



Memorandum

DATE: 15 January, 2013

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THRU: Jerry Weir, Ph.D.

CC: Anissa Cheung, M.S.

SUBJECT: BLA STN 125285, CMC review

PRODUCT: Flublok, influenza vaccine

SPONSOR: Protein Sciences Corporation

Review of the Chemistry, Manufacturing and Control Information Relevant for the drug substance (monovalent bulk rHA for H1, H3 and B strains) and drug product (trivalent formulation) submitted in the BLA application STN 125285 from Protein Science Corporation

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Executive Summary and Recommendations:

This BLA application from Protein Sciences Corporation (PSC) is for licensure of baculovirus-expressed insect cell-derived recombinant hemagglutinin (rHA), under the trade name Flublok. The vaccine is a mixture of purified recombinant hemagglutinins derived from H1, H3 and B influenza viruses recommended for seasonal influenza vaccine production. This trivalent product is a sterile solution with no added preservatives for intramuscular immunization. Each 0.5 ml dose contains 135 µg (45 ug of each strain) rHA and will be for active immunization of adults 18 - 49 yrs. This review focuses on the Chemistry, Manufacturing, and Control information for the drug substance (monovalent bulk rHA for H1, H3 and B strains) and drug product (trivalent formulation) submitted in the original BLA application received on 17 April, 2008 and associated amendments, in addition to responses to CR letters issued 29 August 2008 and 11 January 2010, and associated amendments.

Full-length HA genes are cloned from influenza A subtype H1N1 and H3N2 and influenza B viruses that are recommended for seasonal influenza vaccine production, and inserted into the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) for expression in expresSF+ cells using medium that is free of antibiotics and does not contain serum. The upstream manufacturing process starts with the culture of a working cell bank (WCB) that is used to expand the working virus bank (WVB) and to seed a ----(b)(4)----- . The ---(b)(4)----- manufacturing process begins approximately ---(b)(4)--- after infecting cells in the ---(b)(4)-- with recombinant baculovirus, -----(b)(4)----- . rHA expressed on the surface of the infected cells is extracted by disrupting the cells with Triton X-100. -----(b)(4)----- . The rHA is purified by -----(b)(4)----- chromatographies, -----(b)(4)----- . The monovalent bulk is filtered aseptically through a 0.22 µm filter and ---(b)(4)---- until formulation and filling. The release specifications for the monovalent bulk Drug Substance (DS) are: -----(b)(4)----- ; potency/----- (b)(4)----- .

----- . The potencies of recombinant H1, H3, and B monovalent bulks are measured at -----(b)(4)----- , and shipped to Hospira (McPherson, Kansas) for formulation and filling of the trivalent Drug Product (DP) into 2 ml glass vials. The DP specifications throughout expiry are: potency (≥45 µg/dose), identity (----(b)(4)-----), total protein ----(b)(4)-----, sterility ----(b)(4)-----, endotoxin ----(b)(4)-----, total DNA ----(b)(4)-----, Triton X-100 ---(b)(4)--, general safety test (pass), ----(b)(4)-----, appearance (clear, colorless liquid essentially free of visible particulates), -----(b)(4)----- .

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Reviewer comments: I am satisfied with PSC’s responses to the comments concerning process parameters, and concur with the changes they have made to improve process robustness. Data from downstream process steps of H1, H3 and B (3 lots each) were submitted to support validation of these steps for each strain. I have reviewed these data and concur that they demonstrate consistency of the downstream manufacturing process.

2. Changes in the manufacturing process

- -----

----- (b)(4) -----

- --b(4)-----

- Annual strain change: Conditions need to be optimized to support production of rHA from new strains recommended for vaccine production. -----
----- (b)(4) -----

----- . A list of updated conditions is provided for review in the strain change supplement. I concur with this approach to optimize production.
- Changes in the manufacturing process since pivotal clinical trials: Drug Product used in clinical trial PSC04 in 2007 was manufactured prior to process validation. Although manufactured at a different scale, the approach and consumables were the same except for the --- (b)(4) ----- step. Process improvements that were introduced did not result in qualitative differences in Drug Product.

Reviewer comments: The changes that have been made to the process since 2007 do not have a negative impact on product quality (including purity and potency), and therefore data collected in pivotal trials are valid. I concur with the use of unique conditions for the H1, H3 and B downstream process and agree with optimization of these conditions for strain change.

3. Formulation and fill

There were problems with initial formulation and fill steps conducted by Hospira. The following procedures have been put in place to ensure successful formulation and fill:

- ----- (b)(4) -----

- ----- (b)(4) -----

- ----- (b)(4) -----

- -----(b)(4)-----

- -----(b)(4)-----

Reviewer comments: I have reviewed data from fill runs PV5, PV6 and PV7 and concur that these validation runs demonstrate consistency of the fill process at full scale (b(4)). This shows that the steps taken to improve the formulation and fill process have provided a way to meet release specifications consistently.

