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Introducing Modern Recombinant Technology to the Realm of Seasonal Influenza Vaccine: Flublok® For Prevention of Influenza in Adults

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Abstract

Flublok is the first recombinant hemagglutinin (rHA) vaccine licensed in the US by FDA for the prevention of influenza in adults 18 and older. The rHAs are produced in insect cell culture using the baculovirus expression vector system (BEVS) technology, and are, in contrast to HAs from viruses adapted to growth in hen's eggs, an exact genetic match to the HAs of the influenza strains selected by World Health Organization and FDA for inclusion in the annual seasonal influenza vaccine. These rHAs can be produced rapidly and in large quantity owing to the universal process across different HAs. This universal production process has been scaled successfully to bioreactors ranging up to 21,000L. This review discusses the immunogenicity, efficacy and safety data derived from five main clinical studies that supported licensure of trivalent Flublok for adults 18 and older in the United States. These data are also under regulatory review in other jurisdictions worldwide, including Japan and Mexico.

We show that Flublok results in improved immunogenicity for many influenza strains, likely due to its higher rHA content as compared to conventional inactivated vaccines. Limited data suggest further that efficacy appears to be improved while local reactogenicity is generally less frequent than is observed with conventional inactivated influenza vaccine despite the higher antigen content.

Flublok could include rHAs that are designed to mimic "drifted" influenza viruses as techniques improve for predicting emerging antigenic drift. At a minimum, the BEVS system utilized in the Flublok manufacturing process can address late-appearing influenza viruses to which conventional egg-based manufacturing processes cannot respond in a timely fashion. The implementation of this rapid response approach will require collaboration with and support from regulatory authorities.

Keywords: *Recombinant hemagglutinin; Baculovirus; Flublok; Recombinant DNA technology; Hemagglutinin; Immunogenicity*

Introduction

Flublok is the only influenza vaccine currently produced using modern recombinant DNA technology and is licensed by the US FDA for the prevention of influenza in adults 18 and older. The mechanism of action of Flublok that relies on the humoral immune response to the influenza hemagglutinin surface protein is the same as that of the licensed inactivated influenza vaccines (IIV). However, the IIV products are produced by growth of infectious strains of influenza virus that have been adapted to grow in embryonated chicken eggs. This adaptation to yield a high-growth reassortant is known to have caused mutations in the HA genome that can render the HA antigenically different from the wild-type strain, termed "egg-induced drift". When this happened in 2012-2013, the vaccine produced using this decades-old technology exhibited poor efficacy [1].

Inactivated influenza vaccines are standardized to contain specific quantities of HA, and immune correlates of protection against influenza infection in terms of HAI antibody titers are well established in adults. Regulators worldwide use standardized criteria based on hem agglutination inhibition antibody (HAI) titers to support the licensure of inactivated influenza vaccines. The content of adventitious materials, e.g. ov albumin, antibiotics, formaldehyde, etc., in vaccines produced from growing infectious virus is not as well standardized,

and reflects the relatively crude procedures for inactivation and partial purification used to produce these vaccines. Prior to initiation of registration trials with recombinant HA, these purified proteins were demonstrated in several smaller studies to be well tolerated and to induce a satisfactory level of immune response [2,3]. Flublok was initially licensed by the US FDA in 2013 for the prevention of influenza in adults 18- 49 years of age based on two placebo-controlled clinical studies, the Phase 1/2 PSC01 and the pivotal Phase 3 PSC04. PSC01, a placebo-controlled trial that included one treatment group vaccinated with a trivalent rHA product containing only 15 μg of the H1 and B antigens, whereas the second Flublok group received rHA vaccine containing 45 μg of each of the three antigens. Serologic testing showed that the presence of three times more H3 HA antigen (45 versus 15 microgram (mcg) resulted in improved antibody responses confirming results of an earlier clinical study [4,5]. In addition, limited data from this study suggested an improved efficacy of the higher dose vaccine [4] as has been reported for increased antigen concentration of currently marketed "high-dose" IIVs [6-9]. Study PSC04 confirmed the clinical efficacy of Flublok in adults 18-49 years old despite the circulation of antigenically drifted viruses during the 2007/08 season when the study was conducted [10].

