influenza viruses lethal to domestic fowl have occurred in Ontario, Canada, in 1966 (H5N9); Pennsylvania, United States in 1983 (H5N2); Puebla, Mexico, in 1994 (H5N2); Chile in 2002 (H7N3); Canada in 2004 (H7N3); and Texas, United States, in 2004 (H5N2). [25] All of these outbreaks occurred in domestic poultry and were controlled without further diffusion. Three possible modes are proposed by which highly pathogenic avian influenza H5N1 might gain entry to the New World if birds were to be the introductory hosts: 1) normal interhemispheric migration, 2) vagrancy, and 3) legal and illegal importation of birds as explained in the following sections. [26]

Possible Role of Birds in Arrival of Highly Pathogenic Avian Influenza H5N1 Avian Influenza in the New World

Data based on observations of dead wild birds at sites where infections have broken out and negative results from subsequent extensive screening for seropositive or infected migrants around outbreak sites have indicated that highly pathogenic avian influenza H5N1 was lethal for most wild birds, at least until recently. [26] Nevertheless, some studies have demonstrated that chicken, domestic ducks, and geese infected under laboratory conditions, as well as some wild birds exposed under quasilaboratory conditions (e.g., birds fed, watered, and protected at zoologic parks or gardens), survive infection and shed the virus in active form. [27, 28, 29]

Normal Interhemispheric Migration

Few individual birds within few species undertake regular, interhemispheric migration. However, some do, and the waterfowl (Anseriformes, Charadriiformes, and Ciconiiformes) could be introductory hosts for highly pathogenic avian influenza H5N1 to the New World. Three pathways are used annually by a small number of waterfowl species to travel between the hemispheres: 1) Alaska–East Asia, in which birds that breed in Alaska winter in East Asia; 2) East Asia–Pacific North America, in which birds that breed in northeast Asia winter along the Pacific Coast of North America; and 3) Europe–Atlantic North America, in which birds that breed in Iceland or northwestern Europe winter along the Atlantic Coast of North America (see Figure 1). [26]

Two lines of evidence argue against normal, interhemispheric migration as a likely mode of entry for highly pathogenic avian influenza H5N1 into the Western Hemisphere. First,

as discussed previously, data indicate that most infected individual birds of most species of migrants become extremely ill and either cannot migrate far in their weakened state or die at the place of infection. Second, investigation of the genetics of avian influenza viruses has shown that little natural interchange occurs between the Eastern and Western Hemispheres: each hemisphere appears to have an avian influenza virus community that is largely distinct. [28] This fact is particularly noteworthy when one considers that most avian influenza A viruses appear to be asymptomatic, and migrants readily transport them in infectious form, in stark contrast to the situation for highly pathogenic avian influenza H5N1. Presumably, the distinct nature of the avian influenza A community in each hemisphere results from the fact that the main reservoir for these viruses is migrants, and few migrants move regularly between the hemispheres. [29]

Vagrancy

Perhaps a third or more of Eurasian waterfowl species have traveled into the Western Hemisphere as vagrants; some occur more regularly than others, however, all Eurasian vagrants are, by definition, extremely rare in the New World (a few birds per decade). One mode of interhemispheric vagrancy is tropical storm systems that originate off the West African coast during the Atlantic hurricane season, which lasts from June to November each year. These systems can, and occasionally do, sweep up and transport Old World birds, especially waterfowl, across the Atlantic to the New World (route 4, Figure 1). Vagrancy is much rarer (by several orders of magnitude) than normal interhemispheric migration and seems an even less likely mode of entry for highly pathogenic avian influenza H5N1. [26]

Legal and Illegal Importations

Human traffic in birds and bird products is the sole documented means of highly pathogenic avian influenza H5N1 movement between geographically separate regions to date. [30] While migratory birds have been suspected of involvement, particularly in cases in which no obvious human interchange of infected birds or products has occurred, these conclusions are inferred. [30] Thus, if highly pathogenic avian influenza H5N1 is to be kept out of the Western Hemisphere, control of legal and illegal imports should be the primary focus of prevention efforts.

The legal importation of exotic birds has declined dramatically in the United States since enactment of the 1992 Wild Bird Conservation Act. Nevertheless, 2,770 birds entered the country through the New York port of entry in 1999, including 323 pet birds and 2,447 commercial birds. In addition, 12,931 birds passed through in transit (S. Kaman, US Department of Agriculture [USDA], pers. comm. [26]) Legal importations are controlled by USDA Animal and Plant Health Inspection Service and the US Fish and Wildlife Service. Most imported birds undergo a 30-day quarantine at USDA facilities located near each of the three allowed ports of entry: New York, Miami, and Los Angeles. Quarantine procedures include isolation in indoor, air-filtered cages and standard testing for common poultry diseases, including avian influenza. The number of illegally imported birds is not known. These birds are not subject to quarantine and testing and could be a mode of entry for highly pathogenic avian influenza H5N1. Hawk eagles from Thailand infected with the virus were recently detected while being smuggled into

Belgium. [31] Although these birds were detected and quarantined, they serve as an example of how such imports could spread the virus. [26]

If birds turn out to be responsible for entry of highly pathogenic avian influenza H5N1 into the Western Hemisphere, illegal import of an infected bird or bird product seems the most likely mode of entry. This conclusion is based on the fact that illegally imported birds, unlike infected, free-flying migrants, are provided food and water ad libitum and protected from predators, greatly increasing their chances of survival in an infectious state. Furthermore, these birds often end up in close association with other, similarly protected birds, sharing the same food or water, a situation that provides ample opportunity for viral transmission. [26]

Possible Role of Birds in Movement of Highly Pathogenic Avian Influenza H5N1 in Western Hemisphere

Movement of highly pathogenic avian influenza H5N1 by sale of infected domestic fowl or poultry products in the United States and Canada is unlikely, given existing regulations. Thus, a major mode of highly pathogenic avian influenza spread available in much of Eurasia would be ruled out. Also, most domestic fowl are kept separate from wild migratory waterfowl in both countries, which would rule out a second major mode of introduction and cross-infection. Mixing of wild migratory birds with captive, exotic birds is relatively common, however, at North American zoos. Birds in such exhibits should be screened regularly for H5N1 or whatever highly pathogenic avian influenza virus is in circulation during a given year. [26]

Figure 1: Birds and Influenza H5N1 Virus Movement to and within North America

Map of known routes for natural interhemispheric bird movement: route 1, migrants breeding in Alaska and wintering in East Asia; route 2, migrants breeding in East Asia and wintering along the Pacific Coast of North America; route 3, migrants breeding in Iceland or northwestern Europe and wintering along the Atlantic Coast of North America; route 4, vagrants from West Africa carried by tropical storm systems across the Atlantic to eastern North America

Source: Rappole JH, Hubálek Z. Birds and influenza H5N1 virus movement to and within North America. Emerg Infect Dis [serial on the Internet]. 2006 Oct [11 Jan 2007]. Available from http://www.cdc.gov/ncidod/EID/vol12no10/05-1577.htm

6 Basis of Protective Immunity and Vaccine Development

Developing a vaccine that is protective against H5N1 is a major goal for preparing to reduce the impact of any future pandemic from this potential pandemic virus. The H5 virus itself, like other influenza strains, is mutating, and to date there are two defined clades of the H5N1 virus. [32] The best available evidence on immunity to influenza suggests that antibodies against the hemagglutinin of the virus are protective for humans. For seasonal influenza an antibody titer of ≥ 1:32 or ≥ 1:40 (depending upon the dilutions used in the assay) can protect an individual from infection. [33] Because one cannot perform a clinical trial against a disease like that caused by H5, which has not yet become widespread, the best available correlate of protection will be the development of an antibody titer of ≥ 1:32 or ≥ 1:40.

7 H5N1 Influenza Virus Vaccine Clinical Development Program

A variety of different control measures is expected to be part of an overall integrated and strategic approach for the public health and medical care response if a pandemic were to be declared, and the use of vaccines is universally recognized as essential to its control. Vaccines produced and used to combat the emergence and spread of a new pandemic influenza strain in humans must be safe and effective, able to be produced in large quantities, and delivered quickly enough to make a difference to those at risk of exposure. The overall process from developing influenza reference viruses to delivery of vaccines takes many months. Production and clinical evaluation of investigational lots of vaccines against influenza strains with pandemic potential facilitates establishment of the infrastructure for preparing novel vaccines. This interpandemic activity provides valuable experience that may substantially shorten the time needed for large scale production and health authority or regulatory approval.

7.1 Summary of the clinical program

As a result of the unprecedented spread of influenza A H5N1 clade 1 viruses in poultry in many countries in Southeast Asia and to humans in Thailand and Vietnam in January 2004, NIAID took the lead working with licensed manufacturers to generate clinical data that would help support the overall development of safe and effective vaccines against the H5N1 strain. In May 2004, NIAID awarded a contract to sanofi pasteur for the production of a small scale investigational lot of monovalent inactivated clade 1 H5N1 vaccine for human studies that would be conducted by NIAID. Under the NIAID contract, sanofi pasteur was tasked with producing the H5N1 vaccine using similar processes to those used for U.S. licensed vaccine, Fluzone®. A clade 1 H5N1 reference virus (A/Vietnam/1203/2004 batch #04-067) containing the neuraminidase (NA) gene and a genetically modified hemagglutinin (HA) gene was generated from a 2004 H5N1 human clinical isolate from Vietnam and was produced by St. Jude Children's Research Hospital under NIAID contract. Following an exemption of the reference virus by the USDA as a Select Agent on 07 May 2004, it was provided to NIAID on 02 June 2004. NIAID provided the reference virus to sanofi pasteur the following day.

Because of the need to quickly implement the clinical trials, only two formulations were requested: 30 µg /ml and 90 µg /ml, which allowed the clinical evaluation of a range of doses of the vaccine including 7.5 µg and 15 µg (0.25 ml and 0.5 ml of the 30 µg / ml formulation, respectively,) and 45 µg and 90 µg (0.5 ml and 1.0 ml of the 90 µg / ml, respectively).

The two vaccine formulations were delivered to NIAID by sanofi pasteur in March 2005 and the initial trial (DMID Protocol 04-063) to evaluate two intramuscular doses of the vaccine in healthy adults ages 18 to 64 years was initiated at three NIAID-supported Vaccine and Treatment Evaluation Units in April 2005 with NIAID as the IND sponsor. The adult Phase I/II trial was designed to evaluate the safety of the vaccine and provide initial data on the dose-dependent immune response that would form the basis for additional future clinical trials.

As shown in Table 4, the first stage (Stage I) consisted of the first 118 subjects; 12 control (placebo) group; 28, 25, 25, and 28, in the 7.5 µg, 15 µg, 45 µg, and 90 µg, respectively, of the A/H5N1 Influenza Virus Vaccine groups, followed by a 7-day safety assessment period. The Safety Monitoring Committee (SMC) reviewed the 7-day laboratory results, adverse events and reactogenicity data and recommended to the Division of Microbiology and Infectious Diseases (DMID) that the trial should continue based on pre-defined halting rules detailed in the study protocol.

The Stage II subjects included of the remaining subjects to make up the total sample size of 452; comprising of 48 subjects in the placebo group, 102 in the 7.5 µg, 101 in the 15 µg, 98 in the 45 µg, and 103 in the 90 µg A/H5N1 Influenza Virus Vaccine groups. The SMC performed another review of Stage I safety data 7 days following administration of the second dose of vaccine. As there were no safety issues in the Stage I subjects at the end of the 7-day period following the second vaccination, the Stage II subjects received the second vaccination. The duration of the study treatment for each subject was about 7 months.

Table 4: Study Population

Subjects Enrolled	Total	Study Groups				
		Placebo	**7.5 µg**	**15 µg**	**45 µg**	**90 µg**
Stage I	**118**	12	28	25	25	28
Total	**452**	48	102	101	98	103

NIAID provided sanofi pasteur with information developed during the course of the execution of DMID Protocol 04-063, including both interim and final clinical and serological data sets, a report describing the development of the hemagglutinin inhibition assay used to assess vaccine immunogenicity for the clinical trial, and additional supporting trial documentation prior to and following sanofi pasteur's meeting with CBER/FDA in April 2006 to discuss the submission of a supplement to sanofi pasteur's influenza vaccine license file.

Following the initiation of NIAID's initial trial with the sanofi pasteur H5N1 Influenza Virus Vaccine in healthy adults and a review of the resulting safety and immunogenicity data, NIAID initiated a trial to compare the safety and immunogenicity of either two 45 µg or 90 µg doses of the vaccine in an elderly population (65 years of age and older) and a trial evaluating two doses of the 45 µg vaccine in children (2 to 9 years of age, inclusive).

The study designs and results of the data generated from the NIAID supported and sponsored clinical trials have been coordinated with other ongoing efforts by DHHS, FDA, CDC, and WHO to ensure that the resultant data were widely shared and able to guide other ongoing efforts and to support possible large scale manufacture and implementation of a safe and effective vaccine to protect the public as soon as possible.

The appendices are a comprehensive collection of documents providing full data of the study. Appendix 1: Definition of Safety Parameters as defined in the DMID Protocol 04-063; Appendix 2: the NEJM publication on the study; Appendix 3: the synopsis of clinical data as submitted in the BLA, which will be presented by CBER during the VRBPAC meeting in their briefing document.

This briefing document focuses on the NEJM publication authored by Dr. John Treanor and colleagues. It should be noted that there are slight differences in the immunogenicity data presented in the BLA (See Appendix 3) compared to the NEJM publication on the study [1] (See Appendix 2), these differences include:

- The analyses presented in the BLA were based on the final data, whereas the publication was based on interim data.
- The analyses presented in the BLA used an initial dilution factor of 1:10 in the serologic assay, whereas the publication used an initial dilution factor of 1:20.
- For baseline titers less than Lower Limit of Quantitation (< LLOQ), the analyses presented in the BLA used a fold-rise calculation that considers LLOQ as baseline, whereas the publication considered 0.5 LLOQ as baseline.
- The analyses presented in the BLA considered that a subject with a < 1:10 baseline titer needed to have a ≥ 1:40 post-vaccination titer to be classified as having a four-fold rise; whereas in the publication, a subject with a < 1:20 baseline titer needed to have the same post-vaccination titer (≥ 1:40) to be classified as having a four-fold rise, despite having a higher baseline titer.

7.2 Clinical endpoints - Immunogenicity

7.2.1 Serology methods

Microneutralization assays and hemagglutination-inhibition assays were performed at a central laboratory (Southern Research Institute) with the use of the influenza rgA/Vietnam/1203/2004 × A/PR/ 8/34 influenza (H5N1) vaccine. In addition, a subgroup of samples were also tested with the use of the wild-type influenza A/Vietnam/1203/2004 virus under conditions of enhanced biocontainment (biosafety level 3-plus laboratory).

Microneutralization assays were performed as described. [34, 36] Serum samples were tested at an initial dilution of 1:20, and those that were negative were assigned a titer of 10.

Serum samples were tested separately and in duplicate; if the results showed a difference by a factor of 2, the samples were retested.

Hemagglutination-inhibition assays were performed according to established procedures, [35, 37] using horse erythrocytes. After treatment with receptor-destroying enzyme to remove nonspecific inhibitors of agglutination, the serum samples were tested at an initial dilution of 1:20.

