



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Biostatistics and Epidemiology
Division of Biostatistics

Statistical Review and Evaluation

BLA STN 125285

BLA/Supplement Number: BLA STN 125285/0

Product Name: Flublok

Indication(s): Active immunization against disease caused by influenza virus subtypes A and type B contained in the vaccine

Applicant: Protein Sciences Corporation

Date(s): Submission Date: 4/18/2008
Action Due Date: 1/16/2013

Review Priority: Standard

Statistical Branch: Vaccine Evaluation Branch (VEB)

Primary Statistical Reviewer: Barbara Krasnicka, Ph.D.
Mathematical Statistician

Through: Tsai-Lien Lin, PhD,
Team Leader, Viral and Bioassay, VEB

A. Dale Horne, Dr. P.H.
Branch Chief, VEB

Medical Office/Division: OVRD/DVRPA

Clinical Reviewer(s): Cynthia Nolletti, M.D.

Project Manager: Timothy Fritz, Ph.D. (Chair),
Helen Gemignani (RPM),
Kristina Carroll, Ph.D. (RPM)

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1. EXECUTIVE SUMMARY

1.1 Introduction

The Biologics License Application STN BL 125285/0 was submitted on April 18th, 2008, by Protein Sciences Corporation for licensing of Flublok® (Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine) for active immunization of adults for prevention of influenza.

Trivalent Inactivated Influenza Vaccines (TIVs) are typically manufactured in embryonated hens' eggs. Virions are harvested from the egg allantoic fluid, chemically inactivated and treated with detergent, and the hemagglutinin (HA) and neuraminidase (NA) proteins are partially purified, but Flublok is a recombinant hemagglutinin influenza vaccine produced using the baculovirus expression vector system.

The Flublok vaccine used in the clinical studies contained 45 micrograms (µg) of recombinant influenza hemagglutinin (rHA) representing each of the following 3 seasonal influenza strains: H1N1, H3N2, and B, for a total of 135 µg of rHA per dose.

1.2 Brief Overview of Clinical Studies

The license application for Flublok vaccine included safety and immunogenicity data obtained from one supplemental and three pivotal clinical studies. A summary of the studies carried out is given in Table 1.

Table 1: General summary of submitted studies

Study Number	Location	Objectives	Design	Vaccine	# of Subjects	Population
PSC04	US	Efficacy, safety consistency of 3 lots, and immunogenicity	Double-Blind Randomized, Controlled, Phase III Multi-center	Flublok	2344	Healthy Subjects 18-49y old
				Placebo	2304	
PSC06	US	Efficacy, safety and immunogenicity	Double-Blind, Randomized, Active Controlled, Phase III Multi-center	Flublok	300	Healthy Subjects 50-64y old
				Fluzone	302	
PSC03	US	Safety and immunogenicity	Double-Blind Randomized, Active Controlled, Phase III Multi-center	Flublok	2649	Healthy Subjects 64 yrs or older
				Fluzone	875	
PSC01	US	Safety, immunogenicity, dose escalation	Double-Blind Randomized Controlled Multi-center	Flublok (75)	153	Healthy Subjects 18-49y old
				Flublok (135)	153	
				Placebo	154	

Source: Reviewer's Analysis

1.3 Regulatory History

The BLA was submitted on April 18th, 2008. A Complete Response (CR letter) was issued by the FDA to Protein Sciences Corporation (PSC) on August 29th, 2008, for CMC (Chemistry, Manufacturing, and Controls), Clinical, and Statistical issues. The major statistical issues were related to over 10% missing immunogenicity data for study PSC04 and unexplained variability in the GMTs for all strains by lot and by study. The applicant subsequently submitted responses to this CR letter under amendments 12, 13, and 15 in April, 2009. In the responses, the applicant provided additional clinical efficacy and safety data.

Studies submitted to this BLA in support of Flublok® were conducted in the U.S. under IND 11951.

1.4 Conclusions and Major Statistical Issues

The objective of this BLA submission was to provide evidence that Flublok can be used for active immunization against disease caused by influenza virus subtypes A and B contained in the vaccine. Data, from four clinical trials on safety and efficacy, for surrogate endpoints and the clinical endpoint (prevention of culture-confirmed influenza illness) were supplied to support licensure.

The pre-specified criterion for the efficacy hypothesis was not reached, but the efficacy result was likely influenced by a poor match of the vaccine strains to the viral strains circulating in the 2007-2008 influenza season. It appears that three investigated lots did not meet the pre-defined criteria for lot-to-lot consistency (study PSC04). For pair-wise comparisons of lots, the 95% CI of the ratios of post-vaccination GMTs, for each viral strain in Flublok, should be entirely within the interval (0.67, 1.5). However, for the A/Wisconsin (H3N2) strain, the confidence limits of the GMT ratios are in the range 0.56 to 2.93.

Additionally, the following are to be noted:

- ✓ For all three pivotal studies, the assessments of immunogenicity endpoints were based on the hemagglutination inhibition (HI) antibody level measured by HAI assay utilizing BEVS (baculovirus expression vector system) derived antigens. However, the study which evaluated comparability of the HAI assay against the baculovirus-derived rHA antigens versus egg-derived antigens (prepared from partially purified influenza virus and traditionally used in HAI assay) had some limitations.
- ✓ Because HAI assay using BEVs antigen tends to give substantially higher titer values than for assays using egg-derived antigen, it is difficult to interpret

immunogenicity data and to bridge immunogenicity data from the older adult studies to the clinical efficacy data in adults 18 through 49 years of age.

- ✓ The safety database for subjects ≥ 50 years of age contains about 730 Flublok vaccinees.
- ✓ For study PSC04, over 10% of missing serology data were “recovered” many months after the serology dataset had been locked.

Based on the data and descriptive statistical analyses submitted, no unusual trends, patterns, or safety signals were detected.

2. INTRODUCTION

2.1 Background Information

Flublok is a recombinant hemagglutinin influenza vaccine indicated for active immunization of adults 18 years and older against influenza disease caused by influenza virus subtypes A and B represented in the vaccine. Flublok® utilizes a novel baculovirus / Lepidopteran (*Spodoptera frugiperda*) insect cell line expression system (expresSF+®) to produce recombinant influenza virus hemagglutinin (HA). Baculovirus-expression vector systems (BEVS) and Sf9 cell culture allow the production of recombinant proteins for medical and therapeutic purposes. Eggs are not used for manufacturing Flublok and, therefore, this vaccine may be administered to egg-allergic individuals.

To support licensure, the applicant submitted (to the BLA), Clinical Study Reports for four clinical studies, PSC01, PSC03, PSC04, and PSC06, with relevant datasets. These studies had the following objectives:

- Demonstration of vaccine efficacy based on clinical and surrogate endpoints
- Demonstration of safety as compared to Fluzone and Placebo.
- Demonstration of lot-to-lot consistency.

2.2 Data Sources

The clinical study reports (CSRs) as well as other related materials were provided by the applicant at the time of the BLA STN 125285/0 submission on 04/18/2008. Useable SAS datasets were submitted to the Agency on 05/20/2008. Efficacy datasets, updated datasets for the PSC04 study, and some datasets for study PSC06 were sent to CBER on April 8th, 2009. These various supplied SAS datasets (with proper documentations) were used for verification of the applicant’s results by the statistical reviewer, who also performed independent statistical analyses.

2.3 Material Reviewed

This statistical review is based on the clinical study reports (three pivotal studies and one supportive study), and datasets. The key materials include:

- ✓ STN 125285/0; Module 1 Volume 1; administrative information, labeling
- ✓ STN 125285/0; Module 5 Volumes 1-32; the interim clinical study reports for studies PSC04 and PSC06, and the final report for study PSC03
- ✓ STN 125285/0.2; Final protocols and SAPs for each of the three studies
- ✓ STN 125285/0.3; Studies clinical datasets
- ✓ SSTN 125285/0.12, 0.13, 015; Complete Responses, datasets and final CSRs.

3. STATISTICAL EVALUATION OF IMMUNOGENICITY DATA

3.0 List of Studies

The clinical development program for Flublok was focused primarily on three adult age categories, namely, individuals 18-49 years of age (PSC01 and PSC04), 50-64 years (PSC06), and ≥ 65 years (PSC03).

Effectiveness of Flublok was evaluated based on the immunogenicity data collected during the following clinical trials:

- ✓ PSC01 (2004-2005): a Phase 2 clinical trial to assess dose of vaccine, safety, immunogenicity, and efficacy of Flublok.
- ✓ PSC03 (2006-2007): a comparative clinical trial of the safety and immunogenicity of Flublok versus Fluzone in healthy adults age 65 and older.
- ✓ PSC04 (2007-2008): a clinical trial to evaluate the efficacy, immunogenicity, and safety of Flublok in healthy adults aged 18 to 49.
- ✓ PSC06 (2007-2008): a comparative clinical trial of the safety and immunogenicity of Flublok versus Fluzone in healthy adults 50-64 years of age.

Data on lot-to-lot consistency were supplied by study PSC04.

3.1 Study PSC04

Title of the study: “*Evaluation of the Immunogenicity, Safety, Reactogenicity, Efficacy, Effectiveness and Lot Consistency of Flublok™ Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine in Healthy Adults Aged 18 to 49.*”

Study Period: September 2007 – May 28, 2008

Treatment:

- ✓ Flublok – single dose of 45µg of the following three rHAs:
 - 1) A/Solomon Islands/3/06 (H1N1)
 - 2) A/Wisconsin/67/05 (H3N2)
 - 3) B/Malaysia/2506/04 (B/Victoria)Total dose 135µg.
- ✓ Placebo.

3.1.1 Brief Overview of the Study

Study design

Study PSC04 was a Phase III, multi-center (24 clinical centers), double-blind, and controlled study with the objective to evaluate the efficacy, immunogenicity, safety, reactogenicity, and lot-to-lot consistency of Flublok™ influenza vaccine in healthy adults aged 18 to 49. In total, 4648 subjects were enrolled into the study and randomized into two groups. Subjects received either a single dose of Flublok vaccine (135 µg of rHA₀; 2344 subjects (50.43%)) or placebo (0.5 ml of normal saline; 2304 subjects (49.57%)) administered by intramuscular injection into the deltoid of the non-dominant arm. Enrollment was stratified according to whether subjects received an influenza vaccine during the 2006-07 season or not. Furthermore, subjects in the Flublok group were randomized at a 1:1:1 ratio to receive vaccine either from lot A, lot B, or lot C.

