

MEMORANDUM DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Vaccines Research and Review
Division of Viral Products

Date: October 12, 2012

To: The File STN 125408

From: Haruhiko Murata, DVP

Through: Keith Peden, DVP
Jerry Weir, DVP
Anissa Cheung, DVP

Sponsor: Novartis Vaccines and Diagnostics, Inc.

Subject: Cell Substrate Review for STN125408;
Human Influenza Virus Type A (H1N1; H3N2) and B
Hemagglutinin Vaccine, Purified, Inactivated (Madin Darby
Canine Kidney Cells)

My descriptions/comments are in Arial font, while the texts provided by the Sponsor through the BLA are in Times New Roman font.

The following acronyms/abbreviations are used:

BPL: beta-propiolactone
CTAB: cetyltrimethylammonium bromide
EoP: end of production
FCC: influenza cell culture
MCB: master cell bank
MDCK: Madin-Darby canine kidney (cell)
TSE: transmissible spongiform encephalopathies
VRBPAC: Vaccines and Related Biological Products Advisory Committee
WCB: working cell bank

Source, History, and Generation of the Cell Substrate

The MDCK cell line was initially established from the kidney of an apparently normal, adult, male cocker spaniel in 1958 by Madin and Darby at the University of California, Berkeley, and this cell line was submitted to the ATCC at some time between 1958 and 1967. Cells originating from Madin and Darby (Berkeley, California, USA) were transferred to a colleague, (b)(4) within the (b)(4) for further study. These cells were passaged an undetermined number of times by (b)(4) before transfer to (b)(4) cloned the cell line in 1966 at passage (b)(4). Derivatives of the cloned cell line were provided to (b)(4).

(b)(4) received passage number (b)(4) of the cells cloned by (b)(4) designated the (b)(4) passage as MDCK (b)(4) and then passaged it (b)(4) times (MDCK (b)(4) to establish a Master Cell Stock for use in manufacture of veterinary vaccines. The Master Cell Stock (Bank) was passaged (b)(4) times, which represented the last passage to be used in production (MDCK (b)(4)). Both the Master Cell Bank (MDCK (b)(4) and end-of-production cells (b)(4) were tested in accordance with 9 CFR 113 (United States Code of Federal Regulations) requirements for cell substrates intended for veterinary vaccine use. Approval for use of the MDCK cell line as a production substrate was granted by USDA-APHIS on 24 August 1972 (USDA license No. 263). US veterinary license No. 272 was subsequently granted in 1992 for production of an MDCK-based (b)(4) vaccine. Cellular material derived from the licensed MCB (MDCK (b)(4) was provided to Novartis (formerly Behringwerke) in 1985.

Novartis (formerly Behringwerke) adapted the MDCK (b)(4) cell line to suspension growth and (b)(4) over the course of (b)(4) passages between 1988 and 1992. Novartis's suspension culture-adapted MDCK cell subline was designated MDCK (b)(4). The MDCK (b)(4) subline was further adapted to (b)(4) conditions in 1995 (passages (b)(4)). A major research cell bank was established at passage (b)(4) in 1995. MCB (b)(4) was prepared from the suspension culture, (b)(4) adapted MDCK (b)(4) subline. In 1996, Novartis transferred the MDCK (b)(4) cell line, at (b)(4) for final adaptation to (b)(4) growth. (b)(4) completed the adaptation in 1996 and froze the cells. These cells were designated MDCK (b)(4). In 1998 (b)(4) passaged the MDCK (b)(4) cell line (b)(4) more before returning them to Novartis for preparation of a new master cell bank (b)(4).

Overview of Cell Banks

A (b)(4)-tiered cell bank system represented by (b)(4), master, working, (b)(4) cell banks was established for the FCC (Influenza Cell Culture) vaccine manufacturing process. The first cell bank was generated after the MDCK cell line had been adapted to suspension culture and (b)(4) conditions. Preparation and testing of this early master cell bank (b)(4) is briefly described as the data establish absence

of detectable adventitious agents at this stage of derivation. The current set of cell banks ---(b)(4)---, master, working, and ----(b)(4)----- was established after ---(b)(4)---- adaptation of MDCK ---(b)(4)-. Working cell banks (-----(b)(4)----- derived from --(b)(4)--- MDCK ---(b)(4)---- master cell bank ----(b)(4)---- will be used for commercial production of FCC monovalent bulk and influenza working seed virus. Qualification of the cell banks (MCB, WCB, ---(b)(4)-- as free from adventitious agents included assessment of microbiological purity, mycoplasma, standard *in vivo* and *in vitro* adventitious viral agent (AVA) testing, and additional testing for -----(b)(4)-----
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Reviewer’s Comments:

A diagram depicting the derivation of the cell banks is provided in Appendix Fig. 1.