4. Drug Product shelf life

Three Flublok trivalent drug product batches of the 2007-08 formulation were tested for stability in a ---(b)(4)--- study. The H1, H3, and B monovalent bulk substance batches were unique for each of the 3 trivalent product batches. In terms of appearance, b(4), and sterility, all compliance criteria were met. Total protein content (BCA assay) was well maintained through ---(b)(4)--- at 2-8°C. The 2-8°C SRID potency data on these 3 batches indicate that the H1 and H3 components undergo substantial loss in potency by -(b)(4)--, in fact, the mean potency at 3 months was about 80% of Day 0. -----(b)(4)-----
achieve a shelf life limit of 16 weeks. -----

----- (b)(4) -----

----- Data submitted in Amendment 64 (2 October 2012) toward extension of trivalent Drug Product shelf life to -(b)(4)- are described in section 7 of this review; CBER did not agree to this extension because the data were insufficient to support the change.

Reviewer comments: I am confident that PSC can manufacture product that consistently meets specifications to 16 week expiry. PSC has a protocol in place to monitor stability of product in filled vials from the first 3 lots produced. Potency will be measured every -(b)(4) until the 16 week shelf-life. My recommendation is for approval of a Drug Product shelf life of 16 weeks.

5. Potency measurement

The (b)(4) of the monovalent bulk is usually (b)(4) and therefore the expectation is that the potency measured by SRID should be very similar to -----(b)(4)-----
----- This is not always the case, with SRID values sometimes exceeding the absolute amount of rHA in the product i.e. -----(b)(4)-----
-----.

Resolution: PSC has developed a procedure to evaluate performance of reference reagents at the beginning of each manufacturing season to qualify their use in SRID.

- Egg-grown antigen is not always suitable for use as a reference for rHA SRID assays. This was the case for rHA of A/California/07/09 (H1) in which assay conditions used to generate precipitant rings with egg-grown reference antigen did not allow formation of rings with rHA.

Resolution: After extensive exchange of information (details in section 5), PSC provided rHA to CBER that was lyophilized and calibrated for use as a reference antigen. Both CBER and PSC are monitoring stability of the reference so that fresh reference material can be prepared as soon as it is needed.

Reviewer comments: PSC plans to use CBER-approved SRID reagents and is aware that CBER prefers a conservative approach, requiring extensive characterization of reference antigens and antisera if the usual reference reagents are not suitable. To be prepared for a manufacturing campaign, PSC has initiated SRID reagent qualification testing. PSC will include data from the SRID reagent qualification testing in strain change supplements so that CBER can evaluate these results and provide guidance if necessary.

6. PSC04 lot consistency trial

The manufacturing process had not been validated prior to pivotal clinical consistency trials, including PSC04. In 2007, 3 drug product lots, 50-07010 (Lot A), 50-07011 (Lot B) and 50-07014 (Lot C), each formulated with different monovalent bulk lots, were tested in clinical study PSC04 to evaluate clinical lot consistency. Each drug product batch was formulated to contain 45µg of each antigen as determined by SRID. HAI titers to H1 and B components for individuals vaccinated with different lots were similar, but HAI titers to the H3 (A/Wisconsin/05) component of Flublok Lots B and C were significantly lower than the titers following vaccination with Lot A. Despite this, CBER immune response criteria were met for all three lots. The lower immunogenicity was due to inaccuracy of SRID potency measurements – the H3 monovalent bulks used to formulate Lots B and C had ----(b)(4)----- . Formulation based on SRID values therefore resulted in these lots not containing as much H3 as Lot A which was formulated with a monovalent bulk that had a ----(b)(4)----- .

Resolution: A DS specification for ----(b)(4)----- has been added, together with procedures to -----(b)(4)----- . As a result every Flublok vaccine will contain at least 45 µg HA protein/dose.

Reviewer comments: Clinical consistency was demonstrated for H1 and B components of 3 vaccine lots used in PSC04. I am confident that the root cause of the difference in H3 HAI titers was formulation based on SRID values that were inaccurate for 2 lots with -----(b)(4)----- . In my opinion, since this inaccuracy of potency measurement is now controlled, and there is provision to formulate vaccine with no less than 45 µg HA protein/dose, it is not necessary to verify consistency of the H3 component in clinical studies.