7.3 Summary of pivotal study – Immunogenicity

7.3.1 Study DMID Protocol: 04-063 (sanofi pasteur FUG01)

The following sections describes the results of a phase I/II clinical trial evaluating an egg-grown, subvirion H5N1 vaccine prepared by sanofi pasteur under contract to NIAID.

The study was designed to assess the dose-related safety and immunogenicity of the H5N1 vaccine in a rapid fashion in the face of a potential public health emergency. It is noted that the primary immunogenicity endpoint for this study was the development of a serum neutralizing antibody titer of 1:40 or greater approximately 28 days after the second dose of vaccine, using a microneutralization assay that had been developed in seroepidemiologic studies conducted among persons infected with H5N1 viruses during the Hong Kong outbreak of 1997. [34] Using this assay, there was a clear dose-response to the vaccine, and 54% of recipients of the 90 µg dose (95% CI, 43% to 64%) met the primary immunogenicity endpoint. It should be noted that the potential utility of the hemagglutination-inhibition assay using horse erythrocytes [35] was uncertain at the time this study was originally designed (July 2004), but a decision was subsequently made to include the horse erythrocyte hemagglutination-inhibition assay as a co-primary immunogenicity endpoint. Using the hemagglutination inhibition assay, 58% of recipients of the 90 µg dose (95% CI, 47% to 67%) met the co-primary immunogenicity endpoint.

7.3.2 Immunogenicity results

The immunogenicity results using data of the hemagglutination-inhibition and microneutralization assays are shown in Table 5 and Figure 2. In the majority of subjects, antibody against the A/Vietnam/2004 virus was not detected by either method before immunization, although 15 subjects (3%) had a positive hemagglutination-inhibition test, and 12 (3%) had a positive microneutralization test. The reasons for these positive results are unknown, because none of the subjects reported exposures that would be likely to result in H5 virus infection, and preliminary analysis has not suggested any relationship between H5 antibody and antibody against conventional human influenza viruses.

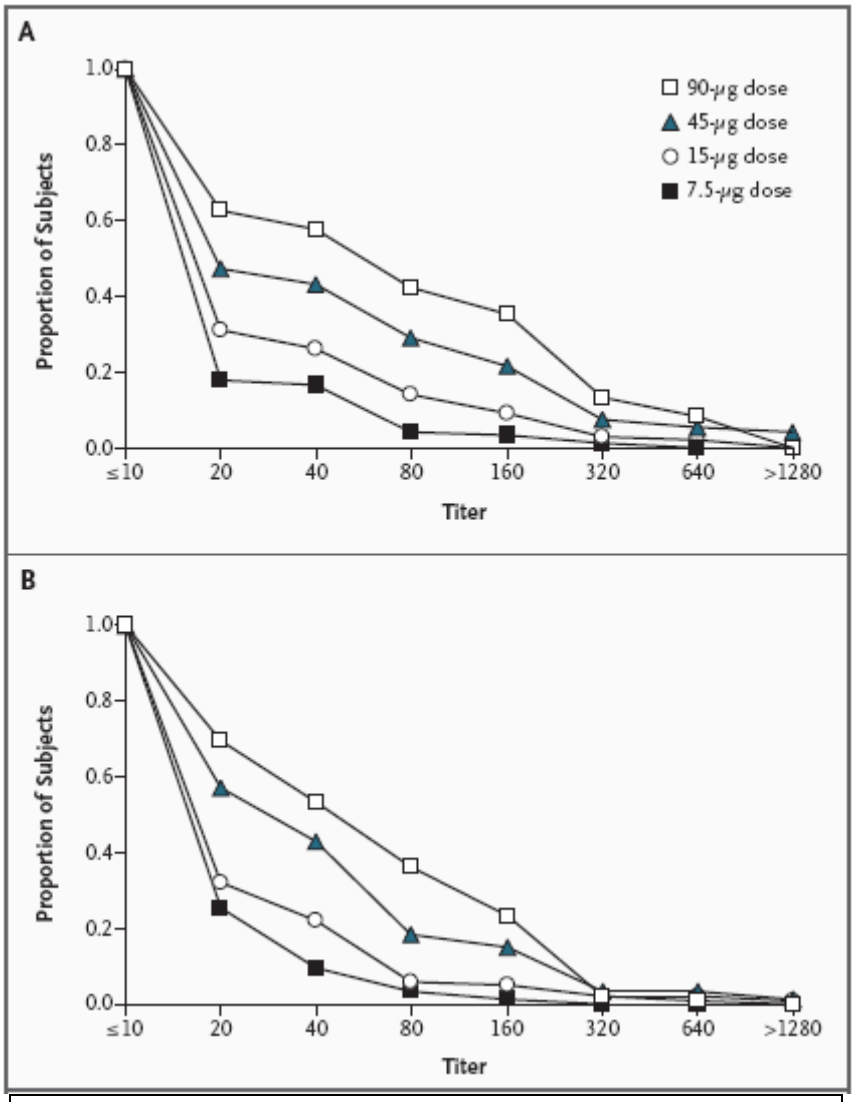
Table 5: Geometric Mean Titers (GMT) of Antibody against the Influenza A/Vietnam/1203/2004 (H5N1) Virus in Subjects Receiving Two Doses of Vaccine, as Assessed by Hemagglutination-Inhibition or Microneutralization*

Assay and Group	Before Vaccination		28 Days after First Dose			28 Days after Second Dose		
	No. of Subjects (N=449)	GMT (95% CI)	No. of Subjects (N=441)	GMT (95% CI)	Antibody Response % (95% CI)	No. of Subjects (N=435)	GMT (95% CI)	Antibody Response % (95% CI)
Hemagglutination inhibition								
90 μg	102	10.4 (9.8–11.0)	99	27.1 (19.6–37.3)†	28 (20–38)‡	99	56.3 (41.2–76.8)‡§	57 (46–67)‡¶
45 μg	98	10.8 (10.1–11.7)	95	22.6 (16.7–30.5)	23 (15–33)	93	34.7 (25.3–47.4)	41 (31–52)
15 μg	101	10.3 (9.9–10.7)	100	14.2 (11.8–17.1)	10 (5–19)	100	20.3 (16.2–25.4)	24 (16–34)
7.5 μg	100	11.4 (10.2–12.9)	99	13.2 (11.3–15.4)	5 (2–11)	95	14.9 (12.5–17.8)	13 (7–21)
Placebo	48	10.6 (9.7–11.6)	48	10.9 (9.7–12.2)	0 (0–7)	48	10.9 (9.6–12.4)	0 (0–7)
Microneutralization								
90 μg	102	10.2 (9.8–10.6)	99	16.6 (13.6–22.6)‖	17 (10–26)‖	99	45.9 (36.0–58.5)‡	53 (42–63)‡
45 μg	98	10.6 (10.0–11.2)	95	17.7 (13.8–22.6)	17 (10–26)	93	32.9 (25.4–42.7)	41 (31–52)
15 μg	101	10.4 (10.0–10.8)	100	12.7 (10.9–14.8)	6 (2–13)	100	18.3 (15.0–22.2)	20 (13–29)
7.5 μg	100	10.8 (10.0–11.7)	99	12.3 (10.9–13.8)	6 (2–13)	95	14.2 (12.4–16.3)	7 (3–15)
Placebo	48	10.3 (9.7–10.9)	48	10.3 (9.7–10.9)	0 (0–7)	48	10.3 (9.7–10.9)	0 (0–7)

* The hemagglutination-inhibition assay was performed with the use of horse erythrocytes. GMTs were compared with the use of the Wilcoxon rank-sum test. Response rates were compared with the use of the Mantel–Haenszel chi-square test. Response was defined by an increase in antibody titer by a factor of 4 or more, as compared with the titer before vaccination. CI denotes confidence interval.
† P=0.001 for the comparison with all other vaccine groups (excluding the placebo group).
‡ P<0.001 for the comparison with all other vaccine groups (excluding the placebo group).
§ P=0.02 for the comparison with the group receiving 45 μg.
¶ P=0.03 for the comparison with the group receiving 45 μg.
‖ P=0.04 for the comparison with all other vaccine groups (excluding the placebo group).

Source: Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M.: Immunogenicity of an Inactivated Subvirion Influenza A (H5N1) Vaccine. N Engl J Med. 2006;354:1343-1351.

Figure 2: Reverse Cumulative Distribution Curves for Serum Samples Collected 28 Days after the Second Vaccination.



The proportions of subjects are based on the total number of subjects tested. Panel A shows the results of hemagglutination-inhibition testing with the use of horse erythrocytes. Panel B shows the results of microneutralization testing.

Source: Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M.: Immunogenicity of an Inactivated Subvirion Influenza A (H5N1) Vaccine. N Engl J Med. 2006;354:1343-1351.

There was a very clear dose–response relationship with the use of either assay ($P < 0.001$), with a large difference in response between the groups receiving vaccine at doses of 45 μg or 90 μg and those receiving lower doses. Only the 90 μg dose was associated with antibody responses (increase titer in antibody titer by a factor of 4 or more) in either hemagglutination-inhibition or microneutralization assays in more than half the subjects (Table 5).

Two doses of 45 μg also resulted in antibody responses in a substantial proportion of subjects, whereas lower doses of vaccine were much less immunogenic. Similarly, there were substantially higher geometric mean titers of both hemagglutination-inhibition and microneutralization antibody after vaccination in the group receiving 90 μg, and there were significantly lower titers of both antibodies in the groups receiving lower doses of vaccine ($P < 0.001$).

The pre-specified primary immunogenicity endpoint chosen for the study was the development of a microneutralization titer of 1:40 or greater after two doses of vaccine. Figure 2 shows the distribution of antibody titers according to the hemagglutination-inhibition assay (Figure 2A) and microneutralization assay (Figure 2B) after vaccination in each group categorized according to dose. Only in the group receiving the 90 μg dose was the primary endpoint reached by more than 50% of the recipients. In this group, 54% of the subjects (95% confidence interval [CI], 43% to 64%) had microneutralization titers of 1:40 or greater and 58% (95% CI, 47% to 67%) had hemagglutination-inhibition titers of 1:40 or greater. The frequency of both these endpoints in the group receiving the 90 μg dose was significantly greater than in the other vaccine groups ($P < 0.001$). Microneutralization titers of 1:20 or greater were seen in 70% of the group receiving the 90 μg dose and 57% of the group receiving the 45 μg dose, but in only 32% of the group receiving the 15 μg dose and 25% of the group receiving the 7.5 μg dose.

Because the highly pathogenic wild-type influenza A/Vietnam/1203/2004 virus can be manipulated only under strict conditions of biocontainment, the majority of serologic tests used the antigenically identical but apathogenic influenza rgA/Vietnam/1203/2004 × A/PR/8/34 vaccine virus. The ability of serum samples from this study to neutralize the wild-type virus was confirmed in a subgroup of 63 samples obtained on day 56 from randomly selected specimens representing a spectrum of antibody titers, which were weighted toward higher responses to the apathogenic vaccine virus and assayed against the wild-type virus. The agreement in the antibody titers assayed against the two viruses was good, with a Spearman's correlation coefficient of 0.74 ($P < 0.001$). Of the 53 samples tested in which the titers of antibody against the vaccine virus were greater than 1:40, 51 also had titers of antibody against the wild-type virus of more than 1:40.

7.4 Conclusions based on the summary of immunogenicity

The study demonstrates that it is possible to generate immunity against H5 influenza with the use of this purified, subvirion vaccine administered in two 90 μg doses. The results are similar to those observed in a study conducted with the use of a purified, recombinant H5 hemagglutinin in humans, [38] in which intramuscular administration of two doses of approximately 90 μg each of a baculovirus-expressed recombinant H5 hemagglutinin resulted in neutralizing antibody titers of 1:80 or greater in 56% of healthy adult recipients, whereas lower doses were considerably less immunogenic.

On the basis of these data, a two-dose schedule of 90 μg of subvirion H5N1 Influenza Virus Vaccine could be effective in preventing H5 influenza in healthy adult recipients.

7.5 Safety – Assessment

7.5.1 Safety Parameters

The following categories of safety information were collected. Definition and severity rating scales for each type of event are detailed in Appendix 1.

- **Immediate reactions (**within 30 minutes**)** following each dose of vaccine
- **Solicited Adverse Events** - Reactogenicity (Days 0-7) following each dose of vaccine:
  a) Injection site (local) reactions including pain, tenderness, redness, and swelling at the injection site.
  b) Systemic reactions including fever, malaise, myalgia, headache, and nausea.
- **Unsolicited Adverse Events**
  a) Nonserious events occurring 28 days following each dose of vaccine (through approximately Day 56).
  b) Nonserious events occurring from Day 29 through Month 6 following the second dose of vaccine (Day 208).
  c) Serious adverse events occurring during the entire study (Day 0 to 208).

7.5.2 Safety Objectives and Statistical Hypotheses Tested

The safety objective was to determine the dose-related safety of subvirion inactivated H5N1 vaccine in healthy adults.

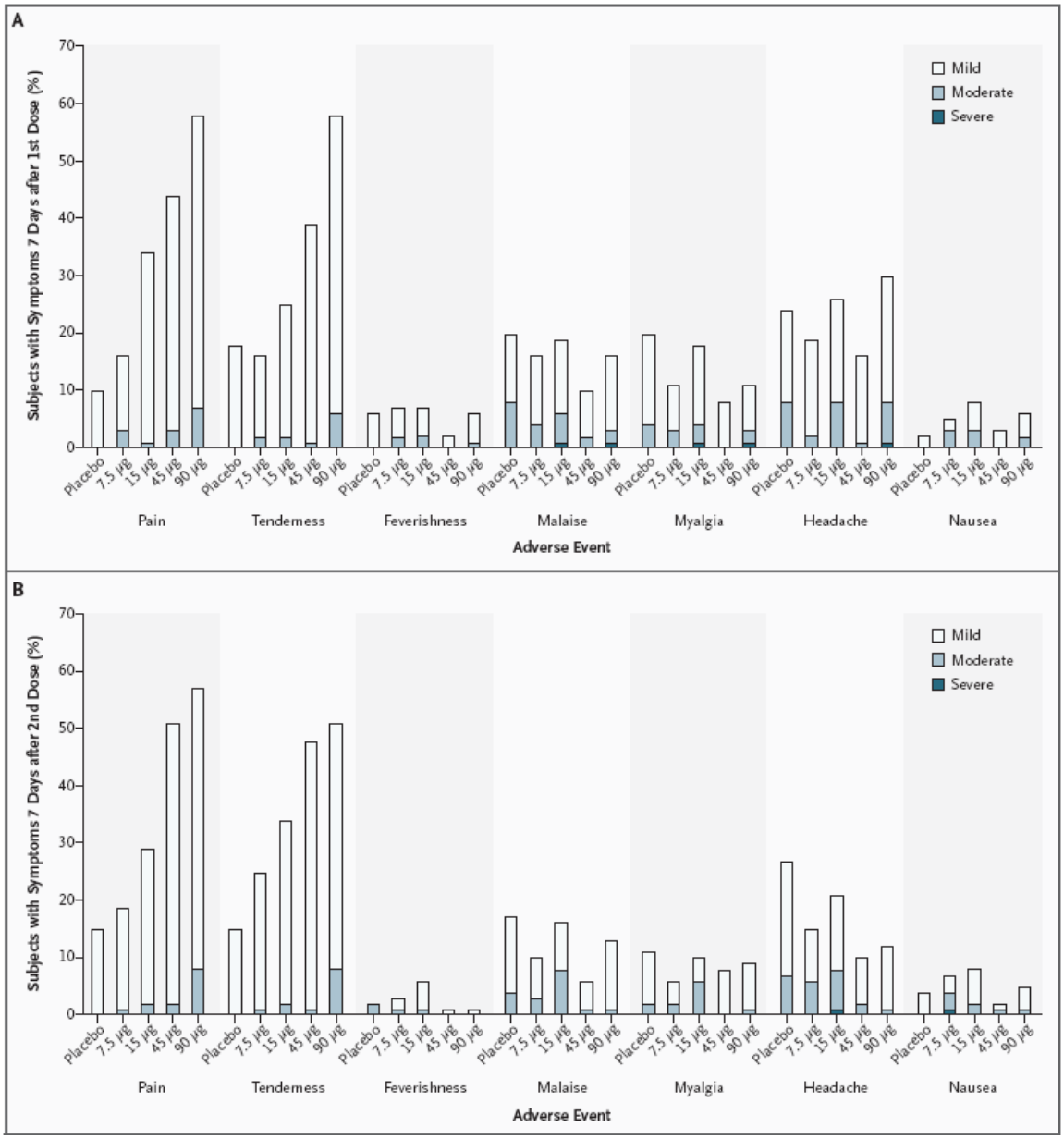
No safety statistical hypothesis was tested.