Safety and immunogenicity data in adults 50 years of age and older who received either Flublok or a comparator licensed IIV3 were collected in three additional Phase 3 and 4 studies. PSC03 compared the safety and immunogenicity of Flublok to that of Fluzone (standard dose) in adults ≥ 65 years of age, while PSC06 compared the same parameters related to Flublok or Fluzone in adults 50-64 years of age [11,12]. Additional safety data for Flublok in older adults were collected in PSC11, the third study of adults older than 50 years [13]. These data complemented the comparative immunogenicity data from earlier active controlled studies that demonstrated improved immunogenicity of Flublok for the influenza A viruses [5].

This review discusses the five main clinical trials (PSC01; PSC03; PSC04; PSC06; PSC11) that supported full approval of Flublok for adults 18-49 and approval for adults over 50 under the "Accelerated Approval" mechanism. The latter regulations refer to approval on the basis of surrogate markers that are "likely to predict clinical benefit". Approval under these regulations requires additional clinical trial(s) to confirm clinical efficacy in order to secure full, traditional approval.

Based on the rapidly evolving transition from trivalent to quadrivalent seasonal influenza vaccines, a large comparative efficacy study in adults over 50 years old is being conducted during the 2014/15 season with a quadrivalent formulation, Flublok Quadrivalent®. Results from this double-blinded efficacy study are expected in the latter half of 2015, and will provide important insights as to whether mutations in the HA protein caused by adaptation of influenza virus to the egg-based manufacturing process (egg-induced drift) were indeed responsible for the reported low effectiveness of the 2014-2015 influenza vaccine [1].

As noted earlier, conventional inactivated influenza vaccines are produced by growing the "egg-adapted" infectious influenza virus in embryonated hen's eggs. The process of adapting the virus to grow efficiently in eggs may yield unpredicted or unexpected genetic changes in the viral surface antigenic protein hemagglutinin that can, in turn, adversely impact the protective efficacy of the vaccine produced from these inactivated viruses. This production process has been transferred recently to in vitro cell culture by one manufacturer (formerly Novartis; recently acquired by CSL-bio) currently growing the influenza viruses in Madin-Darby Canine Kidney (MDCK) cells. The seed virus used for the MDCK cell-based vaccine production process remains the high-growth reassortant generated in eggs. Therefore, the issue of genetic infidelity remains problematic.

Whether grown in eggs or cell culture, whole virions are harvested, chemically inactivated (usually with formaldehyde or a similar preservative) and treated with detergent, to disrupt the virus releasing the surface protein antigens. The HA and neuraminidase (NA) proteins are then partially purified to produce split-product, subvirion, or subunit vaccines [14]. This 60-year old egg-based influenza manufacturing process has served well; however, modern technology enables us to overcome some of the well-recognized disadvantages to the use of eggs as the substrate for vaccine production. In addition to issues addressed above, other limitations include:

1. The time required to produce a high growth reassortant results in a relatively slow cycle time for vaccine production following identification of the strains predicted to circulate in a future season;
2. The manufacture process requires appropriate bio-containment facilities or workers who have appropriate immunity to the viruses they are producing;
3. The virus inactivation process requires the use of undesirable chemicals, including formaldehyde and antibiotics, traces of which may be present in the final product,
4. The endotoxin residue in the final product cannot be carefully controlled and
5. ovalbumin or other residual chemicals or antibiotics required to maintain a low bioburden during processing are known to be present in the final product.

IIVs are standardized to contain 15 mcg of each of three or four HAs, derived from influenza A subtype H1N1, H3N2 and one or two B lineages [15]. HA, the dominant surface glycoprotein on the influenza virus and the recognized key antigen in the host response to influenza virus, in both natural infection and vaccination, is a logical candidate for recombinant vaccine technology [16].