Subjects participated in the study for up to nine months from the moment of vaccination. For a subset of subjects selected at five sites, serology was collected at baseline (Visit 1, before vaccination) and about 28 days after vaccination at Visit 2.

The scheduled follow-up contacts by telephone took place at Days 7 (to collect reactogenicity events) and 28 after vaccination (except for the serology subset from the five sites for which contacts were made in a slightly different way) with the objectives to collect data on concomitant medications, adverse events (AEs), and any changes in health status. At the end of the influenza season (EOIS), final calls were made to record serious AEs (SAEs) and concomitant medications, and to review Flu Symptoms Cards.

Medical evaluations were performed only for some enrolled subjects who required special attention. During the influenza season, subjects maintained Flu Symptoms Cards that were reviewed during telephone calls made by the study site staff.

Subjects who experienced one or more symptoms of influenza like illness (ILI) were to call the clinic and subsequently NS/TS (Nasal Swab/Throat Swabs) were collected for isolation of influenza viruses in cell cultures.

For the immunogenicity component of the study, serology samples were collected at Visit 1 and 2 at the following sites selected by the applicant: #1 - Rochester, NY; #6 - Houston, TX; #11 - St. Louis, MO; #13 - Los Angeles, CA, and #25 - Salt Lake City, UT. Blood

samples (10 mL) were collected from all subjects at these sites. An unblinded staff member at each site segregated serum samples according to the treatment group (Flublok or Placebo) and sent both sets of samples to a central laboratory.

Study Objectives

Primary Objectives:

- ✓ Safety – to determine the rate and severity of solicited and unsolicited adverse events (AEs), and of serious adverse events (SAEs) associated with vaccination
- ✓ Lot consistency
- ✓ Efficacy: to determine the efficacy of Flublok relative to Placebo in the prevention of culture-confirmed influenza.

Secondary Objectives:

- ✓ Immunogenicity: to evaluate the immunogenicity of each vaccine strain.

Study Endpoints

Primary endpoints:

1. Efficacy: Development of an ILI (defined by the CDC) with a positive viral culture for an influenza strain antigenically resembling a strain represented in Flublok.
2. Lot-to-lot consistency: Ratios of post-vaccination GMTs (Lot A vs. B, Lot B vs. C, and Lot A vs. C) for each strain contained in Flublok.
3. Safety: Rates of solicited SAEs reported within seven days after vaccination, all AEs reported within 28 days after vaccination, and all SAEs reported for the duration of the study.

Secondary endpoints:

1. Immunogenicity: For each (influenza) strain represented in Flublok:
 - a. Seroconversion rate
 - b. Seroprotection rate
 - c. Geometric Mean Titer (GMT) collected at Day 28 post-vaccination visit.
2. Frequencies of AEs and SAEs reported from clinics, found via memory aids and phone calls, and during physical examination(s).

For the definitions of seroconversion and seroprotection rates, please see Section 3.1.2, page 16 and 17 of this review.

Hypotheses and sample size considerations

Primary efficacy hypotheses: The efficacy of Flublok vaccine against culture-confirmed CDC-ILI due to influenza strains antigenically resembling the vaccine strains will exceed 40% ($\delta = 0.4$).

In formal form, hypotheses were:

$$\begin{aligned}H_0: VE &\leq \delta \\ H_a: VE &> \delta,\end{aligned}$$

where VE is vaccine efficacy defined as $VE = 1 - \pi_v/\pi_c$ (π_v and π_c are proportions of subjects getting disease in the Flublok and placebo groups, respectively), and the following assumptions are to hold:

$$\begin{aligned}\alpha \text{ (Type I error)} &= 0.025 \\ \beta \text{ (Type II error)} &= 0.20 \\ \delta_0 &= 0.4, \text{ one-sided test.}\end{aligned}$$

Assuming that $P_{\text{observed proportion getting disease in placebo group}} = 0.03$, $VE_{\text{observed/assumed}} = 0.7$, and the above listed assumptions hold, about 2325 subjects per group should supply over 80% power to demonstrate that the lower confidence limit for VE is greater than 40% at the alpha level 0.025.

Data needed for testing the primary efficacy hypotheses were included in the ‘Responses to Complete Response Letter’ submission.

Primary immunogenicity hypotheses (lot-to-lot consistency of immune response):

Primary immunogenicity hypotheses constitute equivalence tests of lots for each strain. Formally, lot-to-lot hypotheses were formulated as follows:

$$H_0: \phi_{ij} \leq 0.67, \text{ or } \phi_{ij} \geq 1.5$$

$$H_a: 0.67 < \phi_{ij} < 1.5 \text{ for all combinations of } i \neq j \text{ and for all strains,}$$

where $\phi_{ij} = \mu_i/\mu_j$ and μ_i, μ_j are GMT values (for Day 28) for the i^{th} and j^{th} lots, respectively.

3.1.2 Evaluation of Study Immunogenicity Results

The study results presented by the applicant in the submission are based on the interim statistical analysis of data pertaining to the Day 28 safety and immunogenicity objectives.

Disposition of Subjects

The disposition of subjects through Day 28 is summarized in Table 2, which is based on the applicant’s Table 4 (Clinical Study Report (Study PSC04), page 56).

Table 2: Disposition of subjects through Day 28 Contact

Disposition	# of subjects Placebo	# of subjects Flublok	# of subjects Flublok Serology Subset
Randomized	2325	2323	480
Vaccinated	2304	2344	480
Completed	2022 (88%)	2049 (87%)	402 (84%)
Discontinued	282 (12%)	295 (13%)	78 (16%)
Due to AE	3 (<1%)	3 (<1%)	0
Lost to follow-up	251 (11%)	295 (13%)	73 (15%)
Withdrew consent	14 (1%)	22 (1%)	5 (1%)
Death	1 (<1%)	1 (<1%)	0
Other reasons	13 (1%)	9 (<1%)	0
Safety Population	2304	2344	

Source: Table 4 on Page 56 in the applicant's CSR for PSC04

In total, 4648 subjects were randomized and vaccinated (100%) and 4071 (88%) subjects completed study procedures through Day 28. Due to a randomization error that occurred at one site, there was no full balance between the treatment groups with respect to the number of vaccinated subjects (2344 subjects were vaccinated with Flublok and 2304 received placebo). As of the Day 28 contact, 577 (12%) subjects were counted as discontinued. The most common reason for discontinuation was loss to follow-up (511 subjects, (11%)). The applicant stated that there were no discontinuations due to AEs (Clinical Study Report, page 55). However, this statement may not be correct; please see the paragraph on safety.

There were no noticeable differences with respect to demographic baseline characteristics among the Flublok group, serology subset, and placebo group of subjects. White subjects constituted 67% and 66% of Flublok and placebo groups, respectively, while females represented 59% of subjects in both groups. The mean age was about 33 years in both groups, and the age range was from 18 to 55 years. At the time of enrollment, four subjects provided an incorrect birth date. Due to this reason, the maximum ages in both study groups were greater than the per protocol age maximum of 49 years.

Subjects were enrolled at 24 sites. On average, 194 subjects were enrolled per site (median 201, standard deviation 81, and range from 50 to 300).

Protocol Deviations

As per the applicant's report, altogether there were 141 protocol deviations. The main violations were: randomization errors (47 subjects), Day 28 visit outside the prescribed time window (16 subjects), and 'not met' inclusion and exclusion criteria (24 subjects).

REVIEWER'S COMMENT

Datasets that constituted bases for the Final Study Report (FSR) and Interim Study Report (ISR) were supplied to the Agency about one year apart. Please note that the ISR should include

- ✓ “complete baseline and 28-day post-vaccination immunogenicity data for all primary, secondary and exploratory immunogenicity endpoints”
- ✓ “7 day post-vaccination reactogenicity data, 28 day unsolicited and/or treatment-emergent adverse event data, and SAE data collected through December 14, 2007.”

The applicant did not submit the relevant data within the frame of the ISR at the time of the BLA submission. It appears that, in preparation for the final immunogenicity and one month safety statistical analysis, the applicant did not carefully check completeness of the dataset. The complete immunogenicity dataset for all primary, secondary, and exploratory immunogenicity endpoints was submitted almost one year later. Due to the long time interval between the two steps of the final immunogenicity dataset preparation, it is unclear whether bias could have been introduced into the study results. Therefore, to examine potential bias, the statistical analyses based on both the ISR and FSR datasets are presented in this statistical review.

Efficacy and Immunogenicity results

I. Primary efficacy hypotheses

A total of 646 swabs from 583 subjects were obtained during the study. Swabs were taken during the 180-day period from subjects with a score of 2 or more on their Flu Symptoms Card. For a total of 178 subjects (64 Flublok and 114 placebo recipients), swabs led to positive cultures of influenza. However, only one (0.04%) and four (0.02%) cases of influenza caused by strains antigenically resembling the vaccine strains and confirmed by positive cultures, were recorded in Flublok and Placebo groups, respectively.

The efficacy of Flublok vaccine was evaluated by utilizing proportions of culture-confirmed CDC-ILI due to influenza strains antigenically resembling the vaccine strains. The vaccine was defined to be efficacious when the lower limit of the 95% CI of VE exceeds 0.4 (40%). Based on the data, the point estimate of VE (vaccine efficacy) for Flublok was 0.755 with 95% CI (- 1.2, 0.97). However, please note that study PSC04 was conducted during seasons when the vaccine strains and the circulating strains were poorly matched and only a small number of influenza cases could be recorded.

In the Final Clinical Study Report, in order to support vaccine clinical efficacy, the applicant considered additionally other types of influenza cases such as: (1) cell culture-confirmed symptomatic influenza (regardless of CDC-ILI) due to strains represented in the vaccine, (2) culture-confirmed, symptomatic influenza satisfying the definition of CDC-ILI due to any strain of influenza regardless of whether the strain was represented in the vaccine, (3) CDC-ILI, regardless of culture results.

As 64 (2.7%) and 114 (4.9%) subjects met this broader definition of the efficacy endpoint in the Flublok and Placebo groups, respectively, the point estimates of vaccine efficacy against culture-positive ILI for all strains regardless of antigenic match was

44%. The lower limit of the 95% CI for VE against culture-positive ILI for all strains regardless of antigenic match was 24.4%.

REVIEWER'S COMMENTS:

The pre-specified criterion for the primary efficacy hypothesis was not technically met. However, this result could be due to the poor match of vaccine strains to viral strains circulating in the 2007-2008 influenza season and by the small number of recorded influenza cases. Based on the secondary and post-hoc analyses, Flublok showed some trend of efficacy.