7.6 Safety – Results

This briefing document as in the publication [1] summarizes the safety and immunogenicity data that were available at the time of the day 28 post vaccine 2 visit for all subjects, i.e., study day 56. The frozen dataset for the analysis included in the publication, therefore, does not include the entire duration of safety evaluation that continued until study day 208. The complete safety follow up represented by the final locked dataset is included in the BLA submission by sanofi pasteur. See Appendix 3 for a full synopsis of the final integrated clinical and statistical report in the BLA submission.

The rates of symptoms reported during the first seven days after administration of each dose of vaccine are shown in Figure 3. Generally, the vaccine was well tolerated at all doses, and 84% of all reported symptoms were graded as mild by the subjects. There was no indication that the frequency or severity of either local or systemic symptoms were greater after the second dose than after the first dose, and there were no instances of anaphylaxis, hives, or other serious allergic reactions.

Figure 3: Rate of Local and Systemic Adverse Events during the Seven Days after Receipt of the First Dose (Panel A) or the Second Dose (Panel B) of Vaccine



Subjects used a subjective scale to grade adverse events. Symptoms were considered '**mild'** if they did not interfere with normal activities; **'moderate'** if they resulted in some interference with normal activities; and **'severe'** if they prevented subjects from carrying out normal daily activities.(See Table 9 in Appendix 1)

Source: Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M.: Immunogenicity of an Inactivated Subvirion Influenza A (H5N1) Vaccine. N Engl J Med. 2006;354:1343-1351.

7.6.1 Solicited Adverse Events

The frequencies of pain and local tenderness at the injection site after each dose were greater among vaccine recipients than placebo recipients in a dose-dependent manner ($P < 0.001$). In addition, moderate pain and tenderness were reported almost exclusively among recipients of the 90 μg dose. In general, reports of local pain were not accompanied by objective findings of erythema or swelling at the injection site. There were no severe local reactions.

Systemic symptoms were relatively less common after either dose in all study groups and were not dependent on the dose; the frequencies of reports of feverishness, malaise, myalgia, headache, and nausea in all groups did not differ significantly from those in the placebo group ($P > 0.05$).

Eleven subjects reported fever (temperature, ≥ 37.8°C; maximum, 38.2°C) after vaccination: 9 after the first dose (2 in the placebo group, 1 in the 7.5 μg group, 3 in the 15 μg group, and 3 in the 90 μg group) and 2 subjects after the second dose (1 in the placebo group and 1 in the 45 μg group).

Clinical laboratory safety tests (hematology and blood chemistry) performed before, and at Day 7 following each vaccination, respectively, on the Stage I subjects did not reveal clinically significant abnormalities.

7.6.2 Unsolicited Adverse Events

In one subject in the 90 μg vaccine group, a rash developed after receipt of the first dose, and the subject did not receive the second dose. This subject noticed a nonpruritic, maculopapular rash over the abdomen and upper arms bilaterally on day 5 after the first dose of vaccine, without involvement of the face, hands or feet, or mucous membranes. The rash faded and resolved completely by day 42. Because the cause of the rash was unclear, the investigator elected not to administer the second dose of vaccine. This subject had no history of reaction to influenza vaccine, including rash.

7.6.3 Serious Adverse Events

There was one serious adverse event in the study, but it was judged by investigators to be unrelated to vaccination. A 52-year-old man in the second stage of the study died 24 days after receipt of the first dose of 45 μg of vaccine. He had a history of alcohol abuse that had not been revealed on enrollment, and the subject was noted to be consuming alcohol heavily. Autopsy revealed marked steatosis of the liver, and the death was determined by the medical examiner to be due to chronic alcoholism.

7.7 Safety-Conclusions

The vaccine was well tolerated with mostly mild to moderate reactogenicity reported. Overall, among the study groups that received the H5N1 vaccine, local reactogenicity tended to be dose related. Nearly all of these reported reactions were of mild to moderate severity. They occurred and resolved without sequelae within 3 to 7 days of vaccination.

Additionally, in the final dataset released to sanofi pasteur for inclusion in the BLA, there were no additional safety issues reported. All local and systemic adverse events resolved without sequelae and no serious adverse events related to the study vaccine occurred.

8 Pharmacovigilance Plan

Preparing for the next influenza pandemic requires support and collaboration from multiple partners at the local, state, national, and international levels. Planning for the prospect of pandemic influenza is one of the most effective steps to mitigate the impacts of such an event. Vaccination remains a critical defense against pandemic influenza, however, the safety profile of such a vaccine will likely be less certain than that of previously released seasonal influenza vaccines. Vaccine safety monitoring is critical and will occur as part of a comprehensive public health surveillance.

Expansion of influenza vaccination in the setting of a pandemic will pose numerous logistical challenges in safety surveillance. Streamlining and prioritization of pharmacovigilance processes and procedures are essential for early detection and communication of potential risks. Routine pharmacovigilance practices will cease with the official announcement of the pandemic by the WHO, and special pharmacovigilance practices could be implemented by the appropriate authorities. Sanofi pasteur proposes a risk management strategy that could be implemented by appropriate authorities to monitor, evaluate, understand, and minimize adverse events of special interest in a pandemic situation by taking the following factors into consideration:

1) Limited clinical data available at time of pandemic.
2) A high volume of safety data is anticipated within a short time frame.
3) Increased public anxiety with adverse events (AEs) reported, regardless of causality, which could potentially be stimulated by media coverage.
4) Limited personnel to handle volume in industry and regulatory agencies.
5) Pharmacovigilance systems may be disrupted during a pandemic period.

The Risk Management Plan is an evolving strategy that will be refined and improved over time. The details have not been finalized at this time, however, the main concepts behind planning safety surveillance activities are presented here.

Post-licensure safety surveillance will address relevant safety issues for H5N1 Influenza Virus Vaccine. The need to detect adverse events in a timely manner will need to be balanced against issues of disease severity and vaccine efficacy. [39] Non-serious adverse events are generally of less importance in a pandemic period. Safety parameters based on biological plausibility of the occurrence of certain adverse events will be investigated in detail. Targeted monitoring may be required for certain types of reactions which can be anticipated for pandemic vaccines on the basis of their relationship to currently licensed or tested influenza vaccines.

The pharmacovigilance activities planned must be efficiently coordinated among the government agencies and with sanofi pasteur. They will all be involved in the design and implementation of a pharmacovigilance plan to address any potentially significant safety

issues that arise during the pandemic. The AE reporting processes must be simplified for patients and healthcare providers. Innovative methods should be developed to accelerate AE reporting; this may include a user-friendly interactive website or a voice-activated call center where data will be stored and automatically sent to a centralized safety database. During the pandemic, Vaccine Adverse Event Reporting System (VAERS) and Vaccine Safety Datalink (VSD) systems will be on the frontline of early signal detection and real-time access needs to be granted for safety signal detection and analyses. [39] A weakness in the current system is the amount of time it takes an AE to reach the centralized database. Robust surveillance systems need to be established for assessing vaccine safety.

The protocol and information-sharing should be tested and harmonized during the forthcoming and subsequent influenza seasons. In the event of an influenza pandemic, only significant safety issues will be reviewed and evaluated on an ongoing basis. Non-urgent activities will be minimized and addressed after the pandemic.

Spontaneous Reports

Expedited reporting will be the basis for safety evaluation. The collection and analysis of the safety data during a pandemic period will be influenced by several factors; therefore, a common collection form should be used by all parties. The level of required safety data needs to be defined. It is recommended that healthcare professionals and patients report serious adverse events, deaths, life-threatening events, and adverse events of special interest (AESI). A system needs to be developed for the triage of consumer reports based on severity and case definitions. Agreed upon case definitions will be used in order to ensure harmonized safety assessment of these events. No actual risk has been observed with H5N1 Influenza Virus Vaccine; however, potential risks have been extrapolated from previous annual influenza vaccines. These potential risks will be considered AESI and include anaphylaxis, Guillain-Barré syndrome, Bell's palsy, optic neuritis, convulsions, and syncope which will be closely monitored.

Expedited reporting of AESI should take place as soon as possible and no later than 15 calendar days. More precise timelines should be implemented. Rapid and open communication between sanofi pasteur and Authorities/Public Health Services (FDA, CDC, state and local authorities) is essential. Electronic communication should be established prior to the pandemic period.

Aggregate Reports

Periodic Safety Update Reports (PSURs) are prepared at defined time intervals or on an ad-hoc basis if required by the Health Authority; however, during the pandemic period due to limited resources, PSURs will not be submitted. Alternatively simplified PSURs, focusing on serious adverse events, deaths, life-threatening events, and AESI can be prepared. An aggregated PSUR will be submitted when the pandemic is declared finished.

Signal Detection and Analysis

Safety evaluation will be based on signal detection taking into account the particular circumstances of a pandemic situation, and its potential impact on assessment of the benefit/risk profile. The use of existing data-mining tools linked to the safety database will be implemented. It is important that all parties involved agree on a set of predefined criteria which would potentially trigger the suspension of the vaccination campaign.

Safety Surveillance Studies

The safety profile of the vaccine is unknown in numerous populations. For example, pregnant women are considered at special risk for influenza infection based on morbidity and mortality from previous pandemics and from intense influenza seasons. [40] There are currently no data on the safety profile of the vaccine in pregnancy. Other populations not evaluated include individuals with underlying medical conditions/high-risk for influenza.

A cohort study should be considered to bridge safety and efficacy data in populations not studied to date. There is a need to consider how patients in these groups could be identified and followed up. The number to be included needs to be established, but it is likely to be in the range of several thousand subjects. These cohorts would still not be of sufficient size to detect rare events (e.g. Guillain-Barré Syndrome). Such studies to detect rare events should be coordinated by national or international public health agencies.

Vaccine failure reports

It is recommended that a true efficacy study should be organized by public health agencies to have a better understanding of the benefit-risk profile of the vaccine in the context of an epidemic. It is essential to determine the mortality/morbidity rate of unvaccinated people and the effectiveness of the vaccine. This information might be attainable through the cohort study mentioned above depending on the size of the study, keeping in mind the potential bias created by the non-randomization between vaccinated and unvaccinated groups.

Vaccine failure will not be requested for routine pharmacovigilance activities, considering that other, more robust means to assess vaccine effectiveness will be made available.

Nevertheless, it is anticipated that a significant number of vaccine failure cases will be reported using the routine pharmacovigilance reporting system. These case reports should be recorded in the safety database.

Risk Minimization Action Plan

In sanofi pasteur's BLA submission, a risk/benefit analysis document was submitted (See Appendix 4). Based on this analysis and the safety profile from the clinical trial, no Risk Minimization Action Plan is deemed necessary.

9 Overall Conclusions

A variety of control measures are expected to be a part of the *National Strategy for Pandemic Influenza Preparedness.* Vaccination is universally recognized as essential to pandemic control and is one of the key components for preparedness. This H5N1 Influenza Virus Vaccine, is the first candidate vaccine produced by sanofi pasteur for the US Government supporting this national strategy.

The license application for H5N1 Influenza Virus Vaccine is based on safety and immunogenicity demonstrated by the DMID study 04-063. In this study, H5N1 Influenza Virus Vaccine was well tolerated and generated neutralizing antibody responses typically associated with protection against influenza. No related Serious Adverse Events were reported and nearly all local and systemic reactions were mild and transient.

Pharmacovigilance activities must be planned and efficiently coordinated among all parties to address any potentially significant safety issues that may arise during a pandemic.

10 Bibliography

1. Homeland Security Council. National Strategy for Pandemic Influenza: Implementation Plan. US Government Printing Office, Washington DC. May 2006
2. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N. Engl. J. Med. 2006; 354:1343-1351.
3. Laver G, Garman E. Pandemic influenza: its origin and control. Microbes Infect. 2002; 4(13): 1309-1316.
4. Nguyen-Van-Tam JS, Hampson AW. The epidemiology and clinical impact of pandemic influenza. Vaccine 2003; 21 (16): 1762-1768.
5. Fox JP, Hall CE, Cooney MK, Foy HM. Influenza virus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. Am J Epidemiol. 1982; 116: 212-227.
6. Fleming DM. The impact of three influenza epidemics on primary care in England and Wales. Pharmacoeconomics 1996; 9 (Suppl 3): 38-45.
7. World Health Organization. WHO Global Influenza Preparedness Plan: the role of WHO and recommendations for national measures before and during pandemics. WHO, Geneva, Switzerland. 2005
8. Wood JM. Selection of influenza vaccine strains and developing pandemic vaccines. Vaccine 2002; 20: B40-B44.
9. Fedson DS. Pandemic influenza and the global vaccine supply. Clin Infect Dis. 2003; 36 (12): 1552-1561.
10. Simonsen L. The global impact of influenza on morbidity and mortality. Vaccine 1999; 17: S3-S10.
11. Centers for Diseases Control and Prevention. Prevention and Control of Influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2002; 51 (No. RR-3): 1-31.
12. Vu T, Farish S, Jenkins M, Kelly H. A meta-analysis of effectiveness of influenza vaccine in persons aged 65 years and over living in the community. Vaccine 2002; 20: 1831-1836.
13. Nicholson KG, Colegate AE, Podda A, et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. The Lancet. 2001; 357:1937-1943.
14. Stephenson I, Nicholson KG, Colegate AE, et al. Boosting immunity to influenza H5N1 with MF59-adjuvanted H5N3 A/Duck/Singapore/97 vaccine in a primed human population. Vaccine. 2003; 21:1687-1693.

15. Guideline of the EMEA on Dossier Structure and Content for Pandemic Influenza Vaccine Marketing Authorisation Application (CPMP/VEG/4717/03).

16. Guideline of the EMEA on Submission of Marketing Authorization Applications for Pandemic Influenza Vaccine Through the Centralised Procedure (CPMP/VEG/4986/03).

17. US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry, Clinical Data needed to Support the Licensure of Pandemic Influenza Vaccines (Draft Guidance). US Government Printing Office, Washington DC March 2006

18. H5N1 avian influenza: timeline 8 May 2006 Previous events in Asia date animals humans 1996 highly pathogenic. Available at: http://www.who.int/csr/disease/avian_influenza/timeline.pdf. Accessed January 11, 2007.

