Live, Attenuated Influenza Vaccine Manufactured in MDCK Cells

VRBPAC

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Silver Spring, Maryland

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To develop a safe, reliable vaccine technology to enhance the nation’s supply of annual influenza vaccine and increase pandemic preparedness
Meeting Goals

- Describe the benefits of switching from egg production to cell-produced influenza vaccines

- Describe the safety of production of LAIV in MDCK cells
  - Characterization of the cell line
  - Manufacturing technologies for high quality vaccines
  - Defined risk assessments

- Enable VRBPAC to recommend moving forward with clinical development of a cell produced live, attenuated influenza vaccine (LAIV)
MedImmune’s robust manufacturing process

Safe & Reliable MDCK Produced LAIV

MedImmune’s MDCK cell substrate

- Extensive characterization & testing
- Banked cells ready for use
Proprietary

+ 

- Removes cells and reduces other cellular components
- Aseptic manufacture
- Comprehensive vaccine testing
Overview of Presentation

Background
- MedImmune overview
- FluMist® (Live, Attenuated Influenza Vaccine)
- Egg and cell based production technologies

Producing a safe, reliable LAIV in cell culture
- Cell line selection
- Cell line testing results
- Manufacturing technology
- Product testing

Defined risk assessment of vaccine safety

Conclusions
Worldwide biologics unit for AstraZeneca

Headquartered in Gaithersburg, MD

Approximately 3,000 employees in the US, UK and the Netherlands

FluMist® (LAIV) licensed in the US since 2003
  - Safety profile supported by nearly 11 million commercial doses distributed
LAIV is an Important Component of Influenza Prevention and Pandemic Preparedness

- **Established efficacy against seasonal influenza**
  - Cross protection against mismatched strains*

- **Strong immune responses seen after a single dose in immuno-naive populations**

- **Considerable manufacturing efficiency**
  - Live vaccine produced in either eggs or cells requires lower manufacturing capacity than inactivated vaccine

- **Innovative intranasal delivery**

* FluMist PI; June 2008
FluMist Shown to be Highly Efficacious in Both Adults and Children

- **57 Completed Clinical Studies***
  - With Pediatric Subjects (38 studies)
    - 6 Studies TIV Controlled
    - 19 Studies Placebo Controlled
    - 13 Studies with Other designs
  - Adult Subjects Only (19 studies)
    - 4 Studies TIV Controlled
    - 9 Studies Placebo Controlled
    - 6 Studies with Other designs

**FluMist efficacy demonstrated:**
- In both adults and children
- Across multiple influenza seasons
- Through trials conducted worldwide

*Company sponsored studies. Sponsors included Aviron, Wyeth and MedImmune*
**Benefits of Cell-Based Production**

<table>
<thead>
<tr>
<th>PRODUCTION SUBSTRATE</th>
<th>Eggs (SPF)</th>
<th>Cell Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposure of flock to environmental agents</strong></td>
<td>Low risk, high impact</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Preproduction characterization</strong></td>
<td>- Limited</td>
<td>- Extensive</td>
</tr>
<tr>
<td></td>
<td>- Inherent contamination</td>
<td>- Sterile</td>
</tr>
<tr>
<td><strong>Manufacturing procedures</strong></td>
<td>Need to control contamination *</td>
<td>Controlled</td>
</tr>
<tr>
<td><strong>Egg allergies limit use</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

* Contributed to significant inactivated vaccine shortages in 2004/05 season

*Proprietary*
Larger quantities of bulk doses are produced more rapidly than in eggs
  - To produce 150 million bulk doses of vaccine
    - > 12 weeks in eggs
    - > 4 weeks with 2 (2,500L) bioreactors

Increasing scale is faster than eggs
  - 12 months needed to increase size of chicken flock & egg production
  - Scale is limited by availability of number of bioreactors
LAIV vaccines are 6:2 reassortants
The internal genes of cell and egg produced vaccines are genetically identical