II. Lot-to-lot consistency

The primary immunogenicity hypotheses are related to clinical lot-to-lot consistency. To support the hypotheses, the applicant should demonstrate that vaccines drawn from three vaccine lots -- Lot A (50-07010), Lot B (50-07011), and Lot C (50-07014) -- elicited equivalent immune responses. For pair-wise comparisons, the 95% CI of the ratios of post-vaccination GMTs, for each viral strain in Flublok, should be entirely within the interval (0.67, 1.5). A summary of the results for lot-to-lot consistency is presented in Table 3 and Table 4.

Results based on the interim database (n = 393 subjects)

Table 3: Day 28 lot-to-lot consistency results; based on the unadjusted statistical analyses and the interim database

Estimation of GMTs (95% CI) per Lot

Strain	Lot A (N=132) Estimated GMT (95% CI)	Lot B (N=131) Estimated GMT (95% CI)	Lot C (N=130) Estimated GMT (95% CI)
H1N1	351.72 (293, 422)	344.61 (289, 411)	393.97 (328, 473)
B	174.024 (143, 212)	197.714 (166, 236)	205.567 (168, 251)
H3N2	396.873 (327, 482)	178.804 (147, 218)	241.225 (197, 295)

Source: Reviewer's Analysis

Estimation of GMTs ratios (95% CI)

Strain	Lot A vs. Lot B	Lot A vs. Lot C	Lot B vs. Lot C
H1N1	1.021 (0.79, 1.31)	0.893 (0.69, 1.15)	0.875 (0.68, 1.13)
B	0.88 (0.68, 1.15)	0.847 (0.64, 1.12)	0.962 (0.74, 1.25)
H3N2	2.220 (1.68, 2.93)	1.645 (1.25, 2.170)	0.741 (0.56, 0.98)

Source: Reviewer's Analysis

Results based on the final database (n = 449 subjects; one subject did not have baseline titer but had Day 28 titer)

Table 4: Day 28 lot-to-lot consistency results; based on the unadjusted statistical analyses and the final database

Estimation of GMTs (95% CI) per Lot

Strain	Lot A (N=151) Estimated GMT (95% CI)	Lot B (N=151) Estimated GMT (95% CI)	Lot C (N=147) Estimated GMT (95% CI)
H1N1	345.97 (292, 411)	322.95 (274, 380)	380.99 (321, 452)
B	182.78 (153, 219)	205.95 (173, 244)	215.34 (179, 259)
H3N2	389.83 (324, 469)	192.25 (1159, 232)	240.01 (200, 288)

Source: Reviewer's Analysis

Estimation of GMTs ratios (95% CI)

Strain	Lot A vs. Lot B	Lot A vs. Lot C	Lot B vs. Lot C
H1N1	1.07 (0.85, 1.35)	0.91 (0.71, 1.16)	0.85 (0.67, 1.07)
B	0.89 (0.69, 1.14)	0.85 (0.66, 1.10)	0.96 (0.75, 1.23)
H3N2	2.03 (1.56, 2.63)	1.62 (1.25, 2.10)	0.80 (0.62, 1.04)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

As per data of clinical study PSC04, three investigated lots did not achieve the pre-defined criteria for lot-to-lot consistency. The criteria required that the 95% CI of the ratio of post-vaccination GMTs, for each viral strain in Flublok, should be entirely within the interval (0.67, 1.5). Especially for the A/Wisconsin (H3N2) strain, the confidence limits of the GMT ratios are in the range 0.56 to 2.93 (based on the interim analyses). Similar results were obtained based on the final study datasets.

Please note that the lot-to-lot consistency results reflect the variability of the means of titers for selected lots of vaccine. The variability between lots is mainly caused by random changes in the manufacturing processes but is also due to subject-to-subject and assay-to-assay variability. Analyses/calculations based on ANOVA (Analysis Of Variance) showed that the variability between lots in study PSC04 was especially noticeable for the H3N2 strain.

II. Secondary immunogenicity hypotheses and exploratory analyses

Secondary immunogenicity endpoints in study PSC04 were related to seroconversion and seroprotection rates.

Seroconversion

Seroconversion rate was defined as the proportion of subjects who had:

- At least four-fold increase in HI antibody titer at Day 28 relative to baseline, for subjects with baseline titer $\geq 1:10$,
- or
- Day 28 minimum titer of 1:40, for subjects with undetectable baseline antibody (HI titer = $<1:10$).

A summary of the seroconversion results, based on both (ISR and FSR) datasets, is given in Table 5.

Table 5: Seroconversion rates at Day 28 Visit in Evaluable Immunogenicity Subset

Strain	Seroconversion rate Flublok (ISR Dataset, N=391) Estimated Endpoint (%) (95% CI)	Seroconversion rate Flublok (FSR Dataset, N=448) Estimated Endpoint (%) (95% CI)
H1N1	78.26 (74, 82)	77.68 (74, 82)
H3N2	80.56 (76, 84)	81.03 (77, 85)
B	53.2 (48, 58)	51.34 (47, 56)

Source: Reviewer's Analysis

As shown in Table 5, the lower limits of the 2-sided 95% CIs of the seroconversion rates, for three antigens, exceeded 40% for both (ISR and FSR) datasets.

Seroprotection

Seroprotection rate was defined as the percentage of subjects achieving an HI antibody titer ≥ 40 measured by HAI assay that used BEVS (baculovirus expression vector system) derived HA antigens.

The statistical analyses related to seroprotection showed (table not shown here) that the lower limits of the 2-sided 95% CIs of the seroprotection rates for all three antigens exceeded 90% for both the ISR and FSR datasets. Therefore, it is apparent that the lower limits of the 2-sided 95% CIs of the seroprotection rates for all three antigens exceeded the seroprotection criterion of $>70\%$ for both the ISR and FSR datasets.

Geometric Mean Titers (GMTs)

The immunogenicity results with respect to the HI antibody responses for subjects in the immunogenicity subset with serology at baseline and at Day 28 are presented in Table 6.

Table 6: Statistical Results for HI Antibody Responses at Day 28 Post-vaccination based on the FSR dataset (N=448 subjects)

Strain	Estimated Pre-vaccination GTM (95% CI)	Estimated Day 28 GTM (95% CI)	Estimated GMFR from Pre-vac (95% CI)
H1N1	31.91 (28, 36)	348.96 (317, 384)	10.94 (9, 12)
H3N2	22.92 (21, 25)	262.1 (235, 292)	11.44 (10, 13)
B	55.27 (49, 62)	200.55 (181, 222)	3.63 (3.2, 4.1)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

Based on Table 6, in the case of the FSR dataset, the GMTs increased relative to baseline 10.94-fold, 11.44-fold, and 3.63-fold, for H1N1, H3N2, and B, respectively, and the results are similar for the ISR dataset. After vaccination, an increase of GMTs was evident for each strain.

GMT fold-increases, based on the FSR dataset, from baseline to Day 28 by strain and lot are presented in Table 7.

Table 7: GMT fold-increases from baseline to Day 28 by lot

Strain	Lot A	Lot B	Lot C
H1N1	9.27 (7.27, 11.83)	10.98 (8.61, 14.00)	12.88 (10.07, 16.47)
B	3.26 (2.64, 4.04)	3.91 (3.16, 4.83)	3.74 (3.02, 4.64)
H3N2	16.22 (13.12, 20.06)	8.38 (6.78, 10.35)	11.02 (8.90, 13.66)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

There is an indication that the immune responses, as measured by GMFR, are dependent on the lot. Especially, there are noticeable differences in the GMFR estimations for the H3N2 strain.

3.1.3 Site, Gender, Race, and Other Special/Subgroup Populations

Exploratory analysis of GMTs and GMFR per site

GMTs at Day 28 and GMT fold-increases (GMFR) from baseline to Day 28 by strain and site are shown in Table 8.

Table 8: GMTs at Day 28 and GMT fold-increases from baseline to Day 28 by strain and per site

Estimation of GMTs

Strain	Site 1 (N=109)	Site 2 (N=62)	Site 3 (N=51)	Site 4 (N=128)	Site 5 (N=98)
H1N1	349 (288,425)	331 (255, 429)	403 (303, 537)	305 (254, 365)	399 (324, 490)
B	172 (140, 211)	219 (166, 288)	283 (209, 383)	202 (167, 245)	187 (150, 233)
H3N2	227 (182, 283)	306 (228, 410)	295 (213, 408)	220 (180, 270)	329 (261, 416)

Source: Reviewer's Analysis

Estimation of GMFR

Strain	Site 1 (N=109)	Site 2 (N=62)	Site 3 (N=51)	Site 4 (N=128)	Site 5 (N=98)
H1N1	9.44 (7, 13)	11.19 (8, 16)	16.44 (11, 25)	10.89 (8, 14)	10.32 (8, 14)
B	3.06 (2, 4)	3.38 (3, 5)	10.5 (7, 15)	4.15 (3, 5)	2.21 (1.7, 3)
H3N2	8.97 (7, 12)	12.94 (9, 18)	17.13 (12, 25)	10.43 (8, 13)	12.94 (10, 17)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

As per Table 8, it appears that estimated GMTs and GMFRs for each site are dissimilar. It can be observed that for site 3 estimated GMTs and GMFRs are always higher than for other sites. Reasons for these differences are not clear. However, it appears that sera may not have been assigned to assay runs at random. The variability between sites may be caused not only by site characteristics (population, etc) but could be also due to assay-to-assay variability. Additionally, please note that these analyses are only exploratory.

Exploratory analysis of GMTs per stratum (vaccination in the previous season)

A comparison of changes of pre- and post-vaccination GMTs for subjects vaccinated and not vaccinated in the previous season is given in Table 9. This table is based only on the ISR dataset.