REVIEWER'S RECOMMENDATION:

PSC has provided data demonstrating consistent production of Drug Substance (monovalent bulk) and trivalent Drug Product, has appropriate specifications for intermediates and Drug Product that are tested in validated assays, is using a validated SRID assay to measure potency with results comparable to those measured at CBER, has sufficiently characterized their product and its stability, and has appropriately addressed inspectional concerns. Based on the CMC data submitted, I recommend approval of PSC's license application for Flublok influenza vaccine, with a shelf-life of 16 weeks.

CMC Review

1. Manufacture sites and contract laboratories

Protein Sciences Corp., Meriden, CT: At this site, rHA monovalent bulk concentrates (drug substance) are manufactured; release tests and stability tests of drug substance are performed; small scale formulation of drug product, potency, DNA content and stability tests of drug product are performed.

Hospira, McPherson, Kansas: At this site, the drug product is formulated, filled, packaged and labeled. Container closure tests and release tests are performed (with exception of potency and DNA quantitation) at Hospira.

----- (b)(4) -----

2. Manufacturing materials and the manufacturing process

2.1 General information

The purified recombinant influenza hemagglutinin (rHA) drug substances included in the Flublok drug product are derived from strains representing influenza A subtypes H1N1 and H3N2 and influenza B. The rHA genes are cloned from the strains approved by FDA on an annual basis. The selected viruses are obtained from CDC and then the full-length HA gene is cloned into the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV). PSC has developed the expresSF+ cell line, which can be propagated in a serum-free medium, as the substrate for recombinant baculovirus infection and rHA production. This is a non-transformed, non-tumorigenic, continuous cell line derived from the fall army worm, *Spodoptera frugiperda*.

Based on PSC's experience with rHA substances of several influenza strains, the monovalent bulk proteins are (b)(4)---, and the purified rHA's migrate on SDS-PAGE -----
----- (b)(4) ----- with molecular weights of about 65 kDa, -----

----- (b)(4) -----

----- By electron microscopy, rosette-like micelle structures are observed. Purified rHA can agglutinate avian red blood cells, which indicates its ability to recognize sialic acid receptors as well as its higher order association into rosettes.

Potency of the vaccine is measured by single radial immunodiffusion (SRID) assay. This assay uses CBER-approved reference antigen and HA-specific sheep antisera that are used to measure the potency of US licensed trivalent inactivated influenza vaccines. However, in instances when these reagents are not suitable, recombinant HA has been prepared and qualified for use in the assay as described in section 5 of this review.

2.2 Raw materials

PSC implements a raw materials and vendor management program in which raw materials containing the highest risk (final formulation components) are under the tightest control (most extensive testing). Each raw material has been assigned a PSC-part number (A through F) to allow for segregation upon receipt.

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Table 6. Flublok Drug Product Specifications: at release and through expiry

Test	Method (Reference)	Acceptance Criteria
Appearance	Visual inspection --(b)(4)--	Colorless, clear liquid essentially free of visible particles
Identity	--b(4)--- -----	--b(4)----- -----
Bacterial Endotoxin	----(b)(4)--- ----(b)(4)---	---(b)(4)-----
Sterility	Membrane Filtration (21 CFR 610.12)	No growth observed
Potency at t=0	SRID conducted within ---(b)(4)-- - (QT0077)	------(b)(4)----- -
Potency throughout shelf-life	SRID (QT0077)	≥45 µg/dose for each HA component (H1, H3, and B) ----- -(b)(4)----- -----
Purity	------(b)(4)-----	(b)(4)
DNA Content	---(b)(4)-- (PSC QT0082)	≤ 10 ng per dose
Total Protein	BCA assay (PSC QT0012)	Mean ₁₀ ≤285 µg/dose
------(b)(4)----- -----	------(b)(4)----- -----	---(b)(4)-----
Triton X-100	---(b)(4)--- -----	(b)(4)
---(b)(4)---	---(b)(4)---	------(b)(94)-----
(b)(4)	----(b)(4)----- ----(b)(4)-----	---(b)(4)---
General Safety	21 CFR 610.11	All animals survive and weigh no less than at time of injection
Fill Volume	--(b)(4)--	Not less than 0.5 mL

Potency: Specifications for Drug Product potency at release and Drug Product potency through the expiry are different because there is significant decay of potency over the 16 week shelf-life. To

ensure the potency specification of $\geq 45 \mu\text{g}/\text{dose}$ is met to expiry, -----
----- (b)(4) -----

Total protein: The specification of $285 \mu\text{g}/\text{dose}$ takes into account clinical safety data. This specification is significantly greater than the potency --- (b)(4) --- since each rHA may be blended at --- (b)(4) --- and in the worst-case scenario, --- (b)(4) --- at time of fill. Protein concentration provides a convenient indication of fill consistency – the protein concentration is measured ----- (b)(4) -----.

