

# Safety, efficacy, and immunogenicity of Flublok in the prevention of seasonal influenza in adults

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**Abstract:** Flublok is the first recombinant hemagglutinin (HA) vaccine licensed by the US Food and Drugs Administration for the prevention of influenza in adults aged 18 and older. The HA proteins produced in insect cell culture using the baculovirus expression system technology are exact analogues of wild type circulating influenza virus HAs. The universal HA manufacturing process that has been successfully scaled to the 21,000L contributes to rapid delivery of a substantial number of doses. This review discusses the immunogenicity, efficacy and safety data from five pivotal clinical studies used to support licensure of trivalent Flublok for adults 18 years of age and older in the United States. The trial data demonstrate that the higher antigen content in Flublok results in improved immunogenicity. Data further suggest improved efficacy and a slightly lower local reactogenicity compared with standard inactivated influenza vaccine, despite the presence of more antigen (statistically significant). Flublok influenza vaccine can include HAs designed to mimic ‘drift’ in influenza viruses as the process of predicting antigenic drift advances and, at a minimum, could address late appearing influenza viruses. The implementation of the latter will require support from regulatory authorities.

**Keywords:** cell culture, hemagglutinin, immunogenicity, influenza, safety, vaccine

## Introduction

Flublok (Protein Sciences Corporation, Meriden, CT, USA) is the only recombinant influenza vaccine licensed by the US Food and Drug Administration (FDA) for the prevention of influenza in adults 18 years of age and older. The mechanism of action of Flublok is the same as that of the licensed egg-grown inactivated influenza vaccines (IIVs). IIVs contain a standardized amount of hemagglutinin (HA) and immune correlates are well established. Regulators around the world use standardized criteria based on HA inhibition (HAI) titers to support the licensure of influenza vaccines.

The FDA initially licensed Flublok in 2013 for the prevention of influenza in adults 18–49 years of age on the basis of two placebo-controlled clinical studies, PSC01 and PSC04. The first study PSC01 demonstrated that including three times more HA antigen (45 *versus* 15 µg) resulted in improved antibody responses, confirming the

results of an earlier clinical study [Treanor *et al.* 2007]. In addition, studies PSC03 and PSC06 demonstrated improved immunogenicity of Flublok for the influenza A viruses [Keitel *et al.* 2010; Baxter *et al.* 2011] as reported for increased antigen concentration of other IIVs [Keitel *et al.* 1994, 1996]. Study PSC04 confirmed the efficacy of the vaccine in adults 18–49 years old despite the circulation of drift viruses during the 2007/08 season when the study was conducted [Treanor *et al.* 2011]. In this review we include a Kaplan–Meijer plot that provides some preliminary evidence for waning vaccine efficacy (VE) later in the season.

Additional safety data for Flublok in older adults were collected in another study of adults older than 50 [ClinicalTrials.gov identifier: NCT01825200].

This review discusses five pivotal clinical studies (PSC01; PSC03; PSC04; PSC06; PSC11)

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supporting full approval of Flublok for adults aged 18–49 and approval under the ‘accelerated approval’ mechanism for adults over 50 (Table 1). The latter means that confirmatory efficacy studies still need to be conducted. A large comparative efficacy study in adults over 50 years old with a quadrivalent formulation is in progress during the 2014/15 season. Results expected from this double-blinded efficacy study will provide important insights as to whether variations in the HA protein caused by the egg-based manufacturing process were indeed responsible for the reported low effectiveness of the 2014/15 influenza vaccine [D’Mello *et al.* 2015; Skowronski *et al.* 2015].

Traditional influenza vaccines are produced by growing the influenza virus in embryonated hen’s eggs. Gradually this production process may be transferred to cell culture with one manufacturer (formerly Novartis; recently acquired by CSL-bio CSL Limited, Parkville, Vicotria 3052, Australia) pioneering the field by growing the influenza viruses in Madin-Darby Canine Kidney (MDCK) cells. Virions are harvested, chemically inactivated and treated with detergent, the HA and neuraminidase (NA) proteins are partially purified to produce split-product, subvirion, or subunit vaccines [Wood, 1998]. The 60-year-old egg-based influenza manufacturing process served well; however, newer technology enables vaccine production to overcome the well recognized egg substrate disadvantages. Limitations include the following: selection or adaptation of influenza virus strains for production at high levels in eggs or cells may result in suboptimal genetic match between the vaccine strains and the disease causing influenza virus strains [Katz *et al.* 1987; Wang *et al.* 1989; Rajakumar *et al.* 1990; Skowronski *et al.* 2014]; of note the MDCK production process uses egg-derived virus seeds in its manufacturing process as well and the mutations associated with adaptation to growth in eggs remain an issue; the time required to produce a high growth reassortant; the manufacture process requires high-level biocontainment facilities or workers with appropriate immune responses against the viruses they produce; the influenza virus inactivation process requires the use of undesirable chemicals; the endotoxin content cannot be carefully controlled; and the presence of ovalbumin or residual chemicals or antibiotics required to maintain a low bioburden during processing.

