

FluBlok, a recombinant hemagglutinin influenza vaccine

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Abstract FluBlok, a recombinant trivalent hemagglutinin (HA) vaccine produced in insect cell culture using the baculovirus expression system, provides an attractive alternative to the current egg-based trivalent inactivated influenza vaccine (TIV) manufacturing process. FluBlok contains three times more HA than TIV and does not contain egg-protein or preservatives. This

review discusses the four main clinical studies that were used to support licensure of FluBlok under the 'Accelerated Approval' mechanism in the United States.

Keywords Baculovirus, cell culture, hemagglutinin, influenza, safety, vaccine.

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Introduction

FluBlok, a trivalent recombinant hemagglutinin (rHA) vaccine, is under development by Protein Sciences Corporation. The mechanism of action of FluBlok is the same as that of the licensed egg-grown trivalent inactivated influenza vaccines (TIV), thereby simplifying the regulatory pathway for product approval. FluBlok is formulated to contain three times the amount of HA as is contained in TIV. Clinical results suggest that FluBlok may provide superior protection against influenza infection especially in at-risk populations (adults over 65 years, immuno-compromised, etc.) as has been reported for increased antigen concentration of TIV.^{1,2} This review discusses in detail the four main clinical studies (PSC01; PSC03; PSC04 and PSC06) that were used to support licensure of FluBlok under the 'Accelerated Approval' mechanism in the United States.

Most current influenza vaccines are generated in embryonated hen's eggs. Virions are harvested from the egg allantoic fluid, chemically inactivated and treated with detergent, and either a whole virion preparation is generated, or the HA and neuraminidase proteins are partially purified to produce split-product, subvirion, or subunit vaccines.³ Although this system has served well for over 50 years, there are several well-recognized disadvantages to the use of eggs as the substrate for vaccine production. TIVs are standardized to contain 15 µg of each of three HAs, derived from influenza A subtype H1N1, H3N2 and

B.⁴ HA, the dominant surface glycoprotein on the influenza virus and recognized key antigen in the host response to influenza virus in both natural infection and vaccination, is a logical candidate for recombinant vaccine technology.⁵

Initially various monovalent HA formulations and one bivalent formulation were tested in six clinical trials.^{6–9} In one study, a pandemic formulation of H5 vaccine (rH5) induced neutralizing antibody in adults at rates roughly similar to that seen with egg-derived subvirion H5N1 vaccine.¹⁰ In this study, neutralizing antibody titers that were considered to be protective (titers >80) were obtained in 52% of the recipients who received two 90 µg doses of the recombinant vaccine,¹⁰ whereas titers >40 were obtained in 54% of recipients who received two 90 µg doses of the inactivated subvirion vaccine.¹¹ Preliminary data also suggest that vaccination with the rH5 can prime for booster responses on revaccination with or exposure to drifted strains of H5.¹² Thus, recombinant approaches may also be extremely valuable in combating future pandemics and further studies of recombinant pandemic vaccines in humans are needed. Subsequently trivalent formulations of FluBlok were evaluated in various clinical studies.^{13–15}

FluBlok contains HA protein antigens that are derived from the three influenza virus strains, which have been selected for inclusion in the annual influenza vaccine by the WHO and are updated on an annual basis. The three proteins are produced in a non-transformed, non-tumorigenic continuous cell line (*expresSF+*[®] insect cells) grown in serum-free medium, which are derived from Sf9 cells of

the fall armyworm, *Spodoptera frugiperda*. Each of the three recombinant HAs is expressed in this insect cell line using a viral vector (baculovirus *Autographa californica* Nuclear Polyhedrosis Virus). The individual HAs are extracted from the cells with buffer and detergent and further purified by column chromatography.

The HA antigens included in FluBlok are full length proteins containing the transmembrane domain and the HA1 and HA2 regions. The HA proteins form trimeric structures under electron microscopy and are not cleaved in insect cells in the absence of exogenously added proteases (with the exception of HAs containing the highly cleavable sequence of basic amino acids at the cleavage site). Therefore, they are sometimes referred to as rHA0. Since the cleavage site is not known to be involved in the immune response, there should be no significant difference between the immune response to cleaved or uncleaved HA. The proteins are typically further purified using a combination of filtration and column chromatography methods. Details on the production and characterization of the rHA are described elsewhere.^{16,17} The mechanism of action of this vaccine candidate is expected to be similar to TIV; namely, the induction of HA inhibition (HAI) antibodies to prevent influenza infection.^{18,19}