Plasmid rescue of 6:2 vaccine strains is part of the current egg produced FluMist® product

Plasmid rescue eliminates the risk from any potential contaminants in the wild type (human) isolate
Vaccines Produced Using Egg and Cell Substrates Are Comparable

- Vaccine traits are encoded in the sequence of the vaccine strains
  - Cell and egg produced strains are genetically identical
- The *ca* and *ts* characteristics are retained that make the vaccine safe

<table>
<thead>
<tr>
<th>Analytical Test</th>
<th>Comparability between egg and cell produced vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Genomic Sequence</td>
<td>✓</td>
</tr>
<tr>
<td>Phenotypic Analysis (<em>ca</em> and <em>ts</em>)</td>
<td>✓</td>
</tr>
<tr>
<td>Host Cell Susceptibility</td>
<td>✓</td>
</tr>
<tr>
<td>Virus Protein Expression</td>
<td>✓</td>
</tr>
<tr>
<td>Virus Morphology and Size</td>
<td>✓</td>
</tr>
<tr>
<td>Replication and Attenuation in Ferrets</td>
<td>✓</td>
</tr>
<tr>
<td>Immunogenicity and Efficacy in Ferrets</td>
<td>✓</td>
</tr>
<tr>
<td>Safety profile in Animal Models</td>
<td>✓</td>
</tr>
</tbody>
</table>
Cell Culture Production of LAIV is a Significant Advance for Public Health

- Cell culture produced LAIV is an important component of influenza prevention and pandemic preparedness
  - Increases reliability of supply of influenza vaccines
  - Accelerates speed and quantity of vaccine supply
  - Retains all the advantages of LAIV
Overview of Presentation

■ Background
  - MedImmune overview
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■ Producing a safe, reliable LAIV in cell culture
  - Cell line selection
  - Cell line testing results
  - Manufacturing Technology
  - Product testing

■ Defined risk assessment of vaccine safety

■ Conclusions
Considerations for Selecting a Safe Cell Line

- Readily characterized to assure product safety
  - No evidence of inherent oncogenic agents

- Supports replication of different influenza serotypes and strains

- Consistent cell growth and high virus productivity at large scale production

- Grows in serum-free media
Of the 13 cell substrates assessed, only MDCK cells had all the requisite characteristics for manufacturing LAIV.

- MRC-5, WI-38; human diploid cells used for other vaccines
- 293, CHO, FRhL-2, MDCK, NIH 3T3, Vero and other mammalian continuous cell lines
- CEF, CEK, DF-1 and other avian cell lines
1958 - Madin-Darby canine kidney (MDCK) cell line was derived from the kidney of a normal cocker spaniel

1964 - Deposited at American Type Culture Collection (ATCC CCL-34)

2001 - MedImmune obtained cells from ATCC CCL-34 for preparation of pre-Master Cell Bank
MDCK Cells Contain Different Subpopulations

- Subclones with differing biochemical properties can be isolated

- Tumorigenicity from different sources are variable

<table>
<thead>
<tr>
<th>Source</th>
<th>Minimum number of cells needed to form tumors in nude mice</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiles, et al (1976)</td>
<td>&gt;10^6</td>
<td>No tumors detected</td>
</tr>
<tr>
<td>Solvay (VRBPAC 2005)</td>
<td>&gt;10^7</td>
<td>ATCC parent cells</td>
</tr>
<tr>
<td>Solvay (VRBPAC 2005)</td>
<td>10^5</td>
<td>Solvay cell bank</td>
</tr>
<tr>
<td>Novartis (VRBPAC 2005)</td>
<td>10^1</td>
<td>Suspension cells</td>
</tr>
</tbody>
</table>
Biological Cloning Isolates Subclones with Specific Properties

Example: Subclone A
- Adherent
- Low tumor potential
- High virus yield

Example: Subclone B
- Suspension
- Moderate tumor potential
- Moderate virus yield