IIVs are standardized to contain 15 µg of each of three or four HAs, derived from influenza A subtype H1N1, H3N2 and more recently two B

lineages [Bridges *et al.* 2002]. HA is the dominant surface glycoprotein on the influenza virus and key antigen in both natural infection and vaccination host response to influenza virus, and the logical candidate for recombinant vaccine technology [Huber and Cullers, 2008]. NA plays an important role in initiation of infection [Matrosovich *et al.* 2004] and antibody to the NA induces an ‘infection permissive’ immunity most likely by a combination of mechanisms: direct inhibition of the enzyme; cross linking individual virions and thereby reducing the number of infectious viruses available to infect host cells; cementing virions to cell associated NA; and inhibiting the detachment of nascent virions from the host cell [Air *et al.* 1989; Huang *et al.* 1980a, 1980b; Kilbourne *et al.* 1968a, 1968b].

Flublok contains HA protein antigens derived from influenza virus strains, selected for inclusion in the annual influenza vaccine by the World Health Organization and updated on an annual basis. The proteins are produced in a proprietary nontransformed, nontumorigenic continuous cell line (*expresSF+* insect cells Protein Sciences Corporation, Meriden, CT USA) grown in serum-free medium, derived from Sf9 cells of the fall armyworm, *Spodoptera frugiperda*. The HAs are expressed in this insect cell line using the baculovirus *Autographa californica* nuclear polyhedrosis virus. The individual HAs are extracted from the cells with buffer and detergent and further purified by column chromatography. Further details on the production and characterization of recombinant HA (rHA) are described elsewhere [Holtz *et al.* 2003; Wang *et al.* 2006; Cox and Hashimoto, 2011]. The mechanism of action of this vaccine candidate is expected to be similar to IIV; namely, the induction of HAI antibodies to prevent influenza infection [Kida *et al.* 1983; Yoden *et al.* 1986].

Earlier development of Flublok was reviewed elsewhere in detail [Cox *et al.* 2008]. Here, we review data derived from five clinical studies and focus on three aspects: safety, immunogenicity and efficacy of Flublok, comparing its performance with other commercially available influenza vaccines.

### Flublok safety

Flublok contains 45 µg of each HA and is well tolerated, whereas the standard IIV contains 15 µg of each HA. The higher HA content offers the potential to provide cross protection for which

**Table 1.** Clinical studies discussed in this review.

ID season	Test product	Comparator	Study population	Endpoints	N	Reference
PSC01 2004–2005 (phase IIb)	Flublok 135 µg A/New Caledonia A/Wyoming B/Jiangsu Flublok 75 µg	Placebo	Healthy adults 18–49 years of age	Safety, immunogenicity clinical efficacy	458	Treanor <i>et al.</i> [2007]
PSC03 2006–2007 (phase III)	Flublok 135 µg A/New Caledonia A/Wisconsin B/Ohio	Fluzone* A/New Caledonia A/Wisconsin B/Malaysia	Healthy adults 65–92 years of age	Safety, immunogenicity	869	Keitel <i>et al.</i> [2010]
PSC04 2007–2008 (phase III)	Flublok 135 µg A/Solomon Islands A/Wisconsin B/Malaysia	Placebo	Healthy adults 19–49 years of age	Safety, immunogenicity clinical efficacy	4648	Treanor <i>et al.</i> [2011]
PSC06 2007–2008 (phase III)	Flublok 135 µg A/Solomon Islands A/Wisconsin B/Malaysia	Fluzone*	Healthy adults 50–64 years of age	Safety, immunogenicity relative clinical efficacy	602	Baxter <i>et al.</i> [2011]
PSC11 2012–2013 (Phase IV)	Flublok 135 µg A/California A/Victoria B/Wisconsin	Afluria <sup>§</sup>	Ambulatory and medically stable adults ≥50 years of age	Safety, solicited and unsolicited hypersensitive adverse events	2640	Izikson <i>et al.</i> [2015]
*Fluzone manufactured by Sanofi-Pasteur, Swiftwater, PA, USA.						
<sup>§</sup> Afluria manufactured by bioCSL Pty Ltd. CSL Limited, Parkville, Victoria 3052, Australia.						

preliminary evidence has been presented, but also the possibility for longer lasting and improved immunogenicity [Treanor *et al.* 2006; Cox and Anderson, 2007]. 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 [Keitel *et al.* 1994, 1996].