Manufacturing in insect cells offers a number of advantages over currently licensed influenza vaccines that are produced in embryonated chicken eggs: (i) the influenza rHA antigens are produced using a scaleable, reproducible, and low bioburden fermentation process in insect cells, which results in a consistent, protein-based vaccine with low endotoxin content^{16,20}; (ii) selection or adaptation of influenza virus strains for production at high levels in eggs is not required, enabling a good genetic match between the vaccine strains and the disease causing influenza virus strains^{16,20}; (iii) the cloning, expression and manufacture of FluBlok can be accomplished within a brief period of time, generally less than 2 months; (iv) the manufacture of FluBlok does not require high-level bio-containment facilities, which may result in more rapid production and lower cost of vaccine in the event of the emergence of a new epidemic or pandemic strain of influenza virus; and (v) purification procedures for rHA do not include influenza virus inactivation or organic extraction procedures, thus avoiding possible denaturing effects and additional safety concerns because of residual toxic chemicals in the vaccine.¹⁶ Perhaps most importantly, from a clinical perspective, FluBlok is highly purified and does not contain ovalbumin or other antigenic proteins present in eggs.^{16,20}

FluBlok is well tolerated, and contains 45 μg of each HA, which is three times more HA antigen than TIV. The higher HA content offers the potential to provide cross protection for which preliminary evidence has been presented, but also the possibility for longer lasting and

improved immunogenicity.^{15,20} Data obtained with FluBlok are consistent with studies that demonstrated increased doses of purified HA and subvirion vaccines produce an enhanced antibody response in both the elderly and healthy adult populations.^{1,2}

Description of trivalent FluBlok vaccine clinical studies PSC01, PSC03, PSC04 and PSC06

PSC01 was a randomized, prospective, double-blind, placebo-controlled multicenter study in which healthy adults age 18–49 years of age were enrolled during the 2004–2005 influenza season. A total of 458 subjects were vaccinated with either a single dose of FluBlok at a total rHA dosage level of 135 μg [containing 45 μg of each antigen (153 subjects)] or 75 μg [containing 45 μg of H3 rHA and 15 μg of B and H1 rHA (151 subjects)], or a saline placebo (154 subjects). The mean age of the subjects receiving FluBlok (135 μg) was 31 years and the majority was female (63%). Additionally, 85% were Caucasian, 6% were African American, 3% were Latino/Hispanic, 3% were Asian, and 2% were Native Americans. The evaluable efficacy population consisted of 451 subjects (150 in the FluBlok 135 μg group, 150 in the FluBlok 75 μg group and 151 in the placebo group) with complete serological data in the per protocol population.¹⁵

PSC03 was a randomized, double-blind, active-controlled study in which 869 medically stable adults age 65–92 years (mean age 73 years) were enrolled during the 2006–2007 influenza season. Participants were randomly assigned to receive either a single dose of FluBlok (135 μg , 436 subjects) or commercially available trivalent influenza vaccine (Fluzone[®], Sanofi Pasteur, Swiftwater, PA, USA; 433 subjects). The majority of subjects receiving FluBlok were female (52%). A majority were Caucasian (99%). The evaluable efficacy population consisted of 431 FluBlok-treated subjects and 430 Fluzone-treated subjects. A total of 854 subjects completed all study procedures.²¹

PSC04 was a randomized, double-blind, placebo-controlled clinical efficacy study in which 4648 healthy adults age 18–49 years (mean age 33 years) were enrolled during the 2007–2008 influenza season. Participants were randomly assigned to receive either a single dose of FluBlok (135 μg , 2344 subjects) or placebo (2304 subjects). The majority of subjects receiving FluBlok were female (59%). Additionally, 67% were Caucasian, 18% were African American, 11% were Latino/Hispanic, 3% were Asian, and <1% were Native American. A total of 4272 subjects completed all study procedures through Day 28. A subset of 391 subjects who received FluBlok served as the evaluable immunogenicity population.²² *This study is an ongoing efficacy study and only data from the first 28 days of study are included herein.*

PSC06 was a randomized, double-blind, placebo-controlled study in which 602 healthy adults age 50–64 years (mean age 56 years) were enrolled during the 2007–2008 influenza season. Participants were randomly assigned to receive either a single dose of FluBlok (135 μ g, 300 subjects) or commercially available trivalent influenza vaccine (Fluzone®, 302 subjects). The majority of subjects receiving FluBlok were female (62%). Additionally, 73% were Caucasian, 4% were African American, 8% were Latino/Hispanic and 12% were Asian. A total of 602 subjects completed all study procedures through day 28. There were 601 subjects in the evaluable population.²³ *This study is an ongoing efficacy study and only data from the first 28 days of study are included herein.*

Vaccine safety

The population for safety analysis from these trials included 6577 adults 18 years of age and older. The four studies included 5106 subjects 18–49 years of age who were randomized to receive FluBlok (2497 subjects received 135 μ g; 151 subjects received 75 μ g) or placebo (2458 subjects), and 1471 subjects age 50 years and older who were randomized to receive FluBlok (736 subjects) or a US-

licensed trivalent, inactivated influenza virus vaccine (Fluzone®) (735 subjects).