Parental Cells

Subclone

Filtration

Subclone
Steps to Isolate a Uniform MDCK Cell Line with Low Tumorigenicity

- Tumorigenicity of MedImmune’s MDCK cell line controlled by focusing on 3 key areas
  - Clonal isolation of a cell line
  - Contact inhibited growth
  - Use of a robust serum-free growth media
Quality Built into the Cell Bank From Multiple Aspects

- Obtain low passage MDCK cells from ATCC
- Biologically clone the cells by limiting dilution
  - Establish a uniform population from a single genetic parent
  - Evaluate and choose a clone which supports vaccine strain replication
  - Enable tracking of exposure of cells to animal derived products
- Transfer cells to serum-free media
  - Elimination of exposure to adventitious agents from animal derived products
- Produce cell banks in compliance with cGMP
A limited number of cell clones supported higher levels of virus productivity

One clone was subsequently expanded in serum free media

Master and working cell banks produced under cGMP in serum-free media

Productivity stable over 25 passages
## Assessing Potential Risks of Using MDCK Cell Lines Through Testing

### Potential risks

- **Adventitious agents**

- **Tumorigenicity**
  - Evaluating whether intact cells can establish a tumor

- **Oncogenicity**
  - Evaluating whether cellular components can induce tumors

### Assessing Potential risks

- in vitro and in vivo testing for specific and general agents

- Evaluate tumorigenicity in nude mice

- Evaluate oncogenicity of MDCK DNA and cell lysate in multiple rodent species

**Based on CBER’s approach for continuous cell lines**
Extensive Testing Did Not Detect Adventitious Agents in MedImmune’s MDCK Cells

General tests (broad detection assays)

- Sterility, mycoplasma, mycobacterium
- in vivo safety
  - Newborn mice, adult mice, guinea pigs and embryonated eggs
- in vitro safety
  - MRC-5, Vero, MDCK, MDBK, HeLa, BHK-21 & RK-13
Additional Testing Did Not Detect Adventitious Agents in MedImmune’s MDCK Cells

- **Specific tests (targeted agents)**
  - Over 30 PCR and other tests
  - Human, simian, canine, rodent, equine, and porcine agents

- **Induction studies**
  - MDCK cells induced with chemical agents
  - Cells evaluated for the presence of latent RNA and DNA viruses
Induction Studies Look for Latent Viruses in the Cell Line

MDCK Cells

Induction with TPA and NaB for DNA viruses

Assays performed:
- TEM
- Broad PCR for detection of:
  - Herpesviruses
  - Polyomaviruses
  - Adenoviruses
  - Papillomaviruses

Induction with AzaC and IdU for retroviruses

Assays performed:
- TEM
- fPERT
- Test articles passaged on multiple indicator cell lines
  - TEM
  - fPERT assays

No latent viruses were detected in MedImmune’s MDCK cells following induction with chemical agents.
MedImmune’s MDCK Cells Contain No Detectable Adventitious Agents

- Multiple testing strategies employed

- Conclusion
  - No evidence of adventitious agents detected in MedImmune’s MDCK cells
Extensive Tumorigenicity & Oncogenicity Tests of MedImmune’s MDCK Cells

- **Studies - overview**
  - MDCK EOP cells (approximately 3 passages beyond manufacture of vaccine)
  - Approximately 40 animals per group
  - Observed for 6 months
  - Conducted in compliance with GLP requirements

- **Tumorigenic potential of intact MDCK cells**
  - Adult athymic nude mice
  - Newborn athymic nude mice

- **Oncogenic potential of MDCK cell lysate and cell DNA**
  - Newborn rodents (athymic nude mice, rats & hamsters)
MDCK Cell Substrate Characterization – Tumorigenicity Testing

Methods: Adult & Newborn Nude Mouse Models

- 10^1 MDCK
- 10^3 MDCK
- 10^5 MDCK
- 10^7 MDCK
- PBS (-)
- HeLa Cells (+)