Flublok has been administered to and safety data collected from 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 five randomized, placebo- or active-controlled clinical trials.

#### 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 45 µg of H3 rHA and 15 µg of B and H1 rHA), Flublok ( $n=153$ ), or placebo ( $n=2304$ ) [Treanor *et al.* 2007].

Study PSC03 included 869 subjects aged 65 years and older for safety analysis, randomized to receive Flublok ( $n=436$ ) or another US-licensed trivalent influenza vaccine (Fluzone, Sanofi-Pasteur, Swiftwater, PA, USA) as an active control ( $n=433$ ) [Keitel *et al.* 2010].

Study PSC04 included 4648 subjects, 18 through 49 years of age, for safety analysis, randomized to receive Flublok ( $n=2344$ ) or placebo ( $n=2304$ ) [Treanor *et al.* 2011].

Study PSC06 included 602 subjects, 50 through 64 years of age, for safety analysis, randomized to receive Flublok ( $n=300$ ) or another US-licensed trivalent influenza vaccine (Fluzone, as an active control) ( $n=302$ ) [Baxter *et al.* 2011].

Study PSC11 included 2627 subjects aged 50 years and older for safety analysis, randomized

to receive Flublok ( $n=1314$ ) or another US-licensed trivalent influenza vaccine (Afluria, manufactured by bioCSL Pty Ltd) as an active control ( $n=1313$ ) [ClinicalTrials.gov identifier: NCT01825200]. 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 findings were specifically solicited by a memory aid used by subjects for the 7-day period following vaccination. All studies collected spontaneous reports of adverse events 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. The frequency of solicited local injection site reactions and systemic adverse reactions within 7 days of administration of Flublok or comparator vaccine are shown in Tables 2 and 3 for adults aged 50–64 and adults aged 65 and older, respectively.

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 were considered vaccine related. Serious adverse events (SAEs) were reported by 32 Flublok recipients and 35 placebo recipients. One SAE in a Flublok recipient was assessed as possibly related to the vaccine: pleuro-pericarditis with effusions requiring hospitalization and drainage. No specific cause was identified. The patient recovered without sequelae.

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, six Flublok recipients and four IIV3 recipients. Vasovagal syncope following injection of Flublok was an SAE considered related to study vaccine, although likely due to the injection procedure 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 four deaths, two in Flublok recipients and two in IIV3 recipients. None were considered related to the study vaccines. SAEs were reported from

**Table 2.** Frequency of solicited local injection site reactions and systemic adverse reactions within 7 days of administration of Flublok or comparator in adults 50–64 years of age, studies PSC06 and PSC11, total vaccinated cohort\*.

	Flublok $N=972$			IIV3* $N=967$		
	Any	Mod <sup>§</sup>	Sev <sup>§</sup>	Any	Mod <sup>§</sup>	Sev <sup>§</sup>
<b>Local</b>	<b>%</b>					
Pain	32	2	<1	37	<1	0
Firmness/swelling	7	2	<1	6	1	<1
Redness	6	2	<1	5	1	<1
<b>Systemic</b>	<b>%</b>					
Headache	17	4	<1	16	3	<1
Fatigue	13	3	<1	17	3	<1
Muscle pain	11	2	<1	11	2	<1
Joint pain	8	2	<1	8	2	<1
Nausea	6	1	0	5	<1	<1
Shivers/chills	5	1	0	4	<1	<1
Fever <sup>‡</sup>	<1	<1	<1	<1	0	0

Data based on the most severe response reported by subjects. Results of at least 1% are reported to nearest whole percent; results greater than 0 but less than 1% reported as less than 1%.

\*Data from studies PSC06 and PSC11 were pooled. For studies PSC06 and PSC11, the US-licensed IIV3 comparators were Fluzone and Afluria, respectively.