In these studies 59% were women; 73% of subjects were White, 8% Hispanic/Latino, 14% Black, <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 50–64 years of age and 13% were 65 years of age and older.

In all studies, a series of symptoms and/or findings were specifically solicited by a memory aid used by subjects for the 7-day period following vaccination (see Table 1). In addition, in all 4 studies, spontaneous reports of adverse events were also collected for 28 days following vaccination (see below) and subjects were actively queried about changes in their health status 6 months after vaccination for studies PSC01 and PSC03.

PSC01 included 458 subjects for safety analysis, ages 18–49 years, randomized to receive FluBlok 75 μ g (151 subjects), FluBlok 135 μ g (153 subjects) or placebo (154 subjects). Serious adverse events (SAEs) reported include safety data reported from day 0 (day of vaccination) through 6 months. Two subjects (1%) in the 135 μ g FluBlok group experienced SAEs that were considered to be unrelated to treatment (one seizure related to hypoglycemia that

Table 1. Solicited adverse events in the first 7 days after administration of FluBlok, placebo, or comparator influenza vaccine

	Study PSC01 Adults age 18–49 years		Study PSC04 Adults age 18–49 years		Study PSC06 Adults age 50–64 years		Study PSC03 Adults age \geq 65 years	
	FluBlok*	Placebo	FluBlok	Placebo	FluBlok	Fluzone	FluBlok	Fluzone
	Number of subjects	153	154	2344	2304	300	302	436
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		<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

Data based on the most severe response reported by subjects on the memory aid. results >1% reported to nearest whole percent; results >0 but <1 reported as <1%.

*Data restricted to 135 μ g formulation.

**NA, data not available (not collected during the study).

***Fever defined as \geq 99.8°F (37.7°C). In PSC03, fever was defined as >100.4°F.

occurred at 26 days post-vaccination and one lobular carcinoma *in situ* at day 55 and syncope at day 125). No subjects discontinued the study because of 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.

PSC04 included 4648 subjects for safety analysis, ages 18–49 years, randomized to receive FluBlok (2344 subjects) or placebo (2304 subjects). Results from an interim analysis are reported herein and include safety data reported from day 0 through the day 28 visit/phone call. A total of 24 SAEs were reported through the day 28 visit/phone call (eight in the FluBlok and 16 in the placebo treatment groups). Of these, five were pregnancies (one FluBlok and four placebo). Only one SAE, ‘pericardial effusion,’ diagnosed 11 days post-vaccination in a FluBlok recipient, was judged to be possibly related to treatment. None of the remaining six SAEs reported in the FluBlok treatment group were considered by the investigators to be related to study treatment.

PSC06 included 602 subjects for safety analysis, ages 50–64 years, randomized to receive FluBlok (300 subjects) or TIV (Fluzone) (302 subjects). Results from an interim analysis are reported herein and include safety data reported from day 0 through the day 28 visit/phone call. One subject receiving FluBlok reported a treatment-related serious adverse event on the day of vaccination (syncope vasovagal) of moderate severity that resolved without sequelae. No subjects died in this study as of the time of the interim analysis and no subjects discontinued the study due to adverse events.

PSC03 included 869 subjects for safety analysis, age 65 years and older, randomized to receive FluBlok (436 subjects) or TIV (Fluzone) (433 subjects). SAEs reported herein include safety data reported from day 0 through 9 months (end of influenza season). A total of 70 (8%) SAEs were reported [36 (8%) for FluBlok and 34 (8%) for Fluzone]. No SAEs were judged to be related to the study treatment by the investigators.

Across the four trials, there were no deaths that were considered as possibly or probably related to treatment. Table 1 shows the solicited adverse events during the first 7 days post-vaccination. In general, local and systemic reactogenicity events occurred with similar frequency across the four clinical studies except in PSC01, where most events tended to be reported more frequently. The only statistically significant difference between the FluBlok group (135 µg dose) and the placebo group was pain at the injection site in study PSC01; 95% of these pain events were reported as mild.

Table 2 summarizes the most common unsolicited adverse events reported during the four clinical studies during the 28 day post-vaccination period. These events were reported either spontaneously or in response to general queries about

changes in health status. The most common events were headache and signs or symptoms of upper respiratory tract infection in the four studies. These, as well as diarrhea and muscle aches, were the only adverse events reported by >1% of subjects. Older subjects were, in general, less likely to report adverse events, despite similar methods of ascertainment in PSC03 compared to the other three studies. The relatively high rates of reactogenicity in Study PSC01 may have been due to an additional clinic visit on study day 2, along with the requirement of a third clinic visit on day 8.