Flublok contains HA protein antigens that are genetically identical to those from the influenza virus strains selected for inclusion in the annual seasonal influenza vaccine by the WHO. The rHAs in Flublok are updated annually. The proteins are produced in a proprietary 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. The rHAs are expressed in this insect cell line using the baculovirus (Autographa californica Nuclear Polyhedrosis Virus) as an expression vector. The individual rHAs are extracted from the cells with buffer and detergent and further purified by column chromatography. The final product is a highly purified soluble protein (>90% rHA) in physiologic phosphate-buffered saline without preservative, antibiotics, formaldehyde or latex. Further details on the production and characterization of rHA are described elsewhere [17-19]. The mechanism of action of this vaccine is the induction of HA inhibition (HAI) antibodies that prevent influenza infection [20,21].

Earlier clinical development of Flublok has been reviewed elsewhere in detail [11,5]. Here, we review data derived from the five clinical studies described above and focus on the three aspects of safety, immunogenicity and efficacy of Flublok. The performance of Flublok with respect to these features is further compared to other commercially available influenza vaccines.

Title	Brief Description	NCT Code	Ref
PSC01	Dose escalation and preliminary efficacy study in 458 subjects 18 through 49 years of age	NCT00328107	4
PSC03	Safety and immunogenicity study in 869 subjects aged 65 years and older randomized to receive Flublok (n = 436) or Fluzone1 as an active control (n = 433)	NCT00395174	11
PSC04	Efficacy Study in 4648 subjects 18 through 49 years of age randomized to receive Flublok (n=2344) or placebo (n=2304)	NCT00539981	10
PSC06	Safety and immunogenicity study in 602 subjects 50 through 64 years of age randomized to receive Flublok (n = 300) or Fluzone1 as an active control	NCT00539864	12
PSC11	Safety study in 2627 subjects aged 50 years and older for randomized to receive Flublok (n=1314) or Afluria[2], as an active control (n = 1313). Among subjects 50 through 64 years of age, 672 received Flublok and 665 received Afluria. Among subjects aged 65 years and older, 642 received Flublok and 648 received Afluria	NCT01825200	13

Table 1 : *[1]Fluzone manufactured by Sanofi Pasteur, Swift water, USA.*

[2]Afluria manufactured by bioCSL Pty Ltd.

Vaccine Safety

Flublok, containing 45 mcg of each rHA, is well tolerated. This protein content is three-fold higher for each HA antigen than is contained in standard-dose conventional IIVs. The higher rHA content offers the potential to provide higher levels of immune response and, potentially, cross-protection, for which preliminary evidence has been presented [10]. By virtue of the generation of higher antibody titers, the possibility of longer lasting immunity has also been speculated [4] [22]. Data obtained with Flublok are consistent with studies that demonstrated an enhanced antibody response to increased doses of purified HA and subvirion vaccines in both the elderly and healthy adult populations [7,8].

In completed clinical trials, Flublok has been administered to 2497 adults 18 through 49 years of age, 972 adults 50 through 64 years of age, and 1078 adults aged 65 years and older enrolled in the five key randomized, placebo- or active-controlled clinical trials. As noted above, safety data were collected from all subjects in the clinical trials, immunogenicity was collected from all subjects in PSC01, PSC03, PSC06 and a subset of subjects in PSC04, and clinical efficacy data (cultures for influenza) were collected from subjects in PSC01 and PSC04. The on-going clinical trials with Flublok Quadrivalent to be completed during 2015 will add ~4500 subjects 50 years of age and older and ~1000 subjects 18-49 years of age to the accumulated safety database for rHA.