<sup>§</sup>Moderate (Mod) = had it, and it was bad enough to prevent a significant part of usual activities; severe (Sev) = had it, and it prevented most or all of normal activities, or had to see a doctor for prescription medicine.

<sup>‡</sup>Fever defined as at least 100.4°F (38°C): mild ( $\geq 100.4^\circ$  to  $<101.1^\circ$ F); moderate ( $\geq 101.2^\circ$ F to  $<102.2^\circ$ F); severe ( $\geq 102.2^\circ$ F). For fever, data for 12 Flublok recipients and 5 IIV3 recipients were missing data, making these denominators 964 and 962, respectively.

**Table 3.** Frequency of solicited local injection site reactions and systemic adverse reactions within 7 days of administration of Flublok or comparator in adults at least 65 years of age, studies PSC03 and PSC11, total vaccinated cohort\*.

	Flublok N= 1078			IIV3 <sup>1</sup> N= 1081		
	Any	Mod <sup>§</sup>	Sev <sup>§</sup>	Any	Mod <sup>§</sup>	Sev <sup>§</sup>
<b>Local</b>	<b>%</b>					
Pain	19	<1	<1	20	<1	<1
Redness	7	1	<1	7	1	1
Firmness/swelling	7	2	<1	7	<1	<1
<b>Systemic</b>	<b>%</b>					
Fatigue	13	3	<1	15	2	<1
Headache	10	<1	<1	9	1	<1
Muscle pain	8	2	<1	8	1	<1
Joint pain	6	1	<1	6	1	<1
Shivers/chills	5	<1	<1	5	<1	<1
Nausea	4	<1	<1	3	<1	<1
Fever <sup>‡</sup>	3	<1	<1	2	0	0

Data based on the most severe response reported by subjects. Results of at least 1% are reported to nearest whole percent; results greater than 0 but less than 1% reported as less than 1%.

\*Data were pooled from studies PSC03 and PSC11. For studies PSC03 and PSC011, the US-licensed IIV3 comparators were Fluzone and Afluria, respectively.

§Moderate (Mod) = had it, and it was bad enough to prevent a significant part of usual activities; severe (Sev) = had it, and it prevented most or all of normal activities, or had to see a doctor for prescription medicine.

‡Fever defined as at least 100.4°F (38°C): mild (≥100.4° to <101.1°F); moderate (≥101.2°F to <102.2°F); severe (≥102.2°F).

80 subjects, 37 Flublok recipients and 43 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 1–2% 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 at least 65 years of age (studies PSC03 and PSC11 pooled), the most frequent unsolicited adverse events, occurring in 1% of subjects, were nasopharyngitis and cough.

Among adults 50 years of age and older (study PSC11) for whom the incidence of rash, urticaria, swelling, nonpitting 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 be likely hypersensitivity reactions were reported from 0.5% and 0.3% of Flublok and IIV3 recipients, respectively.

We compared the incidence of solicited adverse events following Flublok, Fluzone and Fluzone HD from Flublok clinical trials and from the Fluzone package insert [Sanofi-Pasteur, 2014] to evaluate the possible impact of the threefold higher content of HA in Flublok on reactogenicity (Table 4). Solicited adverse events in Flublok were generally similar to Fluzone with the exception of headache, with is slightly more common in Flublok recipients, whereas all solicited adverse events were more common in Fluzone HD recipients.

Pain at the injection site is statistically significantly lower for Flublok in comparison to Fluzone HD.

**Table 4.** Solicited adverse events in the first 7 days after administration of Flublok, Fluzone or Fluzone HD.

	Study PSC03		Sanofi PI high dose*	
	Adults aged $\geq 65$ years		Adults aged $\geq 65$ years	
	Flublok	Fluzone	Fluzone	Fluzone HD
Number of subjects	436	433	2569–2572	1258–1260
<b>Local adverse events</b>				
Pain	22%	23%	24%	36%
RR (95% CI)	0.95 (0.75–1.23)		1.50 (1.36–1.66)	
Redness	10%	12%	11%	15%
RR (95% CI)	0.83 (0.58–1.23)		1.36 (1.15–1.62)	
Swelling	11%	13%	6%	9%
RR (95% CI)	0.85 (0.59–1.22)		1.50 (1.19–1.89)	
<b>Systemic adverse events</b>				
Headache	11%	9%	14%	18%
RR (95% CI)	1.22 (0.82–1.83)		1.28 (1.10–1.49)	
Fatigue <i>versus</i> malaise	9%	10%	14%	18%
RR (95% CI)	0.90 (0.60–1.36)		1.28 (1.10–1.49)	
Muscle + joint pain <i>versus</i> myalgia	12%	15%	18%	21%
RR (95% CI)	0.80 (0.57–1.12)		1.16 (1.02–1.34)	
Data derived from clinical study PSC03 [Keitel <i>et al.</i> 2010] and Fluzone high-dose package insert [Sanofi-Pasteur, 2014]. Relative risk (RR) was calculated using <a href="https://www.medcalc.net/tests/relative_risk.php">https://www.medcalc.net/tests/relative_risk.php</a>				
CI, confidence interval.				