Table 9: Comparison of GMTs for subjects not vaccinated and vaccinated during the previous season

For H1N2 strain

Endpoint	Time Point Related to Vaccination	Not vaccinated in the previous season (N=308) Estimated Endpoint (95% CI)	Vaccinated in the previous season (N=83) Estimated Endpoint (95% CI)	Estimated GMT _{not Vac} /GMT _{Vac} Ratio
GMT	Pre-vaccination	25.79 (20, 30)	63.85 (49, 83)	1.55 (1.21, 1.98)
GMT	Day 28	395.39 (351, 445)	255.4 (211, 310)	
GMFR from Pre-vac	Day28	15.33 (13, 18)	4.0 (3, 5)	

Source: Reviewer's Analysis

For H3N2 strain

Endpoint	Time Point Related to Vaccination	Not vaccinated in the previous season (N=308) Estimated Endpoint (95% CI)	Vaccinated in the previous season (N=83) Estimated Endpoint (95% CI)	Estimated GMT _{not Vac} /GMT _{Vac} Ratio
GMT	Pre-vaccination	18.49 (16, 21)	45.34 (36, 56)	1.58 (1.19, 2.10)
GMT	Day 28	284.02 (248, 325)	179.84 (143, 227)	
GMFR from Pre-vac	Day28	15.36 (13 18)	3.97 (3.12, 5.06)	

Source: Reviewer's Analysis

For B strain

Endpoint	Time Point Related to Vaccination	Not vaccinated in the previous season (N=308) Estimated Endpoint (95% CI)	Vaccinated in the previous season (N=83) Estimated Endpoint (95% CI)	Estimated GMT _{not Vac} /GMT _{Vac} Ratio
GMT	Pre-vaccination	41.94 (37, 48)	93.76 (72, 122)	1.3 (0.99, 1.73)
GMT	Day 28	203.11 (179, 230)	156.04 (125, 195)	
GMFR from Pre-vac	Day28	4.84 (4.16, 5.64)	1.66 (1.39, 2.0)	

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

It is clear from Table 9 that there were differences between two strata with respect to GMTs at baseline and Day 28.

- ✓ At baseline, subjects vaccinated during the previous season had on average higher levels of antibodies than subjects not vaccinated previously.
- ✓ At Day 28, subjects vaccinated during the previous season had statistically lower levels of GMTs than subjects who were not vaccinated previously. As shown in Table 9, the estimated GMT ratios (GMT not vaccinated/GMT vaccinated) were in the range 1.3 to 1.58. It appears that the repeated vaccination produces lower levels of antibody titers in subjects vaccinated in the previous season.

Exploratory analysis of GMTs per gender

GMTs at Day 28 by strain and gender are shown in Table 10.

Table 10: GMTs at Day 28 by strain and gender

Strain	Female (N=242)	Male (N=206)
H1N1	355 (311, 404)	342 (297, 395)
B	201 (175, 232)	200 (172, 232)
H3N2	298 (260, 341)	225 (189, 269)

Source: Reviewer's Analysis

As per Table 10, it appears that estimated GMTs at Day 28 for H1N1 and B strains did not depend on gender, but females had slightly higher GMTs for strain H3N2.

Exploratory analysis of GMTs per race

Day 28 GMTs by strain and race are shown in Table 11.

Table 11: Day 28 GMTs by strain and race

Strain	African-American (N=88)	Asian (N=27)	Latino/Hispanic (N=48)	White/Caucasian (N=285)
H1N1	318 (255, 395)	289 (193, 432)	311 (234, 413)	373 (331, 421)
B	203 (160, 257)	156 (111, 220)	247 (181, 336)	198 (174, 225)
H3N2	230 (180, 293)	312 (203, 480)	258 (194, 342)	269 (234, 310)

Source: Reviewer's Analysis

As per Table 11, it appears that race does not influence the Day 28 GMTs.

3.1.4 Summary of Study PSC04 Immunogenicity Results

In general, the results of study PSC04 demonstrate that the Flublok vaccine elicited an immune response, particularly for the H1 and H3 strains. However, we have the following comments related to assessment of immunogenicity endpoints:

- ✓ For study PSC04, immunogenicity endpoints were assessed using the hemagglutination inhibition (HI) antibody levels measured by HAI assay using BEVS (baculovirus expression vector system) derived antigens. Usually, in the case of immunogenicity assessment for egg derived influenza vaccines, HAI assay using egg derived HA antigens has been used to test sera. Although there is no clearly established immune correlate of protection, the HI response has been considered as an acceptable surrogate marker and the titer (measured by HAI assay against egg-derived antigen) of $\geq 1:40$, suggested by some influenza studies, has been used as a threshold to define the immune response rate for influenza vaccines. The applicant claimed that the HAI assay using BEVS-derived HA antigen produced results in titers that are comparable to the results generated by using egg-derived viral HA in the assay. However, it appears that the method used for showing comparability of the assay using the baculovirus-derived rHA antigens versus egg-derived antigens has limitations.
- ✓ The clinical study results did not provide strong evidence of efficacy. However, the efficacy results could be influenced by a poor match of the vaccine strains to the viral strains circulating in the 2007-2008 influenza season and by a small number of recorded influenza cases.
- ✓ There was noticeable variability in GMTs between lots.
- ✓ No significant numerical differences were noticed across the statistical analyses that were performed, by the reviewer, to assess the impact of the two-step submission of the serology data.

3.2. Study PSC06

Title of the study: “*Evaluation of the Safety, and Reactogenicity of Flublok™, Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine, and Comparison of the Immunogenicity, Efficacy and Effectiveness of Flublok™ to Licensed Egg-Grown Influenza Vaccine in Adults Aged 50 to 64.*”

Study Period: September 25, 2007 – May 30, 2008

Treatment:

- ✓ Flublok: 0.5 mL single dose containing 135µg of rHA (45µg of rHA for each strain) derived from:
 - A/Solomon Islands/03/06 (H1N1)
 - A/Wisconsin/67/05 (H3N2)
 - B/Malaysia/2506/04 (B/Victoria)

- ✓ Fluzone: 0.5 mL single dose containing 45µg of HA (15µg of HA for each strain) derived from:
 - A/Solomon Islands/3/06 (H1N1)
 - A/Wisconsin/67/05 (H3N2)
 - B/Malaysia/2506/04 (B/Victoria).

3.2.1 Brief Overview of the Study

Study design

Study PSC06 was a Phase III, multi-center (six sites), double-blind, and actively controlled clinical trial with the primary objective to compare the immunogenicity, safety, and reactogenicity of Flublok™ and Fluzone in healthy adults 50 to 64 years old.

In total, 602 subjects were randomized into two groups. Subjects in these groups received either Flublok (299 subjects) or Fluzone (302 subjects). Enrollment was stratified according to whether or not subjects received an influenza vaccine during the 2006-07 season. The intervention was a single dose of Flublok vaccine (135 µg of rHA₀) or Fluzone (45 µg of rHA₀) administered by intramuscular injection into the deltoid of the non-dominant arm.

Study Endpoints

Primary endpoints:

1. Frequencies of AEs and SAEs reported from clinics, via memory aids and phone calls, and collected during physical evaluations.
2. Seroprotection rate (see definition in the next section).
3. Seroconversion rate (see definition in the next section).

For each subject, information on AEs and SAEs was collected during the period of 28 days post-vaccination and then throughout the rest of the subject's participation in the study (until the end of the influenza season).

Secondary endpoints:

1. Geometric Mean Titers (GMTs) of serum antibodies against vaccine antigens for each group as measured 28 days after vaccination
2. Differences in seroconversion rates
3. Proportion of subjects in each vaccine group who experienced culture-positive ILI or any ILI during the 2007-2008 influenza epidemic season.

Hypotheses and sample size considerations

Co-primary immunogenicity hypotheses:

I. Seroconversion

Seroconversion rate was defined as the proportion of subjects who had:

- At least four-fold increase in HI antibody titer at Day 28 relative to baseline, for subjects with baseline titer $\geq 1:10$,
- or
- Day 28 minimum titer of 1:40, for subjects with undetectable baseline antibody (HI titer = $<1:10$).

For 3 strain-specific antibody responses to the Flublok vaccine, as measured by HAI assay using BEVS derived antigens, the seroconversion hypotheses were:

$$H_0: \pi_i \leq 0.4$$

$$H_1: \pi_i > 0.4, \text{ for each } i \text{ (H1N1, H3N2 and B) strain,}$$

where π_i is a parameter representing the seroconversion rate for the Flublok vaccination group and the i -th strain.

II. Seroprotection

Seroprotection rate was defined as the percentage of subjects achieving an HI antibody titer ≥ 40 at Day 28.

For 3 strain-specific antibody responses to the Flublok vaccine, as measured by HAI, the seroprotection hypotheses were:

$$H_0: \rho_i \leq 0.7$$

$$H_1: \rho_i > 0.7, \text{ for each } i \text{ (H1N1, H3N2, and B) strain,}$$

where ρ_i is a parameter representing the seroprotection rate for the i -th strain and Flublok group.

Please note that in the protocol, “Sample size consideration” paragraph (page 26), co-primary hypotheses were stated and sample size calculations were performed only for the Flublok group, not for Fluzone group.

Secondary non-inferiority immunogenicity hypotheses

Hypotheses for the immunogenicity comparison between two vaccination groups with respect to 3 strain-specific antibody responses to the influenza vaccine were:

1.

$$H_0: \text{GMT}_1/\text{GMT}_2 \geq 1.5$$

$$H_1: \text{GMT}_1/\text{GMT}_2 < 1.5,$$

where GMT_1 and GMT_2 are the strain-specific GMT parameters for Fluzone and Flublok vaccination groups, respectively.

2.

$$\begin{aligned} H_0: \pi^1 - \pi^2 &\geq 0.1 \\ H_1: \pi^1 - \pi^2 &< 0.1, \end{aligned}$$

where π^1 and π^2 are the parameters for the seroconversion rates for the Fluzone and Flublok vaccination groups, respectively.

3.2.2 Evaluation of Study Immunogenicity Results

The statistical evaluations of the immunogenicity and safety data are based on the interim study (ISR) and final study (FSR) reports that were submitted on April 18th, 2008 and on April 7th, 2009, respectively.

Disposition of Subjects

In total, 602 subjects were randomized and vaccinated. Among them, 601 subjects completed the study procedures through Day 28 and 599 subjects completed the whole study. There were no notable differences with respect to the collected demographic baseline characteristics between the Flublok and Fluzone groups of subjects. White subjects constituted 73% and 70% of the Flublok and Fluzone groups, respectively, while females represented 62% and 64% of subjects in the Flublok and Fluzone groups, respectively. The mean age was about 56 years in both vaccination groups (range 50 to 64 years).

Subjects were enrolled at 6 sites (California and Hawaii). On average, 100 subjects were enrolled per site (median 119, standard deviation 56, range 36 to 159).

Protocol Deviations

As per the applicant's report, there were 18 protocol deviations. Six of them were related to clinic visits occurring outside the protocol-defined time window for respective action. A summary of protocol deviations is presented in Table 12.

Table 12: Summary of protocol deviations

Deviations	# of Subjects Flublok (N=299)	# of Subjects Fluzone (N=301)
Blood collected outside of window	2	4
Day 0 serology missing	1	0
ILI visit outside of window	0	1
Reporting flu symptoms outside of window (no NS/NT)	2	5
Reporting of a flu symptoms; No NS/TS	1	2

Source: Reviewer's Analysis

Immunogenicity results

Primary immunogenicity hypotheses

Seroconversion results

Seroconversion rate and related hypotheses were defined previously (page 24). A summary of the seroconversion results is given in Table 13.