Immunogenicity results

In all four FluBlok studies, hemagglutination-inhibition (HI) antibody titers to each virus strain represented in the vaccine were measured in sera obtained ~28 days after vaccination. Analysis of endpoints was performed for each HA contained in the vaccine, active control and/or placebo according to the criteria specified in the Food and Drug Administration (FDA) Guidance for Industry: ‘Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines’ (May 2007).

In studies PSC04 and PSC06, the following pre-specified co-primary immunogenicity endpoints were assessed: (i) the lower bounds of the two-sided 95% confidence intervals (CI) for the proportion of subjects with HI antibody titers of 1:40 or greater after vaccination (seroprotection rate), which should meet or exceed 70% for each vaccine antigen strain; and (ii) the lower bounds of the two-sided 95% CI for rates of seroconversion (defined as a fourfold increase in post-vaccination HI antibody titers from pre-vaccination titers of 1:10 or greater, or an increase in titers from less than 1:10 to 1:40 or greater), which should meet or exceed 40% for each vaccine antigen strain.

For study PSC03, these endpoints were pre-specified as secondary, except that criteria for subjects ≥65 years of age were applied (seroprotection rate should meet or exceed 60% for each vaccine antigen strain and the seroconversion rate should meet or exceed 30% for each vaccine antigen strain).

For study PSC01, the primary endpoints, as originally specified, were descriptive comparisons of immune response in the various study groups; therefore, a *post hoc* analysis of the endpoints, as described here, with criteria for subjects <65 years of age, was performed. For PSC01 only, seroprotection is defined (*post hoc*) as a post-vaccination (day 28) HI titer of ≥1:64. Based on the serum dilution series used in the HI antibody assay, 1:64 is the first dilution in which the antibody titer would be ≥1:40, the criterion specified in the Center for Biologics and Research (CBER) Guidance Document. Likewise, for PSC01 only, seroconversion is defined as a ≥4-fold increase in HI titer on day 28 in subjects with a pre-vaccination titer of ≥1:4, with a minimum day 28 titer

Table 2. Adverse events reported by $\geq 1\%$ of subjects in any group in the four clinical trials of FluBlok within 28 days of vaccination, irrespective of causality

	Study PSC01 Adults age 18–49 years		Study PSC04 Adults age 18–49 years		Study PSC06 Adults age 50–64 years		Study PSC03 Adults age ≥ 65 years	
	FluBlok	Placebo	FluBlok	Placebo	FluBlok	Fluzone	FluBlok	Fluzone
Number of subjects	153	154	2344	2304	300	302	436	433
Any adverse events (%)	35	42	16	15	14	17	21	20
Diarrhea (%)	1	3	1	1	1	0	1	1
Nasopharyngitis (%)	3	3	1	1	<1	<1	1	2
Upper respiratory tract infection (%)	6	5	1	1	1	<1	1	1
Myalgia (%)	1	3	<1	<1	0	<1	0	0
Headache (%)	8	8	2	2	<1	<1	<1	1
Cough (%)	4	2	2	2	2	<1	1	2
Nasal congestion (%)	3	4	2	1	1	<1	1	1
Pharyngolaryngeal pain (%)	5	5	2	2	1	3	<1	1
Rhinorrhea (%)	1	3	1	1	1	2	<1	1
Fatigue (%)	1	2	1	1	0	<1	<1	<1
Nausea (%)	2	0	1	1	0	0	<1	1
Sinusitis (%)	1	1	1	1	0	<1	1	<1
Pyrexia (%)	0	1	<1	<1	<1	0	<1	0
Back pain (%)	1	2	<1	<1	<1	1	<1	1
Injection site erythema (%)	0	0	0	0	2	<1	2	<1
Injection site hemorrhage (%)	1	0	<1	<1	0	<1	1	1
Injection site swelling (%)	0	0	0	0	0	<1	1	<1
Pain in extremity (%)	0	0	<1	<1	0	<1	1	<1
Tooth abscess (%)	0	0	<1	0	0	0	<1	1
Arthralgia (%)	1	2	<1	<1	<1	<1	<1	<1
Insomnia (%)	1	1	<1	0	0	0	<1	<1
Sinus congestion (%)	1	1	<1	<1	0	0	<1	<1
Hyperhidrosis (%)	1	1	<1	<1	0	0	0	0

of 1:64; or an HI titer of $\geq 1:64$ on day 28 in subjects with a pre-vaccination titer $< 1:4$ [Limit of Detection (LOD) of the HI assay used in PSC01].