0.2 ml of MDCK Cells
0.2 ml of MDCK Cells
0.2 ml of MDCK Cells
0.2 ml of MDCK Cells
0.2 ml of PBS
0.2 ml of HeLa Cells

6-Month Observation

Examined for presence of progressive tumors at the site of inoculation and systemically
### MedImmune’s MDCK Cells Do Not Form Tumors in Nude Mice

<table>
<thead>
<tr>
<th>Study</th>
<th>Test Sample</th>
<th>Number of animals injected</th>
<th>Tumors at site of Injection (SOI)</th>
<th>Tumors at other locations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumorigenicity (Adult Mouse)</strong></td>
<td>Negative Control (DPBS)</td>
<td>33</td>
<td>0</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Positive control (10&lt;sup&gt;7&lt;/sup&gt; HeLa cells)</td>
<td>41</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MDCK cells (10&lt;sup&gt;1,10^3,10^5,10^7&lt;/sup&gt;; 44/grp)</td>
<td>176</td>
<td>0</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Tumorigenicity (Newborn Mouse)</strong></td>
<td>Negative Control (DPBS)</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Positive control (10&lt;sup&gt;7&lt;/sup&gt; HeLa cells)</td>
<td>44</td>
<td>44</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>MDCK cells (10&lt;sup&gt;1,10^3,10^5,10^7&lt;/sup&gt;; ~44/grp)</td>
<td>171</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lymphoma & a bronchiolo-alveolar adenoma: spontaneous murine tumors

<sup>b</sup> Histiocytic sarcoma (10<sup>5</sup> MDCK group): spontaneous murine tumor confirmed by antibody staining and SINE PCR

No MDCK tumors detected
Immunohistochemistry Confirmed Murine Origin of Tumor

- α Canine Ezrin
- α Murine Galectin-3

Histiocytic Sarcoma, Liver (Tumor study, $10^5$ group)

Control MDCK cell pellet
Canine-specific short interspersed nuclear elements (Can-SINE)*
- 130 - 150 bp
- Present approximately every 5 - 8.3 kb in the canine genome
- Constitute about 1.8 - 3% of the genome

*Das M et al. (1998) *Mamm Genome* 9, 64–69

Observation of histiocytic sarcoma

<table>
<thead>
<tr>
<th></th>
<th>Canine</th>
<th>Rodent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINE PCR</td>
<td>─</td>
<td>+</td>
</tr>
</tbody>
</table>

Spontaneous Murine Tumor—Not MDCK Derived
MDCK Cell Substrate Characterization – Oncogenicity Testing

MDCK Lysate (From $10^7$ cells)
- 0.05 ml
- 0.1 ml
- 0.1 ml

MDCK DNA (100 µg; intact)*
- 0.05 ml
- 0.1 ml
- 0.1 ml

Newborn nude mouse
Newborn hamster
Newborn rat

Newborn nude mouse
Newborn hamster
Newborn rat

6 Month Observations

Examine for the presence of tumors at site of inoculation and systemically

* One human dose contains < 1 ng DNA
## MedImmune’s MDCK Cells Do Not Contain Oncogenic Components

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDCK Lysate (10⁷ cell equiv)</th>
<th>MDCK DNA (100 μg)</th>
<th>Tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice</td>
<td>Hamster</td>
<td>Rat</td>
</tr>
<tr>
<td>Non Injected (n=25)</td>
<td>1ᵃ</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PBS (n=45)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Test (n=45)</td>
<td>0</td>
<td>0</td>
<td>1ᵈ</td>
</tr>
</tbody>
</table>

ᵃ Bronchiolo-alveolar adenoma in the lung; spontaneous tumor / no canine DNA by SINE; confirmed rodent origin