Table 13: Seroconversion rates at Day 28 visit for FluBlock group (N=299)

Strain	Estimated Endpoint (95% CI)
H1N1	72.24 (67, 77)
H3N2	61.2 (56, 67)
B	40.8 (35, 47)

Source: Reviewer's Analysis

REVIEWER'S COMMENT:

Based on Table 13, the point estimate of the seroconversion rate for B/Malaysia is about 40%, but the lower confidence limit is 35% and narrowly missed the 40% threshold. From a statistical perspective, the lower confidence limit determines whether the null hypothesis can be rejected or not.

Seroprotection results

Seroprotection rate and related hypotheses were defined previously (page 24). A summary of seroprotection results is presented in Table 14.

Table 14: Seroprotection rates at Day 28 after vaccination for Flublok group (N=299)

Strain	Estimated Endpoint (95% CI)
H1N1	96.32 (94,98)
H3N2	85.28 (81,89)
B	92.98 (89, 96)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

1. Based on Table 14, it is evident that the lower limits of the 2-sided 95% CIs of the seroprotection rates for all three antigens exceeded the criterion of >70%. These results suggest that this immunogenicity criterion was met for all 3 antigens.
2. Assessment of Flublok effectiveness was based on the co-primary endpoints (seroconversion and seroprotection) for HI antibodies against each viral strain contained in Flublok. Both immune response criteria were exceeded for the H1 and H3 strains. However, the statistical criterion for the seroconversion immunogenicity hypothesis was not quite met for the B strain.

Secondary non-inferiority immunogenicity hypotheses

A. GMT ratio

A summary of the results from the non-inferiority analysis of the GMT ratio for each vaccine antigen at Day 28 is given in Table 15.

Table 15: Statistical analysis of non-inferiority of HI antibody responses at Day 28 post-vaccination

Strain	Flublok Group (N=299) Estimated GMT (95% CI)	Fluzone Group (N=302) Estimated GMT (95% CI)	Estimated GMTs Ratio GMT _{Fluzone} /GMT _{Flublok} (95%CI)
H1N1	181.07 (159, 206.)	139.74 (125, 157)	0.77 (0.65, 0.92)
H3N2	105.10 (91, 122)	60.66 (53, 69)	0.58 (0.47, 0.70)
B	110.85 (100, 123)	115.86 (104, 129)	1.05 (0.90, 1.21)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

As can be concluded from Table 15, the upper limit of the 95% CI for the fold differences (GMT ratios), estimated alone or using a regression model (not presented in this review), are <1.5. This means that the antibody responses to Flublok for the H1N1, H3N2, and B

strains are non-inferior to the responses to Fluzone. The model used for estimations of the GMTs and the GMT ratios contained Pre-vaccination Titer, Stratum (vaccinated or not vaccinated in the previous flu season), and ASSAY covariates.

B. Seroconversion rate

The second non-inferiority analysis pertained to the differences in seroconversion rates and yielded 95% CIs for each vaccine antigen at Day 28. A summary of the results of this analysis is given in Table 16.

Table 16: Non-inferiority of HI antibody responses at Day 28 post-vaccination based on the differences in seroconversion rates

Strain	Flublok Group (N=299) Estimated Seroconversion Rate (95 CI%)	Fluzone Group (N=302) Estimated Seroconversion Rate (95 CI%)	Estimated difference in seroconversion rate
H1N1	72.24	66.23	-6.02 (-13.38, 1.35)
H3N2	40.8	41.06	0.26 (-7.61, 8.12)
B	61.2	43.71	-17.50 (-25.36, -9.63)

Source: Reviewer's Analysis

REVIEWER'S COMMENT:

Based on Table 16, it is evident that the upper limits of the 2-sided 95% CIs for the differences in seroconversion rates for all three antigens do not exceed the criterion of 10%. Thus, based on differences in seroconversion rates, it can be concluded that the antibody responses to Flublok are non-inferior to the responses to Fluzone.

Clinical Efficacy Results

The applicant's relative efficacy analyses were based on the pre-specified secondary endpoints, namely, the proportions of subjects who experienced cell-culture confirmed CDC-ILI or non-CDC-ILI associated with isolation of an influenza virus antigenically resembling a vaccine strain. However, none of the influenza isolates obtained in this study from subjects with either CDC-ILI or non-CDC-ILI respiratory illnesses were antigenically matched to the 2007-2008 vaccine strains. Therefore, the descriptive analyses were not performed.

3.2.3 Site, Gender, Race, and Other Special/Subgroup Populations

ANOVA models with covariate adjustment for Site, Stratum (vaccination in the previous season), pre-vaccination log titers, and Assay (runs) were developed by the reviewer. The models yielded estimations of Day 28 GMTs, GMT ratios, and their 95% CIs obtained were generally close to the unadjusted results. Additional univariate analyses were also

performed by the reviewer to explore the potential influence of these factors on GMT or GMT ratio.

I. GMT ratios per site

A summary of the univariate analyses of the GMT ratios per site is given in Table 17.

Table 17: Summary of statistical analyses of GMT ratios* and their 95% CIs per site

Strain	Site 1 (N=159)	Site 2 (N=101)	Site 3 (N=141)	Site 4 (N=137)	Site 5 (N=28)	Site 6(N=36)
H1N1	0.7 (0.5, 1.0)	1.05 (0.7, 1.6)	0.81 (0.6, 1.1)	0.65 (0.5, 0.9)	0.68 (0.2, 2.0)	0.89 (0.4, 1.8)
B	1.09 (0.8, 1.5)	0.89 (0.6, 1.3)	1.13 (0.9, 1.5)	1.13 (0.8, 1.6)	0.75 (0.3, 1.8)	0.93 (0.6, 1.6)
H3N2	0.49 (0.3, 0.7)	0.69 (0.4, 1.1)	0.52 (0.4, 0.8)	0.53 (0.4, 0.8)	0.62 (0.2, 1.7)	1.33 (0.6, 2.9)

Source: Reviewer's Analysis

*Day 28 post-vaccination estimations of GMT ratio (GMT Fluzone/GMT Flublok)

Based on Table 17, the upper limits of the 95% CIs for the fold differences (GMT ratios) are not always <1.5. The estimated GMT ratios and the upper limits of the 95% CIs show some variability across sites. These variations may be partially explained by variation between and within assay runs.

II. GMTs per gender

A summary of the univariate analyses of the GMTs per gender is given in Table 18.

Table 18: Summary of statistical analyses of GMTs per gender

For Female

Strain	Flublok (N=187) Estimated GMT (95% CI)	Fluzone (N=192) Estimated GMT (95% CI)
H1N1	196.44 (166, 233)	142.55 (124, 164)
H3N2	121.63 (101, 147)	59.87 (51, 71)
B	110.72 (97, 127)	116.32 (102, 133)

Source: Reviewer's Analysis

For Male

Strain	Flublok (N=113) Estimated GMT (95% CI)	Fluzone (N=110) Estimated GMT (95% CI)
H1N1	148.69 (128, 173)	155.01 (133, 180)
H3N2	277.28 (231, 333)	177.81 (148, 214)
B	157.71 (163, 218)	188.78 (163, 218)

Source: Reviewer's Analysis

On average, at Day 28 after Flublok vaccination, females had higher titers than males for the H1N1 and H3N2 strains.

Exploratory analysis of GMTs per race

The results of analysis of Day 28 GMTs by strain and race for the Flublok group are shown in Table 19.

Table 19: Day 28 GMTs by strain and race based on the Flublok data group

Strain	African-American (N=12)	Asian (N=35)	White/Caucasian (N=217)	Other (N=12)	Latino/Hispanic (N=48)
H1N1	160 (92, 278)	198 (129, 304)	172 (149, 199)	190 (85, 425)	268 (163, 437)
B	90 (53, 152)	133 (92, 191)	108 (96, 121)	120 (78, 183)	118 (82, 172)
H3N2	70 (28, 175)	69 (42, 112)	114 (96, 134)	85 (37, 193)	134 (78, 230)

Source: Reviewer's Analysis

As per Table 19, at Day 28 after Flublok vaccination, for H1N1 and H3N2 strains, Latino/Hispanic had, on average, higher titers than other race groups. However, please note that Table 19 is based on an exploratory analysis generated on a small number of subjects.

3.2.4 Summary of Study PSC06 Immunogenicity Results

The following issues related to results generated by the clinical trial PSC06:

- ✓ Assessments of immune responses to Flublok were based on the co-primary endpoints (seroconversion and seroprotection) for HI antibodies to each viral strain contained in Flublok. The results exhibited that the pre-defined criteria for testing of the seroconversion hypothesis were not fully met, since the statistical criterion for the seroconversion hypothesis for the B strain was not quite satisfied.
- ✓ As per the protocol, the co-primary hypotheses were stated and sample size calculations were performed only for the Flublok vaccine (group), not for the Fluzone vaccine (group). However, in Tables 5 and 6 (CSR, page 53 and 54), the applicant showed the statistical results based on testing the primary hypotheses, not only for Flublok, but for Fluzone as well. The statistical evaluation of Fluzone with regard to seroconversion and seroprotection rates is *post-hoc* analyses, and thus should be interpreted differently from the pre-specified analyses of Flublok.
- ✓ In the SAP, the sponsor addressed multiplicity with respect to the primary hypotheses, but not with respect to the secondary hypotheses (non-inferiority

hypotheses). Therefore, the results based on testing of the non-inferiority hypotheses (Tables 6b and 7, page 55 and 56) should be treated with caution.

The assessments of immunogenicity endpoints for study PSC06 were based on the hemagglutination inhibition (HI) antibody levels measured by HAI assay utilizing BEVS (baculovirus expression vector system) derived antigens. However, the method used for showing assay comparability of the baculovirus-derived rHA antigens and egg-derived antigens is limited and lacks a strong statistical basis. Therefore, for example, it is unclear to what extent HI titers ≥ 40 correlate with protection against illness.