As shown in Table 3, across all four studies, serum HI antibody responses to FluBlok usually met the pre-specified seroconversion criteria for all three virus strains, and also the pre-specified criterion for the proportion of subjects with HI titers $\geq 1:40$ (seroprotection). In study PSC01, FluBlok did not meet the pre-specified seroprotection criterion for influenza B virus, and in PSC03, FluBlok did not meet the pre-specified seroconversion criterion for the influenza B virus. The clinical relevance of these findings on vaccine-induced protection against illness caused by influenza type B strains is unknown, especially given good responses against type B in young adults in study PSC04, and the lack of a head-to-head comparison for the B vaccine component in study PSC03 (see Table 3). In study PSC04 (subjects age 18–49 years), FluBlok met the pre-specified seroprotection and seroconversion criterion for all three strains. In study PSC06 (subjects age 50–64 years), FluBlok

met the pre-specified seroprotection criterion for all three strains while Fluzone marginally passed the seroprotection criterion for the H3 strain (lower end of two-sided CI was rounded up to 70%). In addition, in PSC06, FluBlok met the seroconversion criterion for the H1 and H3 strains but not for the B strain, while Fluzone failed to meet the pre-specified seroconversion criterion for the H3 and B strains.

In Study PSC03, the following co-primary endpoints were pre-specified for each HA contained in the vaccine and/or active control: (i) the upper bound of the two-sided 95% CI on the ratio of Geometric Mean Titers (GMTs) ($\text{GMT}_{\text{US licensed vaccine}}/\text{GMT}_{\text{FluBlok}}$) should not exceed 1.5; and (ii) the upper bound of the two-sided 95% CI on the difference between seroconversion rates ($\text{seroconversion}_{\text{US licensed vaccine}} - \text{seroconversion}_{\text{FluBlok}}$) should not exceed 10% points. These endpoints were specified as secondary in study PSC06.

As shown in Table 4, for study PSC03, non-inferiority of GMTs (in comparison to Fluzone) were met for all three strains, and non-inferiority of the difference in seroconversion rates was met for the two A strains. In PSC06,

Table 3. Serum hemagglutination-inhibition antibody responses at post-vaccination day 28 in subjects age ≥ 18 years

	Study PSC01 Adults age 18–49 years		Study PSC04 Adults age 18–49 years	Study PSC06 Adults age 50–64 years		Study PSC03 Adults age ≥ 65 years	
	FluBlok	Placebo	FluBlok	FluBlok	Fluzone	FluBlok	Fluzone
Number of subjects	150	151	391	299	302	431	430
A/H1N1	<i>A/New Caledonia</i>		<i>A/Solomon Islands</i>	<i>A/Solomon Islands</i>		<i>A/New Caledonia</i>	
% Seroprotected* (95% CI)	87 (81, 92)	40 (33, 49)	98 (97, 99)	96 (94, 98)	96 (93, 98)	95 (92, 97)	95 (92, 97)
% Seroconversion** (95% CI)	60 (52, 68)	0 (0, 2)	78 (74, 82)	72 (67, 77)	66 (61, 72)	43 (39, 48)	33 (28, 37)
A/H3N2	<i>A/Wyoming</i>		<i>A/Wisconsin</i>	<i>A/Wisconsin</i>		<i>A/Wisconsin</i>	
% Seroprotected* (95% CI)	100 (98, 100)	66 (57, 73)	96 (94, 98)	85 (81, 89)	75 (70, 80)	97 (94, 98)	93 (90, 95)
% Seroconversion** (95% CI)	77 (69, 83)	9 (5, 15)	81 (76, 84)	61 (55, 67)	44 (38, 50)	78 (74, 82)	58 (53, 62)
B	<i>B/Jiangsu</i>		<i>B/Malaysia</i>	<i>B/Malaysia</i>		<i>B/Ohio</i>	<i>B/Malaysia</i>
% Seroprotected* (95% CI)	65 (57, 73)	7 (3, 12)	96 (93, 98)	93 (90, 96)	94 (91, 97)	92 (89, 94)	97 (95, 99)
% Seroconversion** (95% CI)	63 (55, 71)	1 (0, 4)	53 (48, 58)	41 (35, 47)	41 (36, 47)	29 (25, 34)	39 (34, 44)

Values shown for FluBlok are for those subjects who received the 135 μg dose. Numbers in bold meet the criteria listed in the FDA Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (May 2007) (see definitions below). These criteria were specified as secondary endpoints in study PSC03.