17 Pages Determined to be Not Releasable: (b)(4)

Reviewer's Comments:

- The testing program is consistent with 9 CFR regulations as well as the Guidance Document for cell substrate characterization and qualification issued by CBER in 2010.
- In addition to the standard *in vivo* and *in vitro* tests, (b)(4) tests were performed for specific adventitious agents, encompassing an extensive list of -----(b)(4)----- . These tests were demonstrated to have the appropriate level of sensitivity. Many of these tests were performed due to the passage history of the Sponsor's MDCK cells and their past exposure to biological material derived from various species (for example, sera from -----(b)(4)----- sources).
- The Sponsor's cell banks (MCB, WCB, ---(b)(4)---- appear to be free of adventitious agents.

Tumorigenic and Oncogenic Potential of MDCK Cell Substrate

Tumorigenicity and oncogenicity studies performed with either -----
----- (b)(4) ----- prepared from the MDCK cell
line used to produce Novartis's inactivated, trivalent influenza vaccine. The study designs
reflect ICH, CBER, and WHO guidance concerning cell characterization, with the
exception that the study duration was increased from ---- (b)(4) ----- . Studies were
initiated at the request of, and in consultation with, the Center for Biologics Evaluation
and Research (CBER) of the USFDA. The primary series of tumorigenicity and
oncogenicity studies were conducted with material from the --- (b)(4) --- cell bank. (b)(4)
studies were performed by ----- (b)(4) -----
----- . For all studies, the route of administration was by subcutaneous injection into
the flank of the hind limb to allow for ease of palpation/measurement of nodules.

[(b)(4)]

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

tumorigenic MDCK cells could be used for the manufacture of inactivated influenza vaccine.

- More recently, a VRBPAC Meeting held in September 2012 discussed the use of human tumor-derived cell lines (such as HeLa cells) for the manufacture of vaccines. The Committee came to a general consensus that potential safety issues (primarily adventitious agents and DNA oncogenicity) can be adequately addressed. No issues were identified that *a priori* precluded the use of such cells.

10 Pages Determined to be Not Releasable: (b)(4)

4 Pages Determined to be Not Releasable: (b)(4)

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[(b)(4)]

Risk Assessment

A simple method was established to rate the risk of adventitious agents if different cell substrates (embryonated eggs, MDCK cells, ---(b)(4)-- or combinations thereof) were used for influenza virus isolation and subsequently for virus propagation during vaccine manufacturing. Data regarding the replication of various viruses and microbial agents were gathered from published literature or by internal laboratory studies and translated into semi-quantitative scores. Other characteristics of the viruses relevant to vaccine manufacturing (e.g., enveloped or naked, resistance to environmental and processing conditions, stability against chemical inactivation) were also scored. Based on these scores, an algorithm was defined and used to calculate summary scores, which rate the relative risk of occurrence of an adventitious agent in the final influenza vaccine. The methodology and results of this approach were published in a peer-reviewed journal (Vaccine 26:3297, 2008). The main purpose of this scoring system was to rate the risks associated with the introduction of MDCK cells as a new cell substrate to produce influenza vaccines. According to this analysis, MDCK cells were found to reduce contamination risks associated current influenza vaccines. Detailed observations include:

- Avian non-enveloped viruses introduced via embryonated eggs appear to be the most likely virus contaminants to be expected. Low to negligible risks were found for mycoplasma and chlamydia and for almost all enveloped viruses. Of the enveloped viruses only avian retrovirus and herpes simplex virus had risk scores above background.
- Of the mammalian agents, mainly reovirus should be considered a potential source of viral contamination.
- Compared with existing egg-derived vaccines, the MDCK cells used to produce vaccine virus from influenza virus strains isolated in eggs do not introduce new or higher risk, as the host spectrum of MDCK cells is similar to that of embryonated eggs. For all of the viruses scored, MDCK cells used to produce vaccines from egg-derived isolates gave either the same or lower risk scores than embryonated eggs used for isolation and production.