19. Hien TT, Liem NT, Dung NT, et al.: Avian influenza A (H5N1) in 10 patients in Vietnam. N Engl J Med 2004;350:1179.

20. Li KS, Guan Y, Wang J, et al.: Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 2004;430:209-213.

21. de Jong MD, Tran TT, Truong HK, et al.: Oseltamivir resistance during treatment of influenza A (H5N1) infection. N Engl J Med 2005;353:2667-2672.

22. Ungchusak K, Auewarakul P, Dowell SF, et al.: Probable person-to-person transmission of avian influenza A (H5N1). N Engl J Med 2005;352:333-340.

23. WHO: Avian Influenza-Situation in Indonesia: Update 14. Available at: http://www.who.int/csr/don/2006_05_23/en/ Accessed 20 September 2006.

24. Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO. Available at: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_01_11/en/print.html. Accessed January 11, 2007.

25. Werner O. [Highly pathogenic avian influenza--a review.][Article in German.] Berl Munch Tierarztl Wochenschr. 2006;119:140–150.

26. Rappole JH, Hubálek Z. Birds and influenza H5N1 virus movement to and within North America. Emerg Infect Dis [serial on the Internet]. 2006 Oct [11 Jan 2007]. Available from http://www.cdc.gov/ncidod/EID/vol12no10/05-1577.htm

27. Ellis TM, Bousfield R, Bisset L, et al. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathol. 2004;33:492–505.

28. Shortridge KF, Zhou N, Guan Y, et al. Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. Virology. 1998;252:331–342.

29. Hulse-Post DJ, Sturm-Ramirez K, Humberd J, et al. Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proc Natl Acad Sci U S A. 2005;102:10682–10687.

30. Webster RG, Bean W, Gorman O, Chambers T, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev. 1992;56:152–179.

31. Rappole JH, Derrickson S, Hubálek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. Emerg Infect Dis. 2000;6:319–328.

32. Webster RG, Govorkova EA. H5N1 Influenza — Continuing Evolution and Spread. NEJM. 2003;355(21):2174-2177.

33. Oxford JS, Schild GC, Potter CW, Jennings R. Specificity of the anti-hemagglutinin antibody response induced in man by inactivated influenza vaccines and by natural infection. J Hyg (Lond). 1979;82:51-61.

34. Rowe T, Abernathy RA, Hu-Primmer J, et. al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol. 1999;37:937-943.

35. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/Singapore/97 vaccine. Virus Res. 2004;103:91-95

36. Walls HH, Harmon MW, Slagle JJ, Stocksdale C, Kendal AP. Characterization and evaluation of monoclonal antibodies developed for typing influenza A and influenza B viruses. J Clin Microbiol 1986; 23:240-245.

37. Kendal AP, Pereira MS, Skehel JJ, eds. Concepts and procedures for laboratory-based influenza surveillance. Atlanta: Centers for Disease Control, 1982.

38. Treanor JJ, Wilkinson BE, Masseoud F, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. Vaccine 2001;19: 1732-1737.

39. Iskander J, Haber P, Herrera G. Monitoring vaccine safety during an influenza pandemic. Yale J Biol Med. 2005 Oct;78(5):265-75.

40. WHO. Safety of pandemic influenza vaccines. Weekly Epi Record: No. 28, 2006, 81, 273-284

Appendix 1: Definition of Safety Parameters (as defined in the DMID Protocol 04-063)

Safety was assessed by frequency and incidence of AEs and SAEs in each dose group as follows:

- **Immediate reactions** within the 15 to 30-minute period after each vaccination.
- **Solicited local injection site and systemic reactions**

  A solicited injection site (local) or systemic reaction was any reaction listed on the preprinted Memory aid given to the subject that occurred after vaccination on Day 0 through Day 7 post-vaccinations 1 and 2, respectively.
- **Any unsolicited AEs** that occurred during the 28-day following each vaccination period (Day 0 through Day 56).

  An unsolicited AE was any AE spontaneously reported on the Memory Aid or to the study personnel that occurred during Day 0 through Day 56.
- **Any serious events** that occurred during the trial period (through the Day 208 post-vaccination period).

Parameters Measured

The following parameters were measured for the evaluation of the safety objectives, and were categorized as none, mild, moderate, or severe (see Table 6, Table 7, Table 8, and Table 9 for details).

1. Solicited injection site AEs (Reactogenicity) Days 0 through Day 7:
   - Pain
   - Tenderness
   - Redness
   - Swelling.
2. Solicited Systemic AEs (Day 0 through Day 7)
   - Feverishness
   - Malaise
   - Body aches (exclusive of the injection site),
   - Nausea
   - Headache.

Table 6: Solicited Injection Site Adverse Events Severity Scoring

Local Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Does not interfere with activity	Interferes with activity	Prevents daily activity
Tenderness	Does not interfere with activity	Interferes with activity	Prevents daily activity
Erythema/Redness*	Does not interfere with activity	Interferes with activity	Prevents daily activity
Induration/Swelling*	Does not interfere with activity	Interferes with activity	Prevents daily activity

* will be also measured in mm

An oral temperature of 37.7°C (100°F) is considered fever in adults. The severity of Fever was scored as follows (Table 7):

Table 7: Fever Severity Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C)	≥ 37.8 - < 38	≥ 38 - < 39	≥ 39

Method and Timing of Safety Measurement

Immediate reactions:

All subjects were observed for 15 to 30 minutes after each vaccination. Any immediate reaction (e.g., hives, difficulty breathing, or anaphylaxis) and unsolicited adverse event were recorded on the AE page of the CRF.

Solicited Injection Site Adverse Events and Systemic Reactions:

Each subject was given a preprinted Memory Aid card to record their oral temperature for each day, as well as the presence and severity of solicited injection site and systemic reactions on the evening of vaccination and for 7 days after each vaccination.

The solicited injection site reactions were characterized according to the grading in Table 6.
The solicited systemic reactions were characterized as according to the grading in and Table 9.

Table 8: Solicited Systemic Reaction Severity Scoring (Quantitative)

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever (°C)*	≥ 37.8 - < 38° C ≥ 100 - < 100.4° F	≥ 38 - < 39° C ≥ 100.4 - < 102° F	≥ 39° C ≥ 102° F
Tachycardia, beats per minute †	101 - 115	116 - 130	≥ 131
Bradycardia, beats per minute	54 – 50‡	49 - 40	< 40
Hypertension (systolic), mm Hg	141 – 155	156 - 165	≥ 166
Hypertension (diastolic), mm Hg	91 – 95	96 - 100	≥ 101
Hypotension (systolic), mm Hg	89 – 85	84 - 80	≤ 79

* Oral temperature, no recent hot or cold beverages or smoking. [Note: A fever can be considered not product-related if an alternative etiology can be documented and it is confirmed to be not product-related by the Independent Safety Monitor]

† Subject at rest.

‡ Not considered an AE if baseline heart rate is 50 - 54 beats per minute.

Table 9: Solicited Systemic Reaction Severity Scoring (Subjective)

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness	No interference with activity	Some interference with activity	Significant interference, prevents daily activity for ≥ 1 day
Fatigue/Malaise	No interference with activity	Some interference with activity	Significant interference, prevents daily activity for ≥ 1 day
Myalgia/Body Ache	No interference with activity	Some interference with activity	Significant interference, prevents daily activity for ≥ 1 day
Headache	No interference with activity	Some interference with activity	Significant interference, prevents daily activity for ≥ 1 day
Nausea	No interference with activity	Some interference with activity	Significant interference, prevents daily activity for ≥ 1 day

Laboratory Tests (Stage I)

For subjects in Stage I of this study, 20 mL of blood was drawn for safety evaluation, including hemoglobin (Hgb), white blood cells (WBC), platelets (Plt), alanine aminotransferase (ALT), and creatinine levels up to 14 days before the first vaccination and 7 days after vaccination. Laboratory test abnormalities were analyzed based on the grading scale in Table 10.

Additionally, for all female subjects of childbearing potential in Stage I and II of this study, urine pregnancy tests were performed within 24 hours prior to the first and second vaccination.

Laboratory test abnormalities were analyzed based on the grading scale in Table 10:

Table 10: Laboratory Test Values/Severity Scales

Laboratory Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hgb (♀)* – gm/dL	< 11.5 & ≥ 11.0	< 11.0 & ≥ 10.0	< 10.0
Hgb (♀) – change from baseline value in gm/dl	≥ 1.0 & < 1.5	≥ 1.5 & < 2.0	≥ 2.0
Hgb (♂)† – gm/dL	< 12.5 & ≥ 12.0	< 12.0 & ≥ 11.0	< 11.0
Hgb (♂) – change from baseline value in gm/dL	≥ 1.5 & < 2.0	≥ 2.0 & < 2.5	≥ 2.5
WBC – cells/mm^3 (Increase in WBC)	≥ 11,000 & < 15,000	≥15,000 &< 20,000	≥ 20,000
WBC – cells/mm^3 (Decrease in WBC)	< 3500 & ≥ 2500	< 2500 & ≥ 1500	< 1500
Platelets – cell/mm^3	< 135,000 & ≥ 125,000	< 125,000 & ≥ 100,000	< 100,000
ALT (increase by factor)	> 1.0 & < 2.5 x ULN‡	≥ 2.5 & < 4 x ULN	≥ 4 x ULN
Serum creatinine – mg/dL	IN§ - IN+0.2	> IN+0.2 - < 2.0	≥ 2.0

* Female
† Male
‡ ULN is upper limit of normal.
§ IN is institutional normal

Definitions:

Adverse Event (AE): International Conference on Harmonisation (ICH) guideline E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews or by a vaccine recipient presenting for medical care.

All AEs must be graded for severity and relationship to study product.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to study products/vaccines: The investigator's assessment of the relationship of an AE to study drug/vaccine is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their possible relationship to study vaccine assessed using the following terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

Associated – There is a known temporal relationship; and/or, if rechallenge is done, the event abates with dechallenge and reappears with rechallenge; and/or the event is known to occur in association with study product or with a product in a similar class of study products.

Not Associated – The AE is completely independent of study product administration; and/or evidence exists that the event is definitely related to another etiology.

Reactogenicity: Reactogenic events are AEs that are known to occur with this type of vaccine. Reactogenicity was analyzed using the grading systems in Table 6, Table 7, Table 8, and Table 9.

Appendix 2: Study Publication in NEJM

Pages 42 - 50 Available in the paper copy only of the Briefing Document

Appendix 3: Synopsis of the Final Integrated Clinical/Statistical Report

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Title of the trial	A Randomized, Double-Blinded, Placebo-Controlled, Phase I/II, Dose-Ranging Study of the Safety, Reactogenicity, and Immunogenicity of Intramuscular Inactivated Influenza A/H5N1 Vaccine in Healthy Adults
Investigators	1. John Treanor, MD University of Rochester School of Medicine/Dentistry Rochester, NY 14642 2. Ken Zangwill, MD University of California at Los Angeles (UCLA) Center for Vaccine Research Torrance, CA 90502 3. James D. Campbell, MD Division of Infectious Diseases and Tropical Pediatrics Center for Vaccine Development University of Maryland, Baltimore MD 21201
Trial centers	3 Centers in the US
Publications	Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M.: Immunogenicity of an Inactivated Subvirion Influenza A (H5N1) Vaccine. N Engl J Med 2006;354:1343-1351.
Trial period	First Visit First Subject: 04 April 2005 Last Visit Last Subject: 25 January 2006.
Development phase	Phase I/II
Objectives	**Primary Objectives**: • To determine the dose-related safety of subvirion inactivated H5N1 vaccine in healthy adults. • To determine the dose-related immunogenicity of subvirion inactivated H5N1 vaccine in healthy adults approximately 1 month following receipt of 2 doses of vaccine. • To provide information for the selection of the best dose levels for further studies. **Primary Hypothesis:** No primary hypothesis was tested. **Secondary Objective:** To evaluate dose-related immunogenicity and the percent of subjects responding approximately 1 and 7 months after the first vaccination. **Secondary Hypothesis:** No secondary hypothesis was tested.

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Methodology

This is a randomized, double-blinded, placebo-controlled, dose-ranging, Phase I/II study in healthy 18 to 64 years old adults. The study was designed to gather critical information on the safety, reactogenicity, and immunogenicity of the investigational influenza A/H5N1 virus vaccine in healthy adults and was conducted in 2 stages. The first stage (Stage I) consisted of the first 118 subjects; 12 control; 28, 25, 25, and 28, in the 7.5 µg, 15 µg, 45 µg, and 90 µg, respectively, of the influenza A/H5N1 virus vaccine groups, followed by a 7-day safety assessment period.

All Stage I subjects were screened for eligibility laboratory evaluations including hemoglobin (Hgb), white blood cells (WBC), platelets (Plt), alanine aminotransferase (ALT), and creatinine levels up to 14 days before the first vaccination and they also received the same laboratory evaluations 7 days after vaccination. The Safety Monitoring Committee (SMC) reviewed the 7-day laboratory results, adverse events and reactogenicity data and recommended to the Division of Microbiology and Infectious Diseases (DMID) that the trial should continue based on pre-defined halting rules detailed in the study protocol.

The Stage II subjects comprised of the remaining subjects to make up the sample size of 452 in the placebo and the respective study groups were then enrolled and given Vaccination 1. The SMC performed another review of Stage I safety data 7 days following administration of the second dose of vaccine. As there were no safety issues in the Stage I subjects at the end of the 7-day period following the second vaccination, the Stage II subjects received the second vaccination. The duration of the study treatment for each subject was about 7 months.

All subjects, Stage I and II received 2 vaccinations of their assigned vaccine dose level on Day 0 and 28, respectively. Following each vaccination, the subjects remained in the clinic for 15 to 30 minutes during which symptoms and signs were assessed. The subjects also maintained a memory aid for recording their oral temperature and solicited injection site (local) and systemic AEs for 7 days after each vaccination.

Note: Data for the neutralizing antibody assay indicated in the study protocol (DMID protocol 04-063) are not included in this report due to an understanding between the Sponsor, NIH/NIAID/DMID, CBER, and sanofi pasteur (CBER minutes of pre-BLA meeting of 21 April 2006 and DMID minutes of 10 May 2006 teleconference between CBER and DMID).

Sample size

Total: Planned = **450**. Enrolled = **452**.
Stage I: Planned = **113**. Enrolled = **118.**

Subjects	Total	Study Groups				
		Placebo	**7.5 µg**	**15 µg**	**45 µg**	**90 µg**
Total Planned	**450**	50	100	100	100	100
Total Enrolled	**452**	48	102	101	98	103
Stage I Planned	**113**	13	25	25	25	25
Stage I Enrolled	**118**	12	28	25	25	28

This study was not designed to perform any formal statistical hypothesis testing.