3.3. Study PSC03

Title of the study: “*Comparison of the Evaluation of the Immunogenicity, Safety, and Reactogenicity of Flublok™, Trivalent Recombinant Baculovirus-Expressed Hemagglutinin Influenza Vaccine, to Licensed Egg-Grown Influenza Vaccine in Ambulatory Elderly Adults.*”

Study Period: September 9, 2006 – July 9, 2007

Treatment:

- ✓ Flublok: 0.5 mL single dose containing a total of 135µg of rHA derived from:
 - A/New Caledonia/20/99 (H1N1)
 - A/Wisconsin/67/05 (H3N2)
 - B/Ohio/01/05
- ✓ Fluzone: 0.5 mL single dose containing 15µg of HA of each of the following egg-derived vaccine strains:
 - A/New Caledonia/20/99 (H1N1)-like
 - A/Wisconsin/67/05 (H3N2)-like,
 - B/Malaysia/2506/04.

3.3.1 Brief Overview of the Study

Study design

Study PSC03 was a Phase III, multi-center (six clinical centers), double-blind, and active-controlled clinical trial. All together, 870 subjects, elderly adults aged 65 or older, were randomized at a 1:1 ratio into one of two groups: Flublok or Fluzone. The intervention was 0.5 mL single dose of Flublok (135 µg of rHA₀) or Fluzone administered by intramuscular injection into the deltoid of the non-dominant arm.

The scheduled follow-up visits/contacts took place at Day 8 (only telephone contacts), at Day 28 after randomization, and at the end of the influenza season (EOIS). Additionally, subjects maintained Flu Symptoms Cards during the influenza season. Cards were

reviewed during weekly phone calls made by the study site staff. Samples for serology analyses were collected at baseline before the first vaccination, about 28 days after vaccination, and at the EOIS.

It was planned that subjects who experienced one or more symptoms of influenza (ILI) would call the clinic and, subsequently, NS/TS (Nasal Swab/Throat Swab) would be collected for isolation of influenza virus in cell culture.

Study Endpoints

Primary endpoints:

1. Proportion of subjects in each vaccine group who seroconverted
2. Geometric Mean Titer (GMT) collected at the 28th day post-vaccination visit
3. Frequencies of AEs and SAEs reported from clinics, recorded via memory aids, phone calls, and during physical examination(s).

For each subject, AEs and SAEs were collected during the 28 days post-vaccination period and then throughout the rest of subject participation in the study.

Secondary endpoints:

1. Proportion of subjects in each vaccine group who achieved Day 28 post-vaccination serum HI antibody titer of 40 or greater for each vaccine antigen
2. GMTs, seroconversion rates, and proportions of subjects in each vaccine group with serum HI antibody titer of 40 or greater at the end of influenza season
3. Proportion of subjects in each vaccine group who experienced culture-positive ILI or any ILI during the 2006-2007 influenza epidemic season.

Hypotheses

Primary non-inferiority immunogenicity hypotheses

Formal hypotheses for the immunogenicity comparisons between two vaccination groups with respect to 3 strain-specific antibody responses to the influenza vaccine were:

1.
$$H_0: \text{GMT}_1/\text{GMT}_2 \geq 1.5$$
$$H_1: \text{GMT}_1/\text{GMT}_2 < 1.5 \text{ (for each strain),}$$

where GMT_1 and GMT_2 are the strain-specific GMT parameters for the Fluzone and Flublok vaccination groups, respectively.

2.

$$H_0: \pi_1 - \pi_2 \geq 0.1$$

$$H_1: \pi_1 - \pi_2 < 0.1 \text{ (for each strain),}$$

where π_1 and π_2 are the strain-specific seroconversion rate parameters for the Fluzone and Flublok vaccination groups, respectively.

Sample Size Consideration

In the *Sample Size Considerations* paragraph of the protocol, the applicant claimed that 675 subjects per arm were needed to ensure the overall 80% power for testing the non-inferiority hypotheses of Flublok versus U.S. licensed Fluzone.

REVIEWER'S COMMENTS:

The applicant enrolled into the study only about 436, not 675, subjects per arm and claimed that enrollment was stopped after 870 subjects because "slow enrollment created time constraints (i.e., to ensure that all individuals would be vaccinated in time for the forthcoming influenza season)." Based on the dates of baseline visit (enrollment day), the patients enrollment started on 10/09/06 and concluded on 12/20/06. One site (#07) enrolled patients only during one month (November). In comparison to the enrollment to Study PSC06, the enrollment to Study PSC03 appears not to be slow.

3.3.2 Evaluation of Study Immunogenicity Results

Disposition of Subjects

In total, 870 subjects were enrolled into the study and randomized; of that number, 869 subjects were vaccinated and 854 completed all study procedures. Of the 16 subjects who did not complete all study procedures, one (Fluzone arm) discontinued due to an adverse event and 4 died from causes not related to treatments.

Subjects were enrolled at 6 sites. On average, 145 subjects were enrolled per site (median 133, standard deviation 79, range: 53 to 290).

There were no notable differences between study groups with respect to the collected baseline characteristics. Both groups were balanced in terms of age and gender. The majority of subjects were white (98%) and female (53%). The mean age was 73.0 years (range: 65 – 92 years).

REVIEWER'S COMMENTS:

The applicant stated in the PSC03 Clinical Study Report that the Contract Research Organization notified the applicant of several Good Clinical Practice violations

discovered during routine site control at one of the study sites (Site #5). The most important of these violations were: access by blinded study personnel to the randomization code and improper disposal of Study Vaccine after administration. Please note that these violations were not included in the Protocol Deviations Table (Clinical Study Report, page 49).

As the applicant found no significant differences between Site 5 and other study sites regarding the immunogenicity and safety data, the data from this site (n=127) were included in the final analyses.

Immunogenicity results

Validity of Data Pooling

Poolability of data from Site #5 (with several Good Clinical Practice violations) and other sites was examined by checking the impact of Site #5 data on the immunogenicity results. The reviewer performed post-hoc primary and secondary immunogenicity analyses and found that results for Site #5 did not differ significantly from results for remaining sites. However, small differences were noticed for strain H1N1. Additionally, it should be stressed that access by study site staff to the randomization code should not affect HAI results because all laboratory personnel who performed assay analyses remained blinded.

In summary, the reviewer concludes that Site #5 data can be pooled with other sites' data.

Primary non-inferiority immunogenicity hypotheses

A. GMT ratios

A summary of results of the non-inferiority analysis for GMT ratios and each vaccine antigen at Day 28 is given in Table 20.

Table 20: Non-inferiority of HI antibody responses at Day 28 post-vaccination based on GMT ratios

Estimated GMTs per strain

Vaccine Group	H1N1 Estimated GMT (95%CI)	H1N1 Estimated GMT (95%CI)	H1N1 Estimated GMT (95%CI)
Fluzone (N=430)	148.01 (134.2, 163.4)	194.8 (177.5, 213.8)	199.2 (176.8, 224.5)
Flublok (431)	1176.8 (159.4, 1196.1)	149.4 (134.3, 166.2)	338.4 (299.3, 382.4)

GMT ratios ($\text{GMT}_{\text{Fluzone}}/\text{GMT}_{\text{Flublok}}$), and the corresponding 95% CIs

H1N1 Estimated GMT Ratio 95%CI)	B Estimated Ratio GMT (95%CI)	H3N2 Estimated GMT Ratio (95%CI)
0.84 (0.73, 0.97)	1.30 (1.13, 1.50)	0.59 (0.50, 0.70)

Source: Reviewer's Analysis

REVIEWER'S COMMENTS:

As can be concluded from Table 20, the antibody responses (with respect to the fold differences, i.e., GMT ratios) to Flublok were non-inferior to the responses to Fluzone for the H1N1 and H3N2 strains, but Flublok was borderline non-inferior to Fluzone for the B strain. The estimated 95% CI for the GMT ratio for the B strain was (1.13, 1.50), indicating that the upper limit of the fold difference (GMT ratio) was not less than 1.5. However, based on the adjusted calculation (adjusted for pre-vaccination titer and HI ASSAY variables) the estimated 95% CI for the GMT ratio was (1.17, 1.45).

B. Seroconversion rates

The differences in seroconversions rates at Day 28 and the relevant 95% CIs for each vaccine antigen were estimated and the non-inferiority hypotheses were tested. A summary of results of these analyses is given in Table 21.

Table 21: Non-inferiority of HI antibody responses at Day 28 post-vaccination based on the differences in seroconversion rates

Strain	Flublok (N=431) Estimated Seroconversion Rate	Fluzone (N=430) Estimated Seroconversion Rate	Estimated difference in seroconversion rate
H1N1	43.39	32.56	-10.83 (-17.27, -4.39)
B	29.23	39.07	9.84 (3.53, 16.14)
H3N2	77.73	57.67	-20.05 (-26.15, -13.95)

Source: Reviewer's Analysis

REVIEWER'S COMMENT:

Table 21 presents results of non-inferiority analyses that pertained to the differences in seroconversion rates at Day 28 and to the relevant 95% CIs for each vaccine antigen. The pre-specified criteria related to the non-inferiority hypothesis were met with respect to differences in seroconversion rates for the H1N1 and H3N2 strains. For these two antigens, seroconversion rates for the Flublok group were higher in comparison to the Fluzone rates. In contrast to the results for Type A strains, the non-inferiority criterion for the difference in seroconversion rates for the B strain was not achieved for Flublok. However, it is difficult to interpret the immunogenicity result presented in Table 21 for strain B, because the Type B component in the two vaccines differed. The Flublok vaccine used in study PSC03 contained B/Ohio/01/05, but Fluzone contained B/Malaysia/2506/2004 HA proteins. From the statistical perspective, the null hypothesis cannot be rejected because the upper confidence limit of the 2-sided 95% CI for the difference between seroconversion rates for one strain does exceed 10%.

Clinical Relative Efficacy Results

The applicant's planned relative efficacy analyses were based on the pre-specified secondary endpoints, namely, the proportions of subjects who experienced cell-culture confirmed CDC-ILI or non-CDC-ILI associated with isolation of an influenza virus antigenically resembling a vaccine strain. However, out of 53 sets of cultures, only 3 influenza Type A (strains H1N1 or H3N2) cultures were positive (2 Fluzone, 1 Flublok). The number of influenza cases was too small to allow conclusive evidence from study PSC03 regarding the relative risk of influenza illness in recipients of Flublok as compared to Fluzone in adults 65 years of age and older. However, there were no trends giving cause for concern.

3.3.3 Gender, Race, and Other Special/Subgroup Populations

The reviewer performed univariate analyses by "vaccination in the previous flu season," and gender strata, to assess the influence of these factors on the immune response after Flublok vaccination.

I. GMTs per gender

A summary of the univariate analyses of the GMTs by gender stratum is given in Table 22.