Having accumulated data on the virus replication in the cell substrate used, on the inactivation or process removal of various viruses, and with consideration of applicable detection limits for virus-exclusion tests, a process-specific risk assessment was made using quantitative data relative to infectious doses. When necessary, realistic worst-case assumptions were made to adequately cover variables, such as potential virus titers, virus passage numbers, dilutions, or inactivation results with different types of the same virus family. The assessment starts at the earliest relevant step, which is the isolation of an influenza virus strain from a human throat sample, considers egg passages, MDCK cell passages to prepare viral seeds, (b)(4) detection limits for tested materials, and extends through -----(b)(4)-----

----- In contrast to risk analysis described above (which rates the probability of occurrence of a process contaminant), this risk assessment invariably assumed a real contamination. For example contamination by human viruses was assumed in the primary isolate and – because of human manipulation – also in the seed virus. Avian virus contaminants were assumed to occur during egg-passage. Two extreme model cases were also included: a porcine circovirus that survived the double inactivation within the trypsin preparation and a parvovirus introduced from the environment. The analysis was published in a peer reviewed journal (Vaccine 26:3332, 2008). The assessment encompasses 24 different virus types or groups and also include Chlamydia and mycoplasma. The results for all viruses and agents clearly show that any of these modeled contaminants would be reduced to levels that are unable to cause infection. For viruses that are infectious to humans, the results would mean that more than 100,000 doses would need to be administered to one individual to accumulate a single infectious unit. Even if the calculated worst-case contaminant level is considered, the final vaccine product would still be unable to transmit infectious viruses.

modest estimates for combined inactivation (BPL and CTAB splitting/------(b)(4)-----, such as -----(b)(4)-----, are not expected to replicate in the Sponsor's MDCK cells. Importantly, viruses designated to be of concern on the basis of their established ability to replicate in the Sponsor's MDCK cells, were robustly inactivated -----(b)(4)-----.

- Currently, Flucelvax uses seed viruses derived from egg-grown viruses distributed by custodian laboratories on behalf of the WHO Collaborating Centers for Reference and Research on Influenza. -----(b)(4)-----.

- The Sponsor reaches a reasonable conclusion that TSE risks are minimal. Bovine serum is currently not used to propagate the Sponsor's cells during routine manufacturing.
- Key studies and risk analyses associated with adventitious agents have been published in peer-reviewed scientific publications (referenced above). Such efforts by the Sponsor are commendable and ought to be encouraged to the extent possible in order to enhance public confidence in the Sponsor's product.
- Overall, the Sponsor's strategy for mitigation of adventitious agents risk is acceptable. Safety associated with use of the Sponsor's MDCK cells, in terms of adventitious agents, appears to be at least comparable, if not superior, to conventional use of embryonated chicken eggs.

Information Requests

Responses to information requests pertinent to the cell substrate are discussed below.

Amendment 10 (125408/0.9; received by CBER on April 26, 2012)

This amendment contains responses to the following two queries:

Please provide the following documents related to the assessment of risk associated with residual DNA: (1) DRA-Application of Defined Risk Assessment to Use of Cell Bank, (2) Chiron pre-read 5-4188B1_18, (3) Report 228847, (4) Report 235243, (5) Report 229373, (6) Report 250384, and (7) Report 280908.

Reviewer's Comments:

These documents were requested because the Sponsor's risk analysis strategy had undergone a significant change since the 2005 VRBPAC meeting (from an analysis based on DNA size distribution to one based on ---(b)(4)----. The derivation of the estimated ratio of oncogenes to (b)(4) elements ---(b)(4)-- oncogenes/(b)(4) is explained more completely in Report 280908. The evolution of the Sponsor's DNA risk analysis during the course of product development is described more completely in the requested documents.

Please provide the validation report for the host-cell protein (b)(4) (Report 403410).

Reviewer's Comments:

The validation of host-cell protein removal during ---(b)(4)----- processing was described in 3.2.S.3.2.5-1 (study period 04/2004-10/2005); this document references the requested assay validation report 403410 for the host-cell protein (b)(4). The information contained in 403410 is acceptable and corroborates a later supplemental assay validation report included in the original submission (Attachment 3.2.R.3-257228; carried out in 2007/2008).