ᵇ Only 44 animals were available for post-weaning randomization in this group

ᶜ Hepatocellular adenoma & skin hemangiosarcoma; spontaneous tumors

ᵈ Hind leg carcinoma; no canine DNA by SINE; confirmed rodent origin

ᵉ Nephroblastoma; no canine DNA by SINE; confirmed rodent origin

No oncogenicity detected
No Oncogenicity Detected in MedImmune’s MDCK Cells

- All tumors were of rodent origin; no canine DNA detected
- Tumors observed in these studies were spontaneous and observed in other studies in these species
- Balanced frequency between negative control groups and test article groups
Summary – Extensive Data Demonstrates Safety of MedImmune’s MDCK Cells

- **Adventitious Agent Testing**
  - No evidence of adventitious agents by comprehensive testing regimen

- **Tumorigenicity**
  - No evidence of local or systemic tumorigenicity (up to $10^7$ cells)

- **Oncogenicity**
  - No evidence of local or systemic oncogenicity caused by MDCK cellular components (cell DNA or cell lysate)
## Addressing Potential Risks of Using MDCK Cell Lines Through Manufacturing

<table>
<thead>
<tr>
<th>Potential risks</th>
<th>Addressing Potential risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Adventitious agents</td>
<td>■ Control through plasmid rescue and manufacturing processes</td>
</tr>
<tr>
<td>■ Tumorigenicity</td>
<td>■ Removal of all cells through multiple filtration steps</td>
</tr>
<tr>
<td>♦ Evaluating whether intact cells</td>
<td>■ Reduce quantity and size of residual MDCK DNA and quantity of</td>
</tr>
<tr>
<td>can establish a tumor</td>
<td>MDCK proteins</td>
</tr>
<tr>
<td>■ Oncogenicity</td>
<td></td>
</tr>
<tr>
<td>♦ Evaluating whether cellular</td>
<td></td>
</tr>
<tr>
<td>components can induce tumors</td>
<td></td>
</tr>
</tbody>
</table>
Manufacturing Process Removes MDCK Components at Multiple Steps

**MDCK WCB**

- Bioreactor - Cells on Microcarriers -
  - Bioreactor - Infected Cells -
    - Prefiltered Virus Harvest
      - Filtration #1
        - 1.2 µm & 0.45 µm
      - Intact Cell Removal
    - Clarified Virus Harvest
      - Ultrafiltration & Diafiltration
        - MDCK DNA & Protein Removal
      - Intermediate 1
        - Benzonase & Affinity Chromatography
          - MDCK DNA Digestion & DNA and Protein Removal
        - Intermediate 2
          - Diafiltration
            - MDCK DNA & Protein Removal
        - Intermediate 3
          - Filtration #2 (sterile)
            - 0.45 µm / 0.2 µm
          - Intact Cell Removal
    - Clarified Virus Harvest
      - Monovalent Vaccine Bulk

Proprietary
Filtration Removes Intact Cells

- Removal of intact cells occurs at multiple steps
- Process capable of removing at least $10^{21}$ cells
  - This represents 100 billion times more cells than in a typical bioreactor
  - Laboratory studies demonstrate capabilities of the filters
- Multiple filtration steps ensure safety of the process
Reduction of MDCK Quantity and Size
- Multiple steps combine to remove >90% of MDCK DNA
- One dose contains less than 1 ng of MDCK DNA
  > WHO recommends a 10 ng limit for parenteral products produced from continuous cell line substrates
- Median size is reduced to 450 bp
- 90% DNA below 1 kb

Reduction of host cell protein
- Removes >90% of MDCK protein
- One dose contains approximately 0.5 µg of MDCK protein
Rats were given equivalent amount (100 µg) of sheared MDCK DNA
- Tissues measured for residual DNA at various time points
- Route of vaccine administration (intranasal) provides an additional safety barrier
The Bulk Vaccine is Extensively Tested to Ensure Safety and Purity