Table 22: Summary of statistical analyses of GMTs per gender

For Females

Strain	Flublok (N=187) Estimated GMT (95% CI)	Fluzone (N=192) Estimated GMT (95% CI)
H1N1	207.74 (181, 239)	142.34 (125, 162)
H3N2	407.39 (346, 478)	219.32 (188, 256)
B	123.40 (164, 407)	199.99 (177, 226)

For Males

Strain	Flublok (N=113) Estimated GMT (95% CI)	Fluzone (N=110) Estimated GMT (95% CI)
H1N1	148.69 (128, 173)	155.01 (133, 180)
H3N2	277.28 (231, 333)	177.81 (148, 214)
B	157.71 (163, 218)	188.78 (163, 218)

Source: Reviewer's Analysis

On average, at Day 28 after Flublok vaccination, females had much higher titers than males for the H1N1 and H3N2 strains.

II. GMTs dependence on “vaccination in the previous flue season” stratum

It appears (tables not presented here) that the status (yes or no) of influenza vaccination in the previous season has an impact on the GMTs at baseline and Day 28. For the H1N1 and H3N2 strains, data indicated that subjects vaccinated during the previous season had statistically lower levels of GMTs at Day 28. For these strains, it appears that vaccination of subjects vaccinated in the previous year produced lower antibody titers.

Please refer to the clinical review for more detailed discussions on immunogenicity results stratified by ethnic group and gender.

3.4.3 Summary of the Statistical Results for PSC03

The primary objectives were to establish non-inferiority of immunogenicity of Flublok in comparison to Fluzone using two primary endpoints: GMTs and seroconversion rates. For each primary endpoint, the null hypothesis could be rejected and non-inferiority could be concluded only if the non-inferiority criterion was met for all three strains. For seroconversion, the non-inferiority criterion was not quite met for the B strain.

Therefore, from the statistical standpoint, the study success criterion was not strictly satisfied, but there may be non-statistical reasons to conclude otherwise. For example, weaker immunogenicity results for the B strain are a common characteristic of licensed flu vaccines. Additionally, the Type B components in the two vaccines were different and this difference could have influence on the result.

In the SAP, the applicant addressed the multiplicity issue with respect to the primary hypothesis (non-inferiority hypothesis), but not with respect to the secondary hypotheses. Therefore, the results based on tests of the secondary hypotheses with regard to seroprotection and seroresponse (e.g., Table 11, CSR page 60) should be considered with the lack of multiplicity adjustment kept in mind. Additionally, please note that the statistical analyses related to the immunogenicity response to Fluzone vaccine are *post-hoc* statistical analyses.

4. Statistical Evaluations of Safety Data

4.1 Overview of Safety Data

Due to rather small sizes of the studies, only general descriptive assessment of safety is presented in this review. Safety data for Flublok vaccine containing 135µg of rHA (45µg per influenza virus strain) were based on four studies: PSC01, PSC03, PSC04, and PSC06. In studies PSC01 and PSC04, 5106 subjects 18 - 49 years of age were

randomized to receive Flublok (2497 subjects received 135 µg; 151 subjects received 75 µg) or placebo (2458 subjects). In studies PSC03 and PSC06, 1471 subjects aged ≥50 years were randomized to receive Flublok (736 subjects) or a US-licensed trivalent, inactivated influenza virus vaccine Fluzone® (735 subjects). The four study populations differed in age. Therefore, the safety datasets for these studies could not be pooled.

Altogether, the gender/race distribution in the safety data was as follows: 59% of subjects were women; 73% of subjects were Caucasian, 8% Hispanic/Latino, 14% -African-American, < 1% - Native American, and 3% - Asian. The mean age of subjects in the studies was 40 years (range 18-92 years); 9% of subjects were 50 to 64 years of age and 13% were 65 years of age and older.

4.2 Solicited Adverse Events

In all studies, a series of symptoms and/or findings were specifically solicited, applying a memory aid used by subjects for the 7-day period following vaccination. In addition, in all 4 studies, spontaneous reports of adverse events were also collected for 28 days following vaccination, and in studies PSC01 and PSC03, subjects were actively queried about changes in their health status 6 months after vaccination.

The reviewer's Table 23, which was prepared based on the applicant's analyses, presents a summary of the common solicited adverse events that occurred during the 8-day post-vaccination period and were reported during three clinical studies.

Table 23: Summary of solicited adverse events between Day 0 and Day 8 for studies PSC04, PSC06, and PSC03

	PSC01 Flublok N=153	PSC01 Placebo N=154	PSC04 Flublok N=2344	PSC04 Placebo N=2304	PSC06 Flublok N=300	PSC06 Fluzone N=302	PSC03 Flublok N=436	PSC03 Fluzone N=433
Local Adverse Events								
Pain	61%	17%	37%	8%	51%	55%	22%	23%
Redness	5%	2%	4%	2%	8%	8%	10%	12%
Swelling	10%	3%	3%	2%	8%	10%	11%	13%
Bruising	7%	4%	3%	3%	5%	5%	3%	5%
Systemic Adverse Events								
Headache	42%	41%	15%	15%	20%	21%	11%	9%
Fatigue	16%	18%	15%	14%	13%	21%	9%	10%
Muscle Pain	20%	12%	10%	7%	13%	14%	7%	9%
Fever	0%	2%	<1%	<1%	<1%	0	<1%	0%
Joint pain	5%	5%	4%	4%	5%	6%	5%	6%
Nausea	8%	6%	6%	5%	4%	5%	4%	3%
Chills	3%	2%	3%	3%	4%	5%	4%	4%
Sweating	3%	5%	NA [†]	NA	NA	NA	3%	2%

Source: Reviewer's Analysis

The most common events in these three studies were headache, fatigue, and muscle pain. Older subjects were, in general, less likely to report adverse events, despite that similar methods of ascertainment were used in study PSC03 as in two other studies.

REVIEWER'S COMMENTS:

Table 23 shows only the frequencies of subjects with solicited adverse events from Day 0 to Day 8. However, it does not supply information on how long some adverse events lasted, e.g., one or more days. In study PSC03, categories such as tiredness and lack of energy were considered in addition to fatigue. Evidently, definitions of the adverse event 'fatigue' in three studies under consideration were not the same. For more information, see Dr. C. Nolletti's review.

The relatively high rates of reactogenicity in study PSC01 may be associated with an additional clinic visit on study Day 2, along with the requirement of a third visit to the clinic on Day 8.

4.3 Summary of Unsolicited Adverse Events and Serious Adverse Events

Unsolicited AEs were those ascertained during the follow-up clinic visits or the telephone contacts up to and including the Day 28 visit, whether reported spontaneously by the subject or in response to general questions about current or interim health status. Reactogenicity events were also included in this category if the event(s) persisted beyond or was (were) first reported after the period covered by the subject's Memory Aid (Study Days 0-7).

Study PSC04

General information on the unsolicited adverse events reported during the PSC04 clinical study during the 28-days post-vaccination period is given in Table 24.

Table 24: Summary of unsolicited AEs and SAEs for Study PSC04

	Flublok N=2344 # of subjects (%)	Placebo N=2304 # of subjects (%)
Any AE	396 (17)	382 (17)
Treatment related or possibly related AEs	61 (2.6)	67 (3)
SAEs	30	34

Source: Reviewer's Analysis

The most frequently reported AEs overall were pharyngolaryngeal pain (91 subjects, 2%); cough (85 subjects, 2%); and headache (78 subjects, 2%). Cough was the most frequently reported unsolicited AE in Flublok recipients (48 (2%) subjects versus 37 (1%) in placebo).

A total of 132 subjects had unsolicited AEs that were considered as possibly or definitely related to the Study Treatment (Flublok or Placebo): 61 (3%) in the Flublok group, and 67 (3%) in the Placebo group.

Eighty-five SAEs were reported in the PSC04 clinical trial: 41 SAEs were reported in 30 Flublok recipients and 44 SAEs were reported in 34 placebo recipients. Two deaths were reported during the course of the study: one Flublok recipient due to a pulmonary embolism and one placebo recipient due to injuries sustained in a motor vehicle accident.

Nine subjects withdrew from the study due to an AE, not including the two deaths that occurred during the study.

Study PSC06

In study PSC06 (subjects 50 - 64 years of age), 602 subjects were included in the safety analysis (300 subjects from FluBlock group and 302 subjects from Fluzone group). Overall, 96/602 (15%) subjects experienced 1 or more AEs as of the Day 28 visit, including 43/300 (14%) subjects in the Flublok group and 53/302 (17%) subjects in the Fluzone group.

General information on the unsolicited adverse events reported during the course of PSC06 clinical study is given in Table 25.

Table 25: Summary of unsolicited AEs and SAEs for Study PSC06

	Flublok (N=300)	Fluzone (N=302)
	# of subjects (%)	# of subjects
Any AE	43 (14)	53 (17)
Severity of AEs		
Mild	36 (12)	33 (11)
Moderate	7 (2)	18 (6)
Severe	0	2(<1)
Treatment related or possibly related AEs	21 (7)	22 (7)
SAEs	2	2
Vaccine related SAEs	1	0

Source: Reviewer's Analysis

The most frequently reported AEs in the FluBlock group (N=300) were injection site erythema (5 subjects, 2%), cough (5 subjects, 2%), pharyngolaryngeal pain, diarrhea, and rhinorrhea (4 subjects for each symptom, 1% for each symptom).

Overall, 54/602 (9%) subjects in PSC06 had treatment-emergent (unsolicited) AEs considered not to be related to study vaccine, while 31 (5%) subjects experienced 1 or more adverse events that were considered possibly related to study vaccine. Eleven (2%)

subjects experienced 1 or more adverse events that were considered related to the study vaccine.

SAEs in Study PSC06 were captured at the End of Influenza Season (EOIS) by telephone contacts, which took place generally 6-9 months after vaccination.

A total of four SAEs were identified, two in each treatment group (FluBlock group: vasovagal syncope, acute pancreatitis; Fluzone: prostate cancer, Cerebrovascular accident). There were no deaths reported during the study.

No subjects discontinued the study due to adverse events.

Study PSC03

In study PSC03, 869 subjects, age 65 years and older, were randomized to receive Flublok (436 subjects) or Fluzone (433 subjects) and included in the safety analysis.

Unsolicited AEs were “treatment emergent AEs” and included those ascertained at clinic visits, telephone contacts, as well as solicited events that persisted beyond Day 7 or were first reported after Study Days 0-7.

The unsolicited adverse events reported during the clinical study PSC03, based on the dataset submitted by the applicant, are summarized in Table 26.

