



Memorandum

Date: January 14, 2013

From: Arifa S. Khan, Ph.D., DVP

Subject: Cell Substrate Review for STN 125285

Established Name: Influenza Vaccine, Purified Recombinant Influenza
Hemagglutinin (derived from H1, H3, B viral strains)

Proprietary Name: Flublok

Sponsor Name: Protein Sciences Corporation

To: FILE STN 125285

Through: Jerry Weir, Ph.D., DVP

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EXECUTIVE SUMMARY

The *expresSF+* cell line (SF+) used in the production of Flublok is the first insect cell substrate used for development of an influenza virus vaccine. The cells were adapted to grow as a serum-free (SF) cell line starting with the Sf9 insect cell line obtained from the American Type Culture Collection (ATCC), which originated from the fall army worm or *Spodoptera frugiperda*. Although another insect cell line has been used for vaccine production, Sf9 cells are a novel cell substrate in vaccines. The cells have been tested (Master Cell Bank [MCB], Working Cell Bank [WCB], or End of Production cells [EOP]) extensively according to the “Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications, 2010” and by additional assays developed specifically for insect adventitious viruses, insect latent viruses, and insect endogenous retroviruses (also called errantiviruses).

The cell substrate was demonstrated to be non-tumorigenic and free of adventitious agents based upon the recommended testing including *in vivo* and *in vitro* adventitious virus assays and by using additional assays that were specifically developed to investigate for the presence of latent viruses and novel insect viruses. The latter included:

----- (b)(4) -----
-----; testing for (b)(4) insect virus families using ---(b)(4)----- assays;
and testing for ----(b)(4)----- using a virus-specific (b)(4) assay. -----

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

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

FULL REVIEW: Flublok

Influenza Vaccine, Purified Recombinant Influenza Hemagglutinin (derived from H1, H3, B viral strains)

I have reviewed the cell substrate information in the following sections and subsections provided in the Original BLA:

- 3.2.S.2.3.1 Development and History of the Sf9 and *expresSF+*[®] Cell Lines**
 - 3.2.S.2.3.1.1 Historical Summary of the Sf9 Cell Line
 - 3.2.S.2.3.1.2 Generation of the Sf9 Master Cell Bank lot ---(b)(4)-- and Working Cell Bank lot -----(b)(4)-----
 - 3.2.S.2.3.1.3 Qualification Sf9 Cell Banks
 - 3.2.S.2.3.1.4 Generation of the Serum-Free *expresSF+* Cell Line

- 3.2.S.2.3.2 *expresSF+* Master Cell Bank (MCB) Used for rHA Production**
 - 3.2.S.2.3.2.1 Generation of a New Master Cell Bank for Commercial Production of rHA
 - 3.2.S.2.3.2.2 Qualification of MCB lot ----(b)(4)-----
 - 3.2.S.2.3.2.3 Additional Testing on MCB lot ---(b)(4)-----

- 3.2.S.2.3.3 *expresSF+* Working Cell Bank (WCB) Used for rHA Production**
 - 3.2.S.2.3.3.1 Generation of a Working Cell Bank For Commercial Production of rHA
 - 3.2.S.2.3.3.2 Population Doubling Times for WCB lot ----(b)(4)-----
 - 3.2.S.2.3.3.3 Qualification of WCB lot ----(b)(4)-----
 - 3.2.S.2.3.3.4 Additional Testing on WCB lot ----(b)(4)-----

- 3.2.S.2.3.4 *expresSF+* End of Production (EOP) Cells**
 - 3.2.S.2.3.4.1 Generation of *expresSF+* End of Production (EOP) Cells
 - 3.2.S.2.3.4.2 Qualification of WCB lot ----(b)(4)----- at EOP
 - 3.2.S.2.3.4.3 Additional Testing on WCB lot ----(b)(4)----- at EOP

Additionally, I have reviewed the cell substrate and related product information submitted in response to Complete Response (CR) letters and Information Request (IR) letters in the following amendments to the Original BLA:

- **Amendment 6** dated July 1, 2008 in response to Cell Substrate questions submitted in IND 11951, amendment 44: *question 4, (b)(4) screen for ---(b)(4)----- viruses and more extensive ----(b)(4)----- analysis;* and amendment 54: *questions 7/8, (b)(4) screens for ----(b)(4)----- viruses*
- **Amendment 13** dated April 27, 2009 in response to CR letter 8/29/2008: *comment 5a-f, in vitro adventitious agent testing of SF+ cells and (b)(4) testing of cells and monovalent bulks*