Description of Trivalent Flublok Vaccine Clinical Studies PSC01, PSC03, PSC04, PSC06 and PSC11

Study PSC01 included 458 subjects 18 through 49 years of age for safety analysis, randomized to receive Flublok low-dose (n = 151 vaccine contained 45μg of H3 rHA and 15 μg each of B and H1 rHA), Flublok (n = 153) or placebo (n = 150) [4]. Study PSC03 included 869 subjects aged 65 years and older for safety analysis, randomized to receive Flublok (n = 436) or another U.S.-licensed trivalent influenza vaccine (IIV3, Fluzone, manufactured by Sanofi Pasteur, Inc) as an active control (n = 433) [11]. Study PSC04 included 4648 subjects 18 through 49 years of age for safety analysis, randomized to receive Flublok (n = 2344) or placebo (n = 2304) [10]. Study PSC06 included 602 subjects 50 through 64 years of age for safety analysis, randomized to receive Flublok (n = 300) or IIV3 (Fluzone) as an active control (n = 302) [12]. Study PSC11 included 2627 subjects aged 50 years and older for safety analysis, randomized to receive either Flublok (n = 1314) or IIV3 (Afluria, manufactured by bioCSL Pty Ltd.) (n = 1313) (13). among subjects 50 through 64 years of age, 672 received Flublok and 665 received Afluria. Among subjects aged 65 years and older, 642 received Flublok and 648 received Afluria.

In all studies, a series of symptoms and/or findings characteristic of reactogenicity associated with injectable influenza vaccination were specifically solicited by a memory aid used by subjects for the 7-day period following vaccination. In the placebo-controlled study PSC04, the only notable difference between Flublok and saline placebo injections was mild, transient injection site pain that was reported by 37% of Flublok recipients vs. 8% of placebo recipients. In the active-controlled trials involving subjects 50-64 and ≥ 65 years of age, there was no notable difference in the incidence or severity of events of reactogenicity between Flublok and IIV3 recipients. All complaints of reactogenicity, regardless of age category or study vaccine were largely mild in severity.

In addition, in all studies, spontaneous reports of adverse events were also collected for 28 days following vaccination (see below) and subjects were actively queried about serious adverse events for up to six months following vaccination for studies PSC01, PSC03, PSC04 and PSC06.

Among adults 18-49 years of age (Studies PSC01 and PSC04 pooled), through 6 months post-vaccination, two deaths were reported, one in a Flublok recipient and one in a placebo recipient. Both deaths occurred more than 28 days following vaccination and neither was considered vaccine-related. Serious adverse events (SAEs) were reported by 32 Flublok recipients and 35 placebo recipients. One SAE in a Flublok recipient, a case of pleuropericarditis with effusions requiring hospitalization and drainage, was assessed as possibly related to the vaccine, due to the absence of a clear alternative etiology: The patient recovered without sequelae. The investigator considered this event unrelated to study vaccine.

Among adults 50-64 years of age (Studies PSC06 and PSC11, pooled), through up to 6 months post-vaccination, there were no deaths; SAEs were reported by 10 subjects, 6 Flublok recipients and 4 IIV3 recipients. One of the SAEs, an episode of vasovagal syncope following injection of Flublok, was considered related to study vaccine, although it was likely due to the injection procedure itself rather than the vaccine material. Among adults 65 years of age and older (Studies PSC03 and PSC11, pooled), through up to 6 months post-vaccination, there were 4 deaths, 2 in Flublok recipients and 2 in IIV3 recipients. None were considered related to the study vaccines. SAEs were reported from 80 subjects, 37 among Flublok recipients, 43 among IIV3 recipients. None were considered related to the study vaccines.

In Study PSC04 (adults 18-49 years of age), the most frequent unsolicited adverse events, occurring in ≥ 1% of subjects, were nasopharyngitis, upper respiratory infection, headache, cough, nasal congestion, pharyngolaryngeal pain, and rhinorrhea.

Among adults 50-64 years of age (Studies PSC06 and PSC11, pooled), the most frequent unsolicited adverse events, occurring in ≥ 1% of subjects, were diarrhea and cough. Among adults ≥ 65 years of age (Studies PSC03 and PSC11, pooled), the most frequent unsolicited adverse events, occurring in ≥ 1% of subjects, were nasopharyngitis and cough. None of these events in either age category appeared to be related to either study vaccine and there did not appear to be a notable imbalance between study vaccine groups in terms of either frequency or severity.