Company:	Sanofi Pasteur, Inc
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Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Inclusion criteria	Subjects who met all of the following inclusion criteria participated in this study: 1. Male or nonpregnant female (as indicated by a negative urine pregnancy test immediately prior to vaccine administration) between the ages of 18 and 64 years, inclusive. 2. Women of childbearing potential who are at risk of becoming pregnant must agree to practice adequate contraception (i.e., barrier method, abstinence, and licensed hormonal methods) for the entire study period. 3. Is in good health, as determined by vital signs (heart rate, blood pressure, oral temperature), medical history and a targeted physical examination based on medical history. 4. In Stage I subjects, should have normal laboratory values of Hgb, WBC, Plt, ALT, and creatinine prior to the first immunization. 5. Able to understand and comply with planned study procedures. 6. Provides informed consent prior to any study procedures and is available for all study visits.
Exclusion criteria	Subjects that met any of the following exclusion criteria at baseline were excluded from study participation: 1. Has a known allergy to eggs or other components of the vaccine. 2. Has a positive urine pregnancy test prior to vaccination (if female of childbearing potential) or women who are breastfeeding. 3. Is undergoing immunosuppression as a result of an underlying illness or treatment. 4. Has an active neoplastic disease or a history of any hematologic malignancy. 5. Is using oral or parenteral steroids, high-dose inhaled steroids (>800 µg/day of beclomethasone dipropionate or equivalent) or other immunosuppressive or cytotoxic drugs. 6. Has a history of receiving immunoglobulin or other blood product within the 3 months prior to enrollment in this study. 7. Has received any other licensed vaccines within 2 weeks (for inactivated vaccines) or 4 weeks (for live vaccines) prior to enrollment in this study. 8. Has an acute or chronic medical condition that, in the opinion of the investigator, would render vaccination unsafe or would interfere with the evaluation of responses (this includes, but is not limited to: known chronic liver disease, significant renal disease, unstable or progressive neurological disorders, diabetes mellitus, and transplant recipients). 9. Has a history of severe reactions following immunization with contemporary influenza virus vaccines. 10. Has an acute illness, including an oral temperature greater than 100.4°F, within 1 week of vaccination. 11. Received an experimental agent (vaccine, drug, biologic, device, blood product, or medication) within 1 month prior to enrollment in this study, or expects to receive an experimental agent during the 7-month study period. 12. Has any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

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Investigational product	Monovalent subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934 influenza [H5N1] virus) provided in unit-dose vials containing either 30-µg/mL A/H5N1 HA or 90-µg/mL A/H5N1 HA, as determined by single radial immunodiffusion
Form	Liquid
Doses	30-µg/mL A/H5N1 HA or 90-µg/mL A/H5N1 HA. **Dose** / **Volume** 7.5 µg — 0.25 mL from 30 µg/mL vial 15 µg — 0.5 mL from 30 µg/mL vial 45 µg — 0.5 mL from 90 µg/mL vial 90 µg — 1.0 mL from 90 µg/mL vial
Route	Intramuscular (IM)
Batch numbers	[lot no redacted] - 30 µg/mL. [lot no redacted] - 90 µg/mL.
Duration of treatment (Vaccination schedule)	2 vaccinations, Day 0 and Day 28
Duration of follow-up	7 to 8 months
Control product	Physiological Saline (Abbott)
Form	Liquid
Dose	0.5 mL
Route	Intramuscular (IM)
Batch number	23-334-DK
Criteria for evaluation	**The primary endpoints are:** 1. Adverse event or SAE information (solicited in-clinic and via memory aids, concomitant medications, and periodic targeted physical assessments). 2. Proportion of subjects in each dose group achieving a serum neutralizing antibody titer ratio of 1:40 against the influenza A/H5N1 virus 28 days following second dose of vaccine (approximately Day 56). 3. Geometric mean titer and the frequency of 4-fold or greater increases in neutralizing antibody titers in each group 1 month after receipt of each dose, and 7 months after receipt of the first dose of vaccine. 4. Geometric mean titer and the frequency of 4-fold or greater increases in serum HAI antibody titers 1 month after receipt of each dose, and 7 months after receipt of the first dose of vaccine. **The secondary endpoint is:** • Development of serum antibody responses against antigenically drifted variants of H5N1 influenza virus. **Note:** Data for the neutralizing antibody assay indicated in the study protocol and the data for the secondary endpoint are not included in this report.

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Criteria for evaluation: (cont'd)	**Safety:** Safety is based on: **1. Solicited Adverse Events** - Reactogenicity following both vaccinations: a) Injection site (local) reactions including pain, tenderness, redness, and swelling at the injection site. b) Systemic reactions including fever, malaise, myalgia, headache, and nausea. **2. Unsolicited Adverse Events** a) Nonserious events occurring 28 days following each dose of vaccine (through approximately Day 56). b) Serious adverse events occurring during the length of the study. **Immunogenicity:** Immunogenicity is based on H5N1 strain-specific serum neutralizing and HAI antibody titers measured prior to both vaccinations and on Days 56 and 208. **Note:** Data for the neutralizing antibody assay indicated in the study protocol (DMID protocol 04-063) are not included in this report due to an understanding between the Sponsor, NIH/NIAID/DMID, CBER, and sanofi pasteur (CBER minutes of pre-BLA meeting of 21 April 2006 and DMID minutes of 10 May 2006 teleconference between CBER and DMID).
Statistical methods	This is a Phase I/II dose-ranging study and is not designed to test a specific hypothesis. Rather, it is intended to examine the safety of this vaccine and to achieve initial estimates of its dose-dependent immune response for future investigations. As such, the statistical plan is mostly descriptive.

Summary - Conclusions:

A total of 452 subjects were enrolled in the study. There were 48 subjects in the placebo group, 102 in the 7.5 µg, 101 in the 15 µg, 98 in the 45 µg, and 103 in the 90 µg study groups. All except one subject received either placebo or one of the 4 vaccine concentrations.

Overall, 46 subjects were excluded from the per-protocol population for immunogenicity analysis: 5 (10.4%) in the placebo group; 9 (8.8%) in the 7.5 µg study group; 7 (6.9%) in the 15 µg study group; 13 (13.3%) in the 45 µg study group; and 12 (11.7%) in the 90 µg study group. Among these subjects one each did not meet the entry criteria in the 7.5 µg and 15 µg study groups; 1 subject in the 7.5 µg study group did not get vaccinated, the remaining subjects had one or more visits out of window.

A summary of participant disposition and demographics are presented in Table 11 and Table 12.

Company:	Sanofi Pasteur, Inc
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Table 11:Summary of Demographic and Baseline Characteristics of Subjects

	All (N=452)	Placebo (N=48)	7.5 µg (N=102)	15 µg (N=101)	45 µg (N=98)	90 µg (N=103)
Gender n (%)						
Male	210 (46.5)	19 (39.6)	51 (50.0)	36 (35.6)	56 (57.1)	48 (46.6)
Female	242 (53.5)	29 (60.4)	51 (50.0)	65 (64.4)	42 (42.9)	55 (53.4)
Age (years)						
Mean	40.5	40.4	41.2	41.3	40.4	39.4
Median	39.5	38.1	40.0	40.3	38.6	38.1
SD	12.27	12.81	12.71	11.84	12.28	12.12
Min; Max	18; 65	21; 63	19; 65	22; 64	19; 64	18; 64
Race n (%)*						
American Indian / Alaskan Native	3 (0.7)	0 (0.0)	1 (1.0)	2 (2.0)	0 (0.0)	0 (0.0)
Asian	52 (11.5)	5 (10.4)	14 (13.7)	14 (13.9)	11 (11.2)	8 (7.8)
Native Hawaiian or other Pacific Islander	1 (0.2)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)
Black or African American	38 (8.4)	4 (8.3)	5 (4.9)	6 (5.9)	12 (12.2)	11 (10.7)
White	365 (80.8)	41 (85.4)	84 (82.4)	80 (79.2)	76 (77.6)	84 (81.6)
Ethnicity n (%)						
Hispanic or Latino	47 (10.4)	4 (8.3)	13 (12.7)	11 (10.9)	6 (6.1)	13 (12.6)
Non-Hispanic or Non-Latino	405 (89.6)	44 (91.7)	89 (87.3)	90 (89.1)	92 (93.9)	90 (87.4)

* Note: More than 1 race can be checked on the CRF, therefore, N > 452 and (%) >100%.

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Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Table 12: Disposition of Subjects

	Placebo (N = 48) n (%)*	**7.5 µg (N = 102) n (%)**	**15 µg (N = 101) n (%)**	**45 µg (N = 98) n (%)**	**90 µg (N = 103) n (%)**
All Randomized	48 (100.0)	102 (100.0)	101 (100.0)	98 (100.0)	103 (100.0)
Safety Population (Safety Analysis Set)†	48 (100.0)	102 (100.0)	101 (100.0)	98 (100.0)	103 (100.0)
Received Vaccine at Visit 1	48 (100.0)	101 (99.0)	101 (100.0)	98 (100.0)	103 (100.0)
Received Vaccine at Visit 2	46 (95.8)	99 (97.1)	99 (98.0)	92 (93.9)	100 (97.1)
Have Vaccine 1 Reactogenicity Data	48 (100.0)	101 (99.0)	101 (100.0)	98 (100.0)	103 (100.0)
Have Vaccine 2 Reactogenicity Data	46 (95.8)	99 (97.1)	99 (98.0)	92 (93.9)	100 (97.1)
Completed Study	47 (97.9)	96 (94.1)	100 (99.0)	91 (92.9)	100 (97.1)
Subjects Discontinued	1 (2.1)	6 (5.9)	1 (1.0)	7 (7.1)	3 (2.9)
Reasons for withdrawal					
Randomized but not Vaccinated	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Adverse event/SAE (Other than Death)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Adverse Events other than SAE	1 (2.1)‡	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)§
Lost to follow-up	1 (2.1)	3 (2.9)	1 (1.0)	4 (4.1)	2 (1.9)
Non-compliance/Protocol deviation	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
Termination by Site or Sponsor	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Voluntary Withdrawal by Subject	0 (0.0)	2 (2.0)	0 (0.0)	1 (1.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
FAS Population for Immunogenicity†	48 (100.0)	100 (98.0)	101 (100.0)	98 (100.0)	102 (99.0)
Protocol Violators Excluded from PP Population for Immunogenicity**	6 (12.5)	9 (8.8)	7 (6.9)	13 (13.3)	12 (11.7)
Did not meet entry criteria	0 (0.0)	2 (2.0)	1 (1.0)	0 (0.0)	0 (0.0)
Visit out of window	6 (12.5)	7 (6.9)	6 (5.9)	13 (13.3)	12 (11.7)
PP Population for Immunogenicity§	42 (87.5)	93 (91.2)	94 (93.1)	85 (86.7)	91 (88.3)

* Percentages are based on the total number of randomized subjects enrolled in each treatment group.

† Study populations: PP = Per-Protocol; FAS = Full Analysis Set; SAS = Safety Analysis Set.

‡ Subject 06FRO176 (Placebo group) as reported on the CRF, got only the first vaccination, and discontinued vaccination due to adverse reaction to previous vaccination. However, subject completed the protocol, including the Day 208 bleed.

§ Subject 06FLA119 (90 µg group) got only the first vaccination, and terminated early. According to the CRF, she voluntarily withdrew due to 'absence from work following AE'. However, the source document indicated that the reason was "subject decision following grade 3 AE resulting in one day absence from work".

** Protocol violators are counted only once according to their first violation.

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Clinical Safety

Clinical Laboratory Results:

As part of the Halting Rule, hematology and blood chemistry were performed before, and at Day 7 following each vaccination, respectively, on the Stage I subjects. No laboratory results were graded as severe. White blood cell counts (either low or elevated), low hemoglobin readings and changes in hemoglobin from baseline were among the results temporarily associated with the second vaccination that were graded as mild or moderate in severity.

One subject (06FLA022), a 49-year old male in the 7.5 µg study group had a baseline ALT of 14 IU/L on 29 March 2005, experienced an elevated ALT value of 232 IU/L on blood drawn on 12 April 2005. An additional blood drawn from subject on 18 April 2005 was reported as 66 IU/L. This subject was lost to follow up as of 03 May 2005; therefore, no values arising from on Day 28 or Day 7 post-vaccination 2 are available. Other subjects also had repeat blood draws to assess the persistence of out-of-range values.

Immediate Reactions within 15 to 30 minutes after Vaccination:

***Immediate Reactions after Vaccination 1*:** Within 15 to 30 minutes post-vaccination 1, at least one immediate reaction (local injection site or systemic) was reported by 10.4% (5/48) of subjects in the placebo group, 9.9% (10/101) of subjects in the 7.5 µg study group, 11.9% (12/101) of subjects in the 15 µg study group, 4.1% (4/98) of subjects in the 45 µg study group, and 7.8% (8/103) of subjects in the 90 µg study group. Most of these reactions were solicited injection site pain and tenderness. Malaise and myalgia were the most frequently reported immediate systemic reactions during the period, 15 to 30 minutes post-vaccination 1.

***Immediate Reactions after Vaccination 2*:** Within 15 to 30 minutes post-vaccination 2, at least one immediate reaction (local injection site or systemic) was reported by 13.0% (6/46) of subjects in the placebo group, 15.2% (15/99) of subjects in the 7.5 µg study group, 9.1% (9/99) of subjects in the 15 µg study group, 6.5% (6/92) of subjects in the 45 µg study group, and 6.0% (6/100) of subjects in the 90 µg study group. Most of these reactions were solicited injection site pain and tenderness. Malaise, myalgia, and nausea were the most frequently reported immediate systemic reactions during the period 15 to 30 minutes post-vaccination 2.

***Immediate Reactions after any Vaccination*:** Within 15 to 30 minutes following any vaccination, at least one immediate reaction (local injection site or systemic) was reported by 20.8% (10/48) of subjects in the placebo group, 19.8% (20/101) of subjects in the 7.5 µg study group, 16.8% (17/101) of subjects in the 15 µg study group, 9.2% (9/98) of subjects in the 45 µg study group, and 12.6% (13/103) of subjects in the 90 µg study group. Most of these reactions were solicited injection site pain and tenderness. Malaise, myalgia, and nausea were the most frequently reported immediate systemic reactions during the period 15 to 30 minutes following any vaccination.

In addition to the immediate reactions discussed above, the study sites also measured immediate erythema and induration at the injection site within 15 to 30 minutes after each vaccination. As per protocol, these findings were not reported with the other immediate reactions as they were not assessed for severity and the duration was not specifically collected for these events, however, they were captured on the Vaccination Record / Assessment page of the case report form. A total of 887 assessments were conducted for erythema and induration. Of the erythema assessments, approximately 83% had no erythema noted. Fifteen percent of the measurements were $\leq$ 5 mm and 2% were $\geq$ 6 mm with a maximum of 20 mm (1 subject). Of the induration assessments, approximately 94.5% had no induration noted. Five percent of the measurements were $\leq$ 5 mm and 0.5% were $\geq$ 6 mm with a maximum of 28 mm (1 subject).

Solicited Injection Site Reactions within 7 Days after Vaccination:

***Vaccination 1*:** During Day 0 to 7 after vaccination 1, at least one solicited reaction (local injection site or systemic) was reported by 50.0% (24/48) of subjects in the placebo group, 46.5% (47/101) of subjects in the 7.5 µg study group, 66.3% (67/101) of subjects in the 15 µg study group, 69.4% (68/98) of subjects in the 45 µg study group, and 81.6% (84/103) of subjects in the 90 µg study group.

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

During the same period, solicited injection site reactions were reported by 27.1% (13/48) of subjects in the placebo group, 28.7% (29/101) of subjects in the 7.5 µg study group, 50.5% (51/101) of subjects in the 15 µg study group, 62.2% (61/98) of subjects in the 45 µg study group, and 73.8% (76/103) of subjects in the 90 µg study group.