<table>
<thead>
<tr>
<th>Pre-filtration</th>
<th>Post-filtration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma</td>
<td>Sterility</td>
</tr>
<tr>
<td>Microbiological Culture</td>
<td>Potency</td>
</tr>
<tr>
<td>Cell Culture</td>
<td>Genotype</td>
</tr>
<tr>
<td>in vitro Adventitious Agents</td>
<td>Phenotype</td>
</tr>
<tr>
<td>Neutralize influenza</td>
<td>Attenuation</td>
</tr>
<tr>
<td>Multiple indicator cell lines</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>in vivo Adventitious Agents</td>
<td>pH</td>
</tr>
<tr>
<td>Neutralize influenza</td>
<td>Color and Appearance</td>
</tr>
<tr>
<td>Multiple species of sensitive</td>
<td>Residual Host Cell DNA</td>
</tr>
<tr>
<td>host systems</td>
<td>Residual Host Cell Protein</td>
</tr>
<tr>
<td>Potency</td>
<td>Residual Benzonase</td>
</tr>
<tr>
<td>Bioburden</td>
<td>Osmolality</td>
</tr>
</tbody>
</table>

The Bulk Vaccine is Extensively Tested to Ensure Safety and Purity.
Control of all materials
- Minimal exposure to animal derived components
- Highly characterized MDCK cell banks
- Highly characterized vaccine seeds

Production equipment and environment
- Closed systems; isolated from the environment

Multiple purification steps ensure safety of the product
- Removes all intact cells
- Reduces the quantity and size of DNA
- Reduces host proteins
- Sterile filtration
Overview of Presentation

- **Background**
  - MedImmune overview
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- **Producing a safe, reliable LAIV in cell culture**
  - Cell line selection
  - Cell line testing results
  - Manufacturing Technology
  - Product testing

- **Defined risk assessment of vaccine safety**

- **Conclusions**
A defined risk assessment was employed based on CBER guidelines (and in line with other manufacturers) addressing theoretical concerns associated with:

- Intact cells
- Oncogenicity
- Infectivity

Reinforces product safety assurance

Risk Assessment – Tumorigenicity

**Observations**
- Modern processes remove intact cells from the product
  > Filtration removes at least 21 log cells

**Calculation of safety margin**
- Theoretical cells in one dose (no removal or lysis of cells): 5.6 log
  Clearance factor – cell number = safety margin
  21.4 log – 5.6 log = 15.8 log margin of safety

**Conclusion**
- Risk of one dose containing an intact MDCK cell is $1.6 \times 10^{-16}$
- Only 1 out of 6.3 quadrillion ($6.3 \times 10^{15}$) doses may contain a single intact MDCK cell
  > This value represents the risk that one person would receive a cell-containing dose if the entire world population were immunized every 50 minutes for 100 years
- MedImmune’s MDCK cells demonstrate low tumorigenic potential
Oncogenicity assessment – quantitative modeling uses conservative assumptions

- Need to assume an active oncogene in genomic DNA
  - Animal data demonstrated no oncogenicity in MDCK DNA
- Quantitation extrapolated from conservative assumptions
  - 1 ng of oncogenic plasmid DNA elicits tumors in nude mice
- Determine how much genomic DNA would be needed to deliver the same oncogene dose as 1 ng of the plasmids

Safety factor calculation – what does it tell us

- Based on a conservative amount of MDCK DNA in one dose (1 ng)
  - How many doses of vaccine would equal the oncogene dose in the mouse experiments?
  - What is the oncogenicity risk in one single dose?
- Assess quantitative impact of DNA digestion
If cellular DNA contained an active oncogene it would take over 1 million doses to deliver the oncogenic dose used in the mouse studies

- It would take over 5 billion doses assuming 25 µg of plasmid was required*

Benzonase Digestion Genomic DNA
Oncogene Sequence Intact or Non-Intact

Intact DNA

Haploid Canine Genome 2.41 x 10^9 bp

Digested to 1925 bp

Intact Oncogene

Non-Intact Oncogene

DNA in Vaccine 450 bp

Proprietary
Calculate the safety factor including DNA digestion

- Model worst case scenario 1 ng of MDCK DNA digested to 1925 bp in length
- Approximately 1 in every 2000 oncogene fragments is intact