*Seroprotection rate [hemagglutination-inhibition (HI) titer $\geq 1:40$] is defined as the proportion of subjects with a minimum post-vaccination HI antibody titer of 1:40. The lower bound of the two-sided 95% CI for the seroprotection rate should be $\geq 70\%$ for adults age 18–64 years, and $\geq 60\%$ for adults age 65 years and older. However, for PSC01 only, seroprotection rate is defined (*post hoc*) as the proportion of subjects with a minimum post-vaccination HI titer of 1:64. (based on the serum dilution series used in the HI antibody assay in PSC01, 1:64 is the first dilution in which the antibody titer would be $\geq 1:40$.)

**Seroconversion rate is defined as a ≥ 4 -fold increase in post-vaccination HI antibody titer from pre-vaccination titer $\geq 1:10$ or an increase in titer from $< 1:10$ to $\geq 1:40$. The lower bound of the two-sided 95% CI for the seroconversion rate should be $\geq 40\%$ for adults age 18–64 years, and $\geq 30\%$ for adults age 65 years and older. However, for PSC01 only, seroconversion rate is defined (*post hoc*) as a ≥ 4 -fold increase in post-vaccination HI titer from pre-vaccination titer $\geq 1:4$, with a minimum day 28 titer of 1:64; or an increase in titer from $< 1:4$ (LOD of the HI assay used in PSC01) to $\geq 1:64$.

FluBlok, non-inferiority of both the GMTs and the difference in seroconversion rates was met for all three strains.

In an exploratory analysis of PSC03, the proportions of subjects achieving seroconversion and seroprotection were examined in a subset of 322 subjects who were 75 years and older. As shown in Table 5, vaccination with FluBlok yielded similar rates of seroprotection and seroconversion for this subpopulation when compared with the overall population of subjects 65 years of age and older. In addition, FluBlok met the CBER criterion for non-inferiority of GMTs for all three strains in this subpopulation of older subjects.

Study PSC01 also included a pre-specified secondary efficacy endpoint: the development of laboratory documented (culture-confirmed) influenza illness meeting the influenza-like illness case definition specified by the US Centers for Disease Control and Prevention (CDC-ILI), i.e., presence of fever $\geq 99.8^\circ\text{F}$ and either sore throat or cough, or both. Following vaccination, participants in the study were instructed to return to the clinic for illness evaluations if they observed any acute respiratory tract symptoms or fever. During these illness visits, symptoms were reviewed,

a brief physical exam was conducted, and nasopharyngeal swabs for virus culture were obtained.

During the surveillance period in PSC01, culture-confirmed influenza infection was documented in four subjects in the 75 μg FluBlok group (3%), one subject in the 135 μg FluBlok group (1%), and eight subjects in the placebo group (5%). The protective efficacy against all cases of culture-confirmed, symptomatic infection (regardless of whether the subject met the case definition of CDC-ILI) was 68.2% overall (95% CI $-10.1, 91.8$), 49.0% (95% CI $-90.4, 88.8$) for FluBlok 75 μg , and 87.3% (95% CI 5.5, 99.7) for FluBlok 135 μg . Two culture-positive subjects (1%) who received the 75 μg formulation of FluBlok and seven subjects (5%) who received placebo met the case definition for CDC-ILI. There were no cases of culture confirmed CDC-ILI among subjects vaccinated with the 135 μg formulation of FluBlok. The protective efficacy against culture-confirmed CDC-ILI was 85.5% overall (95% CI 23.7, 98.5), including 70.9% (95% CI $-53.1, 97.0$) for FluBlok 75 μg , and 100% (95% CI 29.7, 100) for FluBlok 135 μg . In addition, a *post hoc* analysis using Fisher's exact test

Table 4. Serum hemagglutination-inhibition responses following immunization with FluBlok (135 µg) or Fluzone in studies PSC03 (subjects ≥65 years of age) and PSC06 (subjects 50–64 years of age)