### Flublok efficacy against culture-confirmed influenza

The efficacy of Flublok was evaluated in study PSC04, a randomized, observer-blind, placebo-controlled multicenter trial conducted in the US during the 2007–2008 influenza season. 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 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. ILI was defined as having at least two of three symptoms (no specified duration) in the following categories: fever at least 100°F; respiratory symptoms (cough, sore throat, runny nose/stuffy nose); or systemic symptoms (myalgias, arthralgias, headache, chills/sweats, tiredness/malaise). 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. The VE of Flublok against all strains isolated from any

subject with an ILI regardless of antigenic match, not necessarily CDC-defined ILI, demonstrated an efficacy estimate of 44.8% (95% CI 24.4–60.0). Thus, Flublok is effective in the prevention of influenza illness and provides protection against drift variants [Treanor *et al.* 2011; Cox and Anderson 2007]. The results of this study are summarized in Table 5 for a presentation of VE by case definition and antigenic similarity.

As shown in Figure 1, the majority of influenza isolates were obtained in the period mid January through early February. In the period before and including 8 February, a total of 67 influenza A isolates were obtained, of which 51 were isolated from placebo recipients and 16 from Flublok recipients, resulting in a VE of 69.2% [95% confidence interval (CI) 45.0–83.6]. In the period after 8 February 2008, another 52 influenza A isolates were obtained, 27 in placebo and 25 in Flublok, resulting in an efficacy of only 9% (95% CI –62.9, 49.3). A similar analysis for B isolates showed that in the period before and including 8 February, a total of 14 influenza B isolates were obtained, of which 11 were isolated from placebo recipients and 3 from Flublok recipients, resulting in an efficacy of 73.2% (95% CI –1.5, 95.2).

**Table 5.** Vaccine efficacy against culture-confirmed influenza in healthy adults 18–49 years of age.

Case definition	Flublok (N=2344)		Saline Placebo (N=2304)		Flublok vaccine efficacy*, %	95% confidence interval
	Cases, <i>n</i>	Rate, %	Cases, <i>n</i>	Rate, %		
Positive culture with a strain represented in the vaccine						
CDC-ILI, all matched strains <sup>§</sup>	1	0.04	4	0.2	75.4	[−148.0, 99.5]
Any ILI, all matched strains <sup>‡</sup>	2	0.1	6	0.3	67.2	[−83.2, 96.8]
Positive culture with any strain, regardless of match to the vaccine						
CDC-ILI, all strains <sup>§</sup>	44	1.9	78	3.4	44.6	[18.8–62.6]
Subtype A	26	1.1	56	2.4	54.4	[26.1–72.5]
Type B	18	0.8	23	1.0	23.1	[−49.0, 60.9]
Any ILI, all strains <sup>‡</sup>	64	2.7	114	4.9	44.8	[24.4–60.0]
Subtype A	41	1.7	79	3.4	49.0	[24.7–65.9]
Type B	23	1.0	36	1.6	37.2	[−8.9, 64.5]

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

<sup>§</sup>Meets Centers for Disease Control (CDC) influenza-like illness (ILI) defined as fever of at least 100°F oral accompanied by cough or sore throat, on the same day or on consecutive days.

<sup>‡</sup>All culture-confirmed cases are considered, regardless of whether they qualified as CDC-ILI.

In the period after 8 February 2008, 45 influenza B isolates were obtained, 25 in placebo and 20 in Flublok, resulting in an efficacy of 21.4% (95% CI −47.5, 58.6). The decrease in VE over time does not appear to coincide with an increase in drift viruses.