Triton X-100: Triton X-100 -----
----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

Fill volume: The fill volume specification is “not less than labeled volume”. The labeled volume is 0.5 mL per vial. This specification is appropriate for this product.

General safety test: A general safety test is conducted on each drug product lot, with a specification that all animals inoculated will survive and on day 7 post-vaccination not weigh less than the day of vaccination.

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evidence that all 3 rHA subtypes are more stable in terms of potency when vials are closed with new-style stoppers. However, it should be noted that SRID values at time 0 may be inaccurate as some components had readings 30-50% higher at 1 week than at Day 0.

Stability testing of DP in filled vials was performed on product filled at Hospira in support of fill validation. These data are included in amendment 64 (received 1 October 2012) in which PSC requested extension of Drug Product shelf-life. They provided stability data from each of the 3 drug product fill validation lots: batches --(b)(4)- (PV5), --(b)(4)- (PV6), and --(b)(4)- (PV7) to demonstrate FluBlok specifications (appearance, sterility, b(4), protein concentration, potency) were met out to ---(b)(4)---. PSC requests extension of the shelf life to ---(b)(4)---. In PSC's August 24, 2009 submission, PSC estimated shelf lives for each rHA antigen in the 2007/2008 formulation of FluBlok -----

----- (b)(4) -----

----- Based on this analysis, PSC proposed a shelf life of 16 weeks. ----- (b)(4) -----

Reviewer comments: I do not agree that the data submitted support an extension of Flublok shelf-life for several reasons:

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- -----

----- (b)(4) -----

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-----~~(b)(4)~~-----

9. ----(b)(4)-----

9.1. -----(b)(4)-----:
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9.2. Strain change amendments:

PSC has submitted information to support production of rHA from new strains recommended for manufacture of trivalent influenza vaccines. These amendments include the CoA for the influenza virus from which HA was cloned, information describing the generation of the working virus bank, characterization of the WVB including the HA sequence and its alignment with the reference strain, optimized conditions for manufacture, and antigenic analysis for antigens that are not identical to the reference sequence. PSC has agreed (amendment 66 (16 October 2012)) to including data demonstrating the use of approved potency reagents in SRID assays in future strain change supplements (requested in information request 28 September 2012).

9.2.1. Amendment 34 (10 August 2010): strain change information to include 2 strains, A/California/07/2009 and A/Perth/16/2008, previously not manufactured by PSC for the 2010/2011 vaccine. Since CBER approved reference reagents were not suitable for potency testing, PSC provided well-characterized and freshly prepared rHA to CBER for lyophilization and calibration as described in section 5).

9.2.2. Amendment 53 (27 February 2012): strain change information for 2011/12 vaccine composition. Since serum-free conditions were used at all stages of manufacture, including generation of the WVB, new working virus banks that met all specifications were prepared for each of the 3 strains included in the 2011/12 formulation: A/California/7/2009 (H1N1); A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008.

9.2.3. Amendment 56 (25 June 2012): strain change information to support 2012/2013 vaccine formulation. New working virus banks were prepared for H3 and B components. The genes were cloned from A/Victoria/361/2011 and B/Wisconsin/1/2010, respectively.

Reviewer summary and comments: As for other influenza vaccine manufacturers, submission of strain change supplements is necessary to ensure production of antigen for the recommended vaccine strains. Amendments from PSC to support strain changes have included detailed technical information regarding ~~(b)(4)~~ changes. While these are not essential components of the strain change supplement, the strain change

submission serves as a convenient location to document all supporting information and so we have not discouraged PSC from including this. The essential information needed for review of strain change are: the CoA for the influenza virus from which HA was cloned, information describing the generation of the working virus bank, characterization of the WVB including the HA sequence and its alignment with the reference strain, and antigenic analysis for antigens that are not identical to the reference sequence. We have asked PSC to include data to demonstrate suitability of potency assay reagents (information request 28 September 2012) as during the review cycle we were surprised that PSC did not always approach CBER to resolve potency testing problems. PSC agreed to include this data (amendment 66) to demonstrate reagents are suitably qualified for use in PSC's potency assay. This will give us confidence that CBER reagents are adequate for testing rHA potency of new strains, or will provide the means for us to resolve any problems.

10. Pre-approval inspections

Protein Sciences Corporation manufacturing facility, process development and quality control laboratories were inspected 3 times over the course of this application. Multiple items pertaining to control of the manufacturing process and need for written procedures were identified as 483 items during an inspection in July 2008, inconsistent manufacture of monovalent bulk and need for investigations of deviations were items identified as 483 items during an inspection in October 2009. During a final inspection in November 2012 it was clear that the manufacturing process was controlled and appropriate oversight of the process was in place. All 483 items were appropriately addressed.