Table 26: Summary of Unsolicited AEs and SAEs for Study PSC03 (adults aged >64)

	Flublok (N=436) # of subjects (%)	Flublok (N=436) # of events (est. of rate)	Fluzone (N=433) # of subjects (%)	Fluzone (N=433) # of events (est. of rate)
Any AE	117 (27)	159 (0.41)	113 (26)	148 (0.39)
Severity of AEs				
Mild	70 (16)	89 (0.21)	67 (16)	90 (0.21)
Moderate	41 (9)	56 (0.13)	40 (9)	50 (0.12)
Severe	26 (6)	29 (0.07)	24 (6)	30 (0.07)
Treatment related AEs	33 (8)	45 (0.10)	28 (6)	38 (0.09)
SAEs	36 (8)	45 (0.10)	34 (8)	42 (0.10)
Vaccine related SAEs	0		0	

Source: Reviewer's Analysis

A total of 70 (36 (8%) Flublok recipients and 34 (8%) Fluzone recipients) reported 87 serious adverse events, none of which were judged by the investigators to be related to the study treatment. The most common SAEs were cardiac disorders (2% in each group),

gastrointestinal disorders (1% in each group), infections and infestations (1% in each group), and nervous system disorders (2% for Flublok and 1% for Fluzone). Four subjects (two in each group) died during the study due to causes unrelated to vaccination.

Study PSC01

In study PSC01, 458 subjects, aged 18 to 49 years, were randomized to receive 75 µg of Flublok (151 subjects), or 135 µg of Flublok (153 subjects) or placebo (154 subjects) and were included in the safety analysis.

Unsolicited AEs were those ascertained during the follow-up clinic visits or telephone contacts up to and including the final visit on Day 180, whether reported spontaneously by the subject or in response to general questions about current or interim health. A total of 57 subjects (12%) had unsolicited AEs that were considered as possibly, probably, or definitely related to the study treatment (vaccine or placebo), including 21 (14%) in the Flublok 75µg group, 16 (10%) in the Flublok 135µg group, and 20 (13%) in the Placebo group.

Serious adverse events (SAEs) included safety data reported from Day 0 through at least 6 months later (end-of-influenza-season). Two subjects (1%) in the 135 µg Flublok group experienced SAEs that were considered to be unrelated to treatment: one seizure related to hypoglycemia that occurred at 26 days post-vaccination, one lobular carcinoma in situ at Day 55, and syncope at Day 125. No subjects discontinued the study due to adverse events and no subjects died. Three female subjects became pregnant after vaccination with Flublok. Two pregnancies ended in elective termination and one proceeded normally to full-term, resulting in the live birth of a normal infant.

4.4 Safety Results by Special Population

Please refer to the clinical review for detailed discussions on the influence of factors like race, gender, and age on the adverse events experience.

4.5 Summary of Safety Results

In general, with the exception of injection site pain for Flublok- vs. placebo-treated subjects, local and systemic reactogenicity events occurred with similar frequency across study groups in the four clinical trials. In study PSC01, all events tended to be reported more frequently than in the other studies. The only reactogenicity events in the four studies that occurred more frequently (in comparison to placebo) in the Flublok group were pain at the injection site in studies PSC01 and PSC04, and swelling and bruising in PSC01.

For all four studies, frequencies of AEs for both treatment groups were similar. However, a trend of a small increase in numbers of AEs in the Flublok group as compared to the

Fluzone group for the population 65 years or older and for younger groups in studies PSC04 and PSC06 (please see Table 24, 25, and 26 in this review) was observed. The numbers of any SAEs were 36 (8 %) and 34 (8 %), for Flublok and Fluzone groups, respectively, and they were higher than for the younger population.

The summary of the safety data could be assessed only on a study-by-study and age-group basis, not on the pooled safety datasets, because of:

- ✓ The known variability of adverse event rates across various age groups following influenza vaccination
- ✓ The youngest PSC04 population makes up ~73% of the safety database for the four studies.
- ✓ Some variability among the four studies in methods of collection of the safety data.
- ✓ Due to rather small sizes of studies for subjects 50 years of age or older, as well as the above-mentioned limitations of the safety database, it is difficult to reach meaningful statistical conclusions regarding safety after Flublok vaccine administration in the population of age 50 years or older.

5. Final Conclusions

5.1 Summary of the Statistical Results

The objective of this BLA submission was to provide evidence that Flublok can be used for active immunization of adults 18 years and older for prevention of influenza disease virus subtypes A and type B contained in the vaccine. For consideration of vaccine approval, data from four clinical trials were submitted by the applicant in support of the efficacy, immunogenicity, lot-to-lot consistency, and safety of Flublok.

It appears that the Flublok vaccine elicited immune responses, particularly for the H1 and H3 strains, in all pivotal studies. But it is very important to note that:

- ✓ Results from study PSC04 provide limited support of the Flublok vaccine efficacy, due to the poor match of vaccine strains and circulating strains during the study period. Therefore, statistically bridging efficacy results to the older population must be done carefully.
- ✓ The safety data for subjects of age ≥ 50 years is rather small. The database contains data on only about 730 Flublok vaccinees.
- ✓ The assessments of immunogenicity endpoints were based on the hemagglutination inhibition (HI) antibody levels measured by HAI assay utilizing BEVS (baculovirus expression vector system) derived antigens. However, the study used for showing assay comparability of the baculovirus-derived rHA antigens and egg-derived antigens, prepared from partially purified influenza virus (method traditionally used in HAI assay), had limitations. The main concerns related to the comparability study are: (1) the dataset was based on a small number (14) of serum samples, with apparent spectrum bias (i.e., most titers

were very high), (2) there was only one agreement criterion, which was based on acceptance of two-fold differences between titers obtained from different preparation methods. Therefore, it is unclear to what extent HI titers ≥ 40 correlate with protection against illness.

Additional statistical concerns related to the clinical trial data under review are as follows:

(I) Study PSC04:

- ✓ Due to the small number (only 5) of cases of influenza caused by strains antigenically resembling the vaccine strains (as confirmed by positive cultures), the study was unable to satisfy the primary pre-specified criterion related to the efficacy hypothesis. However, poor strain match should be taken into consideration.
- ✓ Three investigated lots did not quite achieve the pre-defined criteria for lot-to-lot consistency. Especially for the A/Wisconsin (H3N2) strain, the confidence limits for the GMT ratios were in the range 0.56 to 2.93 (based on interim analyses), as opposed to the 0.5 to 1.5 criterion. However, the variability between lots can likely be attributed to random changes in the manufacturing processes, subject-to-subject, and assay-to-assay variability.

(II) PSC06

- ✓ Assessments of immune responses to Flublok were based on the co-primary endpoints (seroconversion and seroprotection) for HI antibodies to each viral strain contained in Flublok. The results suggested that the pre-defined criteria for testing the seroconversion hypothesis were not fully met since the statistical criterion for the seroconversion hypothesis for the B strain was not satisfied. Seroconversion could be concluded, statistically, only if criteria were met for all three strains and for each of the primary hypotheses. However, weaker immunogenicity for the B strain is commonly seen. Thus, there may be non-statistical reasons to find the results acceptable.

(III) PSC03

- ✓ About 36% fewer subjects were enrolled than were planned.
- ✓ The primary objectives were to establish non-inferiority of immunogenicity of Flublok in comparison to Fluzone using two primary endpoints: GMTs and seroconversion rates. For each primary endpoint, the null hypothesis could be rejected and non-inferiority could be concluded only if the non-inferiority criterion was met for all three strains. However, for seroconversion, the non-

inferiority criterion was not met for the B strain, but this finding is common for influenza vaccines. Based on GMTs, the success criteria were met.

Additionally, there were notable differences in geometric mean titers between three studies and between lots for the H3N2 strain in study PSC04. For instance, in studies PSC03 and PSC06, the GMTs for the H3N2 strain were 338.35 and 105.1, respectively. Potential causes of this disparity of results are unclear.

The safety profile of Flublok was evaluated in four studies. A total of 3,384 subjects were exposed to Flublok. In general, with the exception of injection site pain for Flublok- vs. placebo-treated subjects, local and systemic reactogenicity events occurred with similar frequency across the study groups in the four clinical trials. In study PSC01, all events tended to be reported more frequently than in the other studies. The only reactogenicity events in the four studies that occurred more frequently in the Flublok group were pain at the injection site (in comparison to placebo) in studies PSC01 and PSC04, and swelling and bruising in PSC01.

For all four studies, frequencies of AEs for both treatment groups in a given study were similar. However, for the population 65 years or older, there was a trend of a small increase in numbers of AEs in the Flublok group as compared to the Fluzone group and to the younger population Flublok groups from studies PSC03 and PSC06 (please see, Table 24, Table 25 and Table 26).

Please refer to the clinical review for more safety details and for the clinical significance of some observed differences.

5.2. Conclusions/Recommendations

The pre-specified criteria for the primary and secondary efficacy hypotheses (study PSC04), related to the prevention of influenza culture-confirmed against strains included in the Flublok vaccine, were not satisfied. These results were likely influenced by the antigenic mismatch between vaccine and the circulating virus strains. The number of cases caused by antigenically matched strains was very small. However, in the 2007-2008 influenza season characterized by a predominance of antigenically mismatched strains, the protective efficacy of Flublok against culture-confirmed influenza due to any virus strain was 44.8% (LL of CI was 22.4%). These data provide supporting evidence of the efficacy of Flublok.

The pre-defined lot consistency criteria in study PSC04 were reasonably met, except that for the A/Wisconsin (H3N2) strain, the upper confidence limits of the GMT ratios were 2.93, 2.17, and 0.98 for corresponding lots (see Table 3). The criteria required that the 95% CI of the ratio of post-vaccination GMTs for two different lots, for each viral strain in Flublok, should be entirely within the interval (0.67, 1.5).

The pre-licensure safety database was insufficient to detect differences in rare, serious adverse events after Flublok vaccine administration in the population 18 years of age and older. However, based on the data and the descriptive statistics submitted, no unusual trends, patterns, or safety signals were detected.

Due to the uncertain effectiveness of Flublok and small sizes of studies in adults 50 years of age and older, there is not at this time adequate evidence for approval of this vaccine for this population. However, the totality of the data for adults 18–49 years of age suggests that the benefit of vaccination with Flublok in this population likely outweighs known risks and data issues addressed in this review.

6. Distribution List

ChronFile/HFM-210
Cynthia Nolletti, M.D. /HFM-475
Estelle Russek-Cohen, Ph.D. /HFM-215
Amelia D. Horne, Dr. P.H/HFM-217
Tsai-Lien Lin, PhD/HFM-475
Douglas Pratt, M.D., M.P.H./HFM-475
John Scott, Ph.D. /HFM-215
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