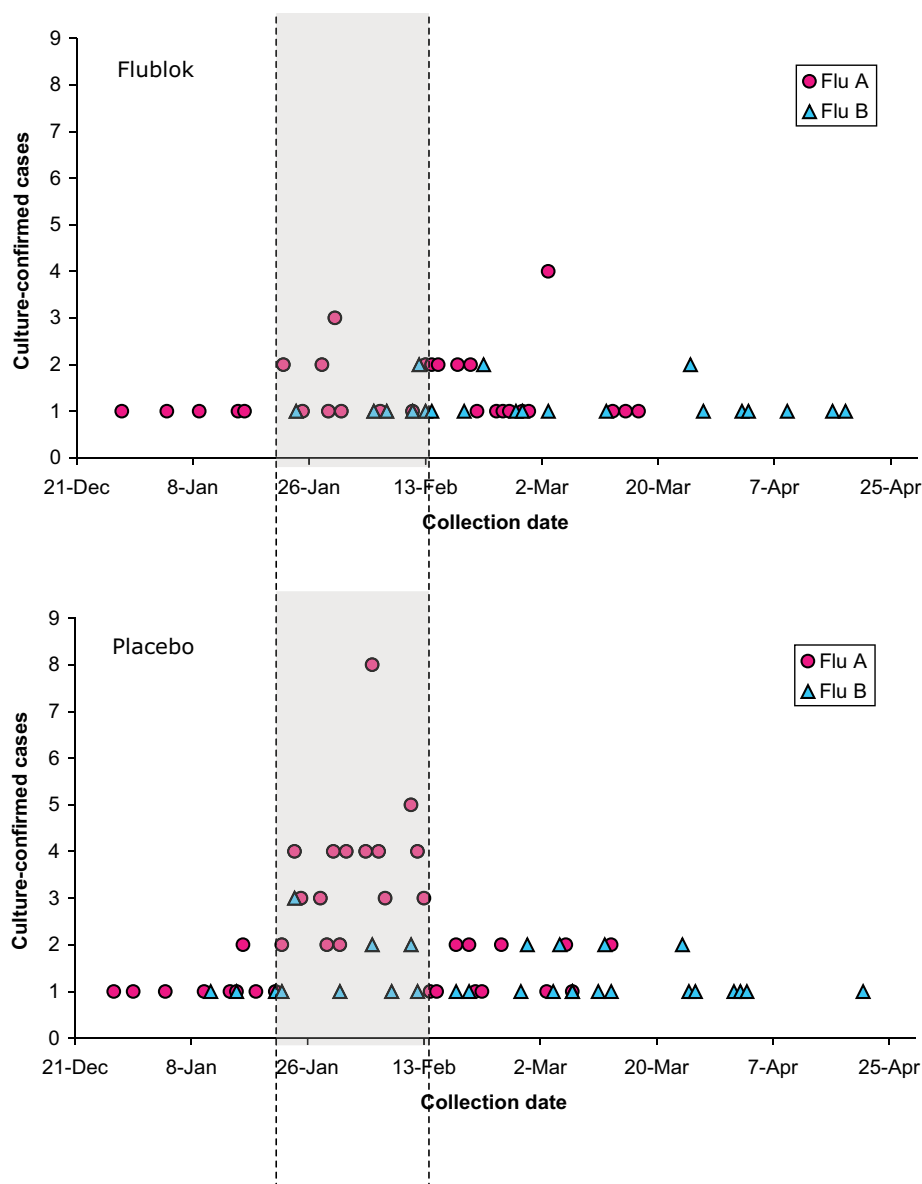
Additional limited efficacy data available from the other studies suggest that the higher antigen content in Flublok may contribute to improved protection. In study PSC01, culture-confirmed influenza infection was documented in four subjects receiving the lower dose (3%), one subject in the Flublok group (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 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 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 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 µg. Fisher's exact test showed a statistically significant reduction in culture-confirmed CDC-ILI between subjects who received Flublok (*versus* placebo;  $p=0.0146$ ). In PSC03,

only three cases of culture-confirmed CDC-ILI symptoms occurred: one in the Flublok group and two in the Fluzone group. In PSC06, 34 subjects reported ILI: 14 (5%) in the Flublok group and 20 (7%) in the Fluzone group. Unfortunately, viral cultures were not obtained uniformly from symptomatic subjects, so the incidence of culture-confirmed CDC-ILI cases could not be assessed.

### Flublok immunogenicity results

In studies PSC01, PSC03, PSC04 (subset) and PSC06, HAI 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, or placebo according to the criteria specified in the FDA *Guidance for Industry* [FDA, 2007].