Overall, among the study groups, the number of subjects reporting injection site reaction tended to increase with increasing vaccine dose following vaccination 1. Injection site tenderness and injection site pain were reported by most of the subjects. Furthermore, most of the solicited injection site reactions were of mild to moderate severity that occurred, and resolved within three days of vaccination 1. One subject in the 7.5 µg study group reported a mild erythema that lasted 10 days and resolved without sequelae.

***Vaccination 2*:** During Day 0 to 7 after vaccination 2, at least one solicited reaction (local injection site or systemic) was reported by 47.8% (22/46) of subjects in the placebo group, 48.5% (48/99) of subjects in the 7.5 µg study group, 56.6% (56/99) of subjects in the 15 µg study group, 68.5% (63/92) of subjects in the 45 µg study group, and 70.0% (70/100) of subjects in the 90 µg study group.

During the same period, solicited injection site reactions we reported by 23.9% (11/46) of subjects in the placebo group, 35.4% (35/99) of subjects in the 7.5 µg study group, 45.5% (45/99) of subjects in the 15 µg study group, 62.0% (57/92) of subjects in the 45 µg study group, and 64.0% (64/100) of subjects in the 90 µg study group.

Overall, among the study groups, the number of subjects reporting injection site reaction tended to increase with increasing vaccine dose post-vaccination 2. Injection site tenderness and injection site pain were reported by most of the subjects. Furthermore, the solicited injection site reactions were of mild to moderate severity that occurred, and resolved within three days of vaccination 2.

***Any Vaccination*:** During Day 0 to 7 after any vaccination, at least one solicited reaction (local injection site or systemic) was reported by 68.8% (33/48) of subjects in the placebo group, 65.3% (66/101) of subjects in the 7.5 µg study group, 78.2% (79/101) of subjects in the 15 µg study group, 78.6% (77/98) of subjects in the 45 µg study group, and 89.3% (92/103) of subjects in the 90 µg study group.

During the same period, solicited injection site reactions were reported by 37.5% (18/48) of subjects in the placebo group, 46.5% (47/101) of subjects in the 7.5 µg study group, 62.4% (63/101) of subjects in the 15 µg study group, 74.5% (73/98) of subjects in the 45 µg study group, and 82.5% (85/103) of subjects in the 90 µg study group. (See Table 13).

Overall, among the study groups, injection site reactions tended to be dose related, increasing with higher vaccine dose following any vaccination. Injection site pain and injection site tenderness were the most frequently reported reactions. Most of the solicited injection site reactions were of mild to moderate severity which occurred, and resolved within three days of vaccination.

Solicited Systemic Reactions within 7 Days after Vaccination:

Vaccination 1: During Day 0 to 7 after vaccination 1, solicited systemic reactions were reported by 43.8% (21/48) of subjects in the placebo group, 29.7% (30/101) of subjects in the 7.5 µg study group, 37.6% (38/101) of subjects in the 15 µg study group, 24.5% (24/98) of subjects in the 45 µg study group, and 39.8% (41/103) of subjects in the 90 µg study group.

The most frequently reported solicited systemic reactions were headache and malaise, reported by 25.0% (12/48) and 18.8% (9/48) of subjects in the placebo group; 18.8% (19/101) and 15.8% (16/101) of subjects in the 7.5 µg study group; 26.7% (27/101) and 19.8% (20/101) of subjects in the 15 µg study group; 16.3% (16/98) and 10.2% (10/98) of subjects in the 45 µg study group, and 30.1% (31/103) and 15.5% (16/103) of subjects in the 90 µg study group, respectively. Most of the solicited systemic reactions were mild to moderate severity. Severe (Grade 3) solicited systemic reactions were reported by 2 subjects: 1 subject in the 15 µg study group reported severe malaise and myalgia and 1 subject in the 90 µg study group reported severe headache, malaise, and myalgia.

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Most of the solicited systemic reactions occurred within three days of vaccination and resolved within seven days of vaccination 1. Two subjects, one each in the 15 µg and 45 µg study groups, reported 3 mild solicited systemic events malaise and myalgia (9 days), and malaise (17 days), respectively, which lasted more than eight days following vaccination 1. The 3 solicited systemic events resolved without sequelae.

***Vaccination 2*:** During Day 0 to 7 after vaccination 2, solicited systemic reactions were reported by 39.1% (18/46) of subjects in the placebo group, 26.3% (26/99) of subjects in the 7.5 µg study group, 30.3% (30/99) of subjects in the 15 µg study group, 19.6% (18/92) of subjects in the 45 µg study group, and 21.0% (21/100) of subjects in the 90 µg study group.

The most frequently reported solicited systemic reactions were headache, malaise, and myalgia reported by 26.1% (12/46), 17.4% (8/46), and 10.9% (5/46), respectively, of subjects in the placebo group; 17.2% (17/99), 10.1% (10/99), and 6.06% (6/99), respectively, of subjects in the 7.5 µg study group; 22.2% (22/99), 17.2% (17/99), and 11.1% (11/99), respectively, of subjects in the 15 µg study group; 9.8% (9/92), 6.5% (6/92), and 7.6% (7/92), respectively, of subjects in the 45 µg study group, and 11.0% (11/100), 12.0% (12/100), and 8.0% (8/100), respectively, of subjects in the 90 µg study group, respectively. Most of the solicited systemic reactions were mild to moderate severity. Severe solicited systemic reactions of nausea and headache were reported by 1 subject each in the 7.5 µg and 15 µg study group, respectively.

Most of the solicited systemic reactions occurred within three days of vaccination and resolved within seven days of vaccination 2. Two subjects one each in the 7.5 µg and 15 µg study group reported 3 mild solicited systemic events, malaise (12 days) and malaise and myalgia (10 days), respectively, which lasted more than eight days following vaccination 2. The 3 solicited systemic events resolved without sequelae.

Any Vaccination: During Day 0 to 7 after any vaccination, solicited systemic reactions were reported by 58.3% (28/48) of subjects in the placebo group, 39.6% (40/101) of subjects in the 7.5 µg study group, 47.5% (48/101) of subjects in the 15 µg study group, 35.7% (35/98) of subjects in the 45 µg study group, and 47.6% (49/103) of subjects in the 90 µg study group.

Overall, the placebo group reported the most solicited systemic reactions. Among the study groups, most of the reports were from subjects in the 15 µg and 90 µg vaccine doses. The group with the least reported solicited systemic events was the 45 µg study group. Headache, malaise and myalgia were reported by most of the subjects after any vaccination.

Unsolicited Adverse Events after Vaccination:

Vaccination 1: During Day 0 to Day 28 after vaccination 1 at least one unsolicited AE was reported by 35.4% (17/48) of subjects in the placebo group, 27.7% (28/101) of subjects in the 7.5 µg study group, 32.7% (33/101) of subjects in the 15 µg study group, 23.5% (23/98) of subjects in the 45 µg study group, and 20.4% (21/103) of subjects in the 90 µg study group. The most frequent unsolicited AEs reported following vaccination 1 were in the System Organ Class (SOC) of gastrointestinal disorders, infections and infestations, and respiratory, thoracic, and mediastinal disorders. Post-vaccination 1, unsolicited AEs classified as severe, life-threatening, or death were rare. They were reported for one subject each in the 15 µg, 45 µg, and 90 µg study groups, respectively.

Vaccination 2: During Day 0 to Day 28 after vaccination 2 (Day 28 to 56 after vaccination 1), at least one unsolicited AE was reported by 31.3% (15/48) of subjects in the placebo group, 22.8% (23/101) of subjects in the 7.5 µg study group, 30.7% (31/101) of subjects in the 15 µg study group, 22.4% (22/98) of subjects in the 45 µg study group, and 25.2% (26/103) of subjects in the 90 µg study group.

The most frequent unsolicited AEs reported following vaccination 2 were in the SOCs of infections and infestations, nervous system disorders, and respiratory, thoracic, and mediastinal disorders.

Post-vaccination 2 unsolicited AEs classified as severe, life-threatening, or death were rare. They were reported by one subject each in the placebo and 90 µg study group, and 2 subjects in the 15 µg study group.

Company:	Sanofi Pasteur, Inc
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Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Any Vaccination: During Day 0 to Day 28 after any vaccination at least one unsolicited AE was reported by 58.3% (28/48) of subjects in the placebo group, 42.6% (43/101) of subjects in the 7.5 µg study group, 52.5% (53/101) of subjects in the 15 µg study group, 39.8% (39/98) of subjects in the 45 µg study group, and 41.7% (43/103) of subjects in the 90 µg study group.

The most frequent unsolicited AEs reported following any vaccination were in the SOCs of gastrointestinal disorders, general disorders and administration site condition, infections and infestations, musculoskeletal and connective tissue disorders, nervous system disorders and respiratory, thoracic, and mediastinal disorders.

After any vaccination, unsolicited AEs classified as severe, life-threatening, or death were rare and did not show a trend by dose or number of vaccinations. They were reported by 7 subjects, 3 following vaccination 1 and 4 following vaccination 2.

Only 2 cases of the reported severe (Grade 3) pharyngitis/infections and infestations (literal term/SOC) occurring 3 days and 4 days, respectively, after vaccination 1 were classified as associated with vaccination by the investigators. The 2 subjects recovered without sequelae. All other reported unsolicited AEs were classified as not associated with the study vaccination. With the exception of the subject that died described below (SAE), the other subjects recovered without sequelae.

Withdrawal due to Adverse Events:

Two subjects, one each in the placebo and the 90 µg dose study group withdrew from the study secondary to adverse events:

1. A 24-year old male in the placebo group reported a mild-Grade 1 Maculopapular Rash Abdomen and Upper Arms Bilateral (Literal Term) 5 days after vaccination 1. It lasted 38 days and resolved without sequelae. The second vaccination was discontinued due to the AE. However, the subject completed other study visits and blood sample draws. The AE was classified by the investigator as related to the placebo product.
2. A 27-year old female in the 90 µg study group reported a severe-Grade 3 pharyngitis which resulted in her missing a day at work 4 days after vaccination 1. The subject decided not to receive any further vaccinations and did not show up for other clinic visits or blood draws. The AE was classified by the investigator as related to the study product.

Serious Adverse Events:

Serious adverse events including 1 death were reported for 4 subjects during the trial. They are SOC reproductive system and breast disorders; neoplasms benign, malignant and unspecified (cysts and polyps); Psychiatric disorders; and nervous system disorders reported by one subject each in the placebo group; 15 µg, 45 µg, and 90 µg study groups, respectively. The SAEs were all deemed unrelated to the vaccination by the investigators.

The subject that died received the study vaccine (45 µg Inactivated Influenza A/H5N1) on 09 May 2005. The subject was found dead at the apartment 23 days later. The cause of death was chronic alcoholism confirmed by the autopsy. The subject had a significant past medical history of well controlled hyperlipidemia and hypertension, as well as alcoholism. Concomitant medications included Lipitor and Adalat. The toxicology report found ethanol in the heart blood, urine and vitreous humor and caffeine in the serum. All other toxicology screens (including barbiturates, analeptics, neural sedatives (sic), benzodiazepams, comprehensive basic drug screen, chloral hydrate, trichloroethanol, opiates, cocaine, cannabinoids and salicylates) were negative. The cause of death was chronic alcoholism with hepatomegaly (2265 grams) and marked hepatic steatosis.

As per the investigator, "although the ultimate cause of this subject's death is not clear to this reviewer, it is apparent that there are multiple possible explanations (sequelae of chronic alcoholism, multiple blunt traumas, hemorrhage related to liver disease, etc.). Given this fact, and the lack of suggestive pathological evidence, there is little reason to suggest a causal relationship of the death to the study article"

The event was classified as not related to the study product by the investigator and the sponsor.

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Table 13: Safety Overview after Any Vaccination - Safety Analysis Set

Subjects with at least one	Placebo (N = 48) n (%)	7.5 µg (N = 101) n (%)	15 µg (N = 101) n (%)	45 µg (N = 98) n (%)	90 µg (N = 103) n (%)
Immediate reaction within 15 to 30 minutes	10 (20.8)	20 (19.8)	17 (16.8)	9 (9.2)	13 (12.6)
Solicited reaction*	33 (68.8)	66 (65.3)	79 (78.2)	77 (78.6)	92 (89.3)
Solicited injection site reaction†	18 (37.5)	47 (46.5)	63 (62.4)	73 (74.5)	85 (82.5)
Solicited systemic reaction	28 (58.3)	40 (39.6)	48 (47.5)	35 (35.7)	49 (47.6)
Unsolicited event‡	28 (58.3)	43 (42.6)	53 (52.5)	39 (39.8)	43 (41.7)
Unsolicited reaction	15 (31.3)	30 (29.7)	32 (31.7)	13 (13.3)	27 (26.2)
AE leading to study discontinuation	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)
Serious Adverse Events§	1 (2.1)	0 (0.0)	1 (1.0)	1 (1.0)	1 (1.0)
Death§	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)

* Reactions are events identified by the investigator in the CRF as related to the study vaccine.

† For solicited reactions, the denominator for percentages is the number of vaccinated subjects with at least one non-missing value for the reaction.

‡ Note: For unsolicited events, the denominator for percentages is the number of vaccinated subjects for whom safety data are available (safety analysis set).

§ Deaths are also included in the count of SAEs.

Note: Immediate reactions are included, except for immediate redness and swelling, as no severity grade was assigned.

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Immunogenicity

Proportion Attaining ≥ 1:40 Titer and Fold Rises in HAI-H5N1 Antibody Titers:

Baseline, Pre-Vaccination 1: At baseline, none of subjects 0.0% (0/42) in the placebo group, 3.2% (3/93) of subjects in the 7.5 µg study group, none of subjects 0.0% (0/94) in the 15 µg study group, 2.4% (2/85) of subjects in the 45 µg study group, and 1.1% (1/91) of subjects in the 90 µg study group had a titer ≥ 1:40.

Twenty eight Days Post-Vaccination 1: Twenty-eight days after receiving the first dose of H5N1 vaccine, 6.5% (6/93) of subjects in the 7.5 µg study group, 8.5% (8/94) of subjects in the 15 µg study group, 22.4% (19/85) of subjects in the 45 µg study group, and 24.2% (22/91) of subjects in the 90 µg study group attained a ≥ 1:40 titer.

Twenty-eight days after receiving the first dose of H5N1 vaccine, 4-fold rise in pre-vaccination titers were achieved by 2.2% (2/93) of subjects in the 7.5 µg study group, 7.4% (7/94) of subjects in the 15 µg study group, 22.4% (19/85) of subjects in the 45 µg study group, and 23.1% (21/91) of subjects in the 90 µg study group.

Twenty eight Days Post-Vaccination 2: Twenty-eight days after receiving the second dose of H5N1 vaccine, 6.5% (6/93) of subjects in the 7.5 µg study group, 17.0% (16/94) of subjects in the 15 µg study group, 34.1% (29/85) of subjects in the 45 µg study group, and 46.2% (42/91) of subjects in the 90 µg study group attained a ≥ 1:40 titer.

Twenty-eight days after receiving the second dose of H5N1 vaccine, 4-fold rise in pre-vaccination titers were achieved by 4.3% (4/93) of subjects in the 7.5 µg study group, 16.0% (15/94) of subjects in the 15 µg study group, 34.1% (29/85) of subjects in the 45 µg study group, and 45.1% (41/91) of subjects in the 90 µg study group.