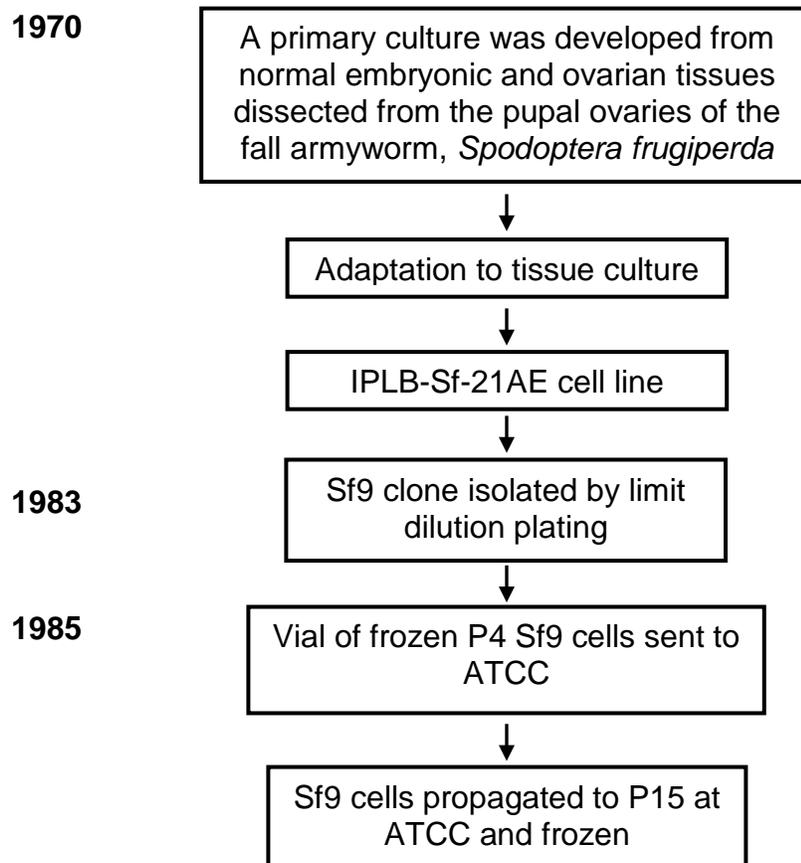
- **Amendment 19** dated August 24, 2009 in response to IR letter 7/30/2009: *comment 5c:-----(b)(4)----- assays and clarification of results of testing MCB and EOP cells and monovalent bulks*
- **Amendment 22** dated October 6, 2009 in response to IR letter 10/1/2009: *comment 5cii: (b)(4) testing of monovalent bulk*
- **Amendment 25** dated December 11, 2009 in response to IR letter 10/1/2009: *information related to ---(b)(4)--- in final trivalent product*
- **Amendment 31** dated June 29, 2010 in response to CR letter 1/11/2010: *comment 2 (5cii), investigations of (b)(4) assays and results of Flublok and comment 3 (5f): insect virus testing results on MCB ---(b)(4)---*
- **Amendments 35** dated September 10, 2010 & **Amendment 37** dated October 6, 2010 in response to CR letter 1/11/2010: *comment 2 (5cii), investigations of (b)(4) assays and results of Flublok*
- **Amendment 39** dated January 5, 2011 in response to discussions during a teleconference on December 10, 2010 regarding IR letter 11/12/2010: *comment 4, (b)(4) investigations and protocols*
- **Amendment 43** dated April 5 2011 in response to IR letter 11/12/2010: *comment 4, revised protocol for investigating ---(b)(4)-- in cell lysate and development of (b)(4) assays for analysis of ---(b)(4)----- in drug product*
- **Amendment 47** dated September 16, 2011 in response to IR letter 5/27/2011: *comment 4, follow up of (b)(4) results in cell lysate and additional information for reviewing ---(b)(4)--- in cell supernatant*
- **Amendment 51:** PSC submitted (b)(4) investigational plans on Feb 15, 2012.
- **Amendment 57** dated July 16, 2012 in response to IR letter 1/17/2012: *comment 4, clarification of results of (b)(4) testing of cell lysate and drug product and information to complete ---(b)(4)-- analysis of drug product for -----(b)(4)-----; In response to IR letter 3/2/2012 and IR letter 4/3/2012: proposed ---(b)(4)----- studies for -----(b)(4)-----*
- **Amendment 71** dated November 26, 2012 in response to IR letter 11/9/2012: *results to complete review of -----(b)(4)----- studies and additional data to complete review ---(b)(4)----- in drug product*
- **Amendment 74** dated December 17, 2012 in response to PMR/PMC letter 12/13/2012: *comment 6, post marketing commitment to -----(b)(4)-----*
- **Amendment 75** dated December 18, 2012 in response to IR letter 12/14/2012: *comment 2, complete summary of (b)(4) investigations*

DEVELOPMENT AND HISTORY OF THE Sf9 AND *expresSF+*[®] CELL LINES

1. Historical Summary of the Sf9 Cell Line

The Sf9 cell line was cloned via dilution plating of the mixed population cell line IPLB-Sf-21AE by C. Cherry and G. Smith at Texas A&M University (unpublished, 1983). At the time the Sf9 line was developed, IPLB-Sf-21AE cells had been in continuous culture since being isolated in 1970 from primary cultures of normal embryonic and ovarian tissues that were dissected from pupal ovaries of the fall armyworm, *Spodoptera frugiperda* (Vaughn et al., 1978). In April 1985, a vial of frozen passage (P) 4 Sf9 cells was sent to American Type Culture Collection (ATCC) where it was propagated and a P15 expansion culture stored in liquid nitrogen for distribution. **Figure 1** below summarizes the generation of the Sf9 cell line deposited at ATCC. As part of ATCC's accessioning process, Sf9 cells were subjected to isoenzyme analysis to verify the species and tested for contamination with bacteria, fungi and mycoplasma. Because the parent cell line IPLB-SF21AE was originally isolated from an explant of mixed ovarian tissues, the exact cell type of the parent line and clone Sf9 is unknown. Morphologically, Sf9 cells are loosely attached rounded cells approximately 15-20 microns in diameter with distinct boundaries. Sf9 cells can be distinguished from the parent IPLB-SF21AE cells and from other lepidopteran and mammalian cell types by a combination of karyology and isoenzyme analysis.

Figure 1. Generation of the Sf9 cell line



75 Pages determined to be not releasable: b(4)