The validation of host-cell protein removal during -----(b)(4)--- processing (described in 3.2.S.3.2.5-1) was performed with process 1.0 samples. In discussion with other CMC reviewers (Zhiping Ye, Xianghong Jing, and Anissa Cheung), this was found to be acceptable because other analytical data (assessment of -----(b)(4)----- corroborate the removal of host-cell proteins, and process 1.1 includes an -----(b)(4)----- step --(b)(4)-. It is notable that the -----(b)(4)----- step assessed for process 1.0 -----(b)(4)----- step) contributed the most to host-cell protein removal ---(b)(4)--

Reviewer's Summary/Conclusions

The Sponsor's MDCK cell substrate is tumorigenic when inoculated into nude mice. A long-standing proscription of the use of tumorigenic cells for vaccine manufacture has been in place since a recommendation in 1954 by the US Armed Forces Epidemiology Board (Nat'l Cancer Inst Monogr 29:463, 1968) to use primary cells for the manufacture of adenovirus vaccines rather than HeLa cells (derived from human cervical cancer). The Sponsor's product (Flucelvax), upon licensure, will be the first vaccine marketed in the US that is produced in tumorigenic cells.

The use of the Sponsor's MDCK cells for manufacture of inactivated influenza vaccine was publicly discussed at the November 2005 VRBPAC meeting. In addition, a related discussion on human tumor-derived cell substrates took place at the September 2012 VRBPAC meeting. While some members of the VRBPAC expressed reservations regarding the use of tumorigenic cells, the Committee came to a general agreement that the Sponsor's approach to risk mitigation was acceptable (documented in the publicly available transcripts for the VRBPAC meetings in November 2005 and September 2012).

The theoretical risks associated with the use of tumorigenic cells (centering on the concern over the potential transfer of factors that might neoplastically transform cells of the vaccine recipient) can be mitigated by (1) comprehensive testing for adventitious agents and (2) reducing or inactivating residual cell-substrate DNA (Biologicals 37:190, 2009). On the basis of the extensive testing performed by the Sponsor, I agree with the conclusion that the Sponsor's MDCK cell banks are free of adventitious agents. The clearance validation studies of multiple model viruses (including viruses known to be resistant to inactivation by physical and chemical means, such as ----(b)(4)----- provide additional safety information. Furthermore, the probability of contamination by adventitious agents during vaccine manufacture is greatly reduced by (1) the testing strategies in place with respect to the vaccine seed and the vaccine bulk harvest, (2) the use of media free of animal-derived components, and (3) the inactivation protocol applied to --- (b)(4) ----- . Finally, the Sponsor provides compelling evidence that the Sponsor's MDCK cells are inherently permissive only to a narrow spectrum of viruses (comparable with chicken eggs), thus forming a basis for a natural obstacle to contamination by adventitious agents. Theoretical risks associated with residual cell-substrate DNA is effectively addressed by the Sponsor through (1) reduction of DNA quantity to a maximal level of 10 ng per dose and (2) reduction of --- (b)(4) --- through the manufacturing process (largely due to BPL). The negative results obtained by in vivo oncogenicity testing of the Sponsor's MDCK cell lysate/DNA provide supportive assurance of safety.

There are substantial benefits to using MDCK cells in the manufacture of influenza vaccine. Cells that are banked and qualified can supply the requisite biomass of vaccine substrate on short demand (as opposed to chicken eggs, which are the conventional substrate for currently licensed influenza vaccines),

thereby allowing a more rapid response in the event of an influenza pandemic. In addition, human influenza viruses are known to undergo adaptations in avian substrates that can impact antigenicity; thus, a vaccine manufactured entirely* in mammalian cells, such as MDCK cells, may have improved efficacy by providing a better antigenic match to circulating human viruses.

On the other hand, the risks associated with tumorigenic cell substrates are largely theoretical, and thus, exceedingly difficult to quantify; they may, in actuality, be non-existent, although such a conclusion can never be empirically established. However, engendering public confidence in vaccination programs necessitates a prudent course and the theoretical risks need to be adequately addressed. It is my conclusion that the Sponsor has done so through extensive characterization of the Sponsor's MDCK cell substrate. With respect to use of the Sponsor's MDCK cells, the known benefits appear to me to outweigh the unknown (theoretical) risks.

On the basis of information pertaining to the MDCK cell substrate, I recommend approval of the Sponsor's licensure application for Flucelvax.

* The Sponsor is presently seeking licensure for a process using egg-grown reassortant virus seed progenitors; -----
----- (b)(4) -----

3 Pages Determined to be Not Releasable: (b)(4)