Six months Post-vaccination 2: Six months after receiving the second dose of H5N1 vaccine 5.4% (5/92) of subjects in the 7.5 µg study group, 6.5% (6/93) of subjects in the 15 µg study group, 22.9% (19/83) of subjects in the 45 µg study group, and 18.7% (17/91) of subjects in the 90 µg study group attained a ≥ 1:40 titer.

Six months after receiving the second dose of H5N1 vaccine, 4-fold rise in pre-vaccination titers were achieved by 2.2% (2/92) of subjects in the 7.5 µg study group, 5.4% (5/93) of subjects in the 15 µg study group, 22.9% (19/83) of subjects in the 45 µg study group, and 17.6% (16/91) of subjects in the 90 µg study group. (See Table 14).

HAI – H5N1 Geometric Mean Titers:

The baseline HAI – H5N1 GMTs were similar for all subjects. Post-vaccination, the GMT values at 28 days post-vaccination 2 were higher than at 28 days post-vaccination 1 and at 6 months post-vaccination 2, respectively. Additionally, the post-vaccination 1 and post-vaccination 2 GMT values tended to be dose related with the highest values reported in the 90 µg study group and the lowest value in the 7.5 µg study group. At 6 months post-vaccination 2, the GMTs in all groups had declined to post-vaccination 1 levels. (See Table 15).

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Table 14:Summary of Proportion Attaining ≥ 1:40 titers and Fold Increases, Hemagglutinin Inhibition - H5N1 (Per-Protocol Population)

Seroresponse criterion	Statistic	Placebo (N*= 42)	7.5 µg (N = 93)	15 µg (N = 94)	45 µg (N = 85)	90 µg (N = 91)
≥ 1:40 Pre-vaccination 1	n/M†	0/42	3/93	0/94	2/85	1/91
	%	0.0	3.2	0.0	2.4	1.1
	95% CI	(0.0; 8.4)	(0.7; 9.1)	(0.0; 3.8)	(0.3; 8.2)	(0.0; 6.0)
≥ 1:40 at 28 Days Post-vaccination 1	n/M	1/42	6/93	8/94	19/85	22/91
	%	2.4	6.5	8.5	22.4	24.2
	95% CI	(0.1; 12.6)	(2.4; 13.5)	(3.7; 16.1)	(14.0; 32.7)	(15.8; 34.3)
≥ 1:40 at 28 Days Post-vaccination 2	n/M	1/42	6/93	16/94	29/85	42/91
	%	2.4	6.5	17.0	34.1	46.2
	95% CI	(0.1; 12.6)	(2.4; 13.5)	(10.1; 26.2)	(24.2; 45.2)	(35.6; 56.9)
≥ 1:40 at 6 Months Post-vaccination 2	n/M	2/41	5/92	6/93	19/83	17/91
	%	4.9	5.4	6.5	22.9	18.7
	95% CI	(0.6; 16.5)	(1.8; 12.2)	(2.4; 13.5)	(14.4; 33.4)	(11.3; 28.2)
2 fold rise‡ at 28 Days Post-vaccination 1	n/M	0/42	5/93	9/94	22/85	26/91
	%	0.0	5.4	9.6	25.9	28.6
	95% CI	(0.0; 8.4)	(1.8; 12.1)	(4.5; 17.4)	(17.0; 36.5)	(19.6; 39.0)
4 fold rise at 28 Days Post-vaccination 1	n/M	0/42	2/93	7/94	19/85	21/91
	%	0.0	2.2	7.4	22.4	23.1
	95% CI	(0.0; 8.4)	(0.3; 7.6)	(3.0; 14.7)	(14.0; 32.7)	(14.9; 33.1)
2 fold rise at 28 Days Post-vaccination 2	n/M	1/42	11/93	22/94	35/85	54/91
	%	2.4	11.8	23.4	41.2	59.3
	95% CI	(0.1; 12.6)	(6.1; 20.2)	(15.3; 33.3)	(30.6; 52.4)	(48.5; 69.5)
4 fold rise at 28 Days Post-vaccination 2	n/M	0/42	4/93	15/94	29/85	41/91
	%	0.0	4.3	16.0	34.1	45.1
	95% CI	(0.0; 8.4)	(1.2; 10.6)	(9.2; 25.0)	(24.2; 45.2)	(34.6; 55.8)
2 fold rise at 6 Months Post-vaccination 2	n/M	1/41	5/92	10/93	21/83	28/91
	%	2.4	5.4	10.8	25.3	30.8
	95% CI	(0.1; 12.9)	(1.8; 12.2)	(5.3; 18.9)	(16.4; 36.0)	(21.5; 41.3)
4 fold rise at 6 Months Post-vaccination 2	n/M	1/41	2/92	5/93	19/83	16/91
	%	2.4	2.2	5.4	22.9	17.6
	95% CI	(0.1; 12.9)	(0.3; 7.6)	(1.8; 12.1)	(14.4; 33.4)	(10.4; 27.0)

* N = number of subjects in dose group.

† M = number of subjects with available data.

‡ All fold rise calculations use Pre-vaccination 1 value as baseline.

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Table 15: Summary of Geometric Mean Titers, Hemagglutinin Inhibition - H5N1 (Per-Protocol Population)

Time	Statistic	Placebo (N*=43)	7.5 µg (N=93)	15 µg (N=94)	45 µg (N=85)	90 µg (N=91)
Day 0 (Pre-vaccination 1)	**M†**	42	93	94	85	91
	GMT	5.4	5.8	5.1	5.5	5.2
	95% CI	(4.8, 5.9)	(5.1, 6.6)	(4.9, 5.4)	(5.1, 6.0)	(4.9, 5.5)
28 Days Post-vaccination 1	**M**	42	93	94	85	91
	GMT	5.5	6.5	6.8	12.0	13.7
	95% CI	(4.8, 6.3)	(5.6, 7.6)	(5.7, 8.2)	(8.6, 16.7)	(9.8, 19.2)
28 Days Post-vaccination 2	**M**	42	93	94	85	91
	GMT	5.5	7.3	9.7	17.8	30.6
	95% CI	(4.8, 6.4)	(6.2, 8.7)	(7.8, 12.2)	(12.7, 24.9)	(22.1, 42.2)
6 Months Post-vaccination 2	**M**	42	92	93	83	91
	GMT	5.6	6.1	6.6	10.6	11.8
	95% CI	(4.8; 6.6)	(5.3; 7.0)	(5.7; 7.7)	(8.0; 14.2)	(8.9; 15.7)

* N = number of subjects in dose group.

† M = number of subjects with available data.

Discussion and Conclusion:

The primary objectives of this trial were to determine the dose-related safety and immunogenicity of subvirion inactivated H5N1 vaccine in healthy adults. As can be demonstrated by the study results, and as will be discussed below, this vaccine shows acceptable reactogenicity and immunogenic profile.

Four hundred and fifty-two subjects, divided into 5 dose groups, including placebo, were randomized at three study centers. Four vaccine doses of HA (rgA/Vietnam/1203/2004 x A/PR/8/1934 influenza (H5N1) virus) were administered at Day 0 and 28. Forty-six subjects, distributed across all study groups, were not included in the per-protocol population mostly due to protocol violations (visit out of window [43/46]). Other than a modest imbalance in the gender distribution in the placebo and 15 µg dose level, the study groups were evenly divided according to age, race and ethnicity. Two subjects, one each in the placebo and 90 µg dose study group withdrew from the study secondary to non-serious adverse events. Four SAEs, including one death, occurred. None were deemed related to study vaccine by the study investigators. The death occurred 23 days after receiving the second vaccination and was attributed to chronic alcoholism.

There were no safety signals of concern raised in this trial. Overall, within 7 days after vaccination, 69% to 89% of all subjects reported at least one injection site reaction. None were severe and generally, most were reported as mild. Pain and tenderness at injection site were most frequently experienced by the subjects. No difference in frequency or reaction type was observed after the first and second vaccinations. Not unexpectedly, the frequency, though not the severity, of the injection site reactions was dose dependent and greater in vaccine versus placebo recipients.

Solicited systemic reactions within seven days after any vaccination were reported with comparable frequency across all study groups and no dose relationship was apparent, especially given that most frequently, these were reported by subjects in the placebo group. Fever was reported infrequently compared to headache, malaise, and myalgia which were most commonly experienced by subjects. No differences were noted in terms of frequency or severity of solicited systemic reactions after either vaccination. However, following vaccination 1 and 2, three and two systemic events, respectively, lasted more than eight days.

Company:	Sanofi Pasteur, Inc
Finished product:	Monovalent Subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
Active ingredient(s):	rgA/Vietnam/1203/2004 (H5N1) x A/PR/8/1934 influenza virus

Unsolicited adverse events were reported by 45.6% of all subjects and were approximately equally distributed across all study groups including placebo. No relationship was noted between dose level received and frequency of unsolicited adverse events. Severe (Grade 3) unsolicited adverse events were rare in all groups. Seven subjects reported severe unsolicited AEs; one subject each in the placebo and 45 µg study group, 2 subjects in the 90 µg study group, and 3 subjects in the 15 µg study group, respectively.

The immunogenicity endpoints reported here were GMTs and frequency of four-fold or greater increase in serum HAI titers in each group one month after receipt of each vaccination and seven months after the initial vaccination. This study was not designed with a particular hypothesis and, consequently, formal statistical testing is not included in the design of this dose ranging and safety study.

Significant increases in GMTs were noted one month after the first and second vaccination at the two highest dose levels. After the second vaccination, a significant increase was also observed at 15 µg. Six months after the second vaccine injection GMTs decreased in all groups but the levels remained at the post-vaccination 1 levels in the 45 µg and 90 µg groups. A four-fold increase in HAI was achieved in a dose dependant manner with a maximum seroconversion rate of 45.1% one month after the second injection in the 90 µg study group. This represents an almost 50% increase over the initial fold-rise 28 days post-vaccination 1. The four-fold increase was sustained in less than half of the subjects initially seroconverting after six months post the two vaccinations. Seroprotection rates followed a similar trend to seroconversion with a maximal effect achieved at 90 µg with a 46.2% response rate. At 28 days post-vaccination 2, 41.2% and 59.3 % of subjects receiving the 45 µg and 90 µg doses, respectively, achieved a greater than 2-fold rise, while at seven months from baseline, 22.9% and 18.7% of subjects still had titers ≥ 1:40 in the 45 µg and 90 µg groups, respectively.

Thus, antibody responses to this H5N1 vaccine were most pronounced after two vaccinations in the two highest dose levels wherein approximately 34% to 45% of a population of healthy female or male adults could be expected to achieve a clinically significant antibody response.

It should be noted that there are differences in the immunogenicity data presented in this report compared to an earlier publication on the study [1] because this report:

- is based on the final data, whereas the publication was based on an interim data.
- uses an initial dilution factor of 1:10, whereas the publication used an initial dilution factor of 1:20.
- for baseline less than Lower Limit of Quantitation (< LLOQ), uses a fold-rise calculation that considers LLOQ as baseline, whereas the publication considered 0.5 LLOQ as baseline.
- considers a subject with a < 1:10 baseline titer needed to have a ≥ 1:40 post-vaccination titer to be classified as having a four-fold rise; whereas in the publication, a subject with a < 1:20 baseline titer needed to have the same post-vaccination titer to be classified as having a four-fold rise, despite having a higher baseline titer.

In conclusion, this study examined the safety and immunogenicity of a monovalent subvirion H5N1 vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934 influenza (H5N1) virus) for potential prophylactic use against avian influenza A viruses of the H5N1 subtype currently circulating on several continents. Over 360 healthy adults received two vaccinations with 7.5 µg to 90 µg of vaccine with no safety signals arising. The vaccine was well tolerated with mostly mild to moderate reactogenicity reported. No serious adverse events related to the study vaccine occurred.

Immunologically, the two highest dose levels induced significant HAI titers and seroconversion. Using conventional influenza immunogenicity criteria, over 45% of healthy adult subjects achieved a potentially protective response following vaccination with 90 µg HA.

Appendix 4: Summary of Benefit Risks

Summary of Benefits and Risks

H5N1 Vaccine

BMF Number:	12132
Product:	Monovalent Subvirion H5N1vaccine (HA of rgA/Vietnam/1203/ 2004 x A/PR/8/1934)
Form/Route:	Liquid/Intramuscular (IM)
Sponsor:	Sanofi Pasteur Inc.
Clinical Team Leader:	Pierre Geoffroy, MDCM, MSc, FCFP
Clinical Program Manager:	Tom LeDuc
Medical Writer	Oladayo Oyelola, PhD
Date of the report:	20 September 2006
Version Number:	1.0

Signatures

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Pierre Geoffroy, MDCM, MSc, FCFP

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Date

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Tom LeDuc

20 SEPT 2006
Date

Medical Writer
Oladayo Oyelola, PhD

20 SEPT 2006
Date

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List of Abbreviations

AE	Adverse event
A/H5N1	Influenza A Virus of the H5N1 Subtype
BL	Biological License
BLA	Biologics License Application
BMF	Biological Master File
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
eCRF	Electronic Case Report Form
GBS	Guillain-Barré syndrome
GMT	Geometric mean titer
HA	Hemagglutinin
HAI	Hemagglutination Inhibition Assay
H5N1 Vaccine	Monovalent Subvirion H5N1vaccine (HA of rgA/Vietnam/1203/2004 x A/PR/8/1934)
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
SAE	Serious adverse event
SOC	System Organ Class
US	United States
WHO	World Health Organization
µg	Micrograms

1 Overview of Risk Benefit

Avian influenza A viruses of the H5N1 subtype are currently causing widespread infections in bird populations throughout Southeast Asia, with spread into Central Asia, Africa, and Europe. (1) There have been a number of instances of transmission of these viruses to humans, resulting in severe disease or death. (2)

These viruses possess a new H5 subtype of hemagglutinin, against which at present there is little immunity in human populations. The A/H5N1 viruses have the potential to cause extremely severe respiratory illness in humans, and have been known to repeatedly "jump the species barrier". Many of the viruses isolated from humans have been found to be genotypically resistant to the adamantanes, (3) and resistance to oseltamivir has also been described. (4)

Although human-to-human transmission appears at present to be rare, (5) a recent bird-flu outbreak in an Indonesian village where seven family members died, has raised the level of concern that the virus may be able to pass directly between people. With no animal identified as yet as the source of infection, the family cluster in Indonesia raises the suspicion of human-to-human transmission. (6)

As of 08 September 2006, the cumulative number of laboratory-confirmed human cases of Avian Influenza A-(H5N1) reported to the World Health Organization (WHO) was 244, including 143 (58.6%) deaths in human adults and children in Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey, and Vietnam. (7)

Pandemic influenza is characterized by the sudden onset of severe typical influenza symptoms, such as high fever, headache, myalgia, arthralgia, anorexia, nausea, vomiting and cough, lasting 2 to 4 days.

Although antiviral drugs exist, vaccines will form the main prophylactic measure against pandemic influenza and will play an important role in the plans to prepare for a pandemic. The WHO Influenza Surveillance Program provides representative influenza viruses for antigenic and genetic analysis and from this information, the WHO is able to make recommendations on vaccine composition. (8)

The development of an effective vaccine against influenza A (H5N1) virus is a matter of considerable urgency. As with influenza vaccines, occasionally, adult recipients of the H5N1 vaccine may develop influenza-like reactions such as fever, body aches, headache, malaise, myalgia, and/or nausea. These may occur more frequently in people who are given the higher dose level of vaccine. These reactions are usually greatest within the first 24 hours after vaccination and last 1 to 2 days. Some subjects may develop reactions at the site of vaccination (redness, swelling, pain, or tenderness). Analgesics (e.g. aspirin or acetaminophen) and rest will generally relieve or moderate these symptoms. These reactions usually resolve in 1 to 4 days and typically do not require additional treatment.