Number of subjects	PSC06		PSC03	
	FluBlok 299	Fluzone 302	FluBlok 431	Fluzone 430
A/H1N1	<i>A/Solomon Islands</i>		<i>A/New Caledonia</i>	
Pre-vaccination GMT*	28.7 (25.6, 32.3)	27.8 (25.1, 30.8)	69.0 (62.1, 76.6)	70.2 (62.8, 78.6)
Post-vaccination GMT*	181.3 (159.6, 206.0)	139.7 (124.6, 156.7)	176.8 (159.4, 196.0)	148.1 (134.2, 163.4)
Post-vax GMT ratio, Fluzone:FluBlok (two-sided 95% CI)	0.77 (0.75, 0.79)		0.84 (0.81, 0.86)	
No. (%) seroconverting** [two-sided 95% CI]	216 (72) [67, 77]	200 (66) [61, 72]	187 (43) [39, 48]	140 (33) [28, 37]
Difference in seroconversion rate, Fluzone–FluBlok® (two-sided 95% CI)	–6% (–13, 1), P = 0.113		–11% (–17, –4), P = 0.001	
A/H3N2	<i>A/Wisconsin</i>		<i>A/Wisconsin</i>	
Pre-vaccination GMT*	18.6 (16.4, 21.1)	18.2 (16.1, 20.6)	42.7 (37.6, 48.4)	44.7 (39.2, 51.0)
Post-vaccination GMT*	105.4 (91.0, 122.1)	60.9 (53.6, 69.2)	338.5 (299.7, 382.5)	199.2 (176.8, 224.4)
Post-vax GMT ratio, Fluzone: FluBlok (two-sided 95% CI)	0.58 (0.53, 0.62)		0.59 (0.57, 0.60)	
No. (%) seroconverting** [two-sided 95% CI]	183 (61) [55, 67]	132 (44) [38, 50]	335 (78) [74, 82]	248 (58) [53, 62]
Difference in seroconversion rate, Fluzone–FluBlok® (two-sided 95% CI)	–18% (–25, –10), P < 0.001		–20% (–26, –14), P < 0.001	
B	<i>B/Malaysia</i>		<i>B/Ohio</i>	<i>B/Malaysia</i>
Pre-vaccination GMT*	48.5 (43.4, 54.2)	49.2 (43.8, 55.3)	79.9 (71.3, 89.5)	80.3 (72.0, 89.5)
Post-vaccination GMT*	110.9 (100.1, 123.0)	116.0 (104.2, 129.3)	149.6 (134.5, 166.3)	194.8 (177.5, 213.7)
Post-vax GMT ratio, Fluzone:FluBlok (two-sided 95% CI)	1.05 (1.01, 1.09)		1.30 (1.26, 1.34)	
No. (%) seroconverting** [two-sided 95% CI]	122 (41) [35, 47]	124 (41) [36, 47]	126 (29) [25, 34]	168 (39) [34, 44]
Difference in Seroconversion rate, Fluzone–FluBlok® (two-sided 95% CI)	–0.3% (–8, 8), P = 1.00		10% (4, 16), P = 0.003	

Numbers in bold meet the non-inferiority criteria listed in the FDA Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (May 2007) (see definitions below).

*Day 0 (pre-vaccination) and day 28 (post-vaccination) geometric mean titers [95% confidence intervals (CI)]. The upper bound of the two-sided 95% CI on the ratio of GMTs ($\text{GMT}_{\text{US licensed vaccine}}/\text{GMT}_{\text{FluBlok}}$) should not exceed 1.5.

**Seroconversion rate is defined as a ≥4-fold increase in post-vaccination HI antibody titer from pre-vaccination titer ≥1:10 or an increase in titer from <1:10 to ≥1:40. The lower bound of the two-sided 95% CI for the seroconversion rate should be ≥40% for adults age 18–64 years, and ≥30% for adults age 65 years and older.

showed a statistically significant reduction in culture-confirmed CDC-ILI between subjects who received FluBlok (135 µg) versus placebo ($P = 0.0146$). Of the 13 influenza isolates detected during the trial, 10 were found to be genetically similar to A/California/7/04 (H3N2), based on complete cDNA sequencing of the HA1 region obtained from reverse transcriptase-polymerase chain reaction amplified Madin-Darby Canine Kidney cell-grown virus.²⁰ These strains were considered to represent significant drift from the vaccine strain, A/Wyoming/3/03. The remaining three isolates were Type B. In PSC03, only three cases of culture-

confirmed CDC-ILI symptoms occurred; one in the FluBlok group and two in the Fluzone group.

Conclusions

FluBlok is a trivalent rHA vaccine with a mechanism of action likely to be similar to that of the trivalent inactivated licensed influenza vaccine, namely the induction of HAI antibodies to prevent influenza infection.^{19,20} The commercial formulation of FluBlok contains three times the amount of HA of the inactivated influenza vaccines and conse-

Table 5. Serum hemagglutination-inhibition responses following immunization with FluBlok (135 µg) or Fluzone in subjects ≥65 years and ≥75 years from study PSC03