Among adults 50 years of age and older (Study PSC11) for whom the incidence of rash, urticaria, swelling, non-pitting edema, or other potential hypersensitivity reactions were actively solicited for 30 days following vaccination, a total of 2.4% of Flublok recipients and 1.6% of IIV3 recipients reported such events over the 30 day follow-up period. A total of 1.9% and 0.9% of Flublok and IIV3 recipients, respectively, reported these events in the 7 days following vaccination. Of these solicited events, rash was most frequently reported (Flublok 1.3%, IIV3 0.8%) over the 30 day follow-up period. The events adjudicated by independent experts to likely represent hypersensitivity reactions (Type 1, IgE-mediated) were reported from 0.5% and 0.3% of Flublok and IIV3 recipients, respectively, yielding similar relative risks for these events with either Flublok or IIV3.

To evaluate the possible impact on reactogenicity of the 3-fold higher content of rHA in Flublok over IIV, we compared the incidence of solicited reactogenicity events following Flublok and Fluzone (standard dose) or Fluzone HD (high dose) from Fluzone (standard dose) clinical trials and from the Fluzone Package Insert [23]. Solicited adverse events in Flublok recipients were generally similar to Fluzone with the exception of headache that was slightly more common in Flublok recipients, whereas all solicited adverse events were more common among Fluzone HD recipients compared with recipients of Fluzone (standard dose). The relative risk of events within each study comparing either Flublok or Fluzone HD to Fluzone show that there was a decreased likelihood of all reported events of reactogenicity, except headache, among Flublok recipients as compared with Fluzone and the difference was even more notable between Flublok and Fluzone High-Dose.

NOTE: Data derived from clinical study PSC03 [11] and Fluzone High Dose Package Insert [23]

Given that Flublok and Fluzone High Dose contain very similar quantities of the active antigenic ingredient, hemagglutinin, it is reasonable to speculate that the higher incidence of side effects associated with Fluzone High Dose can be attributed to residual adventitious materials remaining from the production process in contrast to the pure solution of recombinant hemagglutinin in physiologically buffered saline that is Flublok. Vaccine Efficacy

Data available from the Phase 1/2 PSC01 study suggested that higher antigen content in Flublok may contribute to improved protection (Table 1). In this study one culture-confirmed influenza infection was documented in subjects receiving the full 135 µg dose (1%), while 4 subjects receiving the lower dose (3%), and 8 subjects in the placebo group (5%) experienced culture-confirmed ILI. The protective efficacy against all cases of culture-confirmed, symptomatic infection was 49.0% (95% CI -90.4, 88.8) for the low dose and 87.3% (95% CI 5.5, 99.7) for Flublok. Two culture-positive subjects (1%) who received the low dose formulation and 7 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 full-dose Flublok. The protective efficacy against culture-confirmed CDC-ILI was 70.9% (95% CI -53.1, 97.0) for the low dose, and 100% (95% CI 29.7, 100) for Flublok 135 mcg. Fisher's exact test showed a statistically significant reduction in culture-confirmed CDC-ILI between subjects who received Flublok (vs. placebo (p = 0.0146).