In our previous experience with the H5N1 vaccine, first between November 2005 and January 2006, 83 potentially occupationally exposed workers at the sanofi pasteur Swiftwater, PA facility received two 90 µg doses of the H5N1 vaccine, the vaccine formulation was well tolerated with no untoward safety signals noted; and between May and July 2005, a 2-dose regimen of 7.5 µg, 15 µg or 30 µg, of the H5N1 vaccine

administered with and without adjuvant to 300 volunteers in Europe were well tolerated and elicited neutralizing and hemagglutination-inhibiting antibody responses. (9)

The 1976 swine influenza vaccine was associated with an increased frequency of Guillain-Barré syndrome (GBS), a very rare, acute, and frequently severe polyneuropathy characterized by ascending, fulminant muscle paralysis. Among persons who received the swine influenza vaccine in 1976, the rate of GBS exceeded the background rate of <10 cases/1,000,000 persons vaccinated. (10) Evidence for a causal relationship of GBS with subsequent vaccines prepared from other influenza viruses is unclear. Obtaining strong epidemiologic evidence for such a possible limited increase in risk is difficult for such a rare condition as GBS, which has an annual incidence of 10–20 cases per 1,000,000 adults, and stretches the limits of epidemiologic investigation. The reasons why swine influenza vaccine triggered GBS in 1976 to 1977 have never been discovered. In subsequent annual influenza vaccine programs in the United States, from 1987 to 1991, the overall relative risk estimates for GBS after influenza vaccination were slightly elevated but were not statistically significant in any of the studies. However, in a study of the 1992–1993 and 1993–1994 seasons, the overall relative risk for GBS was 1.7 (95% CI=1.0-2.8; P=0.04) during the 6 weeks after vaccination, representing approximately 1 additional case of GBS for each 1,000,000 persons vaccinated. The combined number of GBS cases peaked 2 weeks after vaccination. Thus, investigations to date indicate that there is no substantial increase in GBS associated with influenza vaccines (other than the swine influenza vaccine in 1976) and that, if influenza vaccine does pose a risk, it is probably slightly more than 1 additional case per 1,000,000 persons vaccinated. Even if GBS were a true side effect of vaccination in the years after 1976, the estimated risk for GBS of approximately 1 additional case/1,000,000 persons vaccinated is substantially less than the risk for severe influenza, which could be prevented by vaccination in all age groups, especially and chiefly persons aged $\geq$ 65 years and those who have medical indications for influenza vaccination.

Neurological disorders temporally associated with influenza vaccination such as encephalopathy, optic neuritis/neuropathy, partial facial paralysis, and brachial plexus neuropathy have been reported rarely. However, no cause and effect has been established. Almost all persons affected were adults, and the described clinical reactions began as soon as a few hours and as late as 2 weeks after vaccination. Full recovery was almost always reported.

Furthermore, vaccine injection into the deltoid muscle causes transient discomfort. Immediate and potentially life-threatening allergic reactions to the vaccine could be manifested by wheals, laryngeal edema, asthma, hypotension, etc. These types of reaction are exceedingly rare and would most likely occur in persons with an allergy to eggs and/or a severe reaction to influenza vaccine in the past.

2 Risk Analysis

As presented in the clinical database created during the H5N1 vaccine clinical program, (11) the safety profile of H5N1 vaccine was documented in a total of 403 subjects (Stage I and II) aged 18 to 64 years who received 2 vaccinations (28 days apart) of their assigned vaccine dose level 7.5 µg, 15 µg, 45 µg, or 90 µg. Overall, the H5N1 vaccine was well tolerated and there were no safety concerns in the adult population studied.

As part of the Halting Rule during the study, hematology and blood chemistry were performed before, and at Day 7 following each vaccination, of the Stage I subjects. No laboratory results were graded as severe. White blood cell counts (either low or elevated), low hemoglobin readings and changes in hemoglobin from baseline were among the results temporarily associated with the second vaccination that were graded as mild or moderate in severity.

Within 15 to 30 minutes of any of the 2 vaccinations, at least one immediate reaction (injection site or systemic) was reported by 19.8% (20/101) of subjects who received 7.5 µg H5N1 vaccine, 16.8% (17/101) of subjects who received 15 µg H5N1 vaccine, 9.2% (9/98) of subjects who received 45 µg H5N1 vaccine, and 12.6% (13/103) of subjects who received 90 µg H5N1 vaccine. Most of these reactions were mild to moderate solicited injection site pain and injection site tenderness. Malaise, myalgia, and nausea were the most frequently reported immediate systemic reactions during this period.

During the 7 days following any of the 2 vaccinations, solicited injection site reactions were reported by 46.5% (47/101) of subjects who received 7.5 µg H5N1 vaccine dose, 62.4% (63/101) of subjects who received 15 µg H5N1 vaccine dose, 74.5% (73/98) of subjects who received 45 µg H5N1 vaccine dose, and 82.5% (85/103) of subjects who received 90 µg H5N1 vaccine dose. The injection site reactions tended to be dose related, increasing with higher H5N1 vaccine dose following any vaccination. These reactions were mostly mild to moderate injection site pain and injection site tenderness that occurred, and resolved without sequelae within three days of vaccination.

During the 7 days after any of the 2 vaccinations, solicited systemic reactions were reported by 39.6% (40/101) of subjects who received 7.5 µg H5N1 vaccine dose, 47.5% (48/101) of subjects who received 15 µg H5N1 vaccine dose, 35.7% (35/98) of subjects who received 45 µg H5N1 vaccine dose, and 47.6% (49/103) of subjects who received 90 µg H5N1 vaccine dose. Mild to moderate headache, malaise and myalgia were the most frequently reported solicited systemic reactions.

During the 28 days after each of the 2 vaccinations, at least one unsolicited adverse event (AE) was reported by 58.3% (28/48) of subjects in the placebo group, 42.6% (43/101) of subjects who received 7.5 µg H5N1 vaccine dose, 52.5% (53/101) of subjects who received 15 µg H5N1 vaccine dose, 39.8% (39/98) of subjects who received 45 µg H5N1 vaccine dose and 41.7% (43/103) of subjects who received 90 µg H5N1 vaccine dose.

The most frequent unsolicited AEs reported following any vaccination were in the system organ class (SOC) of gastrointestinal disorders, general disorders and administration site condition, infections and infestations, musculoskeletal and connective tissue disorders, nervous system disorders and respiratory, thoracic, and mediastinal disorders.

After any vaccination, unsolicited AEs classified as severe life-threatening, or death (Grade 3) were rare and showed no trend by dose or number of vaccinations. They were reported by 7 subjects, 3 post-vaccination 1 and 4 post-vaccination 2. Only 2 cases of

reported severe (Grade 3) pharyngitis/infections and infestations (literal term/SOC) occurring 3 days and 4 days, respectively, post-vaccination 1 were classified as associated with vaccination by the investigators. The 2 subjects recovered without sequelae. All other reported unsolicited AEs were classified as not associated with the study vaccination. Three of the remaining 5 subjects also recovered without sequelae.

Two subjects withdrew from the study secondary to adverse events; a case of mild (Grade 1) Maculopapular Rash Abdomen and Upper Arms Bilateral (Literal Term), 5 days after the first placebo 0.9% saline vaccine, and a severe (Grade 3) pharyngitis (Literal Term) that occurred 4 days after the first vaccination of 90 µg H5N1 vaccine and which resulted in the subject missing a day at work. Both subjects recovered without sequelae and the AEs were classified by the investigators as related to the respective vaccine product.

Serious adverse events including 1 death (confirmed by the autopsy to be due to chronic alcoholism) were reported for 4 subjects during the trial. They are SOC reproductive system and breast disorders; neoplasms benign, malignant and unspecified (cysts and polyps); psychiatric disorders, and nervous system disorders reported by one subject each in the placebo group; 15 µg, 45 µg, and 90 µg H5N1 vaccine groups, respectively. The SAEs were all deemed unrelated to the vaccination by the investigators.

Overall, among the study groups that received the H5N1 vaccine, reactogenicity tended to be dose related, increasing with higher vaccine dose following any vaccination. Most of these reported reactions were of mild to moderate severity. They occurred and resolved without sequelae within 3 to 7 days of vaccination.

3 Benefits Analysis

The only clinical study, FUG01 (11) in this submission generated relevant immunogenicity data in support of the beneficial effects of the H5N1 vaccine in adult population aged 18 to 64 years.

The main criterion for evaluating immunogenicity was the proportion of subjects with ≥ 4-fold rise in HAI titers against the H5N1 virus on Day 28 following each vaccination and at 6 months post vaccination 2 compared to the baseline.

The post-vaccination 1 and post-vaccination 2 titers and fold rises tended to be dose related with the highest values reported in subjects who received 90 µg H5N1 vaccine dose and the least value in subjects who received 7.5 µg H5N1 vaccine dose. Furthermore, at 6 months post-vaccination 2, the proportion maintaining a titer ≥ 1:40 and fold rises were still elevated in the subjects who received either 45 µg or 90 µg H5N1 vaccine dose.

A four-fold increase in HAI was achieved in a dose dependant manner with a maximum seroconversion rate of 45.1% one month after the second injection in subjects who received 90 µg H5N1 vaccine dose. This represents an almost 50% increase over the initial fold-rise 28 days post-vaccination 1. The four-fold increase was sustained in less than half of the subjects initially seroconverting after six months after the two vaccinations. Seroprotection rates followed a similar trend to seroconversion with a maximal effect achieved at 90 µg H5N1 vaccine dose resulting in a 46.2% response rate. At 28 days post-vaccination 2, 41.2% and 59.3 % of subjects receiving the 45 µg and 90 µg H5N1 vaccine doses, respectively, achieved a greater than 2-fold rise, while at seven months from baseline, 22.9% and 18.7% of subjects still had titers ≥ 1:40 in the 45 µg and 90 µg groups, respectively.

The HAI titers of majority of the subjects who received the lower dosages of H5N1 vaccine dose, returned to their baseline (pre-vaccination 1) values, however, 30.8% (28/91) and 17.6% (16/91) of subjects who received the 90 µg H5N1 vaccine dose, respectively, still had 2-fold and 4-fold rises, respectively, at 6 months post-vaccination 2.

Furthermore, significant increases in GMTs were noted one month after the first and second vaccination, respectively. The increase tended to be dose related with the highest values reported in the subjects who received 90 µg H5N1 vaccine dose and the least value in the subjects who received 7.5 µg H5N1 vaccine dose. At 6 months post-vaccination 2, the GMTs in all subjects had declined to the post-vaccination 1 levels.

These parameters demonstrated that the H5N1 vaccine:

1. is immunogenic in the population of adults aged 18 to 64 years
2. has greater immunogenicity at a dose level of 90 µg resulting in significant HAI titers, seroconversion, and antibody persistence at 6 months post-vaccination.

4 Conclusion

The safety of higher than usual doses of inactivated influenza vaccines using purified HA or split-virus vaccine has been confirmed in a number of studies. Doses up to 405 µg of HA were well tolerated when given to healthy younger adults, and doses up to 180 µg of HA were well tolerated when administered to ambulatory subjects who were at least 65 years old. (12) (13) (14) (15) Although higher dose levels were associated with a higher rate of injection site discomfort, there was no increase in the frequency of systemic symptoms. Higher dose levels elicited higher levels of serum hemagglutination inhibition assay (HAI) and neutralizing antibody levels: mean serum antibody titers, and the frequencies of significant titer rises, increased 2- to 3-fold with a 9-fold increase in dose. These studies provide reassurance that the proposed dose level of 90 µg is likely to be safe and well tolerated in the general population.

Our data in healthy adults aged 18 to 64 years demonstrate that the H5N1 vaccine is safe and immunogenic. Antibody responses to this H5N1 vaccine were most pronounced after two vaccinations at the 90 µg dose level wherein approximately 45% of a population of healthy female or male adults could be expected to achieve a clinically significant antibody response. Therefore, the benefits associated with the administration of the 90 µg dose level of the H5N1 vaccine clearly outweigh the minimal risks of experiencing a local reaction or other reversible adverse events following vaccination. No clinically significant adverse events have been identified after a 2-vaccination, 7-month controlled follow-up study in adults aged 18 to 64 years.

5 Additional Studies

5.1 Currently Ongoing Studies

Not Applicable.

5.2 Planned Studies

Not Applicable.

6 Bibliography

1. H5N1 avian influenza: timeline 8 May 2006. Available at: http://www.who.int /csr/disease/avian_influenza/timeline.pdf. Accessed September 13, 2006.
2. Hien TT, Liem NT, Dung NT, et al.: Avian influenza A (H5N1) in 10 patients in Vietnam. N Engl J Med 2004;350:1179.
3. Li KS, Guan Y, Wang J, et al.: Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 2004;430:209-213.
4. de Jong MD, Tran TT, Truong HK, et al.: Oseltamivir resistance during treatment of influenza A (H5N1) infection. N Engl J Med 2005;353:2667-2672.
5. Ungchusak K, Auewarakul P, Dowell SF, et al.: Probable person-to-person transmission of avian influenza A (H5N1). N Engl J Med 2005;352:333-340.
6. WHO: Avian Influenza-Situation in Indonesia: Update 14. Available at: http://www.who.int/csr/don/2006_05_23/en/ Accessed 20 September 2006.
7. Latest Information on Human Avian Influenza Cases: Western Pacific Regional Office of the WHO. Avian Influenza Update Number 60 (12 September 2006). Available at: http://www.who.int/csr/disease/avian_influenza/. Accessed September 13, 2006.
8. WHO Global Influenza Surveillance Network/WHO Collaborating Centres and Reference Laboratories involved in annual influenza vaccine composition recommendations. Available at: http://www.who.int/csr/disease/influenza /surveillance/en/index.html. Accessed September 13, 2006.
9. Bresson J, Perronne C, Launay O, et al.: Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet. 2006;367:1657-1664.
10. Influenza vaccines. WHO position paper. Weekly Epidemiological Record. 2002; 77:230–238. Available at: http://www.who.int/wer/pdf/2002/wer7728.pdf. Accessed September 15, 2006.
11. Sanofi Pasteur, Inc. iCSR of Study FUG01. 12 September 2006.
12. Keitel WA, Couch RB, Cate TR, et al.: High doses of purified influenza A virus hemagglutinin significantly augment serum and nasal secretion antibody responses in healthy young adults. J Clin Microbiol. 1994;32:2468-2473.
13. Keitel WA, Cate TR, Atmar RL, et al.: Increasing doses of purified influenza virus hemagglutinin and subvirion vaccines enhance antibody responses in the elderly. Clin Diag Lab Immunol. 1996;3:507-510.
14. Ruben FL, Potter CW, Stuart-Harris CH. Humoral and secretory antibody responses to immunization with low and high dosage split influenza virus vaccine. Arch Virol. 1975;47:157-166.
15. Keitel WA, Atmar RL, Cate TR, et al.: Increasing doses of standard inactivated influenza virus vaccine enhance antibody responses in ambulatory elderly subjects. Presented at the annual Infectious Diseases Society of America meeting held in San Diego, CA, 2003.