Number of subjects	PSC03			
	Adults age ≥65 years		Adults age ≥75 years	
	FluBlok 431	Fluzone 430	FluBlok 163	Fluzone 159
A/(H1N1)	A/New Caledonia		A/New Caledonia	
Pre-vaccination GMT*	69.0 (62.1, 76.6)	70.2 (62.8, 78.6)	63.3 (53.6, 74.8)	65.5 (55.3, 77.6)
Post-vaccination GMT*	176.8 (159.4, 196.0)	148.1 (134.2, 163.4)	152.7 (128.1, 182.0)	125.3 (107.1, 146.7)
Post-vax GMT ratio, Fluzone:FluBlok (two-sided 95% CI)	0.84 (0.81, 0.86)		0.82 (0.79, 0.85)	
% Seroprotected** (95% CI)	95 (92, 97)	95 (92, 97)	91 (87, 96)	94 (91, 98)
%Seroconversion*** (95% CI)	43 (39, 48)	33 (28, 37)	39 (32, 47)	30 (23, 37)
A/(H3N2)	A/Wisconsin		A/Wisconsin	
Pre-vaccination GMT*	42.7 (37.6, 48.4)	44.7 (39.2, 51.0)	39.7 (32.7, 48.1)	43.1 (35.2, 52.8)
Post-vaccination GMT*	338.5 (299.7, 382.5)	199.2 (176.8, 224.4)	300.2 (244.7, 368.3)	178.4 (147.8, 215.3)
Post-vax GMT ratio, Fluzone:FluBlok (two-sided 95% CI)	0.59 (0.57, 0.60)		0.59 (0.58, 0.61)	
% Seroprotected** (95% CI)	97 (94, 98)	93 (90, 95)	96 (93, 99)	93 (89, 97)
%Seroconversion*** (95% CI)	78 (74, 82)	58 (53, 62)	79 (73, 85)	54 (46, 62)
B	B/Ohio	B/Malaysia	B/Ohio	B/Malaysia
Pre-vaccination GMT*	79.9 (71.3, 89.5)	80.3 (72.0, 89.5)	101.9 (86.7, 119.9)	102.6 (86.1, 122.1)
Post-vaccination GMT*	149.6 (134.5, 166.3)	194.8 (177.5, 213.7)	185.7 (160.8, 214.4)	224.8 (193.2, 261.5)
Post-vax GMT ratio, Fluzone:FluBlok (two-sided 95% CI)	1.30 (1.26, 1.34)		1.21 (1.18, 1.24)	
% Seroprotected** (95% CI)	92 (89, 94)	97 (95, 99)	96 (93, 99)	99 (98, 100)
%Seroconversion*** (95% CI)	29 (25, 34)	39 (34, 44)	26 (20, 33)	35 (28, 43)

Numbers in bold meet the non-inferiority criteria listed in the FDA Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (May 2007) (see definitions below).

*Day 0 (pre-vaccination) and day 28 (post-vaccination) geometric mean titers (95% confidence intervals (CI)). The upper bound of the two-sided 95% CI on the ratio of GMTs (GMT_{US licensed vaccine}/GMT_{FluBlok}) should not exceed 1.5.

**Seroprotection rate [hemagglutination-inhibition (HI) titer ≥1:40] is defined as the proportion of subjects with a minimum post-vaccination HI antibody titer of 1:40. The lower bound of the two-sided 95% CI for the seroprotection rate should be ≥70% for adults age 18–64 years, and ≥60% for adults age 65 years and older.

***Seroconversion rate is defined as a ≥fourfold increase in post-vaccination HI antibody titer from pre-vaccination titer ≥1:10 or an increase in titer from <1:10 to ≥1:40. The lower bound of the two-sided 95% CI for the seroconversion rate should be ≥40% for adults age 18–64 years, and ≥30% for adults age 65 years and older.

quently induces higher antibody titers, which may be of particular importance to those most at risk for influenza (for example, the elderly^{1,2,13} or immunologically compromised¹⁴). The immunogenicity results for the B/strain in the elderly study PSC03 must be interpreted cautiously in the context of a lack of a direct antigen comparison. Although the two strains (B/Ohio and B/Malaysia) were considered to be antigenically related by WHO Reference Laboratories, and therefore interchangeable for purposes of vaccine production, previous studies of influenza vaccines have shown that HI titers achieved following vaccination with different influenza antigens of the same subtype typically differ from each other, often to variable degrees.

While FluBlok contains 135 µg HA per dose, the total amount of protein (HA plus host cell proteins) contained

within one dose of FluBlok is roughly comparable to the total amount of protein contained in FluZone (viral plus egg protein).^{24,25}

The vaccine was shown to be well tolerated and immunogenic in adults older than 18 years. Importantly, this vaccine has demonstrated protective efficacy in a field efficacy trial against drifted influenza viruses.^{15,20} FluBlok received Fast Track Designation from the FDA in December 2006 and Protein Sciences filed its Biological License Application (BLA) in April 2008, and expects to receive FDA approval as early as 2009.

Conflict of interest

The author is an employee of Protein Sciences Corporation.

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