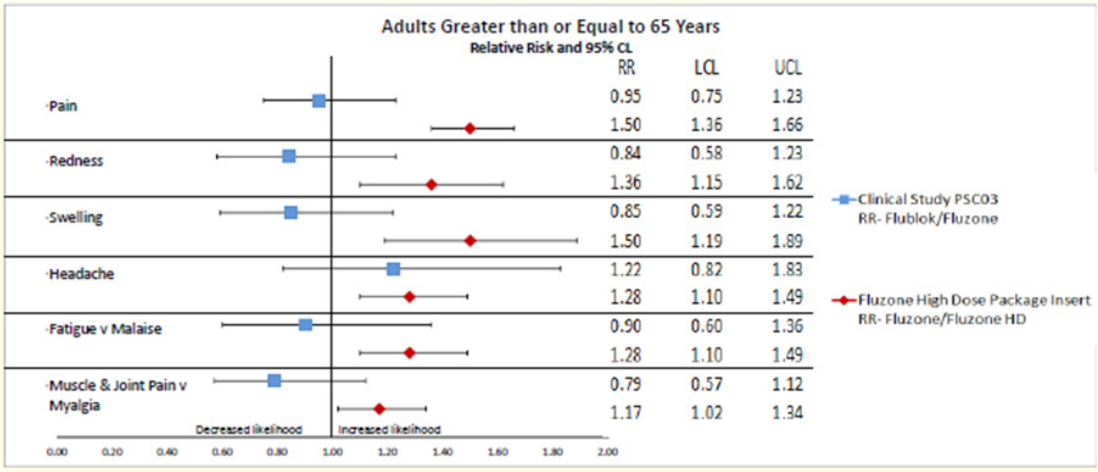

Figure 1: *Relative Risk of Solicited Events of Reactogenicity during Days 0-7 after Administration of Flublok, Fluzone or Fluzone HD.*

Case definition	Flublok 75 µg N = 151	Flublok 135 µg N = 153	Saline Placebo N = 150	Vaccine Efficacy[1] % (95% CI) Low Dose 75 µg	Vaccine Efficacy[1] % (95% CI) High Dose135 µg
	N (%)	N (%)	N (%)		
Positive culture with any influenza strain					
Any ILI, all strains[2]	4 (3)	1 (1)	8 (5)	49.0% (90.4,88.8)	87.3% (5.5, 99.7)
CDC-ILI, all strains[3]	2 (1)	0	7 (5)	70.5% (53.1,97.0)	100% (29.7, 100)

Table 2: *PSC01 -- Vaccine Efficacy against Culture-Confirmed Influenza in Healthy Adults 18-49 Years of Age*

[1]Determined under the assumption of Poisson event rates, according to Breslow and Day, 1987.

[2]All culture-confirmed symptomatic cases are considered, regardless of whether they qualified as CDC-ILI.

[3]Meets CDC influenza-like illness (CDC-ILI) defined as fever of ≥ 100ºF oral accompanied by cough and/or sore throat, on the same day or on consecutive days.

While the numbers of cases of influenza were quite small in this study and the 95% confidence intervals quite wide, there is a strong suggestion of a dose response in efficacy favoring the higher 135 µg dose of Flublok.

The efficacy of Flublok 135 µg was evaluated in the pivotal, Phase 3 Study PSC04, a randomized, observer-blind, placebo-controlled multicenter trial conducted in the U.S. during the 2007-2008 influenza season and determined by protection against culture-confirmed influenza-like illness. In this study 4648 healthy adults (mean age 32.5 years) were randomized in a 1:1 ratio to receive a single dose of Flublok (n = 2344) or saline placebo (n = 2304). The two vaccine groups were similar in demographics. Culture-confirmed influenza was assessed by active and passive surveillance for influenza-like illness (ILI) beginning 2 weeks post-vaccination until the end of the influenza season, approximately 7 months post- vaccination.

Influenza-like illness (ILI) was defined by criteria established by the US Centers for Disease Control and Prevention (CDC): subjects must have symptoms from at least 2 of the following three symptom categories:

a. Fever ≥ 100ºF
b. Respiratory symptoms, including cough, sore throat, runny nose/stuffy nose
c. Systemic symptoms, including myalgias, arthralgias, headache, chills/sweats, fatigue/malaise.

No specified duration of symptoms was required. For subjects with an episode of ILI, nasal and throat swab samples were collected for viral culture.

Most of the influenza isolates obtained from subjects in this study were not antigenically matched to the strains represented in the vaccine, a situation that is well recognized to result in poor effectiveness of the seasonal vaccine [24]. The vaccine efficacy (VE) of Flublok against all strains isolated from any subject with an ILI regardless of antigenic match to the vaccine strains, demonstrated an efficacy estimate of 44.8% (95% CI 24.4, 60.0), suggesting reasonable efficacy against drifted strains. When the analysis was limited to VE against ILI that met CDC-defined criteria for ILI, the VE was similar at 44.6% (95% CI 18.8, 62.6).