Across all studies, serum HAI antibody responses to Flublok usually met the prespecified seroconversion criteria for all three virus strains, and also the prespecified criterion for the proportion of subjects with HAI titers at least 1:40 (seroprotection). This data were reviewed and reported in detail elsewhere [Cox *et al.*, 2008]. Here we compare the data obtained for Flublok in study PSC03 with the Sanofi high-dose (HD) influenza vaccine. As shown in Table 6, the immunogenicity of Flublok exceeds the immunogenicity of Fluzone for the influenza A viruses, but not for



**Figure 1.** Influenza isolates shown by collection date.

the influenza B viruses. Fluzone HD is always more immunogenic than Fluzone. The immunogenicity for the H3 influenza virus is similar between Flublok and Fluzone HD.

#### Discussion and conclusion

Flublok is a trivalent rHA vaccine with a mechanism of action similar to that of the licensed trivalent IIV, namely the induction of HAI antibodies to prevent influenza infection [Kida *et al.* 1983; Yoden *et al.* 1986]. The five pivotal studies support

the vaccine to be safe, immunogenic and effective in the prevention of influenza. The technology used to produce Flublok offers multiple advantages including the following: the vaccine will be an exact genetic match with the disease causing influenza virus; the manufacturing time is shortened; the manufacture process does not require biocontainment as no influenza virus is used in the process; no undesirable chemicals like formaldehyde are used in the process; the endotoxin content is carefully controlled; and no ovalbumin or residual chemicals present or antibiotics are

**Table 6.** Immunogenicity after administration of Flublok, Fluzone or Fluzone HD.

	<b>Study PSC03</b>		<b>Sanofi PI high dose*</b>	
	Adults age $\geq$ 65 years		Adults age $\geq$ 65 years	
	Flublok	Fluzone	Fluzone	Fluzone HD
Number of subjects	436	433	1248–1249	2529–2531
<b>A (H1N1)</b>				
GMT	177	148	67	116
GMT ratio	1.2		1.7	
Seroconversion	43	33	23	49
Difference in seroconversion	10		25	
<b>A (H3 N2)</b>				
GMT	339	199	333	609
<b>GMT ratio</b>	<b>1.7</b>		<b>1.8</b>	
Seroconversion	78	58	51	69
Difference in seroconversion	20		18	
<b>B (Note: different antigen in Flublok versus Fluzone)</b>				
GMT	150	195	52	69
GMT ratio	0.8		1.3	
Seroconversion	29	39	30	42
Difference in seroconversion	–10		12	
Data derived from clinical study PSC03 [Keitel <i>et al.</i> 2010] and Fluzone high-dose package insert [Sanofi-Pasteur, 2014]. The Geometric Mean Titer (GMT) ratio presented in bold shows comparable immunogenicity between Flublok and Fluzone HD.				

required to maintain a low bioburden during processing. The technology has been successfully scaled to the 21,000L bioreactor offering the possibility to respond to late appearing influenza viruses and deal with mismatches in the vaccine that result in poor performance as again reported for the 2014/15 influenza vaccine [D'Mello *et al.* 2015; Skowronski *et al.* 2015]. The production capacity for recombinant proteins is in principle unlimited as any cell culture facility could be used for its manufacturing. At this moment there are two licensed facilities for the manufacturing of Flublok, one in Meriden, CT, USA with a production capacity of approximately 0.5 million doses and another in Pearl River, NY, USA with a production capacity of approximately 2 million doses. The third facility that will be submitted for licensure is located in Japan and offers an immediate 20-fold increase in capacity that could be further expanded threefold. This means that with established yields a production capacity exceeds 1 billion doses of 15 $\mu$ g in a 6–9 month time period.

The commercial formulation of Flublok contains three times the amount of HA compared with the

standard dose IIVs and consequently induces higher antibody titers, which may be of particular importance to those most at risk for influenza (for example, the elderly [Keitel *et al.* 1994, 1996, 2010] or immunologically compromised [Safdar *et al.* 2006]). 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. While Flublok contains 135 $\mu$ g 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) [Renfrey and Watts, 1994; Hehme *et al.* 2003]. The vaccine was shown to be well tolerated and immunogenic in adults older than 18 years. When using standard dose Fluzone as a control to normalize safety data between Flublok and Fluzone HD it is apparent that solicited adverse events in Flublok recipients are generally much less frequent than those observed with Fluzone HD (Table 4). The immunogenicity data obtained for Flublok compare favorably with standard Fluzone and are similar for the H3 component of the vaccine between

Flublok and Fluzone HD. The overall efficacy of Fluzone HD is higher than standard Fluzone as around 24% of the breakthrough illness in Fluzone recipients could be prevented by administration of Fluzone HD [DiazGranados *et al.* 2014].