Final Safety Factor for oncogenicity of residual MDCK DNA

- The risk of an oncogenic event in one dose is $4.2 \times 10^{-10}$
- The safety factor is $2.4 \times 10^9$
  > It would take over 2 billion doses of vaccine to deliver the dose administered to one mouse (equals over 400,000 L of vaccine)
Risk Assessment of Potential Infectivity in Residual DNA

- Infectivity assessment – quantitative modeling uses conservative assumptions
  - Need to assume an active provirus in genomic DNA
    > No evidence of infectious proviruses in MDCK DNA
  - Quantitation extrapolated from in vitro studies*
    > 150 ng of HIV-1 cDNA in a plasmid results in infectious virus
    > Degrading the DNA to a mean of 650 bp abolished infectivity
  - Determine how much genomic DNA would be needed to deliver the same provirus dose as 150 ng of the plasmid

Safety factor calculation – what does it tell us

- Based on a conservative amount of MDCK DNA in one dose (1 ng)
  - How many doses of vaccine would equal the provirus dose in the in vitro experiments
    - OR -
  - What is the risk of infectivity from a provirus in genomic DNA in one single dose

- Assess quantitative impact of DNA digestion
Risk Assessment Outcome of Infectivity of DNA

- Risk of a provirus in one dose is $1.3 \times 10^{-12}$
- Safety factor is $7.2 \times 10^{11}$
  - Extrapolation of in vitro data – no infectivity would be detected in at least 700 billion doses of vaccine
Addressing Potential Risks of Using MDCK Cell Lines

<table>
<thead>
<tr>
<th>Testing</th>
<th>Manufacturing</th>
<th>Risk Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Adventitious agents</td>
<td>■ Adventitious agents</td>
<td>■ Adventitious agents</td>
</tr>
<tr>
<td>♥ General and specific tests on MDCK Cells</td>
<td>♥ Plasmid rescued seeds</td>
<td>♥ Not applicable</td>
</tr>
<tr>
<td>♥ Tests of vaccine bulk</td>
<td>♥ Closed process</td>
<td></td>
</tr>
<tr>
<td>■ Tumorigenicity</td>
<td>■ Tumorigenicity</td>
<td>■ Tumorigenicity</td>
</tr>
<tr>
<td>♥ Adult nude mice</td>
<td>♥ Multiple filtration steps</td>
<td>♥ 6.3 x 10^{15} times excess cell clearance</td>
</tr>
<tr>
<td>♥ Newborn nude mice</td>
<td>♥ remove intact cells</td>
<td></td>
</tr>
<tr>
<td>■ Oncogenicity</td>
<td>■ Oncogenicity</td>
<td>■ Oncogenicity</td>
</tr>
<tr>
<td>♥ MDCK genomic DNA</td>
<td>♥ Reduce DNA size and quantity</td>
<td>♥ Safety factor for DNA oncogenicity: 2.4 x 10^9</td>
</tr>
<tr>
<td>♥ MDCK cell lysate</td>
<td>♥ Reduce protein quantity</td>
<td>♥ Safety factor for provirus infectivity: 7.2 x 10^{11}</td>
</tr>
<tr>
<td>♥ Multiple species</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proprietary
MedImmune’s MDCK cell substrate

- No detectable adventitious agents
- Low tumorigenicity
- No detectable oncogenicity

mediated

= Safe & Reliable MDCK Produced LAIV

MedImmune’s robust manufacturing process

- Acellular vaccine
- Reduction of DNA quantity & size
- Reduction of host protein
- Routine vaccine Testing
Cell Culture Produced LAIV is Safe and Fills a Need for Influenza Vaccine

- Safety of our products is MedImmune’s primary focus
- Scientifically sound advance in influenza vaccine production
- Cell culture production increases the supply and reliability of vaccine
  - Seasonal flu impact
  - Pandemic preparedness
Live, Attenuated Influenza Vaccine Manufactured in MDCK Cells

VRBPAC

September 25, 2008

Silver Spring, Maryland

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