Case Definition	Flublok (N = 2344)		Saline Placebo (N = 2304)		Flublok Vaccine Efficacy[1] %	95% Confidence Interval
	Cases (n)	Rate (%)	Cases (n)	Rate (%)		
Positive culture with any strain						
Any ILI, all strains[3]	64	2.7	114	4.9	44.8	(24.4, 60.0)
Influenza A	41	1.7	79	3.4	49.0	(24.7, 65.9)
Influenza B	23	1.0	36	1.6	37.2	(-8.9, 64.5)
CDC-ILI, all strains[2]	44	1.9	78	3.4	44.6	(18.8, 62.6)
Influenza A	26	1.1	56	2.4	54.4	(26.1, 72.5)
Influenza B	18	0.8	23	1.0	23.1	(-49.0, 60.9)
Positive culture with a strain matching the vaccine						
CDC-ILI, all matched strains[2]	1	0.04	4	0.2	75.4	(-148.0, 99.5)
Any ILI, all matched strains[3]	2	0.1	6	0.3	67.2	(-83.2, 96.8)

Table 3: *PSC04 - Vaccine Efficacy against Culture-Confirmed Influenza in Healthy Adults 18-49 Years of Age*

[1]Determined under the assumption of Poisson event rates, according to Breslow and Day, 1987.

[2]Meets CDC influenza-like illness (CDC-ILI) defined as fever of ≥ 100ºF oral accompanied by cough and/or sore throat, on the same day or on consecutive days.

[3]All culture-confirmed cases are considered, regardless of whether they qualified as CDC-ILI.

Because of the poor match between vaccine strains and the viruses that circulated during the 2007-2008 season, the number of cases of ILI due to strains that matched the vaccine strains was very low; however, the VE calculated for these cases caused by non-drifted strains, recognizing the wide confidence interval due to small numbers, were 67.2–75.4%, well in the range of expected vaccine efficacy for influenza vaccines that have been published over several decades [25,26].

Immunogenicity Results

In studies PSC01, PSC03, a subset of PSC04 and PSC06, hem agglutination-inhibition (HAI) antibody titers to each virus strain represented in the respective vaccines 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 FDA Guidance for Industry [27].

Across all studies, serum HAI antibody responses to Flublok usually met the pre-specified seroconversion criteria for all 3 virus strains, and also the pre-specified criterion for the proportion of subjects with HAI titers ≥ 1:40 (seroprotection). These data were reviewed and reported in detail elsewhere [4-12]. For the purposes of assessing immunogenicity of Flublok in comparison to a conventional IIV developed as a "high dose" product for use in elderly individuals, we compared the data obtained from adults ≥ 65 years of age in Study PSC03 comparing Flublok with standard dose Fluzone and further the data in the same age population administered Fluzone HD dose vs. standard dose Fluzone These two sets of comparison allowed the comparison of Flublok and Fluzone High Dose each to Fluzone standard dose, thus "normalizing" the magnitude of difference observed in separate clinical trials.

As shown in Table 3, the immunogenicity of Flublok provided higher geometric mean antibody titers and higher seroconversion rates against the influenza A strains as compared with the responses to standard dose Fluzone. In a comparable fashion, Fluzone HD was more immunogenic than standard dose Fluzone. The ratios of HAI antibody titers and differences in seroconversion rates vs. Fluzone are very similar between Flublok and Fluzone HD for the A/H3 influenza virus and reasonably comparable for A/H1. Immune responses to the B strains in the Flublok study suggested lesser potency of Flublok, but the discrepancy of B antigens in the two vaccines during the season in which this study was conducted may have contributed to these results.