Importantly, Flublok has demonstrated protective efficacy in a field efficacy trial against drifted influenza viruses [Treanor *et al.* 2011; Cox and Anderson 2007]. In a *post hoc* analysis of this efficacy study, we noted that the efficacy of Flublok was much higher when the efficacy analysis was restricted to the same reference period (21 January–8 February 2008) used in an interim analysis for IIV, with an estimated effectiveness of 44% (95% CI 17–65%) [Belongia *et al.* 2009]. The observed reduction of VE for the interval after 8 February, which declined to 9.0% (95% CI –62.9%, 49.3) for type A and 21.4% (95% CI –47.5%, 58.6%) for type B could not be ascribed to the number of drift variants isolated, which was essentially equivalent in both time periods. The observed decrease in efficacy late in the influenza season remains unexplained, and it is unclear whether this is a finding that might be generally applicable or whether this was unique to this vaccine or influenza season.

In general, comparison of the Flublok efficacy results with the results of other assessments of the protective efficacy of influenza vaccines is complicated by differences in methodologies, populations, and antigenic match between vaccine and circulating strains in the specific year that studies are carried out. Two published studies have evaluated egg-grown inactivated vaccines in healthy adults using a placebo-controlled design during the 2007–2008 influenza season. In one study, conducted primarily in Europe, the overall efficacy of egg-grown inactivated vaccine against culture confirmed illness was 63%, and the lower 95% CI was 46.7% [Frey *et al.* 2010]. The predominant influenza A isolates in that study were H1N1 viruses, which were mostly vaccine like. In another smaller study done on college campuses in Michigan [Monto *et al.* 2009], the protective efficacy of TIV against culture-confirmed illness was 73% (95% CI 51–85%). In that study, 90% of influenza isolates were influenza A (H3N2), but the antigenic characterization of isolates was not reported. In other randomized trials, the protective efficacy of TIV was 22.3% in the 2005–2006 influenza season predominated by influenza B viruses and with overall low attack rates [Beran

*et al.* 2009], and 49.3% over two seasons, 2005–2007, with most cases due to antigenically variant viruses [Jackson, 2009].

Because of the difficulty in conducting placebo-controlled studies of influenza vaccine, especially as the target groups for vaccination have expanded, several recent assessments of influenza vaccine effectiveness have utilized the test-negative, case-control design. In this approach, individuals with a compatible illness are tested by a sensitive PCR diagnostic, and the vaccine histories compared between those with a positive PCR and those with negative diagnostic testing. Vaccine effectiveness is then calculated from the risk-adjusted odds ratio of vaccination. Estimates of overall inactivated vaccine effectiveness in these studies have ranged from 10% to 70% [Belongia *et al.* 2009; Skowronski *et al.* 2007, 2009] and are clearly impacted by antigenic differences between vaccine and circulating viruses, with the highest levels of effectiveness reported for H1 viruses [Skowronski *et al.* 2009] and the lowest levels for influenza B.

A large comparative efficacy study in approximately 9000 adults over 50 years old with a quadrivalent formulation is in progress during the 2014–2015 season to expand on this conclusion. Data from this double-blinded efficacy study will be available at the end of the influenza season and is expected to support approval of quadrivalent Flublok in time for the 2016–2017 influenza season. Furthermore, we anticipate this study to provide important insights regarding the role of mutations in the HA protein caused by adaptation to the egg-based manufacturing process in the low effectiveness of the 2014–2015 influenza vaccine. Mutations in the active site of the HA that occurred during adaptation to growth in eggs were reported in a previous season to be responsible for the ineffectiveness of the H3N2 component of the influenza vaccine [Skowronski *et al.* 2014]. Moreover, it has been previously reported that changes in the HA proteins in egg-grown influenza viruses when compared with primary isolates from infected individuals can result in a less effective vaccine [Katz *et al.* 1987; Wang *et al.* 1989; Rajakumar *et al.* 1990].

Recent studies suggest that the efficacy of influenza vaccine diminishes as the influenza season progresses [Eick-Cost *et al.* 2012]. This information should warn healthcare professionals about vaccinating individuals most at risk for influenza too early in the season.

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