	Study PSC03		**Sanofi PI High Dose***	
	Adults age ≥ 65 yrs		**Adults age ≥ 65 yrs**	
	Flublok	**Fluzone**	**Fluzone**	**Fluzone HD**
Number of Subjects	436	433	1248-1249	2529-2531
A (H1N1)				
GMT	177	148	67	116
GMT Ratio (Flublok: comparator) or (Fluzone HD: comparator)	1.2	1.7		
Seroconversion (%)	43	33	23	49
Difference in seroconversion (%)	10	25		
A (H3/N2)				
GMT	339	199	333	609
GMT Ratio (Flublok: comparator)				
or (Fluzone HD: comparator)	1.7	1.8		
Seroconversion (%)	78	58	51	69
Difference in seroconversion (%)	20	18		
B (Note: Different Antigens in Flublok vs. Fluzone)				
GMT	150	195	52	69
GMT Ratio (Flublok: comparator) or (Fluzone HD: comparator)	0.8	1.3		
Seroconversion (%)	29	39	30	42
Difference in seroconversion (%)	-10		12	

Table 4: *Immunogenicity after Administration of Flublok, Fluzone or Fluzone HD.*
NOTE: Data derived from clinical study PSC03 [11] and Fluzone High Dose Package Insert [23].

Discussion and Conclusions

Flublok is a recombinant trivalent rHA vaccine produced using modern technology that yields a pure hemagglutinin protein solution in physiologic, phosphate-buffered saline. The mechanism of inducing immunity is similar to that of licensed inactivated influenza vaccine, that is the induction of HAI antibodies to prevent influenza infection [21,22]. The modern technology used to produce Flublok offers multiple advantages including:

1) The vaccine antigens are exact genetic matches to the wild-type influenza strains selected for seasonal vaccines in any given year
2) The manufacturing time is shortened, allowing rapid response to late-emerging or pandemic influenza strains
3) The manufacturing process does not utilize infectious influenza virus, thus it requires no bio-containment system
4) No undesirable chemicals, such as formaldehyde, to inactivate infectious virus are used in the process
5) The endotoxin content is carefully controlled and maintained at very low levels
6) No ovalbumin, residual chemicals or antibiotics are present in the final product. Specifically, no antibiotics are commonly used to ensure a low bioburden during processing.
7) The technology has been successfully scaled to 21,000L bioreactors, offering the opportunity to respond to late -appearing influenza strains and to deal with mismatches in the vaccine that may result in poor efficacy as again was reported for the 2014/15 influenza vaccine [28].

The commercial formulation of Flublok contains three times the amount of rHA compared to the standard dose of inactivated influenza vaccines and, as a consequence, may induce higher antibody titers, which may be of particular importance to those most at risk for influenza (for example, the elderly [7,8,11,5] or immunologically compromised [29]. The higher antibody responses are especially notable for the H3 subtype of influenza A.

The immunogenicity results for the B/strain in the study PSC03 in elderly subjects must be interpreted cautiously in the absence of a direct antigen comparison. Although the influenza B strains included in the two study vaccines (B/Ohio and B/Malaysia) were considered by WHO Reference Laboratories to be antigenically related, and therefore interchangeable for purposes of vaccine production, previous studies of influenza vaccines have shown that HAI titers achieved following vaccination with different influenza antigens of the same subtype typically differ from each other, often to substantial degrees. This sort of variability may have confounded the results of immunogenicity testing of the anti-B antibodies in the PSC03 study.

Both Flublok and the representative IIV presented in this review contain comparable quantities of total protein [30,31]. Flublok contains ~135µg rHA per dose, whereas Fluzone (standard dose) contains only ~45 µg HA per dose and the remainder of the total amount of protein include other residual viral and egg protein. Flublok was shown to be equally well tolerated as Fluzone standard dose in all adult age groups, while providing (at least for Influenza A strains), providing immunogenicity in adults older than 65 years comparable to Fluzone High Dose.

Importantly, Flublok has demonstrated protective efficacy in field efficacy trials against drifted influenza viruses [4-10].

In conclusion, production of a seasonal influenza vaccine using modern recombinant DNA technology offers a number of significant advantages over the conventional growth of whole virus in eggs. Because of the purity of the recombinant hemagglutinin vaccine, a number of undesirable elements used to inactivate whole virus and reduce bioburden are absent from the Flublok product. The higher concentration of hemagglutinin in Flublok provides improved immunogenicity comparable to recently introduced "high dose" vaccine without the associated local irritation.

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